QUALITATIVE AND QUANTITATIVE ANALYSIS OF ORGANOSULPHUR COMPOUNDS IN SELECTED FUEL SAMPLES

by

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submitted in accordance with the requirements for
the degree of

MASTER OF SCIENCE

in Chemistry

at the

UNIVERSITY OF SOUTH AFRICA

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December 2022

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KEY TERMS:

Organosulphur compounds, crude oil, diesel, gasoline, kerosene, gas chromatography, time-of-flight mass spectrometry, magnetic solid phase extraction, dichloromethane, nano materials.

ACKNOWLEDGEMENTS

Above all, I would like to first thank God.

My sincere gratitude to my supervisor Dr Nomvano Mketo, who moulded, grounded, encouraged and supported me during this project. I would also like to express my appreciation and recognition to my co-supervisors; Dr Vimbai Mhuka and Prof Nomso Hintsho-Mbita for their support and contribution towards making this project a success.

My sincerest appreciation goes to the University of South Africa Women in Research and NRF-Thuthuka for their financial support during the process of completing the project.

My extended gratitude to Sol Nety and Belete for their help during the project, which includes the learning of GC-HR-ToF-MS amongst other things.

The Analytical Research Group always ensured that I put a smile on my face even when the situation was daunting, is also recognized and applauded. Their warmth and charisma will forever be cherished.

Lastly, I would like to pass my warmest and sincere gratitude to my parents who supported me through thick and thin.

PUBLICATION(S)

1. S. S. Mgiba, V. Mhuka, N. C. Hintsho-Mbita & Nomvano Mketo (2022) *Trends in the Direct and Indirect Chromatographic Determination of Organosulfur Compounds in Various Matrices*, Separation & Purification Reviews, 51:1, 117-129, DOI: 10.1080/15422119.2020.1866011, **published**.
2. S. S. Mgiba, V. Mhuka, N. C. Hintsho-Mbita & Nomvano Mketo, *Selective magnetic solid phase extraction based on Au-*Fe_3O_4 *adsorbent for chromatographic determination of organsulphur compounds in selected fuel oil samples*, **in preparation**.

ABSTRACT

Crude oil is a complex mixture of hydrocarbons, non-hydrocarbons and heteroatom-containing compounds. This fossil fuel undergoes refining processes to produce different fuel oils, which contributes one third to one-half of the world's energy supply. The presence of organosulphur compounds (OSCs) in fuel oils is undesirable because these compounds emit sulphur oxide (SO_x) gases to the atmosphere, corrode vehicle engines and deactivate catalyst during the refinery processes of crude oil. On the other hand, the identification and quantification of OSCs in fuel oils has been a challenging task. This is due to their volatile nature, the substituents structure of some OSCs such as polycyclic aromatic sulphur heterocycles (PASH) and the presence of other polycyclic hydrocarbons in high concentration, which causes interferences during the chromatographic analysis. Therefore, there has been a progress on development of various analytical methods for both qualitative and quantitative determination of OSCs in fuels.

Therefore, this study reports a rapid and efficient method for the qualitative analysis of OSCs in fuel oil samples. A simple sample dilution with dichloromethane was conducted for qualitative analysis of the OSCs in crude oil, kerosene, gasoline and diesel samples using gas chromatographytime of flight-mass spectrometry (GC-T-TOFMS). Diesel sample showed the largest number of OSCs (26) namely: Thiophene, 3-methyl; 2-Thiophenecarboxylic acid, 4-nitrophenyl ester; Acetic acid, mercapto-; Thiazol-2-amine, N-(4-dimethylaminobenzyl)-; benzo[b]thiophene; Thieno [3,4-d]-1,3-dioxole, tetrahydro-2,2-dimethyl-, 5,5-dioxide; dibenzothiophene ; Sulfurous acid, octyl 2-propyl ester; Benz(1,4) oxathiino (2,3-c)pyridine; 3-hydroxy-2-thiabutane; 2-Thiopheneacetic acid, isopropyl ester; 3H-[1,3,4]Oxadiazole-2-thione, 5-(4-fluorophenyl)-3-(piperidin-1-yl)methyl-; 2-Butanone, 3,3-dimethyl-1-thiocyanato-; 3-Acetyl-2,5-dichlorothiophene; 2(3H)-Thiazolethione, 4-methyl-; 1,3-Diphenyl-4H-1,2,4-triazoline-5-thione; Benzothiazole, 2-(m-tolyl)-; 1,3-Diphenyl-4H-1,2,4-triazoline-5-thione; Ethylamine, N,N-dinonyl-2-phenylthio; 1,3-Diphenyl-4H-1,2,4-triazoline-5-thione; 1,3-Diphenyl-4H-1,2,4-triazoline-5-thione; 10H-Phenothiazine, 2-(trifluoromethyl)-; 1,3-Diphenyl-4H-1,2,4-triazoline-5-thione; 1-(4-Methyl-dibenzothiophen-2-yl)-piperidine; 2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate; Difluoro(methylamino)phosphine sulfide followed by crude oil (10), kerosene (11) and gasoline with 8 OSCs, namely: Thiophene, 2-(1,1-dimethylethyl); 3-Picoline, 2-(tert-butylthiol); 2-Thiophenecarboxylic acid, 4-nitrophenyl ester; methanesulfonamide, N-[2-(octylamino)ethyl]; Tetramethyl diphosphane-oxide-sulfide; Sulfurous acid, 2-ethylhexyl hexyl ester; 2-Thiophenecarboxylic acid, 5-tert-butyl and Thiophene, 2-nitro.

The study also describes the development of environmentally friendly, effective, and selective pre-concentration method of OSCs in fuels, followed by GC-TOF-MS for both qualitative and semi-quantitative analysis. The magnetic solid phase extraction (m-SPE) based on Au-Fe_3O_4 adsorbent was selected as the preconcentration method for selected OSCs (thiophene; 3-methylthiophene; benzothiophene and dibenzothiophene). The Au-Fe_3O_4 NPs were synthesized using a co-precipitation method and characterized using XRD which produced diffraction peaks for gold coated magnetite with miller indices of Au (111), (200),(220), (311) and (222). UV-Vis was also used and a surface plasmon resonance peak at around 520 – 523 was produced. TEM and SEM confirmed the spherical shape and the size of 100 nm of the nanoparticles. Lastly, FTIR confirmed the particles with vibrational peaks at 500 cm^{-1} for Fe-O bond, 1500 cm^{-1} and 1750 cm^{-1} for C=O and –OH groups. The most influential parameters (mass of the adsorbent, extraction time, eluent volume and pH of the sample) affecting m-SPE were investigated by using multivariate mathematical tools. The best optimum conditions were found to be 150 mg mass of adsorbent, 100 µL eluent volume of acetonitrile, 50 minutes extraction time and 6,5 pH of the sample. These optimum conditions showed relatively low limits of detection (0,02-0,199 µg/L) and limit of quantification (0,126-0,602 µg/L) for all four of the investigated compounds. Furthermore, precision of 0,2 - 0,8% was obtained with good linearity of 0,9816-0,9961. The extraction efficiency of the proposed m-SPE method ranged from 76 to 95% for the spiked samples (4- 12 µg/L spike concentration level). The optimized m-SPE method was then applied toreal fuel oil samples and the concentration levels of non-target OSCs in crude oil, gasoline, diesel and kerosene ranged from 0,126 – 3,06 µg/L. Therefore, the developed m-SPE method prior to GC-HR-ToF-MS analysis can be applied as an alternative for quantitative determination of OSCs in oily matrices.

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LIST OF ABBREVIATIONS AND ACRONYMS

µ-SPE - Solid phase microextraction

AED - Atomic emission detector

APS cartridge - Aminopropylsilane cartridge

ASE - Accelerated solvent extraction

BT - Benzothiophene

CAR-PDMS - Carboxen polydimethylsiloxane

CCD - Central composite design

DBT - Dibenzothiophene

DCM - Dichloromethane

DI-SDME - Direct single-drop micro-extraction

DLLME - Dispersive liquid-liquid micro-extraction

Dynamic HS - Dynamic headspace

ESI - Electronspray ionization

FID - Flame ionization detector

FLD - Flouresence detector

FM-GC×GC-MS/MS - Flow modulated comprehensive two-dimensional gas chromatography-triple quadrupole mass spectrometry

FPD - Flame photometric detector

FTIR - Fourier transform infrared

GC –HR- ToF-MS - Gas chromatography high resolution time-of-flight spectrometry

GC - Gas chromatography

GC×GC-AED - Comprehensive two-dimensional gas chromatography atomic emission detector

GC×GC-FID - Comprehensive two-dimensional gas chromatography flame ionization detector

GC×GC-MS - Comprehensive two-dimensional gas chromatography mass spectrometry

GC×GC-SCD - comprehensive two-dimensional gas chromatography sulphur chemiluminescence detector

GC×GC-ToF-MS - Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry,

GC-FID - Gas chromatography flame ionization detector

GC-FPD - Gas chromatography flame photometric detector

GC-ICP-MS - Gas chromatography inductively coupled plasma mass spectrometry

GC-MS - Gas chromatography mass spectrometry

GC-MS/MS - Gas chromatography tandem mass spectrometry

GC-MS-NCI - Gas chromatography negative ion chemical ionization mass spectrometry

GC-O-MS - Gas chromatography olfactometric mass spectrometry

GC-PFPD - Gas chromatography pulse flame photometric detector

GC-Q-ToF-MS - Gas chromatography quadruple time-of-flight mass spectrometry

GC-SCD - Gas chromatography sulphur chemiluminescence detector

HF-LLME - Hollow fibre liquid-liquid microextraction

HPLC - High performance liquid chromatography

HPLC-UV - High performance liquid chromatography ultraviolet

HS-SDME - Head space single-drop microextraction

HS-SPME - Head-space solid phase microextraction

HT-GC×GC-SCD - High temperature comprehensive two-dimensional gas chromatography sulphur chemiluminescence

LC - Liquid chromatography

LC-MS - Liquid chromatography mass spectrometry

LLE - Liquid-liquid extraction

LOD - Limit of detection

LOQ - Limit of quantification

MAE - Microwave assisted extraction

MALDI - Matrix assisted laser desorption ionization

MS - Mass spectrometry

MSM - Methylysulphonyl methane

mSPE - Magnetic solid phase extrcation

N/A - Not available

NBT - Naphthobenzothiophene

ND - Not detected

NDBT - Naphthodibenzothiophene

NP – Nanoparticles

NPLC-GC/MS - Normal phase liquid chromatography-gas chromatography mass spectrometry

OSCs - Organosulphur compounds

PDMS - Polydimethylsiloxane

PDMS-DVB - Polymethylsiloxane divinylbenzene

RSD - Relative standard deviation

SBSE - Stir bar sorbtive extraction

SCD - Sulphur chemiluminiscene detector

SEM - Scanning electron microscopy

SFE - Supercritical fluid extraction

SPE - Solid phase extraction

SPME - Solid phase microextraction,

TD-GC-MS - Thermal desorption gas chromatography mass spectrometry

TEM - ransmission electron microscopy

TLC- Thin layer chromatography

TP - Thiophene

UAE-LLME - Ultrasound assisted-emulsification liquid-liquid microextraction

UE - Ultrasonic extraction

UHPLC - Ultra-high-performance liquid chromatography-mass spectrometry

UV - Ultraviolet

XRD - X-ray powder diffraction

CHAPTER I

INTRODUCTION

PREAMBLE

A brief background of crude oil as a fossil fuel and its origin is discussed in this chapter. Under the background section, crude oil refinery process is elaborated to produce other fuel oils. Different examples of selected OSCs are also outlined together with their negative effects. The chapter further confers on the problem statement, aims, hypothesis and the justification of the study. Lastly, the outline of the entire dissertation, which explains the content of each chapter, is summarized.

1.1. Background

Crude oil is a naturally occurring mixture of hydrocarbons and non-hydrocarbons, which makes up fossil fuel in a liquid state. Crude oil is regarded as a fossil fuel because of its origin, as it forms from fossilized buried remains of carbon rich plants and animals that lived decades of years ago [2]. The high pressures, high temperatures, microbiological activities and geological processes of the earth convert these remains into crude oil [3]. It is found in the reservoirs, cracks, narrow openings and pores of the sedimentary rocks underground; thus, it is accessible through drilling. Crude oil is sometimes found on the earth surface in the tart sands [4]. **Figure 1.1** illustrates the formation and the location of crude oil.

Crude oil is regarded as one of the essential energy sources for transportation and other industrial operations around the world. It is considered as such because, the buried crude oil is extracted from the deeper surface of the earth and refined into important products such as kerosene, diesel, gasoline and other petroleum products [5].

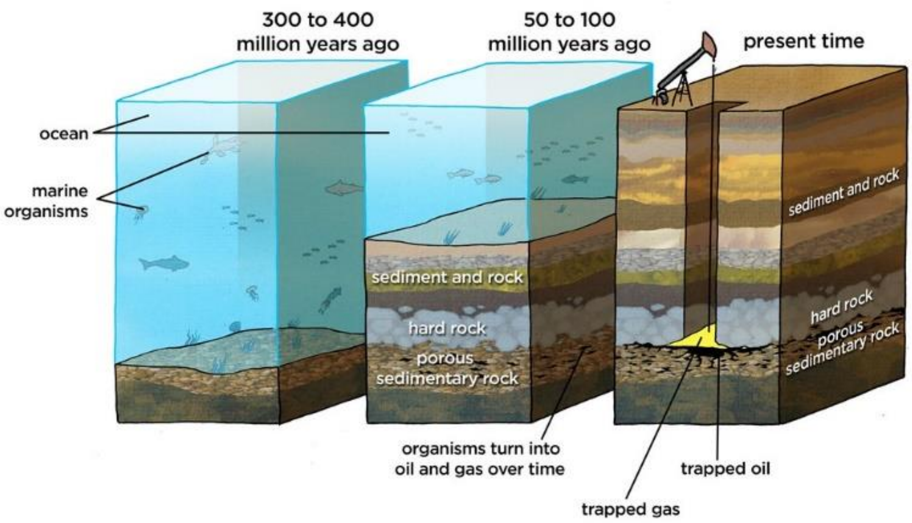


Figure 1.1 Illustration of fossil fuels formation and location underground.

1.2. Crude oil composition

Hydrocarbons are a major component of crude oil and they contain carbon atoms which forms different structures that can either be linear or branched [6]. The most dominant fractions in crude oil are paraffin, naphtha and aromatics (toluene and xylene as derivatives of benzene rings). Furthermore, isoalkanes and methyl cycloalkanes are the main constituents of paraffin hydrocarbons. Naphtha on the other hand, is mostly composed of five to six-membered rings, however, seven membered rings can occasionally be observed [7]. Non-hydrocarbons contain derivatives of nitrogen, sulphur, oxygen and various metal ions in trace concentration levels [8]. **Figure 1.2** represents the different compositions of crude oil.

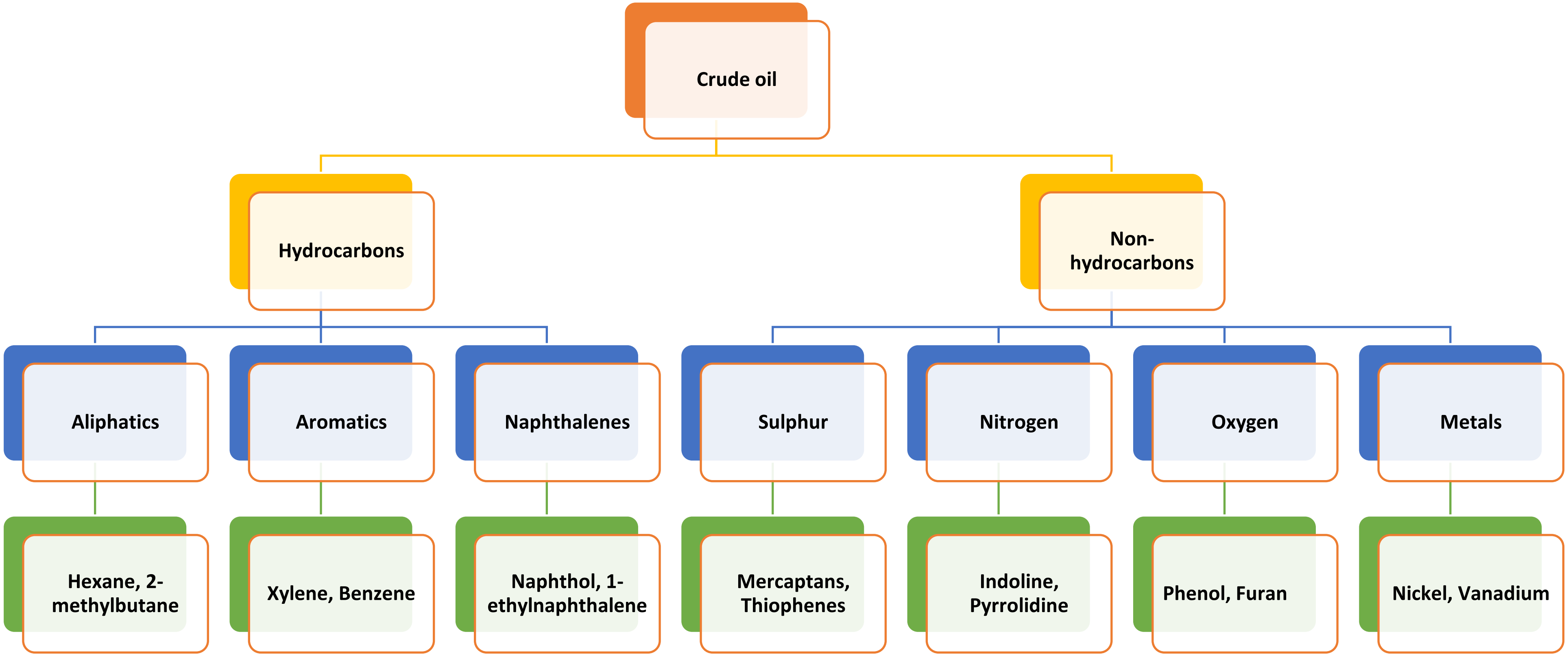


Figure 1. 2 Molecular composition of crude oil [7].

Hydrocarbons contribute up to 60% of crude oil. The dominating paraffin structures contribute to the type of crude oil. When short chained paraffin are contained in crude oil, the crude oil is deemed light crude oil [9]. Aromatics are also unsaturated hydrocarbons that make up to 30% of crude oil [10].

1.3. Classification of crude oil

Crude oil can be classified using both physical and chemical properties. The basic physiochemical properties of crude oil are the same throughout, even though they originate from different geographic locations. However, the location determines the nature of crude oil and its composition [11]. Furthermore, crude oil samples that are extracted from the same well at different times can also differ in terms of chemical composition and physiochemical properties [7]. Fossil fuel can be divided into heavy and light fractions, where heavy oils flow slowly as compared to light oils. Therefore, physical properties such as viscosity and density are normally used to distinguish between the heavy and light crude oils. Light crude oil is less viscous, and its density is less than that of heavy crude oil. Additionally, light crude oil is less expensive to process as compared to the heavy crude oil. This is because, heavy oil contains long chains of hydrocarbons, which are difficult to distillate [12]. Another criterion used for crude oil classification is the American Petroleum Institute (API) degrees and **Table 1.1** shows the classification of crude oil according to API [13].

Table 1. 1 Classification of crude oil by API

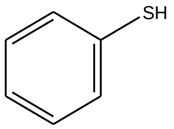
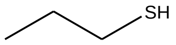
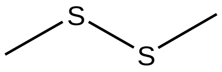
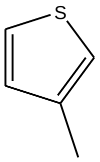
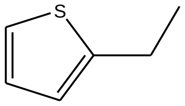
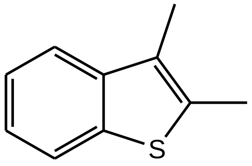
Crude oil	API^{o}
Light crude oil	<31.1^{o}
Medium crude oil	31.1 -22.3^{o}
Heavy crude oil	22.3 -10^{o}
Extra heavy crude oil	>10^{o}

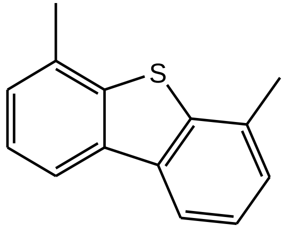
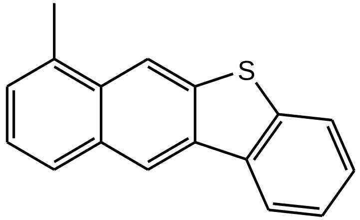
It is well documented that sulphur is the most abundant heteroatom in crude oil with 0.03-6%wt. concentration levels [14]. Therefore, crude oil always contains certain types of OSCs. Crude oil that has higher density generally has a low API degree. This kind of crude oil contains a high content of OSCs. The presence of these OSCs are undesirable/detrimental in crude oil products due to their harmful effects. For example, the presence of OSCs in diesel and gasoline promotes corrosion of engine parts on vehicles, leading to the production of water-containing sulphur dioxide from the exhaust as a result of the combustion process that takes place [15]. Crude oil can be differentiated further with the concentration level of OSCs. Sweet crude oil contains less than 0.5% of sulphur, whilst above 0.5% content, it is regarded as sour crude oil [7]. Crude oil with a low sulphur content is desirable in the finished product thus, sweet crude is always preferable than the sour crude oil. Refining of sweet crude oil is easier compared to sour crude oil, since less sulphur results in less damages in the refineries [16]. The OSCs include mercaptans/thiols, thiophenes, just to name the few. Inorganic sulphur species are sulphates, thiosulphate, sulphides and sulphites [17]. For this study, the focus was on thiophene and its derivatives and **Table 1.2** shows selected structural examples of typical OSCs.

Thiophenes are one group of OSCs contained in crude oil. These are a five-membered electron-rich heteroatomic OSCs that are liquid at room temperature. The thiophene compounds are toxic and flammable. They are insoluble in water but soluble in organic solvents such as alcohol and ether [18]. Polycyclic aromatic heteroatomic sulphur compounds (PASHs) include dibenzothiophenes (DBT), benzothiophenes (BT) and Naphthobenzothiophenes (NBT). These PASHs are the most dominating and challenging OSCs to remove during the process of refining, due to their aromatic ring character [19]. Thiols are also knowns as mercaptans and they are analogous to alcohols. They are identified by the –SH group and characterized with a pungent odour [20].

The concentration levels of OSCs in crude oil varies in relation to the geographical location of the crude oil [21]. For example, Nigeria contains between 0.04 - 0.25 w/w% and Egypt, which is another African country contains 0.04-4.19 w/w% of OSCs in crude oil. European countries such as Norway and Denmark contain between 0.04-0.67 w/w% and 0.2- 0.25 w/w % of sulphur, respectively [22].

Table 1. 2 Examples of selected OSCs [23]

Organosulphur group	Molecular formula	Organosulphur example	Skeletal structure
Thiol/Mercaptan	R-SH	Thiophenol	
		Propanethiol	
Disulphide	R-S-S-R	Dimethyl disulphide	
Thiophene (Th)	C_4H_4S	3-methyl thiophene	
		2-ethyl thiophene	
Benzothiophene (BT)	C_8H_6S	2,3-dimethyl benzothiophene	

Dibenzothiphene (DBT)	$C_{12}H_8S$	4,6-dimethyl dibenzothiophe	
Naphthobenzothiophene (NBT)	$C_{16}H_{10}S$	7-methylbenzo[b]naphtho[2,3-d] thiophene	

1.4. Problem statement

Organosulphur compounds (OSCs) are a major contributor to air pollution. During the refining process of crude oil, OSCs emit oxides of sulphur (SO_X), which contributes to the formation of acid rain, thereby damaging the environment and the surface of building structures [3]. Sulphur emissions can also interact with ozone and other gases to produce sulphur containing smog that can be harmful to human and animal health [8]. Furthermore, the presence of OSCs in crude oil can also lead to the deactivation of the catalyst during the refining processes. The quality of crude oil is the determinant factor in terms of price differentials. Stringent sulphur regulations and specifications have been adopted in various countries to minimize its toxicity to the environment. The United States of America (USA) and European Union (EU) for example, reduced the concentration of sulphur in diesel from 500 to 15 mgL^{-1} and 50 to 10 mgL^{-1}respectively [10].

Therefore, the knowledge of OSCs structures in crude oil is a key factor in the development of effective sulphur removal processes. The development of novel crude-oil desulphurisation catalysts requires detailed knowledge of sulphur-containing compounds present in crude oil and intermediate products. Many of these OSCs are volatile in nature and some are in trace concentration levels in a complex crude oil matrix. The nature of complex crude oil matrix makes it challenging for qualitative and quantitative determination of OSCs [11–13].

Different chromatographic methods have been employed for the determination of OSCs compounds in crude oil. Most of these techniques involves sample pre-treatment prior to analysis. Sample pre-treatment is necessary in order to pre-concentrate and/or to pre-clean the crude oil sample because of its complexity. Liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase micro extraction (SPME), hollow fiber liquid phase extraction (HF-LPME) and dispersive liquid-liquid micro extraction (DLLME) are some of the sample pre-treatment methods that have been reported for the analysis of OSCs in crude oil [24]. Some of these techniques use organic solvents that are halogenated in large amounts. These solvents can be toxic and are not easy to handle, thus can in turn be harmful to the handler. Furthermore, the sample required for some of the techniques should be in large quantities and this is not in-line with green chemistry [25].

1.5. Aim and objectives

The main aim of the study was to develop a rapid and selective analytical method for qualitative and quantitative analysis of organosulphur compounds (OSCs) in selected fuel oil samples using GCTOFMS.

The specific objectives of this project were to develop:

- Dilution pre-treatment method prior to qualitative determination of organosulphur compounds in various crude oil samples using GCTOFMS.
- Magnetic solid phase extraction (m-SPE) followed by GC-TOFMS for semi-quantitative analysis of organosulphur compounds in various fuels.
    - Magnetic gold nano-composites were synthesised and characterised using various techniques such as TEM, SEM-EDX, P-XRD, UV-Vis and FT-IR.
    - The exploration of various parameters (adsorbent mass, eluent type and volume, extraction time and pH) that affect the extraction efficiency of the proposed m-SPE was performed by using multivariate optimization methods.
    - The analytical merit (performance) of the proposed m-SPE method was compared with literature reported pre-concentration procedures.
    - The optimum m-SPE sample preparation procedure was applied in real oil samples for the pre-concentration/extraction of organosulphur compounds.

1.6. Justification of the study

Chromatographic techniques have been used for the determination of OSCs in various samples. These chromatographic techniques include a high performance liquid chromatography (HPLC) [26], Gas chromatography (GC) [27] and a comprehensive two dimensional gas chromatography (GC×GC) [28] amongst others. A sample can be analysed directly using chromatography with any minimal sample pre-treatment [27]. Minimal sample pre-treatment can be a dilution of the sample using organic solvents such as heptane [29], dichloromethane (DCM) [30] *etc*. prior to analysis. HPLC was used by Silveira and co-workers to analyse OSCs in asphalt cement. Toluene and methanol were used as solvents for diluting the samples. This method achieved an accuracy of between 83.85 % and 110.28 %

However, OSCs are found in low concentrations in fuels [14], thus sample preparation or pre-treatment is important prior to analyses in order to improve detection limits [31]. Due to the complexity of a crude oil sample, different sample pre-treatment techniques have been employed for the analysis of OSCs. These techniques include the traditional methods such as LLE [25] and SPE [35] amongst other techniques. The OSCs such as benzothiophene and dibenzothiophenes have been determined using the former technique. From their findings, the extraction of OSCs was between 0.10-250 µg mL^{-1} and a recovery of 77-91% was achieved [36]. This technique also uses solvents, which can be toxic, thus the latter was employed as a solvent less technique. Silveira et al [37] used aminopropyl cartridges as adsorbents and a better recovery of 65.37 to 114.85% was achieved. It also yielded a better extraction of OSCs (0.1–3.5 mgL^{-1}) [37]. The sorbent used was commercially available it can be relatively costly. Other researchers synthesized 8- hydroxyquinoline silica gel impregnated with palladium chloride as an adsorbent which was also expensive. A recovery between 81.5-92.1% was obtained using this technique [38]. The SPE is rapid, and this method was shown to have a high enrichment factor and a high recovery. This has made SPE to be a popular strategy for effective and selective pre-concentration of analytes [39]. Magnetic nano composite adsorbents have received a lot of focus because of their unique structure, properties, high adsorption ability, and reusability [40]. During literature compilation, no information was found in relation to use of the nano composite for OSCs analysis.

Thus, this study seeks to use GC-TOFMS for qualitative and semi quantitative analysis of OSCs in oily matrices after sample pre-treatment. Four target OSCs were chosen for the study due to time limitations. This chromatographic technique has received a lot of attention from researchers because it provides additional separation of overlapping species [41] as well as superior resolution with high sensitivity [42].

1.7. Hypothesis

Dilution (direct analysis) and magnetic solid-phase extraction (indirect analysis) can be used as sample pre-treatment methods for qualitative and quantitative determination, respectively of OSCs in fuel oils using a comprehensive GC-HR-ToF-MS. The gold magnetic nanoparticles can be applied as selective adsorbents for OSCs during m-SPE and the magnetic character will enhance easy separation of adsorbent from the bulk of the solution.

1.8. Research questions

This study seeks to answer the following research questions:

- Does the use of dichloromethane as a solvent aid in separation of OSCs peaks when using a gas chromatography coupled to high resolution time-of-flight mass spectrometry (GC-TOFMS)?
- Is gold coated magnetite selective towards OSCs on the selected fuel samples?
- Does the developed magnetic solid phase extraction method of OSCs applicable to real fuel oil samples?
- How will the figure of merits of this study compare to the previously done studies?

1.9. Dissertation outline

Chapter I (Introduction)

This chapter deals with background information about crude oil. It further explains the origin of crude oil and its composition. An introduction to organosulphur compounds that are present in crude oil is presented. The hypothesis, objectives, problem statement, research questions and the justification of the study are outlined.

Chapter II (Literature review)

The literature on qualitative and quantitative analysis of OSCs in crude oil is discussed extensively in this chapter. Different chromatographic techniques that are associated with the analysis of OSCs in crude oil are highlighted with detailed information on how the analysis were conducted. Sample pre-treatment methods that are used for pre-concentration and sample clean up prior to chromatographic analysis are evaluated. Furthermore, the direct methods of analysis that include dilution are detailed in this chapter. It is worthy to indicate that this chapter has been published as a review article [43].

Chapter III (Methodology)

All the procedures and methodologies that were followed in order to generate data for this study are well defined in this chapter. This includes all general laboratory techniques that were used for qualitative and quantitative analysis of OSCs in crude oil and the characterization techniques for the confirmation of the synthesised adsorbents for m-SPE experiments are described. A detailed description of multivariate optimization methods is also expressed in this chapter.

Chapter IV (Results and discussion)

The qualitative data for the analysis of OSCs in crude oil is presented in this chapter. The discussion of the data is also focussed on. The optimization parameters and results are also presented in this chapter. The precision of the method is also compared against the already published literature reports. Furthermore, this chapter also focuses on the quantitative results and discussions including the figures of merits.

Chapter V (Conclusion and future recommendations)

Overall findings in relation to the aim of the study are summarized in this chapter. Furthermore, recommendations and future work are also presented in this chapter.

CHAPTER II

LITERATURE REVIEW

PREAMBLE

In this chapter, chromatographic analytical techniques that are mostly reported for both qualitative and quantitative determination of OSCs in various matrices are described based on direct (analysis without sample preparation) and indirect (analysis after sample preparation) analysis. The merits and possible limitations associated with direct and indirect analysis are also discussed. Additionally, the analytical performances of the various published sample preparation methods are evaluated in terms of accuracy, the limit of detection, precision, etc. Lastly, this chapter highlights current gaps in chromatographic determination of OSCs and possible future developments.

2.1. Introduction

Organosulphur compounds (OSCs) have a carbon-sulphur bond, exist in different chemical structures and are present in various matrices such as vegetables [44], wine [45], crude oil [46], coal [47], etc. It has to be noted that, OSCs are naturally occurring thus their presence is unavoidable [48]. Some of these sulphur compounds are even found in living organisms as amino acids (e.g. methionine, glutathione and thiamine) [49]. The OSCs are categorised into different classes depending on the functional group of the sulphur atom. The commonly documented OSCs include thiols (mercaptans), sulphides, disulphides, thiophenes, benzothiophenes and dibenzothiophenes [7].

Sulphur compounds such as sulphoxides and sulphones are mostly used as manufacturing products in pharmaceutical industries and other compounds like methylsulphonylmethane (MSM) are supplements that are present in foods for the biological maintenance of hair, skin and nails [50]. The most dominating OSCs present in crushed garlic cloves are sulphoxides like alliin and are good anti-carcinogenic and anti-oxidant reagents [51]–[53]. An investigation by Hall and co-workers was conducted in relation to the presence of OSC in garlic and it was concluded that, OSCs can also reduce inflammation and oxidative stress [54]. It must be noted that, onion is another alliin containing vegetable, with similar biological effects as garlic. Volatile OSCs (diallyl disulphide, allyl methyl trisulphide and dipropyl disulphide) contained in garlic and onion vegetables are mostly responsible for the anti-inflammatory and extra ordinary antimicrobial activities [55].

Alcohol beverages such as whisky, wine and beer also contain OSCs, which are mostly responsible for flavour improvement. However, the concentration levels of these compounds vary from one beverage sample to another. For example, fusel alcohol and whisky contains µg L^{-1} levels of OSCs[56]. The OSCs like thiols, sulphides and disulphides are found in low concentrations in wine and are mainly accountable for the distinct aroma of the wine [57].

The presence of OSCs can either be essential or toxic, depending on their concentration levels, chemical structures and the host sample matrices [51]. OSCs present in environmental samples (water and sediments) and in fossil fuels (coal and crude oil) are mainly detrimental. Gimeno and co-workers reported the presence of polycyclic aromatic sulphur heterocycles (PASHs) in seawater and sediment samples and their potential genetic mutation and cancerous activities [26]. Additionally, it was reported that the commonly found PASH in environmental samples such as spills and discharges (regulated and unregulated) is thiophene. The latter showed some toxicological effects, which include cancer and genetic mutations. [58]. These thiophenic sulphur compounds are abundant in fossil fuels such as crude oil. Burning of the sulphur containing fossil fuels results in the emission of toxic sulphur gases (SO_2 and SO_3) to the atmosphere. These gases are major contributors to the formation of acid rain, which is harmful to plantation, living organisms and building infrastructure [59]. Furthermore, the produced SO_2 can interact with the ozone, results in formation of harmful smog and cause negative respiratory effects to humans [60]. It is also documented that only 1 ppm of SO_3 can lead to coughing and choking in human beings, thereby causing chronic diseases such as bronchitis [61]. Since these fossil fuels are naturally occurring, the amount of OSCs differs depending on geographical location. For example, Australia, Denmark, and Nigeria's fossil fuels contain less than 0.26 %(w/w) of the OSCs and other countries like Cuba contain as high as 7.03 %(w/w) of OSCs [19]. Due to the toxicity of these compounds, different countries adapted stringent regulation conditions to minimize the harmful impact of these compounds to the environment. For example, USA allows the concentration of OSCs to be between 15000 to 500000 µg L^{-1}, while EU is only allowing 1000 to 5000 µg $L^{-1}$of OSCs in diesel oils [62].

It is therefore crucial to monitor the presence of OSCs and their concentration levels in different matrices. In petroleum industries, the presence of OSCs can lead to the deactivation of the catalyst during refining processes. The knowledge of OSC structures is a key in the development of effective desulphurization processes in various industries such as pharmaceutical, agriculture, food and petroleum industries [63]. The development of novel desulphurisation processes requires detailed knowledge of OSCs structure and concentration level.

2.2. Determination of OSCs in different matrices

High performance liquid chromatography (HPLC) [64], gas chromatography (GC) [65] and thin-layer chromatography (TLC) [66] are some of the chromatographic techniques that have been previously reported by various researchers for OSCs determination in various matrices. The TLC relies on stationary phase that is coated on glass or plastic plate. Samples are mounted on the plate in the presence of a liquid mobile phase which is a solvent or a mixture of solvents and placed on a chamber. The sample migrates through the plate on the basis of the analytes' physiochemical properties which determines the interaction with the mobile and stationary phase [67]. Unlike TLC, HPLC uses a column, and the analytes of interest are separated in the column. The packing of the column considers different properties such as the pore sizes, the size of the particle and the functional groups. The normal phase and the reverse phase liquid chromatography are the most popular types of chromatography for OSCs determination [68]. Gas chromatography (GC) is another separation technique that has been used for the determination of volatile OSC in various complex matrices. It must be noted that GC operates similarly to the HPLC. However, GC techniques use inert gasses as mobile phase (helium, nitrogen and hydrogen). This technique offers an opportunity of a wide range of analysis of compounds in different matrices, but more volatile analytes are recommended for efficient chromatographic separation. The stationary liquid phase in GC column is volatized using the temperature gradient. Therefore, easily decomposed organic compounds are preferably analysed by other chromatographic techniques [69], [70].

In order to obtain a more efficient separation, researchers have explored the use of a comprehensive two dimensional chromatography (GC×GC) [41]. The latter is a major analytical tool that overcomes drawbacks that were found in other chromatographic techniques. This technique can overcome challenges such as the presence of OSCs in low concentrations and composition of the OSC in complex matrices [71]. This is because, GC×GC uses dual columns which are coupled to a modulator. The separation on both columns takes place in gaseous phase. The first column is wider and longer than the second column. Two independent steps of separations were combined into one when using this technique. Some of the fraction in the first column go to the second column where they undergo further separation [72]. The GC separation is conducted by using different types of stationary phase combinations [41], [73].Therefore, this chapter discusses literature reported methods applied for OSCs determination using direct (without sample preparation) and indirect (with sample preparation methods followed by analysis) prior to chromatographic analysis, as shown in **Figure 2.1**.

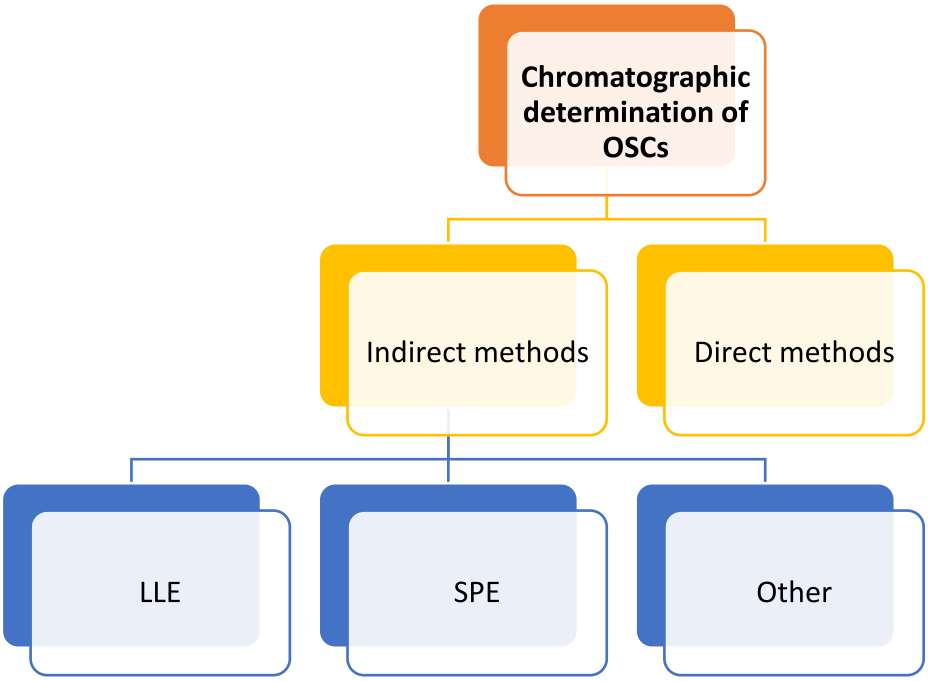


Figure 2. 1 Direct and indirect methods used for chromatographic analysis of OSCs

2.2.1. Direct chromatographic analysis of OSCs in various matrices

In this technique, a sample is analysed directly without undergoing any sample pre-treatment prior to analysis. Direct analysis is fast and simple but is often more suitable for highly concentrated analytes. This analytical strategy does not involve many steps, thus the samples are normally less contaminated [74]. Additionally, direct analysis minimizes the loss of target analytes, which may occur during the sample pre-treatment stages. However, this analysis is only possible when the concentration levels of the OSCs in a particular matrix are higher than the detectors detection limit of the suitable chromatographic technique and when the matrix is less complex [75]. Solvents such as dichloromethane (DCM) [25], n- hexane [76], xylene, toluene [77], 2- propanol and octane [78] are commonly used for dilution of highly viscous samples prior to chromatographic analysis of OSCs. These solvents should be compatible with the proposed chromatographic technique and be able to dissolve the sample and the analyte without affecting the composition of the sample and the chemical structures of the target analytes [79]. It has to be noted that, complex samples like crude oil should be diluted with organic solvents that have low boiling points and high surface tension and the separation between the sample and the solvent should be fast and have chemical stability [80]. Although dilution is a simple technique that is less laborious, it has its own challenges. For example, some of the organic solvents used during sample dilution are difficult to handle, due to their vapour pressure and low viscosity [81]. Additionally, the concentration of the target analyte can increase if using contaminated solvents. It has been reported that, some organic solvents are toxic and hazardous, thereby, require care handling [81]. However, there are samples such as crude oil that poses challenges with regards to solubility [82].

A summary of different direct chromatographic analytical methods for the determination of OSCs in various matrices is presented in **Table 2.1**. It must be noted that, there was either dilution or no sample pre-treatment prior to analysis. The dilution sample pre-treatment was conducted by using organic solvents. This table clearly outlines that GC techniques were the most widely used techniques focusing on both the two dimensional and one-dimensional chromatography from the gathered information. This may be due to advantages of GC techniques towards more volatile compounds and complex samples such as crude oil. It can be further deduced from **Table 2.1** that dibenzothiophene (DBT) was the most determined analyte and naphthodibenzothiophenes (NDBT) was the least determined analyte. Additionally, cyclic OSCs were the most target analytes compared to the others such as sulphoxides and thiols. The favour in DBT analysis may be due to the fact that direct analysis was mostly investigated on petroleum samples, where DBT causes challenges and is difficult to remove [21]. Therefore, samples that contain cyclic OSCs were less favoured in direct analysis, due to the challenges that these compounds bring during GC analysis. Cyclic organosulphur compound, alkylbenzothiophene was the analyte of interest during the study by Zhu et al. in a diesel sample. This compound was directly determined quantitatively without any sample pre-treatment method. The diesel sample was injected into a GC hyphenated to a quadruple time-of-flight mass spectrometry (GC-Q-ToF-MS) using a split/split less injector. This method resulted in a good correlation coefficient that is greater than 0.9920. The method validation included precision and repeatability. The former was calculated using the relative standard deviation (RSD) and was found to be between 1.1-3.4%. The accuracy was 93.1-107.8% for all the dibenzothiophene compounds determined [27]. HPLC coupled to an atmospheric pressure photoionization tandem mass spectrometry for the determination of OSCs in asphalt cement was reported. During this study, Silveira and co-workers used toluene fractions and methanol for dilution of asphalt cement and the HPLC technique detected 14 OSCs (sulphoxides, sulphones and dibenzothiophene) with accuracy between 83.85 and 110,28% for some of those compounds [31]. The DCM solvent was also used by Wilson et al during the qualitative characterization of polycyclic aromatic OSCs by normal phase liquid chromatography [83]. Dichloromethane was used to dilute coal tar, diesel particulate and petroleum extracts. The precision, LODs, LOQs and the recoveries were not reported [83]. Gas chromatography was coupled with a high-resolution mass spectrometry for the determination of alkylbenzothiophenes (alkyl-BT) and alkyl-dibenzothiophenes (alkyl-DBT) in light cycle oil and straight run gas oils. The results were compared to those of GC-SCD and it was reported that the distribution of thiophenic sulphur type was 10% and that of non-thiophenic was 90% in straight run oils. Furthermore, 1.19% of sulphur was contained in gas oil [84]. The percentage of OSCs in crude oil from three geographical locations was characterized. Crude oil that originated from Italy was found to have between 2.1% and 7.1% level of OSCs. The lowest quantity of OSCs was obtained from crude oil that originated in Africa with 0.2%. Oil from Asia had 1.2% and 3.8% of OSC levels. During this study, the limit of quantification of this method was obtained as 2.4 -5 ppb [33]. Guy and co-workers for the identification of benzothiophene, dibenzothiophene and naphthothiophene in light cycle oil used atomic emission detector (AED). The distribution of these OSCs and their retention times were determined by directly injecting the sample at a split ration of 59:1 in GC. The substituted benzothiophene was found to be in majority with 59.3% and 40.7% being shared between dibenzothiophene and naphthothiophene [85]. The one dimensional chromatography was unable to separate and quantify some of the OSCs contained in complex crude oil samples without sample pre-treatment [86]. Therefore, the application of GC×GC, (which is known as a powerful tool for quantitative and qualitative analysis of OSC) was reported. For example, Avila and co-workers reported the use of GC×GC-ToF-MS for both qualitative and semi quantitative determination of OSCs in crude oil. The latter was diluted with DCM solvent prior to OSCs analysis and *n*- tetracosane d_{50} was used as an internal standard. The chromatographic results showed the presence of sulphides, disulphides, and thiols with a total concentration of 1.372 µg L^{-1}. However, researchers did not report the validation of the analytical method [87].

Table 2.1 further indicates that direct sample injection without minimal sample pre-treatment is not often used compared to minimal sample pre-treatment such as dilution and DCM was the mostly used diluent when minimal sample pre-treatment was applied. This can be attributed to the nature of this diluent as it is a polar solvent and miscible with most solvents. Although it is highly volatile which makes it hazardous when inhaled, it is less toxic when compared to other halogen containing solvents [88]. When this diluent was used, a good linear response was also achieved [31]. There are other diluents that were reported, such as toluene [33], isooctane [34] that also yielded good responses for the analysis of these OSCs.

Table 2. 1 Direct analysis of OSCs in different matrices using chromatographic techniques

Matrix	OSCs	Detection Technique	Diluent	LOD (µg/g)	Accuracy (%)	Precision (%RSD)	Linear Range (µg/g)	Correlation coefficient R^2	Ref
Asphalt cement	Sulfoxide, BT, DBT, NBT	UHPLC-MS/MS	DCM	0.28-5.23	83.85-110.28	1.04	50 - 1500	>0.99	[31]
Coal	DBT	NPLC-GC/MS	DCM/Toluene	ND	ND	ND	ND	ND	[83]
Coal tar	DBT	NPLC-GC/MS	DCM/Hexane	ND	ND	ND	ND	ND	[89]
Crude oil	DBT isomers	GC – MS/MS	Toluene	0.7	ND	0.68-2.01	ND	ND	[33]
Crude oil	BT, DBT	GC – MS/MS	Isooctane	0.0099	83.8-103.0	0.2 – 5.6	0,020-9.99	0.9999	[34]
Crude oil	DBT	GC×GC-ToF-MS	DCM	ND	ND	ND	ND	ND	[28]
Crude oil	Thiols, sulphides, TP, BT, DBT, NBT	GC×GC-SCD	N/A	ND	90-102	6.02	ND	ND	[90]
Diesel	DBT	GC-Q-ToF-MS	N/A	3000	93.1-107.8	1.1 – 3.4	20-2000	>0.9920	[27]
Gas Condensate	Thiols, BT, DBT	GC-SCD	Isooctane	ND	ND	5-6	ND	ND	[91]
Gas oil	BT, DBT	GC-SCD	Toluene	ND	<100	ND	ND	0.999	[84]
Heavy crude oil	DBT	GC-SCD	*n*-Hexane/DCM	ND	ND	ND	ND	ND	[92]

Heavy petroleum	TP, BT, DBT, NDBT	HT–GC×GC-SCD	Toluene	ND	ND	ND	ND	>0.9985	[93]
Heavy gas oil	TP, BT, DBT, NBT	FM-GC×GC-MS/MS	DCM	6-90	ND	7-17	1-100	>0.9975	[30]
Light cycle oil	BT, DBT	GC-SCD	*n*-Octane	ND	ND	ND	ND	ND	[94]
Petroleum samples	TP, BT, DBT, NBT	GC×GC-TOF-MS	Tetracosane/ pyrene	ND	ND	ND	ND	ND	[87]
Petroleum products	TP, BT, DBT	GC-ICP-MS	*n*-Hexane	7	ND	ND	ND	ND	[95]
Petrochemical samples	BT, DBT, NBT	GC×GC-AED/MS	N/A	ND	ND	ND	ND	ND	[96]
Shale oil	TP, BT, DBT	GC×GC-FPD	DCM	20	ND	10 - 35	0.025-1	>0.994	[97]

ND = Not determined, N/A = Not available, OSCs = organosulphur compounds, TP = thiophene, BT = benzothiophene, DBT = dibenzothiophene, NBT = naphthobenzothiophene, NDBT = naphthodibenzothiophes, UHPLC = ultra-high performance liquid chromatography-mass spectrometry, DCM = dichloromethane, NPLC-GC/MS = normal phase liquid chromatography-gas chromatography mass spectrometry, GC-MS/MS = gas chromatography tandem mass spectrometry, GC×GC-ToFMS = comprehensive two dimensional gas chromatography time-of-flight mass spectrometry, GC×GC-SCD = comprehensive two dimensional gas chromatography sulphur chemiluminescence detector, GC-Q-ToFMS = gas chromatography quadruple time-of-flight mass spectrometry, HT-GC×GC-SCD = high temperature comprehensive two dimensional gas chromatography sulphur chemiluminescence, FM-GC×GC-MS/MS = flow modulated comprehensive two dimensional gas chromatography-triple quadrupole mass spectrometry, GC-ICP-MS = gas chromatography inductively coupled plasma mass spectrometry, GC×GC-AED = comprehensive two dimensional gas chromatography atomic emission detector

2.2.2. Indirect chromatographic analysis of OSCs in various matrices

Some samples are complex and contain low concentration levels of OSCs, thereby causing various challenges during chromatographic analysis. To circumvent these challenges, sample preparation techniques have been introduced [24]. Depending on the complexity of the sample, sample preparation could involve a couple of steps. The complexity of the sample may include, the concentration levels of the OSCs in the matrix of interest, sample state of the matter (gas, liquid or solid) and the physiochemical properties of target OSCs [100]. Furthermore, variables such as the limit of detection (LOD), limit of quantification (LOQ) linearity, correlation efficient and accuracy are also affected by the choice of the sample preparation methods used [69]. In the current literature review, different sample preparation methods such as solid phase extraction (SPE), liquid-liquid extraction (LLE) and other {supercritical fluid extraction (SFE) and ultrasound assisted extraction (UAE)} are extensively discussed and evaluated according to their performance for chromatographic determination of OSCs in various samples.

2.2.2.1. Liquid-Liquid extraction of OSCs in various matrices

Liquid-liquid extraction (LLE) is one of the traditional sample pre-treatment methods that have been employed with the view of increasing target analyte sensitivity. The LLE is achieved by separating the target analytes from the major sample matrix and other interfering species through partitioning between two immiscible liquids [101]. The LLE was improved to a miniaturized technique called Liquid liquid-microextraction (LLME) technique that is harmonious with GC and liquid chromatography [102]. The LLE procedure incorporates three steps, which are sample clean-up, extraction of analyte and pre-concentration [103]. This method was employed as a sample pre-treatment procedure for the analysis of OSCs in wine samples. The extraction recoveries (91.99-125.87%) were good with effective separation. However, the involvement of numerous steps and large quantities of reagents make LLE time consuming, tedious, expensive, harmful to the user and might introduce cross-contamination [102]. Therefore, flotation assistance was applied together with the LLE method for the extraction and determination of OSCs in water samples. During this study, acetone was used as a homogeneous solvent and toluene was used as an extraction solvent. It was concluded that this method was efficient and the linearity was in the range of 0.25-200 µg L^{-1} with the LODs in the range of 0.05-0.4 µg L^{-1} [1].

Liquid-liquid micro extraction (LLME) uses microliter quantities of solvent for pre-concentration of analyte [104]. This technique was introduced to solve challenges that were experienced in LLE and it is simple, rapid and inexpensive [105]. There are three categories of LLME namely single drop micro extraction (SDME), dispersive liquid-liquid micro extraction (DLLME) and hollow-fibre liquid phase micro extraction (HF-LPME) [106]. Xiong and Hu compared DLLME and HF-LLME for the analysis of OSCs in environmental and beverage samples. During this study, GC was coupled to FPD and the relative standard deviations of 8.5-13.7% were reported for DLLME. The LOD of HF-LLME were found to be 1.16 µg L^{-1}, with the precision of 3.4%. Therefore, it was deduced that the extraction efficiency of HF-LLME was more effective than that of DLLME because it has less extraction time and higher extraction efficiency. Carbon tetrachloride was employed as a solvent for DLLME and *o*-xylene was used as the extraction solvent for HF-LLME [107]. The LLME technique was applied for the determination of OSCs in crude oil, with accuracy between 77-91% [103]. Liquid-liquid extraction methods were compatible for samples that are in liquid phase and the usage of the commercially available fibre increases the cost of HF-LLME methods [108].

Table 2.2 below summarizes different chromatographic analytical techniques employed by researchers for the analysis of OSCs in different sample matrices. The LLE technique demonstrates efficiency on samples that are in liquid phase or aqueous phase. Only one-dimensional gas chromatography was used with LLE as a sample pre-treatment technique. The GC mostly was coupled to a flame ionization detector with DLLE as a sample pre-treatment technique. Acetonitrile and chlorobenzene were used as solvents during a study for the analysis of OSCs in aqueous samples with DLLME employed as a sample pre-concentration technique. The latter was employed as an extraction solvent and the formers was used as dispersive solvent [109]. Lastly, crude oil was the only sample matrix where sulphur specific detector was coupled to GC. However, FPD is not regarded as a specific detector because it is sensitive to both phosphorus and sulphur compounds.

In conclusion, **Table 2.2** shows that the DLLME was the commonly used LLE technique from the extracted literature reports. This technique uses less volume (micro-litres) of solvent, it is simple to operate, it is less expensive and also it a rapid method [102] with high recovery as indicated in **Table 2.2.** The extraction time for this method is the lowest compared to the other LLE techniques, thus it is regarded as rapid. These factors can be the motivation behind the common use of this extraction method compared to the other LLE methods.

Table 2. 2 Indirect analysis of OSCs in different matrices using LLE followed by chromatographic techniques

Sample	Detection Technique	OSC	LLE	LOD (µg L^{-1})	Linear Range (µg L^{-1})	Extraction Time (min)	R^2	Recoveries %	Precision (%RSD)	Ref
Aqueous sample	GC-FID	Sulphides, thiols	DLLME	0.1 – 0.9	1-200	ND	>0.9904	56.4 – 76.8	<10	[109]
		Sulphides	DI - SDME	10.7 -43	100 -1000	2	0.9986	ND	18.3	
Crude oil	GC-SCD	DBT, BT	LLME	1- 50	100 – 250000	5- 60	0.985	77 -91	4.5 -13.3	[103]

ND = Not determined, OSCs = organosulphur compounds, GC-FID = gas chromatography flame ionization detector, , GC-SCD = gas chromatography sulphur chemiluminescence detector,

2.2.2.2. Solid phase extraction of OSCs in various matrices

Solid phase extraction (SPE) is one powerful pre-treatment technique that is widely used for isolation, concentration, clean up, and medium exchange procedures. This extraction method uses cartridges which are filled with the matrix compound and the compounds are extracted in relation to their interaction (ion exchange, polarity or non-polarity interaction and size exclusion) [35] with the stationary phase. Solid phase extraction is a conventional solvent free pre-treatment method that combines both sampling and pre-concentration of analyte in one step [113]. In this technique, the analyte is distributed between the liquid phase and the stationary phase. The stationary phase is the adsorbent, and they exist in different forms. It can be silica-based, modified with NH_2, carbon based sorbents, polymer based and natural sorbents such as cotton [114]. Some of these adsorbents such as the silica-based one are not stable at extreme pH values. The SPE technique has its drawbacks, which includes poor recoveries because of the interaction between the matrix and sorbent, sorbent bed clogging when disks and cartridges are used as sorbents [106]. To minimize some of the drawbacks of SPE, a miniaturized SPE technique was derived, and it was called SPME. This technique has many advantages compared to the conventional SPE. These advantages include high consistency, rapidness, simplicity and compatible with most analyte separation [106]. The SPME technique can be divided into three modes: direct-immersion solid phase micro-extraction (DI-SPME), Head space solid phase micro-extraction (HS-SPME) and the membrane protected [115]

Table 2.3 summarizes SPE and SPME methods applied with different adsorbents for OSCs extraction prior to chromatographic analysis. A reliable quantification of polycyclic aromatic sulphur heterocycles was conducted using GC coupled to a mass selective detector (MSD) and atomic emission detector (AED) on a fossil fuel related sample. During this study, SPE with aminopropylsilane cartridges was used as a clean-up procedure for the removal of polar components in oil. Information such as the molecular weight of thiophenes and dibenzothiophene isomers were reported. The repeatability, linear range and LOD were some of the factors that were not reported [116]. Rudzinski and Rai used tandem mass spectrometry during their study for the detection of polyaromatic sulphur heterocycles in crude oil. In this report, complex crude oil mixtures were separated by using distillation, alumina, ligand exchange chromatography and SPE with LC-NH_2

cartridges. The MS detection confirmed the presence of dibenzothiophene and benzonaphthiophene derivatives in the Arabian crude oil. Post column addition of tropylium cations was performed and the retention time for the polycyclic aromatic heterocycles was 3,28 and 3,51 minutes for dibenzothiophene isomers [73]. Solid phase extraction cartridges are commercially available, thus they can be expensive to obtain [117]. The development of more selective and more easy to use adsorbents has resulted in a simplified procedure [118]. 8-Hydroxyquinoline silica gel impregnated with palladium chloride was synthesized by Yang and co-workers. The silica gel was used as an adsorbent during the determination of polycyclic aromatic sulphur aromatics in sea crude oils. The SPE pre-treatment technique was coupled with GC-MS and it was promising for quantitative analysis of polycyclic aromatic heterocyclic sulphur compounds found in low concentrations. This is because it yielded good accuracy from 81,5 to 92,1% for these compounds. Furthermore, the LOD of benzothiophene was obtained at 0.2777 µg L^{-1}, for dibenzothiophene was at 0.597 µg L^{-1} and finally that for naphthobenzothiophene was 0,597 µg L^{-1} [38]. Da silveira and co-workers employed aminopropyl as a stationary phase for clean up in the analysis of OSCs in petroleum asphalt cement using ultra HPLC. The use of the commercially available aminopropyl cartridges eliminated the modification of stationary phase with metals. This clean-up procedure resulted in identification of at least seven OSCs. The accuracy for these compounds were in the range of 65.37-114.87%. The relative standard deviation was less than 10.11%, this was for all the analytes [119]. The use of aminopropyl was also reported by Anderson and was modified with other metals as a clean-up method during the analysis of OSCs using liquid chromatography [120].

Solid phase micro-extraction is a solvent free technique that involves direct extraction of the analyte of interest. The method uses a diameter silica coated fibre which has a small diameter as the stationary phase [121]. The fibre is used as an interface in chromatography and is used to isolate and concentrate the solute. The analyte, sample and extraction medium are partitioned [122]. When equilibrium is reached, the absorbed solute is then transferred to the chromatographic unit for analysis. This method is less tedious and simple when compared to other sample pre-treatment techniques such as liquid-liquid extraction. The volume of the samples should not be in large amount and also it can be fully automated [115]. Some fibres used for the SPME technique are not sensitive to other mixtures of volatile compounds. This was evident when Pelusio and co-workers analysed OSCs in black and white truffle aroma [123].

The SPME is the most used technique compared to all techniques as it can be seen in **Table 2.3**. The high number of publications on this technique can be attributed to the previously mentioned reasons, which includes the solvent free advantage. Furthermore, different modes of this technique are available. **Table 2.3** further highlights that the most used adsorbent is the commercially available adsorbent compared to the synthesized. The commercially available adsorbent can be relatively costly. Hence, there’s a need for environmentally friendly and recyclable adsorbent for the analysis of the analysis of these OSC in different samples is the SPE.

Table 2. 3 Indirect analysis of OSCs in different matrices using SPE followed by chromatographic techniques

Sample	Detection Technique	Adsorbent	OSC	SPE type	Extraction time (min)	LOD µg L^{-1}	Linear range µg L^{-1}	Correlation coefficient	Accuracy %	RSD %	Ref
Asphalt cement	LC	SiO_2C_3-NH_2, SiO_2/C_3-CN	Sulfoxides, BT, DBT, NDBT	SPE	ND	ND	100-3500	0.991-0.997	65.37-114.58	6.03 - 10.11	[119]
Crude oil	GC-MS	8-HQ-Pd-silica	BT, DBT, NDBT	µ-SPE	2	0.193-0.597	10 - 1000	0.9894–0.994	81.5-92.1	3.23 – 4.20	[38]
Diesel	GC×GC-ToF-MS	Pd-Al_2O_3	Sulphides, BT, DBT	SPE	ND	ND	ND	ND	ND	ND	[128]
Fossil fuel	GC-MS/AED	APS cartridge	NDBT, DBT	On-line SPE	ND	ND	ND	ND	ND	ND	[116]
Gas matrices	GC-MS	CAR-/PDMS	Sulphide	SPME	20	$1x10^{-6}$ – $4.7x10^{-4}$	0.1-100	0.992-1.000	ND	2-12	[132]
Gas samples	GC-FID	CAR-PDMS	Thiol, sulphides	SPME	5	ND	ND	ND	ND	ND	[133]

Lichens	GC-MS/ToF-MS	Glass Cartridge	DBT	μ-SPE	N/D	<1	<2500	0.99	70 -130	16	[134]

ND = Not determined, N/A = Not available, GC-MS = gas chromatography mass spectrometry, LC = liquid chromatography, BT = benzothiophene, DBT = dibenzothiophene, NDBT = naphthodibenzothiophene, SPME = solid phase microextraction, SPE = solid phase extraction, , HS-SPME = head-space solid phase microextraction, μ-SPE = solid phase microextraction, , APS cartridge = aminopropylsilane cartridge, GC-FID = gas chromatography flame ionization detector,

2.2.2.3. Other extraction methods for OSCs in various matrices

Other sample preparation methods used for the extraction of OSCs prior to chromatographic analysis includes desorption, supercritical fluid extraction (SFE), reflux and the use of polymer thin film. **Table 2.4** summarises all the other extraction and the most reported extraction method was the SFE. The latter uses supercritical fluid as a solvent for extraction and is suitable for both the solid and liquid matrices but mostly on the former. The commonly reported sample matrix on extraction of OSCs using SFE is coal. During SFE, supercritical fluid such as carbon dioxide (CO_2) is mostly used and the CO_2 is sometimes altered by co-solvent like ethanol or methanol [142]. However, toluene, 2-propanol and toluene-tetrahydrofuran are some of the organic solvents used in SFE. The latter was used by Gryglewicz and co-workers during the characterization of OSCs in coal extracts prior to GC-MS analysis. The combination of propanol and toluene as co-solvents had a good extraction yield of 39.9 wt.% when compared to other solvents. However, the use of CO_2 has advantages as it is non-corrosive, non- toxic and non-flammable [143]. Furthermore, SFE was used as an extraction method in coal and it was compared with ultrasonic assisted extraction (UAE) and accelerated solvent extraction (ASE). Different solvents for example, hexane and DCM were utilized for both UAE and ASE, while tetrahydrofuran (THF) and toluene combination were used for SFE. This resulted in better yield of 16% whilst ASE achieved 1.3% and UAE attained 1.4%. Although the ASE method achieved the lowest yield, the highest number (150) of OSCs were obtained by using this method [144]. However, the high pressure required for the removal of the supercritical fluid can be costly. Furthermore, CO_2 has its dissolving capacity limited by being a non-polar molecule [142].

The second popular extraction method was ligand exchange chromatography (LEC), which was assisted by $PdCl_2$ for the enrichment isolation of polycyclic aromatic sulphur compounds (PASHs) such as thiophenes (Th), benzothiophenes (BT) and dibenzothiophene (DBT) [143]. This method was as highly efficient and rapid for the separation of PASHs and PAH to avoid interferences when chromatographic analysis was done. The LEC with Pd (II) complex based on 2-amino-1-cyclopentene-1-dithiocaboxylate (ACDA) silica column was used for separation and it yielded excellent separation for the classes of compounds. Gas chromatography analysis of crude oil also showed that non-sulphur compounds were completely isolated [145]. Further investigation was conducted on synthesized larger number of thiophene compounds using the Pd (II) complex. It was found that benzothiophenes are strongly retained when compared to thiophenes [146]. Other metals apart from Pd (II) were evaluated for the separation of OSCs. The ligand compounds such as $CuSO_4$, $HgCl_2$, $AgNO_3$, $PdCl_2$ (aq) and $PdCl_2$ were evaluated for the separation of OSCs. The study revealed that $PdCl_2$ (aq) had the best separation [147].

Polymer thin film is another pre-treatment method highlighted in **Table 2.4**. The polymer films are artificial materials formed from polymerization of monomers in the presence of a target analyte. These polymers are constant over a range of conditions including temperature and pressure [148]. A good reproducibility and a relative standard deviation value of 7.0% was achieved using this method for the selective analysis of thiophene compounds in water samples. During this study, the films were coupled to an HS-GC-SCD for the determination of semi-volatile compounds. Low LODs (0.24-0.82 µg L^{-1}) with good linearity (0.99) and accuracy (89-98%) were obtained [58].

Table 2. 4 Indirect analysis of OSCs in different matrices using other sample pre-treatment followed by chromatographic techniques

Sample	Detection Technique	Extraction method	Extraction time (min)	LOD (µgL^{-1})	Precision (%RSD)	Ref
Asphalt mixture	LC-MS	Reflux	ND	0.5 – 346.5	0.55 – 16.01	[150]
Coal	GC-MS	SFE	ND	-ND	-ND	[143]
Coal	GC-MS	SFE	ND	-ND	-ND	[151]
Coal	GC×GC-ToF-MS	SFE/ASE/UE	ND	-ND	-ND	[144]
Crude oil	GC×GC-FID/SCD	Distillation	ND	-ND	-ND	[71]
Gasoline/Heavy distillates	Size exclusion chromatography	Ligand exchange	ND	ND	ND	[145]

Gas oils distillates	GC	Ligand exchange	ND	ND	ND	[147]
Petroleum	HPLC	Ligand exchange	ND	ND	ND	[146]

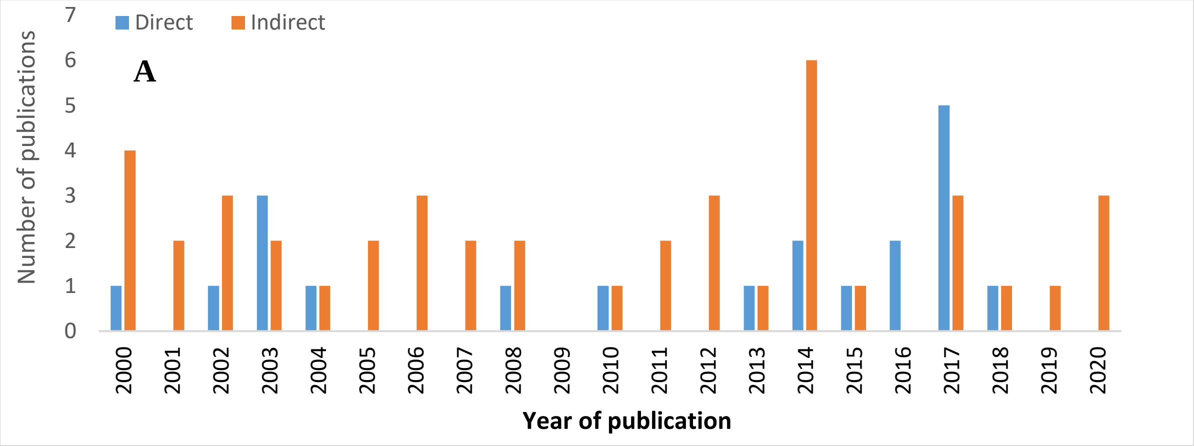
ND = not determined, LC-MS = liquid chromatography mass spectrometry, GC-MS = gas chromatography mass spectrometry, GC×GC-ToFMS = comprehensive two dimensional gas chromatography time-of-flight mass spectrometry,

2.3. Comparison of direct and indirect chromatographic analysis of OSCs

Direct and indirect chromatographic methods have been applied for analysis of OSCs in different samples using direct and indirect analysis. **Figure 2.2A** shows a number of literature publications that were reported for both direct and indirect chromatographic determination of OSCs from 2000 up to 2020 (two decades). Although the direct method eliminates many steps, thereby minimizing the contamination of the sample prior to analysis, it shows low number of publications as compared to indirect method. The observed trend might be due to the fact that, OSCs are commonly found in low concentration levels, thus sample pre-concentration is necessary. Furthermore, due to the complexity of the samples and the volatility of OSCs, sample pre-treatment is crucial, thus, a high number of publications for the indirect method are observed. During the first decade (2000-2010), at least eight publications were obtained on direct analysis of OSCs, followed by 11 publications on the second decade (2010-2020). A slight increase in publication numbers can be attributed to the availability of powerful analytical machines like GC×GC, which can give better separation of OSCs without the requirement of sample preparation steps. It is worthy to indicate that, only two publications described direct analysis of OSCs without sample dilution.

Different sample matrices that were analysed for chromatographic quantitative and qualitative determination of OSCs are illustrated in **Figure 2.2B**. From this figure it can be observed that, no publications were reported for the direct analysis of environmental (wastewater, sediments, and air) and food samples. The low concentrations of OSCs in these samples and the complexity nature of these samples requires extensive sample pre-treatment prior to OSCs chromatographic analysis. The least number of publications (2) was reported for biological sample matrices. It must be noted that these two publications were conducted at the beginning of the two decades, thus a lot of research still needs to be carried out for indirect and indirect determination of OSCs in these samples. Additionally, biological samples preferred direct analysis of OSCs. However, fossil fuel samples have the highest number of publications (16) for the direct approach and most of these samples had to undergo dilution prior to OSCs analysis. The reason for such a high number of publications might be that fossil fuels contain high concentration of OSCs, which can be easily detected without the need of extensive sample preparation.

However, **Figure 2.2B** also shows that, food matrices were mostly analysed for OSCs by using indirect chromatographic methods. The food samples include a wide range of scope such as cheese, beef, and drinks like beer. The main contributor to the highest number of publications in food was wine. This can be due to the afore-mentioned reasons in relation to the presence of OSCs causing undesirable flavours [161]. The category ‘other’ as reflecting in **Figure 2.2B**, includes samples such as Linchens and Lang baiju. Although these classes of matrices were reported to contain a wide range of OSCs, they favoured the indirect approach (7 publications) more than direct methods (2 publications).



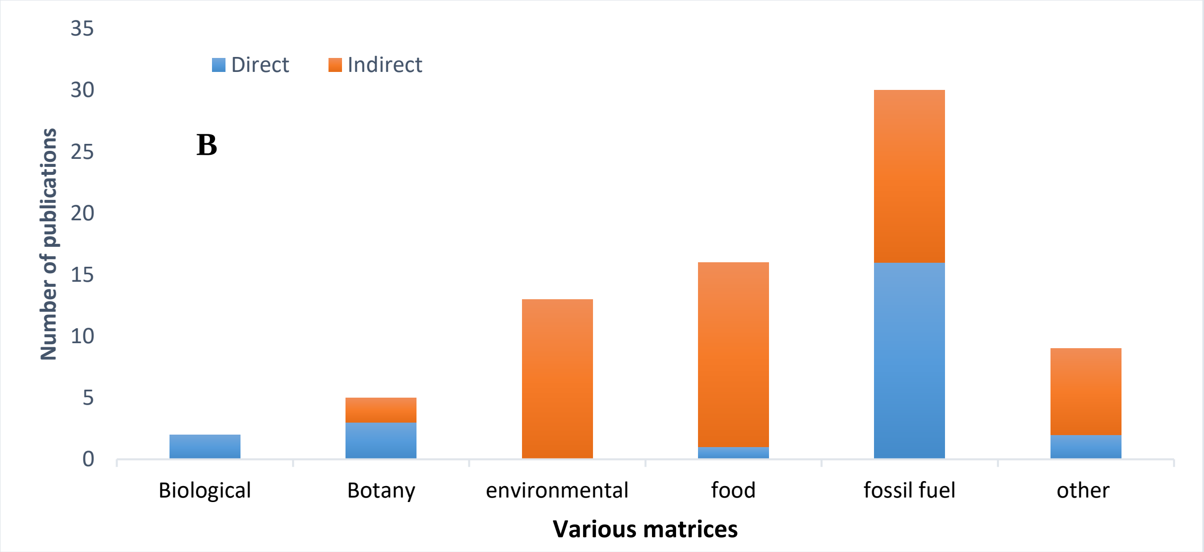


Figure 2. 20 Number of publications for direct and indirect analysis of OSCs over two decades (A) and (B) various matrices

2.4. Comparison of extraction methods (LLE, SPE and other) for determination of OSCs

The indirect chromatographic analysis of OSCs in different matrices involved the use of various sample pre-treatment techniques. From the literature obtained, the sample pre-treatment techniques included solid phase extraction (SPE), liquid-liquid extraction (LLE), and other sample pre-treatment techniques. The 'other' sample pre-treatment category includes techniques such as microwave assisted extractions (MAE), ultrasound assisted extraction UAE), stir bar sorptive extraction (SBSE), accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE). **Figure 2.3** shows that other sample pre-treatment methods have been less favoured for the extraction of OSCs, prior to chromatographic analysis. Furthermore, **Figure 2.3** shows that SPE has the highest number of publications (22) from the past two decades. The highest number of publications for SPE might be due to its simplicity and the wide choice of adsorbents that can be used to improve extraction efficiencies of various OCSs. Additionally, SPE is rapid and minimizes the use of carcinogenic organic solvents that are commonly applied in LLE. The latter technique has three classes of micro techniques namely dispersive liquid-liquid microextraction (DLLME), headspace single drop microextraction (HS-SDME) and hollow fibre liquid-liquid microextraction (HF-LLME). These microextraction methods have immensely high number of publications for the LLE technique. This trend might be due to the use of microliter solvents, which is low quantity when compared to the amount used during traditional LLE methods.

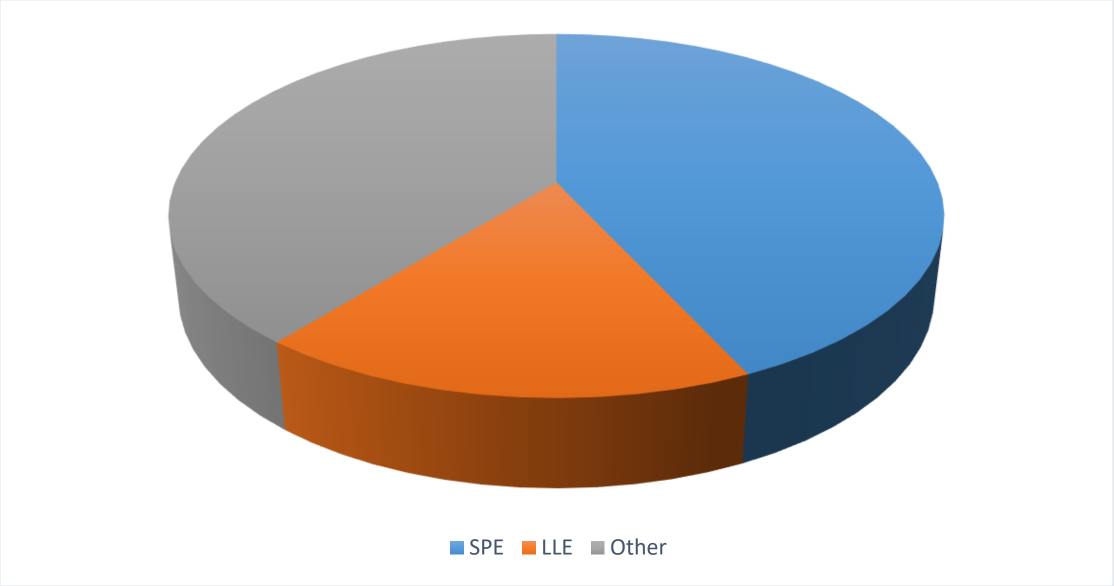


Figure 2. 3 Comparison of sample pre-treatment methods

2.5. The most analysed OSCs in various matrices

Sulfoxides, sulphides, thiophene, and benzothiophene were the reported OSCs in different samples (see **Figure 2.4)**. However, sulfoxides were the least analysed compounds amongst the other three OSCs, with only 5 publications over the two decades. It has to be noted that, this analyte is not as toxic as the other analytes such as the polycyclic aromatic sulphur heterocycles (PASHs) and it is mostly reported in biological samples. Thiols were found in almost all the reported sample matrices; hence the highest number of publications (31) was observed for these sulphur compounds. Furthermore, fossil fuels were reported to be dominated by thiophenes. This can be attributed to the fact that, fossil fuel samples including crude oil are regarded as complex matrices, consist of various aromatic sulphur compounds, which are challenging to analysed, and remove. According to **Figure 2.4**, dibenzothiophene has been an analyte of focus for most researchers with more than 30 publications. This analyte and its derivatives have received much attention from researchers because they are one of the OSCs that contributes immensely to environmental pollution and mostly studied for development of desulphurization strategies.

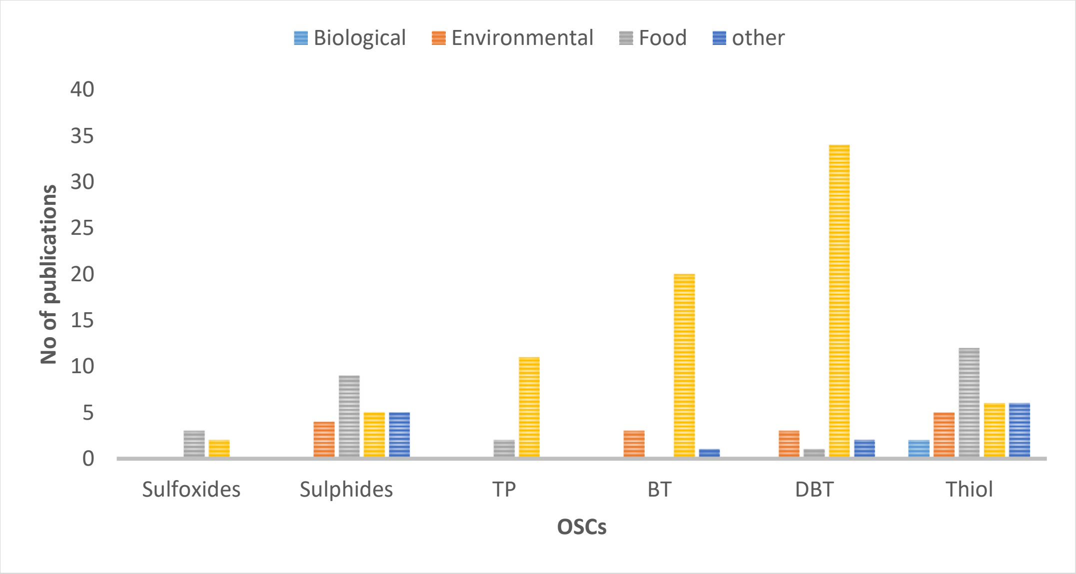


Figure 2. 4 Comparison of OSC analytes in sample matrices

2.6. Comparison of chromatographic techniques for analysis of OSCs

Figure 2.5 summarizes the used chromatographic techniques for the analysis of OSCs for the past two decades. No publications were reported in the year 2009. At the beginning of the first decades (first three years), there were no reports of GC×GC whilst reports on HPLC were published in 2000, 2002 and 2003 with only 1 publication per year. However, HPLC was also reported in 2014 with the highest number of publications (3) and again in 2016 with only 1 publication. This technique was only applied in sample matrices such as garlic, saliva, and water. Additionally, volatile OSCs were not suitable for HPLC, thus a low number of publications were published on this technique. The technique with low publications (4) overall is LC. All the publications reported on LC were done on the second decade, between 2015 and 2018. TLC has also been replaced by LC, because it does not differentiate between individual OSCs [157].

Alternatively, gas chromatographic techniques have been favoured and widely used for the determination of OSCs over the past decades. It has been noted that, GC was used in almost all the sample matrices, hence the high number of publications. Additionally, GC×GC was published more in the second decade with 10 publications, which is eight publications less compared to GC. Because of its increased selectivity towards OSCs and its enhanced power of separations using two columns, GC×GC has gained more popularity from researchers compared to GC [162]. Furthermore, due to the complex nature of the samples, GC cannot offer satisfactory separation especially in the presence of hydrocarbons [163], hence most studies are currently focusing on the use of GC×GC.

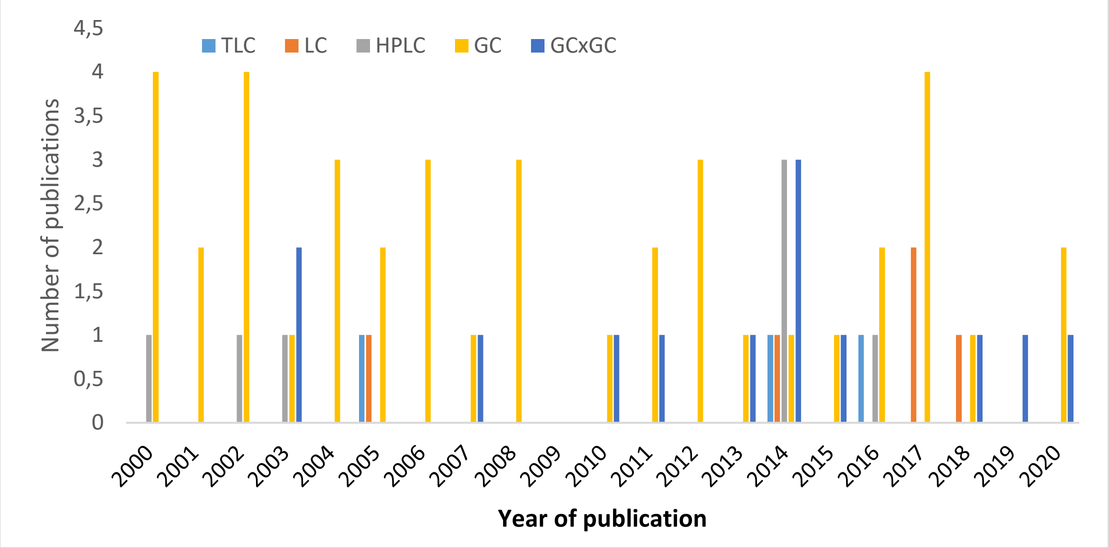


Figure 2. 5 Chromatographic techniques used for OSCs analysis in the past two decades

CHAPTER III

EXPERIMENT AND METHODOLOGY

PREAMBLE

This chapter presents all the experimental procedures that were followed to generate the data reported and discussed in the next chapter. Standard operation procedures, the sample collection procedures, sample pre-treatment and sample analysis followed for both qualitative and quantitative analysis of OSCs are explained in detail. A gas chromatography coupled to a high resolute time-of-flight mass spectrometry (GC-TOFMS) used to generate data for the analysis of OSCs is also detailed in this chapter. Furthermore, different multivariate optimization procedures are discussed, including the one employed for this project. Lastly, physiochemical techniques (UV-VIS, TEM, SEM-EDX, FT-IR and P-XRD) used for the characterization of the proposed adsorbent are also explained in this chapter.

3.1 Standard operation procedure

All the laboratory experiments were conducted in glassware. The glassware was cleaned by soaking in acidic solution (3M HCl in ethanol) for a minimum of 3 hours, then rinsed three times in deionized water followed by drying in an oven overnight before use.

3.2 Sample collection

All crude oil samples were obtained from one of the South African petroleum company, while diesel, gasoline and kerosene samples were purchased from local filling stations.

3.3 Chemicals and standards

Analytical grade dichloromethane (DCM, ≥ 99.8) solvent, was purchased from Sigma-Aldrich (Johannesburg, South Africa

The following salts, iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) ACS reagent, ferrous chloride tetrahydrate ($FeCl_2.4H_2O$), sodium citrate dihydrate ≥ 99 % FG [$HOC(COONa)(CH_2COONa)_2.2H_2O$], tetrachloroauric acid ($HAuCl_4.3H_2O$) ~ 49% Au, and sodium hydroxide (NaOH) were also purchased from Sigma-Aldrich (Johannesburg, South Africa). Organosulphur standards, dibenzothiophene (98 %), 3-methylthiophene (98 %), thiophene (≥99%) and benzothiophene (95%) were also purchased from Sigma-Aldrich (Johannesburg, South Africa). Different solutions including 28 w/v % ammonium solution, hydrochloric acid (98 %) was also purchased from Sigma-Aldrich (Johannesburg, South Africa). Diesel, kerosene and petroleum were purchased from a local filling station (South Africa). Nitric acid (65 %), hydrochloric acid (37 %) was supplied by Merck (South Africa). Ultrapure water which was used during the study with resistivity of 18.2 MΩ.m was obtained from a Milli-Q system. Glassware and GC vials were soaked in nitric acid (6 M) and then rinsed with ultra-pure water.

3.4 Preparation of standards

A 1000 ppm standard stock solution of dibenzothiophene (DBT) was prepared by weighing 10 mg (± 0.1 mg) of DBT. It was transferred into a clean, 10 mL volumetric flask that was half-filled with dichloromethane (DCM) and stored at -5 ^{o}C for later use. Working standards mixture of the target compounds were diluted with dichloromethane.

3.5 Qualitative and quantitative determination of OSCs in fuel samples

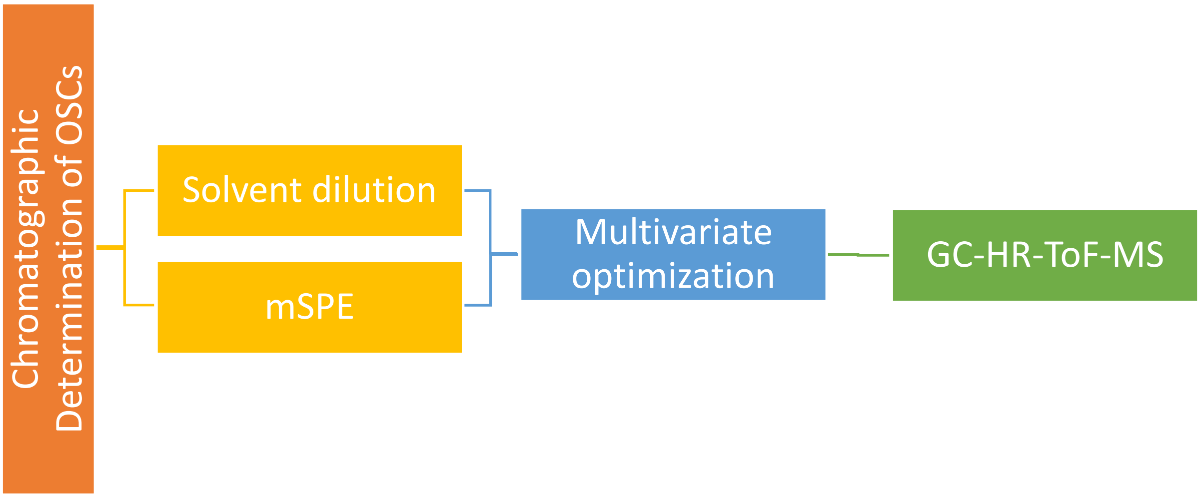


Figure 3. 1 Summary of the overall method

3.6 GC method and qualitative analysis

The prepared stock solution mixture was injected into the GC-TOFMS immediately after preparation. The different concentration (5, 10 and 15 ppm) prepared of the stock solution mixture was injected respectively in triplicate.. Crude oil, diesel, gasoline and kerosene samples were also screened for the OSCs by dissolving 100 µL of each sample in DCM as depicted in **Figure 3.2**. After dilution, each sample was injected in triplicates also.

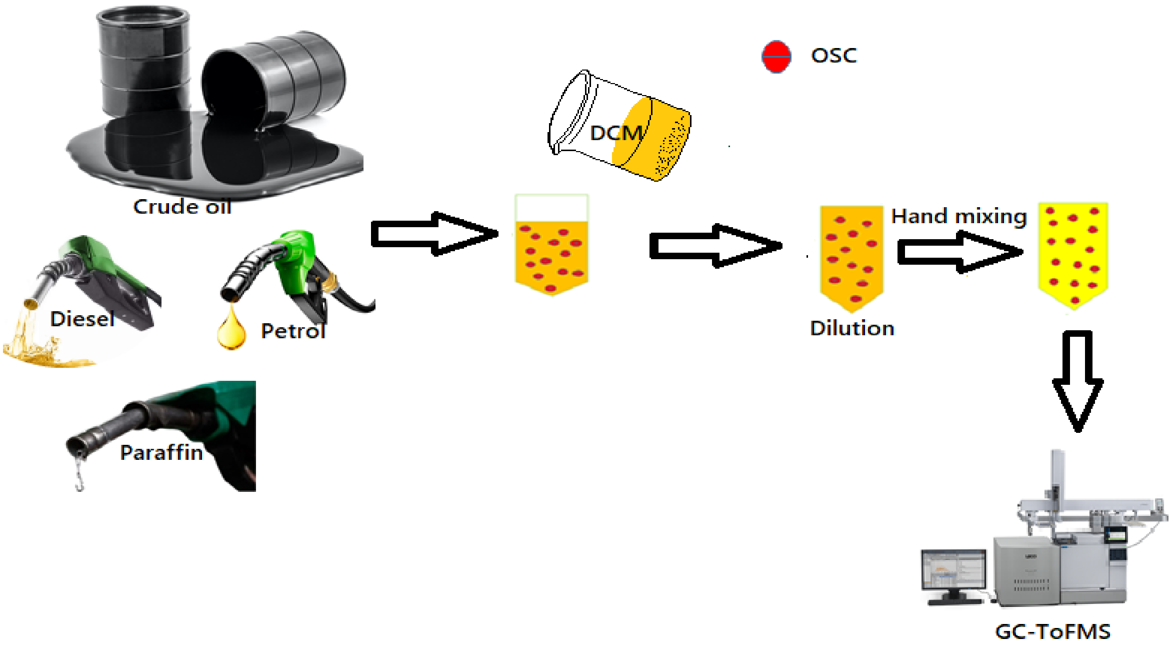


Figure 3. 2 Qualitative analysis of OSCs in crude oil by dilution prior to GCToFMS analysis

3.7 Quantitative analysis

3.7.1 Preparation of Au-Fe_3O_4

Fe_3O_4 preparations

Chemical co-precipitation method was used for the synthesis of Fe_3O_4 nanoparticles. This method was selected because of its simplicity, low production costs, high yield and effectiveness compared to other methods such as microbial incubation which is slow and laborious [164]. There are two types of co-precipitation methods; the first involve the use of ferrous hydroxides and oxidizing agents. The co-precipitation method used was reported by Lancu (2020) et al . [166] and was followed with modifications. A 0.85 mL of conc. HCl (12 M) was diluted with 25 mL ultrapure H_2O. The prepared solution was used to dissolve 5.2 g of $FeCl_3.6H_2O$ (ferric chloride hexahydrate) and 2 g of $FeCl_2.4H_2O$ (ferrous chloride tetrahydrate). The Fe^{2+}/Fe^{3+} ratio was important for the control of morphology and the size of the nanoparticles to be formed [167]. This mixture was conducted in an inert (N_2) environment. Then, 250 mL of NaOH (1.5 M) was added to the iron mixture. A black precipitate was formed and an external magnet then separated it at room temperature. The precipitate was washed four times using ultrapure H_2O. After washing, the back solid was again suspended in 250 mL of ultrapure H_2O mixed with 1 mL of NaOH (1.5 M) for 20 minutes. The synthesized Fe_3O_4-nanoparticles was stabilized by mixing it with 0.1 M (500 µL) glucose [166].

Au-Fe_3O_4 preparations

The as-synthesized Fe_3O_4 NPs were covered with gold (Au) shell by first adding 20 mL of $HAuCl_4.3H_2O$ (50 mM) into 105 mL of deionized water followed by boiling. Then, 5 mL (1mg/ mL) of the as-synthesized Fe_3O_4 was added into the boiling solution. Sodium citrate (5 mL, 80 mmol/L) was added slowly into the solution under constant stirring. The colour of the solution changed from brown to burgundy. The boiling continued for 5 minutes. The heat source was removed and stirring continued until the solution cooled to room temperature. Au-Fe_3O_4 was separated using a permanent magnet [168–170]

3.7.2 Magnetic solid phase extraction of organosulphur compounds

Blank extraction was performed using the solvent firstly. Then m-SPE procedure was done based on study by An (2018) et al. with modifications [171]. samples (10 mL) samples were placed in a beaker followed by 50 mg of Au-Fe_3O_4 nanoparticles. The mixture was then shaken for a certain period (10 min) to ensure complete pre-concentration of the analyte. The adsorbent was then separated using an external magnet. The supernatant was decanted and an eluent (acetonitrile) of 100 µL was introduced to desorb the analyte and mixed with the sample. Separation was done using an external magnet and the supernatant (1,5 ml) was collected and filtered using 0.22 µm (13mm diameter) glass membrane/nylon filter purchased from Merck suppliers. After filtering, the supernatant containing a target analytes was analysed for OSCs using GC-TOFMS. **Figure 3.3**, summarizes the analysis of OSCs using m-SPE and GC-TFMS.

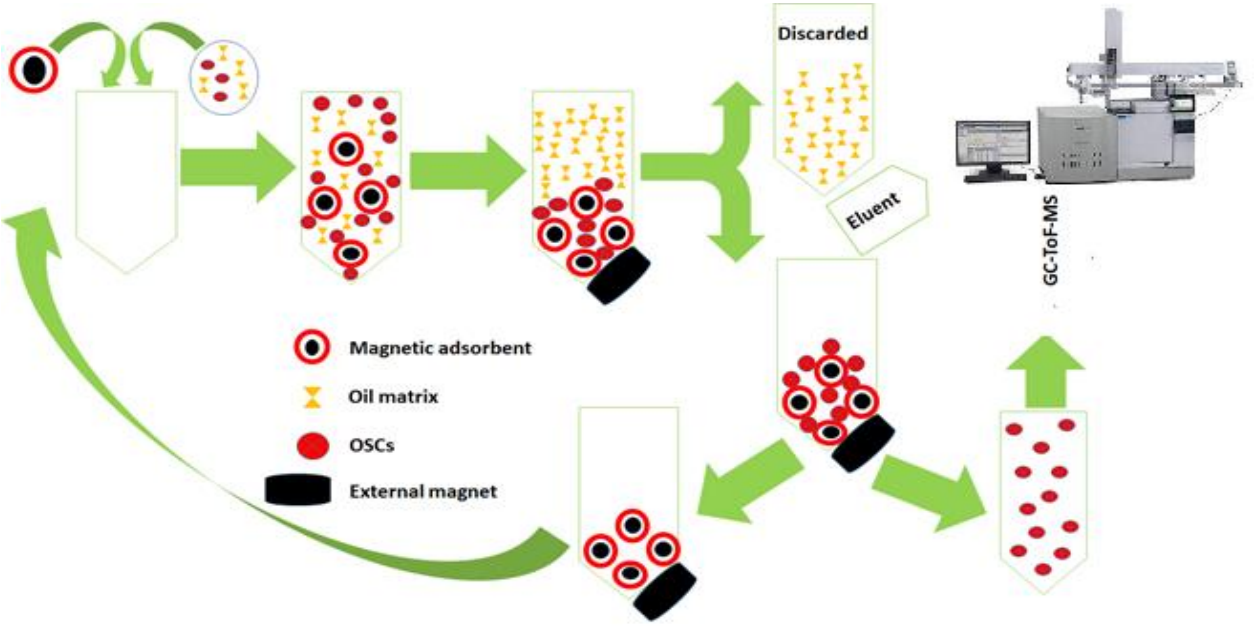


Figure 3. 3 Schematic representation of m-SPE for the quantitative analysis of OSCs in crude oil samples using GC-HR-ToF-MS

3.7.3 Multivariate optimisation

Screening

The screening of the most significant variables was performed using the two level full factorial design (2^4) with three replicates. Each factor was studied at two levels coded -1 for low level and +1 for high level. The factorial levels for the four variables, *viz*, extraction time, adsorbent mass, eluent volume and the pH were selected using a trial and error method. The data was assessed using Mini-Tab 18 software which produced Pareto charts that indicated significant factors at a certain confidence level based on the vertical line [172]. The significant parameters were further optimized using the response surface methodology outlined in the next section.

Response surface methodology

The second order response surface methodology was based on the central composite design (CCD). The significant parameters from the first order design were further optimized while the insignificant parameters were kept constant. The 3D surface plots from MiniTab 18 software were used to interpret analytical results [173]. On these plots, the relationship between the response and the parameters were formulated. Furthermore, the effect of each parameter on the extraction recoveries was observed [174].

3.7.4 Method validation

The m-SPE method was validated by assessing the percentage recovery, the precision in terms of the percentage relative standard deviation, linearity, limit of detection (LOD), limit of quantification (LOQ). A spike recovery strategy was employed for the evaluation of the accuracy at three concentration levels (6 µg/g, 10 µg/g ppm and 25 µg/g) in triplicate to all the four samples (100 µL). Calibration curves were constructed in order to assess the linearity by spiking ultra-pure water with a mixture of target analytes in the range concentration of 0,2 – 20 µg/g for thiophene, 0,05 – 30 µg/g for 3-methylthiophene, 2,6- 19 µg/g for dibenzothiophene and 0,1 – 13 µg/g for benzo[b]thiophene. Furthermore, the LOD and LOQ were calculated as:

$$\text{LOD} = 3\text{SD/m} \text{ and } \text{LOQ} = 10 \text{ SD/m}$$

Where SD is the standard deviation of the peak areas and m is the slope. These are reported on chapter 4.

3.8 Instrumentations

3.8.1 Gas chromatography coupled to a time-of-flight mass spectrometry (GC-ToFMS**)**

The samples were analysed using a LECO Pegasus High Throughput (HT) gas chromatographic time-of-flight mass spectrometry (GC-ToF-MS) (LECO corporation, St Joseph, Michigan, USA) system equipped with Agilent GC model 7890 (Agilent Technology, Santa Clara, California, USA). It was used for qualitative analysis of OSCs.. It was also fitted with jet-cooled modulator. A split/splitless injector set at 300 ^{o}C was also fitted. An HP-5m J&W capillary column with the dimension of 30 m × 0.25 mm × 0.25 µm film thickness with 5%- diphenyl/95% -dimethyl polysiloxane stationary phase supplied by Restek was used for separation. The instrument was controlled by and data acquisition was done using Leco Chroma TOF software . The oven conditions used for separation were as follows: The initial temperature was set at 50^{o}C for a duration of 2 minutes until 140 ^{o}C. It was then ramped at 20 ^{o}C/ min until 200 ^{o}C for 2 minute. The ramping rate was increased to 30 ^{o}C/min and kept constant for 5 minutes until 260^{o}C. The total analysis time was approximately 23 min.

Split/splitless type of front inlet was used and during the analysis, it was used in splitless mode with 1 µL sample injection. The front inlet septum was operated on a flow of 3 mL min^{-1} and the temperature of the injector was 300 ^{o}C. A carrier gas Helium

(99.999%, Afrox, Johannesburg, South Africa) was the carrier gas used under constant flow mode of 1 mL min^{-1}. The GC/ToF-MS interface temperature was set at 250 °C and mass spectra were obtained at 70 eV in full scan mode (scanning m/z ranging from 45 to 450). Data was analysed using Chroma ToF-HRT software.

The next section entails the synthesis of magnetic adsorbent for quantitative analysis of OSCs. Therefore, different characterization techniques including Fourier Transform Infrared spectroscopy (FTIR), Ultraviolet-visible spectrophotometry (UV-VIS), Scanning electron microscope (SEM), Transmission electron microscopy (TEM) and X-ray powder diffraction (P-XRD) were used to confirm the synthesized magnetic adsorbents.

3.8.2 Ultraviolet-visible spectrophotometry

The Genesys 180 UV-Visible spectrophotometer was used for confirmation of the nanoparticles of Fe_3O_4 and Au-Fe_3O_4. The dissolved solid samples (Fe_3O_4 and Au-Fe_3O_4) were first put in centrifuge tubes and diluted to make it less concentrated. Blank samples references were also prepared prior to analysis of real samples using distilled water. The samples were then run at a wavelength range of 200-1200 nm

3.8.3 Scanning electron microscope (SEM-EDX)

The morphology of the nanoparticles was confirmed used SEM-EDX. The scanning electron microscope (SEM) measurements were performed on a Tescan Vega 3 LMH at a voltage of 20 kV, with a secondary electron detector (SED) and energy dispersive X-ray spectroscopy (EDX). Prior to measurement, the samples were carbon-coated with the Agar Turbo Carbon coater to improve their conductivity

3.8.4 Fourier transform infrared spectroscopy (FT-IR)

The functional groups of the nano particles and its structural changes were investigated using the Fourier transform infrared (FTIR) spectra with a Bruker Tensor 27 FTIR spectrophotometer. The synthesized samples were mixed with potassium bromide (KBr) before being compressed into pellets. During the analysis, data was collected from 400 to 4000 cm^{-1} region.

3.8.5 Powder X-ray diffraction (P-XRD)

The crystal structure of the nano particles was determined using the powder X-ray diffraction (P-XRD). The latter measurements were taken with a PANayltical X'Pert Pro powder diffractometer. The diffractometer was fitted with a 1D X'Celerator detector, upper levels of 6.67 and 12.78 keV, respectively, as well as programmable divergence slitting (10 mm radiation length). The measurements were taken with Cu K radiation in the 2 range from 5 to 90^{o} at 40 kV and 40 mA operational conditions, the wavelength was 0.15405 nm. The diffractometer was set up with a sample spinner (Spinner PW3064) and a rotation time of 1 s to get the preferred orientation of crystallites. Predictions for the raw P-XRD patterns were made using High Score (Plus) software and ICDD PDF-4+ 2015.

3.8.6 Transmission electron microscopy (TEM)

The internal structure of the nano particles was studied using Transmission electron microscopy (TEM). It was carried out using a Jeol JEM-2100F Field Emission Electron Microscope at an acceleration voltage of 200 kV (JEOL Inc., Akishima, Japan) equipped with a LaB6 power source. The TEM samples was dispersed in methanol and a drop of the mixture was transferred onto a TEM grid (Cu-grid, 200 mesh) coated with a lacy carbon film.

CHAPTER IV

RESULTS AND DISCUSSION

PREAMBLE

The experimental procedures outlined in the previous section (**CHAPTER III**) produced results that are detailed and discussed in this chapter. Furthermore, the characterization results of the synthesized gold coated magnetite used as an adsorbent are explained and discussed. Lastly, the results and data from a simple and efficient magnetic solid phase extraction (m-SPE) technique was developed under optimum conditions. This was followed by GC-ToFMS analysis for quantitative determination of organosulphur compounds (OSCs) in fuel oil samples including its figure of merits.

4.1 Qualitative analysis of OSCs using sample dilution

4.1.1 Total ion chromatogram

The identification of the OSCs was carried out via their nominal mass. The software was able to produce a total ion chromatogram (TIC) enabling visualization of the targeted OSCs.. The method allowed for the tentative determination of the OSCs including the thiophene isomer as observed on the peaks in **Figure 4.1,** which shows the TIC of targeted OSCs mixture of organic working standards. This mixture was used to develop the gas chromatographic method explained in **Chapter 3**. The retention times of these OSCs are also listed in **Table 4.1**. The chromatogram in **Figure 4.1** shows good separation of benzothiophene and dibenzothiophene compounds. The study by Wilson and co-workers also produced similar peaks for polycyclic aromatic sulphur heterocycles although with use of normal-phase liquid chromatography (NPLC) fractionation prior to analysis [83]. It was anticipated that a co-eluation of polyaromatic hydrocarbons would be happen as explained on literature review. However, it appeared to be more prominent for compounds with high molecular mass or more substituted compounds to have a high retention time. The TIC in **Figure 4.1** showed that dibenzothiophene was eluted late on the chromatogram because it is the most substituted OSCs, which clearly translates to high molecular mass because of the two benzene rings, attached, compared to the other compounds. The chromatogram also showed that thiophene was eluted very close to the solvent peak. This is due to the lower boiling point (85°C) of the compound that makes it to have a higher vapour pressure and shorter retention time since the compound spends much time in the gaseous phase [23]. Ruixiang and co-workers (2004) determined sulphur containing compounds in diesel oil by GC×GC-SCD and the retention times of the sulphur containing compounds also increased with an increase in their boiling points [15]. Furthermore, a study by Lopez Garcia (2002) also showed an increase on the retention times as the substituent on dibenzothiophene ring become bigger which consequently implied an increase on the molecular mass of the compound [24]. The selectivity of the HT-ToF-MS is comparable to that of sulphur chemiluminescence detector [25].

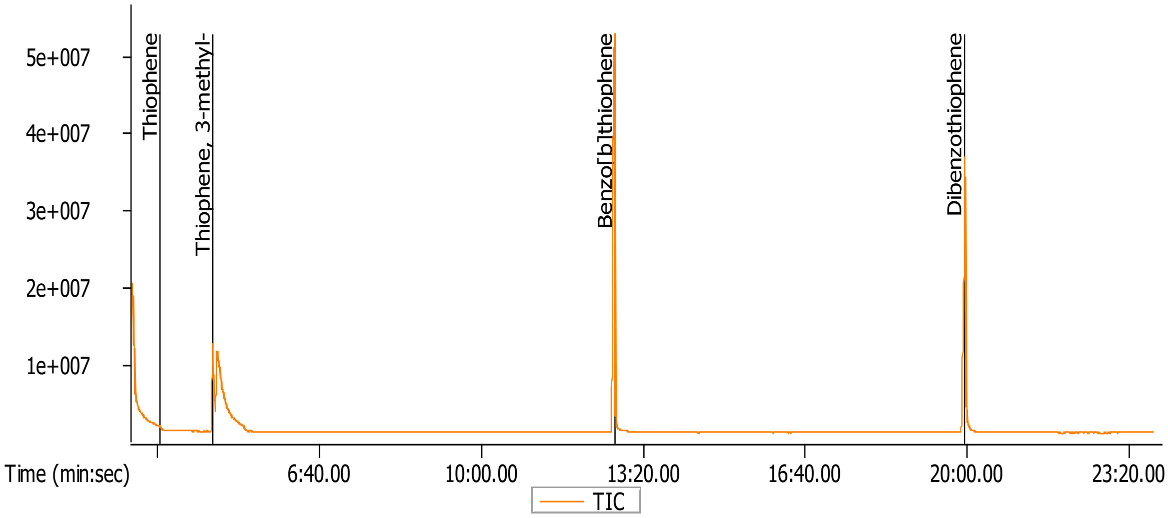
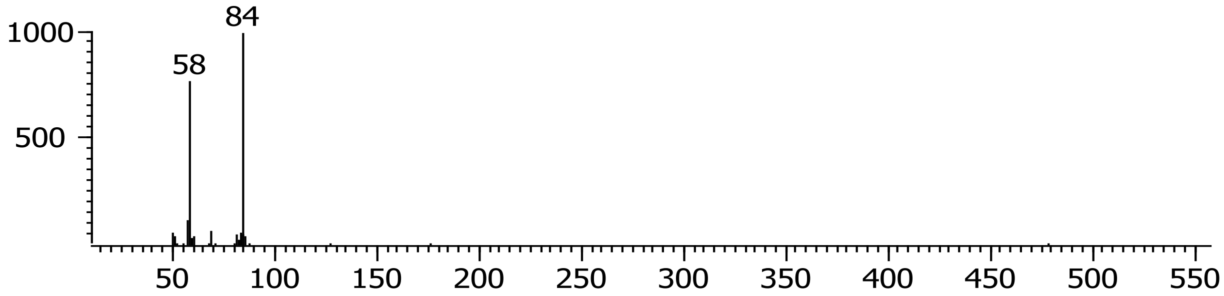


Figure 4. 1 Total ion chromatogram of 10 ppm OSCs compound standards mixture

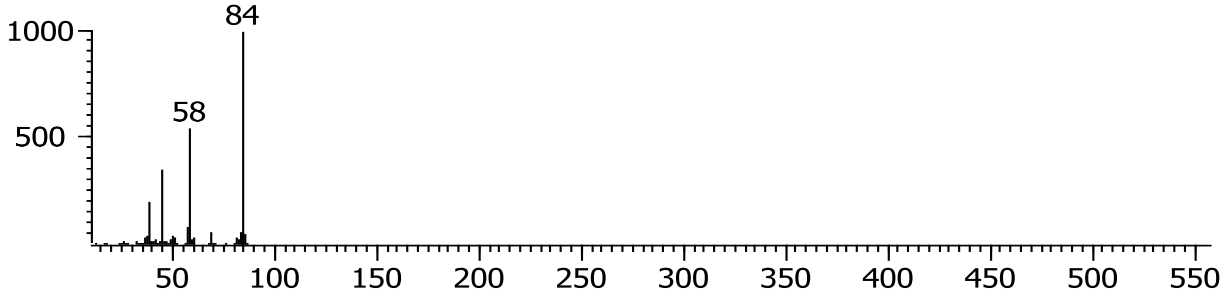
4.1.2 Mass spectrometry

The data from mass spectrometry is displayed in a form of a histogram as shown in **Figure 4.2**. This histogram gives an abundance of ions that reach the detection according to their mass charge ratio (m/z). All the peaks are normalized to the highest peak, i.e. m/z = 84, m/z = 97, m/z = 134 and m/z = 184 as indicated in the figure. The highest peaks are the molecular ion (primary ions) or the quantification peaks for all the target analytes.. This mass spectrum provided evidence of the presence of each analyte. The molecular mass of thiophene for example is 84.14 Da. **Figure 4.2** clearly shows the presence of this compound with a 96,9 % match when compared with the library spectra as indicated. The mass spectrum of dibenzothiophene showed isotopic charged molecular ion at m/z of 134, also providing evidence for the presence of dibenzothiophene. The mass spectra of the other compounds are also attached with **Table 4.1** summarizing all the chromatogram and separation depicted in **Figure 4.1**. Many molecules have been analyzed by mass spectrometry before, and their spectra has been filed with the National Institute of Standards and Technology (NIST). The 2008 NIST library includes over 220,000 mass spectra. When the NIST library is available, it can be used to check for spectra that might match. Similar mass spectrometry was reported by Aloisi and co-workers where the similarity of compound identification was 906 for a substituted benzothiophene [18]. In this study, the similarity/match of benzothiophene was 931, which translated to above 93,1% when compared to the reported study and the other compounds showed a positive match or similarity with high percentage also (> 90 %) confirming the success of this method of analysis.

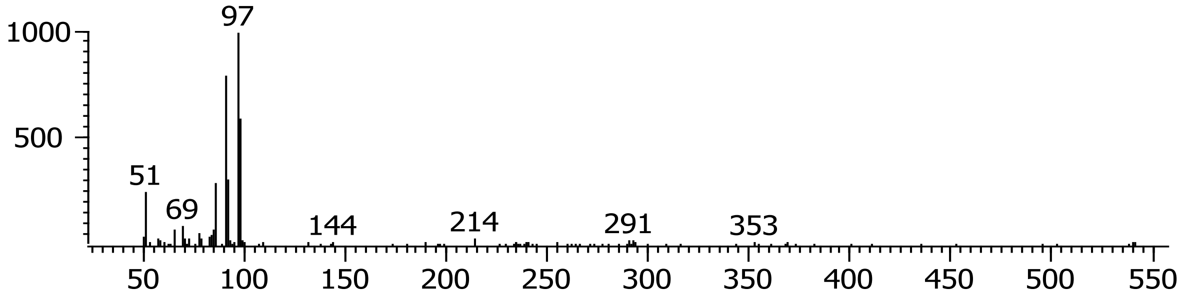
Caliper - sample "5PPM STD:1", 3:22.40 min:sec to 3:22.40 min:sec



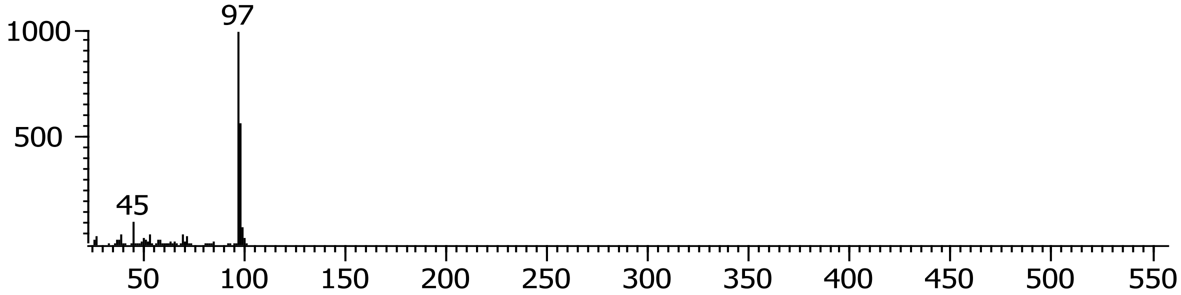
Library Hit - similarity 969, "Thiophene"



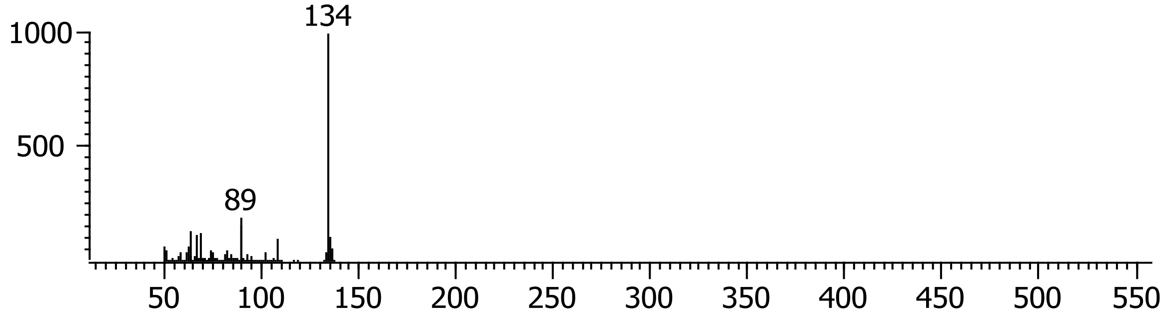
Caliper - sample "5PPM STD:1", 4:18.10 min:sec to 4:18.10 min:sec



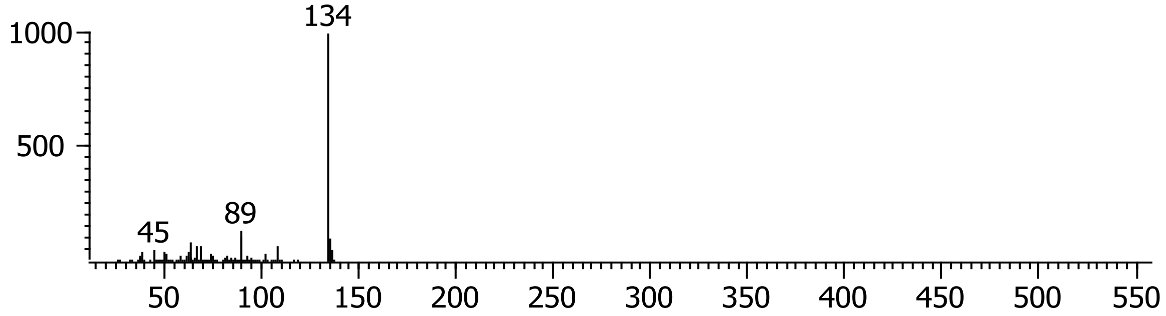
Library Hit - similarity 913, "Thiophene, 3-methyl-"



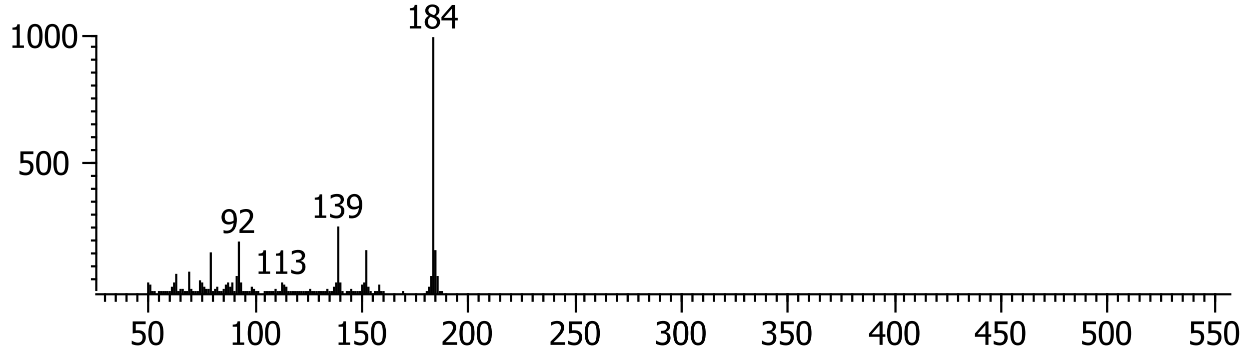
Caliper - sample "5PPM STD:1", 12:44.40 min:sec to 12:44.40 min:sec



Library Hit - similarity 943, "Benzo[b]thiophene"



Caliper - sample "5PPM STD:1", 19:57.70 min:sec to 19:57.70 min:sec



Library Hit - similarity 958, "Dibenzothiophene"

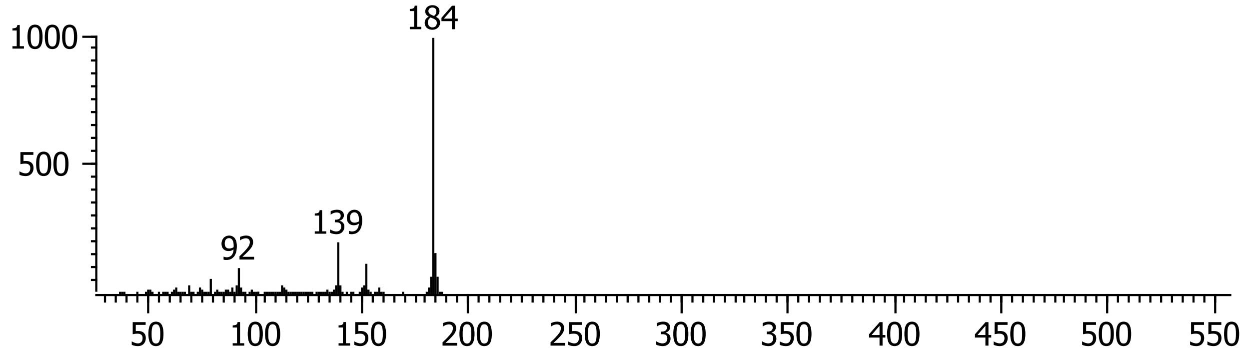


Figure 4. 2 Nominal mass spectrometry of individual OSCs from the mixture with library

Table 4. 1 Organosulphur compounds standard mixture

Standards (5 ppm)	**Retention time (min)**	**Formula**	**Nominal mass**	**Mass spectral similarity**
Thiophene	3:22:40	C_4H_4S	84	974
3-methylthiophene	4:18:10	C_5H_6S	97	991
Benzo[b]thiophene	12:44:40	C_8H_6S	134	931
Dibenzothiophene	19:57:70	$C_{12}H_8S$	184	926

4.1.3 Qualitative analysis of real samples

The screening was conducted on real samples *viz*. crude oil, diesel, kerosene and gasoline. The analysis was conducted through diluting each sample and injecting it according to the chromatographic technique. Different OSCs compounds were tentatively identified in the samples. **Figure 4.3-4.6** shows the chromatogram of the OSCs present in each sample. Compounds of interest were also present on the analysed samples (**Table 4.2**). The table also indicates that the compounds with low molecular mass are eluted at an early stage when compared to other compounds of high molecular masses. The tentative identification of these compounds on real samples was done with high similarity index. **Table 4.2** indicates all the tentative identified compounds in the samples with some of their properties. A similar study of compound identification was conducted on jet oil using GC-AED/MS. The study reported an identification of 234 sulphides isomers [180].. This study identified 11 organosulphur compounds in kerosene sample, 10 OSCs in crude oil, 26 OSCs in diesel sample, 8 OSCs in gasoline with high similarities for some OSCs as indicated on **Table 4.2**. This clearly indicated that sample dilution could be used as an alternative method for analyte screening in different samples. **Table 4.3** compares this study with other more studies conducted for screening or qualitative analysis of OSCs.

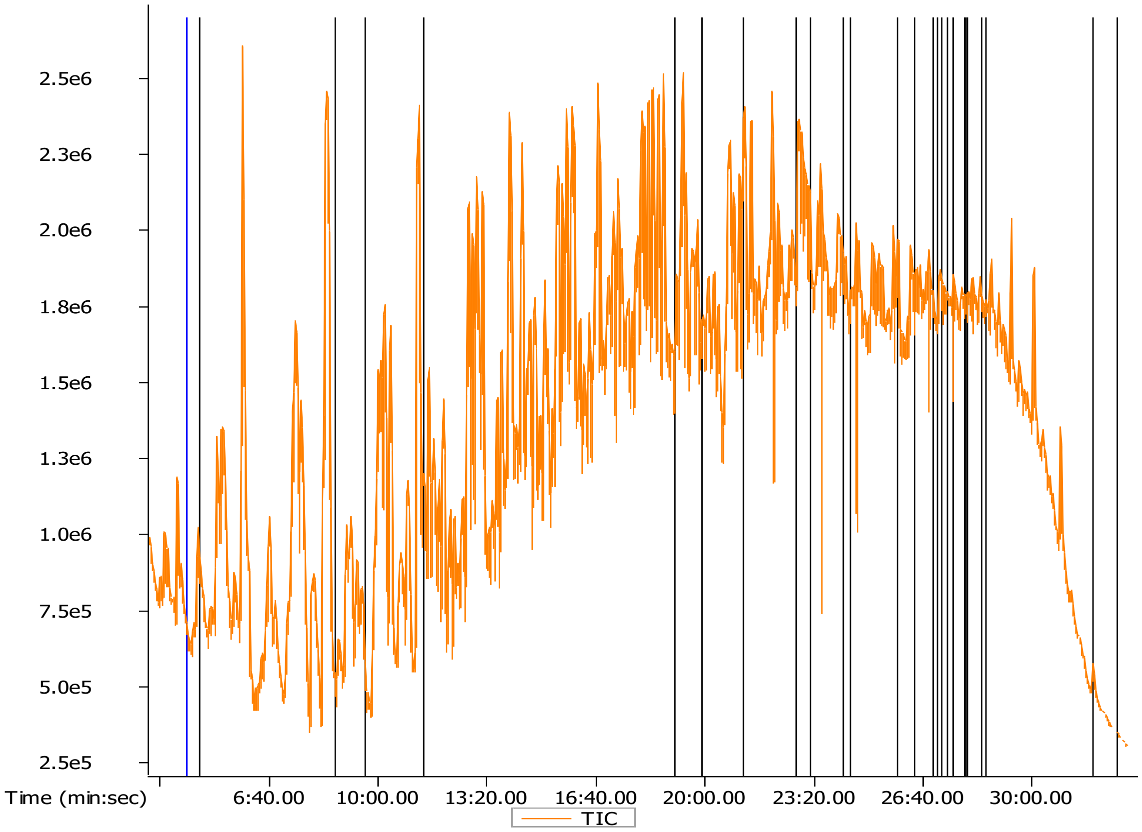


Figure 4. 3 TIC of OSCs contained in diesel sample

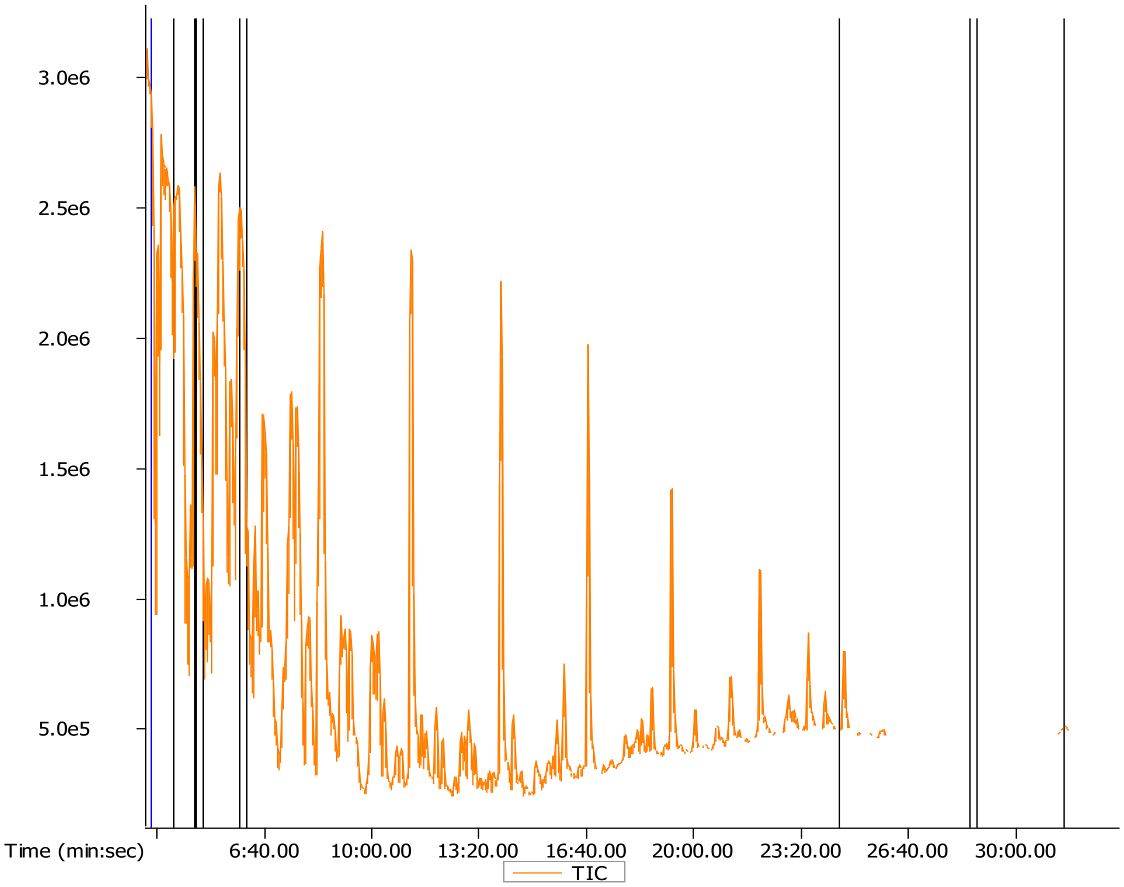


Figure 4. 4 TIC of OSCs contained in crude oil sample

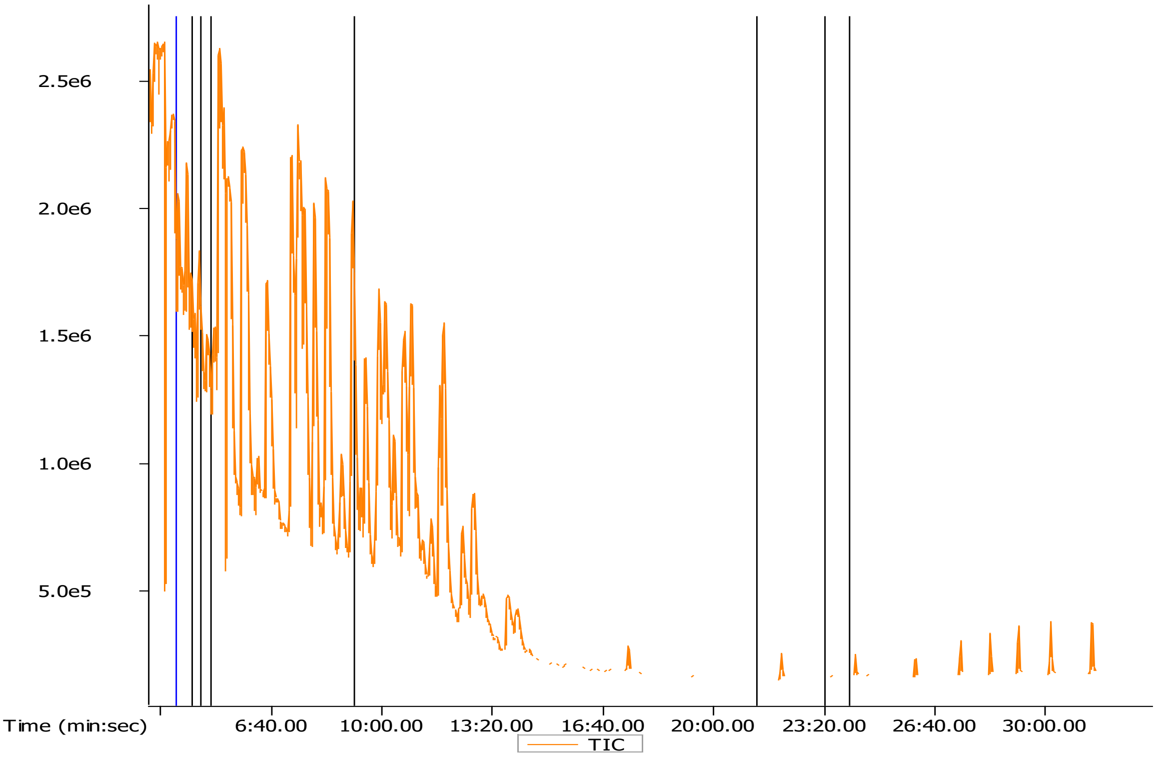


Figure 4. 5 TIC of OSCs contained in gasoline

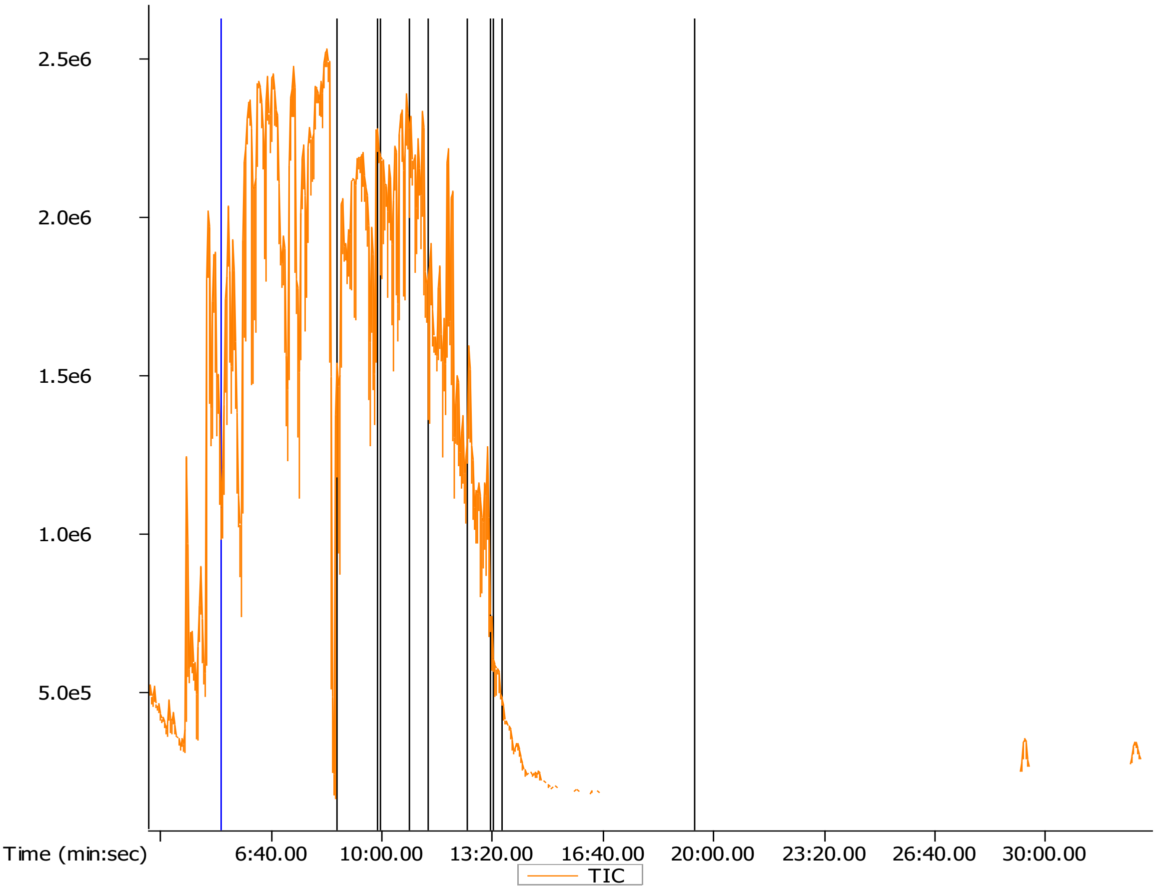


Figure 4. 6 TIC of OSCs compound contained in kerosene

Table 4. 2 Different OSCs contained in crude oil, kerosene, diesel and gasoline samples

Retention times (min)	OSCs	Molecular formula	Peak area	Molecular mass
	Diesel			
04:08,7	Thiophene, 3-methyl	C_5H_6S	433408	98,16
04:33,6	2-Thiophenecarboxylic acid, 4-nitrophenyl ester	$C_{11}H_7NO_4S$	5747829	249,24
08:42,2	Acetic acid, mercapto-	$C_2H_4O_2S$	7796493	92,12
09:35,5	Thiazol-2-amine, N-(4-dimethylaminobenzyl)-	$C_{12}H_{15}N_3S$	5400992	233,33
11:23,4	benzo[b]thiophene	C_8H_6S	7279128	134,2
19:05,5	Thieno[3,4-d]-1,3-dioxole, tetrahydro-2,2-dimethyl-, 5,5-dioxide	$C_7H_{12}O_4S$	6391109	192,23
19:55,4	dibenzothiophene	$C_{12}H_8S$	663300	184,26
21:10,0	Sulfurous acid, octyl 2-propyl ester	$C_{11}H_{24}O_3S$	8290728	236,37
22:45,7	Benz(1,4)oxathiino(2,3-c)pyridine	$C_{11}H_7NOS$	10097397	201,24
23:13,8	3-hydroxy-2-thiabutane	C_3H_8OS	68294015	92,15
24:12,2	2-Thiopheneacetic acid, isopropyl ester	$C_9H_{12}O_2S$	1760520	184,25
24:26,4	3H-[1,3,4]Oxadiazole-2-thione, 5-(4-fluorophenyl)-3-(piperidin-1-yl)methyl-	$C_{14}H_{16}FN_3OS$	49120968	293,36
25:53,4	2-Butanone, 3,3-dimethyl-1-thiocyanato-	$C_7H_{11}NOS$	10672795	157,23

26:25,0	3-Acetyl-2,5-dichlorothiophene	$C_6H_4Cl_2OS$	29360712	195,07
26:59,2	2(3H)-Thiazolethione, 4-methyl-	$C_4H_5NS_2$	49060908	131,22
27:06,2	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	$C_{14}H_{11}N_3S$	1877009	253,32
27:14,8	Benzothiazole, 2-(m-tolyl)-	$C_{14}H_{11}NS$	426584376	225,31
27:23,8	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	$C_{14}H_{11}N_3S$	2012999043	253,32
27:35,0	Ethylamine, N,N-dinonyl-2-phenylthio	$C_{26}H_{47}NS$	2198576738	405,72
27:56,3	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	$C_{14}H_{11}N_3S$	6521420	253,32
27:58,1	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	$C_{14}H_{11}N_3S$	1891188	253,32
28:01,3	10H-Phenothiazine, 2-(trifluoromethyl)-	$C_{13}H_8F_3NS$	443421	267,27
28:27,8	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	$C_{14}H_{11}N_3S$	178087613	253,32
28:35,2	1-(4-Methyl-dibenzothiophen-2-yl)-piperidine	$C_{18}H_{19}NS$	706473792	281,42
31:51,3	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	$C_{10}H_{22}OS_2$	669350617	222,41
32:35,8	Difluoro(methylamino)phosphine sulfide	CH_4F_2NPS	2015304	131,08
	Crude oil			
05:10,0	Thiophene, 2-(1,1-dimethylethyl)-	$C_8H_{12}S$	3009582	140,25
05:39,9	Thiophene, 3-methyl	C_5H_6S	293770482	98,16
08:37,7	Sulfurous acid, 2-ethylhexyl nonyl ester	$C_{17}H_{36}O_3S$	1313395738	320,53
09:52,3	Thiophene, 2-nitro-	$C_4H_3NO_2S$	3584020	129,14
09:56,9	Acetic acid, mercapto-	$C_2H_4O_2S$	1335228412	92,12

10:49,5	Benzenethiol, 4-(1,1-dimethylethyl)-	$C_{10}H_{14}S$	3598037	166,28
11:22,6	2-Thiophenecarboxylic acid, 5-tert-butyl-	$C_9H_{12}O_2S$	1336013527	184,26
12:33,6	benzo[b]thiophene	C_8H_6S	54414	134,2
13:15,4	4-(1-Methylpropylthio)pyrido[3,2-c]pyridazine	$C_{11}H_{13}N_3S$	24117800	219,31
13:22,4	Sulfurous acid, isobutyl pentyl ester	$C_9H_{20}O_3S$	388630153	208,32
13:37,6	dibenzothiophene	$C_{12}H_8S$	1908493197	184,26
	Gasoline			
03:48,7	Thiophene, 3-methyl	C_5H_6S	8339991	98,16
04:16,7	3-Picoline, 2-(tert-butylthio)-	$C_{10}H_{15}NS$	215040643	181,3
04:32,8	2-Thiophenecarboxylic acid, 4-nitrophenyl ester	$C_{11}H_7NO_4S$	961027993	249,24
04:51,1	2-Thiophenecarboxylic acid, 4-nitrophenyl ester	$C_{11}H_7NO_4S$	1192448932	249,24
09:11,4	2-p-Tolyl-N-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yl)acetamide	$C_{12}H_{10}F_3N_3OS$	5759227	301,29
10:40,4	benzo[b]thiophene	C_8H_6S	83611	134,2
21:16,9	methanesulfonamide, N-[2-(octylamino)ethyl]-	$C_{11}H_{26}N_2O_2S$	27964937	250,41
22:40,6	dibenzothiophene	$C_{12}H_8S$	557880573	184,26
23:20,2	Tetramethyl diphosphane-oxide-sulfide	$C_4H_{12}OP_2S$	2489360204	170,15
24:05,6	Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	3135005990	278,45
	Kerosene			
05:10,0	Thiophene, 3-methyl	C_5H_6S	5445,8	98,16

08:37,7	Sulfurous acid, 2-ethylhexyl nonyl ester	$C_{17}H_{36}O_3S$	17150017	320,53
09:52,3	Thiophene, 2-nitro-	$C_4H_3NO_2S$	660253668	129,14
09:56,9	Acetic acid, mercapto-	$C_2H_4O_2S$	2928689230	92,12
10:49,5	Benzenethiol, 4-(1,1-dimethylethyl)-	$C_{10}H_{14}S$	3526779253	166,28
11:22,6	2-Thiophenecarboxylic acid, 5-tert-butyl-	$C_9H_{12}O_2S$	5747829	184,26
12:33,6	2-Ethyl-6-phenyl-1,3,4-thiadiazolo(3,2-a)(1,3,5)-triazine-5,7-dione	$C_{12}H_{10}N_4O_2S$	4302799781	274,3
13:15,4	benzo[b]thiophene	C_8H_6S	433408	134,2
13:22,4	Sulfurous acid, isobutyl pentyl ester	$C_9H_{20}O_3S$	526726563	208,32
13:37,6	1-Butanesulfinamide, 1,1,2,2,3,3,4,4,4-nonafluoro-N-methyl-	$C_5H_4F_9NOS$	2016139509	297,14
19:24,1	dibenzothiophene	$C_{12}H_8S$	2720987822	184,26

4.1.4 Comparisons of studies

The study has showed satisfactory results when compared to other studies as indicated in **Table 4.4**. A total of 56 OSCs from the samples were screened with high degree of similarity above 50 % as indicated on **Table 4.3** without any pre-concentration technique employed. The number of compounds screened are equivalent to the study by Aloisi (2020) as indicated in **Table 4.4** which was done using a two dimensional gas chromatography.

Table 4. 3 Comparison of different studies with current study

Analytical instrument	Sample matrix	Target OSCs	Total OSCs screened	Ref
GC-AED/MS	Jet fuel	Thiols, sulphides and thiophenes	-	[180]
HPLC-FTMS	Crude oil	Thiophene	44	[181]
GC × GC-FID/SCD	Crude oil fractions	Thiols, benzothiophenes, dibenzothiophene	-	[71]
GC-Q-TOFMS	Diesel fuels	Alkyldibenzothiophenes	19	[27]
GC×GC-HRToF-MS	Coal Tar	3-methylthiophene, 2,5-dimethylthiophene, 2-propylthiophene, 3-butylthiophene, benzo[b]thiophene, 2-methylbenzo[b]thiophene , dibenzothiophene (DBT), 4,6-dimethyldibenzothiophene,	60	[182]
GC-ToF-MS	Crude oil, Diesel, gasoline and kerosene	Thiophene, 3-methylthiophene, benzothiophene, dibenzothiophene	19	This work

4.2 Characterization of nanoparticles

4.2.1 UV-VIS

Surface plasmon resonance (SPR) in gold nanoparticles was intense, resulting in an absorption peak in the visible portion of the electromagnetic spectrum as shown in **Figure 4.7**. The presence of superimposed peak represented magnetite and the red peak showed the presence of gold attached to magnetite.. This could be due to the reduced mean free path of the electrons for the small particles. The surface plasmon resonance is visible as a peak in **Figure 4.7** on the visible region, in the range between 520-530 nm. Elbialy et al.,[183]; Thao et al.,[184] and Keung et al., [170] have previously synthesized these nanoparticles and reported the presence of the peak at the same region reported in the current study.

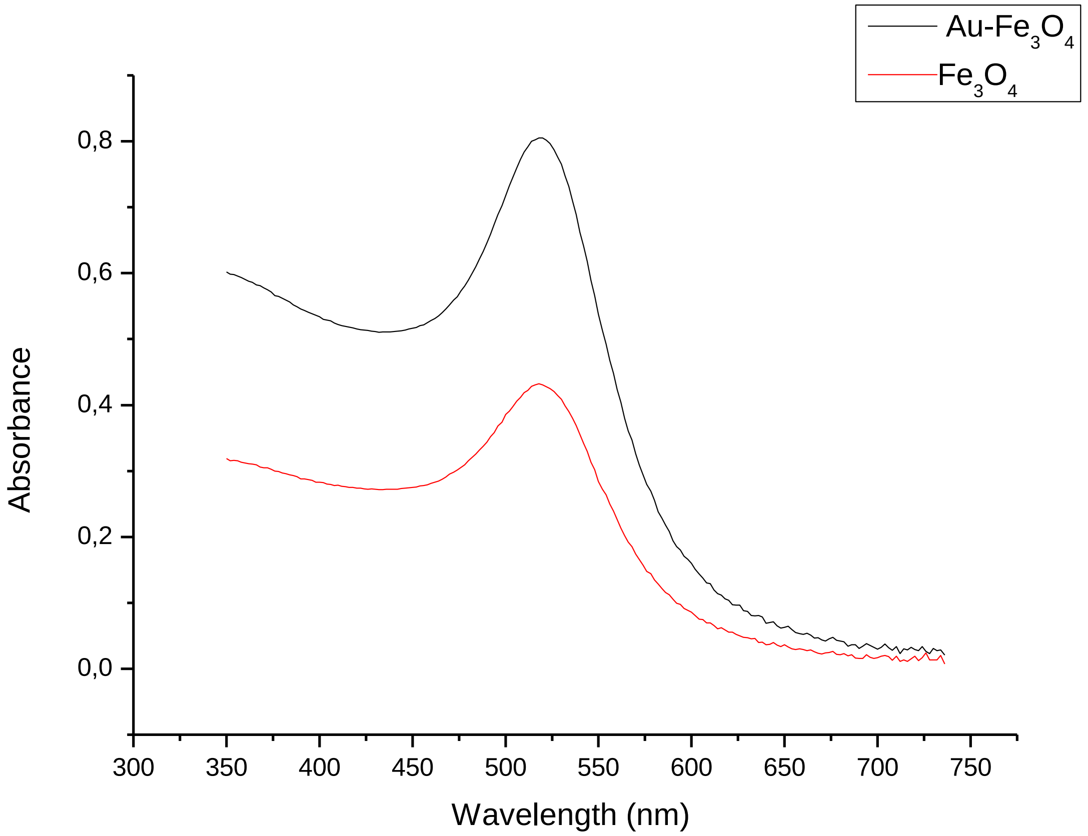


Figure 4. 7 UV-vis spectra of Fe_3O_4 and Au-Fe_3O_4 nanoparticles solution

4.2.2 Fourier transform infrared spectroscopy (FT-IR)

An IR spectra was used to confirm the identity of functional groups attached to the nanoparticles. The spectrums were compared to confirm the functionality. The two spectra in **Figure 4.8** showed similarities and that indicated that the functionality contained on both nanoparticles are similar in composition with major differences brought about the peaks at around 500 cm^{-1}. This peak in Fe_3O_4 is brought about the Fe-O stretching vibrational mode of Fe_3O_4. The peak is also present for the Au-Fe_3O_4 NPs but not sharp and intense because of the presence of Au. **Figure 4.8** shows the peak between 1500 cm^{-1} and 1750 cm^{-1} can be assigned to the presence of C=O vibration. The presence of the C=O band can be attributed to citrate used during the synthesis, thus it can be associated with the –COOH group from the citrate shift. It also indicates the binding of the citrate to the Fe_3O_4 nanoparticles. **Figure 4.8** also shows a sharp peak for both IR spectra at 3492 cm^{-1}. This peak can be assigned to the –OH groups brought about the presence of water on the surface of the nanoparticles. Stein and co-workers (2020) synthesized and characterized citrate stabilized gold-coated magnetite. During their study, characterization of the nano particles using the FT-IR showed a Fe-O between 550 cm^{-1} and 650 cm^{-1} [185] which is in line with what was obtained on current study. In another study, a deprotonated C=O peak was reported at 1710 cm^{-1} [186] which is agreement with the current synthesis of the gold coated magnetite.

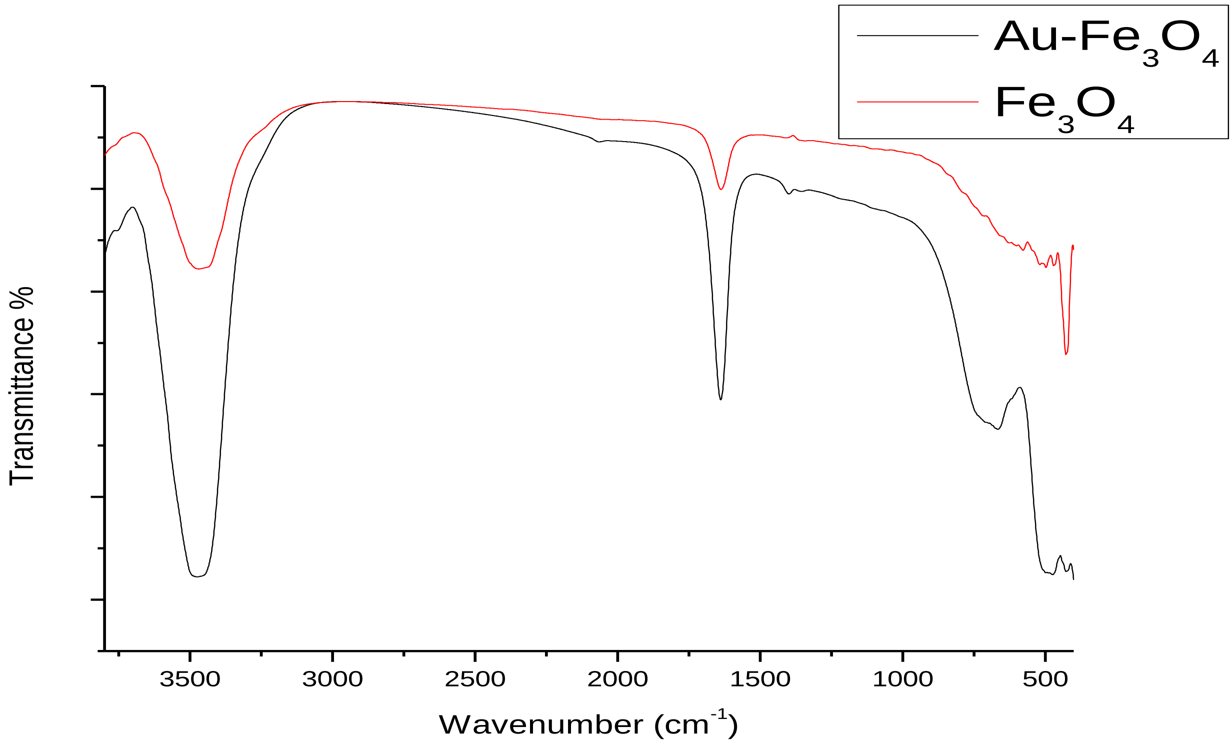


Figure 4. 8 FTIR spectrums of Fe_3O_4 and Au-Fe_3O_4

4.2.3 Scanning electron microscopy and energy dispersive x-ray (EDX)

The prepared Fe_3O_4 and Au-Fe_3O_4 are characterised in **Figure 4.9**. The nanoparticles are well dispersed and uniform in size as observed by SEM, **Figure 4.9A and C**. These images show the spherical shapes of both the Fe_3O_4 and Au-Fe_3O_4 nano particles. Furthermore, EDX confirmed that the prepared nanoparticles contained the elements of Fe, C and O (**Figure 4.9 B)** for the synthesized magnetite. The detected signals of carbon arise from the SEM grid. The Au-Fe_3O_4 nano particles are confirmed with **Figure 4.9 D** with elemental distribution of Fe, O, C and Au at 55, 33.9, 8.8 and 1.8 wt. % respectively, which are elemental composition of Au-Fe_3O_4. The presence of Au element is not visible of the non-coated Fe_3O_4 as expected.

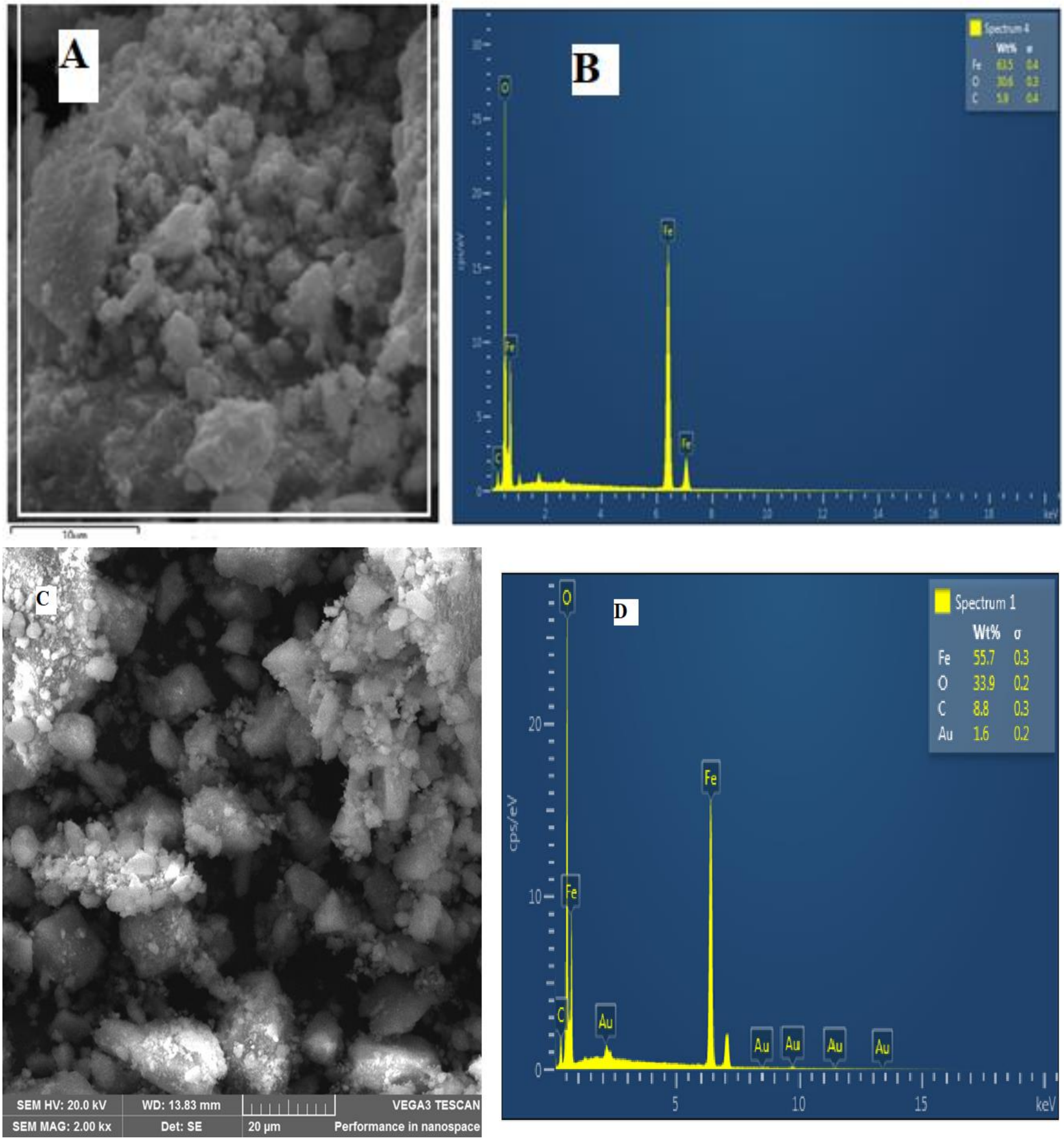


Figure 4. 9 A) SEM for Fe_3O_4, B) EDX for Fe_3O_4, C) SEM for Au-Fe_3O_4 and d) EDX for Au-Fe_3O_4

4.2.4 Transmission electron microscopy

Transmission electron microscope was used to determine the shape and the size of the nanoparticles. The prepared Au-Fe_3O_4 nanoparticles mostly are spherical in shape, well dispersed and uniform in size as observed by TEM in **Figure 4.10**. The nanoparticles on the image look aggregated which can be due to the magnetic properties it possesses. It also shows a shell in a form of gold that has surrounded the Fe_3O_4 nanoparticles. Due to the high electronic density of gold when compared to the magnetite, the shell structure of the particles are not visible on TEM, thus the employment of EDX on the previous section. The congruence of TEM with the SEM and EDX are confirmed as explained in the previous section. The Fe_3O_4 NPs were also spherical in shape with a diameter of about 10 nm. Previous studies of gold coated magnetite produced similar images [169].

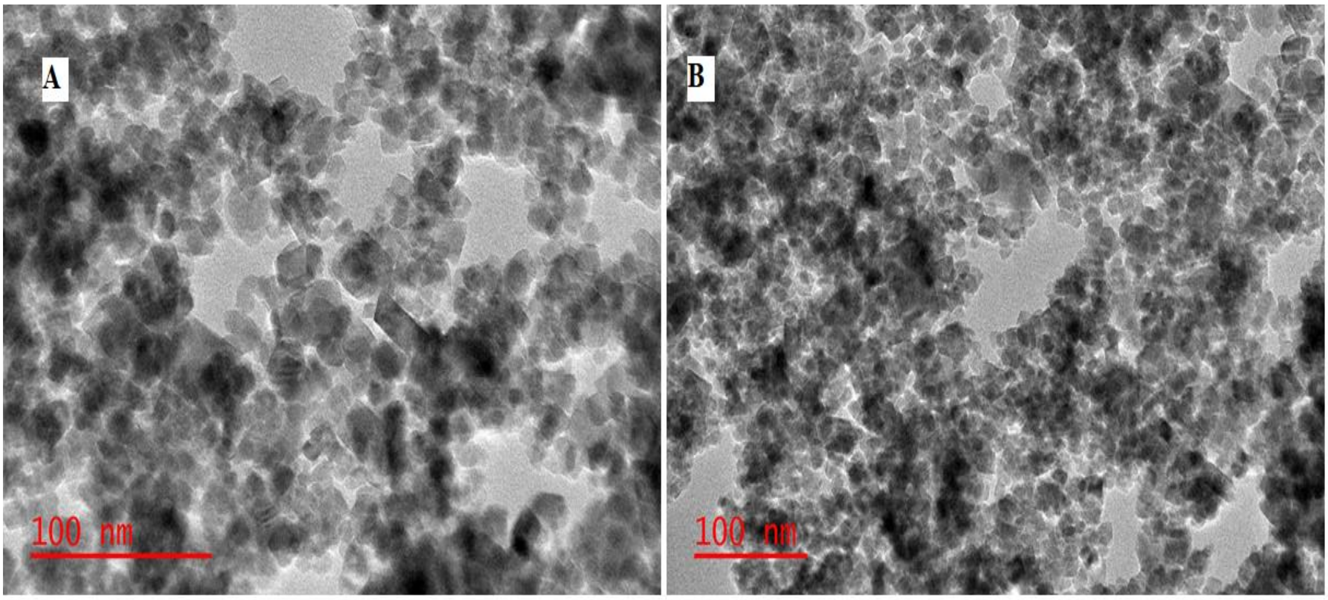


Figure 4. 10 TEM for A) Fe_3O_4 B) Au-Fe_3O_4 core shell magnetic nanoparticles

4.2.5 Powder X-ray diffraction (P-XRD)

Powder X-ray diffraction patterns of Fe_3O_4 nanoparticles depict diffraction peaks (**Figure 4.11**) at 30.05, 35.33, 43.01, 53.39, 56.87, and 62.48u for which can be indexed to the (220), (311), (400), (422), (511), and (440) planes of Fe_3O_4 in a cubic phase. The diffraction peaks of Au-Fe_3O_4 nanoparticles are comparable to those of both Au and Fe_3O_4 nanoparticles. Their diffraction peaks can be assigned to Au (111), (200), (220), (311), and (222), and Fe_3O_4 (220), (311), (511) and (440) planes. The penetration of X-rays through the thin gold-coated layer to the central Fe_3O_4 core can reveal the diffraction peaks for Fe_3O_4. The diffraction patterns for both have been reported in the previous studies such as that conducted by Sedki and co-workers [187].

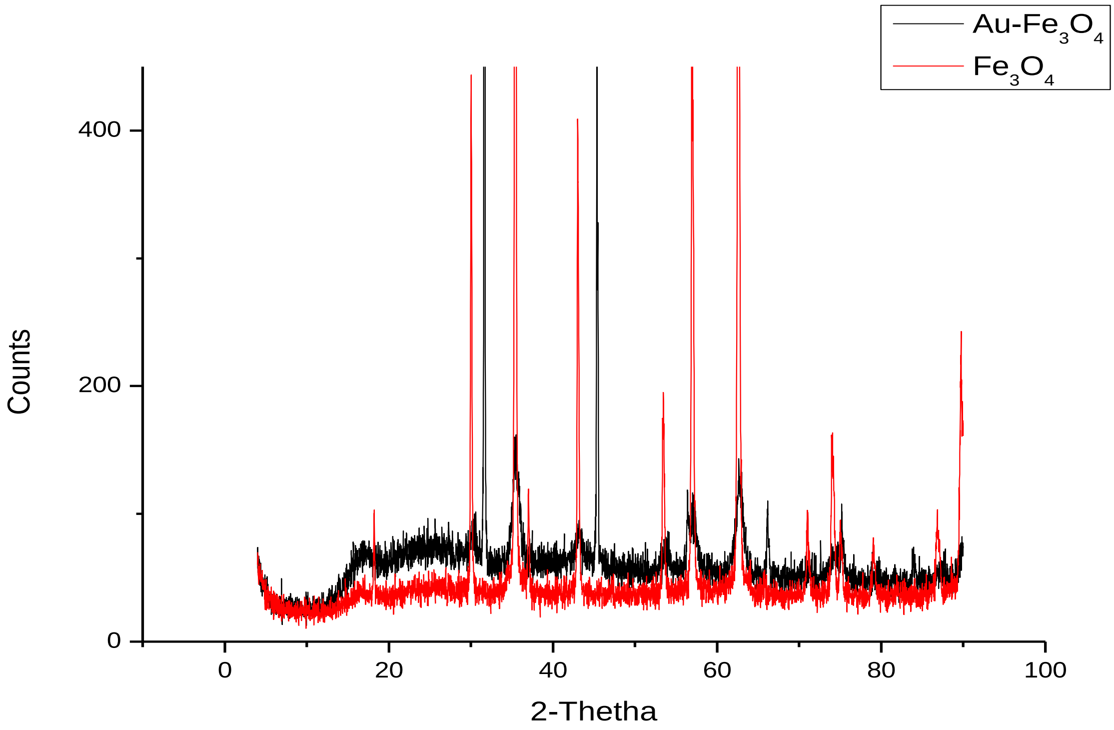


Figure 4. 11 P-XRD for Fe_3O_4 (black) and Au-Fe_3O_4 (red)

4.3 Quantitative analysis of OSCs using m-SPE extraction method

4.3.1 Optimization of the extraction procedure

The screening process of most significant factors was conducted using a full factorial design with four independent variables. Mass of the sorbent, pH, eluent volume and time of extraction were chosen as the parameters to be varied. The minimum and maximum amount of each factor was chosen based on the previous studies. **Table 4.4** shows all the factors with the maximum and minimum amount for each factor.

Table 4. 4 Minimum and maximum amount used for different parameters for screening

Variable	Minimum (-)	Maximum (+)
Mass of sorbent (mg)	10	100
Volume of Acetonitrile eluent (µL)	100	1000
Extraction time (min)	10	30
pH	4	9

The screening of significant parameters was done using atwo level full factorial design. The analytical response of each OSCs was given in a form of percentage recovery and the design matrix is displayed in **Figure 4.12.** The design of experiment (DOE) results produced Pareto charts form ANOVA that shows significant parameters on the preconcentration of OSCs using the magnetic solid phase extraction (m-SPE) procedure. The Pareto charts were constructed from four terms combination. The significant parameters are represented by the blue bars showed in **Figure 4.12** for each individual factor. The red-line indicates the 95% confidence level, thus from **Figure 4.12** it can be seen that extraction time is the only parameter that passed the 95% confidence level on thiophene, 3-methylthiophene and benzothiophene. Since the other parameters' bar length suggest that they are significant, further optimization is required thus the respond surface methodology is employed in the next section. Mass of the sorbent is another parameter which has a long bar length, thus in the next section, the two parameters i.e. extraction time and the mass of the sorbent will be further optimized to improve the recoveries of the OSCs extraction using the m-SPE.

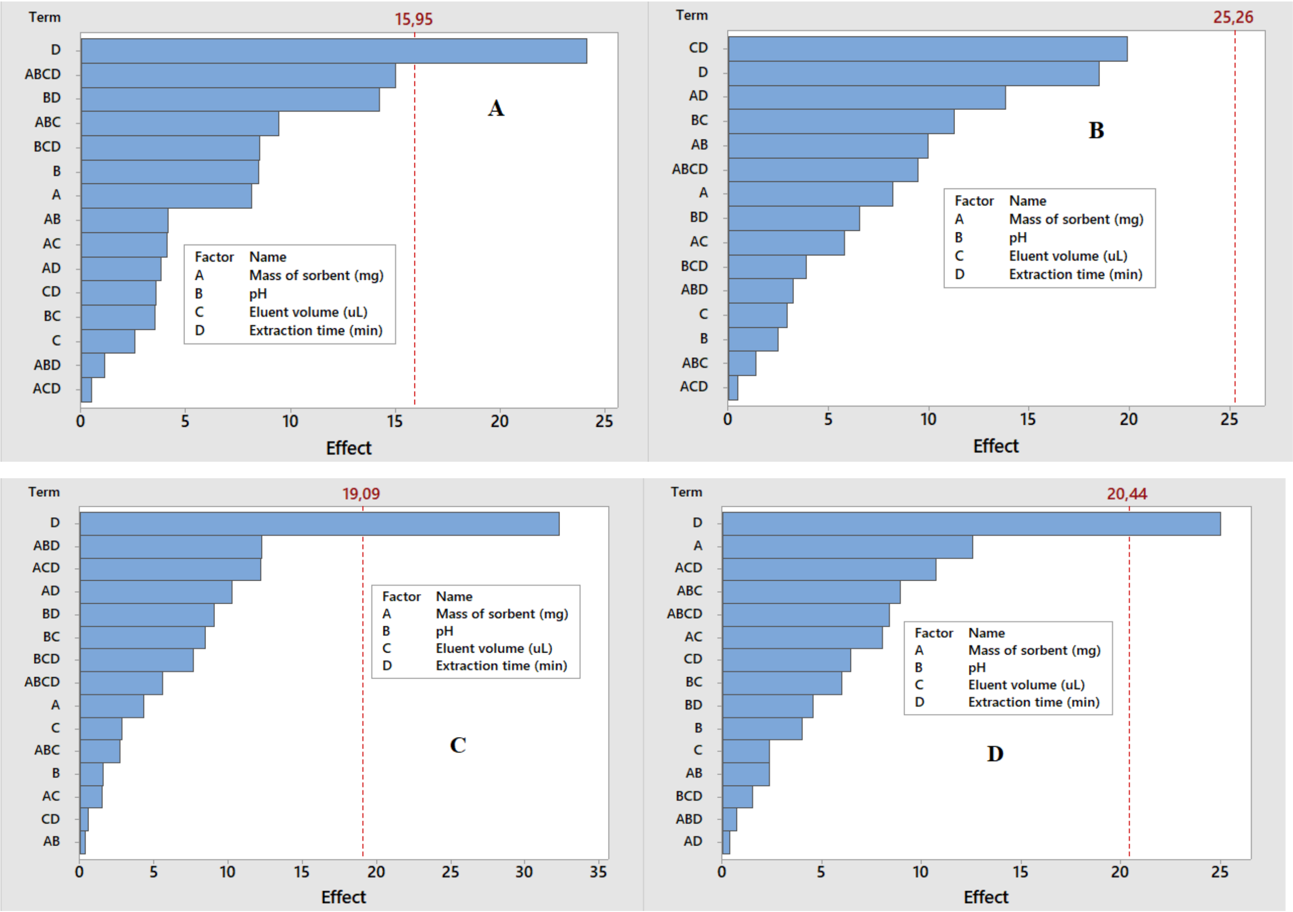


Figure 4. 12 Full factorial design Pareto charts (n = 3) of A) thiophene, B) 3-methylthiophene, c) dibenzothiophene and D) benzothiophene for the optimization of mass of sorbent, pH, eluent volume and extraction time at 95 % confidence level

Respond surface methodology

Central composite design (CCD) was used for robust mapping during the study in order to optimize further the parameters that are significant for the pre-concentration of the OSCs in crude oil samples. **Table 4.5** shows the central composite design matrix and its responses in terms of relative peak areas. The CCD deduced the interaction between the parameters under investigation and it helped in formulating the quadratic effects of the parameters, which are shown in equations 1-4. In this study, two experimental parameters were investigated at a two level full factorial design with 4 axial points and 5 centre points in cube. The experiments were conducted in triplicate for precision and accuracy. Each run produced 13 base runs with only base block. The central composite designs surface plots (**Figure 4.13 A-C**) were produced under optimum conditions in order to investigate the effect of mass of sorbent (40-120 mg) and the time of extraction (20-60 min). These conditions were informed by the results obtained from the previous section during the screening process. In **Figure 4.13** it can be clearly seen that recoveries of above 75% for all the OSCs was obtained. For example, **Figure 4.13 c** shows that a recovery of more than 90% was obtained with a mass of 100 -200 mg and extraction time of around 60 minutes. The recoveries were satisfactory as they were above 75%. The conditions that yielded this satisfactory recovery were used in the next section for other sample pre-concentration using the magnetic solid phase extraction.

$$Thiophene = -32{,}3 + 20{,}4\,A + 0{,}943\,B - 1{,}436\,A^2 - 0{,}00462\,B^2 - 0{,}0142\,A\mathrm{B} \qquad \text{Equation 1}$$

$$3 - methylthiophene = 18{,}8 + 9{,}2\,A + 0{,}733\,B - 0{,}780\,A^2 - 0{,}00515\,B^2 + 0{,}0156\,AB \quad \text{Equation 2}$$

$$Benzothiophene = -26{,}6 + 24{,}1\,A + 1.199\,B - 1{,}393\,A^2 - 0{,}00247\,B^2 - 0{,}1369\,AB \quad \text{Equation 3}$$

$$Dibezothiophene = -53{,}2 + 23{,}1A + 2{,}050\,B - 1{,}44\,A^2 - 0{,}00629\,B^2 - 0{,}0927\,AB \quad \text{Equation 4}$$

Table 4. 5 Respective percentage recoveries of central composite design matrix

Experiment	Extraction time (min)	Mass of sorbent (mg)	Thiophene	3-methylthiophene	Benzothiophene	Dibenzothiophene
1	40	110	80,3	79,3	87,9	90,1
2	60	200	99,1	85,6	92,5	96,1
3	40	110	68,5	73,6	77,9	81,3
4	20	20	72,1	67,2	55,4	56,3
5	11	110	77,2	75,4	89,3	91,3
6	40	237	61,3	56,4	89,1	66,2
7	68	110	50,2	70,8	70,1	55,5
8	40	-17	0	0	0	0
9	60	20	68,5	73,6	77,9	81,3
10	40	110	70,2	67,4	69,3	70,9
11	20	200	51,4	64,2	55,6	60
12	40	110	71,9	77,5	81,9	90,5

13	40	110	77,9	71,9	83,9	76,5

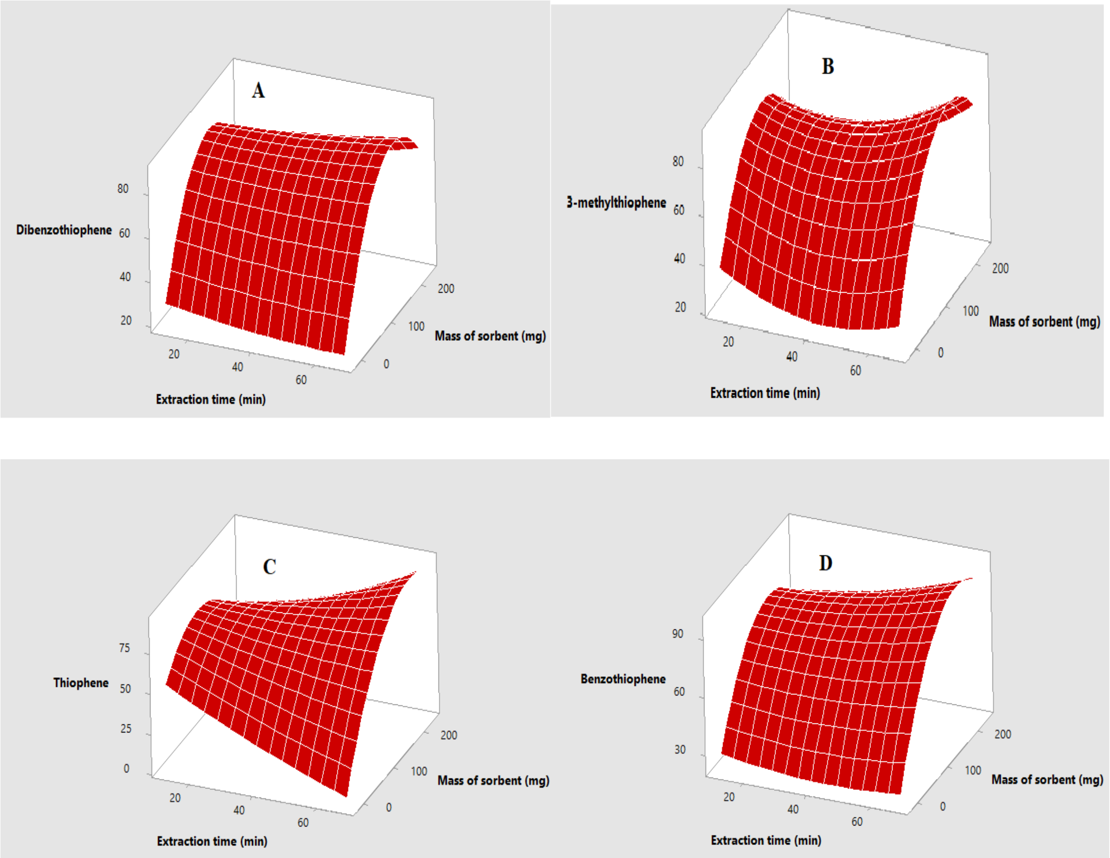


Figure 4. 13 Central composite design optimization method for mass of sorbent and extraction time

4.3.2 Validation of magnetic solid phase extraction method

The proposed m-SPE method was validated by using five concentration levels of spiked and unspiked crude oil samples with three replications for each concentration level. The spike concentration levels for the sample were 6, 10, 17, 22 and 25 ppm. The concentration of the samples was plotted with peak areas to provide method calibration. The calibration was used to deduce figures of merit such as the linearity, precision, limit of detection, limit of quantification and the linear equations in order to validate the m-SPE on the OSCs analysis by GC-ToF-MS. The validation data is summarized in **Table 4.6.** The correlation factor for the proposed procedure ranged from 0,9816-0,9961 for all the investigated OSCs. Furthermore, the limit of detection and the limit of quantification ranged from 0,02-0,199 and 0,602-0,126 - 0,602 ug/g respectively. The precision of the method was also validated through the relative standard deviation as it was in the range of 0,9-2,3 %. Since the RSD is less than 4%, this indicates that the method relatively has excellent reproducibility.

Table 4. 6 Figures of analytical merits for OSCs analysis in fuel oil samples at 25 µg/g

Compound	**LODs (µg/g)**	**LOQs (µg/g)**	**linear correlation coefficients(R^2)**	**Recovery (%)**	**Linear equation**	**RSD %**
Thiophene	0,02	0,131	0,9816	76	y = 2E+06x + 2E+06	1,8
3-Methylthiophene	0,0265	0,0806	0,993	93	y = 4E+07x + 8E+07	1,1
Benzothiophene	0,199	0,602	0,9961	88	y = 1E+08x + 7E+08	0,8
Dibenzothiophene	0,0416	0,126	0,9829	95	y = 1E+08x + 1E+09	2,3

LOD, limit of detection for = 3SD/m ; LOQ limit of quantification = 10SD/m for S/N =10; RSD, relative standard deviation (n = 3)

4.3.3 Comparison with reported literature

Several reports have been compiled on different extraction and pre-concentration methods for the OSCs in different samples. **Table 4.7** gives the summary of the analytical figure of merits of the compiled reports with those of the current study. From the complied tabled, the current study has linearity of 0,9816-0,9961. Furthermore, it has a relatively good precision (0,8-2,3) when compared to other study such as study by , Yang et al [38]. The developed method can be used as an alternative with high precision for the analysis of OSCs. The lowest concentration of OSCs that can be detected was found to be between 0.08-0.602 µg/g.. Although the limit of detection is higher, the methods that yield relatively low limit of detection requires high extraction time, use of commercially available fibres and low selectivity of the analyte [186]. Thus, the extraction time of the current study is lower which makes a rapid and low-cost method since the adsorbent can be easily synthesized.

Table 4. 7 Comparison of analytical merits for developed method with reported literature

Samples	OSCs	Method	Chromatography	LODs (ppm)	% Recovery	Correlation R^2	RSD %	Ref
Crude oil and diesel	Benzothiophene, dibenzothiophene and derivatives	LPME	GC-SCD	0,001 – 0,111	77 -91	0,98 – 0,99	4,5-13,8	[36]
Crude oil	Benzothiophene, dibenzothiophen, and benzo[b]naphtho[1,2-d]thiophene and their derivatives	µ-SPE	GC-MS		81.5 -92.1	0.9894 to 0.9994	3.23 – 7,52%	[38]
Coal tar	Benzo[b]thiophene, Thiophene and its derivatives	Dilution	GC × GC-ToF-MS	8,0 – 30,0	-	0,97414 – 0,9882	1 – 4	[182]
Crude oil	Thiophene, dibenzothiophen, 3-	m-SPE	GC-HR-ToF-MS	0,02 - 0,199	76 - 95	0,9816 – 0,9961	0,8 – 2,3	This work

methylthiophene and
benzathiophene

4.3.4 Application of m-SPE in real fuel oil samples

The developed extraction method was then applied on diesel, gasoline and kerosene samples to detect the amount of OSCs present including the target analytes. This was conducted using the previously deduced optimum conditions applied on crude oil for validation. The amount of these OSCs was detected in some samples as depicted in **Table 4.8**. Furthermore, it can clearly be seen that the semi-quantification of kerosene sample contains the highest concentration of the OSCs ranging from 1,01-3,6 ppm. Crude oil sample was the second highest with semi- quantified concentration of OSCs ranging from 0,13-4,09 ppm. On the other hand, the kerosene sample did not show any presence of a lot of OSCs compounds as compared to the other samples. During the analysis of the samples, other OSCs compounds, which were not a target during the study, were visible.

Table 4. 8 Semi-quantified Concentration of OSCs in crude oil, diesel, gasoline and kerosene samples using m-SPE followed by GC-ToF-MS

Organosulphur compounds	Crude oil (ppm)	Diesel (ppm)	Gasoline (ppm)	Kerosene (ppm)
Acetic acid, mercapto-	0,3 ± 0,005	0,02 ± 0,001	1,06 ± 0,007	ND
Disulfide, diphenyl	ND	0,1 ± 0,02	3,06 ± 0,02	ND
Benzo[b]naphtho[2,3-d]thiophene, 9,10-dihydro-6,7-dimethyl-	1,06 ± 0,004	0,012 ± 0,1	3,05± 0,04	1,01 ± 0,05
Disulfide, bis(4-methylphenyl)	ND	0,41 ± 0,06	ND	5,02 ± 0,0001
Benzenemethanethiol, TMS derivative	0,13 ± 0,08	ND	0,46 ± 0,003	3,6 ± 0,2
2-Thiophenecarboxylic acid, 5-tert-butyl-	1,02 ± 0,05	3,06 ± 0,03	2,06 ± 0,01	1,9 ± 0,003
Acetic acid, mercapto-	1,93 ± 0,03	ND	ND	ND
Benzo[b]thiophene, 3-phenyl-	0,35 ± 0,02	2,15 ± 0,009	ND	2,45 ± 0,004
Thiophene, 2-iodo-	2,05 ± 0,07	ND	1,04 ± 0,005	ND
5-Bromo-2-thiophenecarboxaldehyde	4,09 ± 0,1	2,01 ± 0,003	2, 65 ± 0,03	1,43 ± 0,4
Thioxanthene	ND	0,9 ± 0,006	1,12 ± 0,05	ND

Experimental conditions: volume of eluent, 100 µL; mass of sorbent, 150 mg; time of extraction, 50 min; and pH of sample, 6,7.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

. Sample dilution and an intensive magnetic solid phase extraction (m-SPE) were the methods developed. The sample dilution method was used for screening of the different OSCs contained in crude oil, diesel, gasoline and kerosene samples thus qualitative analysis.

During the qualitative analysis of the OSCs in the fuel oils, dichloromethane (DCM) was used as a solvent for the sample dilution prior to chromatographic analysis. The good properties of the solvent such as the good solubility of the solvent made it easy for the sample to be well dissolved. Moreover, DCM has a low boiling point and low molecular mass, thus peak interference between the analyte of interest and the solvent peak was eliminated. A total of 56 OSCs were obtained through screening of the samples. These compounds include thiols, disulphides, thiophenes and polycyclic aromatic sulphur heterocycles, just to name the few.

The quantitative analysis of OSCs in crude oil, diesel, gasoline, and kerosene was performed using the m-PSE procedure for preconcentration method prior to chromatographic analysis. During the m-SPE, a magnetic sorbent in a form of gold coated magnetite nano particles was used. The synthesized nano particles were successfully characterized to confirm the formation of the Au-Fe_3O_4 nano particles. UV-Vis peak at around 520 – 5230 nm which is a typical characteristic of Au or Fe3O4 was observed. The SEM and TEM showed that the particles have a spherical shape with particle size of 100 nm, while XRD showed that they have magnetite phase. FTIR showed Fe-O peak at 500 cm^{-1} which proved that Fe_3O_4 nanoparticles were successfully synthesized Moreover, factors affecting m-SPE were evaluated using full factorial design. These were the volume of the eluent (100-1000 µl), the pH of the sample (4-9), the extraction time (10-30 min) and the mass of the adsorbent (10-100 mg). Through Pareto charts produced from the full factorial design, it was found that extraction time and mass of adsorbent were the most significant parameters. These significant factors were further optimized using the central composite design (CCD) as the response surface methodology to find the optimum conditions. The optimum conditions were found to be 150 mg for the mass of sorbent and 50 min for the time of extraction. The other conditions that were kept constant were eluent volume which was 100 µL of acetonitrile and the pH of the sample at 6.7.

Coupling the GC-ToF-MS with the developed sample pre-treatment method yielded an improved correlation factor of 0,9816-0,9961, better sensitivity of 0,8-2,3 % and acceptable recoveries of 76-95 %. The limit of quantification and the limit of detection were found to be in the range of 0,126-0,602 ppm and 0,02-0,0416 ppm respectively. The developed methods can be used as alternative methods for determination of OSCs in oily matrices. Upon analysing, the concentration levels of OSCs in crude oil, gasoline, diesel, and kerosene were found to be in ranged of 0,3-4,09; 1,06-1,65; 0,02-3,06 and 1,01-5,02 ppm, respectively. The concentrations of OSCs in the investigated fuel samples were in acceptable level according to the South African Petroleum regulations. The dilution method was rapid, cost-effective, and environmentally friendly, while m-SPE was simple and selective toward OSCs thus recommended for qualitative and semi-quantitative analysis of OSCs.

5.2 Recommendations for future studies

. In future, a comparison between this study and a study using a two-dimensional gas chromatography can be conducted to compare these studies since this instrument provides an extra dimension due to the presence of the dual columns. The number of targeted analytes can be increased to include more of the polycyclic aromatic sulphur compounds, since they contain different substituents, which can cause interferences during analysis. Furthermore, other parameters can be investigated instead of the four that this study focused on, this includes factors as such as the spiking concentration, different eluent etc. Other studies can also incorporate the use of a bio-adsorbent for the m-SPE because they are also inexpensive and ecologically friendly. These bio-adsorbents can be further used to remove the OSCs contained in different oily matrices.

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