UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. **Nama Ketua Penyelidik:** *Name of Research Leader* NURZALINA BT. ABDUL KARIM KHAN

☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr/ *Dr* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):** *School/Department* PUSAT PENGAJIAN SAINS FARMASI

3. **Nama Penyelidik Bersama:** *Name of Co-Researcher* PROF. MADYA DR. YUSRIDA DARWIS

4. **Tajuk Projek:** *Title of Project* Design and Evaluation of Orally Disintegrating Tablets of Water Soluble and Water Insoluble Drugs

5. Ringkasan Penilaian/*Summary of Assessment*	Tidak Mencukupi *Inadequate* 1	2	Boleh Diterima *Acceptable* 3	Sangat Baik *Very Good* 4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☑	☐
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	☑	☐
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☐	☑	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☐	☐	☑	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☐	☐	☑
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☐	☑	☐

6. **Abstrak Penyelidikan**
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**. Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Sila lihat Lampiran 1 dan 2.

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Sila lihat Lampiran 3

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research

Bahasa Malaysia	*Bahasa Inggeris*
Tablet berkecai oral, Terlindung rasa, Ondansetron Sumatriptan suksinat, Farmakokinetik	Orally disintegrating tablets, Taste masked, Ondansetron, Sumatriptan succinate, Pharmacokinetics

8. **Output dan Faedah Projek**
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

Ravi, S., Khan, N. and Darwis, Y. (2011) Development and validation of an RP-HPLC–UV method for the determination of ondansetron in rabbit plasma: Application to a pharmacokinetic study. *Acta Chromatographica*, **23(4)**, 579–593.

Sheshala, R., Khan, N., Chitneni, M. and Darwis, Y. (2011) Formulation and in vivo evaluation of ondansetron orally disintegrating tablets using different superdisintegrants. *Arch. Pharm. Res.*, **34(11)**, 1945-1956.

Ravi Sheshala, Nurzalina Khan, and Yusrida Darwis (2011) Formulation and optimization of orally disintegrating tablets of sumatriptan succinate. *Chemical & Pharmaceutical Bulletin*, **59(8)**, 920-928 .

Sheshala, R., Darwis, Y. and Khan, N. (2009) Development and validation of an RP-HPLC–UV method for analysis of sumatriptan succinate in pharmaceutical dosage forms. *Acta Chromatographica*, **21(3)**, 421–432.

Sheshala, R., Darwis, Y. and Khan, N. (2009) Development and validation of an RP-LC-UV method for the determination of ondansetron: Application to pharmaceutical dosage forms. *Chromatographia*, **70**, 75-81.

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

Hasil penyelidikan dapat dikomersilkan oleh syarikat farmaseutikal

* Sila berikan salinan/*kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Sheshala Ravi, PhD, Graduasi Dis 2010

ii) Lain-lain:
Others:

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

Tiada.

Tandatangan Penyelidik
Signature of Researcher

07/03/2012
Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

Disokong.

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

Professor Dr. Syed Azhar Syed Sulaiman
Dekan
Pusat Pengajian Sains Farmasi

8/8/12
Tarikh
Date

Abstrak

Tujuan penyelidikan ini dijalankan adalah untuk memformulasikan tablet berkecai oral terlindung rasa (ODTs) bagi drug tidak larut air (ondansetron) dan drug larut air (sumatriptan suksinat). Tablet disediakan melalui tiga teknik berlainan iaitu Wowtab, Orasolv dan pengeringan sejuk beku. Rasa pahit ondansetron telah diselindung dengan penambahan pemanis (aspartam), manakala bagi sumatriptan, drug tersebut telah disalut dengan Eudragit EPO menggunakan pengeringan semburan. Kekerasan tablet telah dikekalkan dalam julat 2-3 kg dan kerapuhan adalah <1% untuk semua kelompok. Dalam teknik Wowtab, beberapa jenis superdisintegran yang berbeza dimasukkan ke dalam sediaan untuk mencapai pengecaian tablet yang lebih cepat. Mengikut teknik Orasolv, tablet disediakan dengan menambah agen effervesen samada bersendirian atau bergabungan, manakala dalam teknik pengeringan sejuk beku, agen pembentuk matriks yang berlainan (gelatin, HPMC-3cp, PVP K-90, Cryogel dan Instagel) telah dimasukkan. Formulasi teroptimumkan yang memenuhi syarat rasmi bagi tempoh pengecaian (<10 saat bagi ondansetron dan <60 saat bagi sumatriptan) telah dikenakan ujian kandungan air dan ujian pelepasan in vitro. Kandungan air bagi ondansetron dan sumatriptan didapati <4% dan <6.5% masing-masing. Drug yang terlepas tidak kurang daripada 90% dalam tempoh 5 min bagi ondansetron dan 15 min untuk sumatriptan. Tablet teroptimumkan tersebut didapati mempunyai rasa yang enak dengan rasa mulut yang baik, dan berkecai dalam mulut dalam tempoh 12 dan 41 saat bagi ondansetron dan sumatriptan masing-masing. ODTs yang diformulasi bagi kedua-dua drug menunjukkan profil pelepasan in vitro yang serupa dengan produk komersil dan juga biosetara bagi kadar dan takat penyerapan in vivo. Secara kesimpulan, ODTs terselindung rasa bagi ondansetron dan sumatriptan telah berjaya disediakan dan formulasi tersebut dapat menjadi alternatif berguna kepada produk yang sedia ada secara komersil.

Abstract

The aim of the present research was to formulate taste masked orally disintegrating tablets (ODTs) for the water insoluble (ondansetron) and water soluble (sumatriptan succinate) drugs. The tablets were prepared by three different techniques such as Wowtab, Orasolv and freeze drying. The bitter taste of ondansetron was masked with the addition of sweetener (aspartame), whereby for sumatriptan, the drug was coated with Eudragit EPO using spray dryer. The tablets hardness was maintained in the range of 2-3 kg and friability was <1% for all the batches. In Wowtab technique, different types of superdisintegrants were incorporated in the formulations to achieve faster disintegration of the tablets. In Orasolv technique, tablets were prepared by addition of effervescent agents either singly or in combination, whereby in freeze drying technique, different matrix forming agents (gelatin, HPMC-3cp, PVP K-90, Cryogel and Instagel) were incorporated. The optimized formulations which met the official requirements for in vitro disintegration time (<10 sec for ondansetron and <60 sec for sumatriptan) were subjected to water content and in vitro release studies. The resulting water content values for ondansetron and sumatriptan were <4% and <6.50%, respectively. The drug was released not to be less than 90% within 5 min for ondansetron and 15 min for sumatriptan. The optimized tablets were found to have a pleasant taste with good mouth feel and disintegrated in the mouth within 12 and 41 sec for ondansetron and sumatriptan, respectively. Formulated ODTs for both drugs showed similar in vitro release profiles with that of a commercial product and also bioequivalent in their rate and extent of absorption in vivo. In conclusion, the taste masked ODTs for ondansetron and sumatriptan were successfully prepared and these formulations could be a useful alternative to commercially available products.

LAMPIRAN 3

DESIGN AND EVALUATION OF ORALLY DISINTEGRATING TABLETS OF WATER SOLUBLE AND WATER INSOLUBLE DRUGS

Introduction

The most important drug delivery route is undoubtedly the oral route which have wide acceptance up to 50-60% of total dosage forms. Drugs that are administered orally, solid oral dosage forms in general and tablets in particular, represent the preferred class of product. Tablet is the most popular among all dosage forms existing today because of its convenience of self administration, compactness and ease of manufacturing (Bharawaj *et al.*, 2010). However, hand tremors, dysphasia (difficulty in swallowing) in case of geriatric patients, underdeveloped muscular and nervous systems in young individuals and in case of uncooperative patients, the problem of swallowing is a common phenomenon which leads to poor patient compliance. One study showed that 26% out of 1576 patients experienced difficulty in swallowing tablets due to their large size, followed by their surface, shape and taste (Andersen *et al.*, 1995). The difficulty also applies to people who are ill in bed, active working patients who are busy or travelling, those who have no access to water and patients with nausea, vomiting and motion sickness complications. To overcome these drawbacks, orally disintegrating tablets (ODTs) has emerged as a viable alternative oral dosage form.

United States Food and Drug Administration (USFDA) defined ODT as "A solid dosage form containing medicinal substance or active ingredient which disintegrates/dissolves rapidly within a matter of seconds when placed upon the tongue without need of water." The disintegration time for ODTs generally ranges from several seconds to about a minute. These tablets are also termed orodispersible, quick disintegrating, mouth dissolving, fast disintegrating, rapid dissolving and porous tablets. All of these terms were approved by United States Pharmacopoeia (USP 30, 2007).

Recent market studies indicate that more than half of the patient population prefers ODTs to other dosage forms and most consumers would ask their doctors for ODTs (70%), purchase ODTs (70%), or prefer ODTs to regular tablets or liquids (>80%). These responses may be attributed to the ODT advantages such as ease of administration, ease of swallowing, pleasant taste, and the availability of several flavours. ODTs also offer clinical advantages such as improved safety, and, in some cases, improved efficacy. In addition, several business needs

are driving ODT technology development and the commercialization of new products such as the need for expanded product lines, improved life-cycle management, extended patent life, and marketing advantages (Brown, 2001; Seager, 1998).

Advantages of ODTs

- Administration to patients who cannot swallow, such as the elderly, stroke victims, bedridden patients and patients who refuse to swallow such as pediatric, geriatric & psychiatric patients.
- Rapid drug therapy intervention.
- Achieve increased bioavailability/rapid absorption through pregastric absorption of drugs from mouth, pharynx & oesophagus as saliva passes down the GIT.
- Convenient for administration for disabled, bedridden patients and for travellers and busy people, who do not always have access to water.
- Good mouth feel property helps to change the perception of medication as bitter pill particularly in pediatric patients.
- New business opportunities from product differentiation, product promotion, patent extension and life cycle management of existing active pharmaceutical ingredients (APIs).

Disadvantages of ODTs

Despite these advantages, the application of this technology is limited by the following disadvantages:

- Drugs with relatively larger doses are difficult to formulate into ODTs e.g. antibiotics like ciprofloxacin with an adult dose containing about 500 mg of drug.
- Patients with Sjögren's syndrome or dryness of mouth due to decreased saliva production may not be good candidates for these formulations.
- Due to rapid dissolution, ODTs cannot provide controlled or sustained release profile of drugs, except those containing slow-dissolving, microparticulate-coated drugs, which quickly disperse and are swallowed.
- Fragile products require special unit-dose packaging, which may add to the cost. Few technologies are available that can produce tablets that are sufficiently hard and durable to allow them to be packaged in multi-dose bottles.

Challenges in the formulation of ODTs

1. Mechanical strength and disintegration time:

ODTs are formulated to obtain disintegration time usually less than a minute. Whilst doing so, maintaining a good mechanical strength is a primary challenge. Many ODTs are fragile and there are many chances that such fragile tablets will break during packaging, transport or handling by the patients. Tablets based on technologies like Zydis (freeze dried) need special type of packaging. It is a fundamental phenomenon that increasing the mechanical strength will delay the disintegration time. Hence, a good compromise between these two parameters is always essential.

2. Taste masking:

Many drugs are bitter in taste. A tablet of bitter drug which disintegrates in the mouth will seriously affect patient compliance and acceptance for the dosage form. Therefore, effective taste masking of bitter drugs must be done so that the taste of the drug is not felt in the oral cavity.

3. Mouth feel:

The ODT should not disintegrate into larger particles in the oral cavity. The particles generated after disintegration of the ODTs should be as small as possible. ODTs should leave minimal or no residue in mouth after oral administration. Moreover, addition of flavours and cooling agents like menthol improve the mouth feel.

4. Sensitivity to environmental conditions:

ODTs generally should exhibit low sensitivity to environmental conditions such as humidity and temperature as most of the materials used in an ODT are meant to dissolve in minimum quantity of water.

5. Cost:

The technology used for an ODT should be acceptable in terms of cost of the final product. Methods like Zydis and Orasolv that require special technologies and specific packaging increase the cost to a remarkable extent.

Objectives of research

The objective of the present study was to formulate a "patient-friendly dosage form" of ondansetron (water insoluble) and sumatriptan succinate (water soluble) ODTs having adequate hardness and fast disintegration with pleasant taste and mouthfeel in the oral cavity. The study was performed in various stages encompassing the following objectives:

1. To formulate ondansetron and sumatriptan succinate (SS) ODTs using different manufacturing techniques such as Wowtab, Orasolv and freeze drying.
2. To evaluate the tablets for weight variation, thickness, hardness, friability, drug content, water content, in vitro disintegration time and in vitro dissolution study.
3. To evaluate the optimized formulation which produces desired ODT characteristics for its taste, mouth feel and in vivo disintegration time in healthy human volunteers.
4. To conduct stability studies for optimized formulation.
4. To develop and validate individual HPLC methods for the determination of ondansetron and SS in plasma.
5. To conduct in vivo study to evaluate the performance of the final optimized formulation of ondansetron and SS using rabbits as animal model in comparison to the respective commercial products available in the market.

Materials and Methods

Materials

Ondansetron was purchased from Symed Labs (Hyderabad, India). Sumatriptan succinate was purchased form Nosch Labs (Hyderabad, India). Mannitol was purchased from Merck (Darmstadt, Germany). Microcrystalline cellulose (MCC; Avicel PH 112 and PH 113) and croscarmellose sodium (CCS) were purchased from FMC Biopolymer (Newark DE, USA). Crospovidone (polyplasdone XL and XL-10) was obtained from ISP Technologies (NewJersey, USA). Sodium starch glycolate (SSG) was purchased from DMV International (NewJersey, USA). Kollidon CL and Kollidon CL-SF were obtained from BASF (Ludwigshafen, Germany). Low substituted hydroxypropyl cellulose (L-HPC, LH11) was obtained from Shin-Etsu Chemicals (New York, USA). Aspartame and flavours (strawberry,

orange, mango, pineapple and banana) were purchased from Nutrasweet (Chicago, USA). Magnesium stearate was purchased from Beijing Jingqiu Chemicals (Beijing, China). Sodium stearyl fumarate (SSF) was purchased from Micro Orgo Chem (Mumbai, India). Aerosil was purchased from Cabot Corporation (Boston, USA). Calcium silicate was obtained from Huber Chem (Mumbai, India). Citric acid was purchased from R&M Chemicals (Essex, UK). Sodium carbonate and sodium bicarbonate, sodium hydroxide, potassium dihydrogen phosphate was purchased from Systerm® (Selangor, Malaysia). Gelatin, Methylparaben sodium and propylparaben sodium were purchased from Sigma-Aldrich (St. Louis, USA). Cryogel and Instagel were purchased from PB Gelatins (Vilvoorde, Belgium). Hydroxypropyl methyl cellulose-E3 (HPMC-E3) was obtained from Colorcon (Pennsylvania, USA). Eudragit EPO was purchased from Degussa (GmbH, Germany). Methanol and acetonitrile (HPLC grade) were purchased from J.T.Baker (Phillipsburg, USA). All the excipients used in this study are well known as GRAS (Generally Recognised As Safe) for human use and were also used within the acceptable limits of USFDA (USFDA, 2009).

Methods

Preparation of the tablets

The orally disintegrating tablets of ondansetron and SS were prepared using 3 different manufacturing techniques as described below:

1. Wowtab technique

Ondansetron: Orally disintegrating tablets containing ondansetron were prepared by wet granulation technique. In brief, the drug and intragranular ingredients (mannitol, Avicel PH113 and superdisintegrant) were weighed, passed through 0.8 mm sieve and mixed intimately by geometric dilution. Water (granulating agent) was added to the mixture to form granules which were then dried in an oven at 40 °C for 1 hr. The dried granules and extragranular ingredients (Avicel PH112, aspartame and strawberry flavour) were screened through 0.8 mm sieve and blended for 5 min. The obtained blend was lubricated with SSF and aerosil before compression. A 75 mg of blend was compressed into tablets using laboratory single station tableting machine (Korsch, Berlin, Germany) equipped with 5.5 mm round shaped punches.

SS: The taste masked sumatriptan succinate ODTs were prepared by direct compression method followed by sublimation technique. The spray dried powder containing SS and Eudragit EPO, Avicel PH 112, superdisintegrant, calcium silicate, ammonium bicarbonate, aspartame, and strawberry flavour were passed through 0.8 mm sieve and mixed intimately by geometric dilution. The obtained blend was lubricated with Mg. stearate and aerosil before compression. The tablets were compressed at 320 mg weight on a single station tableting machine (Manestry Liverpool, UK) with 10 mm concave punches. The tablets were kept in an oven at 40 °C for 15-21 hr to facilitate sublimation of sublimating material (ammonium bicarbonate) to increase the porosity of tablets which could decrease the disintegration time of tablets.

2. Orasolv technique

Ondansetron: Orally disintegrating tablets containing ondansetron were prepared by direct compression. Ondansetron and Eudragit EPO complex (1:0.5) powder and all other excipients (mannitol, Avicel PH112, citric acid, sodium bicarbonate, aspartame, SSF and aerosil) were weighed and passed through 0.8 mm sieve and mixed intimately by geometrical dilution. This uniformly mixed blend was compressed into tablets using laboratory single station tableting machine (Korsch, Berlin, Germany) equipped with 5.5 mm round shaped punches to produce ODTs weighing 100 mg each.

SS: The taste masked sumatriptan succinate ODTs were prepared by direct compression method. The spray dried powder containing SS and Eudragit EPO, Avicel PH 112, calcium silicate, aspartame, strawberry flavour and different concentrations of effervescent agents (citric acid, tartaric acid, sodium bicarbonate and sodium carbonate) were passed through 0.8 mm sieve and mixed intimately by geometric dilution. The blend was lubricated with magnesium stearate and Aerosil before compression. A blend of 300 mg was compressed into tablets using single station tableting machine (Manesty, Liverpool, UK) with 10 mm concave punches.

3.Freeze drying technique

Ondansetron: Ondansetron ODTs were prepared by dissolving mannitol, methylparaben sodium, propylparaben sodium, aspartame and strawberry flavour in water while maintaining stirring rate at 750 rpm. Different matrix forming agents (gelatin, hydroxypropyl methylcellulose (HPMC-E3), polyvinyl pyrrolidone (PVP K-29/32, PVP K-90) and

hydrolyzed gelatin (Cryogel and Instagel)) in a concentration range of 20-40% were added to above solution and then ondansetron was added. The solution was stirred for another 5 min to prepare a fine and uniform suspension. The suspension containing drug (0.25 ml) was filled in PVC blister cavities and frozen at -70 °C for 8 hr and then freeze dried for 24 hr. The tablet weight was 25 mg.

SS: The SS ODTs were prepared by dissolving mannitol, methylparaben sodium, propylparaben sodium, aspartame and strawberry flavour in water while maintaining stirring rate at 750 rpm. Different matrix forming agents (gelatin, hydroxypropyl methylcellulose (HPMC-E3), polyvinyl pyrrolidone (PVP K-29/32) and hydrolyzed gelatin (Cryogel and Instagel)) in a concentration range of 1 to 5% were added to above solution and then the spray dried powder containing SS and Eudragit EPO was added. The solution was stirred for another 5 min to prepare a fine and uniform suspension. The suspension containing drug (1 ml) was filled in PVC blister cavities and frozen at -70 °C for 8 hr and then freeze dried for 24 hr. The tablet weight was 200 mg.

Evaluation of ondansetron and SS tablets

1. Weight variation

20 tablets were selected randomly and average weight was determined using an electronic balance. Tablets were weighed individually and compared with average weight. The maximum percentage different allowed of the weight variation are 10% for the weight of tablets 130 mg or less and 7.5% for 130 to 324 mg (USP 30, 2007).

2. Thickness

Ten tablets were selected randomly and thickness was assessed using digital caliper (Neiko, USA).

3. Hardness

Hardness is the force required to break a tablet by radial compression. It was determined using Vanguard hardness tester in the units of kg (YD-2 model, Vanguard, USA). The mean hardness of 10 tablets was calculated and reported.

4. Friability

The friability of 20 tablets was measured using a friability test apparatus (CS-1 tablet friability tester, USA). Twenty pre-weighed tablets were rotated at 25 rpm for 4 min. The tablets were then dedusted, reweighed and loss in weight (%) was calculated. The test was run once for each tablet formulation.

5. Drug content

Ten tablets from each formulation were randomly selected and pulverized to a fine powder. A portion of powder equivalent to a single dose of drug was accurately weighed and assayed for the drug content using UV spectrophotometer (UV/Vis Spectrophotometer, Hitachi, Japan) at a wavelength of 310 nm for ondansetron and at 227 nm for SS. The experiment was run for three replicates.

6. Water content

The tablets were analyzed for their water content using Karl Fischer titrator (Metrohm 703 Ti Stand, Germany). The formulations which produced in vitro disintegration time <10 sec for ondansetron and < 60 sec for SS were evaluated for water content. The tablet was pulverized, inserted in the titration vessel containing dried methanol (Karl Fischer grade) and titrated with Hydranal Composite 5 reagent (Riedel-de-Haen, Germany) after a stirring time of 3 min. The samples were analyzed in triplicate. According to USP limits, the water content for ondansetron ODTs should not be more than 4% (USP 30, 2007) and there was no specific limitation for SS ODTs.

In vitro taste masking evaluation of SS spray dried microspheres

The in vitro taste masking evaluation was conducted by the method adopted from Shukla et al. (2009). The required amount of powder equivalent to a single dose of drug was placed in 25 ml beaker. A volume of 5 ml phosphate buffer solution pH 6.8 (USP) was added and allowed to stand for 60 sec. The 5 ml of pH 6.8 phosphate buffer has been used to mimic the salivary fluid volume and pH. After the specified time, the suspension was filtered through 0.45 μm nylon filter. The filtrate was analyzed for drug content using UV at 227 nm for SS. The experiment was run in triplicate.

Thermal analysis

Thermal analysis was carried out using Differential scanning calorimetry (DSC) (Perkin Elmer, Pyris 6 DSC, USA) to determine the interaction between SS and Eudragit EPO microspheres. DSC experiments were performed on plain drug and Eudragit EPO and drug complex. Accurately weighed samples (5-7 mg) were hermetically sealed in flat bottom aluminium pans and thermograms were recorded at a constant rate of 10 °C/min over a temperature range of 30-300 °C. Inert atmosphere was provided by purging nitrogen gas at a flow rate of at 20 ml/min. An empty pan sealed in same way as the sample was used as a reference. The experiment was run in triplicates.

In vitro disintegration time

In vitro disintegration time (In vitro DT) of ODTs was determined using USP tablet disintegration test apparatus (Pharma Test, Germany). The tablet was placed in the ≠ 10 basket. The test was carried out in 900 ml of distilled water maintained at 37 °C and agitation speed of 30 shakes per min. Only one tablet at a time was tested. The tablet was considered disintegrated completely when all the particles passed through the screen. The DT of 6 individual tablets were recorded and the average was reported. According to USP limits, the in vitro DT for ondansetron ODTs should not be more than 10 sec (USP 30, 2007). According to USFDA, for SS ODTs should be < 60 sec.

In vitro dissolution studies

In vitro dissolution studies of ondansetron and SS ODTs were performed using USP XXIV type-II dissolution test apparatus (Distek Premiere, 5100, USA) equipped with an autosampler and fraction collector. Ondansetron and SS formulations which produced in vitro DT <10 sec and <60 sec, respectively were selected for dissolution studies. Ondansetron dissolution study was conducted in 500 ml of 0.1 N HCl as dissolution medium with paddle speed of 50 rpm at the temperature of 37 °C (USP 30, 2007). SS dissolution study was conducted in 900 ml of 0.01 N HCl as dissolution medium with paddle speed of 30 rpm at the temperature of 37 °C (USP 30, 2007). Aliquots of dissolution medium (4 ml) were withdrawn at specified intervals, 5, 10, 15, 30, 45 and 60 min and replaced with an equal volume of fresh medium. The samples were analyzed for drug concentration spectrophotometrically at 310 nm for

ondansetron and at 227 nm for SS. Cumulative percent of drug release was calculated and plotted against time.

Taste, mouth feel and in vivo disintegration time evaluation of ondansetron and SS optimized formulation in human volunteers

The commercial product and optimized formulation was assessed for taste, mouth feel and in vivo disintegration time in 12 healthy human volunteers in the age group of 25 to 33 years. Prior to the test, all the volunteers were informed of the purpose and protocol of the study. The institutional ethics committee approved the study protocol and each volunteer gave his/her written consent to participate in the study. As per the protocol, all volunteers were asked to rinse their mouth with water before placing the tablet on the tongue and immediately a stopwatch was started. Volunteers were allowed to move the tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. The taste and mouth feel were evaluated based on the volunteers' spontaneous verbal judgments immediately after the tablet was placed in their mouth and also after 3-4 min and data was recorded. The taste and mouth feel were rated on a scale of 1 through 5. In taste evaluation, '1' was considered to be "good" while a '5' was considered as 'awful". In mouth feel evaluation, '1' was considered to be "good" while a '5' was considered as 'high grittiness". Time taken for the volunteer to feel that the last noticeable granule or fragment had disintegrated in the oral cavity was considered as the in vivo disintegration time. The swallowing of saliva was prohibited during the test and the mouth was rinsed after measurement.

Stability studies for optimized formulations of ondansetron and SS

The stability of optimized formulation was conducted at 40±2 °C/75±5% RH (accelerated) and 25±2 °C/65±5% RH (short term) for a period of 6 months. The ODTs were packed and sealed in 30cc HDPE bottles (Shukla *et al.*, 2009). Samples were withdrawn at 1, 3 and 6 months and evaluated for appearance, weight variation, thickness, hardness, friability, drug content, water content, in vitro DT and in vitro dissolution study. The drug was assayed using our previously reported HPLC-UV methods (Ravi *et al.*, 2009).

Results and Discussion

Ondansetron

1. Wowtab technique

Wowtab technology utilizes conventional granulation and tableting methods to produce ODTs employing low (mannitol and sucrose) and high (sorbitol) compressibility saccharides. In order to produce ODTs with commonly used production methods and equipments, the formulation excipients should have a quick disintegration rate in the mouth and a high compressibility in order to yield an adequate hardness when compressed.

Physical properties of the ondansetron tablets

All the prepared formulations produced tablets which were white in colour and spherical in shape with smooth surface with zero defects. The average weight and thickness of tablets for all the formulations was found to be in the range of 73.34 to 76.88 mg and 2.45 to 2.83 mm, respectively. The weight of the tablets is within the acceptable limits. The tablets provided good weight uniformity as indicated by the very low relative standard deviation obtained (RSD <1%) for all formulations. The hardness of the tablets was maintained in the range of 2-3 kg in order to produce tablets with satisfactory strength that can withstand the mechanical shocks in handling, packaging and at the time of administration. The friability of all the formulations was within acceptable limits and it indicates the ODTs ability to withstand abrasion in handling, packaging and shipment. All the formulations and the commercial tablets demonstrated uniformity in the assay and drug content varied from 97.59 to 102.71%.

Formulation rationale:

In preliminary studies, ondansetron tablets were prepared by different granulation techniques such as wet granulation, direct compression and dry granulation and found that wet granulation is a suitable technique. The hardness of the tablets was tested at three different levels 1-2, 2-3 and 3-4 kg. An inverse relationship was observed between hardness and friability. The friability of the formulations decreased from 1.25 to 0.27% with an increase in the hardness from 1-2 to 3-4 kg. The DT of the formulations increased from 11.83 to 49.33 sec with an increase in the hardness from 1-2 to 3-4 kg. There is a positive correlation between hardness and DT of the tablets i.e. as the hardness of the tablets was increased there was also an increase in the disintegration time. The hardness, 2-3 kg was chosen which

produced a good balance over friability and DT. Microcrystalline cellulose was incorporated in the formulations as disintegrant and diluent. The partly intragranular and extragranular incorporation of MCC produced faster disintegration of the tablets compared to intragranular or extragranular. It may be due to immediate disruption of the tablet into previously compressed granules by the extragranular MCC, while the disintegrating agent within the granules (intragranular) produces further erosion of the granules to the original powder particles (Bagul *et al.*, 2006). Mannitol is water soluble, non-hygroscopic and provides a semi-sweet, smooth and cool taste due to its negative heat of solution (Rowe *et al.*, 2003). The higher the amount of mannitol present in the formulations produced higher DT values due to the increase in polyol quantity in the tablet formulation. As polyols are readily soluble in water, there exists a competition between mannitol and disintegrant for water penetrating into the tablet. The disintegration is hindered by the dissolution process of mannitol, consequently leading to poor swelling of disintegrant with subsequent delay in disintegration (Schiermeier and Schmidt, 2002; Khan *et al.*, 2007). Preliminary studies were performed in healthy human volunteers for the taste characterization of ondansetron ODTs prepared with different amount of aspartame (1 to 9%) as a sweetener in the formulations. From the data obtained concluded that incorporation of 7% aspartame successfully masking the bitter taste of the ondansetron. Sodium stearyl fumarate was chosen as a lubricant in ondansetron ODTs as it produced lower DT values compared to magnesium stearate.

Superdisintegrants are added to the tablet formulations to aid the disintegration of the tablet at a faster rate. This part of the study was done to chose the type and concentration of superdisintegrant based on the tablet properties obtained. The disintegration time of various formulations studied varied form 5.83 to 33.00 sec and that of the commercial product (Zofer MD 8) was 8.53 sec. The disintegration time of the tablets containing 5% polyplasdone XL and polyplasdone XL-10 was 10.17 and 11.17 sec, respectively. Increase in the concentration of polyplasdone XL and polyplasdone XL-10 to 10% resulted in a decrease in the DT of the tablets. However, there was no significant effect ($p > 0.05$) on the DT, when the concentration of polyplasdone XL and polyplasdone XL-10 was further increased to 15%.

When the superdisintegrants, polyplasdone XL and XL-10 were replaced with CCS and SSG at a concentration level of 5%, the resultant disintegration time was 12.67 and 16.33 sec, respectively. Further increase in the concentration of CCS and SSG from 5 to 10 and 15% resulted in a significant increase ($p < 0.05$) in the DT of the tablets from 12.67 to 18.50 and

28.50 sec for CCS and from 16.33 to 26.00 and 33.00 sec for SSG. It can be observed from the results that the disintegration times of crospovidone (polyplasdone XL, polyplasdone XL-10) containing tablets were comparatively lower than those containing CCS and SSG. This dissimilarity in the effect of concentration of crospovidone, CCS and SSG on the disintegration time can be attributed to the difference in their mechanism of disintegration. Increasing the concentration of crospovidone resulted in faster disintegration of tablets, which may be due to rapid capillary activity and pronounced hydration with little tendency for gel formation (Rowe *et al.*, 2003; Setty *et al.*, 2008). On the contrary, when the concentration of CCS and SSG was increased it had a negative effect on the disintegration of the tablets. This negative effect may be due to the formation of a viscous gel layer by CCS and SSG which may impede further penetration of the disintegration medium and hindered the disintegration of tablet contents (Swamy *et al.*, 2007; Setty *et al.*, 2008). The obtained results were similar to the findings of Khan *et al.* (2007).

The tablets containing L-HPC as a superdisintegrant disintegrates the tablets based on its swelling property in water (Bi *et al.*, 1996). L-HPC, when used at concentrations of 1, 3 and 5%, resulted in a disintegration time of 21.17, 15.33 and 15.67 sec, respectively. There was no significant difference ($p > 0.05$) in the DT of the tablets when L-HPC concentration was increased from 3 to 5%. The superdisintegrants, Kollidon CL and Kollidon CL-SF exhibited their disintegrant effect by wicking action without forming a gel. They increase the porosity and provide pathways for the penetration of fluids into tablets, which in turn would result in wicking through capillary action facilitating the disintegration of tablets (Mishra *et al.*, 2006). The disintegration time of the tablets was decreased significantly ($p < 0.05$) from 13.67 to 5.83 sec with the increase in the concentration of Kollidon CL from 5 to 10%. There was no significant difference ($p > 0.05$) in the DT of tablets containing 1.25 (14.00 sec) and 2.5% Kollidon CL-SF (14.50 sec), respectively. However, the tablets containing Kollidon CL–SF-5% did not disintegrate into particles, but tended to separate axially into upper and lower sections. The formulations which produced DT <60 sec were selected for water content and in vitro dissolution studies.

The commercial product and all the ODT formulations with a disintegration time of less than 10 sec showed that the water content of the ODT formulations was less than 4% which was within the acceptable limits.

In vitro drug dissolution studies conducted with 0.1 N HCl at a paddle speed of 50 rpm showed that the commercial product (Zofer MD 8®) and promising ODT formulations (polyplasdone XL-10%, polyplasdone XL-15%, polyplasdone XL-10-10%, polyplasdone XL-10-15% and Kollidon CL-10%) released more than 90% of drug in 10 min except the formulation F31 which contained Kollidon CL (10%). The dissolution studies were also conducted with a paddle speed of 25 rpm to select the optimized ODT formulation due to lower paddle speeds yield more discriminating dissolution profiles as ODT formulations disintegrate rapidly. The release profiles results showed that only the formulation prepared with polyplasdone XL-10-15% released more than 90% of the drug in 10 min and the results were comparable with the commercial product. Hence, this formulation was selected as optimized one in this technique.

2. Orasolv technique

This technique involves use of effervescent disintegrating agents compressed with low pressure to produce the ODTs. In this technique, the disintegration is aided by the evolution of carbon dioxide by the reaction of acid and a base, when the tablet comes into contact with the saliva. For this purpose effervescent disintegrating pairs usually include an acid source (citric acid, tartaric acid) and a carbonate source (sodium carbonate, sodium bicarbonate).

At an initial formulation, the sweetener, aspartame was incorporated at the same level used in Wowtab technology and the obtained tablets were found to be very bitter. Ondansetron base is less bitter compared to its salt form owing to low solubility of the former (Ahmed *et al.,* 2008). Ondansetron being basic in nature might react with citric acid present in the formulation to yield a salt which is expected to be extremely bitter. So, addition of ondansetron in the free form with citric acid may not be feasible from the taste masking point of view. Considering this obstacle in the taste masking, ondansetron and Eudragit EPO complexation was prepared by precipitation technique. This complexation powder was used for the preparation of the tablets.

Physical properties of ondansetron ODTs

All the prepared formulations produced tablets which were white in colour and spherical in shape with smooth surface with zero defects. The average weight and thickness of tablets for all the formulations was found to be in the range of 99.89 to 101.22 mg and 3.48 to 3.57 mm,

respectively. The tablets provided good weight uniformity as indicated by the very low relative standard deviation obtained (RSD < 1.5%) for all the formulations. The hardness of the tablets was maintained in the range of 2-3 kg. The friability of all the formulations were within acceptable limits (<1%). The drug content of all the formulations was varied from 99.67 to 101.53%.

The DT obtained for the formulations containing 1:1, 2:1 and 3:1 ratios of citric acid and sodium bicarbonate effervescence agents, together accounting to a weight of 20 mg was on an average 5.17, 6.27 and 7.35 min respectively. The DT values increased with increase in the citric acid and decrease in sodium bicarbonate concentration. One molecule of citric acid requires three molecules of sodium bicarbonate for complete reaction and production of maximum carbon dioxide (CO_2). The production of carbon dioxide is responsible for the disintegration of tablet. So, the presence of more or equal amount of citric acid in these formulations compared to sodium bicarbonate may result in lower amounts of CO_2 release and slower disintegration.

Further experiments were carried out by reducing the amount of citric acid and increasing the amount of sodium bicarbonate. The ratios of citric acid to sodium bicarbonate studied were 1:2 and 1:3. The mean DT values obtained were 4.36 and 3.13 min, respectively. The DT values decreased with increase in sodium bicarbonate and decrease in citric acid concentration.

Next step of formulation development was to increase the amount of citric acid and sodium bicarbonate combination in a ratio of 1: 3 from 20 mg to 30, 40 and 50 mg while keeping the tablet weight constant at 100 mg. Compensation in weight was made in these formulations by decreasing the amount of Avicel. The mean DT values obtained for formulations prepared with 30, 40 and 50 mg were 1.26, 0.82 and 2.14 min respectively. It can be observed from the results that as the amount of sodium bicarbonate and citric acid was increased up to 40 mg there was a decrease in the DT value. Further increase in the amount to 50 mg, there was an increase in the DT value. It might be due to decrease in the amount of Avicel in the formulation compared to the prior formulations. The formulation prepared with 40 mg of the total amount of citric acid and sodium bicarbonate combination in a ratio of 1:3 was the promising formulation among the ones studied as the DT value obtained was <1 min. Further modification was made in this formulation to achieve a DT value of less than 10 sec.

The formulation which produced DT less than 1 min was modified by replacing part of sodium bicarbonate with sodium carbonate by maintaining the ratio of acid to base at 1:3. The total amount of carbonate (sodium carbonate and sodium bicarbonate) was maintained at 30 mg. The resulted DT values for the formulation prepared with the 5:1, 3:1 and 2:1 ratios of sodium bicarbonate to sodium carbonate were 0.82, 0.77 and 0.83 min, respectively and no significant ($p>0.05$) difference in DT values. Nonetheless, all the formulations studied were not able to achieve DT limitations set by USP.

By using all possible combinations of excipients, the least DT obtained was 0.77 min which is well within the limit for an ODT but not within the limits for ondansetron ODTs (<10 sec). Hence, water content and dissolution studies were not carried out.

3. Freeze dried technique

Lyophilization is a process which includes the removal of water from a frozen suspension/solution of drug with structure forming additives. Freeze drying of drug with additives imparts glassy amorphous structure resulting in highly porous and light weight product. The resulting dosage form has a rapid disintegration and dissolution when placed on the tongue. The influence of different matrix forming agents on the DT of the prepared ODTs was investigated in this part of the study. The matrix forming agents that were investigated were gelatin, polyvinyl pyrrolidone (PVP K-29/32, PVP K-90), hydroxypropyl methyl cellulose (HPMC-E3) and hydrolyzed gelatin (Cryogel and Instagel) in the concentration range of 20-40% using mannitol as a bulking agent. The matrix forming agents used in these formulations may impart glassy amorphous structure which provides the required strength and resilience during handling. Mannitol incorporated in the formulation induces crystallinity, imparts hardness, prevent collapse of structure, mask the bitter taste and provides elegance to the final dosage form. Water is used in the manufacturing process to ensure the production of porous tablets which disintegrate rapidly on the tongue. Preservatives, methylparaben sodium and propylparaben sodium were used to prevent microbiological growth of the aqueous solutions during the manufacturing process. When the product has been freeze dried, the preservative has no further action. The water within the final formulation is expected to be too low to support the growth of micro-organisms. The sweetener (aspartame) and flavour (strawberry) were added in all the formulations to mask the bitter taste of ondansetron (Seager, 1998; Manivannan, 2005). The prepared tablets were evaluated for weight variation

and in vitro DT. The formulations that resulted in tablets with DT less than 10 sec were studied for water content and in vitro dissolution.

Physical properties of freeze dried tablets

All the formulations resulted in successfully dried and produced elegant tablets that were strong enough to be easily handled except formulations prepared with PVP K-29/32. Hence, these formulations were not included in the further study. The average weight of tablets for all the formulations was found to be in the range of 24.37 to 26.03 mg. The relative standard deviation of the tablet weight ranged from 0.77 to 1.95%, indicating good weight uniformity in the tablets. The mean percent drug content in all formulations was found to be in the range of 98.78 to 102.54%.

The choice of matrix forming agents is an important parameter in the formulation of these products. For the first set of formulations, gelatin was used as the matrix forming agent. The DT values obtained for formulations prepared with 20, 30 and 40% of the gelatin were 4.17, 2.00 and 2.00 sec, respectively. Irrespective of the concentration of gelatin used in the formulations the DT values obtained was <10 sec. There was no significant difference ($p>0.05$) in DT values of the formulations of 30 and 40% gelatin.

The tablets prepared with PVP K-29/32 were very fragile and difficult to handle. Hence, further formulations were prepared using higher viscosity grade of PVP. The DT values of 20, 30 and 40% PVP K-90 were 11.83, 12.67 and 30.17 sec, respectively. There was an increase in DT values as the concentration of PVP was increased. It was attributed to formation of a viscous jelly like structure of PVP K-90 at higher concentrations.

The DT values of the freeze dried tablets were influenced by the concentration of HPMC-E3 (3 cP). The DT values obtained for formulations prepared with 20, 30 and 40% were 11.33, 26.83 and 47.50 sec, respectively. A similar trend to that observed with PVP K-90 was found with HPMC-E3. There was an increase in the DT value with increase in its concentration and observed a statistically significant difference ($p < 0.05$) among the formulations.

Hydrolyzed gelatins, Cryogel and Instagel were used as matrix formers in further experiments. Cryogel and Instagel both are cold water soluble gelatins. The DT values obtained for formulations prepared with concentrations of 20, 30 and 40% were 3.33, 35.67

and 54.83 for Cryogel and 2.33, 6.67 and 7.33 sec, for Instagel, respectively. The trend could be observed in the influence of concentration of the matrix forming agents on the DT of the tablets; increasing the concentration resulted in an increase in the disintegration time. When a comparison was made between Cryogel and Instagel; Instagel containing freeze dried tablets disintegrated faster. The probable reason for this difference in the DT may be due to the bloom strength of both the hydrolysed gelatins. Instagel has lower bloom strength (50-70 g) so it would not form a thick gel upon exposure to water rendering faster disintegration, while in case of Cryogel it forms a thick gel upon exposure to fluids because of its high bloom strength (180-200 g). It was observed that there was no statistically significant difference ($p>0.05$) in DT values of the formulations prepared with Instagel 30 and 40%.

The promising formulations (gelatin-20, 30 and 40%, Cryogel-20% and Instagel-20, 30 and 40%) that resulted in DT of <10 sec were subjected to determine the water content and the results were <4% in compliance with USP limits (USP 30, 2007).

In vitro drug dissolution studies conducted with 0.1 N HCl at a paddle speed of 50 rpm. The commercial product (Zofer MD 8®) and all the promising ODT formulations released more than 90% of drug in 5 min. In addition, the dissolution studies were also conducted with a paddle speed of 25 rpm and found that formulations prepared with Cryogel-20% and Instagel-20, 30 and 40% released more than 90% of the drug in 10 min and results were comparable with commercial product. The drug release was decreased with increase of gelatin concentration from 20 to 40% at a lower paddle speed and it might be due to the increase in viscosity with increase in gelatin amount in tablets. Further dissolution studies were conducted with phosphate buffer pH 6.8 at a paddle speed of 25 rpm and found that formulations prepared with Cryogel-20% and Instagel-20% produced the similar release profiles with the commercial product and released more than 90% of the drug in 30 min. Hence, these two formulations were chosen as optimized formulations in this technique.

Based on the findings in these 3 techniques, formulation prepared with polyplasdone XL-10-15% in Wowtab technique and formulations prepared with Cryogel-20% and Instagel-20% in freeze dried technique were the optimized formulations and met the official requirements. There was no formulation that met the USP limits in terms of disintegration time in Orasolv technique. Formulation prepared with polyplasdone XL-10-15% in Wowtab technique was selected for the evaluation of taste, mouth feel and in vivo disintegration time in human

volunteers with the consideration of freeze dried technique disadvantages such as cost intensive and time consuming production process, lack of physical resistance in standard blister packs and insufficient hardness of the tablet for the aged patients to handle (Schiermeier and Schmidt, 2002; Mizumoto *et al.*, 2005).

In taste evaluation, 10 and 11 out of 12 volunteers rated the commercial product and optimized formulation as sweet and good. In evaluation of mouth feel, 9 out of twelve volunteers experienced a good mouth feel without any grittiness for commercial product and optimized formulation. The in vivo DT results showed that commercial product and optimized formulation on average disintegrated in the oral cavity within 12.83 and 11.67 sec, respectively. Hence, this formulation was selected for stability study.

In stability study, samples of optimized formulation were examined after 1, 3 and 6 months storage and found that there were no significant change in appearance of the tablets, weight variation, thickness, hardness, friability, water content and disintegration time. The results of water content and disintegration time after 6 months of storage were within the USP limits (USP 30, 2007). The drug content in the tablets stored for 6 months at 40±2 °C/75±5% RH and 25±2 °C/65±5% RH were 99.87 and 100.80%, respectively and found that there was insignificant loss ($p > 0.05$) in the drug content at the end of 6 months. There were no significant changes in the in vitro release profiles of ondansetron from the optimized formulation stored for 0, 1, 3 and 6 months at 40±2 °C/75±5% RH and at 25±2 °C/65±5% RH. Thus, the optimized formulation prepared with superdisintegrant, polyplasdone XL-10-15% was proven to be stable at least for 6 months. Therefore, this formulation was used for the pharmacokinetic study in rabbits.

HPLC-UV method for determination of ondansetron in rabbit plasma

Prior to pharmacokinetic study, a new, sensitive and specific RP-HPLC-UV method with simple sample preparation procedure was developed and validated for the determination of ondansetron in the rabbit plasma. Chromatographic analysis was performed on a Shimadzu liquid chromatographic system (Kyoto, Japan) equipped with an LC-10AT VP solvent delivery pump, SPD-10A VP UV-VIS detector, SIL-10AD VP autosampler and Class VP Chromato software for data acquisition and processing. The mobile phase was consisted a mixture of 50 mM ammonium acetate adjusted to pH 3.5 with glacial acetic acid and acetonitrile (35:65, v/v) and delivered at a flow rate of 1.0 ml/min. The detector was set at a

wavelength of 310 nm. Chromatographic separation of the analyte was performed using a cyano column (Phenomenex, 250 x 4.6 mm i.d., 5 μm particle size) fitted with a cyano guard cartridge (Phenomenex, 4 x 3 mm i.d., 5 μm particle size). The injection volume was 100 μl.

The plasma samplers were extracted as follows: To 0.5 ml of plasma, 25 μl of 1 mg/ml of IS (risperidone) and a mixture of 1 ml of acetonitrile and 50 μl of 10% w/v zinc sulphate solution was added and vortexed for 2 min. The denatured protein precipitant was separated by centrifugation at 10,000 rpm for 20 min. The supernatant was transferred to autosampler vials and an aliquot of 100 μl was injected into the HPLC.

The calibration curve exhibited an excellent linearity over the concentration range of 25-1000 ng/ml of ondansetron with a correlation coefficient of 0.9999. There was no interference from endogenous substances at the retention times of analyte and IS and were well resolved with the retention times of 6.16 min for ondansetron and 7.83 min for IS. The LOQ of the present method was 25 ng/ml. The intra-day accuracy ranged between -3.63 and 1.01% with a precision of 0.93 to 3.41%. The inter-day accuracy ranged between -1.01 and 1.47% with a precision of 1.53 to 2.62%. The results were within the acceptable limits (±15%). The mean extraction recoveries of ondansetron at concentrations of 75, 400 and 900 ng/ml and IS were 83.78, 85.63 and 88.21% and 99.80%, respectively. Ondansetron was found to be stable at -20 °C for 14 days.

In vivo pharmacokinetic study in rabbits

The in vivo study was conducted to evaluate the performance of the optimized ondansetron ODT formulation (Test) in comparison with the Reference product (Zofer MD 8®) using rabbits as an animal model. The study was conducted in accordance with Animal Ethical Guidelines for investigations in laboratory animal and the study protocol was approved by the Animal Ethics Committee of Universiti Sains Malaysia. Six healthy male New Zealand white rabbits weighing between 2.8 to 3.2 kg were used in the study. The rabbits were randomly divided into two groups of three rabbits in each group. The rabbits were fasted for 12 hr with free access to water prior to the experiment. One group received Reference product whereas the other group received Test product. After a washout period of one week, the animals were crossed-over and received the alternate products. Blood samples of 2 ml was withdrawn from marginal ear vein at predetermined time intervals of 0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4,

6, 8, 12, 16 and 24 hr post administration. The blood was immediately centrifuged at 4000 rpm for 15 min and the obtained plasma was stored at -20° C until further analysis.

The mean C_{max}, T_{max}, $AUC_{0-\infty}$, $t_{1/2}$, and K_e values 299.09 ± 12.90 ng/ml, 1.75 ± 0.27 hr, 1960.32 ± 146.91 ng.hr/ml, 4.02 ± 0.23 hr and 0.1730 ± 0.010 hr^{-1} for Reference and 308.40 ± 15.93 ng/ml, 1.58 ± 0.20 hr, 1910.65 ± 162.69 ng.hr/ml, 3.87 ± 0.25 hr and 0.1795 ± 0.012 hr^{-1} for Test formulation, respectively. From the results it was concluded that the Test formulation and Reference product (Zofer MD 8®) have similar plasma concentration-time curves and pharmacokinetic parameter values. The two formulations are bioequivalent in their rate and extent of absorption and thus, may be used interchangeably. The Test formulation could be a useful alternative to commercially available formulations.

Sumatriptan succinate (SS)

Tate masking of the SS

Spray drying technique was used for the preparation of SS taste masked microspheres. Eudragit EPO was used as a taste masking agent because it dissolves in pH < 5 so the polymer dissolves fast in stomach (pH 1-3) without influence the bioavailability, but keep intact in buccal cavity (pH 5.8-7.4) with good taste masking.

The polymer, Eudragit EPO was dissolved in ethyl acetate and then added drug to prepare a suspension. The composition of final mixture in the ratio of drug: polymer: organic solvent ratio is 1:1:50. The prepared suspension was sprayed through nozzle (diameter of 0.7 mm) using spray dryer (Lab Plant SD-04, Huddersfield, UK) to obtain tasted masked SS powder. The suspension was stirred using Heidolph stirrer at 500 rpm to maintain uniformity in suspension and also to prevent suspending the drug particles in Eudragit EPO solution while spray through nozzle. The obtained powder was transferred into a tightly closed container and stored over silica gel until further use. The spray dryer was operated under the following conditions: inlet and outlet temperatures of 80 and 55-61 °C, respectively, blower setting at 70 and peristaltic pump at 30%.

The yield of the spray dried microspheres was about 56.34%. The entrapment efficiency of the microspheres was 92.86%. The taste masking property of the spray dried microspheres

was confirmed by the in vitro taste masking experiment with the simulation of salivary conditions in the mouth and only 4.57% (3.2 mg) in 60 sec in 5 ml of pH 6.8 phosphate buffer. It could be further decrease as the disintegration time of the ODT is less than 60 sec, which could be insufficient to impart bitter taste. The Thermal analysis (DSC) of spray dried microspheres of drug and polymer revealed negligible change in the melting point of Eudragit EPO and SS, indicating no modification or interaction between the drug and polymer. It concludes that drug is compatible with the polymer used and does not undergo any change during spray dry processing. In the gas chromatographic (GC) analysis, it was found that there was no residual solvent of ethyl acetate present in the microspheres. Hence, the produced microspheres were safe to oral ingestion of SS.

1. Wowtab technique

Physical properties of the SS tablets

All the prepared formulations produced tablets which were white in colour and concave in shape with smooth surface with zero defects. The average weight and thickness of tablets for all the formulations was found to be in the range of 316.29 to 323.45 mg and 6.34 to 6.89 mm, respectively. All the prepared formulations passed weight variation test, with percent weight variation within the pharmacopoeial limits of ±7.5% of the average weight. The tablets provided good weight uniformity as indicated by the very low relative standard deviation obtained (RSD <1%) for all formulations. The hardness of the tablets was maintained in the range of 2-3 kg. The friability of all the formulations were found to be within acceptable limits (<1%). The drug content was varied in the range of 98.11 to 102.56%.

Formulation rationale

The SS ODTs were prepared with direct compression and wet granulation techniques and found that direct compression is suitable technique. In the optimization of hardness, the similar trend of results as that of ondansetron was produced. The hardness of 2-3 kg was selected for further experiments as it resulted in tablets with a good balance over hardness, friability and DT. The lubricants, magnesium stearate and SSF did not show any difference in the DT values due to high water solubility of SS. Hence, in all the formulations magnesium stearate was used as a lubricant.

In the formulations, calcium silicate was incorporated as a pore forming agent to increase the pores and fasten the DT of the tablets. The pore forming agent was used in a concentration range of 1 to 5%. The DT of the tablets was 354.00, 264.83 and 229.50 sec for the formulations prepared with 1, 3 and 5%, respectively. The DT of the tablets was decreased with the increase in the concentration of calcium silicate. It might be due to the increase in the porous nature of the tablets with respect to the calcium silicate concentration which could be attributed to faster absorption of water through the pores by wicking action and disintegration of the tablets. Although, the DT of the tablets did not meet the requirements set by USP.

To decrease the disintegration time further and to achieve the desired DT, a sublimation technique was used in further experiments. Ammonium bicarbonate (ABC) was used as a subliming agent in a concentration range of 5 to 10%. The resulted DT of the tablets were 164.83 (5%), 121.33 (7.5%) and 71.50 sec (10%), respectively. The results indicate that concentration dependent disintegration was observed in the formulations prepared using ABC as subliming agent. During drying, the ABC was sublimed with the formation of a porous structure on the surface of the tablets. The porous structure is responsible for faster water uptake; hence it facilitates wicking action of polyplasdone XL in bringing about faster disintegration. The formulation containing 10% ABC showed the least disintegrating time and used for further evaluation.

To further decrease in the DT of the tablets, different types of superdisintegrants at different concentration levels were studied. The disintegration time of various formulations studied varied form 33.83 to 190.33 sec. The disintegration time of the tablets were decreased with the increase in the concentration of polyplasdone XL from the 5 to 15% and the results were 71.50 (5%), 55.67 (10%) and 39.17 sec (15%). There was a statistically significant difference ($p < 0.05$) in the DT of ODTs among the formulations. Increase in the concentration of polyplasdone XL-10 from 5 (63.67 sec) to 10% (37.50 sec) resulted in a significant decrease in the DT of the tablets. However, further increase in the concentration of polyplasdone XL-10 to 15% (38.50 sec) did not show any significant effect ($p > 0.05$) on the DT. The formulations prepared with the superdisintegrants of CCS and SSG at a concentration level of 5%, produced the DT values was 64.33 and 111.83 sec, respectively. Further increase in the concentration of CCS and SSG from 5 to 10 and 15% resulted in a significant increase ($p < 0.05$) in the DT of the tablets from 64.33 to 86.33 sec and 136.00 sec for CCS and from 111.83 to 148.00 sec and 190.33 sec for SSG. It can be observed from the results that the

disintegration times of crospovidone (polyplasdone XL and polyplasdone XL-10) containing tablets were comparatively lower than those containing CCS and SSG. The similar trend of the results was observed with the DT of ondansetron ODTs. As described in the preparation of ondansetron ODTs, this dissimilarity in the effect of concentration of crospovidone, CCS and SSG on the disintegration time can be attributed to the difference in their mechanism of disintegration. The disintegration time of the tablets were decreased with increase in the concentration from 5 to 10% for Kollidon CL and 1.25 to 5.00% for Kollidon CL-SF. The resulted DT of the tablets were 75.00 (5%), 64.33 (7.5%) and 48.17 sec (10%) for Kollidon CL and 73.00 (1.25%), 62.83 (2.5%) and 38.17 sec (5%) for Kollidon CL-SF. The superdisintegrants, Kollidon CL and Kollidon CL-SF exhibited their disintegrant effect by wicking action without forming a gel. There was a statistically significant difference ($p < 0.05$) in the DT of the ODTs among the formulations prepared with Kollidon CL and Kollidon CL-SF. The superdisintegrant, L-HPC at concentrations of 1, 3 and 5%, resulted in a disintegration time of 155.50, 133.33 and 112.00 sec, respectively. The DT of the tablets was decreased with increase in the concentration of superdisintegrant from 1 to 5%. The tablets prepared with L-HPC disintegrate based on its swelling property in water (Bi *et al.*, 1996). There was a statistically significant difference ($p > 0.05$) in the DT of the tablets among the formulations.

To decrease the disintegration time further, combination of superdisintegrants were evaluated in the preparation of SS ODTs. The formulations prepared with the superdisintegrants, polyplasdone XL (10 and 15%), polyplasdone XL-10 (10 and 15%), Kollidon CL (10%) and Kollidon CL-SF (5%) were produced the desired DT with < 60 sec. The DT obtained for formulations prepared with the combination of polyplasdone XL-5% and polyplasdone XL 10-10%, polyplasdone XL-10% and polyplasdone XL-10-5% and polyplasdone XL 10-10% and Kollidon CL-SF-5% were 35.50, 37.33 and 35.67 sec, respectively. The incorporation of superdisintegrants in combination did not produce any satisfactory results in further decrease in the DT of the tablets. There was no significant difference ($p > 0.05$) in the DT among the formulations prepared with different combinations of superdisintegrants.

Aspartame was used as a sweetener in the present study. Preliminary studies were performed in healthy human volunteers for the taste characterization of SS ODTs prepared with different amount of aspartame (1 to 3%) as a sweetener in the formulations. The optimum amount of sweetener was determined based on the taste perception. The disintegration time of the tablets

was not affected with the increase in the amount of aspartame from 1 to 3% in the ODTs. All the volunteers rated the formulations prepared with 2 and 3% aspartame as sweet and acceptable and 2% aspartame was used in further evaluations.

The promising sumatriptan ODT formulations with a disintegration time of less than 60 sec (polyplasdone XL-10 and 15%, polyplasdone XL-10-10 and 15%, Kollidon CL-10%, Kollidon CL-SF-5%, polyplasdone XL-5% & polyplasdone XL-10-10%, polyplasdone XL-10% & polyplasdone XL-10-5% and polyplasdone XL-10-10% & Kollidon CL-SF-5%)were subjected for water content determination. The results showed that the water content of all the ODT formulations was less than 9%.

In vitro drug dissolution studies conducted with 0.01 N HCl at a paddle speed of 30 rpm showed that the commercial product (Suminat®) and promising ODT formulations released more than 90% of drug in 15 min except the formulation which contained Kollidon CL (10%). There was no statistically significant difference ($p > 0.05$) in the release profiles of all the promising ODT formulations compared to commercial product. The superdisintegrant, Kollidon CL-SF used in this technique had smaller particle size (10-30 μm) since it produces excellent mouthfeel without any grittiness in the mouth when the patient administer the tablet. Hence, it fulfils the one of the ideal requirements of ODTs, good mouth feel. The tablets prepared with this superdisintegrant produced lower values of the water content. Hence, formulation prepared with Kollidon CL-SF-5% was chosen as an optimized formulation in this technique.

2. Orasolv technique

Physical properties of the tablets

All the prepared formulations produced tablets which were white in colour and concave in shape with smooth surface with zero defects. The average weight and thickness of tablets for all the formulations was found to be in the range of 298.97 to 302.49 mg and 6.15 to 6.29 mm, respectively. All the prepared formulations passed weight variation test, with percent weight variation within the pharmacopoeial limits of ±7.5% of the average weight. The tablets provided good weight uniformity as indicated by the very low relative standard deviation obtained (RSD <1%) for all formulations. The hardness of the prepared tablets was maintained in the range of 2-3 kg. The friability of all the formulations was between 0.28 to

0.62% and the results were found to be within acceptable limits (<1%). All the formulations demonstrated uniformity in the assay and drug content varied from 99.55 to 101.97%.

At an initial experiment, formulations were prepared with different ratios of acid (citric acid) and base (sodium bicarbonate) as a pair of effervescent agent including 1:1, 1:2 and 1:3 and resulted DT values were 161.50, 129.17 and 125.33 sec, respectively. The DT of the tablets was decreased with increase in the ratio of acid and base from 1:1 to 1:2. It might be due to the decrease in the amount of citric acid and increase in sodium bicarbonate in the formulations. However, further increase in the ratio of acid and base to 1:3, did not show any effect ($p > 0.05$) on the DT of the tablets. The DT values obtained for the formulations were more than 60 sec and did not produce desired specification set by the USFDA for ODTs. Therefore, formulation prepared with 1:2 was used for further modification in the formulations.

To further decrease in the DT of the tablets, next step of experiments were carried out with the combination of two bases, combination of two acids and combination of two pairs of acid and base. In combination of two bases, sodium bicarbonate and sodium carbonate was used. The amount of sodium bicarbonate was kept constant and incorporated different concentrations of sodium carbonate in the range of 1 to 3%. The resulted DT values were 126.00 (1%), 115.83 (2%) and 100.67 sec (3%), respectively. The incorporation of 1% sodium carbonate did not aid in decrease of DT of the tablets. The DT of tablets was decreased to 115.83 and 100.67 sec with further increase in the concentration of sodium carbonate to 2 and 3%. Nevertheless, these formulations were also not able to attain the DT specifications set for the ODTs.

In combination of two acids, citric acid and tartaric acid was examined to decrease the DT of the tablets. The amount of citric acid was kept constant and incorporated different concentrations of tartaric acid in the range of 1 to 3%. The resulted DT values were 126.83 (1%), 108.83 (2%) and 94.50 sec (3%), respectively. The incorporation of 1% citric acid did not aid in decrease of DT of the tablets. The DT of tablets was decreased to 108.83 and 94.50 sec with further increase in the concentration to 2 and 3%. Nevertheless, these formulations were also not able to attain the DT specifications set.

The formulations which were produced lower DT of the tablets with the combination of two bases and two acids at a concentration of 3% were combined to prepare the tablets with the combination of two pairs of acid and base. In this formulation, tartaric acid and sodium carbonate were incorporated each at a concentration of 3% and resulted DT was 92.83 sec. The DT values were decreased with the combination of two bases and two acids alone as well as combination with two pairs of acid and base compared to one pair of acid and base. From the results observed that combination of two acids played a significant role in the decrease of DT of the tablets compared to combination of two pairs of acid and base. However, the DT values obtained were not within the limits. Further modification was made to achieve a DT value of less than 60 sec.

In the next step of formulation development, calcium silicate was incorporated in the formulation prepared with combination of two acids to further decrease in the DT of the tablets. Calcium silicate was incorporated as a pore forming agent in a concentration range of 5.0 to 12.5% in the formulations. Calcium silicate has many pores and a large pore volume with characteristic porous structure of the tablets (Yuasa *et al.*, 1996). The resulted DT values for the formulations prepared with calcium silicate at a concentration of 0, 5.0, 7.5, 10.0 and 12.5% were 94.50, 81.17, 69.33, 56.67 and 49.67 sec, respectively. The DT of the tablets was decreased with the increase in the concentration of calcium silicate from 5 to 12.5%. As explained earlier, it might be due to the increase in the porous nature of the tablets with respect to the calcium silicate concentration which could be attributed to faster absorption of water through the pores by wicking action and disintegration of the tablets. From the study it can be observed that there was a statistically significant difference ($p < 0.05$) among the formulations in the DT of the tablets. The formulations prepared with a concentration of 10 and 12.5% of calcium silicate were produced the DT of the tablets with < 60 sec and were well in the specifications set by USFDA for orally disintegrating tablets. Hence, these two formulations were used for water content determination and in vitro dissolution studies. The resulted water content values for the formulations prepared with 10 and 12.5% of calcium silicate were 5.35 and 5.87%, respectively.

The commercial product (Suminat®) and promising ODT formulations dissolution studies were conducted in 0.01 N HCl medium at a paddle speed of 30 rpm. The commercial product and formulation prepared with calcium silicate at a concentration of 12.5% were released more than 90% of drug in 10 min whereas, the formulation prepared with 10% calcium

silicate was released 90% of drug in 15 min. There was no statistically significant difference ($p>0.05$) in the release profiles of the commercial product and formulation prepared with 12.5% calcium silicate. Hence, this formulation was chosen as an optimized formulation in this technique.

3. Freeze dried technique

Physical properties of the tablets

All the formulations resulted in successfully dried and produced elegant tablets that were strong enough to be easily handled. The average weight for all the formulations was found to be in the range of 198.71 to 203.10 mg. The tablets provided good weight uniformity as indicated by the very low relative standard deviation obtained (RSD $\leq$ 1.33%) for all the formulations. The mean percent drug content in all formulations was found to be in the range of 99.37 to 102.21%.

The in vitro disintegration time results for all the freeze dried formulations prepared with different matrix forming agents were studied in the range of 1 to 5%. At an initial study, the formulations were prepared using gelatin as the matrix forming agent. The resulted mean DT values for the formulations prepared with 1, 3 and 5% were 299.67, 225.33 and 140.17 sec, respectively. The obtained DT results were inversely proportional to the concentration of gelatin in the formulations i.e. disintegration time decreased with increase in the amount of gelatin. There was a statistically significant difference ($p<0.05$) among the formulations. However, the produced DT results were not within the acceptable specifications set by USFDA for ODTs. Further experiments were carried out using PVP K-90 as a matrix forming agent. The resulted DT values were 74.00 (1%), 82.83 (3%) and 95.67 sec (5%), respectively. As the concentration of PVP K-90 was increased there was an increase in the DT values. It could be attributed to the formation of a viscous jelly like structure at higher concentrations of PVP K-90. There was a significant difference ($p<0.05$) among the formulations in the DT values. Nevertheless, the prepared formulations did not produce the satisfactory DT results.

In the next step of experiments, PVP K-90 was replaced with the HPMC-E3 as a matrix forming agent. The formulations prepared with HPMC-E3 at different concentrations of 1, 3 and 5% were produced the DT results of 123.00, 93.83 and 76.50 sec, respectively. The DT of the tablets was decreased with increase in the concentration of HPMC-E3. There was a

significant difference ($p<0.05$) in the DT values of among the formulations. The produced DT results were not within the acceptable limits for the ODTs.

The cold water soluble gelatins (hydrolyzed gelatins), Cryogel and Instagel were used as a matrix forming agents in further experiments. The resulted DT values of 1, 3 and 5% were 52.17, 82.67 and 94.00 sec for Cryogel and 40.17, 68.83 and 77.17 sec for Instagel, respectively. The DT values of the tablets were affected by Cryogel and Instagel concentrations. As the concentration of both Cryogel and Instagel was increased there was an increase in the DT of the tablets. As explained earlier, the formulations prepared with Instagel disintegrated faster due to their lower bloom strength compared to Cryogel. There was a statistically significant difference ($p<0.05$) among the formulations prepared with different concentrations of Cryogel and Instagel. The two formulations prepared with 1% Cryogel and 1% Instagel were produced the DT of the tablets <60 sec and the results were within the acceptable limits of USFDA set for the ODTs. Hence, these formulations were subjected for water content and in vitro dissolution studies.

The resulted water content values for the formulations prepared with Cryogel and Instagel at a concentration of 1% were 9.16 and 6.88%, respectively. The tablets prepared with Instagel matrix forming agent were produced the lower values of the water content compared to Cryogel matrix former.

The commercial product (Suminat®) and promising ODT formulations dissolution studies were conducted in 0.01 N HCl medium at a paddle speed of 30 rpm. The commercial product and formulation prepared with 1% Instagel were released more than 90% of drug in 10 min whereas; the formulation prepared with 1% Cryogel was released 90% of the drug in 15 min. It might be attributed to the higher bloom strength of the Cryogel which can form a thick gel upon exposure to dissolution medium and hindered the drug release profile. There was no statistically significant difference ($p>0.05$) in the release profiles of the commercial product and formulation prepared with 1% Instagel. Hence, this formulation was chosen as an optimized formulation in this technique.

Based on the findings in these 3 techniques, formulation prepared with Kollidon CL-SF-5% in Wowtab technique, formulation prepared with 12.5% calcium silicate in Orasolv technique and formulation prepared with 1% Instagel in freeze dried technique were the optimized

formulations and met the official requirements for the ODTs. All these formulations were comparable with the commercial product in the in vitro release profiles. The optimized formulations in Wowtab and freeze dried techniques were produced almost similar DT values, 38.17 and 40.17 sec, respectively. Moreover, these two formulations produced lower DT values compared to Orasolv technique (49.67 sec). Hence, with the consideration of freeze dried technique disadvantages, the optimized formulation in Wowtab technique was selected for the evaluation of taste, mouth feel and in vivo disintegration time in human volunteers and for stability studies.

In taste evaluation, the ODTs prepared with uncoated drug used as a reference. All the volunteers rated the reference formulation as '5' which indicates that the tablets were very bitter and awful. Ten out of 12 volunteers rated the optimized formulation as sweet and good. In evaluation of mouth feel, 11 out of 12 volunteers experienced a good mouth feel without any grittiness for optimized formulation. Data collected from the in vivo DT showed that optimized formulation on average disintegrated in the oral cavity within 41 sec. Due to the intensely bitterness of the reference formulation prepared with uncoated drug, the volunteers immediately spitted out the tablet. Hence, it was not able to determine the mouth feel and in vivo DT of reference formulation.

In stability study, samples of optimized formulation were examined after 1, 3 and 6 months storage and found that there were no significant change in appearance of the tablets, weight variation, thickness, hardness, friability, water content and disintegration time. The results of DT after 6 months of storage were within the USFDA limits (< 60 sec). The drug content in the tablets stored for 6 months at 40±2 °C/75±5% RH and 25±2 °C/65±5% RH were 99.01 and 99.83%, respectively and found that insignificant loss ($p>0.05$) in the drug content. There was no significant difference ($p>0.05$) in the in vitro release profiles between fresh and samples stored for 6 months. Thus, this formulation was proven to be stable and selected for the in vivo pharmacokinetic study.

HPLC-Fluorescence method for the determination of sumatriptan in rabbit plasma

Prior to pharmacokinetic study, a new, sensitive and specific HPLC method with fluorescence detection was developed and validated for the determination of sumatriptan in rabbit plasma. The HPLC system consisted of a Shimadzu chromatographic system (Kyoto, Japan) equipped with an LC-20AD solvent delivery binary pump, RF-10AXL fluorescence detector, SIL-

20AHT autosampler, CTO-10AS VP column oven and LC Solution software for data acquisition and processing.

Chromatographic separations were performed using a reversed-phase C4 analytical column (Phenomenex Kromasil, 250 x 4.6 mm i.d., 5 μm particle size) fitted with a C4 guard column (Phenomenex Kromasil, 10 x 4 mm i.d., 5 μm particle size). The mobile phase used for the analysis was 25 mM ammonium acetate (pH 6.5) and acetonitrile (85:15, v/v) delivered at a flow rate of 0.9 ml/min. Fluorescence detection was performed with excitation and emission wavelengths of 225 and 350 nm. The column oven temperature was maintained at 40°C. The injection volume was 50 μl.

The plasma samples were extracted as follows: To 0.5 ml aliquot of plasma, 20 μl of 1 μg/ml of IS (sulpiride), 0.5 ml of 1 M sodium hydroxide and 7 ml mixture of TBME, DCM and EA (2:2:3, v/v) as an extraction solvent was added. The mixture was vortexed for 2 min and centrifuged at 4000 rpm for 15 min. The supernatant was transferred to reacti-vial and evaporated to dryness at 50°C under nitrogen gas. The residue was reconstituted with 0.2 ml of 10% v/v methanol, transferred to autosampler vials and injected 50 μl of the sample.

The calibration curve exhibited an excellent linearity over the concentration range of 1-300 ng/ml of sumatriptan with a correlation coefficient of 0.9999. The chromatograms of blank plasma and plasma spiked with sumatriptan shows that the blank rabbit plasma had no interference from endogenous substances at the retention times of the analyte and I.S. A good resolution between analyte and IS was achieved with the retention time of 7.13 min for IS and 8.73 min for sumatriptan. The LOQ of the present method was 1 ng/ml. The intra-day accuracy ranged between -1.10 and 1.53% with a precision of 2.25 to 3.49%. The inter-day accuracy ranged between 0.16 and 2.86% with a precision of 2.24 to 4.28%. The results were within the acceptable limits (±15%). The mean extraction recoveries of sumatriptan from rabbit plasma at the concentrations of 5, 150 and 250 ng/ml were 90.65, 88.68 and 90.42%, respectively. The mean extraction recovery of IS was 91.03%. Sumatriptan was found to be stable at -20 °C for 14 days.

In vivo pharmacokinetic study in rabbits

The in vivo study was conducted to evaluate the pharmacokinetic parameters of the optimized sumatriptan ODT formulation (Test) in comparison with the Reference product (Suminat®)

using rabbits as an animal model. Six healthy male New Zealand white rabbits weighing between 2.8 to 3.2 kg were used in the study. The rabbits were randomly divided into two groups of three rabbits in each group. The rabbits were fasted for 12 hr with *ad libitum* access to water prior to the experiment. One group received Reference product whereas the other group received Test product. After a washout period of one week, the animals were crossed-over and received the alternate products. Blood samples of 2 ml were withdrawn from marginal ear vein at predetermined time intervals of 0 (pre-dose), 0.25, 0.5, 0.45, 1, 2, 3, 4, 6, 8, 12 and 16 hr post administration. The blood was immediately centrifuged at 4000 rpm for 15 min and the obtained plasma was stored at -20° C until further analysis.

The mean C_{max}, T_{max}, $AUC_{0-\infty}$, $t_{1/2}$, and K_e values for 523.32 ± 346.17 ng/ml, 1.42 ± 0.96 hr, 2147.42 ± 1529.06 ng.hr/ml, 1.70 ± 0.23 hr and 0.4128 ± 0.056 hr^{-1} for Reference and 510.00 ± 222.57 ng/ml, 1.54 ± 0.84 hr, 2232.35 ± 1203.24 ng.hr/ml, 1.39 ± 0.26 hr, and 0.5124 ± 0.082 hr^{-1} for Test, respectively. From the results it was concluded that the Test formulation and Reference product have similar plasma concentration-time curves and pharmacokinetic parameter values. The two formulations are bioequivalent in their rate and extent of absorption and thus, may be used interchangeably.

Conclusions

The "patient friendly dosage form" of water soluble and water insoluble orally disintegrating tablets were successfully prepared. The prepared tablets had an adequate hardness and produced faster disintegration with pleasant taste and mouth feel in the oral cavity. The in vivo performance of the optimized formulations was evaluated in rabbits and found that results were comparable with commercial product. These two formulations could be useful alternatives to commercially available formulations.

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Development and Validation of an RP-HPLC–UV Method for the Determination of Ondansetron in Rabbit Plasma: Application to a Pharmacokinetic Study

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Summary. A new sensitive and specific isocratic RP-HPLC–UV method was developed and validated for the determination of ondansetron in rabbit plasma using risperidone as an internal standard (IS). The sample preparation involved a simple deprotenization procedure with a mixture of 1 mL of acetonitrile and 50 μL of 10% *w/v* zinc sulfate. Analysis was performed on a Phenomenex CN column (250 mm × 4.6 mm, 5 μm) with 50 mM ammonium acetate (pH 3.5) and acetonitrile (35:65, *v/v*) as mobile phase at a flow rate of 1.0 mL min^{-1}. Column eluent was monitored at 310 nm. The calibration curve was linear over the concentration range of 25–1000 ng mL^{-1} (r^2 = 0.9999) with a limit of quantification (LOQ) 25 ng mL^{-1}. The intraday and interday precision and accuracy were between 0.93% and 3.41% and –3.63% and 1.01%, respectively. The mean recoveries of ondansetron and risperidone were 85.87% and 99.80%, respectively. Ondansetron-containing plasma samples were stable at –20°C for 14 days. The validated method was successfully applied for a pharmacokinetic study after a single oral administration of ondansetron (8 mg) to rabbits.

Key Words: ondansetron, RP-HPLC–UV, method validation, rabbit plasma, pharmacokinetics

Introduction

Chemotherapy-induced nausea and vomiting (CINV) has a severe impact on quality of life of cancer patients. The generation of 5-HT3 serotonin antagonists (ondansetron, granisetron, dolasetron) represents an important progress in the management of CINV [1]. Ondansetron is a basic compound (pK_a 7.70) and chemically known as {1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one} *(Fig. 1a)*. It is a white to off-white powder that is soluble at pH 1.2. The partition coefficient (log *P*) of the ondansetron base in *n*-octanol/water is 2.14. It is used in the treatment of emesis and nausea associated with cancer-related chemotherapy and radiation. As an antiemetic, the usual dose is in the range of 8–

32 mg per day [2, 3]. Oral ondansetron is well absorbed with a bioavailability of approximately 60–70%.

Several analytical methods have been developed previously for the determination of ondansetron in biological fluids. Most studies included HPLC with UV detection [3–9]. Some researchers such as Depot et al. [3], Bauer et al. [4], and Chandrasekar et al. [5] have developed sensitive HPLC–UV methods to quantify ondansetron in plasma with limits of quantification (LOQ) of 0.5, 0.62, and 10 ng mL^{-1}, respectively. However, these methods achieved the sensitivity by concentrating large volumes of samples (1–2 mL) into lower volumes (100 μL). Moreover, all of the above-mentioned studies used a liquid–liquid extraction (LLE) technique with large volumes of solvent and involved laborious and time-consuming extraction steps. Other drawbacks were the higher flow rate (1.5 mL min^{-1}) and long run time of analysis (15 min), which were not suitable for the analysis of a large number of samples present in pharmacokinetic studies. To date, there has been only one publication reported by Sutariya and Mashru [9] on ondansetron extraction with a smaller plasma volume (0.3 mL) with an LOQ of 25 ng mL^{-1}. The plasma sample preparation using the LLE technique and long run time of analysis (18 min) limit its use in pharmacokinetic studies. Expensive solid-phase extraction (SPE) procedures with multiple steps of column conditioning, washing, and sample extraction have been reported by some authors [6–8]. In addition, Kelly et al. [7] and Liu and Stewart [8] employed a specific cellulose-based chiral analytical column in analysis of ondansetron.

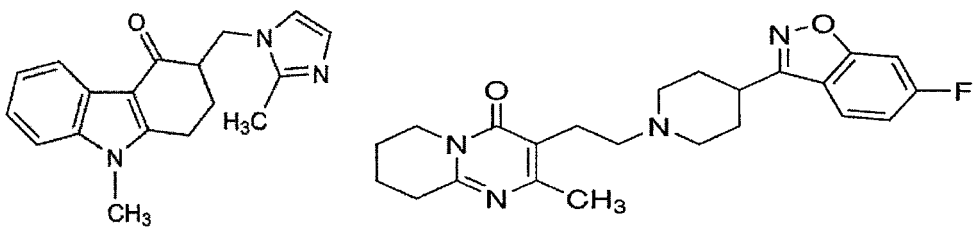

Fig. 1. Chemical structures of (a) ondansetron and (b) risperidone (IS)

Other researchers used liquid chromatography coupled with mass spectrometry for the determination of ondansetron in biological samples [10–13]. Although mass spectrometry provides greater sensitivity and specificity with short analysis times, it might not be universally applicable in laboratories because of cost implications.

The extraction technique employed in sample preparation plays a vital role in the development of a suitable assay for the analysis of analytes. In bioanalytical methods, the protein precipitation technique (PPT), LLE, and SPE were the commonly used techniques for the extraction of analytes from

biological matrices. PPT is a simple sample preparation technique that removes proteins from biological fluids prior to analysis. PPT procedures are considered fast, are convenient to handle biological samples, and can be applied to a wide range of analytes. To our knowledge, no method is reported to date for the extraction of ondansetron plasma samples using PPT. Therefore, in the present study, a new sensitive and specific RP-HPLC-UV method with simple sample preparation procedure was developed and validated for the determination of ondansetron in rabbit plasma after a single oral administration of ondansetron (8 mg) to the animals.

Experimental

Chemicals and Reagents

Ondansetron was purchased from Symed Labs (Hyderabad, India). Zofer MD 8® mouth dissolving tablets were purchased from Sun Pharmaceuticals (Vapi, India). Risperidone was purchased from Janssen-Cilag (New Jersey, USA). Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (Phillipsburg, USA). Zinc sulfate was purchased from R&M Chemicals (Essex, UK). Ammonium acetate was purchased from Nacalai Tesque (Kyoto, Japan). Glacial acetic acid was purchased from QRec (Selangor, Malaysia). Blank rabbit plasma was collected from the marginal ear veins of several New Zealand rabbits and stored at –20°C until further use.

HPLC Instrumentation and Chromatographic Conditions

The HPLC system consisted of a Shimadzu chromatographic system (Kyoto, Japan) equipped with an LC-10AT VP solvent delivery pump, an SPD-10A VP UV-Vis detector, an SIL-10AD VP autosampler, and the Class VP Chromato software for data acquisition and processing. The analysis was performed on a reversed-phase cyano (CN) column (Phenomenex, 250 × 4.6 mm i.d., 5 μm particle size) with an isocratic mobile phase consisting of 50 mM ammonium acetate adjusted to pH 3.5 with glacial acetic acid and acetonitrile (35:65, *v/v*). The mobile phase was delivered at a flow rate of 1.0 mL min^{-1}. The detector was set at the wavelength of 310 nm. The injection volume was 100 μL.

Preparation of Stock Solutions, Standards, and Quality Control Samples

Stock solutions were prepared in acetonitrile at a concentration of 1 mg mL^{-1} for ondansetron and risperidone (internal standard (IS), *Fig. 1b*). Ondansetron stock solution was subsequently diluted in the same diluent to obtain working standard solutions in the range of 0.5–20 μg mL^{-1}. These solutions were added (25 μL) to blank plasma to produce final concentrations of 25–1000 ng mL^{-1} for ondansetron. Quality control (QC) samples were prepared at three concentration levels of 75 (low), 400 (medium), and 900 ng mL^{-1} (high). For each solution, the IS was added at a constant level of 25 μL of 1 mg mL^{-1} stock solution. All solutions were stored under refrigeration at 4°C prior to use.

Sample Treatment

To 0.5 mL aliquot of plasma, 25 μL of 1 mg mL^{-1} IS were added and deproteinized with a mixture of 1 mL of acetonitrile and 50 μL of 10% *w/v* zinc sulfate. The mixture was vortexed for 2 min and centrifuged at 10,000 rpm for 20 min. The supernatant was transferred to autosampler vials, and 100 μL were injected into the HPLC system.

Pharmacokinetic Study in Rabbits

Six healthy male New Zealand rabbits (2.8–3.2 kg) were used for the study. The study was conducted in accordance with Animal Ethical Guidelines for investigations in laboratory animal, and the study protocol was approved by the Animal Ethics Committee of University Sains Malaysia. After an initial period of acclimatization for 1 week to laboratory conditions, the rabbits were randomly divided into two groups of three each. All rabbits were fasted for 12 h with *ad libitum* access to water prior to the experiment. One group received the reference product (Zofer MD 8®), whereas the other group received the test product (orally disintegrating tablets prepared in our research laboratory). The tablets were administered at the back of the pharynx using a gastric intubation tube (made of silicone rubber) with one tablet set on the tip of tube; immediately, 5 mL of water was administered through the tube to facilitate swallowing of the tablet and to prevent it from sticking to the rabbit's throat. The animal had access to food 4 h after dose administration. About 2 mL of blood sample was withdrawn from marginal ear vein into heparinized eppendorf tubes at time intervals of 0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, and 24 h post administration. Accord-

ing to US Food and Drug Administration (USFDA) and European Agency for the Evaluation of Medicinal Products (EMEA) regulations, the sampling schedule should be planned to provide a reliable estimation of the extent of absorption [14, 15]. This can be achieved if AUC_{0-t} is at least 80% of $AUC_{0-\infty}$. Usually, the sampling time should extend to at least three terminal elimination half-lives of the active ingredient. Time periods between samplings should not exceed one terminal half-life [16]. From the pharmacokinetic data reported in the existing literature, it was found that the half-life ($t_{1/2}$) of ondansetron is approximately 3-4 h. Hence, in the present study, the samples were collected up to 24 h after drug administration so as to cover a minimum of three half-lives of ondansetron. The time interval between the sample collections was also maintained not to exceed more than one terminal half-life, until covering the three $t_{1/2}$ of ondansetron. The plasma was separated by centrifugation at 4000 rpm for 15 min and was stored at –20°C until analysis. After a wash-out period of 1 week, the animals were crossed over and administered the alternate product. According to the standard for bioequivalence tests [14], the wash-out period should be at least 5 times the half-life of the active ingredient after administration. Considering ondansetron $t_{1/2}$, 7 days should be sufficient for the drug to be eliminated completely from the body.

The pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}), were obtained directly from the data. The area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was calculated by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling time to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula and the latter by dividing the last measurable plasma drug concentration with the terminal elimination rate constant (K_e). The value of K_e was calculated using the least-squares regression analysis of the terminal portion of the log plasma concentration versus time curve. The elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 by K_e.

Statistical Analysis

The results are reported as mean ± standard deviation. An analysis of variance (ANOVA) was performed on the pharmacokinetic parameters $AUC_{0-\infty}$, C_{max}, and $t_{1/2}$, which distinguishes the effects due to subjects, periods, and treatment [17]. The *p*-value was calculated from the obtained *F*-value using the software GraphPad Prism, version 5.02 (GraphPad Prism software, San Diego, CA). The values of $AUC_{0-\infty}$ and C_{max} were logarithmic-transformed before analysis. The T_{max} values were analyzed using the Wilcoxon signed

rank test for paired samples. A statistically significant difference was considered at $p < 0.05$.

Results and Discussion

Several variables of the HPLC method with respect to their effect on the separation of ondansetron and IS from the matrix were investigated. In extensive preliminary experiments, parameters such as the choice of analytical column, composition of the mobile phase (i.e., organic modifier, pH, and molarity of buffer salt), and flow rate were optimized in order to provide a good performance of the assay for the determination of ondansetron in the rabbit plasma.

Method Development and Optimization

Ondansetron has optimum sensitivity at wavelengths of 212, 248, and 310 nm. Baseline drift was observed at 212 nm and more interfering peaks emerged from the plasma at both 212 and 248 nm. Hence, the detection wavelength of 310 nm was selected for the quantification of ondansetron in rabbit plasma.

Several reversed-phase analytical columns such as C18, C8, C4, and CN were tested with the mobile phase composition of 50 mM ammonium acetate (pH 5.0) and acetonitrile (35:65, *v/v*) for the separation of ondansetron. Ondansetron is a basic nonpolar compound and insoluble in water. For organic nonpolar molecules, the sample retention increases with increase in the length of the bonded phases. However, in this study, the analyte was eluted at earlier retention time from the C18 (Phenomenex, 250 mm × 4.6 mm, 5 μm), C8 (Phenomenex, 250 mm × 4.6 mm, 5 μm), and C4 (Thermo-Hypersil 250 mm × 4.6 mm, 5 μm) than from the CN column. Moreover, C18, C8, and C4 analytical columns were not able to give a good resolution between the analyte and IS. In the Cyano (CN, Phenomenex 250 mm × 4.6 mm, 5 μm) column, ondansetron eluted at a longer retention time with good chromatographic response and peak shape and also well resolved from the IS. On the basis of these findings, the analytical column CN was found to be the most appropriate for the determination of ondansetron in plasma.

Mobile phases at different ratios of buffer and organic modifier (20:80, 30:70, 35:65, 40:60, 50:50, 60:40, and 65:35 (*v/v*)) were tested using the CN analytical column. At the ratio of 20:80 (*v/v*), the IS peak was not eluted until 15 min, whereas at the ratio of 30:70 (*v/v*) IS was eluted at 9.72 min but the peak shape was not optimal. Further decrease in the content of the organic

modifier from 30:70 to 65:35 (*v/v*) resulted in an increase in the peak tailing and retention time of the both analytes. However, the ratio of 35:65 (*v/v*) provided a more symmetric peak shape with reasonable retention time for both analytes. Acetonitrile was found to be a suitable organic modifier compared to methanol which produced high column pressure and late elution of analytes with peak tailing.

The selection of buffer pH mainly depends on the pK_a of the analyte. For basic compounds, pH needs to be selected approximately 2.5 pH units below the pK_a. The pK_a values for ondansetron and risperidone are 7.70 and 7.89, respectively. No considerable changes in peak symmetry and chromatographic response of analytes were observed while varying the buffer pH from 3.5 to 5.0. However, a change in buffer pH from 3.5 to 6.0 caused a slight increase in the ondansetron retention time from 6.16 to 7.25 min. Hence, pH 3.5 was considered to be optimal, as it gave a good compromise between retention time and peak shape.

In evaluation of buffer molarity, a poor resolution between the analytes was observed at 20 mM. However, no significant differences were found in the retention time and the peak shape of both analytes at 50 and 100 mM. Thus, 50 mM were considered optimal for the elution of analytes with short run times. The effect of the mobile phase flow rate was investigated at 0.9, 1.0, and 1.1 mL min^{-1}. The optimum flow rate was found to be 1.0 mL min^{-1} since it yielded good peak shapes without endogenous peak interference at the retention time of both analytes.

After several trials, the mobile phase consisting of a mixture of 50 mM ammonium acetate (pH 3.5) and acetonitrile (35:65, *v/v*) was finally adopted at a flow rate of 1.0 mL min^{-1}. The described chromatographic conditions achieved satisfactory resolution and symmetrical peak shape for ondansetron and IS with the retention time of 6.16 and 7.83 min, respectively. No interference from the endogenous compounds present in plasma was observed at the retention time of ondansetron and IS.

Choice of Internal Standard

Several substances were tested for selection of an IS. Risperidone was selected as the most suitable IS because the plasma samples showed no interference during its retention time and the peak was also well resolved from that of the ondansetron. Moreover, it is a stable compound and does not exist endogenously in the plasma.

Sample Preparation

In the present study, PPT was tested to investigate the effect of the plasma matrix and also to obtain satisfactory values for the recovery of ondansetron and risperidone. This is because the method offers obvious advantages such as a shorter processing time, consumption of less organic solvent, fewer steps, and a good cleanup of plasma samples.

Different solvents were investigated to precipitate protein in plasma samples such as acids (perchloric acid, PCA and trichloroacetic acid, TCA), organic solvents (acetonitrile and methanol), metal (zinc sulfate, $ZnSO_4$), and combination of organic solvents (acetonitrile and methanol), organic solvent and acid (acetonitrile/PCA and acetonitrile/TCA), and organic solvent and metal (acetonitrile and $ZnSO_4$). The PPT using PCA and TCA alone or using a mixture of acetonitrile/PCA or acetonitrile/TCA was simple and rapid without any interference from endogenous substances at the retention times of ondansetron and IS, but poor recovery was observed for ondansetron. The PPT using methanol alone or with a mixture of acetonitrile and methanol did not successfully remove all of the protein in the plasma, which resulted in incomplete precipitation. A significant interference was observed at the retention time of ondansetron when the samples were treated with acetonitrile or $ZnSO_4$ alone as precipitating agents. There was no interference from endogenous substances present in plasma at the retention times of the analytes when the blank plasma sample was treated with a mixture of acetonitrile and $ZnSO_4$. Moreover, the sample also produced a good recovery for both analytes. Thus, a mixture of acetonitrile and $ZnSO_4$ was selected in the present method to precipitate protein in the plasma samples.

Bioanalytical Method Validation

The method was validated according to USFDA guidance for bioanalytical method validation [18] for specificity, linearity, sensitivity, accuracy, precision, recovery, and stability.

Specificity

Specificity is the ability of a method to discriminate the analyte from all potentially interfering substances. The method specificity was investigated by comparing the chromatograms of the blank plasma from six rabbits with those of plasma samples spiked with ondansetron and IS. The chromatograms of the blank plasma, plasma spiked with ondansetron, and plasma obtained 1.5 h after oral administration are depicted in *Fig. 2*. *Fig. 2(a)*

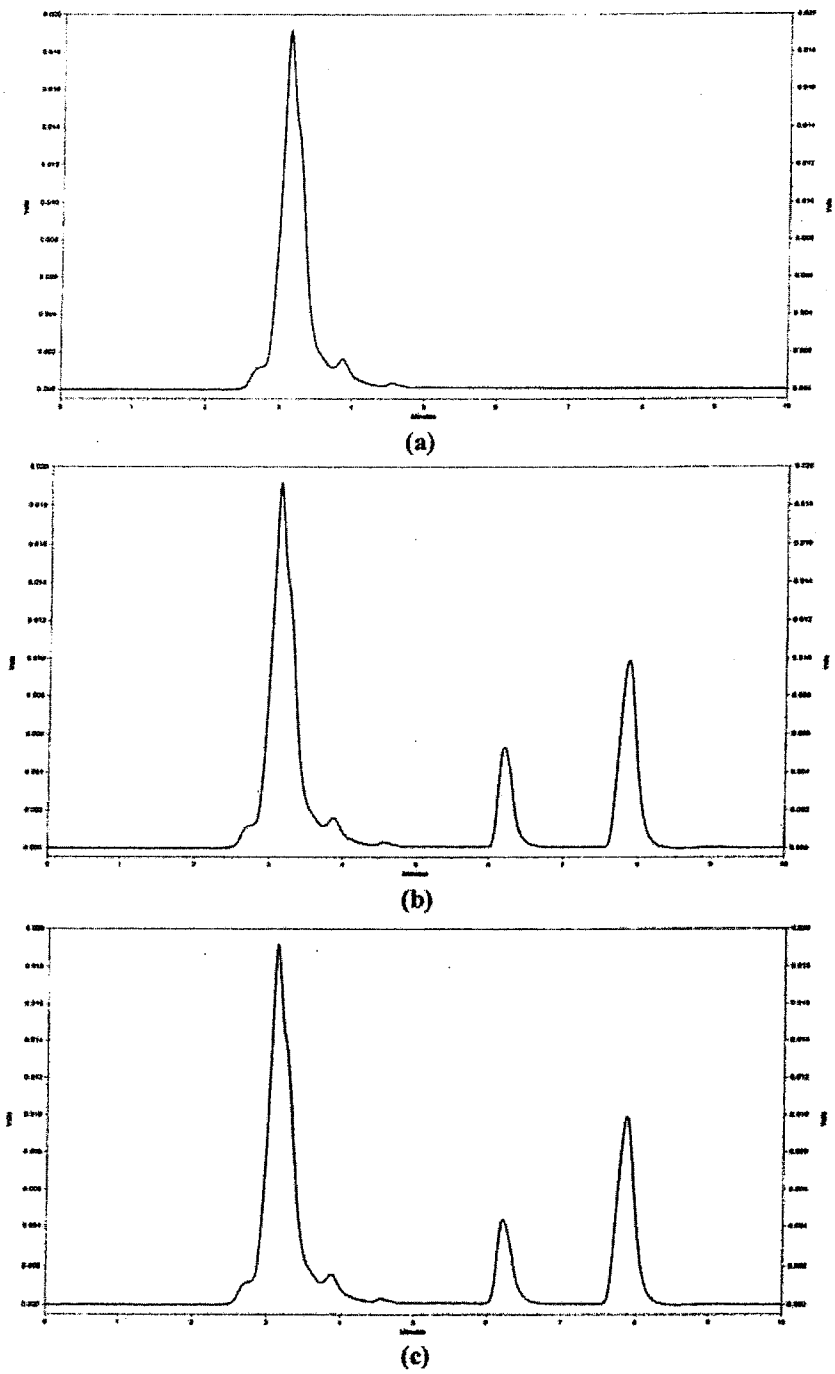

Fig. 2. Representative HPLC chromatograms: (a) blank rabbit plasma; (b) rabbit plasma spiked with 300 ng mL^{-1} ondansetron (6.16 min) and IS (7.83 min); (c) rabbit plasma collected at 1.5 h after oral administration of ondansetron

shows that the blank rabbit plasma had no interference from endogenous substances at the retention times of the analyte and IS. *Fig. 2(b)* indicates a good resolution between the analyte and IS under the optimized chromatography conditions. The retention time was 6.16 min for ondansetron and 7.83 min for IS with a precision of 0.24% and 0.19%, respectively. The developed method was therefore found to be selective for ondansetron in the presence of endogenous matrix components.

Linearity

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to the concentration of analyte in the sample. To evaluate the linearity of the method, five calibration plots at seven concentration levels consisting of 25, 50, 100, 300, 500, 700, and 1000 ng mL^{-1} were determined in rabbit plasma. The linearity of each calibration curve was determined by plotting the peak area ratio of ondansetron to IS of plasma standards versus the nominal concentration using linear regression analysis. The calibration curve showed excellent linearity over the concentration range 25–1000 ng mL^{-1} of ondansetron. The mean linear regression equation from five calibration plots was $y = 0.0004\ (\pm 0.0000)x + 0.0030\ (\pm 0.0006)$ with a correlation coefficient of 0.9999 (±0.0001). The linearity results are shown in *Table I.*

Table I. Summary of the calibration curve results for ondansetron. Mean ± SD, $n = 5$

Concentration added (ng mL^{-1})	Concentration found (ng mL^{-1})	Precision (%RSD)[a]	Accuracy (%RE)[b]
25	24.57 ± 0.59	2.39	–1.70
50	48.44 ± 1.44	2.97	–3.12
100	99.05 ± 2.19	2.21	–0.95
300	293.99 ± 8.78	2.99	–2.00
500	503.73 ± 16.08	3.19	0.75
700	695.00 ± 21.67	3.12	–0.71
1000	989.13 ± 17.01	1.72	–1.09

[a]Relative standard deviation
[b]Relative error

Limit of Detection and Limit of Quantification

The limit of detection (LOD), defined as the amount for which the signal-to-noise ratio was 3:1, was 10 ng mL^{-1}. The LOQ of the assay is defined as the

lowest concentration on the calibration curve that can be reproducibly quantified with acceptable precision and accuracy (±20%). The present assay method had an LOQ of 25 ng mL^{-1} with an accuracy of –1.70% and precision of 2.39% ($n = 5$), which was sufficient for monitoring ondansetron plasma levels over a period of 16 h after a single oral administration dose of 8 mg to rabbits.

Intraday and Interday Precision and Accuracy

Intraday and interday precision and accuracy were evaluated by analyzing QC samples at low, medium, and high concentrations of 75, 400, and 900 ng mL^{-1}. For the intraday variation, sets of five replicates were analyzed on the same day, and for the interday validation five replicates of three concentration levels were analyzed on three different days. To be acceptable, the measures should be within ±15% at all concentrations. The intraday accuracy (%RE) ranged between –3.63% and 1.01% with a precision (%RSD) of 0.93–3.41%. The interday accuracy ranged between –1.01% and 1.47% with a precision of 1.53–2.62%. All the results for precision and accuracy were within the acceptable limits. The results are shown in *Table II*.

Table II. Experimental values of mean concentration, %RSD, and %RE presented for validation parameters of ondansetron

Study	Concentration added (ng mL^{-1})	Concentration found (ng mL^{-1})	%RSD[g]	%RE[h]
Intraday[a]	75	72.28 ± 2.47	3.41	–3.63
	400	387.45 ± 12.19	3.15	–3.14
	900	909.10 ± 8.47	0.93	1.01
Interday[b]	75	74.98 ± 1.54	2.06	–0.02
	400	405.86 ± 10.63	2.62	1.47
	900	890.93 ± 13.59	1.53	–1.01
Benchtop[c]	75	74.23 ± 1.46	1.97	–1.02
	400	398.64 ± 5.67	1.42	–0.34
	900	901.26 ± 12.24	1.36	0.14
Freeze and thaw[d]	75	73.79 ± 0.72	0.98	–1.62
	400	394.32 ± 3.20	0.81	–1.42
	900	897.18 ± 3.31	0.37	–0.31

Table II. (continued)

Study	Concentration added (ng mL^{-1})	Concentration found (ng mL^{-1})	%RSD[g]	%RE[h]
Autosampler[e]	75	73.29 ± 0.29	0.39	−2.28
	400	392.96 ± 1.73	0.44	0.88
	900	887.36 ± ± 7.82	0.88	−1.40
Short term[f]	75	72.40 ± 1.44	1.99	−3.46
	400	389.87 ± 4.45	1.14	−2.53
	900	882.99 ± 3.75	0.42	−1.89

[a]Intraday accuracy and precision were determined with five replicates for each concentration

[b]Interday accuracy and precision were determined with 15 replicates (day 1, $n = 5$; day 2, $n = 5$; day 3, $n = 5$) for each concentration

[c]After 6 h at room temperature (25 ± 2°C), $n = 3$

[d]After three freeze and thaw cycles at −20°C, $n = 3$

[e]After 24 h at room temperature (25 ± 2°C), $n = 3$

[f]14 days at 4 °C, $n = 3$

[g]Relative standard deviation

[h]Relative error

Extraction Recovery

The recoveries of ondansetron at three QC levels were determined by comparing the mean peak area of extracted QC samples with that obtained from direct injections of a standard solution containing the same concentration of ondansetron. Five replicates were prepared at each concentration level. The mean extraction recoveries of ondansetron at concentrations of 75, 400, and 900 ng mL^{-1} were 83.78%, 85.63%, and 88.21%, with a precision of 1.21%, 3.67%, and 2.91%, respectively. The mean extraction recovery of IS was 99.80%. The extraction recovery of the analytes was shown to be consistent and reproducible.

Stability Studies

Stability experiments were performed with low, medium, and high QC samples to evaluate the ondansetron stability under different conditions. Experiments were performed in triplicate to determine benchtop (6 h) and autosampler (24 h) stability at room temperature (25 ± 2 °C), freeze–thaw stability (three cycles at −20°C and room temperature), and short-term stability (−20 °C) for 14 days. The drug was found to be stable in all the above-

mentioned conditions and assay values were within the acceptable limits (±15%). The stability results are shown in *Table II.*

Pharmacokinetic Study in Rabbits

The oral pharmacokinetics of reference and test formulations was compared in terms of rate (C_{max} and T_{max}) and extent (AUC_{0-t} and $AUC_{0-\infty}$) of absorption. A representative chromatogram of a sample obtained for the pharmacokinetic study is shown in *Fig. 2(c).*The plasma concentration–time profiles following oral administration of reference and test are depicted in *Fig. 3,* which indicates the suitability of the current method for pharmacokinetic studies of ondansetron in rabbit plasma. The pharmacokinetic parameter results are shown in *Table III.* There was an increase in plasma concentration, which reached a maximum in all the rabbits at 1.5–2.0 h for both preparations. Thereafter, the plasma drug concentration declined gradually over a period of 16 h. The resulting $t_{1/2}$ values demonstrated that a wash-out period of 1 week was sufficient due to the fact that no plasma sample showed any ondansetron levels at 0 h of blood collection in the phase 2 experiment. The $AUC_{t-\infty}$ values were found to be less than 10% of the $AUC_{0-\infty}$ *(Table III).* It indicates that the sample collection duration was sufficient for calculating at least 80% of $AUC_{0-\infty}$ and provided a reliable estimation of extent of absorption. When compared statistically, there was no significant difference ($p > 0.05$) between the pharmacokinetic variables of two formulations. Thus, reference and test formulations were bioequivalent in their rate and extent of absorption.

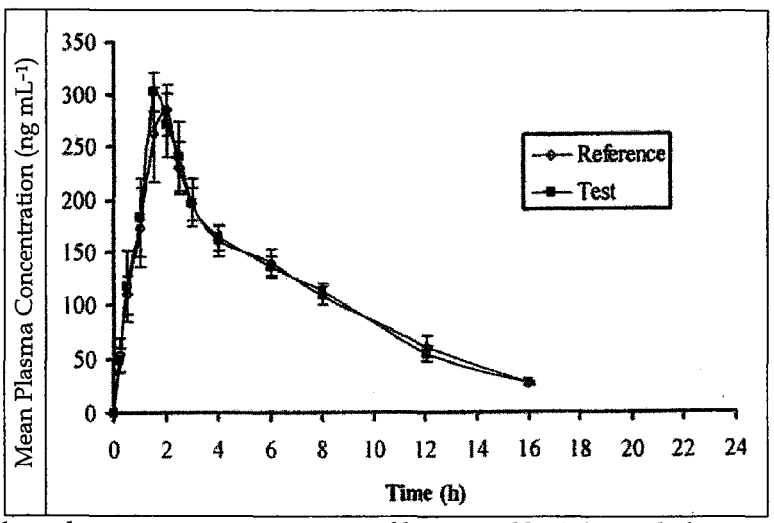

Fig. 3. Mean plasma concentration–time profile in six rabbits obtained after a single oral administration (8 mg) of reference and test formulations of ondansetron

Table III. Pharmacokinetic parameters of ondansetron after a single oral administration (8 mg) of reference and test formulations to rabbits. Mean ± SD, $n = 6$

Pharmacokinetic parameters	Reference	Test
T_{max} (h)	1.75 ± 0.27	1.58 ± 0.20
C_{max} (ng mL^{-1})	299.09 ± 12.90	308.40 ± 15.93
$t_{1/2}$ (h)	4.02 ± 0.23	3.87 ± 0.25
AUC_{0-t} (h ng mL^{-1})	1802.16 ± 143.78	1760.08 ± 166.65
$AUC_{t-\infty}$ (h ng mL^{-1})	158.15 ± 16.17	150.57 ± 19.85
$AUC_{0-\infty}$ (h ng mL^{-1})	1960.32 ± 146.91	1910.65 ± 162.69

Conclusions

A new simple, sensitive, and isocratic HPLC–UV method was developed and validated for the determination of ondansetron in rabbit plasma. The validated method showed satisfactory data for all the validation parameters tested. Because of the added advantages of the simple and inexpensive protein precipitation extraction procedure with no drying and reconstitution steps and short analytical run time (10 min), the method is easy and fast to perform compared to the previously reported HPLC methods. The developed method was validated over the concentration range of 25–100 ng mL^{-1} for ondansetron. This range is suitable for measuring ondansetron in plasma samples after an oral administration of 8 mg orally disintegrating tablets in the pharmacokinetic study in rabbits. The results of pharmacokinetic variables were comparable between the two formulations. Thus, reference and test formulations are bioequivalent in their rate and extent of absorption.

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Formulation and *In Vivo* Evaluation of Ondansetron Orally Disintegrating Tablets Using Different Superdisintegrants

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The aim of this study was to formulate cost effective taste-masked orally disintegrating tablets of ondansetron, a bitter drug using different superdisintegrants by a wet granulation technique. Microcrystalline cellulose (Avicel) as a diluent and disintegrant in addition to aspartame as a sweetener were used in all formulations. The prepared tablets were evaluated for weight variation, thickness, hardness, friability, drug content, water content, *in vitro* disintegration time and *in vitro* drug release. The tablets' hardness was maintained in the range of 2-3 kg and friability was <1% for all batches. All tablet formulations disintegrated rapidly *in vitro* within 5.83 to 33.0 sec. The optimized formulation containing 15% Polyplasdone XL-10 released more than 90% of drug within 5 min and the release was comparable to that of a commercial product. In human volunteers, optimized formulation was found to have a pleasant taste and mouth feel and they disintegrated in the oral cavity within 12 sec. The stability results were also satisfactory. A pharmacokinetic study with the optimized formulation was performed in comparison with a reference (Zofer MD 8®) and they were found to be bioequivalent. In conclusion, a cost effective ondansetron orally disintegrating tablet was successfully prepared with acceptable hardness, desirable taste and rapid disintegration in the oral cavity.

Key words: Ondansetron, Orally disintegrating tablets, Superdisintegrants, Wet granulation, Disintegration time, Pharmacokinetic study

INTRODUCTION

Oral tablets are the most widely used solid dosage form because of its convenience in terms of self-administration, ease in manufacturing, accurate dosing and good physical and chemical stability (Marshall and Rudnic, 1990; Joshi and Duriez, 2004). Many patients particularly pediatric, geriatric, bedridden, nauseous and developmentally disabled patients find it difficult to swallow conventional tablets and thus do not comply with their recommended dose schedule, resulting in ineffective therapy (Chang et al., 2000; Jeevanandham et al., 2010). To overcome this problem and improve treatment compliance of such patients, orally disintegrating tablets (ODTs) dosage form is a better alternative for oral administration. United States Food and Drug Administration (US FDA) defined ODT as "A solid dosage form containing active ingredient which disintegrates rapidly usually within a matter of seconds when placed upon the tongue." When an ODT is placed in the oral cavity, saliva quickly penetrates into the pores causing rapid disintegration of the tablet. Their major advantage is that they can be taken without water at any time, which is ideal for pediatric and geriatric patients. Further, increased bioavailability and good stability make ODTs the dosage form of choice in the current market (Swamy et al., 2007; Patel and Patel, 2008). From the perspective of industry, ODTs may provide new business opportunities in the form of product differentiation, patent life extension and cost effective drug development (Battu et al., 2007).

Ondansetron is a selective 5-hydroxytryptamine type 3 (5-HT_3) receptor antagonist used to treat chemotherapy- and radiotherapy-induced nausea and emesis.

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The mean bioavailability of ondansetron in humans is about 60%. In general, emesis is preceded by nausea and in such a condition, ingestion of conventional dosage forms with water leads to vomiting and expulsion of a portion or the entire dose administered. Thus, it is beneficial to administer ondansetron as an ODT dosage form (Khan et al., 2007).

Ondansetron is a water insoluble and bitter taste drug. If incorporate this drug directly into an ODT dosage form, the objective of the formulation will definitely be futile due to the bitterness of ondansetron. Hence, the characteristics of ondansetron such as insolubility in water and bitter taste make it an ideal drug candidate for ODT drug delivery system.

Ondansetron freeze-dried formulation is currently marketed by GlaxoSmithKline (UK) under the trade name Zofran ODT®, but due to its high cost, many patients do not have access to it. Although, a few USA-based manufacturers like Teva, Sandoz and Mylan have launched generic equivalents of Zofran ODT® on the market, the cost of tablets are still very high due to the manufacturing process, lyophilization. After extensive research, some of the Indian generic formulation companies including Sun Pharma launched cost-effective conventional ODTs in the place of costly freeze-dried tablets. However, these tablets have yet reached the market in most of the developing countries. Hence, it is necessary to develop a cost-effective ondansetron ODT formulation for global utility.

The key parameters need to be considered in the process of formulating ODTs are taste masking and disintegration time. Masking the bitter taste of some drugs can be challenging for this dosage form to achieve patient acceptability. Addition of sweeteners and flavours in the formulation is the foremost and the simplest approach for taste masking. Many studies have evaluated this technique to mask the bitter taste of drugs (Malke et al., 2007; Mohapatra et al., 2008) and our study also used this technique to mask the bitter taste of ondansetron.

The basic approach used in the development of fast disintegrating ODTs is the use of superdisintegrants like crospovidone, croscarmellose sodium and sodium starch glycolate which provide instantaneous disintegration of tablet after contact with the tongue, thereby releasing the drug in saliva (Indhumathi and Rathnam, 2010). A few studies were reported investigating the formulation of ondansetron using different superdisintegrants. Ahmed et al. (2008) published a patent (US Patent 7,390,503) on the preparation of ondansetron ODTs using different superdisintegrants. In the patent, the disintegration behavior of ondansetron ODTs was studied only with Polyplasdone XL as a superdisintegrant from the crospovidone family rather than with different grades. It is well-known that ODTs with a good "feel" in the mouth has better compliance and provides a more pleasant consumer experience. The particle size of the superdisintegrant selected is a key factor that impacts the textural attributes of the finished product. Large particles tend to provide a gritty feeling while smaller particles tend to support a smoother texture in the mouth. Hence, Polyplasdone XL used in the patent could produce grittiness in the mouth due to larger particle size (110-140 μm) (Jeong et al., 2008). Moreover, the authors did not confirm the taste and mouth feel of the prepared tablets using human volunteers. Khan et al. (2007) formulated ondansetron HCl ODTs using different concentrations of Polyplasdone XL-10, Ac-Di-Sol and Primojel. Although, the tablets prepared with Polyplasdone XL-10 disintegrated within 10 sec, masking the bitter taste of drug by complexing with Eudragit EPO by a precipitation method is a tedious and time consuming process and would increase the cost of the product. Moreover, a stability study and *in vivo* evaluation of optimized tablets were not carried out. Goel et al. (2009a, 2009b) formulated ondansetron HCl ODTs by direct compression and wet granulation methods using glycine-chitosan mixture as a superdisintegrant. The resultant disintegration time for optimized formulation was about 30 sec which is out of the official requirements for ondansetron (≤10 sec) (USP 30, 2007). Hence, the aim of present study was to formulate a cost-effective taste masked conventional ondansetron ODTs which could be commercially viable for global utility using different superdisintegrants and also to demonstrate the *in vivo* performance of the optimized formulation.

MATERIALS AND METHODS

Materials

Ondansetron was purchased from Symed Labs, India. The commercial product, Zofer MD 8® (Generic Zofran ODT®) was purchased from Sun Pharma. Mannitol was purchased from Merck. Microcrystalline cellulose (Avicel PH 112 and PH 113) and croscarmellose sodium (CCS) were obtained as a gift from FMC Biopolymer, USA. Crospovidone (Polyplasdone XL and XL-10) was provided as a gift from ISP Technologies, USA. Sodium starch glycolate (SSG) was provided as a gift from DMV International, USA. Kollidon CL and CL-SF were obtained as gifts from BASF, Germany. Low substituted hydroxypropyl cellulose (L-HPC, LH11) was obtained as a gift from Shin-Etsu Chemicals, USA. Aspartame and strawberry flavor were provided as gifts from Nutrasweet, USA. Sodium stearyl fumarate

was purchased from Micro Orgo Chem. Aerosil (Cab-O-Sil®) was purchased from Cabot Corporation. Ammonium acetate was purchased from Nacalai Tesque. Methanol and acetonitrile (HPLC grade) were purchased from J.T.Baker.

Formulation of ondansetron ODTs

Orally disintegrating tablets containing ondansetron and different concentrations of superdisintegrants were prepared by wet granulation technique. The composition of various ODT formulations containing different concentrations of aspartame and different types and concentrations of superdisintegrants are shown in Table I and Table II, respectively. In brief, the drug and intragranular ingredients were weighed, passed through a 0.8 mm sieve and mixed thoroughly by geometric dilution. Water was added to the above mixer to form granules which were then dried in an air oven at 40°C. The moisture content of the dried granules was determined by an infrared moisture balance (Mettler PM480 Delta Range®). The granules were considered dry, if their moisture content was less than 2.0%, which was equal to the moisture content of the pre-granulated blend. The dried granules and extragranular ingredients (Avicel PH 112, aspartame and strawberry flavor) were screened through a 0.8 mm sieve and blended for 5 min. The obtained blend was lubricated with sodium stearyl fumarate and Aerosil before compression. Seventy-five (75) mg of blend was compressed

Table I. Composition of ODT formulations containing different concentrations of aspartame

Ingredients (mg/Tablet)	A1	A2	A3	A4	A5
Intragranular					
Ondansetron	8.00	8.00	8.00	8.00	8.00
Mannitol	22.50	22.50	22.50	22.50	22.50
Avicel PH113	18.87	18.87	18.87	18.87	18.87
Polyplasdone XL	3.75	3.75	3.75	3.75	3.75
Extragranular					
Avicel PH112	18.88	17.38	15.88	14.38	12.88
Aspartame	0.75 (1%)	2.25 (3%)	3.75 (5%)	5.25 (7%)	6.75 (9%)
Strawberry flavour	0.75	0.75	0.75	0.75	0.75
SSF	0.75	0.75	0.75	0.75	0.75
Aerosil	0.75	0.75	0.75	0.75	0.75

Table II. Composition of ODT formulations containing different types and concentrations of superdisintegrants (F1 to F21)

Ingredients (mg/Tablet)	Polyplasdone XL			Polyplasdone XL-10			Croscarmellose sodium			Sodium starch glycolate		
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Intragranular												
Ondansetron	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Mannitol	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50
Avicel PH113	18.87	15.12	11.37	18.87	15.12	11.37	18.87	15.12	11.37	18.87	15.12	11.37
Superdisintegrant	3.75 (5%)	7.50 (10%)	11.25 (15%)	3.75 (5%)	7.50 (10%)	11.25 (15%)	3.75 (5%)	7.50 (10%)	11.25 (15%)	3.75 (5%)	7.50 (10%)	11.25 (15%)
Extragranular												
Avicel PH112	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38
Aspartame	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25
Strawberry flavor	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
SSF	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Aerosil	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75

Ingredients (mg/Tablet)	L-HPC (LH11)			Kollidon CL			Kollidon CL-SF		
	F13	F14	F15	F16	F17	F18	F19	F20	F21
Intragranular									
Ondansetron	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Mannitol	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50
Avicel PH113	21.87	20.37	18.87	18.87	17.00	15.12	21.68	20.75	18.87
Superdisintegrant	0.75 (1%)	2.25 (3%)	3.75 (5%)	3.75 (5%)	5.63 (7.5%)	7.50 (10%)	0.98 (1.25%)	1.88 (2.5%)	3.75 (5%)
Extragranular									
Avicel PH112	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38	14.38
Aspartame	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25
Strawberry flavor	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
SSF	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Aerosil	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75

machine (Korsch EKO) equipped with 5.5 mm round-shaped punches.

Evaluation of tablets

Weight variation

Twenty tablets were selected randomly and the average weight was determined using an electronic balance (P/PI-203MDS model, Denver instruments). Tablets were weighed individually and compared with the average weight.

Thickness

Ten tablets were selected randomly and thickness was assessed using a digital caliper (01407A model, Neiko).

Hardness

Hardness is the force required to break a tablet by radial compression. It was determined using a Vanguard hardness tester in the units of kg (YD-2 model, Vanguard). The mean hardness of 10 tablets was calculated and reported.

Friability

The friability of 20 tablets was measured using a friability test apparatus (CS-1 tablet friability tester). Twenty preweighed tablets were rotated at 25 rpm for 4 min. The tablets were then dedusted, reweighed and loss in weight (%) was calculated. The test was run once for each tablet formulation.

Drug content

Ten tablets from each formulation were randomly selected and pulverized to a fine powder. A portion of powder equivalent to a single dose (8 mg) of ondansetron was accurately weighed and assayed for drug content using a UV-visible spectrophotometer (Hitachi) at a wavelength of 310 nm. Drug content was calculated using a standard calibration curve. The mean percent drug content was calculated as an average of three determinations.

Water content

The tablets were analyzed for their water content using a Karl Fischer titrator (Metrohm 703 Ti Stand). Formulations which produced an *in vitro* disintegration time less than 10 sec were evaluated for water content. The tablet was pulverized, inserted in a titration vessel containing dried methanol and titrated with Hydranal Composite 5 reagent (Riedel-de-Haen) after a stirring time of 3 min. The samples were analyzed in triplicate. According to USP limits, the water content for ondansetron ODTs should not be more than 4% (USP 30, 2007).

In vitro disintegration time

In vitro disintegration time was determined using a USP tablet disintegration test apparatus (Type DIST 3, Pharma Test). The test was carried out in 900 mL of distilled water maintained at 37 ± 0.5°C with an agitation speed of 30 shakes per min. Only one tablet at a time was tested. The tablet was considered disintegrated completely when all the particles passed through the screen. The disintegration time of 6 individual tablets were recorded and the average was reported. According to USP limits, the *in vitro* disintegration time for ondansetron ODTs should not be more than 10 sec (USP 30, 2007).

In vitro dissolution studies

In vitro dissolution studies for commercial product (Zofer MD 8®) and ODT formulations were performed using USP XXIV type-II dissolution test apparatus (Distek Premiere, 5100) equipped with an autosampler and fraction collector. The formulations which produced an *in vitro* disintegration time less than 10 sec were selected for dissolution studies. The dissolution medium and sampling time intervals were chosen according to USP official dissolution specification for ondansetron (USP 30, 2007). The study was performed at a paddle speed of 50 rpm using 500 mL of 0.1 N HCl as the dissolution medium, at 37 ± 0.5°C. Aliquots of dissolution medium (4 mL) were withdrawn at specified intervals, 5, 10, 15, 30, 45 and 60 min and replaced with an equal volume of fresh medium. The dissolution study was also carried out at a lower paddle speed of 25 rpm. The concentration of drug in samples was analyzed using a UV spectrophotometer at a wavelength of 310 nm. Cumulative % of drug release was calculated and plotted against time. The drug release profile of formulations was compared with that of the commercial product. Dissolution studies were performed in replicates of six. The release profiles were characterized by dissolution efficiency (% DE). DE is defined as the area under a dissolution curve up to a certain time (t), expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time (Barakat et al., 2009). A constant time interval should be chosen for the comparison of dissolution data, and in this study DE_{10min} values were selected. DE was calculated by the following equation:

$$\text{Dissolution efficiency (DE)} = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100\%$$

y: drug percent dissolved at time t

Evaluation of taste, mouth feel and *in vivo* disintegration time in human volunteers

In preliminary study, taste masking of the ondansetron tablets were performed using 6 healthy human volunteers by preparing formulations (A1 to A5) containing five different amounts of aspartame (Table III). The taste, mouth feel and *in vivo* disintegration time evaluation of the optimized formulation (F6) and commercial product were carried out in 12 healthy human volunteers (25 to 33 years). The study protocol was approved by the Joint Ethics Committee of School of Pharmaceutical Sciences, Universiti Sains Malaysia and Hospital Lam Wah Ee. Prior to the test, all volunteers were informed of the purpose and protocol of the study; each volunteer gave his/her written consent to participate in the study. As per protocol, all volunteers were asked to rinse their mouth with water before placing the tablet on the tongue and then immediately, a stopwatch was started. Volunteers were allowed to move the tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. The taste and mouth feel were evaluated based on the volunteers' spontaneous verbal judgments immediately after the tablet was placed in their mouth as well as after 3 to 4 min. The taste and mouth feel were rated on a scale of 1 through 5. In taste evaluation, '1' was considered to be "good" while a '5' was considered as 'awful". In mouth feel evaluation, '1' was considered to be "good" while a '5' was considered as 'high grittiness". The time taken for the volunteer to feel that the last noticeable granule or fragment had disintegrated in the oral cavity was considered as the *in vivo* disintegration time. The swallowing of saliva was prohibited during the test and the mouth was rinsed after measurement.

Stability studies

The optimized formulation (F6) was examined at 40 ± 2°C/75 ± 5% relative humidity (RH) for accelerated stability and at 25 ± 2°C/65 ± 5% RH for short term stability for a period of 6 months. The ODTs (F6) were packed and sealed in 30cc HDPE bottles (Shukla et al., 2009). Samples were withdrawn at 3 and 6 months and evaluated for appearance, weight variation, thickness, hardness, friability, drug content, water content, disintegration time and dissolution time. The drug was assayed using our previously reported HPLC-UV method (Sheshala et al., 2009). The mobile phase consisted of 50 mM KH_2PO_4 adjusted to pH 3.5 with orthophosphoric acid and acetonitrile (30:70, v/v) delivered at 1 mL/min in C4 Hypersil column (250 × 4.6 mm, 5 μm). The detector wavelength was set at a wavelength of 310 nm and the injection volume was 20 μL.

In vivo study

Six healthy male New Zealand white rabbits weighing between 2.8 to 3.2 kg were used for the study. The rabbits were randomly divided into two groups of three rabbits each. All rabbits were fasted for 12 h with *ad libitum* access to water. One group received the commercial product (Zofer MD 8®) whereas the other group received test formulation (F6). The study was conducted according to a 2-period, 2-sequence crossover design with one week wash out period between the phases. The study was conducted in accordance with Animal Ethical Guidelines for investigations in laboratory animals and the study protocol was approved by the Animal Ethics Committee of Universiti Sains, Malaysia. Orally disintegrating tablets were administered to the rabbits using a procedure reported by Ishikawa et al. (2001). The tablets were administered at the back of the pharynx using a gastric intubation tube (made of silicone rubber) with one tablet set on the tip of the tube and immediately 5 mL of water was administered through the tube to facilitate swallowing of the tablet and to prevent it from sticking to the rabbit's throat. About 2 mL of blood sample was withdrawn from the marginal ear vein into heparinized eppendorf tubes at time intervals of 0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16 and 24 h post administration. The blood was immediately centrifuged at 4000 rpm for 15 min to separate the plasma, which was stored at −20°C until HPLC analysis.

To a 0.5 mL aliquot of plasma, 25 μL of 1 mg/mL of risperidone (internal standard) was added and the sample was then deproteinized with a mixture of 1 mL of acetonitrile and 50 μL of 10% w/v zinc sulfate. The mixture was vortexed for 2 min and centrifuged at 10,000 rpm for 20 min. The supernatant was transferred to autosampler vials and injected into the HPLC system. The HPLC system was performed on a Shimadzu chromatographic system equipped with an LC-10AT VP solvent delivery pump, SPD-10A VP UV-VIS detector and Class VP Chromato software. Ondansetron was analyzed using a cyano (CN) column (Phenomenex, 250 × 4.6 mm ID, 5 mm). The mobile phase consisted of 50 mM ammonium acetate adjusted to pH 3.5 with glacial acetic acid and acetonitrile (35:65, v/v). The analysis was run at a flow rate of 1.0 mL/min, the detector was set at a wavelength of 310 nm and the injection volume was 100 μL. The calibration curve exhibited an excellent linearity curve over the concentration range of 25-1000 ng/mL of ondansetron with a correlation coefficient of 0.9999.

The pharmacokinetic parameters, namely, maximum

plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained directly from the data. The area under the plasma concentration-time curve from 0 to the last measurable concentration (AUC_{0-t}) was calculated by the trapezoidal rule. $AUC_{0-\infty}$ was the summation of area under plasma concentration-time curve from zero to time t (AUC_{0-t}) and area under plasma concentration-time curve from time t to infinity ($AUC_{t-\infty}$). $AUC_{t-\infty}$ was calculated by dividing the last measurable plasma concentration with the terminal elimination rate constant (K_e). The value of K_e was calculated using the least-squares regression analysis of the terminal portion of the log plasma concentration *vs* time curve. The elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 with K_e.

Statistical analysis

The results are reported as means ± S.D. The *in vitro* disintegration time, % DE values for *in vitro* release profiles of ondansetron and all the physical properties of ondansetron in stability studies were treated statistically using a one-way analysis of variance (ANOVA). When there was a statistically significant difference, a Tukey's HSD (honestly significant difference) post hoc test was performed. The pharmacokinetic parameters, $AUC_{0-\infty}$, C_{max}, $t_{1/2}$, K_e were analyzed statistically using one way analysis of variance (ANOVA) which distinguishes effects due to subjects, periods and treatment (Wagner, 1975). The p value was calculated from the obtained F value using GraphPad Prism, version 5.02 (GraphPad Prism software). The values of $AUC_{0-\infty}$ and C_{max} were logarithmically transformed before analysis. The T_{max} values were analyzed using Wilcoxon Signed Rank test for paired samples. A statistically significant difference was considered when $p < 0.05$.

RESULTS AND DISCUSSION

Formulation rationale

Formulation development for ODTs with commonly used production methods and equipment is a challenging task, since the formulator should select raw materials which have a quick disintegration rate in the mouth and a high compressibility in order to yield an adequate hardness when compressed. The use of superdisintegrants for preparation of ODTs is highly effective and commercially feasible. The superdisintegrants, crospovidone (Polyplasdone XL, Polyplasdone XL-10, Kollidon CL and Kollidon CL-SF), CCS, SSG, L-HPC (LH11) were used to achieve a fast disintegration of tablets. These superdisintegrants accelerate disintegration of tablets by virtue of their ability to absorb a large amount of water when exposed to an aqueous environment. The absorption of water results in the breaking of tablets and therefore, a faster disintegration. This disintegration is reported to have an effect on dissolution characteristics as well. Sugar based excipients such as mannitol are used to improve the palatability of the tablets by its cool, sweet mild taste and provides a pleasing mouth feel due to its negative heat of solution (Rowe et al., 2003). It has also been used as a diluent to achieve a desired tablet weight. Avicel PH 113 and PH 112 were selected as a disintegrant and diluent due to their very low moisture content. Avicel PH 113 (particle size 50 μm) and Avicel PH 112 (particle size 100 μm) were added intragranularly and extragranularly, respectively. The intra- and extra-granular addition of Avicel was necessary not only to favor the disintegration of the tablets but also to promote the deaggregation of the granules, and the dissolution of the drug. Aspartame and strawberry were included as sweetening and flavoring agents, respectively to impart a pleasant taste and improved mouth feel. Sodium stearyl fumarate was employed as a lubricant instead of magnesium stearate not only because of the metallic taste of the latter, but also due to its hydrophobicity. The results also showed that the disintegration time of tablets with magnesium stearate was 6 to 7 sec longer than sodium stearyl fumarate (data not shown). This might be due to the formation of a hydrophobic lubricating film with magnesium stearate on the surface of the excipient particles which would have hindered the penetration of water into the tablet, resulting in slower disintegration (Battu et al., 2007; Kuno et al., 2008). Aerosil acts as a glidant and also helps in reducing tablet friability by restoring the bonding properties of the excipients (Shasaku, 1999).

Evaluation of tablets

Physical properties of tablets

The prepared tablets were evaluated for physical parameters such as weight variation, thickness, hardness, friability, drug content, water content and *in vitro* disintegration time. The results are shown in Table IV. All the prepared tablets were white in color and spherical in shape with a smooth surface without any defects. The average weight and thickness of tablets for all the formulations were found to be in the range of 74.43 to 76.88 mg and 2.46 to 2.83 mm, respectively. The hardness of the tablets was maintained in the range of 2 to 3 kg. The friability for all formulations was between 0.28 to 0.62% and found to be within acceptable limits (<1%). These results suggested that the ODTs were able to withstand abrasion during handling, packaging and shipment. All the

Table III. Evaluation of taste and mouth feel of commercial product and ODTs using human volunteers

Formulation	No. of volunteers	Aspartame concentration (%)	No. of volunteers rating the formulation taste as[a]					No. of volunteers rating the formulation mouth feel as[b]				
			1	2	3	4	5	1	2	3	4	5
Zofer MD 8	12	-	10	-	2	-	-	9	-	3	-	-
A1	6	1	-	-	-	-	6	-	-	-	-	-
A2	6	3	-	-	-	-	6	-	-	-	-	-
A3	6	5	-	-	1	-	5	-	-	-	-	-
A4	6	7	6	-	-	-	-	-	-	-	-	-
A5	6	9	6	-	-	-	-	-	-	-	-	-
F6	12	7	11	-	1	-	-	9	1	2	-	-

[a]Taste: 1 = Sweet and good; 2 = Tasteless; 3 = Slightly bitter; 4 = Bitter; 5 = Awful
[b]Mouth feel: 1 = Good; 2 = No feeling; 3 = Slight grittiness; 4 = Moderate grittiness; 5 = High grittiness

Table IV. Physical characteristics of various formulations of ondansetron orally disintegrating tablets. Mean ± S.D.

Formulation	Weight variation (mg); n = 20	Thickness (mm); n = 10	Hardness (kg); n = 10	Friability (%); n = 20	Drug content (%); n = 3	*In vitro* disintegration time (sec); n = 6	Water content (%); n = 3
Zofer MD 8®	180.74 ± 1.75	2.97 ± 0.07	2.86 ± 0.13	0.38	101.68 ± 0.89	8.53 ± 0.65	3.51 ± 0.24
F1	75.33 ± 0.26	2.60 ± 0.01	2.23 ± 0.11	0.44	99.66 ± 1.14	10.17 ± 1.17	-
F2	75.11 ± 0.57	2.57 ± 0.01	2.68 ± 0.12	0.38	101.55 ± 1.21	7.33 ± 1.03	3.29 ± 0.07
F3	74.43 ± 0.34	2.77 ± 0.02	2.59 ± 0.19	0.40	100.88 ± 1.47	6.00 ± 0.89	3.35 ± 0.21
F4	74.76 ± 0.35	2.72 ± 0.06	2.46 ± 0.16	0.53	101.37 ± 0.86	11.17 ± 0.41	-
F5	74.80 ± 0.59	2.74 ± 0.01	2.53 ± 0.02	0.48	102.25 ± 1.61	7.17 ± 0.75	3.52 ± 0.43
F6	75.22 ± 0.51	2.83 ± 0.01	2.32 ± 0.01	0.59	100.14 ± 1.77	7.00 ± 0.89	3.59 ± 0.31
F7	75.62 ± 0.36	2.67 ± 0.01	2.62 ± 0.11	0.37	99.94 ± 1.37	12.67 ± 0.82	-
F8	76.88 ± 0.60	2.78 ± 0.01	2.74 ± 0.07	0.31	99.62 ± 1.60	18.50 ± 1.05	-
F9	75.56 ± 0.78	2.71 ± 0.01	2.79 ± 0.12	0.30	102.45 ± 0.85	28.50 ± 1.38	-
F10	75.22 ± 0.29	2.68 ± 0.01	2.40 ± 0.16	0.49	101.13 ± 1.30	16.33 ± 0.82	-
F11	75.08 ± 0.32	2.67 ± 0.01	2.41 ± 0.13	0.54	98.81 ± 0.70	26.00 ± 0.89	-
F12	75.22 ± 0.22	2.66 ± 0.01	2.33 ± 0.15	0.62	100.31 ± 1.76	33.00 ± 1.41	-
F13	74.77 ± 0.33	2.54 ± 0.02	2.54 ± 0.10	0.55	102.23 ± 0.80	21.17 ± 1.33	-
F14	75.05 ± 0.22	2.46 ± 0.02	2.54 ± 0.17	0.46	100.31 ± 1.76	15.33 ± 1.21	-
F15	75.31 ± 0.32	2.70 ± 0.01	2.74 ± 0.25	0.39	98.32 ± 0.56	15.67 ± 1.03	-
F16	74.68 ± 0.23	2.54 ± 0.01	2.65 ± 0.22	0.29	100.03 ± 0.73	13.67 ± 0.82	-
F17	75.42 ± 0.40	2.62 ± 0.02	2.66 ± 0.29	0.33	102.71 ± 1.11	11.17 ± 0.41	-
F18	75.61 ± 0.36	2.50 ± 0.01	2.63 ± 0.29	0.31	99.47 ± 0.97	5.83 ± 0.75	3.11 ± 0.09
F19	74.51 ± 0.29	2.51 ± 0.01	2.61 ± 0.18	0.28	100.29 ± 0.29	14.00 ± 0.89	-
F20	75.43 ± 0.40	2.51 ± 0.02	2.42 ± 0.04	0.41	101.04 ± 0.69	14.50 ± 0.55	-
F21	74.99 ± 0.29	2.49 ± 0.01	2.64 ± 0.11	0.34	98.54 ± 0.68	Broken	-

formulations and commercial tablets demonstrated uniformity in drug content which varied from 98.32 to 102.71%.

In vitro disintegration time

The most important parameter that needs to be optimized in the development of ODTs is the disintegration time of tablets. Unlike in the patent (US Patent 7,390,503), the present study describes the disintegration behavior of ondansetron ODTs with different grades of crospovidone (Polyplasdone XL, Polyplasdone XL-10, Kollidon CL and Kollidon CL-SF) including other superdisintegrants CCS, SSG and L-HPC. The disintegration time of various formulations studied varied from 5.83 to 33.00 sec and that of the commercial product was 8.53 sec. The results are presented in Table IV. The disintegration times of the tablets containing 5% Polyplasdone XL (F1) and XL-10 (F4) were 10.17 and 11.17 sec, respectively. Increases in the concentration of Polyplasdone XL and XL-10 to 10% (F2 and F5) resulted in a decrease in the disintegration time of the tablets. However, there was no sig-

nificant effect ($p > 0.05$) on the disintegration time, when the concentration of Polyplasdone XL and XL-10 was further increased to 15% (F3 and F6). Hahm and Augsburger (2008) reported that higher levels of superdisintegrants do not necessarily produce faster disintegration, where as much as 15% of superdisintegrants may be beneficial in ODTs. Furthermore, when the superdisintegrants, Polyplasdone XL and XL-10 were replaced with CCS and SSG at a concentration of 5% (F7 and F10), the disintegration times were 12.67 and 16.33 sec, respectively. Further increase in the concentration of CCS and SSG from 5 to 10 (F8 and F11) and 15% (F9 and F12) resulted in a significant increase ($p < 0.05$) in the disintegration time of the tablets from 12.67 (F7) to 18.50 (F8) and 28.50 sec (F9) for CCS, respectively and from 16.33 (F10) to 26.00 (F11) and 33.00 sec (F12) for SSG, respectively. This dissimilar behavior of crospovidone, CCS and SSG on the disintegration time can be attributed to the difference in their mechanism of disintegration. The concentration of the superdisintegrant, crospovidone had a positive effect on the disintegration of tablets. Increasing the concentration of crospovidone resulted in faster disintegration of tablets, which may be due to a rapid capillary activity and pronounced hydration with little tendency for gel formation (Rowe et al., 2003; Setty et al., 2008). On the contrary, when the concentration of CCS and SSG was increased, it had a negative effect on the disintegration of the tablets. This negative effect may be due to the formation of a viscous gel layer by CCS and SSG which may impede further penetration of the disintegration medium and hinder the disintegration of tablet contents (Swamy et al., 2007; Setty et al., 2008). Moreover, at the same concentration level, the disintegration time of the tablets formulated using crospovidone was lower than those containing CCS and SSG. This might be due to crospovidone's rapid water absorbing nature involving both capillary and swelling mechanisms which builds up the pressure internally leading to the faster disintegration (Battu et al., 2007). The obtained results were similar to the findings of Khan et al. (2007) and Patel et al. (2004). The tablets containing L-HPC as a superdisintegrant disintegrates the tablets based on its swelling property in water (Bi et al., 1996). L-HPC, when used at concentrations of 1 (F13), 3 (F14) and 5% (F15), resulted in disintegration times of 21.17, 15.33 and 15.67 sec, respectively. There was no significant difference ($p > 0.05$) in the disintegration time of tablets when the L-HPC concentration was increased from 3 to 5%. The superdisintegrants, Kollidon CL and Kollidon CL-SF exhibit their disintegrant effect by a wicking action without gel formation. They increase the porosity and provide pathways for penetration of fluids into the tablets, which in turn, results in wicking through the capillary, hence facilitating the disintegration of tablets (Mishra et al., 2006). The disintegration time of the tablets was decreased significantly ($p < 0.05$) from 13.67 to 5.83 sec with the increase in the concentration of Kollidon CL from 5 (F16) to 10% (F18). In general, the group of crospovidones (Polyplasdone XL, Polyplasdone XL-10 and Kollidon) acts as disintegrants by absorbing water and subsequent swelling. However, the speed of disintegration is not only based on the swelling but also a combination of various properties including particle size, surface area, swelling pressure and volume and hydration capacity (BASF, 2010). The tablets prepared with different grades of crospovidone, disintegrated in the following order: Kollidon CL (F18) < Polyplasdone XL (F3) < Polyplasdone XL-10 (F6). Kollidon CL had the highest swelling pressure and lowest time to reach 90% maximum swelling pressure (171 kPa and 6.9 sec) compared to Polyplasdone XL (110 kPa and 21.9 sec) and Polyplasdone XL-10 (94 kPa and 85.4 sec) (Quadir and Kolter, 2006). Therefore, from the results obtained, it can be predicted that these two factors might play a significant role in the faster disintegration of Kollidon CL. The attained results are similar to the findings of Quadir and Kolter, 2006; BASF, 2010. There was no significant difference ($p > 0.05$) in the disintegration time of tablets F19 (14.00 sec) and F20 (14.50 sec) containing 1.25 and 2.5% Kollidon CL-SF, respectively. However, the tablets containing Kollidon CL-SF 5% (F21) did not disintegrate into particles, but tended to separate axially into upper and lower sections.

Water content

Water content determination is an important parameter in the selection of optimized formulation of ODTs. Corveleyn and Remon (1999) reported a clear negative correlation between water content and hardness. An increase in water content of the tablets decreases tablet hardness due to the weakening in intermolecular attraction forces between the particles in the tablets. Hence, water content in all ODT formulations with a disintegration time of less than 10 sec (F2, F3, F5, F6 and F18) was evaluated. The results (Table IV) demonstrated that the water content of all ODT formulations was less than 4% which was within the acceptable USP limits for ondansetron ODTs (USP 30, 2007).

In vitro dissolution studies

In vitro drug dissolution studies conducted with a

paddle speed of 50 rpm showed that the commercial product (Zofer MD 8®) and promising ODT formulations F2, F3, F5 and F6 containing Polyplasdone XL (10%), Polyplasdone XL (15%), Polyplasdone XL-10 (10%) and Polyplasdone XL-10 (15%), respectively released more than 90% of drug in 10 min except formulation F18, which contained Kollidon CL (10%) (Fig. 1). The DE_{10min} values for commercial product, F2, F3, F5, F6 and F18 were 69.96, 67.16, 67.29, 69.88, 69.33 and 63.08%, respectively. There was no significant difference ($p > 0.05$) in the release profiles of the ODTs formulations F2, F3, F5 and F6 compared to commercial product except for formulation F18. But, the *in vitro* disintegration time of the tablets prepared with formulation F18 was shorter (5.83 sec) than F5 (7.17 sec) and F6 (7 sec) and the DE_{10min} value for F18 was lower than that of F5 or F6 at a paddle speed of 50 rpm. Te Wierik and Bolhuis (1992) shown that dissolution from tablets of poorly soluble and hydrophobic drugs (such as ondansetron) can be strongly improved by solid deposition of the drug upon the surface of the hydrophilic and strong swelling superdisintegrant. The swelling volume of Polyplasdone XL-10 is higher (5.4 L/kg) than Kollidon CL (4.3 L/kg) (Quadir and Kolter, 2006). The lower swelling volume of Kollidon CL could be a possible reason for slow dissolution of the tablets. It was observed from the results that the dissolution of ODTs occurred very fast at paddle speed of 50 rpm. Typically, the dissolution of ODTs is very fast when using USP monograph conditions. Hence, lower paddle speeds might yield more discriminating dissolution profiles (Klancke, 2003; Battu et al., 2007). Therefore, *in vitro* dissolution studies were also conducted at a lower paddle speed of 25 rpm to select the optimized ODT formulation. The release profiles results (Fig. 2) showed that only commercial product and formulation F6 released more than 90% of the drug in 10 min. The DE_{10min} values for commercial product, F2, F3, F5 and F6 were 61.52, 47.35, 51.88, 54.35 and 63.01%, respectively. A difference among the DE_{10min} values in the formulations prepared with both types of crospovidone (Polyplasdone XL and XL-10) at a similar concentration level (F2 and F5 as well as F3 and F6) were observed at a paddle speed of 25 rpm. Although similar in particle morphology, both crospovidone types differ in their particle size. Polyplasdone XL-10 has the smallest particle size and highest surface area (1.4 m^2/g) compared to Polyplasdone XL (0.7 m^2/g). The high surface area increases interfacial activity that can aid in drug dissolution. Thus, it could be a possible reason for faster dissolution of Polyplasdone XL-10 at both concentrations at a lower paddle speed (Balasubramaniam and Bee, 2009). There was no significant difference ($p > 0.05$) in the release profiles of commercial product and formulation F6 at the lower paddle speed. Thus, the results indicated that the dissolution profiles of commercial product and formulation F6 were similar. Therefore, formulation F6 as an optimized formulation was selected for the evaluation of taste, mouth feel and *in vivo* disintegration time in human volunteers, stability and *in vivo* studies.

Evaluation of taste, mouth feel and *in vivo* disintegration time in human volunteers

The results are shown in Table III. All the volunteers rated the preliminary study formulations prepared with 1 (A1) and 3% (A2) aspartame as '5' which indicates that tablets were awful and bitter in taste. Five

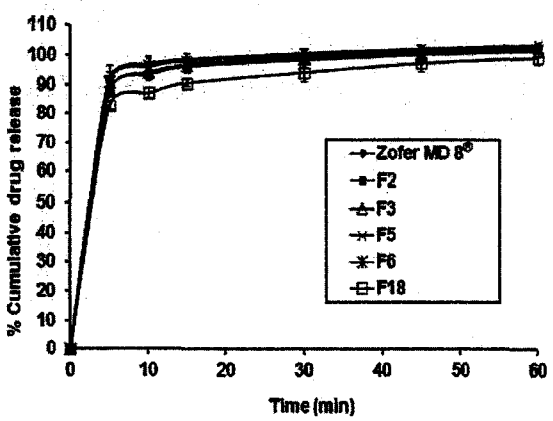

Fig. 1. Comparison of *in vitro* release profiles of formulations F2 (10% Polyplasdone XL), F3 (15% Polyplasdone XL), F5 (10% Polyplasdone XL-10), F6 (15% Polyplasdone XL-10), F18 (10% Kollidon CL) and commercial product (Zofer MD 8®) at a paddle speed of 50 rpm. Mean ± S.D., n = 6.

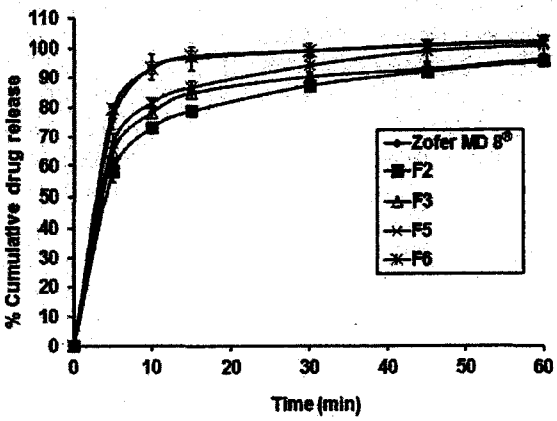

Fig. 2. Comparison of *in vitro* release profiles of formulations F2 (10% Polyplasdone XL), F3 (15% Polyplasdone XL), F5 (10% Polyplasdone XL-10), F6 (15% Polyplasdone XL-10) and commercial product (Zofer MD 8®) at a paddle speed of 25 rpm. Mean ± S.D., n = 6.

Table V. Physical properties of optimized formulation F6 after 3 and 6 months storage at different temperatures and humidities. Mean ± S.D.

Tablet Properties	n	F6 0 month	25°C/65% RH		40°C/75% RH	
			3 months	6 months	3 months	6 months
Weight variation (mg)	20	74.91 ± 0.45	75.03 ± 0.35	75.15 ± 0.48	75.02 ± 0.35	75.28 ± 0.71
Thickness (mm)	10	2.75 ± 0.01	2.76 ± 0.01	2.76 ± 0.01	2.76 ± 0.02	2.77 ± 0.01
Hardness (kg)	10	2.41 ± 0.10	2.43 ± 0.06	2.38 ± 0.11	2.38 ± 0.07	2.33 ± 0.07
Friability (%)	20	0.46	0.48	0.53	0.51	0.53
Drug content (%)	3	101.20 ± 1.39	101.24 ± 1.86	100.80 ±1.14	100.82 ± 0.82	99.87 ± 0.61
Disintegration time (sec)	6	7.17 ± 0.75	7.00 ± 0.89	6.83 ± 0.75	7.00 ± 0.63	6.50 ± 0.84
Water content (%)	3	3.62 ± 0.06	3.63 ± 0.03	3.69 ± 0.09	3.66 ± 0.07	3.73 ± 0.06

out of six volunteers rated the formulation containing 5% (A3) aspartame as '5' and only one volunteer reported as '3', which was slightly bitter in taste. All the volunteers rated the formulations prepared with 7% (A4) and 9% (A5) aspartame as '1', suggesting that these formulations were sweet and acceptable. From these data, it was concluded that formulations prepared with 7 and 9% of aspartame successfully masked the bitter taste of ondansetron. As these were not the optimized formulations, mouth feel and *in vivo* disintegration time were not evaluated. The amount of aspartame used in these two formulations was within the acceptable limits (36 mg/day) (US FDA, 2009). The formulation A4 prepared with 7% aspartame (5.25 mg) was chosen for further studies as it contained a lower amount of aspartame.

Comparing commercial product and optimized formulation (F6), eleven out of twelve volunteers rated formulation F6 as '1' while only one volunteer reported as '3', whereas for commercial product ten out of twelve volunteers rated the product as '1', suggesting that the product was sweet. Hence, it was concluded that the addition of sweetener to ODTs suppressed the bitter taste and provided a pleasant sweet taste. Nine out of twelve volunteers experienced a good mouth feel without any grittiness for formulation F6 and commercial product. In the formulation F6, Polyplasdone XL-10 was used as a superdisintegrant and the majority of the volunteers reported that there was no grittiness in the mouth. The result could be expected due to the smaller particle size of Polyplasdone XL-10. This might be advantageous from the consumer point of view when compared to the patented formulation (US Patent 7,390,503). Data collected from the *in vivo* disintegration time showed that formulation F6 and commercial product on an average disintegrated in the oral cavity within 12 and 13 sec, respectively. The results demonstrated that formulation F6 had a pleasant taste with good mouth feel and rapid disintegration in the oral cavity. Hence, formulation F6 was comparable with that of commercial product.

Stability study

In stability study, samples of optimized formulation (F6) were examined after 3 and 6 months storage and there were no significant changes in appearance of the tablets, weight variation, thickness, hardness, friability, water content and disintegration time (Table V). The results of water content and disintegration time after 6 months of storage were within the USP limits (USP 30, 2007). In HPLC assay, the blank tablets did not show any interfering peaks from the excipients at the retention time of ondansetron. Drug content in the tablets after 6 months storage at 40 ± 2°C/75 ± 5% RH and 25 ± 2°C/65 ± 5% RH were 99.87 and 100.80%, respectively. The *in vitro* release profiles of ondansetron from formulation F6 stored for 0, 3 and 6 months are shown in Fig. 3. The DE_{10min} values for 0, 3 and 6 months at 40 ± 2°C/75 ± 5% RH were 69.27, 68.44 and 67.60%, respectively whereas at 25 ± 2°C/65 ± 5% RH were 69.27, 68.39 and 67.64%, respectively. There was no significant difference ($p > 0.05$) in the release profiles between fresh and stored samples and thus, the

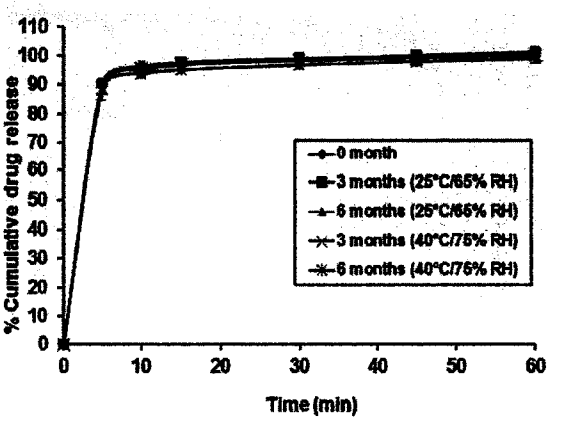

Fig. 3. *In vitro* release profiles of formulation F6 after 3 and 6 months storage at different temperatures and humidities. Mean ± S.D., n = 6.

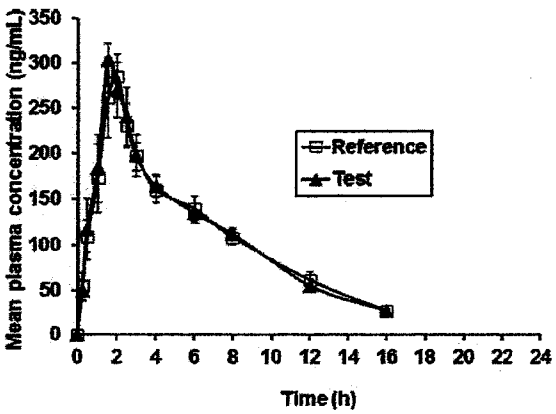

Fig. 4. Mean plasma ondansetron concentration profiles after oral administration of reference product (Zofer MD 8®) and test formulation (F6) in rabbits. Mean ± S.D., n = 6.

Table VI. Pharmacokinetic parameters following oral administration of reference product (Zofer MD 8®) and test formulation (F6) in rabbits. Mean ± S.D., n = 6

Parameter	Reference	Test
AUC_{0-t} (ng·h/mL)	1802.16 ± 143.78	1760.08 ± 166.65
$AUC_{t-\infty}$ (ng·h/mL)	178.07 ± 52.09	150.57 ± 19.85
$AUC_{0-\infty}$ (ng·h/mL)	1980.23 ± 109.53	1910.65 ± 162.69
C_{max} (ng/mL)	299.09 ± 12.90	308.40 ± 15.93
T_{max} (h)	1.75 ± 0.27	1.58 ± 0.20
$t_{1/2}$ (h)	4.02 ± 0.23	3.87 ± 0.25
K_e (1/h)	0.1730 ± 0.010	0.1795 ± 0.012

formulation F6 was proven to be stable at least for 6 months.

In vivo study

The plasma-concentration time profiles following oral administration of the commercial product and test formulation (F6) are depicted in Fig. 4. The data obtained for pharmacokinetic parameters, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{1/2}$ and K_e for the reference were 1960.32 ng·h/mL, 299.09 ng/mL, 1.75 h, 4.02 h and 0.1730 h^{-1}, respectively whereas for formulation F6, they were 1910.65 ng·h/mL, 308.40 ng/mL, 1.58 h, 3.87 h and 0.1795 h^{-1}, respectively (Table VI). There was no statistically significant difference ($p > 0.05$) in the pharmacokinetic parameters of the two formulations. The data indicated that the reference and F6 were bioequivalent in their rate and extent of absorption and thus, may be used interchangeably.

Orally disintegrating tablets of ondansetron with Polyplasdone XL-10 (15%) as a superdisintegrant was successfully prepared using wet granulation technique. This "patient-friendly dosage form" is useful in administration of ondansetron in a more acceptable and palatable form without water during emesis. It had a good taste, mouth feel and rapidly disintegrated in the mouth. Formulated ODTs showed similar *in vitro* release profiles with that of a commercial product and were bioequivalent in their rate and extent of absorption. The stability results were also satisfactory. In conclusion, a cost effective taste masked ondansetron ODTs (formulation F6) was successfully prepared with conventional equipment and commonly available excipients and the formulation F6 may be a useful alternative to commercially available formulations.

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Formulation and Optimization of Orally Disintegrating Tablets of Sumatriptan Succinate

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The aims of the present research were to mask the intensely bitter taste of sumatriptan succinate and to formulate orally disintegrating tablets (ODTs) of the taste masked drug. Taste masking was performed by coating sumatriptan succinate with Eudragit EPO using spray drying technique. The resultant microspheres were evaluated for thermal analysis, yield, particle size, entrapment efficiency and *in vitro* taste masking. The tablets were formulated by mixing the taste masked microspheres with different types and concentrations of superdisintegrants and compressed using direct compression method followed by sublimation technique. The prepared tablets were evaluated for weight variation, thickness, hardness, friability, drug content, water content, *in vitro* disintegration time and *in vitro* drug release. All the tablet formulations disintegrated *in vitro* within 37—410 s. The optimized formulation containing 5% Kollidon CL-SF released more than 90% of the drug within 15 min and the release was comparable to that of commercial product (Suminat®). In human volunteers, the optimized formulation was found to have a pleasant taste and mouth feel and disintegrated in the oral cavity within 41 s. The optimized formulation was found to be stable and bioequivalent with Suminat®.

Key words sumatriptan succinate; orally disintegrating tablet; superdisintegrant; sublimation; disintegration time; *in vivo* study

Migraine is a common disorder characterized by a unilateral headache that is often associated with nausea, vomiting, gastrointestinal disturbance and extreme sensitivity to light and sound.[1,2] Sumatriptan succinate is the first member of a new class of antimigraine compounds that act as a specific and selective 5-hydroxytryptamine-1 receptor agonist. Consequently, it is a novel and effective acute treatment for migraine *via* the oral route in the tablet form, parenteral route as a subcutaneous injection and nasal route as nasal spray.[3] Injected sumatriptan works the fastest of all the dosage forms available and is the most effective but it is inconvenient due to pain at the injection site and it also requires a trained person to administer the dose. The nasal spray bypasses the stomach, gets absorbed more quickly than the oral form and relieves the pain within 15 min after administration. However, it is less effective when the patient has nasal congestion from cold or allergy and it also leaves a bad after taste. The oral administration in the form of conventional tablet is a convenient method but in some instances, such as during travel where the patients have little or no access to water, administration of drug is not feasible and carries a risk of choking. Moreover, a substantial proportion of patients suffer from severe nausea or vomiting during their migraine attack. In such condition, even if the patient has access to water, ingestion of conventional tablet could lead to vomiting and expulsion of a portion or the entire dose administered which leads to treatment failure.[4] All these factors limit the utility of conventional tablets. Thus, the orally disintegrating tablet (ODT) delivery system for sumatriptan succinate may be a viable alternative for self-administration, whereby these limitations could be overcome.

Sumatriptan succinate is a highly water soluble and intensely bitter drug. If it is incorporated directly into ODTs, the main objective behind formulation of such a dosage form will definitely be futile due to the bitterness of the drug. Therefore, its high solubility in water and bitter taste makes sumatriptan succinate a challenging drug candidate for ODT drug delivery system.

Sumatriptan succinate is not available in the dosage form of ODT in the market world over. To the best of our knowledge, there is only one published article on the formulation of sumatriptan succinate ODTs[5] but the researchers have not performed taste masking experiments for the drug which is a prime parameter to improve the patient compliance and the quality of treatment. Thus, in the present study, an attempt has been made to mask the intensely bitter taste of sumatriptan succinate and to formulate ODTs with a pleasant taste and mouth feel in the oral cavity so as to prepare a "patient-friendly dosage form." The *in vivo* performance of the optimized formulation was also demonstrated using rabbits as an animal model.

Experimental

Materials Sumatriptan succinate was purchased from Nosch Labs (Hyderabad, India). Microcrystalline cellulose (Avicel PH 112) and croscarmellose sodium (CCS) were obtained as gift samples from FMC Biopolymer (Newark, U.S.A.). Crospovidone (Polyplasdone XL and XL-10) was provided as gift samples from ISP Technologies (New Jersey, U.S.A.). Sodium starch glycolate (SSG) was provided as a gift sample from DMV International (New Jersey, U.S.A.). Kollidon CL and CL-SF were obtained as gift samples from BASF (Ludwigshafen, Germany). Low substituted hydroxypropyl cellulose (L-HPC, LH11) was obtained as a gift sample from Shin-Etsu (New York, U.S.A.). Calcium silicate was obtained as a gift sample from Huber Chem (Mumbai, India). Ammonium bicarbonate was purchased from Sigma-Aldrich (St. Louis, U.S.A.). Aspartame and pineapple flavor were provided as gift samples from Nutrasweet (Chicago, U.S.A.). Magnesium stearate was purchased from Micro Orgo Chem (Mumbai, India). Aerosil was purchased from Cabot Corp. (Boston, U.S.A.). Ammonium acetate was purchased from Nacalai Tesque (Kyoto, Japan). *tert*-Butyl methyl ether (TBME) was purchased from Acros Organics (New Jersey, U.S.A.). Dichloromethane (DCM) was purchased from R&M Chemicals (Essex, U.K.). Ethyl acetate (EA) was purchased from Lab Scan (Bangkok, Thailand). HPLC grade of methanol and acetonitrile were purchased from J.T.Baker (Phillipsburg, U.S.A.).

Preparation of Spray Dried Microspheres Sumatriptan succinate taste masked microspheres were prepared by spray drying technique at drug : polymer : organic solvent ratios of 1 : 0.5 : 50, 1 : 0.75 : 50 and 1 : 1 : 50. The polymer, Eudragit EPO was dissolved in ethyl acetate and then drug was added to prepare a suspension. The prepared suspension was stirred

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using Heidolph stirrer at 500 rpm to maintain uniformity and sprayed through a nozzle (diameter of 0.7 mm) using a spray dryer (Lab Plant SD-04. Huddersfield, U.K.). The spray dryer was operated under the following conditions: inlet and outlet temperatures of 80 and 55—61 °C, respectively, blower setting at 70% and peristaltic pump setting at 30%. The resultant taste masked microspheres stored in a tightly closed container over silica gel until further use.

Evaluation of Spray Dried Microspheres. *In Vitro* Taste Masking The study was conducted in accordance to the method adopted from Shukla *et al.*[6] The required amount of spray dried microspheres equivalent to 70 mg sumatriptan succinate was placed in a 25 ml beaker. A volume of 5 ml phosphate buffer solution pH 6.8 (United States Pharmacopeia (USP)) was added and the mixture was allowed to stand for 60 s. A 5 ml volume of phosphate buffer pH 6.8 was used to mimic the salivary fluid volume and pH. After the specified time, the suspension was filtered through 0.45 μm nylon membrane filter. The filtrate was analyzed for drug content using UV/Visible spectrophotometer (Hitachi, Japan) at 227 nm. The experiment was run in triplicate.

Thermal Analysis Differential Scanning Calorimetry (DSC) (Perkin Elmer, Pyris 6 DSC. California, U.S.A.) was used to evaluate the compatibility between sumatriptan succinate and Eudragit EPO. The DSC experiments were performed on plain drug, Eudragit EPO and spray dried drug loaded microspheres. Accurately weighed samples (5—7 mg) were sealed in flat bottom aluminium pans and thermograms were recorded at a constant rate of 10 °C/min over a temperature range of 30—300 °C. Inert atmosphere was provided by purging helium gas at a flow rate of 20 ml/min. An empty pan sealed in the same way as the sample was used as a reference. The experiment was run in triplicate.

Particle Size The analysis was performed using a Mastersizer S (Malvern Instruments, U.K.) fitted with MS1 small volume sample dispersion unit connected to a dispersion unit controller. The spray dried microspheres were dispersed in water and sonicated for 2 min using bath sonicator (Branson 5200, Branson Ultrasonics, Danbury, U.S.A.) to prevent aggregation before measuring particle size. Samples were analyzed in triplicate.

Drug Entrapment Efficiency, Loading and Yield The entrapment efficiency and drug loading in microspheres was estimated by dissolving 50 mg of spray dried powder in methanol and further diluted with 0.01 N HCl. The samples were analyzed using UV/Visible spectrophotometer (Hitachi, Japan) at a wavelength of 227 nm. Entrapment efficiency, drug loading and yield were calculated using the following equations

$$\text{drug entrapment efficiency (\%)} = \frac{\text{weight of drug in microspheres}}{\text{weight of drug fed initially}} \times 100\%$$

$$\text{drug loading (\%)} = \frac{\text{weight of drug in microspheres}}{\text{weight of microspheres}} \times 100\%$$

$$\text{yield (\%)} = \frac{\text{weight of microspheres}}{\text{weight of drug and Eudragit EPO}} \times 100\%$$

Preparation of Tablets The ODTs were prepared by direct compression method. The ODTs containing uncoated sumatriptan succinate (formulation F0) were used as control. The taste masked sumatriptan succinate ODTs (formulation F1 to F31) consisted of the spray dried microspheres containing drug and polymer at a ratio of 1 : 1 (150 mg equivalent to 70 mg of sumatriptan succinate), Avicel PH 112, superdisintegrant, pore forming agent (calcium silicate), subliming agent, aspartame and pineapple flavor (Table 1). The ingredients were passed through 0.8 mm sieve and mixed intimately by geometric dilution. The obtained blend was lubricated with magnesium stearate and Aerosil was added as a glidant before compression. The blend was compressed on a single station tableting machine (Manesty, Liverpool, U.K.) using 10 mm concave punches. The tablets weight was kept at 320 mg and hardness was maintained in the range of 2—3 kg. The tablets (F5 and F8 to F31) were kept in an oven at 40 °C (15—21 h) until a constant weight was obtained to facilitate sublimation of subliming agent (ammonium bicarbonate).

Evaluation of Tablets. Physical Properties of Tablets Twenty tablets were selected randomly to determine the tablets weight variation. Tablets were weighed individually using an electronic balance (Denver instruments, U.S.A.) and compared with an average weight. Thickness of the tablets was assessed using digital caliper (Neiko, U.S.A.). Hardness of the tablets was determined using a Vanguard hardness tester in the units of kg (YD-2 model, Vanguard, U.S.A.). The mean hardness of 10 tablets was calculated and reported. Twenty preweighed tablets were rotated at 25 rpm for 4 min in friability test apparatus (CS-1 tablet friability tester, U.S.A.) to measure the friability of the tablets. The tablets were then dedusted, reweighed and loss in weight (%) was calculated. The test was run once for each formulation.

Drug Content Ten tablets from each formulation were randomly selected and pulverized to a fine powder. A portion of powder equivalent to a single dose (70 mg) of sumatriptan succinate was accurately weighed and assayed for the drug content using UV/Visible spectrophotometer (Hitachi, Japan) at a wavelength of 227 nm. The mean percent drug content was calculated as an average of three determinations.

Water Content The tablets which produced *in vitro* disintegration time of less than 60 s were evaluated for water content using Karl Fischer titrator (Metrohm 703 Ti Stand, Germany). The tablet was pulverized, inserted in the titration vessel containing dried methanol (Karl Fischer grade) and titrated with Hydranal Composite 5 reagent (Riedel-de-Häen, Germany) after a stirring time of 3 min. The samples were analyzed in triplicate.

***In Vitro* Disintegration Time** The test was carried out using USP tablet disintegration test apparatus (Pharma Test, Germany). The tablet was placed in 900 ml distilled water maintained at 37 °C and agitation speed of 30 shakes per min. Only one tablet at a time was tested. The tablet was considered disintegrated completely when all the particles passed through the screen. The disintegration time of 6 individual tablets were recorded and the average was reported. The disintegration time set by U.S. Food and Drug Administration (FDA) for all the ODT formulations (<60 s) were considered as a specification limit for sumatriptan succinate ODTs.

***In Vitro* Dissolution Studies** *In vitro* dissolution studies of commercial product (Suminat®, Sun Pharma, India) and ODT formulations were performed using USP XXIV type-II dissolution test apparatus (Distek Premiere, 5100, U.S.A.). The formulations, F10, F11, F15, F16, F25 and F28 which produced *in vitro* disintegration time less than 60 s were selected for dissolution studies. The dissolution medium and sampling time intervals were chosen according to USP official dissolution specification for sumatriptan succinate.[7] The study was conducted in 900 ml of 0.01 N HCl as a dissolution medium with paddle speed of 30 rpm at a temperature of 37±0.5 °C. In addition, dissolution studies were also performed in acetate buffer pH 4.5 and phosphate buffer pH 6.8 at a similar paddle speed. Aliquots of dissolution medium (5 ml) were withdrawn at specified intervals, 5, 10, 15, 30, 45 and 60 min and replaced with an equal volume of fresh medium. The concentration of drug in samples was analyzed using UV/Visible spectrophotometer (Hitachi, Japan) at a wavelength of 227 nm. Cumulative percent of drug release was calculated and plotted against time. The drug release profile of formulations was compared with that of the commercial product (Suminat®). Dissolution studies were performed in replicates of six. The release profiles were characterized by dissolution efficiency (%DE). A constant time intervals should be chosen for the comparison of dissolution data, whereby in this study $DE_{15\,min}$ values were selected. DE was calculated by the following equation

$$\text{dissolution efficiency (DE)} = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100\%$$

y = drug percent dissolved at time t

Stability Studies The optimized formulation (F28) was examined at 40±2 °C/75±5% relative humidity (RH) for accelerated stability and at 25±2 °C/65±5% RH for short term stability for a period of 6 months. The ODTs (F28) were packed and sealed in 30 cc high density polyethylene (HDPE) bottles.[6] Samples were withdrawn at 1, 3 and 6 months and evaluated for appearance, weight variation, thickness, hardness, friability, drug content, water content, disintegration time and dissolution. The drug was assayed using the previously reported HPLC-UV method.[8] The mobile phase was consisted of 20 mM KH_2PO_4 (pH 4.0) and acetonitrile (65 : 35, v/v) delivered at 1.0 ml/min in C4 Hypersil column (250×4.6 mm, 5 μm). The detector wavelength was set at a wavelength of 227 nm and the injection volume was 50 μl.

Evaluation of Taste, Mouth Feel and *in Vivo* Disintegration Time in Human Volunteers The control formulation (F0) and optimized formulation (F28) containing superdisintegrant Kollidon CL-SF (5%) were selected to assess taste, mouth feel and *in vivo* disintegration time in 12 healthy human volunteers at the age group of 25 to 33 years. The study protocol was approved by the Joint Ethics Committee of School of Pharmaceutical Sciences, Universiti Sains Malaysia and Hospital Lam Wah Ee. Prior to the test, all volunteers were informed of the purpose and protocol of the study and each volunteer gave his/her written consent to participate in the study. As per the protocol, all volunteers were asked to rinse their mouth with water before placing the tablet on the tongue and immediately a stopwatch was

Table 1. Composition of Sumatriptan Succinate ODT Formulations F1 to F31

Ingredients (mg/tablet)	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Sumatriptan (SS)	70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mannitol	50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SS: Eudragit EPO	—	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Avicel PH 112	54	140.4	137.2	130.8	124.4	108.4	108.4	108.4	100.4	92.4	76.4	60.4	63.6	57.2	92.4	76.4
Polyplasdone XL	10	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0 (5%)	32.0 (10%)	48.0 (15%)	48	48	—	—
Polyplasdone XL-10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16 (5%)	32 (10%)
Calcium silicate	—	—	3.2 (1%)	9.6 (3%)	16.0 (5%)	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Ammonium bicarbonate	—	—	—	—	—	16.0 (5%)	—	—	24.0 (7.5%)	32.0 (10%)	32.0	32.0	32.0	32.0	32.0	32.0
Menthol	—	—	—	—	—	—	16.0 (5%)	—	—	—	—	—	—	—	—	—
Camphor	—	—	—	—	—	—	—	16.0 (5%)	—	—	—	—	—	—	—	—
Aspartame	10	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4 (2%)	3.2 (1%)	9.6 (3%)	6.4	6.4
Pineapple flavour	2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Magnesium stearate	2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Aerosil	2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4

Ingredients (mg/tablet)	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	F29	F30	F31
SS: Eudragit EPO	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Avicel PH 112	60.4	92.4	76.4	60.4	92.4	76.4	60.4	92.4	84.4	60.4	104.4	100.4	92.4	105.2	98.8	92.4
Polyplasdone XL-10	48 (15%)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CCS	—	16 (5%)	32 (10%)	48 (15%)	—	—	—	—	—	—	—	—	—	—	—	—
SSG	—	—	—	—	16 (5%)	32 (10%)	48 (15%)	—	—	—	—	—	—	—	—	—
Kollidon CL	—	—	—	—	—	—	—	16 (5%)	24 (7.5%)	32 (10%)	—	—	—	—	—	—
Kollidon CL-SF	—	—	—	—	—	—	—	—	—	—	4.0 (1.25%)	8.0 (2.5%)	16.0 (5%)	—	—	—
L-HPC	—	—	—	—	—	—	—	—	—	—	—	—	—	3.2 (1%)	9.6 (3%)	16 (5%)
Calcium silicate	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Ammonium bicarbonate	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0
Aspartame	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
Pineapple flavour	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Magnesium stearate	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Aerosil	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4

started. Volunteers were allowed to move the tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. The taste and mouth feel were evaluated based on the volunteers' spontaneous verbal judgments immediately after the tablet was placed in their mouth as well as after 3—4 min. The taste and mouth feel were rated on a scale of 1 through 5. In taste evaluation, '1' was considered to be "good" while a '5' was considered as "awful." In the mouth feel evaluation, '1' was considered to be "good" while a '5' was considered as "high grittiness." Time taken for the volunteer to feel that the last noticeable granule or fragment had disintegrated in the oral cavity was considered as the *in vivo* disintegration time. The volunteers were prohibited swallowing of their saliva during the test and instructed to rinse their mouth after measurement.

***In Vivo* Study** The study was carried out in accordance with Animal Ethical Guidelines for investigations in laboratory animal and the study protocol was approved by the Animal Ethics Committee of Universiti Sains Malaysia. Six healthy male New Zealand white rabbits weighing between 2.8—3.4 kg were used for the *in vivo* study. The study was conducted according to a 2-period, 2-sequence crossover design with one week wash out period between the phases. The rabbits were randomly divided into two groups of three rabbits each. All the rabbits were fasted for 12 h with *ad libitum* access to water. One group received Reference product (Suminat®) whereas the other group received Test formulation (F28).

The tablets were administered to the rabbits using a procedure reported by Ishikawa *et al.*[9] The tablets were administered at the back of the pharynx using a gastric intubation tube (made of silicone rubber) with one tablet set on the tip of the tube and immediately 5 ml of water was administered through the tube to facilitate swallowing of the tablet and to prevent it from sticking to the rabbit's throat. Two milliliters of blood sample was withdrawn from marginal ear vein into heparinized Eppendorf tubes at time intervals of 0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 16 h post administration. The blood was immediately centrifuged at 4000 rpm for 15 min and plasma was stored at −20 °C until HPLC analysis.

To 0.5 ml aliquot of plasma, 20 μl of 1 μg/ml of sulpiride dissolved in methanol was added as an internal standard and then added 0.5 ml of 1 M sodium hydroxide and 7 ml mixture of TBME, DCM and EA (2 : 2 : 3, v/v) as an extraction solvent. The mixture was vortexed for 2 min and centrifuged at 4000 rpm for 15 min. The supernatant was transferred to a reacti-vial and evaporated to dryness at 50 °C under a gentle stream of nitrogen gas. The residue was reconstituted with 0.2 ml of 10% v/v methanol and 50 μl of the sample was injected into the HPLC system. The HPLC system consisted of a Shimadzu chromatographic system (Kyoto, Japan) equipped with an LC-20AD solvent delivery binary pump, RF-10AXL fluorescence detector, SIL-20AHT autosampler, CTO-10AS VP column oven and LC Solution software for data acquisition and processing. Chromatographic separations were performed using a reversed-phase C4 analytical column (Phenomenex Kromasil, 250×4.6 mm, 5 μm) fitted with a C4 guard column (Phenomenex Kromasil, 10×4 mm, 5 μm). The mobile phase was 25 mM ammonium acetate (pH 6.5) and acetonitrile (85 : 15, v/v) and delivered at a flow rate of 0.9 ml/min. Fluorescence detection was performed with excitation wavelength 225 nm and emission wavelength 350 nm. The column oven temperature was maintained at 40 °C. The calibration curve exhibited an excellent linearity over a concentration range of 1—300 ng/ml of sumatriptan with a correlation coefficient of 0.9999.

The pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained directly from the plasma concentration–time data. The area under the plasma concentration–time curve from 0 to 16 h (AUC_{0-16h}) was calculated by the trapezoidal rule.

Statistical Analysis The results are reported as mean±standard deviation (S.D.). The *in vitro* disintegration time, %DE values for *in vitro* release profiles of sumatriptan succinate and all the physical properties of stability samples were treated statistically using one-way analysis of variance (ANOVA). When there was a statistically significant difference, a *post hoc* Tukey's honestly significant difference (HSD) test was performed. The pharmacokinetic parameters, C_{max} and AUC_{0-16h} were analyzed statistically using one way analysis of variance (ANOVA) which distinguishes effects due to subjects, periods and treatment.[10] The p value was calculated from the obtained F value using GraphPad Prism, version 5.02 (GraphPad Prism software, San Diego, CA, U.S.A.). The values of C_{max} and AUC_{0-16h} were logarithmic transformed before analysis. The T_{max} values were analyzed using Wilcoxon Signed Rank test for paired samples. A statistical significant difference was considered at $p<0.05$.

Results and Discussion

Taste Masking of Sumatriptan Succinate The two key parameters that need to be considered in the development of ODTs are taste masking of bitter drug and the disintegration time. In the development of ODT formulations, the prime challenges are to mask the bitter taste of an active, especially a water-soluble compound and to optimize a formulation that will dissolve in the oral cavity in less than a minute.

Sumatriptan succinate is a highly water soluble and intensely bitter drug. Spray drying technique was used for the taste masking of sumatriptan succinate by coating the drug with Eudragit EPO polymer because it requires only a one-step process and can be easily controlled and scaled up. Eudragit EPO was used as a taste masking agent because it dissolves at a pH of less than 5. Therefore, it does not dissolve in the buccal cavity (pH 5.8—7.4) and keeps the coated drug intact to produce good taste masking, but the polymer dissolves in the stomach (pH 1—3) to release the drug.[11] The prepared microspheres were evaluated for *in vitro* taste masking in 5 ml of phosphate buffer pH 6.8. The results showed that about 13.82±0.43, 7.62±0.50 and 4.57±0.27% of the drug was released from the microspheres in 60 s at the drug to polymer ratios of 1 : 0.50, 1 : 0.75 and 1 : 1, respectively. Among the three ratios of drug to polymer, the microspheres prepared with a ratio of 1 : 1 released less than 5% of drug in 60 s. The drug release would further decrease as the disintegration time of the ODT is less than 60 s. Hence, the microspheres prepared with a ratio of 1 : 1 could be sufficient to mask the bitter taste of sumatriptan succinate in the ODT preparations and used for further evaluation.

Evaluation of Spray Dried Microspheres. Thermal Analysis Figure 1 shows the characteristic endothermic peaks of Eudragit EPO and sumatriptan succinate which represent their melting points. The reported sumatriptan succinate melting point in literature is 169—171 °C.[12] The DSC analysis of spray dried microspheres at a drug to polymer ratio of 1 : 1 revealed negligible change in melting points of Eudragit EPO and sumatriptan succinate, indicating no interaction between the drug and polymer (Fig. 1C). Thus, the findings suggest that the drug was compatible with the polymer used and it did not undergo any changes during the spray drying process.

Particle Size The mean particle size of the spray dried microspheres was 7.77±0.22 μm. The particle size of the microspheres was considered to be suitable for preparation of orally disintegrating tablets as it would produce a smooth mouth feel without grittiness in the mouth when administered in the form of a tablet.

Entrapment Efficiency, Drug Loading and Yield The entrapment efficiency of the microspheres was found to be 92.86±0.68% with a drug loading of 46.43±0.56%. The yield of the spray dried microspheres was about 56.34%. The low yield could be due to a smaller portion of small and light particles which escaped through the exhaust of the spray dryer during the spray-drying process. The yield of the microspheres may be further improved if the loss of particles through the exhaust of the spray dryer apparatus can be prevented.[11]

Evaluation of Tablets The average weight and thickness of tablets for all the formulations was found to be in the range of 316.29 to 323.45 mg and 6.34 to 6.58 mm, respec-

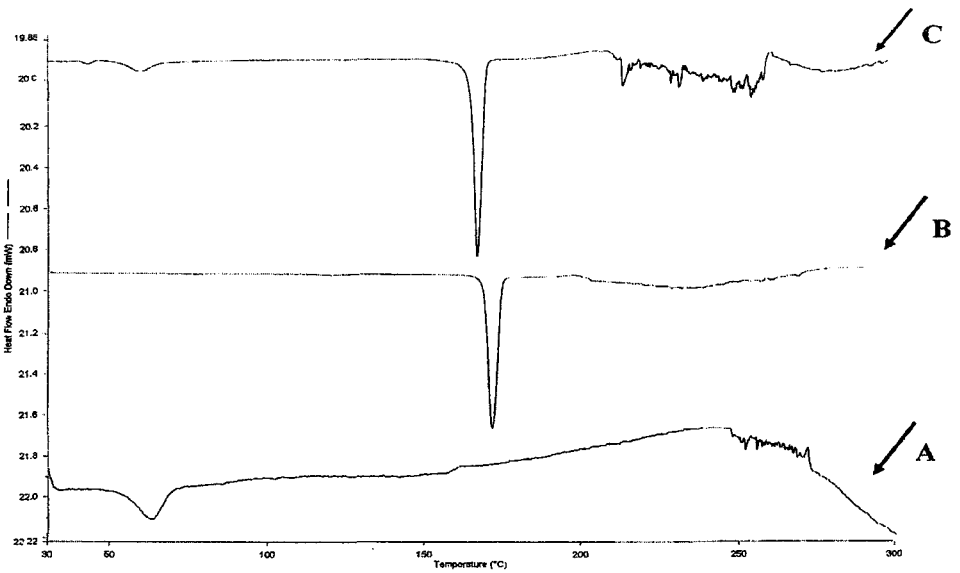

Fig. 1. DSC Thermograms of (A) Eudragit EPO, (B) Sumatriptan Succinate, (C) Sumatriptan Succinate and Eudragit EPO Spray Dried Microspheres

Table 2. Results of Various Formulations of Sumatriptan Succinate ODTs. Mean±S.D.

Formulation	Weight variation (mg); $n=20$	Thickness (mm); $n=10$	Hardness (kg); $n=10$	Friability (%); $n=20$	Drug content (%); $n=3$	*In vitro* DT[a] (s); $n=6$	Water content (%); $n=3$
F1	319.39±3.01	6.38±0.09	2.67±0.14	0.33	100.77±1.23	409.17±10.91	—
F2	320.14±1.11	6.42±0.03	2.70±0.13	0.29	100.55±0.89	354.00±5.29	—
F3	321.11±1.50	6.49±0.07	2.55±0.14	0.51	101.24±1.36	264.83±5.98	—
F4	318.70±1.05	6.35±0.04	2.49±0.09	0.55	98.87±0.91	229.50±6.50	—
F5	318.63±1.74	6.36±0.02	2.60±0.13	0.42	99.09±1.12	164.83±4.36	—
F6	—	—	—	—	—	—	—
F7	—	—	—	—	—	—	—
F8	319.96±0.87	6.37±0.08	2.46±0.10	0.59	99.69±0.99	121.33±2.16	—
F9	317.21±1.58	6.48±0.09	2.72±0.12	0.33	98.56±1.05	71.50±1.87	—
F10	316.29±2.13	6.47±0.05	2.47±0.16	0.65	98.23±1.48	55.67±2.07	7.67±0.06
F11	319.85±1.43	6.37±0.06	2.53±0.15	0.42	100.77±1.14	39.17±1.83	7.83±0.06
F12	320.85±0.99	6.41±0.06	2.51±0.07	0.68	99.96±0.74	38.00±1.10	—
F13	320.34±1.41	6.44±0.05	2.36±0.05	0.72	100.33±0.81	39.50±1.64	—
F14	317.64±1.87	6.41±0.05	2.51±0.21	0.62	99.11±0.57	63.67±1.63	—
F15	321.93±2.11	6.37±0.08	2.49±0.24	0.69	100.43±1.09	37.50±1.52	8.19±0.05
F16	317.28±1.59	6.56±0.08	2.53±0.19	0.59	99.19±0.73	38.50±1.38	8.72±0.04
F17	320.14±1.73	6.40±0.11	2.71±0.10	0.37	100.47±1.16	64.33±1.75	—
F18	322.18±1.19	6.53±0.03	2.74±0.15	0.30	102.31±1.48	86.33±1.97	—
F19	316.49±1.80	6.37±0.08	2.40±0.17	0.64	98.50±0.71	136.00±2.19	—
F20	319.33±1.93	6.44±0.05	2.42±0.14	0.59	100.27±0.84	111.83±3.19	—
F21	323.32±1.52	6.39±0.08	2.56±0.11	0.59	102.56±1.31	148.00±2.37	—
F22	318.46±1.48	6.58±0.09	2.55±0.22	0.61	100.27±0.62	190.33±2.16	—
F23	321.76±2.04	6.34±0.02	2.54±0.17	0.56	100.93±0.95	75.00±1.90	—
F24	319.11±1.75	6.47±0.10	2.49±0.13	0.61	100.38±1.21	64.33±1.21	—
F25	317.76±2.22	6.48±0.07	2.67±0.06	0.39	99.39±1.03	48.17±2.32	6.35±0.05
F26	316.29±1.92	6.39±0.06	2.56±0.20	0.39	98.11±0.36	73.00±1.55	—
F27	323.45±1.04	6.41±0.03	2.63±0.19	0.46	102.45±1.17	62.83±1.33	—
F28	317.98±1.10	6.43±0.03	2.46±0.18	0.54	99.05±0.79	38.17±1.47	6.41±0.04
F29	317.43±1.44	6.54±0.02	2.41±0.16	0.63	99.83±0.68	155.50±2.74	—
F30	317.28±1.28	6.47±0.04	2.65±0.15	0.42	100.06±1.22	133.33±2.73	—
F31	322.09±1.82	6.50±0.09	2.76±0.09	0.28	101.89±0.73	112.00±1.90	—

a) *In vitro* DT: *In vitro* disintegration time.

tively (Table 2). All the formulations exhibited low weight variation which lies within the USP pharmacopoeial limits of ±7.5% of the average weight.[7] The hardness of the tablets was maintained in the range of 2—3 kg and the friability results were found to be within the acceptable limits (<1%) which suggested that ODTs ability to withstand abrasion in handling, packaging and shipment. All the formulations demonstrated uniformity in the assay and drug content varied from 98.11 to 102.56%. The formulations with a disintegration time of less than 60 s were subjected to water content de-

termination. The water content of all the ODT formulations was found to be less than 9%.

Effect of Pore Forming Agent The disintegration time of the tablets prepared with formulation F1 was very high and not within the acceptable limits (<60 s) of ODT specifications set by USFDA. Hence, calcium silicate, a highly porous, lightweight powder was incorporated as a pore forming agent in the next formulations to decrease the disintegration time of the tablets. Calcium silicate was used in a concentration range of 1 to 5% (F2 to F4). The concentration of calcium silicate had no effect on the physical properties of the tablets but influenced the disintegration time (Table 2). The disintegration time of tablets were decreased significantly ($p<0.05$) with respect to increase in the concentration of calcium silicate from 1 to 3 and 5%. It might be due to the increase in the amount of calcium silicate in the formulations increasing the porous nature of the tablets which lead to a faster absorption of water through the pores by wicking action and disintegration of the tablets.[13] However, formulations F2 to F4 did not meet the disintegration time requirements set by USFDA. Therefore, in the next experiments, sublimation technique was used to achieve the desired disintegration time of <60 s.

Effect of Subliming Agent Type and Concentration The effect of subliming agent type was studied by preparing the formulations containing ammonium bicarbonate (F5), menthol (F6) and camphor (F7) at a concentration of 5%. Many researchers reported that the sublimation of tablet containing subliming agent provided faster disintegration time compared to sublimation directly from the granules.[14—16] It could be explained that a compaction process during tableting might have caused breakage of porous granules and subsequent reduction in porosity.[17,18] Hence, in the present study, sublimation process was carried out from the tablets instead of from the granules. The tablets prepared with ammonium bicarbonate (F5) were disintegrated in 164.83 s and produced a pronounced decrease in disintegration time of the tablets when compared to formulation F4 (229.50 s). It could be due to the addition of ammonium bicarbonate increased the porosity of the tablet. Surprisingly, the formulations containing menthol (F6) and camphor (F7) formed wet mass which may be due to formation of eutectic mixture between one of the excipients or drug itself or their combination with camphor or menthol.[19] As the wet mass was not suitable to produce tablets, ammonium bicarbonate was therefore selected for the next formulations.

Ammonium bicarbonate in different concentrations, 5% (F5), 7.5% (F8) and 10% (F9) were incorporated as a subliming agent. Sublimation process was carried out for 15—21 h at 40 °C depended on the amount of ammonium bicarbonate present in the formulations. Increasing the concentration of subliming agent in the formulations did not show any effect on physical properties of the tablets, but decreased the disintegration time of the tablets significantly from 164.83 (F5) to 121.33 (F8) and 71.50 s (F9). The tablets containing 10% ammonium bicarbonate produced faster disintegration which could be due to higher porosity of tablets. During drying, ammonium bicarbonate sublimed and could be formed porous structure on the surface of the tablets.[20] The porous structure is responsible for faster water uptake which facilitates wicking action of Polyplasdone XL in bringing about faster disintegration of tablet.[18,21] Although, sublimation method produced lower disintegration time results, the tablets have yet to achieve the desired disintegration time specified for ODTs by USFDA.

Effect of Type and Concentration of Superdisintegrant The different concentrations and types of superdisintegrants used in the formulations did not show any effect on the physical properties but influenced the disintegration time of the tablets (Table 2). The disintegration time of various formulations prepared with different types and concentrations of superdisintegrants varied from 37.50 to 190.33 s. The disintegration time of the tablets decreased significantly with the increase in the concentration of Polyplasdone XL from 5 (F9) to 10 (F10) and 15% (F11). Increase in the concentration of Polyplasdone XL-10 from 5 (F14) to 10% (F15) resulted in a significant decrease ($p<0.05$) in the disintegration time of the tablets. However, further increase in the concentration to 15% (F16) did not show any significant effect ($p>0.05$) on the disintegration time of the tablets.

When the superdisintegrants, Polyplasdone XL and XL-10 were replaced with CCS and SSG at a concentration level of 5% (F17 and F20), the disintegration time of the tablets was 64.33 and 111.83 s, respectively. Further increase in the concentration of CCS and SSG from 5 to 10 (F18 and F21) and 15% (F19 and F22) resulted in a significant increase ($p<0.05$) in the disintegration time of the tablets. It can be observed from the results that the disintegration of tablets containing crospovidone (Polyplasdone XL and Polyplasdone XL-10) were comparatively faster than those containing CCS and SSG at the same concentration level. It might be attributed to rapid water absorbing nature of crospovidone, involving both capillary and swelling mechanisms which build up the pressure internally leading to the faster disintegration.[22] This dissimilarity in behaviour of crospovidone, CCS and SSG on the disintegration time can be attributed to the difference in their mechanism of disintegration. The concentration of crospovidone had a positive effect on the disintegration of tablets. Increasing the concentration of crospovidone resulted in a faster disintegration of tablets, which may be due to rapid capillary activity and pronounced hydration with little tendency for gel formation.[23,24] On the contrary, when the concentration of CCS and SSG was increased it had a negative effect on the disintegration of the tablets. This negative effect may be due to the formation of a viscous gel layer by CCS and SSG which may impede further penetration of the disintegration medium and hindered the disintegration of tablets.[24,25] The obtained results were similar to the findings of Khan *et al.*[26] and Patel *et al.*[27]

The disintegration time of the tablets was significantly decreased ($p<0.05$) with increase in the concentration of Kollidon CL from 5 to 10% (F23 to F25) and Kollidon CL-SF from 1.25 to 5% (F26 to F28). The tablets prepared with formulation F28 produced shorter disintegration time results compared to formulation F25. These superdisintegrants exhibited their disintegrant effect by wicking action without forming a gel. They increase the porosity and provide pathways for the penetration of fluids into tablets, which in turn resulted in wicking through capillary action facilitating the disintegration of tablets.[28] An increase in the superdisintegrant, L-HPC concentration from 1 (F29) to 3 (F30) and 5% (F31) resulted in a significant decrease in the disintegration

time of tablets. The tablets prepared with L-HPC disintegrated based on its swelling property in water.[29]

Amount of Aspartame in Preparation of ODTs Aspartame was incorporated as a sweetener in the formulations to produce sufficient sweet taste of the tablets. In the process of formulation optimization, the first formulation which produced disintegration time of less than 60 s was selected for the incorporation of different concentrations of sweetener. Although, the formulation F10 was the first formulation which was able to disintegrate in less than 60 s (55.67 s), the formulation F11 was selected for this study as this formulation produced lower disintegration time (39.17 s) compared to the former. Preliminary studies were performed in healthy human volunteers for the taste characterization of sumatriptan succinate ODTs prepared with different amount of aspartame (1 to 3%) as a sweetener in the formulations F12, F11 and F13. The physical properties of the tablets including disintegration time were not affected by the concentration of aspartame in the formulations (Table 2). The optimum amount of sweetener was determined based on the taste perception. A single blind study was designed for the taste masking test. Six healthy human volunteers in the age group of 25—33 years participated in the test. The evaluation was based on the extent to which subjects liked the taste of each ODT. Among the 6 volunteers, 4 volunteers rated the formulation F12 containing 1% aspartame as '2,' indicating the formulation had no taste, 1 volunteer rated as '1' indicating a sweet taste and 1 volunteer rated as '3' indicating a slight bitterness of tablets. All the volunteers rated the formulation F11 and F13 as '1,' suggesting that these formulations were sweet and acceptable. Hence, it was concluded that formulations F11 and F13 with 2 and 3% of aspartame successfully produced sweet taste of sumatriptan succinate ODTs and the amount of aspartame used was also well within the limits of USFDA.[30] Thus, 2% aspartame was incorporated in all other formulations.

***In Vitro* Dissolution Studies** The commercial product (Suminat®) released more than 90% of drug in 15 min in 0.01 N HCl and phosphate buffer pH 6.8 and 10 min in acetate buffer pH 4.5. All the ODT formulations (F10, F11, F15, F16, F25 and F28) exhibited similar release profiles with commercial product (Fig. 2) and showed no significant difference in the $DE_{15\,min}$ values irrespective of the dissolution media (Table 3).

Although all the formulation release profiles were comparable with the commercial product irrespective of the dissolution media, formulation F28 was selected as an optimized formulation due to the smaller particle size (10—30 μm) of the superdisintegrant, Kollidon CL-SF compared to other superdisintegrants used in this study. The smaller particle size of Kollidon CL-SF would produce a tablet with smooth mouth feel without grittiness in the mouth when administered to the patient. Moreover, the tablets prepared with Kollidon CL-SF produced lower water content results compared to Polyplasdone XL and Polyplasdone XL-10 and similar to Kollidon CL (Table 2). The amount of water content present in the ODTs significantly affects the stability of the final drug product. Hence, this lower water content could be an added advantage in increasing the stability of the final drug product.

Evaluation of Taste, Mouth Feel and *in Vivo* Disinte-

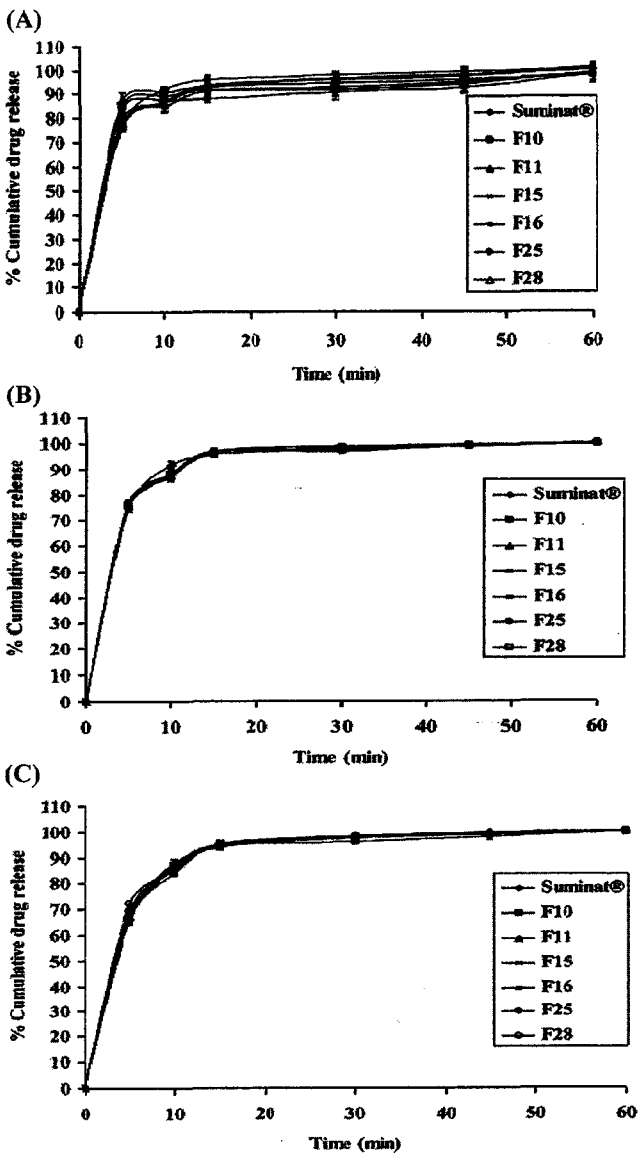

Fig. 2. *In Vitro* Release Profiles of Formulations F10 (10% Polyplasdone XL), F11 (15% Polyplasdone XL), F15 (10% Polyplasdone XL-10), F16 (15% Polyplasdone XL-10), F25 (10% Kollidon CL), F28 (5% Kollidon CL-SF) and Commercial Product (Suminat®) in (A) 0.01 N HCl, (B) Acetate Buffer pH 4.5, (C) Phosphate Buffer pH 6.8

Mean±S.D., n=6.

Table 3. Dissolution Efficiency (DE) Results of Sumatriptan Succinate ODTs in Different Dissolution Media at a Paddle Speed of 30 rpm

Formulation	$DE_{15\,min}$ (%)		
	0.01 N HCl	Acetate buffer pH 4.5	Phosphate buffer pH 6.8
Suminat®	72.19±0.44	71.45±1.04	66.94±0.73
F10	73.66±0.46	70.23±0.89	65.85±0.54
F11	74.95±1.43	70.31±0.61	67.02±0.70
F15	70.15±1.04	70.42±0.73	66.77±1.04
F16	70.46±2.57	71.13±0.72	66.17±1.21
F25	71.70±1.40	70.15±0.71	67.84±1.00
F28	69.76±0.48	70.53±0.72	68.17±0.93

Mean±S.D., n=6.

gration Time in Human Volunteers All the volunteers reported that the control formulation (F0) was very bitter in taste and immediately spitted out the tablet. Hence, mouth feel and *in vivo* disintegration time of the control formulation was not determined. The optimized formulation (F28) was found to be pleasant taste with good mouth feel without any grittiness and rapidly disintegrated in oral cavity in 40.50±2.07 s (Table 4).

Stability Study The stability results demonstrated that there was no significant change in appearance of the tablets, weight variation, thickness, hardness, friability, water content and disintegration time (Table 5). The results of disintegration time after 6 months of storage were within the USFDA limits (<60 s) for ODTs. No significant loss was found in the drug content at the end of 6 months. The *in vitro* release profiles of optimized formulation (F28) stored for 0, 1, 3 and 6 months are shown in Fig. 3. There was no significant difference in $DE_{15\,min}$ values of fresh and stored samples and thus, the formulation F28 was proven to be stable for at least 6 months.

***In Vivo* Study** The mean sumatriptan plasma concentration *versus* time profiles of reference and test formulation are depicted in Fig. 4. The plasma profile of each rabbit showed was highly variable with some of them displayed double peaks or multiple peaks. The results are in line with the findings reported by other researchers.[31—34] The possible reason for the appearance of double peaks in many rabbits could be to the presence of two compartment absorption phases with only one disposition phase.[35,36] The probable

Table 4. Evaluation of Taste, Mouth Feel and *in Vivo* Disintegration Time of Sumatriptan Succinate ODTs Using Human Volunteers

Volunteers	Taste		Mouth feel		*In vivo* disintegration time (s)	
	Control	F28	Control	F28	Control	F28
1	5	1	—	1	—	40
2	5	1	—	1	—	45
3	5	3	—	1	—	38
4	5	1	—	2	—	39
5	5	1	—	1	—	40
6	5	1	—	1	—	39
7	5	1	—	1	—	41
8	5	1	—	1	—	38
9	5	1	—	1	—	42
10	5	3	—	1	—	40
11	5	1	—	1	—	43
12	5	1	—	1	—	41
Mean	—	—	—	—	—	40.50
S.D.	—	—	—	—	—	2.07

n=12. Taste: 1, sweet and good: 2, tasteless; 3, slightly bitter; 4, bitter; 5, awful. Mouth feel: 1, good: 2, no feeling; 3, slight grittiness; 4, moderate grittiness; 5, high grittiness.

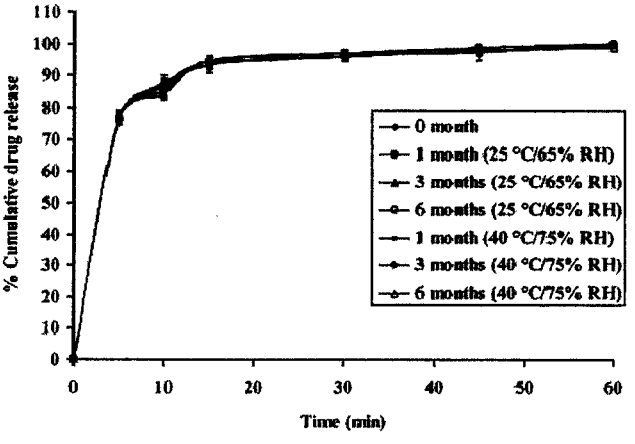

Fig. 3. *In Vitro* Release Profiles of Optimized Formulation F28 after 1, 3 and 6 Months Storage at Different Temperatures and Humidities

Mean±S.D., n=6.

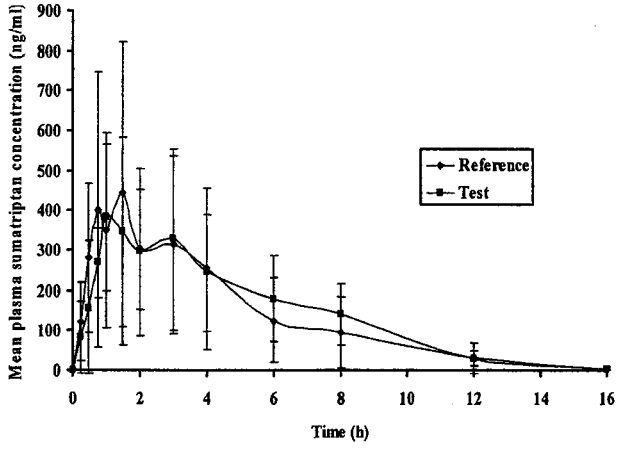

Fig. 4. Mean Plasma Sumatriptan Concentration Profiles after Oral Administration of Reference Product (Suminat®) and Test Formulation (F28) in Rabbits

Mean±S.D., n=6.

Table 5. Stability Study Results of Optimized Formulation (F28) after 1, 3 and 6 Months Storage at Different Temperatures and Humidities

Tablet properties	n	25 °C/65% RH				40 °C/75% RH		
		0 month	1 month	3 months	6 months	1 month	3 months	6 months
Weight variation (mg)	20	319.56±1.22	320.22±1.24	320.76±0.53	321.31±0.69	320.93±0.50	321.47±1.10	321.50±1.48
Thickness (mm)	10	6.42±0.03	6.43±0.02	6.43±0.03	6.45±0.02	6.41±0.01	6.44±0.03	6.45±0.02
Hardness (kg)	10	2.54±0.05	2.56±0.06	2.51±0.08	2.51±0.09	2.52±0.07	2.49±0.06	2.48±0.04
Friability (%)	20	0.40	0.37	0.41	0.39	0.40	0.43	0.45
Drug content (%)	3	100.86±0.70	100.77±1.40	100.12±0.98	99.83±0.93	100.59±1.20	99.92±1.13	99.01±0.85
In vitro DT (s)	6	38.83±1.47	38.33±1.63	37.67±1.86	37.50±1.38	38.00±1.41	37.17±1.17	36.67±1.63
Water content (%)	3	6.40±0.05	6.42±0.06	6.48±0.04	6.52±0.07	6.45±0.06	6.51±0.08	6.54±0.05
Dissolution efficiency (%)	6	69.92±1.03	69.60±1.21	70.17±1.74	69.98±1.32	70.76±0.99	69.63±0.82	69.85±0.77

Mean±S.D.

reasons for multiple peaks may be due to alteration in the gastric motility by sumatriptan[37,38] and/or presence of multiple absorption sites for sumatriptan in the gastrointestinal tract of rabbits.[39] The data demonstrated that plasma concentrations of sumatriptan increased rapidly then fluctuated and reached maximum in all the rabbits at approximately 0.5 to 3 h for both preparations, thereafter declined gradually over a period of 16 h. The pharmacokinetic parameters, C_{max}, T_{max} and AUC_{0-16h} values were 523.32±346.17 ng/ml, 1.42±0.96 h and 2135.87±1515.89 ng·h/ml, respectively for Reference and 510.00±222.57 ng/ml, 1.54±0.84 h and 2227.44±1204.11 ng·h/ml, respectively for Test formulation (F28). There was no significant difference between pharmacokinetic parameters values of Reference product and Test formulation (F28). Thus, both preparations were bioequivalent in their rate and extent of absorption.

Conclusion

The taste masked orally disintegrating tablets of sumatriptan succinate containing Kollidon CL-SF (5%) as a superdisintegrant and ammonium bicarbonate (10%) as a subliming agent was successfully prepared using direct compression method followed by sublimation technique. The optimized formulation (F28) had sweet taste, smooth mouth feel and rapidly disintegrated in the mouth within 41 s and also had good stability. Formulated ODTs showed similar *in vitro* release profiles with that of a commercial product and also bioequivalent in their rate and extent of absorption. Hence, this "patient-friendly dosage form" could be a useful alternative to commercially available conventional tablets.

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Development and Validation of an RP-HPLC-UV Method for Analysis of Sumatriptan Succinate in Pharmaceutical Dosage Forms

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Summary. An isocratic RP-HPLC-UV method for analysis of sumatriptan succinate in pharmaceutical dosage forms has been developed and validated. Best separation was achieved on a Thermo Hypersil C4 column (250 mm X 4.6 mm, 5 μm) using a mobile phase of 20 mM potassium dihydrogen phosphate adjusted to pH 4.0 with orthophosphoric acid and acetonitrile (65:35, *v/v*) at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 227 nm. The method was validated for specificity, linearity, precision, accuracy, limit of quantification, limit of detection, robustness, and solution stability. The calibration plot was linear over the concentration range 25–600 ng mL^{-1} (r^2 = 0.9998) and the limits of detection and quantification were 10 and 25 ng mL^{-1}, respectively. Intra-day and inter-day precision and accuracy were between 1.25 and 2.95% and between −1.15 and 2.47%, respectively. The method was successfully used for analysis of sumatriptan succinate, in the presence of excipients, in orally disintegrating tablets prepared in our laboratory and in commercially available tablets (Imigran) and nasal spray (Suminat).

Key Words: sumatriptan succinate, RP-HPLC-UV, method validation, pharmaceutical dosage forms

Introduction

Migraine is a chronic, episodic, neurological disorder, which usually begins in childhood, adolescence, or early adult life, characterized by unilateral headache often accompanied by nausea and/or vomiting [1]. Sumatriptan succinate (SS; {3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulphonamide succinate}; *Fig. 1*) is a highly selective 5-hydroxytryptamine-1 receptor agonist used for treatment of migraine headache. It is a basic (pK_a 9.63) white to off-white powder which is readily soluble in water and

in saline [2]; the *n*-octanol/water partition coefficient ($P_{o/w}$) of the sumatriptan base is 0.65 [3]. Clinical routes of administration are oral, subcutaneous, and intranasal, with absolute bioavailabilities of approximately 14, 96, and 15%, respectively. The lower bioavailabilities are primarily because of presystemic first-pass metabolism and partly because of incomplete absorption [4, 5].

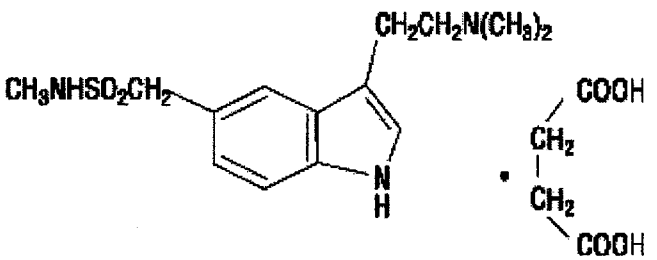

Fig. 1. The chemical structure of sumatriptan succinate

Orally disintegrating tablets (ODT), which disintegrate and dissolve in the saliva without water within 60 s or less, are a widely used new pharmaceutical dosage form. ODT preparations are formulated with UV-absorbing components, specifically flavours and sweeteners. These excipients could decrease the signal of the drug to background (excipients) in the UV because the excipient to drug ratio is higher in formulation of ODT. High ratio of excipient to drug in ODT formulation is necessary to impart good taste and mouth feel [6]. Hence, development of a suitable HPLC method is required for routine and in-process quality-control analysis, dissolution, or similar studies.

Literature review reveals that few methods have been published for analysis of SS in the bulk form and in pharmaceutical preparations. Available methods include HPLC [2, 4, 5, 7–10], HPLC with colorimetric detection [11], HPTLC with spectrophotometric [12], densitometric, and spectrophotometric detection [13], voltametry [14], and capillary electrophoresis [15, 16]. The reported HPLC methods [4, 5] which are modifications of the method of Nozal et al. [2], have not been directly applied to analysis of SS in pharmaceutical preparations. The disadvantages of other HPLC methods [7–10] include low sensitivity, long analysis time, and unreported solution stability data. To the best of our knowledge, there are no published reports of analysis of SS in ODT in the presence of excipients.

The objective of this work was to develop and validate an isocratic RP-HPLC–UV method for quantitative analysis of SS in an ODT dosage form prepared in our research laboratory. The validated method was also used for analysis of SS in commercially available tablets and nasal spray.

Experimental

Chemicals, Reagents, and Solutions

Sumatriptan succinate was purchased from Nosch Laboratories (Hyderabad, India). Imigran tablets were purchased from GlaxoSmithKline (Middlesex, UK). Suminat nasal spray was purchased from Sun Pharmaceuticals (Gujarat, India). Potassium dihydrogen phosphate (anhydrous) was obtained from R&M Chemicals (Essex, UK). Orthophosphoric acid was purchased from Ajax Chemicals (New South Wales, Australia). Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (Phillipsburg, USA).

Primary standard stock solution of SS was prepared in methanol at a concentration of 1.0 mg mL^{-1} and further diluted with mobile phase to furnish working standard stock solution of 10 µg mL^{-1}. The working standard stock solution was used to prepare calibration samples in the concentration range 25–600 ng mL^{-1} and quality control samples at low, medium, and high concentrations of 75, 300, and 500 ng mL^{-1}. These solutions were stored under refrigeration at 4°C prior to use.

HPLC Instrumentation and Chromatographic Conditions

HPLC was performed with a Waters (France) 510 delivery pump, a Rheodyne (Cotati, California, USA) 7725i six-port sample-injection with 50-µL sample loop, a Jasco (Tokyo, Japan) 875-UV UV–visible detector, and a Hitachi (Tokyo, Japan) D-2500 Chromato-Integrator. Chromatographic response was measured in microvolts (µV).

Chromatographic separation of SS was achieved at ambient room temperature (25 ± 2°C) using Thermo Hypersil C4 (250 mm X 4.6 mm, 5 µm) analytical column with a mobile phase containing mixture of 20 mM potassium dihydrogen phosphate (pH 4.0 adjusted with ortho phosphoric acid) and acetonitrile (65:35, *v/v*) at a flow rate of 1.0 mL min^{-1}. Before use, the mobile phase was filtered through a 0.45 µm Nylon membrane filter (Whatman, UK), under vacuum, and degassed. The detector was set at 227 nm and the injection volume was 50 µL.

Analysis of the Pharmaceutical Preparations

For ODT and Imigran tablets assay, ten tablets were weighed and finely powdered, an amount of powder equivalent to 50 mg drug was accurately weighed into each of five 50-mL volumetric flasks, and 25 mL methanol was added to each flask. The flasks were sonicated for 15 min to dissolve SS completely and the solutions were then diluted to volume with methanol. The solutions were filtered through 0.45-μm PTFE syringe filters and 0.1 mL filtrate was diluted with mobile phase to yield a concentration of 500 ng mL^{-1} SS.

For Suminat nasal spray assay, one actuation of nasal spray solution, equivalent to 20 mg SS, was transferred into each of five 25-mL volumetric flasks, diluted to volume with mobile phase, mixed well, and 0.1 mL of the solution was diluted with mobile phase to yield a concentration of 500 ng mL^{-1} SS.

Results and Discussion

Method Development and Optimization

Development of RP-HPLC–UV methods for analysis of drugs has received much attention in recent years because of their importance in routine quality-control analysis. To validate an efficient method for analysis of a drug in pharmaceutical formulations, preliminary tests are performed with the objective of selecting optimum conditions. In this work analytical column, mobile phase composition, organic modifier, pH, molarity of buffer salt, and mobile phase flow rate were optimized to achieve an assay of good performance for analysis of SS in pharmaceutical dosage forms.

The optimum wavelength for detection of SS with adequate sensitivity was 227 nm. Several reversed-phase analytical columns (C_{18}, C_8, C_4, and CN, in order of increasing polarity) were tested with a 60:40 (*v*/*v*) mixture of 20 mM potassium dihydrogen phosphate solution (pH 4.0) and acetonitrile as mobile phase. A drug concentration of 500 ng mL^{-1} was used for optimization.

Initial separation studies were performed with C18 (Phenomenex, 250 mm X 4.6 mm, 5 μm) column. For organic polar molecules, sample retention decreases with increasing length of the bonded phase. SS is a basic polar compound and freely soluble in water, for this reason SS was eluted rapidly from the C_{18} column. At acidic pH, basic compounds are protonated; this also might promote rapid elution of the analyte from the C_{18} column. Under such conditions, SS was not well separated from the excipients present in

the pharmaceutical dosage forms. The SS was eluted more quickly with good peak shape from the C_8 column (Phenomenex, 250 mm × 4.6 mm, 5-µm particle) but resolution between the SS and solvent-front peak was poor. The C_4 column (Thermo-Hypersil, 250 mm × 4.6 mm, 5-µm particle) is less hydrophobic than C_8 and C_{18} columns. Polar compounds have longer retention on C_4 than on C_8 and C_{18}. SS was eluted at a longer retention time with good chromatographic response and peak shape, and was also well resolved from the excipients and solvent-front peaks, because of the polar nature of the analyte. The cyano (CN, Phenomenex, 250 mm × 4.6 mm, 5-µm particle) chromatographic column is used for polar basic compounds in both reversed- and normal-phase modes. Retention of SS on CN was greater than on the other columns (C_{18}, C_8, and C_4), with good chromatographic response and optimum separation, but peak shape was not optimum. On the basis of these findings, the C_4 analytical column was selected as most appropriate for analysis of SS.

Mixtures of 20 mM potassium dihydrogen phosphate (pH 4.0) and acetonitrile in the proportions 40:60, 60:40, 65:35, 70:30, and 80:20 (*v/v*) were tested as mobile phases with the C_4 column. Variation of the composition of the mobile phase led substantial changes in chromatographic performance. Increasing the organic modifier content resulted in a decrease in the retention time of the analyte but had no effect on analyte response. The peak shape of drug was poor and shoulder peak was observed along with analyte for the highest proportion of acetonitrile in the mobile phase (40:60, *v/v*). The most symmetric peak shape with reasonable retention time were achieved by use of a 65:35 (*v/v*) mixture of buffer and acetonitrile.

When experiments were performed with methanol instead of acetonitrile as organic modifier in the mobile phase, late elution of analyte with peak tailing and increased column pressure were observed. Hence, experiments were performed with acetonitrile as an organic modifier.

There were no substantial changes in retention time, peak symmetry and chromatographic response of the analyte when mobile phase pH was varied in the range 2.5–6.0 by using potassium dihydrogen phosphate as buffer salt. A negative baseline for the last-eluting part of the peak was observed when mobile phase pH was 7.0. pH 4.0 was selected as optimum because this achieved a good compromise between retention time and peak shape.

Buffer molarity of 10, 20, and 50 mM was tested. There were no significant changes in the chromatographic response and peak symmetry with change in buffer molarity. A buffer molarity of 20 mM was selected for further analysis.

After several trials, a mixture of 20 mM potassium dihydrogen phosphate (pH 4.0) and acetonitrile (65:35, v/v), at a flow rate of 1.0 mL min^{-1},

was finally adopted as mobile phase. These chromatographic conditions achieved satisfactory resolution, reasonable retention, and symmetric peak shape for SS with a retention time of 4.50 min. No interference from the sample solvent and dosage form excipients was observed at the retention time of SS.

Method Validation

To confirm its suitability for its intended purpose, the method was validated in accordance with ICH guidelines Q2 (R1) [17], for system suitability, linearity, specificity, precision, accuracy, limit of detection, limit of quantification, robustness, and solution stability.

System Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (R_T), number of theoretical plates (N), tailing factor (T), and peak asymmetry (A_S) were evaluated for five replicate injections of the drug at a concentration of 300 ng mL^{-1}. The results shown in *Table I* were within acceptable limits [18].

Table I. System-suitability data. Mean ± SD, n = 5

Property	Value	RSD (%)	Required limits
Retention time (R_T)	4.51 ± 0.01	0.31	RSD ≤ 1%
Theoretical plates (N)	7918.20 ± 191.62	2.42	N > 2000
Tailing factor (T)	1.06 ± 0.02	1.76	$T \leq 2$
Asymmetry (A_S)	1.11 ± 0.02	1.74	$A_s \leq 1.5$

Linearity

Five calibration plots were constructed in the concentration range 25–600 ng mL^{-1} (25, 50, 100, 200, 400, and 600 ng mL^{-1}) by plotting analyte concentration against peak-area response. The regression equation was $y = 431.97x + 1263.7$ and the mean values of the slope, intercept, and correlation coefficient were 431.97 ± 12.74, 1263.7 ± 57.46, and 0.9998 ± 0.0001, respectively. The standard error (SE) of the slope and intercept were 5.70 and 25.70, respectively. These results show there was an excellent correlation be-

tween peak area and analyte concentration. The linearity results are presented in *Table II.*

Table II. Summary of calibration data for sumatriptan succinate

Theoretical amount (ng mL^{-1})	Experimental amount (ng mL^{-1})[a]	RSD (%)	RE (%)
25	24.68 ± 1.19	4.81	−1.28
50	48.68 ± 1.22	2.52	−2.63
100	100.57 ± 2.24	2.23	0.57
200	203.76 ± 1.58	0.78	1.88
400	395.61 ± 1.91	0.48	−1.10
600	601.70 ± 1.60	0.27	0.28

$n = 5$

Specificity

To determine the specificity of the method, the absence of interference from the excipients present in the pharmaceutical dosage forms (placebo sample) was investigated. The placebo solution contained mannitol, microcrystalline cellulose, polyplasdone XL, aspartame, strawberry flavour, sodium stearyl fumarate, aerosol, lactose, croscarmellose sodium, methylhydroxypropylcellulose, triacetin, titanium dioxide, magnesium stearate, iron oxide, benzalkonium chloride, and aqueous buffered vehicle. The placebo sample was prepared in methanol and the quantity of excipients was equivalent to the maximum potency/day/dose reported by the USFDA [19]. The placebo

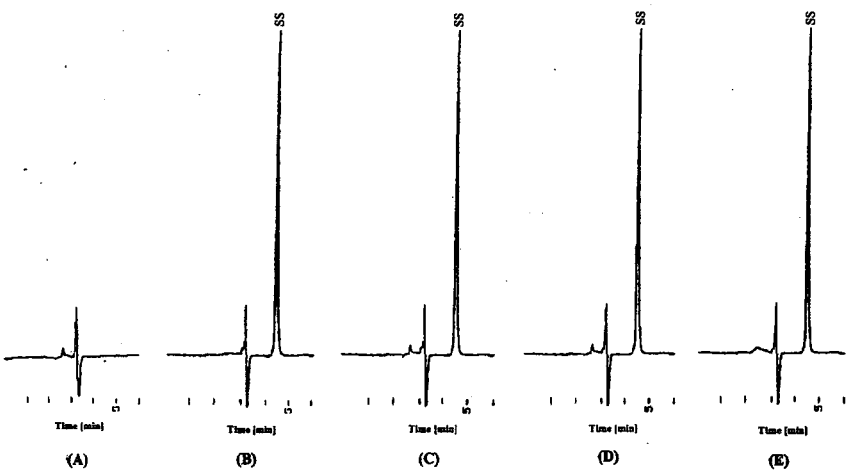

Fig. 2. Representative chromatograms obtained from sumatriptan succinate. (A) Placebo, (B) Pure drug (4.50 min.), (C) ODT, (D) Tablets, (E) Nasal spray

sample was analyzed in six replicates. No interfering peaks from the excipients were found at the retention time of SS (4.50 min). The chromatograms obtained from placebo and pure drug, ODT, tablets and nasal spray at concentrations of 500 ng mL^{-1} are shown in *Fig. 2*. It is apparent from the figure that the analytical method was specific for the analysis of SS in pharmaceutical dosage forms.

Intra-Day and Inter-Day Precision and Accuracy

Intra-day and inter-day precision and accuracy were evaluated by analyzing quality-control samples containing low, medium, and high concentrations of SS of 75, 300, and 500 ng mL^{-1}. For intra-day variation, sets of five replicates of the three concentrations were analyzed on the same day; for inter-day variation, five replicates were analyzed on three different days. Intra-day accuracy (RE, %) ranged between −1.15 and 1.96% with a precision (RSD, %) of 1.41 to 2.77%. Inter-day accuracy ranged between 0.34 and 2.47% with a precision of 1.25 to 2.95%. All the results obtained during assessment of precision and accuracy (shown in *Table III*) were within the acceptable limits.

Table III. Intra-day and inter-day precision and accuracy of analysis of sumatriptan succinate

Concentration (ng mL^{-1})	Intra-day[a]			Inter-day[b]		
	Mean ± SD (ng mL^{-1})	RSD (%)	RE (%)	Mean ± SD (ng mL^{-1})	RSD (%)	RE (%)
75	74.14 ± 2.05	2.77	1.15	75.25 ± 2.22	2.95	0.34
300	297.96 ± 4.19	1.41	-0.68	301.56 ± 5.12	1.70	0.52
500	509.81 ± 7.84	1.54	1.96	512.35 ± 6.40	1.25	2.47

[a]Intra-day accuracy and precision were determined by five replicate analyses for each concentration

[b]Inter-day accuracy and precision were determined by fifteen replicate analyses (day 1, $n = 5$; day 2, $n = 5$; day 3, $n = 5$) for each concentration

Limits of Detection and Quantification

The LOD, defined as the amount for which the signal-to-noise ratio was 3:1, was 10 ng mL^{-1}. The LOQ was 25 ng mL^{-1}, with precision and accuracy of 4.81 and −1.28%, respectively.

Robustness

A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the detection wavelength by ±2 nm (225 nm and 229 nm), mobile phase buffer to acetonitrile ratio (66:34 and 64:36, *v/v*), mobile phase pH by ±0.2 units (pH 3.8 and 4.2), and mobile phase flow rate by 0.1 mL min^{-1} (0.9 and 1.1 mL min^{-1}) had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. The results are shown in *Table IV*.

Table IV. Results from testing the robustness of the method (n = 3, concentration = 300 ng mL^{-1})

Condition	Modification	Mean area ± SD	RSD (%)	Mean R_T ± SD (min)
Detector wavelength (nm)	225	131400 ± 1265.853	0.964	4.507 ± 0.017
	227	131627 ± 971.778	0.741	4.502 ± 0.012
	229	130573 ± 988.171	0.759	4.514 ± 0.019
Mobile phase pH	3.8	132571 ± 1021.340	0.852	4.494 ± 0.016
	4.0	131216 ± 888.316	0.673	4.500 ± 0.013
	4.2	129679 ± 934.938	0.737	4.523 ± 0.021
Mobile phase composition (*v/v*)	66:34	132092 ± 811.751	0.938	4.530 ± 0.023
	65:35	131830 ± 966.245	0.615	4.508 ± 0.018
	64:36	132679 ± 1106.512	1.150	4.497 ± 0.011
Mobile phase flow rate (mL min^{-1})	0.9	130123 ± 955.869	0.970	4.521 ± 0.016
	1.0	132385 ± 771.033	0.561	4.503 ± 0.028
	1.1	130757 ± 996.196	0.814	4.491 ± 0.014

Stability Studies

Experiments were performed with the low, medium, and high-concentration quality-control samples to evaluate the stability of SS under different conditions. Experiments were performed to determine stability at room temperature (RT; 25 ± 2°C) for 6 h, freeze–thaw stability (three cycles), post-preparative stability at RT (25 ± 2°C) for one day. Short-term stability was measured at RT exposed to light and in darkness, under refrigeration

Table V. Results from study of the stability of sumatriptan succinate.
Mean ± SD, $n = 3$

Stability conditions	Storage time (days)	Theoretical amount (ng mL^{-1})	Experimental amount (ng mL^{-1})	RE (%)[a]
Bench-top	6 h	75	72.26 ± 0.57	−3.66
		300	304.25 ± 7.46	1.42
		500	490.99 ± 4.44	−1.80
Freeze and thaw at −20°C (three cycles)	-	75	77.77 ± 0.69	3.70
		300	290.53 ± 2.86	−3.16
		500	514.30 ± 4.70	2.86
Post-preparative	1	75	77.90 ± 0.85	3.86
		300	294.36 ± 3.59	−1.88
		500	504.34 ± 3.32	0.87
Short-term stability				
RT Darkness	0[b]	75	74.51 ± 1.36	−0.66
		300	299.31 ± 0.39	−0.23
		500	517.00 ± 2.96	3.40
	7	75	72.88 ± 0.67	−2.82
		300	288.30 ± 2.65	−3.90
		500	494.39 ± 4.62	−1.12
	14	75	69.50 ± 1.17	−7.34
		300	276.27 ± 5.27	−7.91
		500	465.60 ± 3.30	−6.88
RT Light				
	7	75	71.52 ± 0.43	−4.64
		300	287.98 ± 1.92	−4.01
		500	483.85 ± 3.23	−3.23
	14	75	67.65 ± 2.22	−9.79
		300	267.41 ± 2.07	−10.86
		500	456.93 ± 2.88	−8.61
Darkness (4°C)				
	7	75	73.48 ± 1.42	−2.02
		300	292.58 ± 2.46	−2.47
		500	501.27 ± 5.31	0.25
	14	75	72.50 ± 0.76	−3.33
		300	286.36 ± 1.51	−4.55
		500	492.25 ± 2.83	−1.55
Darkness (−20°C)				
	7	75	74.96 ± 1.45	−0.05
		300	298.60 ± 1.18	−0.47
		500	512.51 ± 4.71	2.50
	14	75	73.42 ± 0.88	−2.11
		300	296.72 ± 1.94	−1.09
		500	509.43 ± 1.89	1.89

[a]Relative error (%)
[b]0 day results for short-term stability under all conditions

(4°C), and in the freezer (−20°C) in darkness for 14 days. Stability was assessed by comparing the chromatograms obtained from the solutions after storage with those obtained from the freshly prepared solutions. The analyte was found to be stable at RT for 6 h, through repeated freeze-thaw cycles, and post-preparation for one day. The analyte was found to be stable at room temperature in both light and darkness for seven days and under refrigeration and in the freezer for 14 days (darkness). The analyte was found to be unstable at RT in light and darkness for 14 days. The results from stability testing are shown in *Table V*.

Analysis of Pharmaceutical Preparations

Mean recovery of the drug from ODT, Imigran tablets and Suminat nasal spray were 99.94, 101.96, and 100.45% with precision of 0.93, 0.15, and 1.07%, respectively. The results obtained (*Table VI*) were in good agreement with the label claims. The recovery values also indicated non-interference from excipients present in pharmaceutical dosage forms. The results obtained from the validation studies proved the method is suitable for quantification of SS in pharmaceutical dosage forms.

Table VI. Results from analysis of sumatriptan succinate in pharmaceutical dosage forms

Product	Label claim (mg per dose)	Amount found (mg per dose)	Recovery (%)	RSD (%)
Orally disintegrating tablets	50	49.97	99.94	0.93
Imigran tablets	50	50.98	101.96	0.15
Suminat nasal spray	20	20.09	100.45	1.07

Conclusions

An isocratic RP-HPLC-UV method for analysis of SS in pharmaceutical dosage forms has been developed and validated in accordance with ICH guidelines. Validation of the method was satisfactory. The short analytical run time of 4.50 min leads to a cost-effective and rapid chromatographic procedure. Formulation excipients did not interfere with the method. The method was successfully used for quality-control analysis of SS in pharmaceutical dosage forms. The method is suitable for routine analysis of SS in pharmaceutical formulations.

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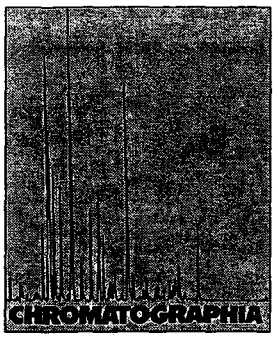
Development and Validation of an RP-LC-UV Method for the Determination of Ondansetron: Application to Pharmaceutical Dosage Forms



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Abstract

A new, simple, rapid, sensitive and specific isocratic RP-LC-UV method was developed and validated for the determination of ondansetron in pharmaceutical dosage forms of orally disintegrating tablets, oral solution and injection. The LC separation was achieved on a Hypersil C4 column (250 × 4.6 mm, 5 µm) using a mobile phase of 50 mM potassium dihydrogen phosphate anhydrous adjusted to pH 3.5 with orthophosphoric acid and acetonitrile (30:70, *v/v*) at a flow rate of 1.0 mL min^{-1} and UV detection at 310 nm. The method was validated for specificity, linearity, precision, accuracy, limit of quantification, limit of detection, robustness and solution stability. The calibration curve was linear over a concentration range of 100–1,000 ng mL^{-1} ($r^2 = 0.9996$) with limit of detection and limit of quantification 50 and 100 ng mL^{-1}, respectively. The intra-day and inter-day precision and accuracy were between 0.79 and 2.37% and −0.64 and 1.65%, respectively. The method was successfully applied for analysis of ondansetron in the presence of excipients in commercially available pharmaceutical dosage forms.

Keywords

Column liquid chromatography
Pharmaceutical dosage forms
Method validation
Ondansetron

Introduction

Chemotherapy-induced nausea and vomiting (CINV) has a severe impact on the quality of life of cancer patients. The generation of the 5-HT3 serotonin antagonists (ondansetron, granisetron, dolasetron) have represented an important progress in the management of CINV [1]. Ondansetron is a basic compound (pK_a 7.70) and chemically known as {1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4*H*-4-one} (Fig. 1). It is a white to off-white powder that is soluble at pH 1.2. The partition coefficient (log *P*) of the ondansetron base in *n*-octanol/water is 2.14. It is used in the treatment of emesis and nausea associated with cancer related chemotherapy and radiation. As an antiemetic, the usual dose is in the range of 8–32 mg per day, whereas preliminary results from clinical investigations in panic disorder support the use of doses as low as 2–4 mg per day [2]. Seynaeve et al. [3], Ruff et al. [4] and the Italian Group for Antiemetic Research (IGAR) [5] reported that an 8 mg dose was showing an equal efficacy to 32 mg dose, particularly in cisplatin induced acute emesis [6].

Orally disintegrating tablets/mouth dissolving tablets (ODT/MDT) are widely used new pharmaceutical dosage form, which disintegrate and dissolve in the saliva without water within 60 s or less. ODT preparations are formulated with UV-absorbing components specifically flavors (e.g. strawberry, mint and pineapple) and sweeteners (e.g. aspartame, acesulfame potassium and sodium saccharin). These excipients could decrease the signal of the drug to background (excipients) in the UV because the excipient to drug ratio is higher in the formulation of ODT. High ratio of excipient to drug in ODT formulation is necessary to impart good taste and

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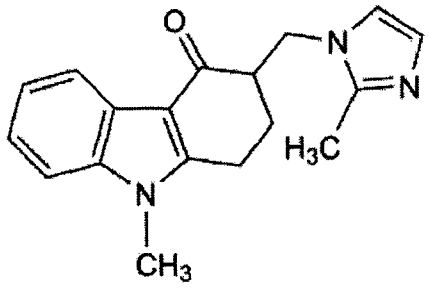

Fig. 1. Chemical structure of ondansetron

mouthfeel [7]. Hence, development of a suitable LC method is required for routine and in-process quality control analysis, dissolution or similar studies.

Literature survey reveals that some analytical methods have been reported for determination of ondansetron in biological fluids by LC [2, 8–12] and LC-MS [13–16]. Although, Bauer et al. [10] and Depot et al. [11] developed a sensitive LC–UV method for the determination of ondansetron in plasma the sensitivity was achieved by concentrating a large volume of samples (1–2 mL) into a lower volume (100 µL) and injecting the higher volume (75–100 µL). The drawbacks of the above methods were a higher flow rate (1.5 mL min^{-1}) and longer run time of analysis (15 min) which were not suitable for routine quality control analysis of pharmaceutical dosage forms.

Few methods were reported in the literature for the analysis of ondansetron alone or in combination with other drugs by LC in pharmaceutical dosage forms [17–24]. These methods suffer from disadvantages of low sensitivity, higher flow rate and late elution of the analyte. Raval et al. [25] reported a validated HPTLC method for simultaneous estimation of ondansetron combinations in solid dosage form with omeprazole and rabeprazole. To our present knowledge, no LC method has yet been developed and validated for the determination of ondansetron in the dosage form of ODT in the presence of their excipients.

The aim of the present work was to develop and validate a new, sensitive, specific and robust RP–LC–UV method for the quantitative determination of ondansetron in commercially available pharmaceutical dosage form of ODT (Zofer MD 8). In addition, the validated method was also applied for the determination of ondansetron in commercially available oral solution (Onset) and injection (Onditron 2).

Experimental

Materials

Ondansetron was purchased from Symed Labs (Hyderabad, India). Zofer MD 8 mouth dissolving tablets were purchased from Sun Pharmaceuticals (Vapi, India). Onset oral solution was purchased from Panther Health Care (Roorkee, India). Onditron 2 injection was purchased from Nitin Lifesciences (Sirmour, India). Potassium dihydrogen phosphate anhydrous (KH_2PO_4) was obtained from Wako Pure Chemical Industries (Chuo-Ku, Japan), orthophosphoric acid was purchased from Ajax Chemicals (Auburn, Australia), methanol and acetonitrile (LC grade) were purchased from J.T.Baker (Phillipsburg, USA).

Instrumentation

The LC system consisted of Waters 510 delivery pump (Waters, France) equipped with a 6-valve sample injection port (7725i Rheodyne, Cotati, California, USA) fitted with 20 µL sample loop, a UV/Vis is detector (875-UV, Jasco, Tokyo, Japan) and a Chromato-Integrator (D-2500, Hitachi, Tokyo, Japan).

Chromatographic Conditions

The chromatographic separation of the analyte was achieved at room temperature (25 ± 2 °C) using a Hypersil C4 (250 × 4.6 mm, 5 µm) analytical column. The mobile phase contained a mixture of 50 mM KH_2PO_4 adjusted to pH 3.5 with orthophosphoric acid and acetonitrile (30:70, *v/v*). The mobile phase was filtered through 0.45 µm nylon membrane filter (Whatman, UK) under vacuum and degassed prior to use. The analysis run at a flow rate of 1.0 mL min^{-1}. The detector was set at a wavelength of 310 nm. The injection volume was 20 µL.

Preparation of Standard and Quality Control Solutions

Primary standard stock solution of ondansetron was prepared in methanol with a concentration of 1.0 mg mL^{-1}. Working standard solution (10 µg mL^{-1}) was prepared by diluting stock solution with mobile phase and was used to prepare calibration and quality control samples. These solutions were stored under refrigeration at 4 °C prior to use.

Calibration samples were prepared by diluting working standard solution with mobile phase to give concentrations in the range of 100–1,000 ng mL^{-1}. The quality control samples were prepared at low, medium and high concentrations of 300, 500 and 900 ng mL^{-1} ondansetron.

Analysis of the Pharmaceutical Preparations

For Zofer MD 8 tablets assay, ten tablets were weighed, finely powdered and a portion of powder equivalent to 8 mg of drug was accurately weighed into each of five 50 mL volumetric flasks and 25 mL of methanol was added to each flask. The volumetric flasks were sonicated for 15 min to dissolve ondansetron completely and the solutions were then made up to the volume with methanol. The solution was filtered through a 0.45 µm PTFE syringe filter and 0.5 mL of the filtrate was diluted with mobile phase to yield a concentration of 800 ng mL^{-1} ondansetron. The samples were analyzed in five replicates.

For Onset oral solution and Onditron 2 injection assay, an aliquot of solution equivalent to 0.4 mg of ondansetron was transferred separately into each of five 50 mL volumetric flasks, made up to the volume with the mobile phase and mixed well. An aliquot of 1 mL of the solution was diluted with mobile phase to yield a concentration of 800 ng mL^{-1} ondansetron. The samples were analyzed in five replicates.

Results and Discussion

Method Development and Optimization

The development of the RP–LC–UV method for the determination of drugs has received considerable attention in recent years because of their importance in routine quality control analysis. In order to validate an efficient method for drug analysis in pharmaceutical formulations, preliminary tests were performed with the objective to select adequate and optimum conditions. Parameters, such as choice of analytical column, composition of the mobile phase, organic modifier, pH and molarity of buffer salt in addition to mobile phase flow rate were optimized in order to provide a good performance of assay for the determination of ondansetron in pharmaceutical dosage forms.

The selection of wavelength is a prerequisite for the determination of a drug without interference from the UV-absorbing excipients, specifically flavors and sweeteners present in the dosage form of ODT. An ultraviolet spectrophotometer scan in the range of 200–400 nm showed detection of ondansetron at the wavelengths of 212, 248 and 310 nm. It was observed that ondansetron had maximum sensitivity at the wavelength of 212 nm. The estimation of ondansetron from the pharmaceutical dosage forms was found to be complex due to the baseline drift at 212 nm and unresolved peaks caused by the excipients co-eluting with the analyte of interest at both wavelengths of 212 and 248 nm. Hence, detection wavelength 310 nm was selected for the quantification of ondansetron since there was no absorbance from the excipients.

Several reversed-phase analytical columns such as C18, C8, C4 and CN (in the order of increasing polarity of the stationary phase) were tested with the mobile phase composition of 20 mM KH_2PO_4 solution (pH 3.5) and acetonitrile (30:70, *v/v*) for the separation of ondansetron. Initial separation studies were performed with C18 (Phenomenex, 250 × 4.6 mm, 5 µm) column. Ondansetron is a basic non polar compound

Table 1. System suitability parameters

	Retention time (R_T)	Theoretical plates (N)	Tailing factor (T)	Asymmetry (A_S)
	4.42 ± 0.01	4,593 ± 104	1.16 ± 0.02	1.22 ± 0.02
% RSD	0.23	2.27	1.72	1.64
Required limits	RSD ≤ 1%	N > 2,000	$T \leq 2$	$A_s \leq 1.5$

Mean ± SD, $n = 5$

Table 2. Summary of the calibration curve results for ondansetron

Theoretical amount (ng mL^{-1})	Experimental amount (ng mL^{-1})	% RSD	% RE
100	102.63 ± 2.32	2.26	2.63
200	204.63 ± 5.66	2.76	2.31
400	392.98 ± 4.67	2.71	−1.75
600	588.41 ± 4.02	0.68	−1.93
800	807.13 ± 6.95	0.86	0.89
1,000	1,002.73 ± 8.12	0.81	0.27

Mean ± SD, $n = 5$

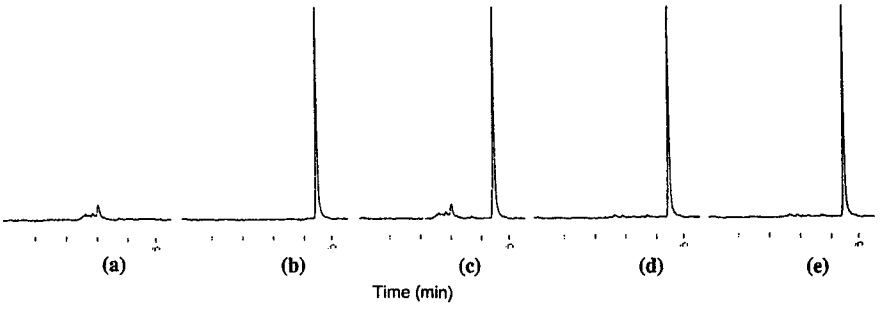

Fig. 2. Typical LC chromatograms of ondansetron. **a** Placebo sample, **b** pure drug (4.42 min), **c** ODT, **d** oral solution and **e** Injection

and insoluble in water. For organic non polar molecules the sample retention increases with the increase in length of the bonded phases, but the analyte was eluted at an earlier retention time from the C18 and C8 columns compared to CN and C4. Moreover, C18 and C8 analytical columns were not able to give a good resolution between analyte and excipients present in the pharmaceutical dosage forms. The analytical column, C4 (Thermo-Hypersil 250 × 4.6 mm, 5 µm) is more hydrophobic than CN and elutes the non polar compounds at longer retention time. Ondansetron was eluted at the longer retention with good chromatographic response, peak shape and also well resolved from the excipients and solvent front peaks due to the non polar nature of the analyte. Cyano (CN, Phenomenex 250 × 4.6 mm, 5 µm) chromatographic column is used for polar basic compounds in both reversed and normal phase modes. Ondansetron has earlier elution compared to the C4 column with good chromatographic response and optimum separation but peak shape was not optimal. Based on these findings, the analytical column C4 found to be the most appropriate for the determination of ondansetron.

In the preparation of the mobile phase, several combinations of buffer and organic modifier were tested using C4 as an analytical column at the ratios of 20:80, 30:70, 40:60, 50:50 and 60:40 (*v/v*). Variations in the mobile phase lead to considerable changes in the chromatographic parameters. A decrease in the content of the organic modifier resulted in an increase in the peak tailing and retention time of the

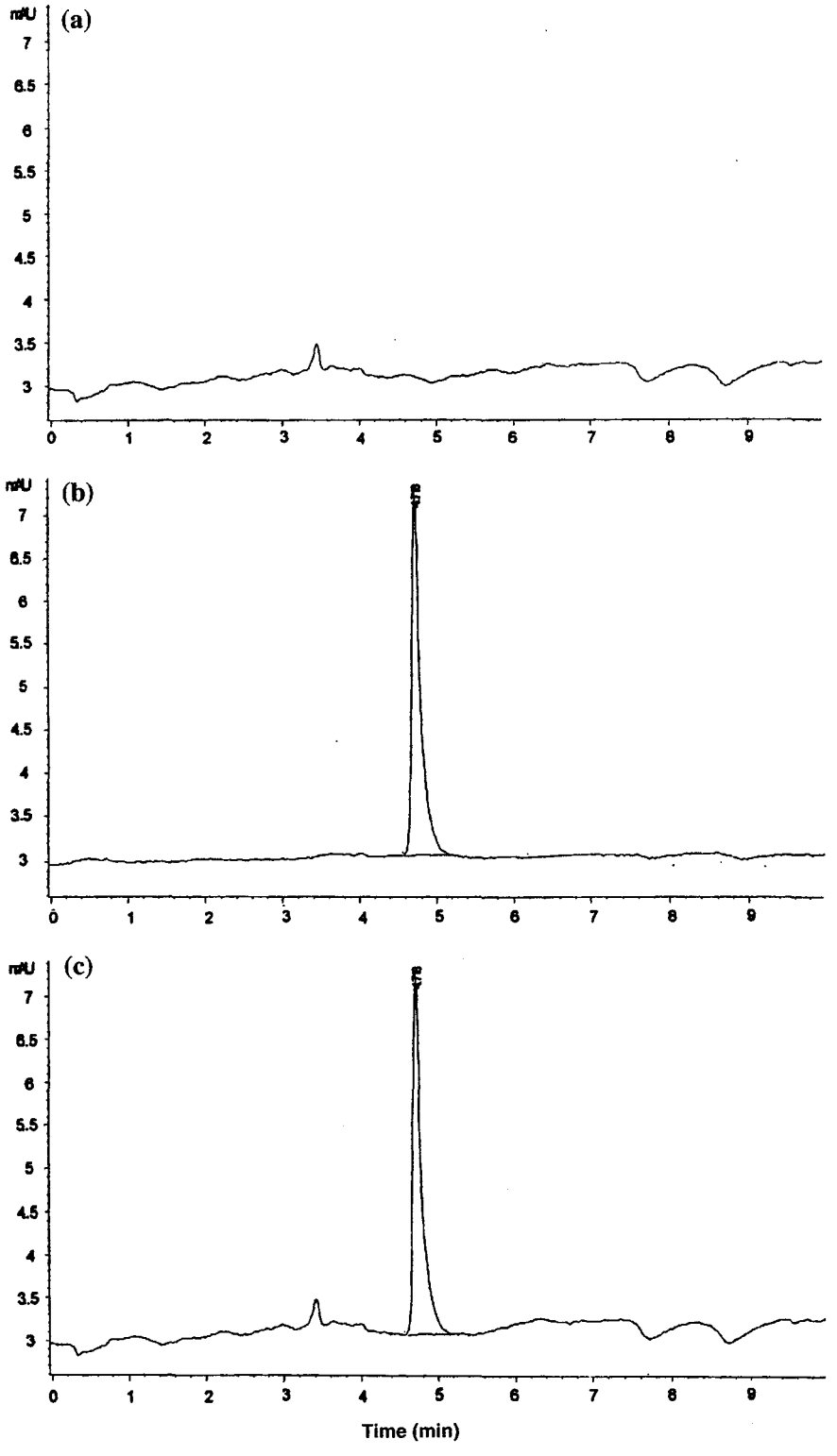

Fig. 3. Typical DAD chromatograms of ondansetron. **a** Placebo sample, **b** pure drug and **c** drug spiked in placebo sample

analyte. However, the ratio of mobile phase at 30:70 (*v*/*v*) provided the more symmetric peak shape with reasonable retention time.

When experiments were performed with methanol instead of acetonitrile as the organic modifier in the mobile phase, late elution of analyte with peak tailing and high column pressure were observed. Hence, the experiments were carried out with acetonitrile as an organic modifier.

The selection of buffer pH mainly depends on the pK_a of the analyte. For the basic compounds, pH needs to be selected approximately 2.5 pH units below the pK_a. The pK_a value for the ondansetron is 7.70. Hence, no considerable changes were observed in the retention time, peak symmetry and chromatographic response of analyte when pH of the mobile phase was varied in the range of 2.5–4.0. Peak tailing was observed at pH 5.0 and above. The pH value of 3.5 was considered to be optimal as it gave a good compromise between retention time and peak shape.

The buffer molarity was tested at 10, 25, 50 and 100 mM. It was found that the buffer molarity affected the retention time and peak symmetry of the analyte. At 10 and 25 mM, analyte was eluted late at 8.60 and 6.21 min, respectively and the peak shape was also not optimal. There was a slight difference in the retention time of analyte with 50 mM (4.42 min) and 100 mM (3.96 min), but the peak shape was found to be symmetrical. A buffer molarity 50 mM was optimal for the elution of analyte with good chromatographic response and symmetrical peak shape in a short run time of analysis.

After several trials, the mobile phase consisted of a mixture of 50 mM KH_2PO_4 (pH 3.5) and acetonitrile (30:70, *v*/*v*) was finally adopted at a flow rate of 1.0 mL min^{-1}. The described chromatographic conditions achieved satisfactory resolution and symmetrical peak shape for ondansetron with the retention time of 4.42 min. No interference from the sample solvent and dosage form excipients was observed at the retention time of ondansetron.

Method Validation

The newly developed LC method was validated to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2 (R1) [26]. The described method was

extensively validated in terms of system suitability, linearity, specificity, precision, accuracy, limit of detection, limit of quantification, robustness and solution stability.

System Suitability

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters, retention time (R_T), theoretical plates (N), tailing factor (T) and peak asymmetry (A_S) were evaluated using five replicate injections of the drug at a concentration of 500 ng mL^{-1}. The results are shown in Table 1 and were found to be within the acceptable limits [27].

Linearity

To evaluate the linearity of the method, five calibration curves in a concentration range of 100–1,000 ng mL^{-1} (100, 200, 400, 600, 800 and 1,000 ng mL^{-1}) were determined. The calibration curves were plotted for peak area of the analyte against the corresponding concentration using linear regression analysis. The mean linear regression equation was $y = 58.377(\pm 3.15)x + 366.18(\pm 48.08)$ with the correlation coefficient of 0.9996 (± 0.0002). The result shows that an excellent correlation existed between the peak area and concentration of the analyte. The result of linearity is presented in Table 2.

Specificity

The specificity of the proposed method was performed by the excipients present in the pharmaceutical dosage forms (placebo sample). The following excipients are present in the pharmaceutical dosage forms: Zofer MD 8 (aspartame, colloidal silicon dioxide, croscarmellose sodium, glycerol distearate, magnesium stearate, mannitol, talc and strawberry flavor), Onset oral solution (citric acid anhydrous, glycerin, sodium saccharin, sodium benzoate, sodium citrate, and strawberry flavor) and Onditron 2 injection (sodium chloride, citric acid anhydrous, sodium citrate dihydrate as buffers in water for injection). The placebo sample was analyzed in six replicates. No significant interfering peaks from the excipients were found at the retention time of ondansetron (4.42 min). It showed that the developed analytical method was specific for the analysis of ondansetron in pharmaceutical dosage forms. The chromatograms of placebo sample and pure drug, ODT, oral solution and injection at a concentration of 800 ng mL^{-1} are shown in Fig. 2.

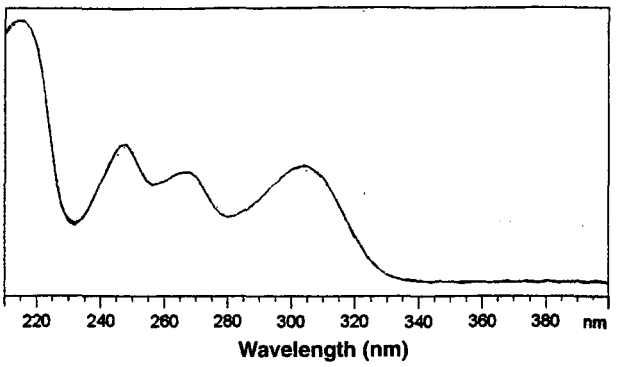

Fig. 4. Typical DAD peak spectrum of ondansetron spiked in placebo sample in the region, 200–400 nm

The specificity of the analyte in the presence of excipients was also evaluated by the diode array detector (DAD) which confirms the singularity of the peak component. Chromatography was performed with an Agilent 1100 LC (Madrid, Spain) coupled with an Agilent 1100 DAD detector. The pump, autosampler, and degasser were also series 1100 from Agilent. Data acquisition and analysis were performed using ChemStation workstation. The peak determined was considered pure when the purity factor value was above the threshold value across the entire peak as specified by the workstation. The analyte was found to be pure as the purity factor value (999.968) above the threshold value (999.353) and chromatogram also displayed a single peak in peak purity spectrum (in the UV region, 200–400 nm). It indicates that there was no interference from the sample solvent and dosage form excipients at the retention time of ondansetron. The typical DAD chromatograms of placebo sample, pure drug (800 ng mL^{-1}) and drug spiked in placebo sample are shown in Fig. 3. The typical DAD peak spectrum of ondansetron spiked in placebo sample in the region of 200–400 nm is shown in Fig. 4.

Intra-Day and Inter-Day Precision and Accuracy

Intra-day and inter-day precision and accuracy were evaluated by analyzing quality control samples at low, medium and high concentrations of 300, 500 and 900 ng mL^{-1}. For the intra-day variation, sets of five replicates were analyzed on the same day and for the inter-day validation, five replicates of three concentration levels were analyzed on three different days. The intra-day accuracy (% RE) ranged between −0.64 and 1.65% with a precision (% RSD) of 0.85–2.37%. The inter-day accuracy ranged between −0.17 and 0.85% with a precision of 0.79–2.08%. All the results for precision and accuracy were within the acceptable limits. The results are shown in Table 3.

Limit of Detection and Limit of Quantification

The limit of detection was found to be 50 ng mL^{-1} at a signal to noise ratio of 3:1. The limit of quantification was found to be 100 ng mL^{-1} with a precision and accuracy of 2.26 and 2.63%, respectively.

Robustness

The robustness of a method is the ability to remain unaffected by small changes in operating conditions. To determine robustness of the method, experimental conditions were deliberately altered and evaluated for retention time and

Table 3. Experimental values of mean concentration, % RSD and % RE presented for validation parameters of ondansetron

Study	Theoretical amount (ng mL^{-1})	Experimental amount (ng mL^{-1})	% RSD	% RE
Intra-day[a]	300	302.48 ± 7.17	2.37	0.83
	500	496.81 ± 6.08	1.22	−0.64
	900	914.89 ± 7.80	0.85	1.65
Inter-day[b]	300	299.49 ± 5.48	1.83	−0.17
	500	504.25 ± 10.51	2.08	0.85
	900	905.79 ± 7.14	0.79	0.64
Bench top[c]	300	298.15 ± 5.77	1.93	−0.62
	500	505.04 ± 5.66	1.12	1.01
	900	905.38 ± 7.09	0.78	0.60
Freeze and thaw[d]	300	292.36 ± 4.83	1.65	−2.55
	500	507.98 ± 6.03	1.19	1.60
	900	901.16 ± 6.91	0.77	0.13
Post preparative[e]	300	297.29 ± 2.14	0.72	−0.90
	500	501.50 ± 6.00	1.20	0.30
	900	897.95 ± 9.59	1.07	−0.23
Short-term[f]	300	303.14 ± 5.16	1.70	1.05
	500	505.99 ± 6.42	1.27	1.20
	900	906.13 ± 5.29	0.58	0.68

[a] Intra-day accuracy and precision was determined with 5 replicates for each concentration
[b] Inter-day accuracy and precision was determined with 15 replicates (day 1, $n = 5$; day 2, $n = 5$; day 3, $n = 5$) for each concentration
[c] After 6 h at room temperature (25 ± 2 °C), $n = 3$
[d] After 3 freeze and thaw cycles at −20 °C, $n = 3$
[e] After 24 h at room temperature (25 ± 2 °C), $n = 3$
[f] 14 days at 4°C, $n = 3$

Table 4. Results from testing the robustness of the method ($n = 3$, concentration = 500 ng mL^{-1})

Condition	Modification	Mean area ± SD	% RSD	Mean R_T (min) ± SD
Detector wavelength (nm)	308	30,561 ± 273.48	0.895	4.421 ± 0.014
	310	31,198 ± 151.77	0.486	4.430 ± 0.018
	312	30,811 ± 295.14	0.958	4.428 ± 0.021
Mobile phase pH	3.3	31,571 ± 193.34	0.612	4.422 ± 0.016
	3.5	31,316 ± 218.16	0.696	4.421 ± 0.010
	3.7	30,889 ± 203.48	0.659	4.428 ± 0.024
Mobile phase composition (*v*/*v*)	31:69	31,936 ± 311.51	0.975	4.430 ± 0.017
	30:70	31,639 ± 175.66	0.555	4.426 ± 0.011
	29:71	30,892 ± 186.15	0.602	4.419 ± 0.023
Mobile phase flow rate (mL min^{-1})	0.9	31,645 ± 255.88	0.808	4.428 ± 0.015
	1.0	31,336 ± 191.59	0.611	4.421 ± 0.012
	1.1	31,803 ± 236.14	0.742	4.418 ± 0.020

Table 5. Results from analysis of ondansetron in pharmaceutical dosage forms, $n = 5$

Product	Label claim (mg per dose)	Amount found (mg per dose)	Recovery (%)	% RSD	% RE
Zofer MD 8 ODT	8	7.97	99.66	0.31	−0.38
Onset oral solution	4	4.07	101.83	0.69	1.75
Onditron 2 injection	4	4.06	101.58	0.52	1.50

chromatographic response. Variation of the detector wavelength by ±2 nm (308 and 312 nm), pH of the mobile phase by ±0.2 units (3.3 and 3.7 buffer pH), composition of mobile phase at buffer and acetonitrile ratio (31:69, *v*/*v*) and (29:71, *v*/*v*) and flow rate of mobile phase by 0.1 units (0.9 and 1.1 mL min^{-1}) had no significant effect on the retention time and chromatographic response of the method, indicating that the developed method was robust. The results are shown in Table 4.

Solution Stability

Stability experiments were performed with low, medium and high quality control samples to evaluate the ondansetron solution stability under different conditions. Experiments were performed to determine stability of bench top (6 h) and post preparative (24 h) samples at room temperature (25 ± 2 °C), freeze thaw stability (three cycles) and short-term stability in refrigerator (4 °C) for 14 days. The drug was found to be stable in all the above mentioned conditions. The solution stability results are shown in Table 3.

Analysis of the Pharmaceutical Preparations

The mean recoveries of drug at a concentration of 800 ng mL^{-1} from Zofer MD 8 ODT, Onset oral solution and Onditron 2 injection were 99.66, 101.83 and 101.58% with a precision of 0.31, 0.69 and 0.52%, respectively. The results are shown in Table 5. The percent recovery values obtained also indicate non-interference from the excipients present in dosage forms of ODT, oral solution and injection. The results obtained from the validation studies proved that the developed method is suitable for quantification of ondansetron in pharmaceutical dosage forms.

Conclusions

A new, isocratic RP–LC–UV method developed for the determination of ondansetron in pharmaceutical dosage forms was found to be simple, specific, sensitive and robust. The validated method showed satisfactory data for all the validation parameters tested. The short retention time of 4.42 min allows the analysis of a large number of samples in a short period of time and is therefore more cost effective. Furthermore, the

developed method showed no interference from the formulation excipients and was successfully applied for the quality control of ondansetron in commercially available pharmaceutical dosage forms. The proposed method is suitable for routine estimation of ondansetron in pharmaceutical formulations.

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