Thesis

No.	Authors, Year, Title of Thesis, University, Date of Convocation
1.	Ahmad Makahleh, PhD 2011, "Green approaches involving liquid phase microextraction, monolithic column and capacitively coupled contactless conductivity detection in flow techniques"
2.	Gan Hui Siang, MSc 2012," "Development of liquid phase microextraction technique for the gas chromatographic determination of fatty acids in vegetable oils"
3.	
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Determination of underivatized long chain fatty acids using RP-HPLC with capacitively coupled contactless conductivity detection

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method with capacitively coupled contactless conductivity detector (C^4D) has been developed for the separation and the simultaneous determination of five underivatized long chain fatty acids (FAs), namely myristic, palmitic, stearic, oleic, and linoleic acids. An isocratic elution mode using methanol/1 mM sodium acetate (78:22, v/v) as mobile phase with a flow rate of 0.6 mL min^{-1} was used. The separation was effected by using a Hypersil ODS C_{18} analytical column (250 mm × 4.6 mm × 5 μm) and was operated at 45 °C. Calibration curves of the five FAs were well correlated ($r^2 > 0.999$) within the range of 5–200 μg mL^{-1} for stearic acid, and 2–200 μg mL^{-1} for the other FAs. The proposed method was tested on four vegetable oils, i.e., pumpkin, soybean, rice bran and palm olein oils; good agreement was found with the standard gas chromatographic (GC) method. The proposed method offers distinct advantages over the official GC method, especially in terms of simplicity, faster separation times and sensitivity.

1. Introduction

Fatty acids (FAs) are widely found in nature (e.g., food products, vegetable oils, and living organisms) and are vital as nutritional substances and metabolites in living organisms. They form the basic components of most naturally occurring lipids in both animals and plants. The diversity of the chain length, degree of unsaturation, geometry and position of double bonds, as well as the presence of other groups, render their composition the most definitive characteristic of these lipids and their origin [1]. Analysis of FAs is important in the control of technical products, in medical diagnostics, cancer research and in the testing of purity, origin, and shelf-life studies of food products, and in biodiesel projects [2].

The most commonly used method for the analysis of FAs involves the determination of the corresponding methyl esters using capillary gas chromatography (GC) with flame ionization detector (FID) [2]. A derivatization procedure is mandatory to increase the volatility and overcome adsorption of the polar functional groups to the GC column [3]. In general, GC provides excellent separation and quantification together with acceptable sensitivity. However, there is a growing interest in the use of high-performance liquid chromatography (HPLC) for studying FAs. The major advantages of HPLC over GC are the lower temperatures required during the analysis (reduce the risk of isomerisation of double bonds) and the possibility of collecting fractions for further analysis. Apart from that, HPLC is considered more flexible as the retention characteristics can be easily modified by varying the composition of mobile phase.

HPLC analysis of the FAs is complicated as these analytes are neither UV nor fluorescence-active. Thus, derivatization procedures allow the "tagging" of chromophore or fluorophore to the analyte, rendering them to be detected using UV–vis or fluorescence detector [4–8].

While the derivatization procedure used to increase the HPLC sensitivity markedly (especially for fluorescence detection), the derivatization process itself, however, is not preferred. Inherent problems include not only the longer analysis time required, but also the possibility of inaccurate results due to incomplete or unstable reaction with the derivatization compounds; unselective labelling that leads to interfering by-products; and the expensive and unstable nature of some derivatization reagents. There is thus, a strong emphasis lately on developing alternative methods to the traditional HPLC methods that require UV and fluorescence [4–8] for detection. HPLC methods for the determination of underivatized FAs involving detectors such as mass spectrometer [3,9], chemiluminesce [1,10], electrochemical [1], refractive index [11], and evaporative light scattering detectors (ELSD) [2,12] have been reported.

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Flow injection determination of free fatty acids in vegetable oils using capacitively coupled contactless conductivity detection

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ABSTRACT

A single line flow injection analysis (FIA) method that incorporated a preconcentrator column packed with C_{18} particles and capacitively coupled contactless conductivity detector (C^4D) was developed for the determination of free fatty acid (FFA) in vegetable oils. The carrier stream was methanol/1.5 mM sodium acetate (pH 8) 80:20 (v/v) at a flow rate of 1.0 mL min^{-1}. Calibration curve was well correlated ($r^2 = 0.9995$) within the range of 1–200 mg L^{-1} FFA (expressed as palmitic acid). Sampling rate of 40–60 h^{-1} was achieved. Good agreement was found between the standard non-aqueous titrimetry method and the proposed method when applied to the determination of FFA in palm (crude, olein, and refined, bleached and deodorised) and other vegetable (soybean, rice bran, walnut, corn and olive) oils. The proposed method offers distinct advantages over the official method, especially in terms of simplicity, high sampling rate, economy of solvents and sample, offering considerable promise as a low cost automated system that needs minimum human intervention over long periods of time.

1. 1. Introduction

Oil acidity, usually determined as free fatty acid (FFA) is an important quality parameter in the oleochemical industry. It is a measure of the extent to which hydrolysis has liberated the fatty acids from their ester linkage of the parent triglyceride molecule and is routinely measured during production and storage stages [1,2]. FFA is commonly determined by non-aqueous titrimetry, using phenolphthalein as indicator. The method, however, uses large amounts of organic solvents and involves manual operation. Flow approaches (e.g., flow injection analysis (FIA) [3–5], sequential injection analysis (SIA) [6]) have been introduced as alternatives, mainly to automate the analysis, thus enhancing laboratory productivity. FIA methods have been developed for the determination of acidity in vegetable oils (olive [3,4] and palm [5]). A SIA method for the determination of acidity in vegetable oils, although consume less chemicals and sample, but the sampling rate was rather low (12 h^{-1}) [6]. These SIA and FIA methods use spectrophotometric detection and their performance can be affected by samples of different viscosities.

Acidity is also an important quality indicator in other samples such as fruit juices, soft drinks, etc. As an example, a slight change in the acidity of coffee resulted in marked differences in its taste and aroma [7]. In biodiesels, FFA is one of the main factors that affect the transesterification process [6]. FIA methods, either using spectrophotometric or conductimetric detectors have been applied for the determination of fruit juice [8–10], coffee [7], vinegar [11,12] and soft drink [12] acidities.

The determination of individual FFA is traditionally done by derivatization to increase its volatility, followed by GC separation. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) methods have also been used [13,14]. Since fatty acids (FAs) lack chromophores, either derivatization or indirect UV detection is necessary. Ion chromatography and CE coupled with conductivity detection for the analysis of short chain and long chain FAs, respectively, were also reported [14,15]. CE with C^4D detection for analysis of FA was also described [16]. Recently, we reported a HPLC method with C^4D detection for the profiling of FAs in vegetable oils [17]. These HPLC methods require long analysis time and consume large amounts of solvent.

In this paper, we describe a new FIA method for the rapid determination of FFA using C^4D. The analytical practicality of the method was demonstrated in the determination of FFA in a few types of vegetable oils.

2. 2. Experimental

2.1. Chemicals and reagents

Myristic acid (99%), palmitic acid (≥99%), stearic acid (≥99%), oleic acid (99%), linoleic acid (99%), sodium hydroxide and potassium hydrogen phthalate were purchased from Sigma-Aldrich

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ANA-O7

BIODIESEL POTENTIAL OF MILO (*THESPESIA POPULNEA*) SEED OIL IN PAKISTAN

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The production of biodiesel from conventional vegetable oils is leading to food versus fuel dilemma. This has encouraged the researchers to explore some non-conventional and lesser known seed oils as feedstock for biodiesel production. In the present study, Milo (*Thespesia populnea*) seed oil was evaluated for the first time as a potential feedstock for preparation of biodiesel. The hexane-extracted crude oil from *T. populnea* seeds was transmethylated under basic catalytic conditions using a lab scale biodiesel reactor. The optimum conditions elucidated for the transesterification of the investigated oil: 6.5:1.0 molar ratio of methanol/oil, 65 °C temperature and 0.90 % (oil weight basis) $NaOCH_3$ catalyst offered 98.1 % yield of *T. populnea* methyl esters (TPMEs)/biodiesel. GC-MS analysis of the biodiesel produced revealed the presence of mainly four fatty acid methyl esters: linoleic acid (40.5 %), oleic acid (27.8 %), palmitic acid (25.7 %) and stearic acid (4.95 %). A small amount of 2-hexyl cyclopropaneoctanoic acid and arachadic acid with contribution 0.54 and 0.50 % respectively was also examined. The ^1H-NMR spectrum of TPMEs was also recorded for authentification purposes. The fuel quality characteristics of TPMEs which included density, kinematic viscosity, cetane number, flash-, cloud-, pour-, and cold filter plugging- points, sulfur and water contents and acid value compared well with ASTM D 6751 and EN 14214 specifications, where applicable. These properties of the tested biodiesel were also found to be rather comparable with those of biodiesel derived from conventional oilseed crops suggesting its utilization as an acceptable substitute for petro-diesel.

ANA-O8

CAPACITIVELY COUPLED CONTACTLESS CONDUCTIVITY AS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETECTOR

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Capacitively coupled contactless conductivity detection (C^4D) in the axial electrode configuration was introduced in 1998 as a quantification method for capillary electrophoresis. Its universality allows the detection of small inorganic ions as well as organic and biochemical species. Due to its robustness, minimal maintenance demands and low cost, the popularity of this detector has been steadily growing. Applications have recently been extended to other analytical methods such as ion chromatography, high-performance liquid chromatography and flow-injection analysis. The main objective of this presentation is to provide an up-date on this fascinating technology. It will also focus on the working principles and selected applications in HPLC. Research activities within our group for the determination of fatty acids, betulinic acid, and sodium glutamate will be demonstrated.

EEFA15P (Poster)

Solid-Phase Microextraction-Comprehensive Gas Chromatography Combined with Very Fast Quadrupole Mass Spectrometry for the Analysis of Water Pesticides

Giorgia Purcaro [1,2], Flavio Franchina [1], Peter Q. Tranchida 1, Paola Dugo [1,3], Rosaria Costa [4,1] and *Luigi Mondello [1,3]
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[2] Dipartimento di Scienze degli Alimenti, Università di Udine, Italy
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A spin-off of the University of Messina, Italy

Comprehensive two-dimensional gas chromatography (GC×GC) is a novel approach for the analyses of complex samples and, over the past decade, its applicability has been demonstrated for a varied range of samples types. The implementation of GC×GC requires fast detector acquisition rates to provide sufficient data density for accurate definition of the narrow 2D peaks, which are often reported to be less than about 100 ms wide. Herein the performance of a novel rapid-scanning quadrupole mass spectrometer (qMS) detector, characterized by 20 000 amu/s scan speed, using a 50-440 m/z mass range and a 33 Hz scan frequency, was evaluated in a GC × GC analysis of a series of water pesticides. Analytes were manually extracted from water by using solid-phase microextration (SPME). The qMS performance was evaluated considering: a) number of data points per peak; b) mass spectral quality; c) extent of peak skewing; d) consistency of retention times. Standard solutions at eight concentration levels, from 5 to 1000 ug/L, were prepared, and employed for method validation. Spectral similarity and, in particular, the use of a linear retention index (LRI) filter greatly simplified the identification procedure. Finally, ten different tap water samples were subjected to SPME-GC×GC-qMS analysis, fortunately giving negative results.

Food and Flavour Chemistry

FFC01

Capacitively Coupled Contactless Conductivity as Flow Injection Analysis Detector for the Determination of Free Fatty Acids in Vegetable Oils

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A single line flow injection analysis (FIA) method that incorporated a preconcentrator column packed with C_{18} particles and capacitively coupled contactless conductivity detector was developed for the determination of free fatty acid (FFA) in vegetable oils. The carrier stream was methanol/1.5 mM sodium acetate (pH 8.0) 80:20 (v/v) at a flow rate of 1.0 mL min^{-1}. Calibration curve was well correlated (r^2 = 0.9995) within the range of 1–200 mg L^{-1} FFA (expressed as palmitic acid). Sampling rate of 40–60 h^{-1} was achieved. Good agreement was found between the standard non-aqueous titrimetry method and the proposed method when applied to the determination of FFA in palm (crude, olein, and refined, bleached and deodorised) and other vegetable (soybean, rice bran, walnut, corn and olive) oils. The proposed method is superior over the official method, especially in terms of simplicity, high sampling rate, economy of solvents and sample.

FFC02

Simultaneous Detection of Type A and Type B *trichothecenes* in cereals by Liquid Chromatography with Quadrupole Time of Flight Mass Spectrometry (LC/QTOF-MS/MS)

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A method based on Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique followed by liquid chromatography coupled with quadrupole time of flight mass spectrometry (LC/QTOF-MS/MS) with electrospray ionization (ESI) was developed for the determination of eight(8) type-A and type-B trichothecenes in cereal samples. This method excludes the use of dispersive solid-phase extraction (dSPE) extraction cleanup step to reduce time and

DEVELOPMENT OF A LIQUID PHASE MICROEXTRACTION TECHNIQUE FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF FATTY ACIDS IN VEGETABLE OILS

by

GAN HUI SIANG

Thesis submitted in fulfillment of the
requirements for the degree of
Master of Science

February 2012

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LAPORAN AKHIR DAN PENUTUPAN AKAUN PROJEK PENYELIDIKAN RU

"Innovative Methods in the Analytical Determination of Fatty Acids"
Nombor Akaun: 1001/PKIMIA/811148

Sukacita bersama ini saya sertakan laporan akhir untuk projek penyelidikan di atas. Dengan ini juga, saya mohon dengan resminya untuk akaun projek ini ditutup.

Sekian, terima kasih.

Yang benar,

Prof Bahruddin Saad

Sk Timbalan Dekan, Pengajian Siswazah dan Penyelidikan
PP Sains Kimia

UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT
FINAL REPORT
Geran Penyelidikan Universiti
Laporan Akhir

A.	**PARTICULARS OF RESEARCH** / *MAKLUMAT PENYELIDIKAN:*
(i)	**Title of Research: Innovative Methods in the Analytical Determination of Fatty Acids** *Tajuk Penyelidikan:*
(ii)	**Account Number:** ○ *Nombor Akaun: 1001/PKIMIA/811148*
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader: Prof Bahruddin Saad** *Nama Ketua Penyelidik:*
	Name of Co-Researcher: Assoc Prof Afidah Abdul Rahim *Nama Penyelidik Bersama:*
(ii)	**School/Institute/Centre/Unit : Chemical Sciences** *Pusat Pengajian /Institut/Pusat/Unit :*

E. 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)

Fatty acid is an important parameter that is routinely measured in many industries (e.g., oleochemicals, biofuels, food). It is normally carried out using the official method involving gas chromatography (GC) method. Prior to the analysis, a liquid-liquid extraction step employing large volumes of organic solvent is required to extract the fatty acids from the sample. The GC analysis itself takes a long time (40-60 min), and derivatization of the fatty acids is required to increase volatility. This project focusses on some innovative analytical methodologies to improve and simplify the analysis. We investigated the viability of using a robust, low cost detector (i.e., the capacitively coupled contactless conductivity detector (C4D)) when used in a flow injection analysis (FIA) mode, or when used as HPLC detector. The proposed FIA method offers distinct advantages over the official method, especially in terms of simplicity, high sampling rate, economy of solvents and sample, offering considerable promise as a low cost automated system that needs minimum human intervention over long periods of time. The proposed HPLC method also offers distinct advantages over the official GC method, especially in terms of simplicity,faster separation times and better sensitivity. Furthermore, derivatization is not required, thus significantly simplifying the analysis. A hollow fiber liquid-phase microextraction technique, followed by gas-chromatography-flame ionization detection (GC-FID) for the profiling of the fatty acids (FAs) (lauric, myristic, palmitic, stearic, palmitoleic, oleic, linoleic, linolenic and arachidic) was also successfully developed. Enrichment factors varying from 37 to 115 were achieved. The method was successfully applied to the profiling of the FAs in palm oils (crude, olein, kernel, and carotino cooking oil) and other vegetable oils (soybean, olive, coconut, rice bran and pumpkin). The encouraging enrichments achieved offer an interesting option for the profiling of the minor and major FAs in palm and other vegetable oils, a feature that is not possible using the official method.

F. SUMMARY OF RESEARCH FINDINGS

Ringkasan dapatan Projek Penyelidikan

We have successfully automate the determination of fatty acids using a single line flow injection an a new detector (capacitively coupled contactless conductivity detector).

We have also successfully developed a new sample preparation technique based on a two-phase hollow fibre for the selective isolation of the fatty acids from vegetable oil samples. The isolated fatty acids are then analysed using gas chromatography method.

A new HPLC method using C4D detector was also successfully developed for the determination of fatty acids.

The main advantages of these developed methods are the significant reduction in the use of extracting solvent, low-cost, rapid and offer green features in determination.

H.

a) Results/Benefits of this research
Hasil Penyelidikan

No. *Bil:*	**Category/Number:** *Kategori/ Bilangan:*	**Promised**	**Achieved**
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)*	2	3
2.	**Human Capital Development**		
	a. Ph. D Students	**1**	**1**
	b. Masters Students		**1**
	c. Undergraduates (Final Year Project)		
	d. Research Officers		
	e. Research Assisstants		
	f. Other: Please specify		
3.	**Patents** *Paten*		
4.	**Specific / Potential Applications** *Spesifik/Potensi aplikasin*		
5.	**Networking & Linkages** *Jaringan & Jalinan*		
6.	**Possible External Research Grants to be Acquired** *Jangkaan Geran Penyelidikan Luar Diperoleh*		

- Kindly provide copies/evidence for Category 1 to 6.

b) Equipment used for this research.
Peralatan yang telah digunakan dalam penyelidikan ini.

Existing HPLC and GC equipments at the School of Chemical Sciences was used.

Items Perkara	Approved Equipment	Approved Requested Equipment	Location
Specialized Equipment Peralatan khusus			
Facility Kemudahan			
Infrastructure Infrastruktur			

- Please attach appendix if necessary.

H. COMMENTS OF PTJ'S RESEARCH COMMITTEE
KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ

Fatty acids (FAs) are the basic components of most naturally occurring lipids in both animals and plants. The diversity of the chain length, degree of unsaturation, geometry and position of double bonds determine the characteristic of these lipids and their origins. The most commonly used method for the analysis of FAs involves the determination of the corresponding methyl esters (FAMEs) using capillary gas chromatography (GC) with flame ionization detector (FID). The derivatization procedure (especially for the longer chain FAs) is mandatory to increase the volatility and overcome adsorption of the polar functional groups to the GC column. Prior to the GC-FID determination, the FAMEs were extracted using liquid-liquid extraction (LLE). A major problem of the LLE technique is the gross consumption of organic solvents, lack of sensit ivity, time consuming, labour intensive, and the extra evaporation step required prior to analysis to remove the excess solvent. This research project is directed towards finding alternative methods that can overcome many of the problems associated with the presently used standard method.

Currently, there is growing interest in the use of high performance liquid chromatography (HPLC) for studying FAs. The major advantages of HPLC over GC are the lower temperatures required during the analysis (reduce the risk of isomerisation of double bonds) and the possibility of collecting fractions for further analysis. A HPLC method using a new detector, the capacitively coupled contactless conductivity detector (C4D) for the separation and the simultaneous determination of five underivatized long chain FAs, namely myristic, palmitic, stearic, oleic, and linoleic acids was proposed. The separation was effected by using a Hypersil ODS C18 analytical column and was operated at 45 °C. An isocratic elution mode using methanol/1 mM sodium acetate (78:22, v/v) as mobile phase with a flow rate of 0.6 mL min^{-1} was used. Calibration curves of the five FAs were well correlated (r2 > 0.999) within the range of 5 – 200 gm L^{-1} for stearic acid, and 2 – 200 g mL^{-1} for the other FAs. The proposed method was tested on palm olein oils, other vegetable oils (pumpkin, soybean, rice bran). Good agreement was found with the standard GC method. The proposed method offers distinct advantages over the official GC method, especially in terms of simplicity (no derivatization required), faster separation times and better sensitivity.

A new method for the rapid determination of the total FAs was also developed using flow injection analysis (FIA) method that incorporated a preconcentrator column packed with C18 particles. The C4D was used as detector. The carrier stream was methanol/1.5 mM sodium acetate (pH 8) 80:20 (v/v) at a flow rate of 1.0 mL $\text{min}^{-1.}$ Calibration curve was well correlated (r^2 = 0.9995) within the range of 1 – 200 mg L^{-1} FFA (expressed as palmitic acid). Sampling rate of 40 – 60 h^{-1} was achieved. Good agreement was found between the standard non-aqueous titrimetry method and the proposed method when applied to the determination of FFA in palm (crude, olein, and refined, bleached and deodorised) and other vegetable (soybean, rice bran, walnut, corn and olive) oils. The FIA method is advantageous over the official method, especially in terms of simplicity, high sampling rate, economy of solvents and sample, offering considerable promise as a low cost automated system that needs minimum human intervention over long periods of time.

PROJECT OUTPUT

1. Postgraduate Students

(i) Gan Hui Siang, MSc 2012, "Development of liquid phase microextraction technique for the gas chromatographic determination of fatty acids in vegetable oils".

(ii) Ahmed Makahleh, PhD 2011 "Green approaches involving liquid phase microextraction, monolithic column and capacitively coupled contactless conductivity detection in flow techniques".

2. Presentations at Conferences

(i) Ahmad Makahleh, Bahruddin Saad and Hasnah Osman, *"Capacitively coupled contactless conductivity as high performance liquid chromatographic detector,"* 3rd International Conference for Young Chemists, Penang, 23-25 June 2010.

(ii) Gan Hui Siang, *Bahruddin Saad, Ahmad Makahleh and Boey Peng Lim, *"Liquid-phase microextraction (lpme) coupled with gas chromatography for determination of fatty acids in palm oil and other vegetable oils",* 3rd International Conference for Young Chemists, Penang, 23-25 June 2010.

(iii) Bahruddin Saad, *"Some developments in the analysis of fatty acids in palm and other vegetable oils",* 13 International Symposium on Advances in Extraction Technologies, Kuala Lumpur, 27-29 September 2011 **(invited lecture).**

(iv) Ahmad Makahleh, Bahruddin Saad, *"Capacitively coupled contactless conductivity as flow injection analysis detector for the determination of free fatty acids in vegetable oils",* 13 International Symposium on Advances in Extraction Technologies, Kuala Lumpur, 27-29 September 2011.

3. Publications

(i) Ahmad Makahleh, **Bahruddin Saad**, 2011, "*Flow injection determination of free fatty acids in vegetable oils using capacitively coupled contactless conductivity detection,* **Analytica Chimica Acta**, 694, 90–94(2010 Impact Factor: (2010 Impact Factor: 4.311)

(ii) Gan Hui Siang, Ahmad Makahleh, **Bahruddin Saad**, Boey Peng Lim, 2010 "*Hollow fiber liquid-phase microextraction coupled with gas chromatography-flame ionization detection for the profiling of fatty acids in vegetable oils",*
Journal of Chromatography A, *1217, 8073-8078* (2009 impact factor: 4.101)

(iii) Ahmad Makahleh, **Bahruddin Saad**, Gan Hui Siang, Muhammad Idiris Saleh, Hasnah Osman, Baharuddin Salleh, 2010 *"Determination of un-derivatized long chain fatty acids using RP-HPLC with capacitively coupled contactless conductivity detection",* **Talanta,** 81, 20-24 (2009 impact factor: 3.29)

DEVELOPMENT OF A LIQUID PHASE MICROEXTRACTION TECHNIQUE FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF FATTY ACIDS IN VEGETABLE OILS

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Thesis submitted in fulfillment of the
requirements for the degree of
Master of Science

February 2012

ANA-O7

BIODIESEL POTENTIAL OF MILO (*THESPESIA POPULNEA*) SEED OIL IN PAKISTAN

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The production of biodiesel from conventional vegetable oils is leading to food versus fuel dilemma. This has encouraged the researchers to explore some non-conventional and lesser known seed oils as feedstock for biodiesel production. In the present study, Milo (*Thespesia populnea*) seed oil was evaluated for the first time as a potential feedstock for preparation of biodiesel. The hexane-extracted crude oil from *T. populnea* seeds was transmethylated under basic catalytic conditions using a lab scale biodiesel reactor. The optimum conditions elucidated for the transesterification of the investigated oil: 6.5:1.0 molar ratio of methanol/oil, 65 °C temperature and 0.90 % (oil weight basis) $NaOCH_3$ catalyst offered 98.1 % yield of *T. populnea* methyl esters (TPMEs)/biodiesel. GC-MS analysis of the biodiesel produced revealed the presence of mainly four fatty acid methyl esters: linoleic acid (40.5 %), oleic acid (27.8 %), palmitic acid (25.7 %) and stearic acid (4.95 %). A small amount of 2-hexyl cyclopropaneoctanoic acid and arachadic acid with contribution 0.54 and 0.50 % respectively was also examined. The ^1H-NMR spectrum of TPMEs was also recorded for authentification purposes. The fuel quality characteristics of TPMEs which included density, kinematic viscosity, cetane number, flash-, cloud-, pour-, and cold filter plugging- points, sulfur and water contents and acid value compared well with ASTM D 6751 and EN 14214 specifications, where applicable. These properties of the tested biodiesel were also found to be rather comparable with those of biodiesel derived from conventional oilseed crops suggesting its utilization as an acceptable substitute for petro-diesel.

ANA-O8

CAPACITIVELY COUPLED CONTACTLESS CONDUCTIVITY AS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETECTOR

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Capacitively coupled contactless conductivity detection (C^4D) in the axial electrode configuration was introduced in 1998 as a quantification method for capillary electrophoresis. Its universality allows the detection of small inorganic ions as well as organic and biochemical species. Due to its robustness, minimal maintenance demands and low cost, the popularity of this detector has been steadily growing. Applications have recently been extended to other analytical methods such as ion chromatography, high-performance liquid chromatography and flow-injection analysis. The main objective of this presentation is to provide an up-date on this fascinating technology. It will also focus on the working principles and selected applications in HPLC. Research activities within our group for the determination of fatty acids, betulinic acid, and sodium glutamate will be demonstrated.

ANA-P16

LIQUID-PHASE MICROEXTRACTION (LPME) COUPLED WITH GAS CHROMATOGRAPHY FOR DETERMINATION OF FATTY ACIDS IN PALM OIL AND OTHER VEGETABLE OILS

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In the present work, hollow fibre liquid-phase microextraction (LPME) followed by gas-chromatography-flame ionization detector (GC-FID) determination of the fatty acids (FAs), namely methyl laurate, methyl tridecanoate, methyl myristate, methyl palmitate, methyl palmitolate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate and methyl arachidate in vegetable oils parameters such as type of extraction solvent, temperature, extraction time, stirring speed and salt addition were studied and optimized. For the best conditions (extraction solvent, *n*-tridecane; extraction time, 35 min; extraction temperature ambient; without addition of salt), enrichment factors varying from 37.0 to 115 were achieved. The method was successfully applied to mainly crude palm, palm olein, palm kernel and other vegetable oil (soybean, olive, coconut, rice bran and pumpkin), offering an interesting alternative to liquid–liquid extraction and solid phase extraction for the analysis of FAs in palm and other vegetable oils samples.

ANA-P17

PRELIMINARY REPORT ON SEPARATION OF MONILIFORMIN BY HPLC USING HILIC COLUMN

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In this project a detection method of Moniliformin (MON) by HPLC using hydrophilic interaction chromatography (HILIC) column was developed. The mobile phase composition, of (MeCN: water) was studied and the best composition was found at 85:15 v/v with HETP equal to 0.12. The retention time of about 3.9 min is the reasonable time for multisample analysis in the application in biological sample in future application. The sharp peak was obtained with UV detector at 5 ppm without having buffer in the mobile phase.

EEFA15P (Poster)

Solid-Phase Microextraction-Comprehensive Gas Chromatography Combined with Very Fast Quadrupole Mass Spectrometry for the Analysis of Water Pesticides

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Comprehensive two-dimensional gas chromatography (GC×GC) is a novel approach for the analyses of complex samples and, over the past decade, its applicability has been demonstrated for a varied range of samples types. The implementation of GC×GC requires fast detector acquisition rates to provide sufficient data density for accurate definition of the narrow 2D peaks, which are often reported to be less than about 100 ms wide. Herein the performance of a novel rapid-scanning quadrupole mass spectrometer (qMS) detector, characterized by 20 000 amu/s scan speed, using a 50-440 m/z mass range and a 33 Hz scan frequency, was evaluated in a GC × GC analysis of a series of water pesticides. Analytes were manually extracted from water by using solid-phase microextration (SPME). The qMS performance was evaluated considering: a) number of data points per peak; b) mass spectral quality; c) extent of peak skewing; d) consistency of retention times. Standard solutions at eight concentration levels, from 5 to 1000 ug/L, were prepared, and employed for method validation. Spectral similarity and, in particular, the use of a linear retention index (LRI) filter greatly simplified the identification procedure. Finally, ten different tap water samples were subjected to SPME-GC×GC-qMS analysis, fortunately giving negative results.

Food and Flavour Chemistry

FFC01

Capacitively Coupled Contactless Conductivity as Flow Injection Analysis Detector for the Determination of Free Fatty Acids in Vegetable Oils

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A single line flow injection analysis (FIA) method that incorporated a preconcentrator column packed with C_{18} particles and capacitively coupled contactless conductivity detector was developed for the determination of free fatty acid (FFA) in vegetable oils. The carrier stream was methanol/1.5 mM sodium acetate (pH 8.0) 80:20 (v/v) at a flow rate of 1.0 mL min^{-1}. Calibration curve was well correlated (r^2 = 0.9995) within the range of 1–200 mg L^{-1} FFA (expressed as palmitic acid). Sampling rate of 40–60 h^{-1} was achieved. Good agreement was found between the standard non-aqueous titrimetry method and the proposed method when applied to the determination of FFA in palm (crude, olein, and refined, bleached and deodorised) and other vegetable (soybean, rice bran, walnut, corn and olive) oils. The proposed method is superior over the official method, especially in terms of simplicity, high sampling rate, economy of solvents and sample.

FFC02

Simultaneous Detection of Type A and Type B *trichothecenes* in cereals by Liquid Chromatography with Quadrupole Time of Flight Mass Spectrometry (LC/QTOF-MS/MS)

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A method based on Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique followed by liquid chromatography coupled with quadrupole time of flight mass spectrometry (LC/QTOF-MS/MS) with electrospray ionization (ESI) was developed for the determination of eight(8) type-A and type-B trichothecenes in cereal samples. This method excludes the use of dispersive solid-phase extraction (dSPE) extraction cleanup step to reduce time and

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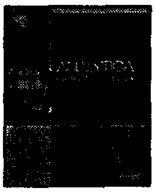

Flow injection determination of free fatty acids in vegetable oils using capacitively coupled contactless conductivity detection

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ABSTRACT

A single line flow injection analysis (FIA) method that incorporated a preconcentrator column packed with C_{18} particles and capacitively coupled contactless conductivity detector (C^4D) was developed for the determination of free fatty acid (FFA) in vegetable oils. The carrier stream was methanol/1.5 mM sodium acetate (pH 8) 80:20 (v/v) at a flow rate of 1.0 mL min^{-1}. Calibration curve was well correlated ($r^2 = 0.9995$) within the range of 1–200 mg L^{-1} FFA (expressed as palmitic acid). Sampling rate of 40–60 h^{-1} was achieved. Good agreement was found between the standard non-aqueous titrimetry method and the proposed method when applied to the determination of FFA in palm (crude, olein, and refined, bleached and deodorised) and other vegetable (soybean, rice bran, walnut, corn and olive) oils. The proposed method offers distinct advantages over the official method, especially in terms of simplicity, high sampling rate, economy of solvents and sample, offering considerable promise as a low cost automated system that needs minimum human intervention over long periods of time.

1. 1. Introduction

Oil acidity, usually determined as free fatty acid (FFA) is an important quality parameter in the oleochemical industry. It is a measure of the extent to which hydrolysis has liberated the fatty acids from their ester linkage of the parent triglyceride molecule and is routinely measured during production and storage stages [1,2]. FFA is commonly determined by non-aqueous titrimetry, using phenolphthalein as indicator. The method, however, uses large amounts of organic solvents and involves manual operation. Flow approaches (e.g., flow injection analysis (FIA) [3–5], sequential injection analysis (SIA) [6]) have been introduced as alternatives, mainly to automate the analysis, thus enhancing laboratory productivity. FIA methods have been developed for the determination of acidity in vegetable oils (olive [3,4] and palm [5]). A SIA method for the determination of acidity in vegetable oils, although consume less chemicals and sample, but the sampling rate was rather low (12 h^{-1}) [6]. These SIA and FIA methods use spectrophotometric detection and their performance can be affected by samples of different viscosities.

Acidity is also an important quality indicator in other samples such as fruit juices, soft drinks, etc. As an example, a slight change in the acidity of coffee resulted in marked differences in its taste and aroma [7]. In biodiesels, FFA is one of the main factors that affect the transesterification process [6]. FIA methods, either using spectrophotometric or conductimetric detectors have been applied for the determination of fruit juice [8–10], coffee [7], vinegar [11,12] and soft drink [12] acidities.

The determination of individual FFA is traditionally done by derivatization to increase its volatility, followed by GC separation. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) methods have also been used [13,14]. Since fatty acids (FAs) lack chromophores, either derivatization or indirect UV detection is necessary. Ion chromatography and CE coupled with conductivity detection for the analysis of short chain and long chain FAs, respectively, were also reported [14,15]. CE with C^4D detection for analysis of FA was also described [16]. Recently, we reported a HPLC method with C^4D detection for the profiling of FAs in vegetable oils [17]. These HPLC methods require long analysis time and consume large amounts of solvent.

In this paper, we describe a new FIA method for the rapid determination of FFA using C^4D. The analytical practicality of the method was demonstrated in the determination of FFA in a few types of vegetable oils.

2. 2. Experimental

2.1. Chemicals and reagents

Myristic acid (99%), palmitic acid (≥99%), stearic acid (≥99%), oleic acid (99%), linoleic acid (99%), sodium hydroxide and potassium hydrogen phthalate were purchased from Sigma–Aldrich

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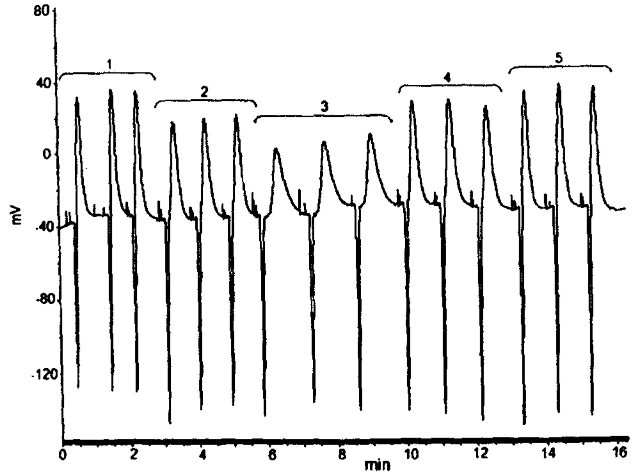

Fig. 2. Response of different fatty acids using the FIA-C⁴D system; peak label; (1) myristic; (2) palmitic; (3) Stearic; (4) oleic; (5) linoleic acid; fatty acids concentration, 0.4 mM; FIA-C⁴D conditions: carrier stream, methanol: 1 mM sodium acetate '8.22, v/v); flow rate, 1.0 mL min⁻¹; injection volume, 10 μL; amplitude of C⁴D, 200 V and at frequency, 100 KHz.

affects the conductivity for short chain FAs [19]. However, other independent studies show that there are no significant differences in the conductivity as the chain length increases (chain length, C > 12) [20,21]. The tested FAs show differences in peak height and shape (Fig. 2), which is dependent on the chain length and number of unsaturated sites. Taking into consideration of the time required to record three peaks for every FA, it is also interesting to note that the elution time is in agreement to the elution order that was found for the previously reported HPLC-C⁴D work (i.e., myristic > linoleic > palmitic > oleic > stearic acids) [17].

3.1. Optimisation of FIA parameters

For optimum detection using the C⁴D detector, particular attention must be paid to two conditions. Firstly, the carrier stream should have as low conductivity as possibly can [19,22], and secondly; the analyte should be charged [20,23]. As palmitic and other FAs are weak acids (pKa ~4.7); a basic carrier stream with low conductivity will be necessary to ensure that the FAs exist in the ionised form [17]. Sodium acetate, commonly used as HPLC mobile phase component, is suitable as it is a weak base and a weakly conductive salt. It can be expected to provide suitable pH environments to ionise all the FAs [17].

The C⁴D detector frequencies (50–1000 KHz) were studied for five different concentrations of sodium acetate (0.1–2.0 mM) (Fig. 3). The highest peak area was achieved when sodium acetate concentration was 1.5 mM and frequency of 150 KHz was used. As the concentration of sodium acetate increase (>1.5 mM), peak area was found to decrease due to the increase of the conductivity of the carrier stream. However, when the concentration of sodium acetate decreased (≤1.5 mM) the sensitivity start to decrease due to the lower ionisation of the FAs (Fig. 3). pH of the carrier stream was also investigated from pH 6.5 to 8.0. The peak area slightly increased as the pH was increased from 6.5 to 7.5, followed by an abrupt increase in peak area when pH >7.5. pH greater than 8.0 was not recommended due to the negative effects on the preconcentrator column. Therefore pH 8.0 was selected.

The effect of the amount of methanol (78–84%) in the carrier stream on the peak area was also investigated. A slight increase in peak area when methanol more than 80% was used was observed. Therefore, 80% methanol in the carrier stream was selected for subsequent experiments. The effect of flow rate (0.6–1.4 mL min⁻¹) was also investigated. The peak area decreased as the flow rate was increased, while when the flow rate was less than 1.0 mL min⁻¹, peak tailings were observed. Therefore, a carrier stream flow rate of 1.0 mL min⁻¹ was used.

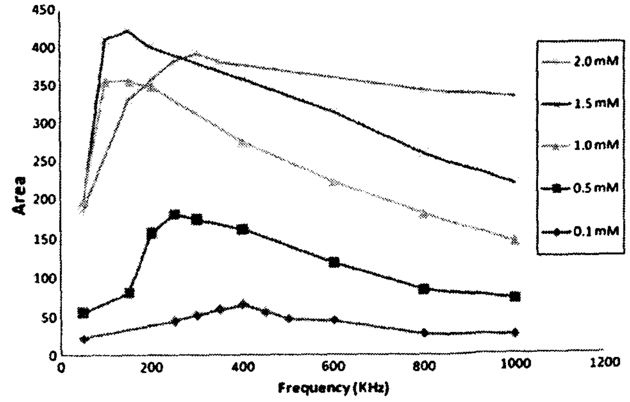

Fig. 3. Effect of the C⁴D frequency on the peak area for different buffer concentrations; palmitic acid concentration, 100 mg L⁻¹; FIA-C⁴D conditions: carrier stream, methanol: sodium acetate (78:22, v/v); flow rate, 1.0 mL min⁻¹; injection volume, 10 μL; amplitude of C⁴D, 200 V.

3.2. Method validation

3.2.1. Linear range

Eight standard solutions of palmitic acid (1–200 mg L⁻¹) were prepared in methanol; each concentration was injected thrice. Good linearity over this concentration range with regression equation ($y = 6.0869x$; where y is the area and x is the concentration in mg L⁻¹) and correlation coefficient 0.9995 was obtained.

3.2.2. Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were calculated at signal/noise ratio of 3 and 10, respectively, using methanol as blank. The LOD and LOQ were 0.28 and 0.85 mg L⁻¹, respectively. The method exhibits the required sensitivity to be tested for all grades of palm oil, including extra pure oil (typical FFA ~0.05%). The LOD value is lower than the reported methods using sequential injection analysis (1.49 mg L⁻¹) or the non aqueous FIA titrimetry methods (6–10 mg Kg⁻¹) [5,6].

3.2.3. Intra- and inter-day precision

Intra- and inter-day precision were performed using three different concentrations of palmitic acid (5, 100, 150 mg L⁻¹). Each concentration was prepared thrice and each preparation was injected thrice. Intra-day precision was tested by injecting the palmitic acid standards in the same day, while inter-day precision was performed over six days. Good precision was obtained as reflected in the low relative standard deviation values (<2.7%, Table 2).

3.3. Adopted FIA conditions

As a compromise between sensitivity and sample throughput, the adopted conditions are: carrier stream, methanol: sodium acetate (1.5 mM, pH 8), (80:20 v/v); flow rate, 1.0 mL min⁻¹;

Table 2
Precision data of peak area (% RSD) for the proposed method.

	Concentration (mg L⁻¹)		
	5	100	150
Intra-day precision ($n = 9$)	2.3	0.93	1.5
Inter-day precision ($n = 54$)	2.7	1.8	1.6

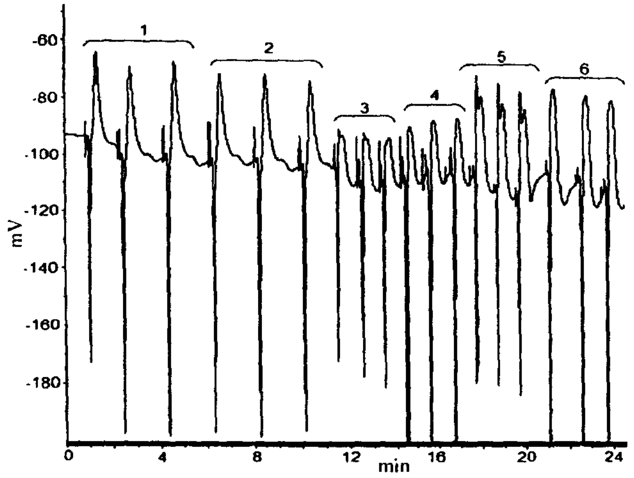

Fig. 5. FIA output from the injections of different oil samples under the optimised conditions as was stated in Fig. 4; Peak label: (1) palm olein oil using LLE, (2) palm olein oil diluted with IPA; (3) walnut oil using LLE; (4) walnut oil diluted with IPA; (5) rice bran oil using LLE; (6) rice bran oil diluted with IPA.

Table 4
Comparison of FFA values of oil samples obtained by FIA-C^4D and MPOB methods ((mg palmitic acid/100 mg oil) ± SD).

Sample	FIA-C^4D	MPOB Method
PO1	0.0622 ± 0.001	0.0612 ± 0.005
PO2	0.0524 ± 0.0003	0.0531 ± 0.006
PO3	0.0579 ± 0.001	0.0590 ± 0.002
CPO1	2.11 ± 0.008	2.33 ± 0.4
CPO2	3.89 ± 0.005	3.69 ± 0.4
SCPO1	19.6 ± 0.007	20.5 ± 0.9
SCPO2	10.0 ± 0.02	10.4 ± 0.6
SRBD palm oil	1.73 ± 0.006	1.83 ± 0.1
Soybean oil 1	0.107 ± 0.005	0.118 ± 0.01
Soybean oil 2	0.102 ± 0.003	0.112 ± 0.01
Corn oil 1	0.0873 ± 0.001	0.0902 ± 0.008
Corn oil 2	0.103 ± 0.002	0.106 ± 0.01
Extra virgin olive oil 1	0.436 ± 0.004	0.442 ± 0.02
Extra virgin olive oil 2	0.871 ± 0.006	0.893 ± 0.009
Rice bran oil	0.120 ± 0.001	0.118 ± 0.003
Walnut oil	0.052 ± 0.002	0.055 ± 0.002

PO, palm olein; CPO, crude palm oil; SCPO, stored crude palm oil; SRBD palm oil, stored refined bleached deodorised palm oil.

carrier stream that is effective in desorbing any adsorbed materials on the surface of the sorbent.

Good agreements of results were obtained from the proposed FIA-C^4D and the MPOB official methods (Table 4). The proposed method is clearly superior over the official method in terms of solvent consumption, speed and the uncertainty associated with the visual detection of end-points for highly coloured samples (e.g., crude palm oil). Moreover, this method has the advantage over the other reported methods using FIA and SIA [3–6] in chemical and solvent consumption, detection limit and acidity ranges. The sampling rate is comparable with the other reported FIA methods but much higher than the SIA method (Table 3). The fact that the sensing components are not in direct contact with the sample and due to the robustness-nature of C^4D detectors, the system offer interesting options for the implementation at various plant locations for long unattended periods. With minor modifications, the method can be easily converted to a fully automated system.

4. Conclusion

A single line FIA method for the rapid determination of FFA in vegetable oils using C^4D has been realised. The success of the method is mainly contributed by the incorporation of a preconcentrator column and the judicious choice of carrier stream conditions. Although the detector shows good reproducibility in the peak height and peak area, peak shapes among some of the oils are different (e.g., myristic and stearic acids). While exhibiting desirable analytical features (e.g., sensitivity, accuracy, precision, high sample throughput), the robustness, simplicity and low maintenance nature of C^4D detectors are additional factors that are superior over the optical flow-through detectors. Furthermore, the performance of the C^4D is not affected by background colour and viscosity changes between oil types. Although the analytical usefulness of the technique was demonstrated for mainly palm oil-based samples, it can be readily extended to not only other vegetable oils but also to biofuels.

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Hollow fiber liquid-phase microextraction coupled with gas chromatography-flame ionization detection for the profiling of fatty acids in vegetable oils

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ABSTRACT

The development of a two phase hollow fiber liquid-phase microextraction technique, followed by gas-chromatography-flame ionization detection (GC-FID) for the profiling of the fatty acids (FAs) (lauric, myristic, palmitic, stearic, palmitoleic, oleic, linoleic, linolenic and arachidic) in vegetable oils is described. Heptadecanoic acid methyl ester was used as the internal standard. The FAs were transesterified to their corresponding methyl esters prior to the extraction. Extraction parameters such as type of extracting solvent, temperature, extraction time, stirring speed and salt addition were studied and optimized. Recommended conditions were extraction solvent, n-tridecane; extraction time, 35 min; extraction temperature, ambient; without addition of salt. Enrichment factors varying from 37 to 115 were achieved. Calibration curves for the nine FAs were well correlated ($r^2 > 0.994$) within the range of 10–5000 μg L^{-1}. The limit of detection (signal:noise, 3) was 4.73–13.21 ng L^{-1}. The method was successfully applied to the profiling of the FAs in palm oils (crude, olein, kernel, and carotino cooking oil) and other vegetable oils (soybean, olive, coconut, rice bran and pumpkin). The encouraging enrichments achieved offer an interesting option for the profiling of the minor and major FAs in palm and other vegetable oils.

1. Introduction

Fatty acids (FAs) are the basic components of most naturally occurring lipids in both animals and plants. The diversity of the chain length, degree of unsaturation, geometry and position of double bonds determine the characteristic of these lipids and their origins [1]. The properties of oils are very much dependent on the FAs profile, which provide information on the chain length, percent saturation, monounsaturation and polyunsaturation. Based on these information, the recommended usage of each oil can be proposed. Profile determination of unsaturated FAs is useful in health care management (e.g., towards the prevention of diseases [2–7].

The most commonly used method for the analysis of FAs involves the determination of the corresponding methyl esters (FAMEs) using capillary gas chromatography (GC) with flame ionization detector (FID) [8]. For lipids, fats and oils, often a transesterification procedure involving the direct conversion of FAs to alkyl esters (particularly methyl esters) by alcohol in the presence of a catalyst is often carried out. The derivatization procedure (especially for the longer chain FAs) is mandatory to increase the volatility and overcome adsorption of the polar functional groups to the GC column [9]. The thermally labile FAs can also be separated using high performance liquid chromatography (HPLC), capillary electrophoresis and supercritical fluid chromatography [10].

Prior to the analytical determination, the FAs or the FAMEs need to be isolated from the sample. This is commonly done using liquid–liquid extraction (LLE), and is used in the official methods recommended by regulatory bodies, e.g., the American Oil Chemists Society (AOCS) and the Malaysian Palm Oil Board (MPOB). Major problems of the LLE technique are the gross consumption of organic solvents, lack of selectivity, time consuming, labour intensive, and the extra evaporation step required prior to analysis to remove the excess solvent. This can lead to contamination problems and possible loss of analytes [11–13].

To overcome these problems and to meet the increasing demands for green approaches in analytical determinations, a considerable amount of effort has been directed to address these issues. In the early 1970s, the solid phase extraction (SPE) technique was introduced [11] and many reports utilized this technique for the analysis of FAs (e.g., determination of the composition of long chain FAs in transesterified palm oil [14], determination of methyl linolenate and methyl linoleate in soy-derived biodiesel [15], and free FAs in beer [16]). More recently, several microextraction and solvent minimized extraction techniques such as the solid phase microextraction (SPME) and liquid phase microextraction (LPME) were introduced. In particular, the hollow fiber LPME, originally

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Sigma-Aldrich (St. Louis, MO, USA). Heptadecanoic acid methyl ester (IS, ≥99.5%) was purchased from Fluka (Buchs, Switzerland). Methanol (HPLC grade), boron trifluoride and n-hexane were purchased from Merck (Darmstadt, Germany). Vegetable oil samples were purchased from local supermarkets and crude palm oil samples were kindly donated by Carotino Sdn. Bhd. (Johor Bahru, Malaysia).

2.2. Materials

Q3/2 Accurel polypropylene hollow fiber membrane (600 μm inner diameter, 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). 4 cm of the hollow fiber was used. The hollow fiber was discarded after single use. A 25 μL Hamilton microsyringe (model 702SNR) with a blunt needle tip was used to introduce the acceptor phase, support the hollow fiber and acts as an injector for the analysis after the extraction. The syringe with the attached hollow fiber was clamped to a retort stand during the extraction. A hot plate stirrer (model GLHPS-G) purchased from Global Lab (Penang, Malaysia) was used for stirring during the extraction.

2.3. Chromatographic conditions

GC analysis was performed using a Clarus 500 GC unit that was purchased from Perkin Elmer (Shelton, CT, USA). A Supelcowax 10 fused silica capillary column of 30 m × 0.32 mm I.D., film thickness 0.25 μm from Supelco (Bellefonte, PA, USA) was used. The chromatographic conditions were performed according to the AOCS procedure (Ce 1e-91) [8]. The oven temperature was programmed as follows: 80 °C (hold for 2 min) at 20 °C min^{-1} to 125 °C (hold for 1 min) then at 3 °C min^{-1} to 220 °C (hold for 5 min). The injector and the FID were operated at 240 °C. Nitrogen was used as carrier gas at a flow rate of 1 mL min^{-1}. A split ratio of 1:10 was applied. Chromatographic data were processed using Total Chrom Workstation version 6.3.1 software. Quantification was done using an eight-points external standard calibration assay.

2.4. Preparation of standards

A stock solution (1000 mg L^{-1}) was prepared by dissolving the nine FAs (lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidic) in boron trifluoride in methanol (14%). The mixture was heated at 80 °C with continuous stirring for 60 min. The transesterified mixture was stored at 4 °C. Working solutions were prepared fresh every day in methanol after spiking with IS (1000 μg L^{-1} for LPME method and 100 mg L^{-1} for LLE method, prepared by direct dilution in methanol).

2.5. Transesterification of samples

Prior to the LPME-GC-FID analysis, a transesterification process was carried out by mixing oil (15 mg) with 0.5 M sodium methanoate (1.25 mL) in a vial (16 mL). The vial was tightly capped and was heated for 5 min at 60 °C. The vial was then cooled and 14% boron trifluoride in methanol (2 mL) was added, and heated (80 °C) with continuous stirring for 60 min. The vial was allowed to cool and the mixture was than diluted to the 100 mL mark with methanol after spiking with IS (1000 μg L^{-1}). For the LLE, 30 g of oil was mixed with 2.5 mL of 0.5 M sodium methanoate. Similar procedure was used except that 4 mL of 14% boron trifluoride was used. The mixture was spiked with IS (100 mg L^{-1}).

2.6. Extraction procedures

For the LPME method, transesterified standard or sample (10 mL) were transferred to a sample vial (16 mL). A magnetic stirrer (15 mm × 5 mm) was placed in the solution. Next, n-tridecane (5 μL) was withdrawn using a syringe. The syringe needle was then inserted into the hollow fiber segment and the assembly was immersed in n-tridecane for 20 s to impregnate the pores of the hollow fiber. Subsequently, the acceptor phase (n-tridecane) was injected into the lumen of the hollow fiber. The hollow fiber was than placed immediately in the sample solution and the sample vial was agitated at 1700 rpm. After a prescribed time of extraction, the magnetic stirrer was switched off and the acceptor phase was carefully withdrawn into the syringe and the hollow fiber was discarded. Finally, the acceptor phase was injected into the GC system. For the LLE method, 2.5 mL of water and 2.5 mL of n-hexane were added to the transesterified cooled mixture, vortexed for 15 min and the upper layer was injected into the GC system.

2.7. Validation of the LPME procedure

Linearity of the calibration curve was established after the extraction of the FAMEs standard solutions at eight different concentrations and three replicates were prepared at each level. The limits of detection (LOD) were calculated as the minimum concentration providing chromatographic signals three times higher than the background noise. The repeatability of the procedures was evaluated by carrying out six replicates of a mixture of the FAMEs that was prepared in methanol within the same day at three concentrations (50, 500, 5000 μg L^{-1}). The mixtures were subjected to the LPME procedure and analysed using GC-FID.

3. Results and discussion

Generally, the chemical analysis of vegetable oils is difficult due to the complexity of the sample as they contain a diverse range of major and minor components. The intense colour of some oils (e.g., crude palm and carotino cooking oils) and its viscosity adds to the complication.

3.1. LPME conditions

Several parameters (e.g., type of organic solvent, temperature, extraction time, stirring speed and salt addition) that influence the extraction efficiency were studied and optimized. To evaluate the effects of these parameters, a mixture that contained 500 μg L^{-1} of each FAME and spiked with IS (1000 μg L^{-1}) was used.

3.1.1. Selection of organic solvent

The choice of extracting organic solvent is generally important in extraction work. For the hollow fiber LPME technique, the selected solvent should be effectively impregnated in the pores of the fiber. In addition, the solvent must be of low volatility, immiscible with methanol (used in the transesterification step) and suitable for the GC analysis. Some common solvents such as n-hexane, cyclohexane, n-heptane, iso-octane and n-octane were not considered due to their volatility and difficulty to sample from the hollow fiber lumen. The use of n-decane resulted in low enrichments for all the FAMEs studied. However, significant improvements in the extraction were found when n-tridecane and n-hexadecane were used, but between these two solvents, n-tridecane is preferred as it offered higher enrichments (Fig. 2). The use of n-tridecane and n-hexadecane resulted in better extraction efficiencies due to their low polarity (log P = 7.5 and 8.8, respectively) when compared to n-decane (log P = 5.6) [25,26]. The low affinity for n-hexadecane to the

Table 1
Analytical characteristics of the proposed LPME-GC-FID method.[a]

FA	r^2	LOD (ng L^{-1})	Enrichment factor
$C_{12:0}$	0.9945	13.2	36.9
$C_{14:0}$	0.9999	7.92	72.5
$C_{16:0}$	0.9994	6.31	69.2
$C_{18:0}$	0.9984	5.78	115.0
$C_{20:0}$	0.9983	5.53	112.2
$C_{16:1}$	0.9997	6.50	91.9
$C_{18:1}$	0.9987	4.73	66.5
$C_{18:2}$	0.9993	6.01	51.6
$C_{18:3}$	0.9998	9.47	76.5

[a] Linearity range of FAs studied, 10–5000 μg L^{-1}.

time, 35 min at ambient temperature without the addition of salt.

The overall extraction efficiency was evaluated by the enrichment factor (EF). As it is impossible to detect the target analytes in the donor phase at very low concentrations, a reference standard (50 mg L^{-1} in methanol) was directly injected into the GC system. EF was calculated according to the formula:

$$EF = \frac{C_a}{C_d}$$

where C_a is the concentration of analyte in the reference standard (50 mg L^{-1}) and C_d is the concentration of analyte obtained from the calibration curve of the analyte after the LPME extraction. Under these extraction conditions, respectable enrichment factors ranging from 37 to 115 were achieved (Table 1).

3.3. Method validation

3.3.1. Linearity

Eight different concentrations (10–5000 μg L^{-1} for LPME and 10–150 mg L^{-1} for LLE) of standard solutions, each containing a mixture of nine FAMEs spiked with IS were injected into the GC column. Each mixture was injected thrice. The ratio of peak area of FAME to that of the IS was plotted against concentration of the FAME. The LPME method shows good linearity with correlation coefficient ($r^2 > 0.994$) (Table 1).

Table 2
Repeatability (%RSD) in the determination of the nine FAs using the proposed LPME-GC-FID method.

FA (μg L^{-1})	%RSD								
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
50	6.84	9.17	10.0	10.2	10.4	10.8	12.5	10.2	5.37
500	6.66	2.45	1.76	2.37	3.43	2.31	2.07	1.51	2.91
5000	3.72	1.97	1.67	1.48	1.78	1.43	1.12	1.01	1.89

$n = 6$.

3.3.2. Limit of detection (LOD)

The proposed LPME method gave comparable LODs for the nine FAMEs (4.73–13.2 ng L^{-1}) compared to the ion-pair dynamic fiber LPME-GC-MS (9.3–15 ng L^{-1}) [13], but is significantly lower compared to the reported methods using LLE-GC-FID (700–800 μg L^{-1}) [31], the dispersive liquid–liquid microextraction-GC-FID (0.67–1.06 μg L^{-1}) [24] and the SPME-GC–MS (0.51–170 μg L^{-1}) [12].

3.3.3. Repeatability

A satisfactory relative standard deviation (RSD) of the peak areas was obtained (1.01–12.5%) for the tested FAs (Table 2).

3.4. Analysis of vegetable oils

The proposed method was applied for the profiling of FAs in palm oils (crude, olein, kernel and carotino cooking oils) and other vegetable oils (soybean, olive, coconut, rice bran and pumpkin oils). Quantification was done using an eight points external standard calibration assay of the ratio of peak area to that of the IS versus concentration of analyte. Qualitative analysis was done by comparing the retention times to those of the standards. Using the LLE in conjunction with GC-FID as suggested in the standard AOCS and MPOB methods, only the major FAs ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$) can be determined in crude and olein palm oils (Table 3). The minor FAs ($C_{12:0}$, $C_{20:0}$, $C_{16:1}$, $C_{18:3}$) cannot be determined using the standard methods. Reasonable agreement of the major FAs was found between the LPME results and the standard method for the samples tested. The enormous complexity of the matrix especially the crude palm and carotino cooking oils do affect the extraction process. The

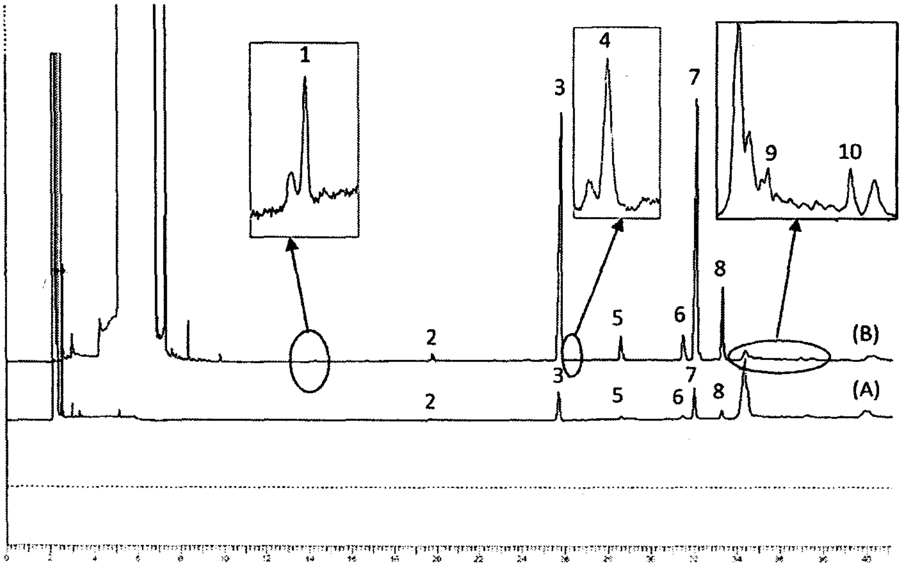

Fig. 6. Profiling of FAs of palm olein sample after (A) LLE, and (B) LPME. Peak assignments: (1) lauric, (2) myristic, (3) palmitic, (4) palmitoleic, (5) IS, (6) stearic, (7) oleic, (8) linoleic, (9) linolenic, and (10) arachidic acids.

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Determination of underivatized long chain fatty acids using RP-HPLC with capacitively coupled contactless conductivity detection

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method with capacitively coupled contactless conductivity detector (C^4D) has been developed for the separation and the simultaneous determination of five underivatized long chain fatty acids (FAs), namely myristic, palmitic, stearic, oleic, and linoleic acids. An isocratic elution mode using methanol/1 mM sodium acetate (78:22, v/v) as mobile phase with a flow rate of 0.6 mL min^{-1} was used. The separation was effected by using a Hypersil ODS C_{18} analytical column (250 mm × 4.6 mm × 5 μm) and was operated at 45 °C. Calibration curves of the five FAs were well correlated ($r^2 > 0.999$) within the range of 5– 200 μg mL^{-1} for stearic acid, and 2–200 μg mL^{-1} for the other FAs. The proposed method was tested on four vegetable oils, i.e., pumpkin, soybean, rice bran and palm olein oils; good agreement was found with the standard gas chromatographic (GC) method. The proposed method offers distinct advantages over the official GC method, especially in terms of simplicity, faster separation times and sensitivity.

1. Introduction

Fatty acids (FAs) are widely found in nature (e.g., food products, vegetable oils, and living organisms) and are vital as nutritional substances and metabolites in living organisms. They form the basic components of most naturally occurring lipids in both animals and plants. The diversity of the chain length, degree of unsaturation, geometry and position of double bonds, as well as the presence of other groups, render their composition the most definitive characteristic of these lipids and their origin [1]. Analysis of FAs is important in the control of technical products, in medical diagnostics, cancer research and in the testing of purity, origin, and shelf-life studies of food products, and in biodiesel projects [2].

The most commonly used method for the analysis of FAs involves the determination of the corresponding methyl esters using capillary gas chromatography (GC) with flame ionization detector (FID) [2]. A derivatization procedure is mandatory to increase the volatility and overcome adsorption of the polar functional groups to the GC column [3]. In general, GC provides excellent separation and quantification together with acceptable sensitivity. However, there is a growing interest in the use of high-performance liquid chromatography (HPLC) for studying FAs. The major advantages of HPLC over GC are the lower temperatures required during the analysis (reduce the risk of isomerisation of double bonds) and the possibility of collecting fractions for further analysis. Apart from that, HPLC is considered more flexible as the retention characteristics can be easily modified by varying the composition of mobile phase.

HPLC analysis of the FAs is complicated as these analytes are neither UV nor fluorescence-active. Thus, derivatization procedures allow the "tagging" of chromophore or fluorophore to the analyte, rendering them to be detected using UV–vis or fluorescence detector [4–8].

While the derivatization procedure used to increase the HPLC sensitivity markedly (especially for fluorescence detection), the derivatization process itself, however, is not preferred. Inherent problems include not only the longer analysis time required, but also the possibility of inaccurate results due to incomplete or unstable reaction with the derivatization compounds; unselective labelling that leads to interfering by-products; and the expensive and unstable nature of some derivatization reagents. There is thus, a strong emphasis lately on developing alternative methods to the traditional HPLC methods that require UV and fluorescence [4–8] for detection. HPLC methods for the determination of underivatized FAs involving detectors such as mass spectrometer [3,9], chemiluminesce [1,10], electrochemical [1], refractive index [11], and evaporative light scattering detectors (ELSD) [2,12] have been reported.

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and 14% boron trifluoride in methanol (1.5 mL) and transferred to a 15 mL vial. The vial was capped and was heated at 80 °C with stirring for 60 min. Water (2.5 mL) and hexane (2.5 mL) were added to the cooled mixture. The mixture was vortexed for 15 min and the upper layer was separated and injected into the GC column.

2.5. Optimization C^{4}D

Many variables (e.g., sodium acetate concentration and mobile phase composition) can affect the performance of the C^{4}D detector. Since different concentrations of sodium acetate (0.1–10 mM) were investigated, the mobile phase composition was also investigated over the range of 78–83% methanol. The amplitude was fixed at 100 V, while the frequency was optimized from 50 to 500 kHz.

3. Results and discussion

In order to separate the FAs several parameters were considered. Initially, different columns (Chromolith RP-18e 100 mm × 4.6 mm, Chromolith RP-18e 100 mm × 3 mm, Inertsil ODS-3 C_{18}, 250 mm × 4.6 mm × 3 μm, and Hypersil ODS C_{18}, 250 mm × 4.6 mm × 5 μm) were investigated for the separation of the FAs. Most of these columns do not result in the satisfactory separation of OA and PA. Furthermore, tailing or fronting was observed for some peaks [13]. However, more promising results were obtained when the Hypersil ODS C_{18} column was used as it resulted in not only good separation among the FAs but also the peaks were more symmetrical. Thus this column was used.

Since the C^{4}D detector response is very much influenced by variation in the mobile phase composition, isocratic elution was applied. Gradient elution was attempted by some authors, but sloping of baseline was observed [18]. Nevertheless, this problem was overcame by the authors using suitable softwares [18].

3.1. Optimization of chromatographic conditions

3.1.1. Selection of mobile phase

An important strategy in the method development involving C^{4}D detector is that it is important for the analytes to be charged under the operated conditions; moreover the mobile phase should have low conductivity as possibly can [18]. As the long chain FAs are weak acids (pK_a around 4.7), a basic mobile phase will ensure that the FAs will be in the ionised form and at the same time possess low conductivity [18]. Acetic acid and its salts are frequently used as HPLC mobile phase with organic solvents due to its low conductivity and can provide suitable pH environments to ensure that the analytes are in the ionized form [13,18]. It had been suggested that buffers with specific conductivity less than 1 mS cm^{-1} are able to provide the necessary background conductivity [18,20]. Sodium acetate seems to fit these criteria and was thus selected. Different concentrations of sodium acetate (0.1–10 mM) were investigated under the optimum frequency and amplitude for each concentration of sodium acetate. The best sensitivity and separation was achieved using 1 mM sodium acetate for all the FAs. As the concentrations of sodium acetate decrease ($\leq$10 mM), peaks areas were found to increase due to the decrease of the conductivity of the mobile phase. However, when the concentration of sodium acetate was less than 1 mM the sensitivity start to decrease due to the environment that do not favour the ionization of the FAs. Peak fronting was also observed when less than 1 mM sodium acetate was present (Fig. 2).

Methanol and acetonitrile are among the most widely used mobile phase components in HPLC separations. Using methanol resulted in better sensitivity than acetonitrile, thus methanol was used. The effect of varying methanol composition 78–83% was also investigated. Slightly higher peak area for the FAs was achieved using 81% methanol, but 78% methanol was chosen as it offers better separation between PA and OA, and thus this composition was used for the remaining studies.

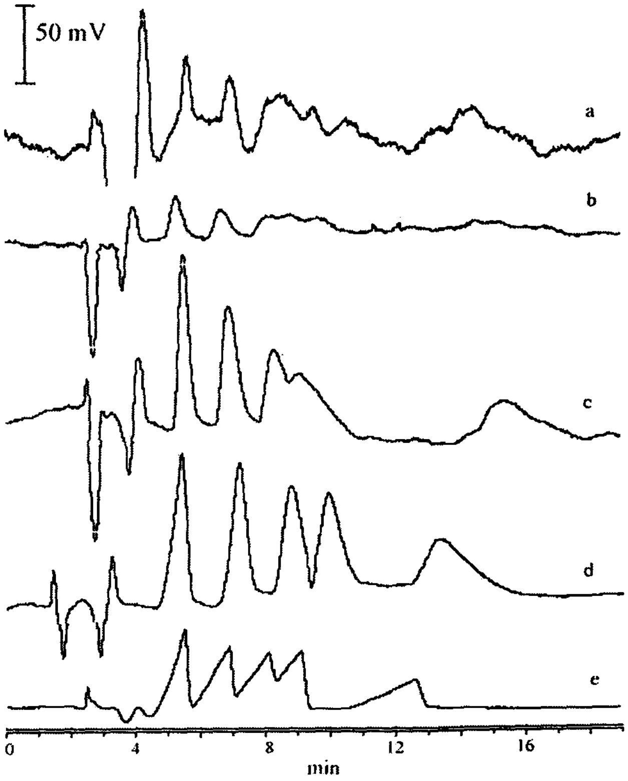

Fig. 2. Influence of sodium acetate concentration on the separation of five FAs using HPLC-C^{4}D method. Column, Hypersil ODS 250 mm × 4.6 mm × 5 μm; flow rate 0.6 mL min^{-1}; mobile phase composition 78% methanol, 22% sodium acetate. Concentration of FAs; 80 μg mL^{-1}, sodium acetate concentration: (a) 10 mM, (b) 5 mM, (c) 2.5 mM, (d) 1 mM, and (e) 0.1 mM.

3.1.2. Selection of detector conditions

The detector should be operated at its optimum frequency, which is as low as possible to minimize the effect of stray capacitance [18]. The detector amplitude was fixed at 100 V, while the frequency was varied from 50 to 500 kHz. The highest signals of the FAs were found using 100 kHz, thus 100 kHz was used. Furthermore, more stable baseline was found. Typical chromatogram when operated under the adopted conditions is shown in Fig. 3. The FAs are all separated in about 15 min.

3.2. Method validation

3.2.1. Linearity of calibration

Linearity was checked by preparing standard solutions of FAs at eight different concentrations using the stock solution. Each solution was injected thrice. The method showed a good linearity over 5–200 μg mL^{-1} for SA, and 2–200 μg mL^{-1} for the other four FAs (Table 1).

3.2.2. Limit of detection (LOD)

The LOD was estimated at a signal-to-noise ratio of 3. The HPLC-C^{4}D method exhibited significantly lower LOD values for the five FAs (0.1–0.6 μg mL^{-1}) (Table 1) compared to the GC-FID (0.7–0.8 μg mL^{-1}) and the reported methods using HPLC-ELSD (1.5–5 μg mL^{-1}), and CE-C^{4}D (10.5–12.3 μg mL^{-1}) [2,17]. The low LOD was attributed to the low baseline noise of the detector for the selected mobile phase and frequency used.

3.4. Analysis of vegetable oil samples

The proposed method was applied to the analysis of FAs in soybean (*Glycine max* L.), rice bran (*Oryza sativa* L.), pumpkin seed (*Cucurbita pepo* L.) and palm olein oil (*Arecaceae Elae*) oil samples. MA was found only in palm olein oil, consistent with literature values [21]. Soybean and palm olein oils were selected as they are among the most widely used vegetable oils whereas pumpkin oil and rice bran oils were selected as they are commonly added in functional foods. The levels of the FAs were in good agreement with the GC–FID method (Table 3). The tailing of the stearic acid peak does not seem to affect the results. It can be readily seen that the HPLC-C^4D method gave better reproducibility than the GC–FID method. Typical chromatograms are shown in Fig. 4. The separation time was significantly shorter (~15 min) compared to the GC–FID method (~40 min).

4. Conclusion

An alternative analytical method for the determination of underivatized FAs using reversed-phase HPLC using C^4D detection was developed using methanol/1 mM sodium acetate (78:22, v/v) as mobile phase. Under the adopted conditions, separation of the analytes was achieved in about 15 min. The method was superior to the standard GC–FID method both in terms of speed and sensitivity. Furthermore, the GC–FID method requires the mandatory liquid–liquid extraction step and derivatization to methyl esters prior to the analysis. Good agreement of the levels of FAs was found between the proposed method and the GC–FID method when applied to the analysis of soybean, palm olein, pumpkin and rice bran oils.

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