INVESTIGATION OF *IN VITRO* PERMEATION OF MITRAGYNINE THROUGH THE SKIN

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INVESTIGATION OF *IN VITRO* PERMEATION OF MITRAGYNINE THROUGH THE SKIN

by

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
-AA	0.01%w/v of ascorbic acid is added to the permeation medium
ACN	Acetonitrile
ANOVA	Analysis of variance
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
BHT	Butylated hydroxytoluene
CPE	Chemical penetration enhancer
DMSO	Dimethyl sulphoxide
DSC	Differential scanning calorimetry
DTT	Dithiothreitol
FA	Formic acid
FDA	Food and Drug Administration
GRAS	Generally recognised as safe
HF	Hair follicles
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
ICH	International Council for Harmonisation
ID	Inner diameter
LOD	Limit of detection
LOQ	Limit of quantification
MTG	Mitragynine
NTP	Number of theoretical plates
OECD	Organisation of Economic Cooperation and Development
PBS	Phosphate buffer saline
PG	Propylene glycol

rpm	Rotations per minute
RS	Remaining skin
RSD	Relative standard deviation
SC	Stratum corneum
SD	Standard deviation
US	United States
UV	Ultraviolet
XRD	X-ray diffraction

PENYIASATAN PERESAPAN MITRAGINA MELALUI KULIT SECARA *IN VITRO*

ABSTRAK

Mitragina adalah alkaloid yang diasingkan daripada daun tumbuhan *Mitragyna speciosa* dan telah diambil secara tradisional dalam bentuk ramuan teh. Kajian-kajian terbaru mengemukakan teori bahawa mitragina merupakan calon yang berpotensi untuk melegakan kesakitan dan merawat gejala sarak opiat sebagai terapi gantian. Oleh sebab biokeperolehan oral mitragina yang rendah, penghantaran mitragina melalui kulit telah dicadangkan dalam kajian ini. Walau bagaimanapun, penyiasatan resapan mitragina *in vitro* melalui kulit adalah terhad. Kajian ini bertujuan untuk menyiasat potensi penyampaian mitragina melalui kulit menggunakan pelbagai pelarut dengan kepentingan untuk memperbaiki, mengoptimumkan dan mengesahkan kaedah untuk menilai resapan mitragina melalui kulit. Ujian pencirian mitragina menggunakan kalorimetri pengimbasan perbezaan, jumlah pemantulan terlemah spektroskopi inframerah transformasi Fourier dan belauan sinar-X. Mitragina yang digunakan dalam eksperimen ini mungkin bersifat amorfus. Kaedah analisis kromatografi cecair berprestasi tinggi yang dipasangkan dengan pengesan ultraungu telah dioptimumkan dan disahkan mengikut garis panduan *International Council of Harmonisation*. Kaedah analisis ini didapati jitu, tepat dan teguh dan berjaya menghasilkan puncak mitragina yang tajam pada ~4 min dengan faktor pengekoran yang memuaskan iaitu 1.32. Kaedah eksperimen untuk kajian resapan *in vitro* menggunakan sel resapan Franz telah dioptimumkan dan asid askorbik digunakan sebagai antioksidan dalam kompartmen reseptor. Kajian resapan *in vitro* telah dijalankan menggunakan mitragina yang dilarutkan dalam pelbagai pelarut yang dipanggil sebagai sistem pelarut mudah. Pelarut yang digunakan dalam kajian ini didapati mempunyai keupayaan untuk melarutkan dan membantu penyerapan mitragina melalui kulit. Keputusan kajian resapan *in vitro* menunjukkan bahawa

Transcutol® dan dimetil sulfoksida (DMSO) mempunyai potensi sebagai peningkat penembusan kulit untuk membantu penghantaran mitragina melalui kulit, diikuti oleh Maisine® dan propelina glikol (PG). Kajian kestabilan mendedahkan bahawa PG dan DMSO menunjukkan peratus pemulihan mitragina tertinggi (68 – 74%) sepanjang 96 jam manakala Labrasol® dan Maisine® menunjukkan pemulihan paling rendah (5 – 20%). Penambahan 0.01%w/v butil hidroksitoluena (BHT) dapat mengurangkan degradasi mitragina dalam pelarut dengan signifikan selepas 96 jam. Peningkatan kepekatan BHT (0.05%w/v) didapati lebih berkesan untuk mengurangkan peratus degradasi mitragina dalam sistem pelarut mudah. Berdasarkan kajian resapan dan kestabilan *in vitro*, Transcutol®, DMSO dan PG telah dipilih sebagai pelarut yang berpotensi dengan kebolehan meningkatkan penembusan kulit dan menunjukkan peratus pemulihan mitragina tertinggi. Formulasi gel menggunakan pelarut-pelarut ini telah berjaya digunakan sebagai formulasi model untuk menilai potensi formulasi untuk penghantaran mitragina melalui kulit dengan hidroksipropil metilselulosa sebagai asas gel. Kajian seterusnya melaporkan bahawa semua formulasi gel berjaya melepaskan mitragina (> 50%), membenarkan ~ 4 – 5 μg/cm^2 mitragina meresap melalui kulit sepanjang 48 jam dan menunjukkan pemulihan mitragina sebanyak > 80%. Kesimpulannya, mitragina telah berjaya dihantar melalui kulit dengan bantuan pelarut dengan kebolehan meningkatkan penembusan kulit terutamanya PG dan Transcutol®.

INVESTIGATION OF *IN VITRO* PERMEATION OF MITRAGYNINE THROUGH THE SKIN

ABSTRACT

Mitragynine is an alkaloid isolated from the leaves of the *Mitragyna speciosa* plant and has been traditionally consumed in the form of tea concoctions. Recent studies theorised mitragynine to be a potential candidate for pain relief and the treatment of opiate withdrawal symptoms as a replacement therapy. Due to mitragynine's poor oral bioavailability, the delivery of mitragynine through the skin is proposed in this study. However, the investigation of *in vitro* permeation of mitragynine through the skin is deemed to be lacking. This study aims to investigate the potential of delivering mitragynine using various solvents through the skin, with emphasis to improve, optimise and validate methods to evaluate the permeation of mitragynine across the skin. Mitragynine was characterised using differential scanning calorimetry, attenuated total reflectance-Fourier transform infrared spectroscopy and x-ray diffraction. Mitragynine utilised in this experiment was found to be likely amorphous. A high-performance liquid chromatography analytical method with an ultraviolet detector was optimised and validated according to the International Council of Harmonisation guidelines. The analytical method was found to be precise, accurate and robust which successfully eluted a sharp mitragynine peak at ~4 min with an acceptable tailing factor of 1.32. The experimental method for the *in vitro* permeation studies using Franz diffusion cells was optimised and ascorbic acid was used as an antioxidant in the receptor compartment. *In vitro* permeation studies were conducted using dissolved mitragynine in simple solvent systems of various solvents. The solvents employed in this study were found to have the ability to solubilise and assist the permeation of mitragynine through the skin. From the results obtained in the *in vitro* permeation studies, Transcutol® and dimethyl sulphoxide (DMSO) showed

promise as a skin penetration enhancer to aid the delivery of mitragynine through the skin, followed by Maisine® and propylene glycol (PG). The stability studies revealed that PG and DMSO showed the highest percentage recovery of mitragynine (68 – 74%) over 96 h while Labrasol® and Maisine® showed the least (5 – 20%). The addition of 0.01%w/v of butylated hydroxytoluene (BHT) was able to significantly minimise the degradation of mitragynine in solvents after 96 h. An increase in concentration of BHT (0.05%w/v) was found to be more effective to minimise the percentage of mitragynine loss in simple solvent systems. Based on the *in vitro* permeation and stability studies, Transcutol®, DMSO and PG were shortlisted as promising solvents with penetration enhancing abilities that displayed the highest percentage recovery of mitragynine. Gel formulation using these solvents was successfully employed as a model formulation to evaluate the feasibility of a formulation for transdermal mitragynine delivery with hydroxypropyl methylcellulose as a matrix for the gel base. Subsequent studies of gels reported that all gel formulations successfully released mitragynine (> 50%), allowed ~ 4 – 5 μg/cm^2 of mitragynine permeated over 48 h and demonstrated a total of > 80% of mitragynine recovered. In conclusion, mitragynine was successfully delivered through the skin with the aid of solvents with penetration enhancing abilities especially PG and Transcutol®.

CHAPTER 1 INTRODUCTION

1.1 Skin structure and function

The skin is the largest organ in the human body, which represents roughly 10% of a human adult's total body mass and covers a total surface area of roughly 1.7 m^2 (Walters and Roberts, 2002; Benson, 2012). The skin primarily acts as a barrier that functions as a protective shield between the human body and the external environment (Gorzelanny et al., 2020). This physiological barrier mainly comprises three layers, namely the epidermis, dermis and subcutaneous layer and also consists of several appendages such as hair follicles, eccrine and apocrine glands.

The epidermis is a multi-layered outermost region of the skin with thickness varying from 0.06 – 0.8 mm depending on the location of the skin (Benson, 2012). The epidermis can be further divided into five main layers in terms of the stages of cell differentiation and morphology. Starting from the bottommost layer – the stratum basale, followed by the stratum spinosum, stratum granulosum, stratum lucidum and the outermost layer – stratum corneum (SC). Aside from the SC, the other four layers make up the viable epidermis. During the process of differentiation, the cells of the viable epidermis are continuously flattened and enucleated while moving upwards to replace the routine shedding of the SC (Bouwstra et al., 2021).

The SC, also labelled as the horny layer, is the outermost layer of the skin. This layer has a thickness of roughly 10 – 15 layers of cells in depth (10 – 20 μm thick) and is the primary barrier of the skin (Walters and Roberts, 2002; Benson, 2012). The 'bricks and mortar' analogy has been commonly adopted to describe the structural composition of the SC with 'bricks' referring to the cells or corneocytes in a 'mortar' of intercellular lipoidal matrix as shown in Figure 1 (Michaels et al., 1975).

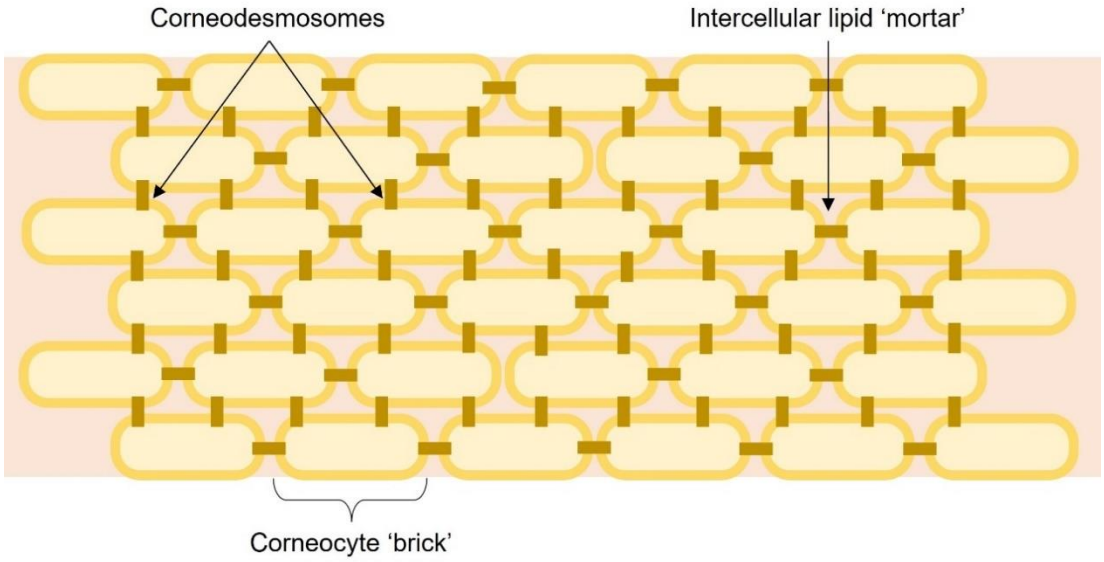


Figure 1 'Brick and mortar' model of the SC

Corneocytes are composed of 70 – 80% keratin and ~ 20% lipids with ~40 – 50 µm in length and 0.5 – 1.5 µm in width. They are riveted to each other by specialised protein structures called corneodesmosomes (Walters and Roberts, 2002; Harding, 2004; Benson, 2012). The 'mortar' of the SC contains no phospholipids but mainly comprises a special mixture of ceramides (~ 50%), fatty acids (~ 26%) and cholesterol (~ 20%) (Yardley and Summerly, 1981). This special arrangement of corneocytes within the intercellular lipids contributes to the integrity of the barrier function of the SC (Benson, 2012; Gorzelanny et al., 2020; Bouwstra et al., 2021).

The dermis is the layer that lays beneath the epidermis ranging about 1 – 5 mm in thickness (Walters and Roberts, 2002). This layer is mainly composed of connective tissue made up of collagen and elastin fibres in a semi-gel matrix of mucopolysaccharides and is responsible for the mechanical support, elasticity and flexibility of the skin (Walters and Roberts, 2002). In this layer, the fundamental cells are the fibroblasts, mast cells and melanocytes. The fibroblasts are responsible for the proliferation of the connective tissue components including collagen, laminin,

fibronectin and vitronectin (Walters and Roberts, 2002; Bouwstra et al., 2021). Mast cells are involved in the immune and inflammatory response while melanocytes produce the melanin pigments which are responsible for the skin's pigmentation and colour (Walters and Roberts, 2002; Benson, 2012). Additionally, the dermis layer also contains an extensive network of blood vessels and appendages such as hair follicles, eccrine and apocrine glands (Benson, 2012; Gorzelanny et al., 2020). This extensive vascular network is engaged in the thermoregulation and removal of toxins and waste products. Besides, the vascular network serves to provide oxygen and nutrients to the skin for repair and immune response. For transdermal drug delivery, drugs applied on the surface of the skin have to reach the vascular network in this layer to be transported systemically.

The subcutaneous layer, also known as the hypodermis, lies beneath the dermis and is made up of a layer of adipose cells. This layer which helps to anchor the skin to the underlying muscle is joined to the dermis by interconnecting collagen and elastin fibres. The integral function of this layer is to provide heat insulation and cushions against physical shock, as well as acting as an energy storage (Walters and Roberts, 2002; Benson, 2012).

1.2 Drug delivery to the skin

In recent years, aside from the generic routes of drug delivery such as oral and parenteral routes, drug delivery through the skin is also an emerging contender as one of the most attractive routes for drug delivery. This in turn, has drawn the interest of formulation scientists throughout the world to investigate and evaluate drug administration of various compounds to the skin.

Drug administration to the skin usually results in three different end goals – epidermal, topical or transdermal absorption (Trommer and Neubert, 2006; Lane et al., 2012). Epidermal delivery of drugs allows the active compound to remain on the surface of the skin or the epidermis. The topical route allows the drug to be delivered to the deeper layers of the skin to exert a local action without being absorbed systemically while transdermal delivery allows the drug to be transported through the blood circulation and exerts a systemic effect.

There are usually three main routes of drug penetration through the skin (Figure 2): across the epidermis (transepidermal), through the eccrine glands or the hair follicles (transappendageal) (Wiechers, 1989; Barry, 1991; Roberts, 1997).

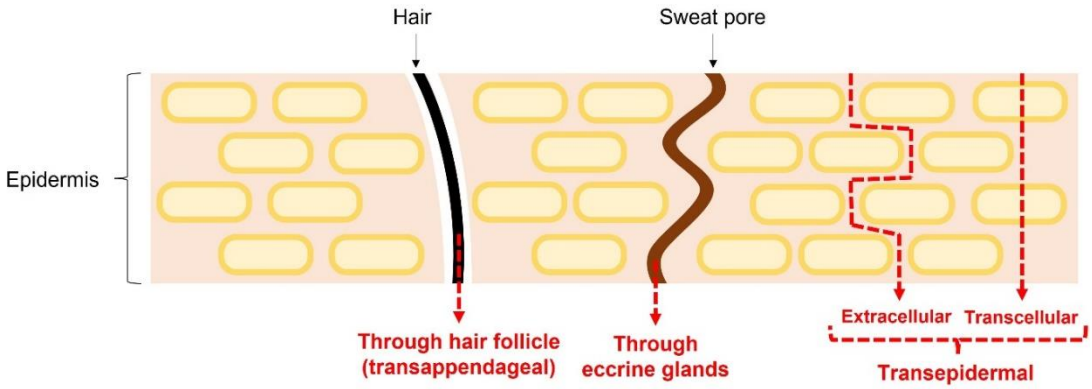


Figure 2 Routes of drug penetration through the skin

The transepidermal route can be further divided into two routes, namely the transcellular and extracellular routes (Ng and Lau, 2015). In the transcellular route, drugs transverse the corneocytes and the intercellular lipids. This route typically permits the delivery of mostly lipophilic and some amphiphilic compounds (Benson, 2012). On the other hand, for the extracellular route, the drug travels between the

corneocytes in the lipoidal matrix. Most hydrophilic compounds are transported through the skin via this route.

The transappendageal route involves the transport of compounds across the eccrine glands through the hair follicles. Although the appendages only constitute a total percentage of 0.1 – 1% of the overall skin structure, a modest amount of highly lipophilic compounds can be delivered through the skin via the transappendageal route (Benson, 2012).

1.3 Principles of drug delivery through the skin

Drug delivery through the skin is typically described using Fick's diffusion laws. In order to evaluate drug permeation through the skin, two dosing methods are generally used: infinite and finite dosing. Although finite dosing is closer to mimic real-life dosing situations, infinite dosing allows to investigate the permeation profile and the influence of the dose. In the current research, infinite dose is employed where the evaluation of steady-state flux can be conducted using Fick's diffusion laws (Lau and Ng, 2017).

Fick's first diffusion law is frequently employed to describe the rate of diffusion of compounds (flux) between two compartments across a membrane at a given time. Fick's first law of diffusion is equated as follows:

$$Flux = -D\left(\frac{\partial C}{\partial x}\right) \qquad \textbf{Equation 1}$$

where

D = diffusion coefficient of the drug

C = concentration of the drug

x = drug location in the system

$\frac{\partial C}{\partial x}$ = concentration gradient

$-$ (negative sign) = diffusion occurring in the opposite direction of increasing concentration

Typically, after a significant period of time, the flux profile becomes constant in which a steady-state concentration gradient of the delivered compound can form across the membrane (Lau and Ng, 2017). This observation is consistent with the assumptions made in the Fickian diffusion model, where it is assumed that steady-state conditions have been attained by the system (Hadgraft and Guy, 2003). This condition is known as an isotropic environment and the foremost mentioned Fick's first law of diffusion (Equation 1) is most commonly applied to describe the behaviour of the system (Crank, 1975; Hadgraft and Guy, 2003).

Experimentally, the steady-state flux can be determined with *in vitro* permeation experiments using infinite dose with 'ideal' sink conditions (Lau and Ng, 2017). By plotting a graph of cumulative amount of drug permeated against time, J_{ss} can be estimated from the linear portion of the graph. J_{ss} can be expressed as:

$$J_{ss} = \frac{DC_o}{h} \qquad \textbf{Equation 2}$$

where

J_{ss} = steady-state flux

D = diffusion coefficient of the drug

C_o = concentration of drug in the outermost first layer of the skin

h = diffusion path length of the SC (usually the thickness of SC)

Realistically, the measurement of C_o is exceedingly challenging in common practise. However, the drug concentration in the donor vehicle, C_v is typically known. Since C_o and C_v are connected to the partition coefficient between the donor and the membrane (K_m), C_o can be futher expressed as:

$$C_o = K_m C_v \qquad \textbf{Equation 3}$$

By substituting Equation 3 into Equation 2, this gives rise to Equation 4, which is the most widely applied mathematical model in examining skin permeation data using the infinite permeation method (Hadgraft and Guy, 2003; Lau and Ng, 2017):

$$J_{ss} = \frac{DK_m C_v}{h} \qquad \textbf{Equation 4}$$

1.3.1 Transdermal drug delivery

For transdermal drug delivery, a drug has to travel through the skin via a series of steps: drug release from the formulation, partition and diffusion before reaching the vascular network of blood vessels to be transported throughout the body (Lane et al., 2012).

Transdermal drug delivery offers several advantages over the conventional routes of drug delivery such as oral and parenteral routes (Guy et al., 1987; Guy, 2010; Benson, 2012). One of the main advantages of transdermal drug delivery over oral delivery is the evasion of gastrointestinal and hepatic first-pass metabolism which permits the administration of a lower daily dose and a reduced dosing frequency. Inter- and intra-

patient variability can be reduced through the transdermal delivery of drugs. This advantage allows the possibility of decreased undesirable side effects and in turn, provides a chance of improved patient compliance (Guy et al., 1987; Guy, 2010). Transdermal drug delivery also offers the luxury to provide a steadier controlled and prolonged drug release which is non-invasive (Benson, 2012). Besides, as the drug is administered externally, this delivery system allows the user to have control over the drug input, which is facilitated by the easy removal of the dosage form (Guy et al., 1987). In this study, transdermal delivery allows a long dosing interval which reduces the dosing frequency for MTG as a pain reliever. Transdermal formulations such as patches with certain specifications such as a rate-controlling membrane allow the control of the rate of drug being released which allows an extended period of pain relief (Pastore et al., 2015). Also, this route has been widely studied and utilised to deliver drugs across the skin barrier into the systemic circulation such as estradiol, fentanyl, lidocaine, nicotine, nitroglycerin, scopolamine and testosterone (Graybiel et al., 1976; Graybiel, 1979; Graybiel et al., 1981; Prausnitz and Langer, 2008).

For a drug to be considered as a potential candidate for transdermal drug delivery, scientists have deduced the prerequisites of several physicochemical properties this drug should fulfil. The multiple hydrophilic (polar heads of the lipids) and lipophilic (hydrocarbon chains of the lipids) layers of the SC pose as a limiting factor to most drugs for permeation before reaching the vascular network. Highly hydrophilic drugs are deemed unlikely to partition into the lipoidal layers of the SC, au contraire to the highly lipophilic drugs which are most likely to remain in these lipoidal layers without partitioning into the deeper layers of the skin (Guy et al., 1987; Lane et al., 2012). This can be indicated by the drug's log *P* value which is calculated by the ratio of the drug concentration between organic (octanol) and aqueous phases at equilibrium. Having a log *P* value of less than 3 is preferred for transdermal drug delivery (Lipinski et al., 1997; Korting and Schäfer-Korting, 2010; Pastore et al., 2015).

Aside from having an appropriate hydro/lipophilic balance, the transdermal route is a suitable route for potent drugs (Guy, 2010). While MTG is calculated to have only one-fourth the potency of morphine, MTG is reported to have milder withdrawal symptoms and is also less addictive (Halpenny, 2017).

Additionally, drugs intended for transdermal delivery should have a molecular weight of less than 500 Dalton (Lipinski et al., 1997; Guy, 2010; Lane et al., 2012; Pastore et al., 2015). Considering that the penetration of drugs through the skin transpires through a passive diffusion mechanism, small molecules can penetrate through the skin faster than larger molecules (Scheuplein et al., 1969; Lane et al., 2012).

Drug solubility is also an integral factor influencing drug permeation into the skin. Lane et al. (2012) mentioned that the melting point of drugs influences the drug's solubility in the intercellular lipids of the SC. Pastore et al. (2015) recommended a melting point of 250 °C or less for drugs to be considered suitable for transdermal delivery.

1.3.2 Mitragynine as a transdermal drug candidate

Mitragynine (MTG) is an indole alkaloid extracted from the ketum or *Mitragyna speciosa* Korth., a plant which is native to parts of Southeast Asia (E. Adkins et al., 2011; Idayu et al., 2011; Hassan et al., 2013) (Figure 3).

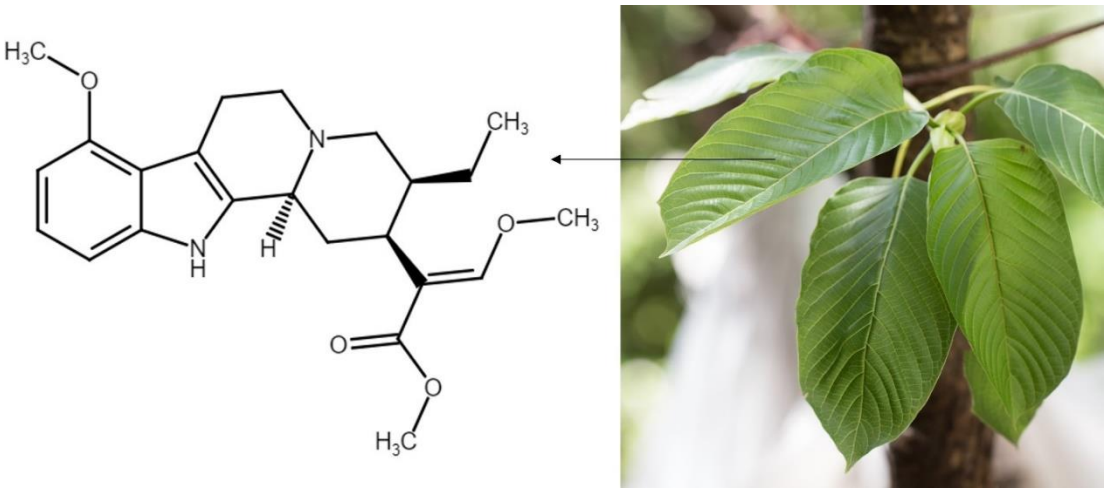


Figure 3 **Molecular structure of MTG obtained from the leaves of *Mitragyna speciosa* [obtained from NCCIH (2018)]**

Historically, the ketum plant is known for its ability to combat fatigue and pain in addition to increasing tolerance, endurance and work performance of the consumers (Reanmongkol et al., 2007). The reported pharmacological effects of MTG include muscle relaxation, anti-diarrheal, anti-pyretic, anti-diabetic and analgesic effects (Veltri and Grundmann, 2019; Ramachandram et al., 2020). These opiate-like actions of MTG were identified due to MTG's affinity to bind to μ, κ and δ-opioid receptors in the nervous system (Matsumoto et al., 1996a; Matsumoto et al., 1996b; Thongpradichote et al., 1998; Kumarnsit et al., 2007; Kruegel and Grundmann, 2018). In recent years, MTG is identified as a possible nominee in the treatment of opiate withdrawal symptoms as a replacement therapy (Parthasarathy et al., 2010).

MTG is most usually consumed as a traditional herbal medication by chewing the *Mitragyna speciosa* leaves or brewing the leaves into beverages such as tea (E. Adkins et al., 2011; Nelson et al., 2014; Veltri and Grundmann, 2019; Ramachandram et al., 2020). However, this conventional method of consuming MTG through the oral route brings several disadvantages such as low oral bioavailability due to hepatic metabolism of the drug, MTG's low aqueous solubility and acid lability (Ramachandram et al., 2020). The hepatic metabolism of the drug causes a lower

amount of drug to enter the bloodstream despite a high oral dose given. The human pharmacokinetic study of MTG by Trakulsrichai et al. (2015) showed a low oral bioavailability with the calculated pharmacokinetic parameters C_{max} (0.0185 – 0.105 µg/mL), T_{max} (0.83 ± 0.35 h) and AUC_{0-24h} (0.062 – 0.67 µg h/mL) after consuming ketum tea (MTG consumed: 9.96 – 23 mg) for 7 days. Similarly, pharmacokinetic studies of MTG in rats also reported low oral bioavailability of MTG ranging from 3 – 26% (Janchawee et al., 2007; de Moraes et al., 2009; Parthasarathy et al., 2010). This low bioavailability of MTG might cause an insufficient amount of drug in the blood to reach the desired therapeutic level of the drug.

Comparing MTG to the desired characteristics of a potential drug candidate for transdermal drug delivery, MTG fulfils most of these physicochemical requirements with a molecular weight of 398.5 Dalton, a log *P* value of 1.73 and a melting point of 104°C (Ramanathan et al., 2015). Therefore, transdermal delivery of MTG is proposed here as a promising administration route.

1.4 Investigation of skin permeation using simple formulations

Although the transdermal delivery of drugs offers several attractive advantages, these advantages are counterbalanced by a number of downsides due to the unique composition of the skin barrier (Guy et al., 1987). Due to the excellent barrier properties of the skin, transdermal delivery of drugs faces a fair share of difficulties that limit the penetration of drugs across the skin barrier (Stoughton, 1972). Various strategies have been proposed, explored and developed to expedite drug penetration through the skin, namely passive and active penetration enhancement strategies. Passive penetration enhancement strategies include increasing the thermodynamic activity of the drug (i.e. supersaturated drug systems) or using chemical penetration enhancers (CPE) in aiding the drug to overcome the skin barrier (Lane et al., 2012).

On the other hand, active penetration enhancement strategies involve using an external force/energy to induce physical changes to weaken the SC barrier (i.e. iontophoresis, microneedles) (Vitorino et al., 2015).

For the preliminary investigation of the potential penetration of a novel drug compound such as MTG through the skin, simple formulations with one or more penetration enhancement strategies are usually employed. In this study, solvents with skin penetration enhancing abilities will be employed as a formulation itself (simple solvent systems) and as penetration enhancers.

1.5 Simple solvent systems

Simple solvent systems are basic formulations consisting of only solvents. Single (monophasic) or a mixture of solvents (bi- or triphasic) have been extensively used for the investigation of the delivery of drugs through the skin, notably in the pre-formulation stages. In the current research, only single solvents are utilised for the pre-formulation investigations of the penetration of MTG via the skin.

1.5.1 Types of solvents used

The incorporation of solvents with skin penetration enhancing abilities into formulations is a well-known passive enhancement strategy for the transdermal delivery of drugs. Many groups of solvents have been identified as CPEs such as alcohols, amides, esters, glycols and sulphoxides (Lane et al., 2012; Williams and Barry, 2012). However, the safety of including these solvents into transdermal formulation is crucial, especially in clinical uses. Hence, an effort to identify solvents that fall under the Generally Recognised As Safe (GRAS) category has been made based on several ideal properties includes being pharmacologically inert, non-

irritating, non-damaging and nontoxic, physically and chemically compatible with drugs and excipients in the dosage forms (Lane et al., 2012; Vitorino et al., 2015). Such solvents can interact with the skin constituents and modify the penetration of active substances and substantially influence the *in vivo* performance of transdermal formulations, more notably increasing the drug flux across the skin (Lane et al., 2012; Roussel et al., 2015). These solvents are found to exert their effects mainly at three sites of the lipid bilayer which are the polar head groups of the lipids, the aqueous domain of the lipid bilayer and the lipid alkyl chains (Lane et al., 2012).

In the current study, solvents such as dimethyl sulphoxide (DMSO), Labrasol®, Lauroglycol™, Maisine®, propylene glycol (PG) and Transcutol® are chosen for *in vitro* permeation studies. These solvents are chosen as they have different mechanisms of action and are also widely used to improve permeation through the skin. Table 1.1 shows the chemical name, category and chemical structure of each solvent used in this study.

Table 1.1 Solvents used in this study

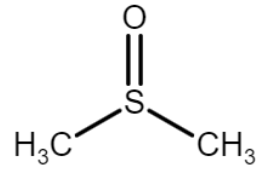
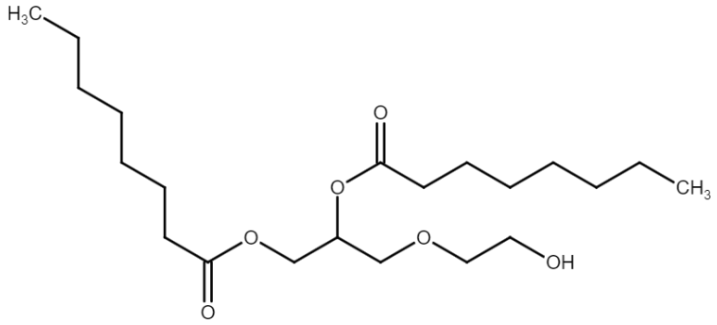
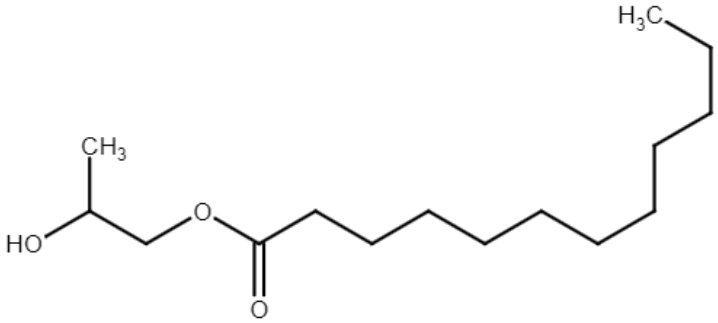
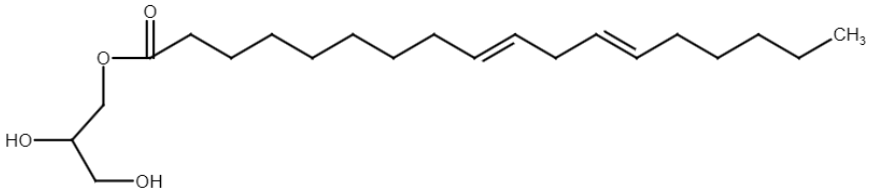
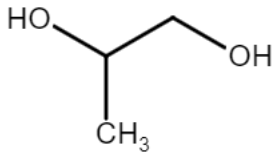
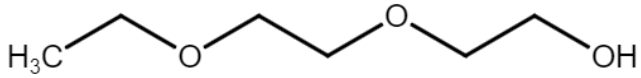
Solvent / Trade name	Chemical name	Category	Chemical structure
DMSO	Dimethyl sulphoxide	Sulphoxide	
Labrasol®	Caprylocaproyl macrogol-8-glyceride	Non-ionic surfactant	
Lauroglycol™	Propylene glycol monolaureate	Non-ionic surfactant	

Table 1.1. Continued

Solvent / Trade name	Chemical name	Category	Chemical structure
Maisine®	Glyceryl/glycerol monolinoleate	Fatty acid ester	
PG	Propylene glycol	Glycol	
Transcutol®	Diethylene glycol monoethyl ether	Ether	

1.5.1(a) DMSO

DMSO is a colourless, odourless and hygroscopic liquid that is completely miscible in both water and organic solvents (Martin et al., 1967). DMSO is a strong polar aprotic solvent that is well known for its ability to increase the permeability of membranes, thus increasing the delivery of both lipophilic and hydrophilic compounds through the skin. Although the exact mechanism of action of DMSO on the skin membrane remains ambiguous, there are speculations that DMSO enhances drug permeation by denaturing proteins, modifying the keratin conformation in the SC and also extracting skin lipids (Williams and Barry, 2012; Lane, 2013).

The penetration enhancement effects of DMSO are found to be dependent on the concentration of DMSO used in the formulation, where a marked increase in drug penetration occurring usually at concentrations of 60% of DMSO or higher (Rowe et al., 2009). However, higher concentrations of DMSO used is usually associated with unwanted adverse reactions such as skin irritation (Kligman, 1965; Malten and den Arend, 1978; Williams and Barry, 2012; Lane, 2013).

1.5.1(b) Labrasol®

Labrasol® is a colourless and odourless liquid that is miscible in aqueous medium which helps in solubilising compounds which are poorly soluble (Rowe et al., 2009; Gattefossé, 2023). Labrasol® consists primarily of caprylic (C8) and capric acid (C10) as the main fatty acids, as well as mono- and di- fatty acid esters of polyethylene-8 glycol. Labrasol® is a non-ionic surfactant that is frequently used in self-micro-emulsifying drug delivery systems which consist of an aqueous phase and a lipid phase as a solvent, co-surfactant or surfactant.

In dermal formulations, Labrasol® has been used at concentrations ranging at 10 – 55% (Rowe et al., 2009). As a solvent, Labrasol® is found to facilitate and improve the penetration of drugs across biological membranes by forming micelles and increasing the solvation of drugs within the SC (Koga et al., 2006). A further study conducted by Yeoh et al. (2022) speculated that Labrasol® causes a possible disordering of the lipid bilayer in the SC, which creates a pathway to allow an increase of drug permeation through the skin layer.

1.5.1(c) Lauroglycol™

Lauroglycol™ is another example of a non-ionic surfactant that is employed in the current research. Lauroglycol™ is a liquid consisting mostly of propylene glycol mono- and di- esters of lauric (C12) acid, which are composed of monoesters and a small fraction of diesters (Gattefossé, 2022). Similar to Labrasol®, Lauroglycol™ is commonly used in most self-emulsifying drug delivery systems as a solvent or cosurfactant.

1.5.1(d) Maisine®

Maisine® is a solvent consisting of fatty acid that is also commonly used in self-emulsifying drug delivery systems. According to the European Pharmacopoeia (PhEur 6.3), Maisine® is a blend of mono-, di-, and triglycerides made by glycerol esterification or partial glycerolysis of vegetable oils. Maisine® occurs as a viscous lightly amber-coloured oily liquid with a characteristic odour which could be partially solidified at room temperature (Rowe et al., 2009).

A study done by Ogiso et al. (1995) showed that Maisine® successfully enhanced the permeation of drugs such as indomethacin through the skin after being pre-treated

with Maisine®. Additional research discovered that Maisine® has the ability to fluidise SC lipids, extract intercellular lipids (ceramides) from the SC and also enhance drug partitioning into the SC (Ogiso et al., 1995). Recent research revealed Maisine®'s ability to perturb the highly ordered arrangement of the intercellular lipids of the SC and interact with the keratin of the SC simultaneously, which in turn, promotes drug penetration through the skin layer (Liu et al., 2019). This fluidisation, extraction and disturbance of the intercellular lipids allow Maisine® to not only act as a solubilising agent, but also to enhance the permeation of drugs through the skin in the current research.

1.5.1(e) PG

PG is one of the most popular and extensively studied solvent which is frequently employed as a solvent or co-solvent in a variety of pharmaceutical formulations, cosmetics, and food products (Rowe et al., 2009). PG is a slightly viscous, virtually odourless liquid that is clear, colourless, and has a sweet slightly acrid taste similar to that of glycerine which is miscible in both aqueous and organic mediums (Rowe et al., 2009). In topical formulations, PG is commonly used at a concentration of 5 – 80% as a solvent to dissolve numerous drugs that are poorly soluble (Barry and Bennett, 1987; Bouwstra et al., 1991; Rowe et al., 2009). Aside from the solubilising properties, PG can also be employed as a preservative as well as a humectant in various pharmaceutical formulations (Rowe et al., 2009). The exact mechanism of action of PG which allows penetration enhancement of drugs through the skin is not well documented, but various effects of PG on the skin have been proposed.

With the ability to fluidise the lipid layers, PG can affect the skin's barrier which improves drug partitioning into the SC (Barry and Bennett, 1987; Lane et al., 2012; Carrer et al., 2020). Another proposed and studied mechanism of action of PG is the

dehydrating effect of PG on the SC keratin (Bouwstra et al., 1991; Goh et al., 2017). PG has been found to displace the water regions of the polar heads of the lipid bilayer in the SC, which in turn decreases the barrier properties of the skin. A study conducted by Yeoh et al. (2022) showed a possibility that the decreased barrier property could be due to the complete perturbation of the lipid polar regions of the SC by PG. Furthermore, the study also found that PG may alter the conformation of the protein structures of the SC. This decreased skin barrier property is speculated to be one of the major contributing factors that allow an increase in the permeated amount of drug through the skin when PG is incorporated into the formulation.

1.5.1(f) Transcutol®

Transcutol® (diethylene glycol monoethyl ether) is a notable solvent from the ether group. Transcutol® can be divided into three different grades based on their purities after the manufacturing process, namely Transcutol® CG (99.5% purity), Transcutol® P (99.8% purity) and Transcutol® HP (99.9% purity). Transcutol® CG is used for cosmetic manufacturing while Transcutol® HP is suitable for use in oral pharmaceuticals. In the current research, Transcutol® P is used and is referred to simply as Transcutol® in all future parts of this thesis.

Transcutol® is a non-volatile, colourless liquid with low viscosity that is nearly odourless with good miscibility in water and common solvents such as ethanol (Osborne, D. W., 2011; Osborne, David W. and Musakhanian, 2018). In the United States (US), Transcutol® has been successfully formulated in Food and Drug Administration (FDA) approved products at concentrations of up to 49.9% for transdermal and topical application (Osborne, David W. and Musakhanian, 2018). Aside from being GRAS, Transcutol® has the ability to solubilise both lipophilic and

hydrophobic drugs, which makes Transcutol® a favourable solvent with skin penetration enhancing abilities.

Transcutol® is proposed to have a similar mechanism of action on the skin as Maisine® by their ability to extract ceramides from the SC (Ogiso et al., 1995). Yeoh et al. (2022) observed that Transcutol® was able to increase drug permeation through the skin by affecting and altering the lateral lipid packing and protein conformation in the SC. Additionally, it has been reported that Transcutol® exhibits a dehydration effect on the skin which potentially enhances the mobility of the SC (Osborne, David W. and Musakhanian, 2018). Ganem et al. (1997) came to a conclusion that Transcutol®'s ability to increase drug permeation through the skin may be attributed to the permeation of Transcutol® into the skin, alteration of the partition of drug through the skin and the absorption of water from the air and skin (dehydrating ability) by Transcutol®.

1.6 Gel formulation

Currently, there are a variety of pharmaceutical dosage forms for transdermal and topical delivery such as creams, lotions, ointments, gels and patches. Gels are a type of semisolid formulations which are usually formed by incorporating drugs or active components into a three-dimensional polymer matrix suspended in an external fluid solvent while still maintaining sufficient flowability (Rehman and Zulfakar, 2014; Mayba and Gooderham, 2018). The incorporated polymer functions as a structural network with the ability to retain the drug in the matrix of the gel.

Typically, gels can be differentiated into two main types based on the nature of their external fluid phase, namely organogels/oleogels (contains an organic solvent as the external fluid phase) and hydrogels which contain water (Rehman and Zulfakar, 2014).

Other types of gels aside from organogels and hydrogels include niosomal gels (contains liposomes), emulgels (contains organogels/hydrogels with an emulsion and a surfactant), bigels (mixture of organogels and hydrogels without a surfactant) and aerogels (contains silica and produced by drying). Gels have been formulated to incorporate various drugs in the current market such as diclofenac sodium (Voltaren Emulgel™), terbinafine (Tefin), adapalene (Differin® Gel), lignocaine (Axcel®) and testosterone (Androgel®).

1.6.1 Advantages of gel formulation

Transdermal application of gels for drug delivery through the skin offers a variety of advantages. Gels are well known for their ease and comfort with minimal to no pain caused during the application process (Rehman and Zulfakar, 2014). Texturally, after application on human skins, gels usually dry off as a greaseless non-occlusive film (with the exception of organogels) and is more cosmetically elegant (Mayba and Gooderham, 2018).

Although the stability of gels is usually stated as a disadvantage of gels (typically hydrogels and gels with a high-water content) as microbial contamination is possible, this problem can be easily overcome with the addition of a preservative into the formulation.

1.6.2 Polymer used in gel formulation

In the current study, Hypromellose or hydroxypropyl methylcellulose (HPMC) was chosen as a polymer for the formulation of gels to investigate the delivery of MTG through the skin. HPMC is an inert hydrophilic and non-ionic propylene glycol ether of methylcellulose. The chemical structure of HPMC is illustrated in Figure 4.

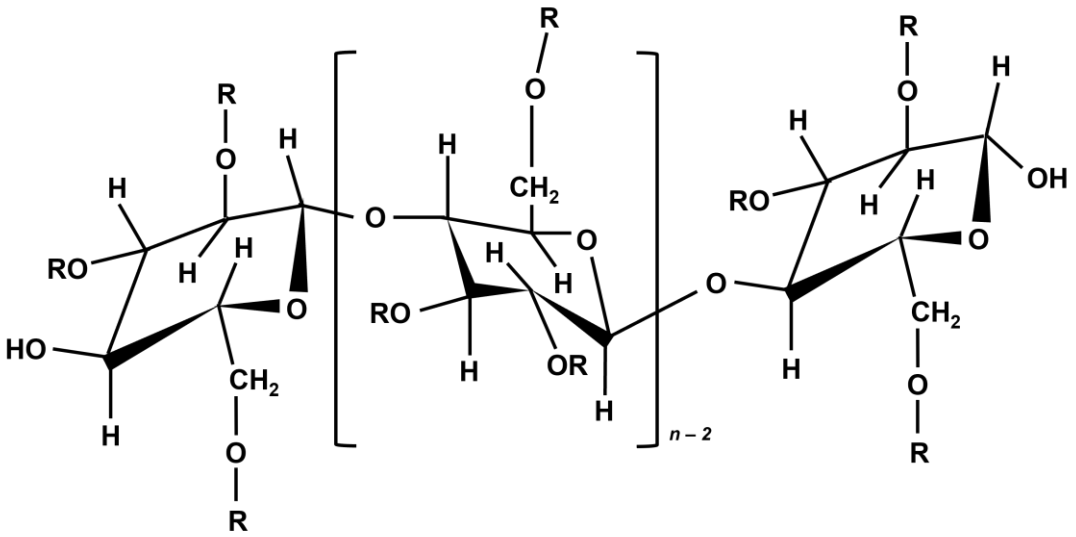


Figure 4 **Chemical structure of HPMC (adapted from Deshmukh et al. (2017))**

Gels formed through the hydration of HPMC falls under the category of hydrogels which has been commonly used in oral, ophthalmic, nasal, topical, transdermal, subcutaneous, vaginal and rectal formulations (Peppas et al., 2000; Rowe et al., 2009; Rehman and Zulfakar, 2014).

For most pharmaceutical formulations, purified HPMC is usually sold as an odourless and tasteless, white or sometimes off-white powder that can be hydrated or dissolved in aqueous alcohols (alcohol content < 50%) (Rowe et al., 2009). As a gelling agent, HPMC forms a clear, colourless gel when thoroughly hydrated and solubilised. HPMC is generally regarded as a nontoxic and non-irritating material for the production of pharmaceuticals by the World Health Organisation (Rowe et al., 2009). These desired traits make HPMC a suitable candidate for formulating a transdermal gel base as a model formulation in this study.

1.7 *In vitro* studies using Franz diffusion cells

In vitro studies are typically carried out using Franz diffusion cells based on the guidelines set by the Organisation of Economic Cooperation and Development (OECD) (OECD, 2004a, 2004b). Although various designs of Franz diffusion cells exist depending on the manufacturer and user's needs, Franz diffusion cells usually comprise of two main parts: a donor compartment and a receptor compartment with a sampling port. The Franz diffusion cell setup for *in vitro* permeation studies employed in the current experiment is as shown in Figure 5.

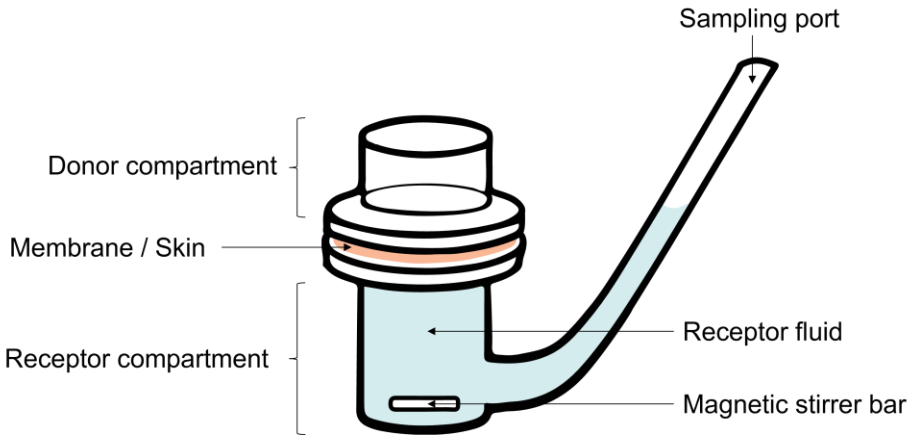


Figure 5 Setup of Franz diffusion cell for *in vitro* studies

The two compartments are separated by a layer (i.e. human skin, porcine skin, selectively permeable synthetic membranes such as polydimethylsiloxane membrane or simple membranes such as nylon or cellulose membrane) used for the permeation evaluation (Firpo et al., 2015). The use of either a membrane or skin depends on the nature of the *in vitro* study being carried out. An *in vitro* drug release study is carried out with a simple membrane such as nylon membrane mounted between the two compartments while on the other hand, an *in vitro* drug permeation study uses skins such as human cadaver skin or alternative skins such as porcine, rabbit or rat skins.

For both types of *in vitro* studies, drug formulations are loaded into the donor compartment while the receptor compartment is filled with fluids (receptor fluid) mimicking the human body conditions. A solubilising agent (i.e. PG, DMSO or BRIJ™ O20) may be added into the receptor fluid to aid dissolution of the drug for poorly soluble drugs or if the solubility of the drug in the receptor fluid is < 10µg/mL (Bronaugh and Stewart, 1984). A magnetic stirrer bar is loaded into the receptor compartment to ensure good mixing throughout the entire time of the permeation study. Aliquots are removed from the sampling port at predetermined time points for further drug analysis.

1.8 High-performance liquid chromatography (HPLC)

The detection and quantification of drugs in the receptor compartment of Franz diffusion cells during *in vitro* permeation studies are typically quantified using liquid chromatography methods such as HPLC (Goh et al., 2019; Kung et al., 2019; Otterbach and Lamprecht, 2021; Teoh et al., 2021). HPLC coupled with an ultraviolet (UV) detector has been widely used to identify and determine the MTG content in various samples including MTG spiked in solvents, plasma, urine samples and MTG beverages (Janchawee et al., 2007; Mudge and Brown, 2016). A UV detector has advantages such as being non-destructive, reliable, relatively easy to use and is compatible with gradient analysis (Dong and Jedrzej, 2019). Also, using HPLC-UV requires a less amount of sample for analysis (usually volume of 10 – 25 µL) compared to the amount needed for UV spectroscopy (~2 mL). Currently, there are a total of 11 reports analysing the MTG content in samples using HPLC-UV as summarised in Table 1.2.