Method development and applications - hollow fiber supported liquid membrane extraction of mono and dicarboxylic acids and analysis of aerosol samples after ultrasonic extraction.

Degree Project in Analytical Chemistry, 30 ECTS credits

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Abstract:

A highly sensitive (LOD= 0.04-0.4 ng/ml) method was developed for the detection and quantification of monocarboxylic and dicarboxylic acids (C_3 -C_{10}) by GC-MS. These compounds exist in trace amount as essential components of secondary organic aerosols i.e. they are important constituents of atmospheric aerosols. Membrane extraction technique was utilized for selective enrichment (1-4300 times) of the target compounds. Good repeatability (RSD% ≤ 10%) using a selective organic phase (10% TOPO in DHE) was achieved with three-phase HF-LPME. Target compounds in real samples (aerosols), after ultrasonic assisted extraction were quantified through GC-MS. Effective derivatization of each target compound was performed with BSTFA reagent. Gas chromatography, having a capillary column and interfaced with mass spectrometry was used for detection, separation and quantification of the target compounds.

Key words: HF-LPME, Derivatization, BSTFA, UAE samples, TOPO, DHE, TMS

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1. Introduction:

The impact of atmospheric aerosols on human health and the effect on Earth's atmosphere is getting importance and the phenomena have been well understood. [1]. Atmospheric aerosols can harm respiratory and cardiovascular system of human.
The impact of secondary organic aerosols (SOA) as biogenic and anthropogenic antecedents is identified [1]. Low molecular dicarboxylic acids (C_3-C_9) are vital tracers of SOA [2]. Short chain fatty acids are found as SOA, which are also supposed to originate from long chain fatty acids [1]. The importance of organic aerosol has been well established and carboxylic acids are of great interest for environmental studies [1]. Several studies and mechanisms were proposed to understand the production of these SOA precursors [1]. Short chain carboxylic acids (C_3-C_9) are found extensively in the troposphere [2]. SOA are formed in the atmosphere by gas particle conversions. Organic matter present in aerosols constitutes more than 90% of troposphere's aerosols [3, 4].

Dicarboxylic acids are found in nature as polymeric compounds such as suberin and cutin [5]. Aromatic acids (phthalic acid, 4-hydroxy benzoic acid, syringic acid, etc.) are generally emitted through anthropogenic sources, like reminiscent of solvent evaporations and automobile exhaust [6]. Dicarboxylic acids are found in plant oils, which have greater interest for the cosmetic and pharmaceutical industries [7]. Short chain dicarboxylic acids, having aliphatic chain possess strong cyclotoxicity and antineoplastic activities [8]. Dicarboxylic acids are consumed by industry in high scale. These acids, as important intermediates are also used in biosynthesis for the production of biological compounds [9].

Many analytical techniques are used to determine the composition of SOA, so keeping in view these techniques a new method for the determination of fatty acids (common in SOA) has been developed. Membrane extraction is used in this work due to its increasing importance for high selectivity and enrichment factor [10].

1.1. Description of target analytes:

Important properties (source, structure and existence in aerosols) of monocarboxylic and dicarboxylic acids target compounds (C_3-C_{10}) are discussed further (sections; 1.1.1-1.1.12). These compounds (C_3-C_{10}) were the target analytes in this diploma work and were extracted through liquid phase micro-extraction and detected by GC-MS system. Fig. 1.1-1.12 represent structures of these target analytes.

1.1.1- Malonic acid:

Malonic acid is a metabolite of plants and tissues (Malonyle-CoA) [11]. Malonic acid is an intermediate for the preparation of fatty acids from plants and other tissues [12]. It is also present in aerosols as an important constituent of short chain fatty acids [13]. It is present in beet roots as a calcium salt [14 (A)].

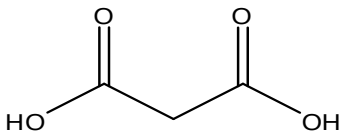

Fig. 1.2- Structure of malonic acid

1.1.2- Succinic acid:

Succinic acid is found in the atmosphere as a water soluble compound and as a compound of SOA [15]. Succinic acid exists as solid crystals, anciently called spirit of amber. Succinic acid is an important intermediate in the citric acid cycle. This biological cycle has basic importance for the living organism [14 (B)].

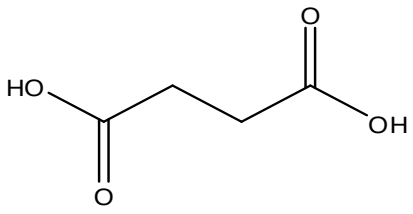

Fig. 1.3- Structure of succinic acid

1.1.3- Adipic acid

Adipic acid is a product of lipid peroxidation. It does not hydrolyse in the environment, perhaps due to the lack of hydrolysable functional groups [3].

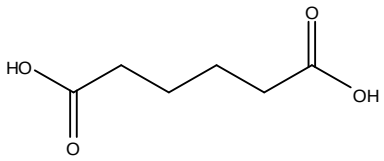

Fig. 1.1- Structure of adipic acid

1.1.4- Glutaric acid:

Glutaric acid is found as SOA in aerosols [13]. It is sparingly soluble in water [14 (C)] and is used to prepare plasticizers for polyesters [14 (C)].

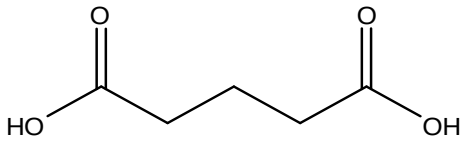

Fig. 1.4- Structure of glutaric acid

1.1.5- Pimelic acid:

Derivatives of pimelic acid are used for the biosynthesis of amino acids, typically lysine [14 (C)]. Pimelic acid is produced when nitric acid is heated with oleic acid as a ‘‘secondary sublimation product’’ and pimelic acid is not crystallized in this reaction [22].

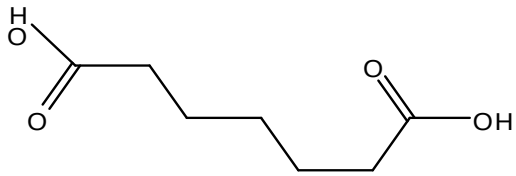

Fig. 1.5- Structure of pimelic acid

1.1.6- Suberic acid:

Suberic acid is normally produced from suberine [13]. Suberic acid can also be produced by vigorous reaction condition of the natural oil with nitric acid [13].

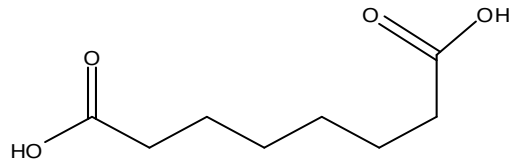

Fig. 1.6- Structure of suberic acid

1.1.7- Azelaic acid:

Azelaic acid is an important constituent of SOA. It produces short chain fatty acids upon photo oxidation. Azelaic acid is produced during oxidation of unsaturated acids i.e. those are found in oleic acid [17].

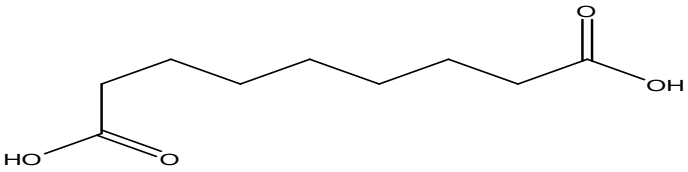

Fig. 1.7- Structure of azelaic acid

1.1.8- Cis-pinonic acid:

Cis-pinonic acid is produced in atmosphere by photo oxidation of α-pinene in the presence of ozone [18].

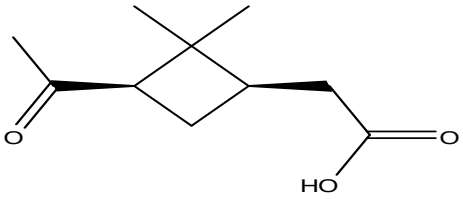

Fig. 1.8- Structure of cis-pinonic acid

1.1.9- Pinic acid:

Pinic acid is a derivative of α-pinene and is found in SOA. It is generated by the photo oxidation of α-pinene with ozone as given in this chemical reaction; ($C_{10}H_{16}$ + 5/3 O_3 ----> $C_9H_{14}O_4$ + HCHO). Pinic acid is used to prepare plasticizers in industry [18].

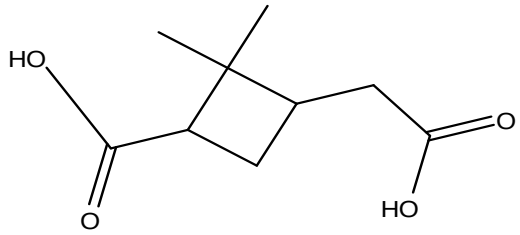

Fig. 1.9- Structure of pinic acid.

1.1.10- 4-hydroxybenzoic acid

4-hydroxy benzoic is used to derive parabens and is also used as antioxidant [14 (D)].

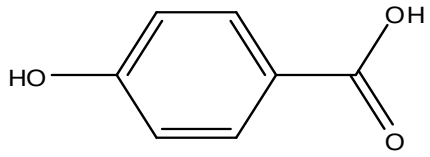

Fig. 1.10- Structure of 4-hydroxy benzoic acid.

1.1.11- Phthalic acid:

Phthalic acid is an aromatic dicarboxylic acid. It is found purely in crystalline state [14 (E)]. It is found abundantly in the atmosphere and has toxic properties [6].

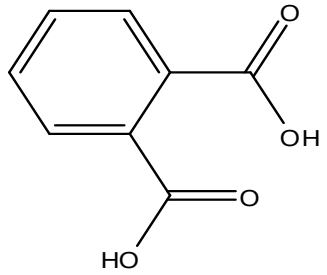

Fig. 1.11- Structure of phthalic acid

1.1.12- Syringic acid:

Syringic acid is found as humic substance in theenvironment [19].

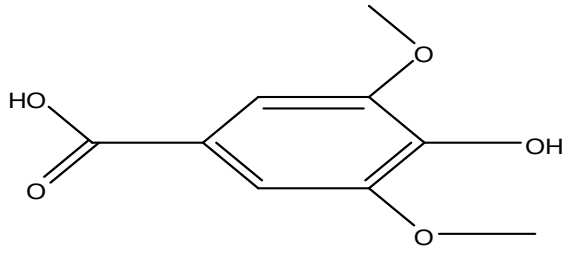

Fig. 1.12- Structure of syringic acid.

1.2. Ultrasonic assisted extraction:

A detection procedure by GC-MS was established through a series of injections containing the reference standards of the target analytes (pure). After validating the method with standard injections on the GC-MS, unknown real (aerosol) samples after ultrasonic assisted extraction (UAE) were quantified. A theoretical description is given in section 1.2 for “UAE”.

1.2.1- Principle and applications of UAE:

The name ‘Ultrasonic’ is derived from ultrasound. Ultrasound refers to the sound that has a higher frequency than a normal human can hear. Ultrasonic techniques are used in chemistry in several aspects and due to their application in chemistry, it is known as “sonochemistry” [20].

Ultrasound is used in sample preparation in analytical chemistry like extraction, filtration, sample purification and dissolution. When the ultrasonic technique is used for assistance in extraction, this assistance in extraction is called “UAE” [20].

There are many advantages with UAE because it requires less organic solvent , it is non destructive, less expensive and less time consuming compared with other sample preparation techniques like soxhlet [21].

The normal range of ultrasound frequencies, used in the laboratory are in the range of 20-40 KHz. Practical use of UAE is very simple, a sample solution in a vessel is placed inside an ultrasonic bath at a desired temperature and sound waves stir the sample [22].

When UAE is used in an experiment, it increases the speed of mass transport by the vibration of mechanical transport from the sample matrix through a process called “cavitation” [21].

1.2.2- Theory of UAE:

There are two theoretical aspects of sonication for the sample preparation i.e. physical and chemical aspects. Physical and chemical aspects of UAE are described (section 1.2.2.1-1.2.2.2) in order to understand its practical use in analytical chemistry.

1.2.2.1- Physical aspects of UAE:

Ultrasonic intensity produces cavitations in a liquid sample during extraction (UAE). Two types of US cavitations are produced known as "transient cavitations" and "permanent cavitations" [21].

The life time of the "transient bubble" (produces due to the transient caviations) is so short that no mass transport or the diffusion of gas is possible with in the sample [21]. Transient bubble is believed to be produced at US intensity (10 W/cm^2) and a permanent bubble at intensity (1-3 $Watt/cm^2$). Sonochemical effects are intense inside the bubble because energy (numerous amounts) is produced during bubble eruption and production [21].

1.2.1.2 Chemical aspects of UAE:

When US radiation strikes a water molecule free radicals, OH^* and H^* are produced due to the collapsing of cavitations bubbles. These cavitation bubbles exhibit high temperature and pressure. Many other radicals can be produced in the same solution under analysis [21]. The radical 'OH^*' is believed to be more stable and can begin many new reactions, while the "H^*" radical is not stable.

The second effect of the US radiation is the "pyrolytic reactions". These reactions occur inside the bubble and can degrade compounds under analysis [19, 20].

1.3. Liquid Phase micro Extraction (LPME):

The applications of membrane extractions in analytical chemistry have taken the intentions of analysts during recent time. The goal of utilizing membrane extraction is to achieve a high enrichment, a selective extraction and an environment friendly procedure [10]. A small quantity of solvent (usually in microliters) is required compared with the old techniques of extractions (soxhlet) [10]. Clean extracts are obtained and after extraction, recovered compounds are shifted to another analytical instrument like gas chromatography for further quantitative or qualitative analysis [10].

1.3.1 Hollow fiber membrane extraction:

Two types of membranes are used in liquid phase micro extractions (LPME). One type of membrane is a flat sheet porous and a second is a polypropylene hollow fiber. In this diploma work polypropylene hollow fiber was used as membrane support in the membrane extractions due to its limited cost and less carry over problems [10].

1.3.1.1 HF-LPME Technique:

When a hollow fiber is used in LPME, the technique is called hollow fiber liquid phase micro extraction (HF- LPME). In the HF- LPME technique, a hollow fiber is used containing a thin film of immobilized liquid membrane inside the pores, while the fiber is dipped into an aqueous phase containing objective analytes. Objective (target) analytes transport through the membrane into a liquid filled inside the lumen of the fiber, which is termed as an acceptor phase [24].

Extraction of the target analytes (C_3-C_{10}) was carried through three-phase HF- LPME during this diploma work. The donor phase contained analytes in an acidic aqueous phase, a suitable organic solvent i.e. dihexyl ether (TOPO mixture) was used in the pores of the hollow fiber as a stationary liquid membrane support (SLM). The acceptor contained a basic aqueous phase [24]. Target analytes were recovered into the acceptor phase after the evaporation of water. Acetonitrile was added in a small dried glass flask along with a derivatizing reagent (BSTFA). After derivatization, these samples were injected into a gas chromatographic system.

1.3.2 Basic Principle of LPME:

In three-phase HF- LPME, the "donor" in an aqueous solution containing sample and is filled in a flask. A short piece of hollow fiber is normally used and the "acceptor" is injected inside the fiber through a micro syringe. After injecting the acceptor one end of the hollow fiber is closed and the other end contains a syringe needle. The fiber containing solutions is inserted in an appropriate organic solvent having less polarity (dihexyl ether) to create a SLM. The pH of the donor is adjusted such that it can restrain the ionization of the target analytes [24].

The process of a three-phase extraction [24] can be explained in Eq. 1.1.

$$A_{\text{Sample (in aqueous)}} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} A_{\text{Org}} \underset{K_4}{\overset{K_3}{\rightleftharpoons}} A_{\text{aqueous acceptor}} \qquad \text{..... 1.1}$$

Where 'A' is the target analyte, 'K_1', 'K_2', 'K_3' and 'K_4' are the first order extraction rate constants. In order to obtain the combined distribution coefficient, at equilibrium recovery, Eq. 1.2 is derived [24].

$$D_{\text{acceptor/sample}} = C_{\text{eq acceptor}} / C_{\text{eq sample}}$$

$$= C_{\text{Org sample}} / C_{\text{eq acceptor}}$$

$$= \alpha_D . \mathbf{K}_{\text{org/sample}} / \alpha_a . \mathbf{K}_{\text{org/acceptor}} \qquad \text{.....1.2}$$

In Eq. 1.2, $C_{\text{eq acceptor}}$, $C_{\text{eq sample}}$ and $C_{\text{Org sample}}$ are the concentration of analytes at equilibrium, in acceptor phase, in the aqueous sample phase and in the organic phase respectively. Here '$\mathbf{K}_{\text{org/sample}}$' and '$\mathbf{K}_{\text{org/acceptor}}$' are the partition ratios between organic phase to the sample phase and between the organic phase to the acceptor phase, respectively [24]. 'α_D' and 'α_a' are the extractable fraction of the total concentration of target analytes in the sample and in the acceptor respectively.

If the extraction conditions between the sample and acceptor are similar, other than the ionization of the analytes in the sample phase from the interpretation of Eq. 1.2 it is obvious that equilibrium is independent on the partition ratio of the SLM in the three-phase LPME i.e. it depends mainly on the ionization of the analytes in the sample (donor) [24].

Extraction efficiency (E) can be calculated from Eq. 1.3 [24].

$$E = \frac{D_{acceptor/sample} \cdot V_{acceptor}}{D_{acceptor/sample} V_{acceptor} + D_{Org/sample} \cdot V_{mem} + V_{sample}} \quad \text{..... 1.3}$$

'V_{sample}', '$V_{acceptor}$' and 'V_{mem}' in Eq. 1.3 are the volume of donor sample phase, aqueous acceptor phase and organic immobilized liquid membrane phase respectively. $D_{acceptor/sample}$ and $D_{Org/sample}$ are the individual distribution coefficients relative to the sample and acceptor, and the sample to the organic phase respectively [24]. Eq. 1.3 is derived for three-phase LPME. It is evident from the interpretation of Eq. 1.3 that the efficiency is mainly controlled by the individual distribution coefficients. Individual distribution ratios are directly dependent on the partition coefficients, so by increasing the partition ratios efficiency can be improved [24]. The ratio of the partition coefficients can be improved by properly adjusting the pH of the donor or acceptor and by using an appropriate organic solvent. The concentration of the sample in the donor phase and the volume of organic phase should be very small (Eq. 1.3) to develop better efficiency [24].

1.3.3- Mass transfer in LPME:

The enrichment factor (E_e) of three-phase LPME is given in Eq. 1.4.

$$E_e = C_{acceptor}/C_{initial} = V_{sample.} E / V_{acceptor} \quad \text{..... 1.4}$$

In Eq. 1.4, $C_{acceptor}$ is the concentration of the target analyte present in the final stage inside the acceptor [24].

When an acidic analyte is ionized in an aqueous solution, the total extractable fraction of the analytes (α) is given in Eq. 1.5 [10].

$$\alpha = [AH]/ [A^-][AH] = 1/[1+10^{(pH-pKa)}] \quad \text{..... 1.5}$$

In the context of Eq. 1.3, the overall distribution constant (D) at equilibrium can be rearranged, as given in Eq. 1.6 [10].

$$D = 1+10^{s(pH-pKa)}.K_D/1 + 10^{s(pH-pKa)}. K_A \quad \text{..... 1.6}$$

's' is equal to 1 for acidic analytes (Eq. 1.6). 'pKa' is the dissociation constant and 'pH' refers to the donor or acceptor (Eq. 1.6) [10].

$$\Delta C = \alpha_D .C_s - \alpha_a C_A.K_A/K_S \quad \text{..... 1.7}$$

Eq. 1.5-1.7 are derived from the Henderson-Hasselbalch relation, in this equation 'α' represents the extractable fraction of analytes [10].

The driving force for the extraction in neutral conditions of three-phase LPME is the concentration gradient (ΔC) from the sample to the acceptor [25]. The concentration gradient between the two phases, between donor and acceptor, is described in Eq. 1.7. 'K' is the partition ratio of uncharged analyte between the membrane and aqueous phase. 'C_A' and 'C_s' are the concentrations of analytes in the acceptor and sample phase respectively [25].

1.3.4 End point for extraction:

Three end points are normally considered for the extraction [24].

1. Exhaustive extraction. 2. Kinetic extraction. 3. Equilibrium extraction.

1.3.4.1 Exhaustive extraction:

The exhaustive end point is the specific point (time) when the entire amount of analytes is exhausted (which can be practically possible) present in the donor [24]. In this diploma work, exhaustive end point was focused mainly in (LPME) extractions. The enrichment factor is increased by growing the concentration of analytes in the acceptor by the passage of time and at a certain point it reaches a stable value [25, 26]. The enrichment factor can be improved by increasing the value of α_D preferably close to unity and decreasing the value of α_A to zero. Such conditions for the α_D and α_A values are called "infinite sink" conditions i.e. are required normally for exhaustive extractions [24].

1.3.5 Rate of LPME:

Two parameters govern the rate of extraction (when extraction approaches equilibrium conditions) i.e. the "membrane controlled" and the "diffusion controlled extractions" [10, 26]. Maximum concentration, E_e, can be obtained when the concentration gradient (ΔC) is zero, described in Eq. 1.8 [10, 26].

$$E_{e\,(max)} = (C_a / C_d)_{max} = \alpha_D / \alpha_A \qquad \text{..... 1.8}$$

In membrane controlled extractions, the rate limiting step is the diffusion of target analytes. When analytes pass through the organic phase the mass transfer (K_m) is given in Eq. 1.9 [10, 26].

$K_m \propto K.D_m/h_m$ …….. 1.9

In Eq. 1.9, K is the partition coefficient, 'D_m' is the membrane diffusion coefficient and 'h_m' is the thickness of the membrane [10, 26].

1.3.6 Addition of trioctyl phosphine oxide (TOPO):

Mass transfer can be improved for acidic analytes by using different concentrations (w/v) of TOPO in the organic solvent typically for short chain carboxylic acids. Interaction of TOPO with polar acids in solution takes place efficiently due to hydrogen bonding [27].

1.3.7 Trapping of Analyte in Three-phase LPME [10]:

The concentration enrichment of analytes in three-phase LPME is achieved by stable mass transfer through the membrane to the acceptor phase. Back diffusion of the analytes is prevented by the trapping of analytes in the acceptor phase. In order to achieve high enrichment of the acidic analytes, the pH of the acceptor is fixed enough basic so that when acidic analytes reached to the acceptor becomes charged i.e. the analytes can not be driven back to the donor, this trapping of analytes due to the pH adjustment is called ''direct trapping''. The buffer capacity of an acceptor should be sufficiently high so that during extraction, protons from the acidic donor should not be neutralized by the concentration gradient between the two aqueous phases during the three-phase LPME [10].

1.3.8 Selection for organic phase:

The choice of proper organic solvent is very importance in method validation of LPME because the SLM solvent directly affects the partition coefficient. The organic phase solvent should have low solubility in water [24] and low volatility to prevent solvent losses during the extraction process [27]. The organic phase should have a high distribution coefficient, between donor to organic phase and between organic to acceptor phase to achieve a high enrichment. The organic phase should have adequate affinity to the hollow fiber. The organic phase should be immobilized sufficiently to cause efficient trapping of the analytes in the pores through polarity matching [24]. A mixture of organic solvents can also be used as mobile phase [27]. In this project the organic solvent was either pure DHE or DHE was mixed with different amounts of TOPO (section 1.3.6) to achieve a high stability of the organic phase [10, 26].

1.3.9 Agitation of sample:

Extraction kinetics can be improved by agitation. Agitation increases the diffusion of analytes in the donor. The organic membrane solution (DHE) is very stable inside the pores of the

membrane. Shaking by a magnetic stirrer helps the transfer of analyte from the donor to the acceptor [28]. When the donor containing analytes is stirred at high speed, the probability of fresh solution contact with the organic phase is enhanced [29]. In order to enhance mass transfer, all membrane extractions in this project were assisted through agitation by a magnetic stirrer. A membrane extraction assembly used in this diploma work is shown in Fig. 1.13.

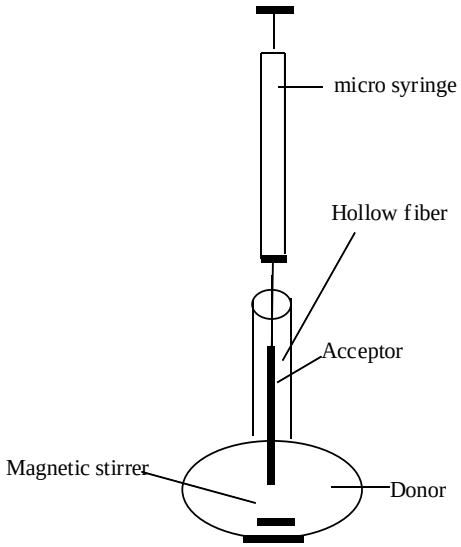

Fig. 1.13- Extraction assembly for three-phase HF- LPME

1.3.10 Volume of donor and acceptor

The volume of the acceptor should be minimal compared with that of the donor to get better sensitivity [28]. The volume of the acceptor should be sufficient enough to achieve better quantification by GC-MS. The volume of the acceptor should be enough to fill the lumen of the hollow fiber appropriately [28].

1.3.11 Adjustment of pH (donor and acceptor)

High partition ratios can be obtained in three-phase LPME by the proper adjustment of pH for the donor and the acceptor [28]. According to Eq. 1.7 the efficiency can be improved by an increase in the concentration gradient, which depends mainly on pH. In this project, three-phase LPME was used to extract acidic analytes (C_3-C_{10}) so the donor pH was adjusted lower than the pKa values of the target analyte to suppress the ionization of the target analytes in the donor [28].

1.4. Detection and quantification of Analytes

1.4.1- GC-MS analysis

GC-MS is a powerful detection technique for environmental trace analysis due to its high sensitivity [30]. Aerosols exist in trace levels so their detection requires a highly sensitive device having a low limit of detection. GC-MS suffers less matrix effects and is usually cost effective and highly selective [30]. Analytes are separated, after passing through the mass spectrometer according to their charge to mass (m/e) ratio. Scan mode is used for the identification of each target compound [30].

When gaseous analytes reach the mass spectrometer, these analytes are converted to their respective molecular ions. Electron ionization in the mass spectrometer strikes the molecules of the target compound into fragments [8]. These molecular ions are representative for each analyte, sensitivity and selectivity of GC-MS is improved through the selection of these specific molecular ions. This method is called the “selected ion chromatogram” (SIM) method [30]. “Signal to noise ratio” (SNR) is improved through “extracted ion chromatogram” (XIC). XIC is used through the SIM (MS) mode [30]. The SIM mode is used for qualitative and quantitative analysis [30].

Target analytes (C_3-C_{10}) in this project were polar and non volatile, so these analytes could not be detected and separated directly in pure state by gas chromatographic column. A derivatization step is necessary to convert these analytes into volatile substances. Derivatization was done to convert carboxylic and hydroxyl functional groups to their respective ester functional group [30].

1.5. Derivatization:

Two derivatization reagents; ''N, O-bis(trimethylsilyl) trifluoroacetamide'' (BSTFA) and ''N-(tertbutyldimethylsilyl)-N-methyltrifluoroacetamide'' (MSTFA) are commonly used for the esterification of the compounds containing hydroxyl and carboxylic functional groups, before injecting these compounds to GC-MS system[30]. Both derivatizing reagents were applied separately and compared prior to the quantification of samples by GC-MS.

1.5.1- **Silylation:**

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When BSTFA reagent is used as a derivatization reagent, a nucleuophilic attack is taken place by a hetero atom to the silicon atom [30]. BSTFA is very efficient to convert hydroxyl groups to the irrespective silyl esters [31].

The advantage with BSTFA is that its derivatives can be injected directly without purification and they can be used for very sensitive detection [8]. BSTFA is non polar and its efficiency can be improved by using BSTFA in acetonitrile [32]. The chemical structure of BSTFA is shown in Fig. 1.14 below.

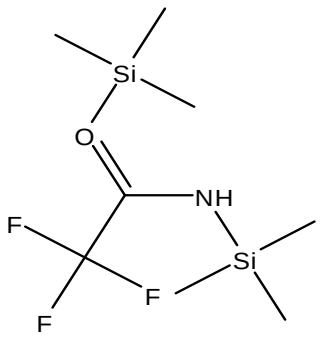


Fig 1.14: structure of BSTFA derivatizing reagent

Analytes, containing carboxylic acids (C_3-C_{10}) were introduced to the GC-MS system after derivatization. Carboxylic acids were converted to their respective trimethyl silyl ester (TMS derivative) by BSTFA.

Due to the use of BSTFA reagent in the reaction, a common peak appears at m/z= 73, due to the $[Si\ (CH_3)_3]^+$ molecular ion and at m/z=145 due to $[OH{=}Si\ (CH_3)_2]^+$ molecular ion. When analytes containing dicarboxylic acids are used for mass spectrometric analysis, ion peak appears at m/z=147. Ion peak at m/z=147 appears due to the $[(CH_3)_2Si{=}Si\ (CH_3)_2]^+$ molecular ion [8].

2. Method:

2.1. Membrane extraction:

The three-phase HF- LPME method was used for the extraction of the target analytes. The method for the three-phase HF-LPME is described (section 2.1.1-2.1.2) below.

2.1.1 Equipment and reagents for the HF-LPME:

The hollow fiber 'Accurel PP polypropylene' (Q3/2) was purchased from Membrana (Wuppertal, Germany). The wall thickness of the membrane was 200 µm, the inner diameter 600 µm and the pore size was 0.2 µm. Before extraction a 7.5 cm membrane was cut carefully with a fine cutter. After cutting the membrane, it was washed in acetone and dried overnight.

A magnetic stirrer containing multiple stations, model (Ika-werke, Germany) was used for the agitation of the donor. A 50 µl micro syringe (Agilent, Australia) was used to push the acceptor inside the lumen of the membrane and for holding the membrane. A pH meter (Mettler Toledo) was used to measure the pH of the donor and the acceptor. A volumetric flask (Kebo, Germany) was used to hold the donor.

Milli-Q water was obtained from Millipore gradient system (Millipore, USA). Hydrochloric acid (37%, Fluka) and Sodium hydroxide monohydrate (Fluka) were used to prepare further solutions. Dihexyl ether (97%) was purchased from Sigma Aldrich. TOPO (99%; Aldrich) was used to prepare solutions in DHE (%, w/v).

2.1.2- Set up for the membrane extraction:

2.1.2.1 Preparation of the donor:

The pH of the aqueous donor was adjusted to 2.0. All aqueous solutions were prepared in Milli-Q water and pH was adjusted by adding HCl (0.1M). All samples were spiked in a dried 100 ml volumetric flask. This flask was then, filled up to the mark with donor. Further 5 ml of the donor was added in the same flask to dip the whole of the fiber inside the donor phase. The total volume of the donor was adjusted to 105 ml (Fig. 1.13). A clean magnet was dropped in this flask and then this spiked solution was allowed to stir for 30 min at a fixed revolutions/min (800 rpm) of the magnetic stirrer.

2.1.2.2 Preparation of the acceptor:

The acceptor was prepared in Milli-Q water and pH 12 was adjusted by addition of sodium hydroxide (0.5 M, 5 M). The acceptor was injected inside the lumen of the dried membrane through the micro syringe. 24 µl of the acceptor was injected inside the lumen of the hollow fiber via the micro syringe. A specific volume (24 µl) of the acceptor was fixed after several adjustments to achieve the best compatibility with the 7.5 cm hollow fiber and to achieve good repeatability and enrichment.

2.1.2.3 Preparation of organic phase:

The hollow fiber containing the acceptor phase was dipped for 15 s (±3 s) into the organic solvent (pure DHE or TOPO% solutions in DHE) to impregnate the fiber with organic solvent and to establish an organic phase. The solvents, immobilized in the pores of the hollow fiber were; pure DHE, 1%, 5%, 10%, 15% and 19% TOPO in DHE (w/v). All solutions (TOPO in DHE) were prepared and mixed by shaking and sonicating, although 15% and 19% TOPO in DHE solutions were prepared by vigorous shaking and were put inside a sonicator for more than 20 min to achieve efficient mixing of TOPO.

2.2. Sample preparations:

All primary solutions were prepared in methanol. Primary solutions were prepared by transferring a specific amount (10 mg) of the analytes to a sample flask having air tight cap. This solution was diluted with methanol to prepare a solution of 100 µg/ml. Table 2.1 represents physical and chemical properties of the target analytes i.e. molecular weight (Mw), molecular (Molec) formula, source (chemicals were purchased from), pK_a values (in water) and purity (as labeled on each chemical) of each analyte.

Table. 2.1- Analytes source (purchased from), purity and properties

Sr. No.	Chemical name	Mw	Molec formula	Purchased from	pk_a Values	Purity (%)
1	Malonic acid	104.06	$C_3H_4O_4$	Aldrich	2.83, 5.69 [33]	99
2	Succinic Acid	118.09	$C_5H_6O_4$	Fluka	4.19, 5.48 [33]	99.9
3	Glutaric Acid	132.04	$C_5H_8O_4$	Aldrich	4.34, 5.42 [33]	99
4	Adipic Acid	146.14	$C_6H_{10}O_4$	Fluka	4.34,5.44 [33]	99.5
5	Pimelic Acid	160.17	$C_7H_{12}O_4$	Aldrich	4.48, 5.42 [33]	98
6	Suberic Acid	174.2	$C_8H_{14}O_4$	Aldrich	4.52, 5.40 [33]	98
7	Azelic Acid	188.22	$C_9H_{16}O_4$	Aldrich	4.55, 5.41 [33]	98
8	Cis-Pinonic Acid	184.23	$C_{10}H_{16}O_3$	Sigma Aldrich	N/A	98
9	Pinic Acid	186.2	$C_9H_{14}O_4$	Sigma Aldrich	N/A	99
10	Syringic Acid	198.17	$C_9H_{10}O_5$	Sigma	N/A	-
11	Pthalic Acid	166.03	$C_8H_6O_4$	Sigma Aldrich	2.98,528[14 (E)]	99.5
12	4- Hydroxy benzoic Acid	138.03	$C_7H_6O_3$	Aldrich	4.52,9.23 [34]	99

Primary solutions, (100 µg/ml, solution A) containing individual analytes were used to prepare multi-component standard mixture of 5 µg/ml. This solution was then called 'solution B'. Solution B was used to prepare further (dilute) solutions of different concentrations (Table. 2.2).

Multi-component standards were the mixtures containing all target analytes. Multi-component standards were further diluted to solutions of different concentrations (2000, 1000, 500, 250, and 50 ng/ml). These dilutions were prepared through 'solution B'. All standard solutions were prepared and diluted with methanol (solvent). The method for the preparation of dilute solutions is presented in Table 2.2.

Table 2.2- Multi-component standard mixtures of different strengths (concentrations)

Sr. No.	Amount of solution A(µl)	Methanol(µl)	Total volume	Solution concentrations
1	800	1200	2 ml	2000 ng/ml
2	400	1600	2 ml	1000 ng/ml
3	200	1800	2 ml	500 ng/ml
4	80	1920	2 ml	250 ng/ml
5	40	1960	2 ml	100 ng/ml
6	20	1980	2 ml	50 ng/ml

2.2.1 Sample preparation after extraction for the injection to GC-MS:

15 µl of the standard solutions (2000 ng/ml), was spiked to the donor in a volumetric (100 ml, Fig. 1.13) flask before insertion into the hollow fiber. This solution was stirred for 20 min to mix the sample solution thoroughly into the donor. After stopping the extraction, 24 µl (almost) of sample was collected from the acceptor via a syringe and this solution was transferred to a 1.5 ml GC flask (small pear shape glass flask), 24 µl of 0.1 M HCl was transferred to the flask to neutralize the basic pH of acceptor. This flask, containing neutralized solution was then put under a stream of nitrogen, at a specific temperature (30-40 $^{\circ}$C) to evaporate all solvent. Extreme care was required to evaporate the solvent under the stream of nitrogen. After drying of the solvent, 20 µl of internal standard (in acetonitrile) along with 10 µl of BSTFA were poured into the same flask. Finally, this sample flask was put in an oven at 80 $^{\circ}$C for 60 min. Derivatization was accompanied during this time and then 2 µl of sample from the sample flask was injected directly into the GC-MS system**.**

2.3. Chromatographic analyses:

Chromatographic analyses were performed on a 6890 series gas chromatographic system interfaced with an Agilent 5973-N mass selective detector. The Gas chromatographic system was equipped with an auto sampler and a 7683 injector. An EI source was used at -70 eV to produce fragmented ions. EI was operated in positive mode. Full scan mode from 50 m/z to 600 m/z was used to study the ion fragments of each analyte. Quantitative analyses were done by selecting characteristic molecular ions of each analyte through selected ion monitoring (SIM) technique [30]. The extracted ion chromatogram (XIC) method was used through data analysis window to get the information about the retention time of the characteristic ions. Peak area of each characteristic ion was selected and calculated for quantification purpose [30].

A factor four capillary column (30 m x 0.25 mm) having a phase thickness of 0.25 µm and 5% phenyl cross linked (Varian, Germany) was used. The column was fitted with a retention gap.

Ultra pure helium (99.9995% pure) was used as carrier gas. Table 2.3 shows the schedule of the oven (GC) temperature that was programmed for gas chromatographic analysis.

Table 2.3 – GC Temperature parameters

Ramp	Rate C/Min	Temperature C	Hold Min.	Time total
	-	60	2	2
Ramp 1	2.5	120	0	24
Ramp 2	10	220	0	34
Ramp 3	20	300	0	38

Gas (He; 99.999% pure) flow rate (1.5 ml/min) was used with the splitless injection mode. A fixed amount (volume) of sample "2 µl" was injected into the GC Injector (285 °C) throughout this project. Acetonitrile (HPLC grade), acetone (HPLC grade) and methanol (HPLC grade) were purchased from Fisher Scientific (USA).

2.3.1 Sample preparation for GC-MS analysis:

15 µl of a multi-component standard solution was transferred to a conical glass flask. This sample was put in an oven at 80 °C for 20 min to evaporate methanol. After solvent evaporation, 20 µl of an internal standard (in acetonitrile), along with 10 µl of BSTFA reagent were shifted to the previously dried flask. After pouring BSTFA and acetonitrile the flask was tightly capped and put in oven at 80 °C for 60 min. After 60 min of derivatization reaction the same flask was put to the auto sampler and the sample was injected directly to the GC-MS system**.** Multi-component standard solutions were injected in duplicate and in the concentrations range of 16.7 - 666.7 ng/ml (absolute quantity) to check linearity. Table 2.4 shows different standard amounts that were used to obtain the regression line (calibration curve).

Table 2.4- Concentration (ng/ml) of standard solutions in the injection flask.

Sr. No.	1	2	3	4	5	6
Sample Injected	666.7 ng/ml	333.3 ng/ml	166.7 ng/ml	83.3 ng/ml	33.3 ng/ml	16.7 ng/ml

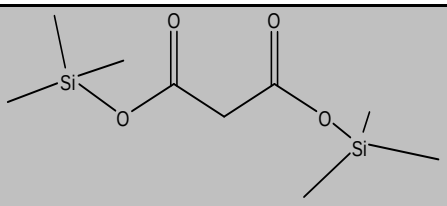
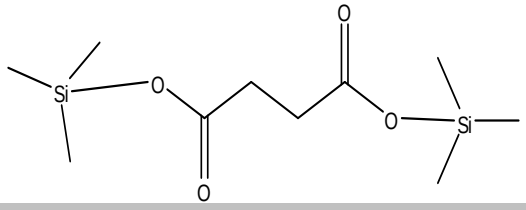
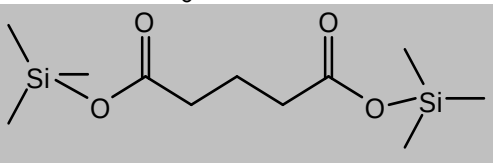
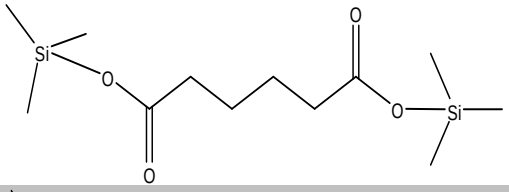
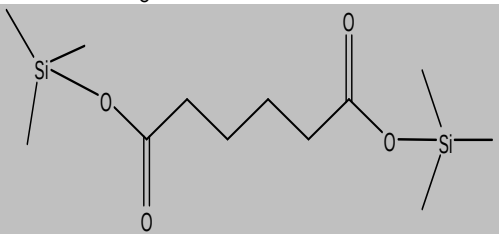
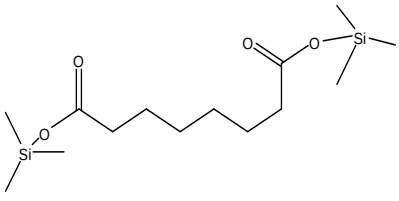
2.3.2- Set up for retention time to confirm mass spectra of analytes (GC-MS Analysis):

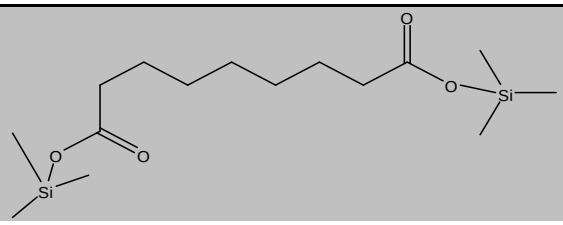
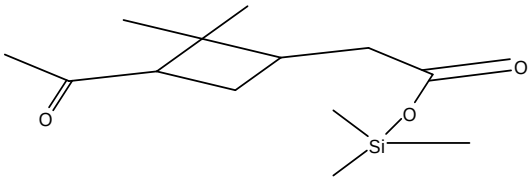
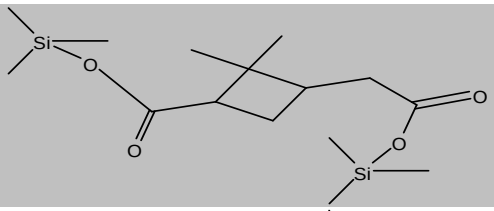
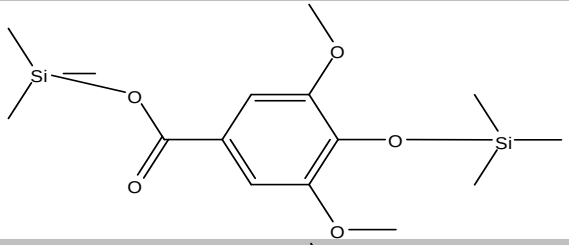
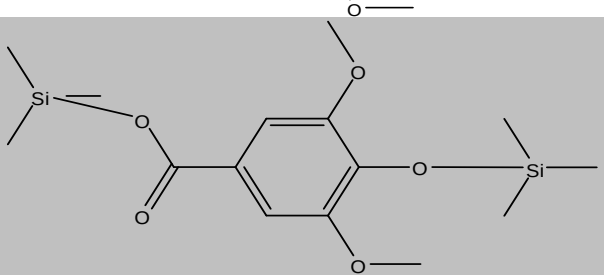
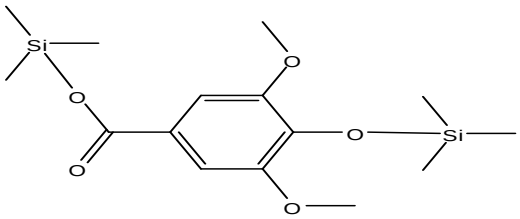
10 µl of each analyte, containing individual analyte (solution A), were poured to a flask and this flask was put in oven at 80 °C for 20 min to evaporate methanol. Respective retention time and fragmented ions were listed in Table 2.5.

2.3.3 Derivatization:

BSTFA and MSTFA were purchased from Sigma. Both reagents were used for the derivatization and compared for best selectivity. Trimethyl silyl (TMS) derivatives were produced after reaction with target analytes in acetonitrile in the reaction medium. Acetone and n-hexane were also used as solvents for the derivatization. The TMS derivatives are presented in Table 2.3 [8, 18, 23, 32, 35]. New (abbreviation) names to the respective TMS esters of each target analyte were given; new names consist of maximum three words only. These TMS esters (table 2.3) were purposed to be produced through the derivatization reaction before injecting into the GC-MS system.

Table 2.5 - Structures of TMS derivatives of the derivatized target analytes.

Sr. No.	TMS ester (Name)	TMS derivative of analytes	Structure of TMS derivative	Molecular weight
1	Mal	$C_9 H_{20} O_4 Si_2$		248.05
2	Suc	$C_{10} H_{22} O_4 Si_2$		262.11
3	Glu	$C_{11} H_{24} O_4 Si_2$		276.12
4	Ad	$C_{12} H_{26} O_4 Si_2$		290.14
5	Pim	$C_{13} H_{28} O_4 Si_2$		304.15
6	Sub	$C_{14} H_{30} O_4 Si_2$		318.17

Sr. No.	TMS ester (Name)	TMS derivative of analytes	Structure of TMS derivative	Molecular weight
7	Aze	$C_{15}H_{32}O_4Si_2$		332.18
8	Pin	$C_{13}H_{24}O_3Si$		256.15
9	Pnc	$C_{15}H_{30}O_4Si_2$		330.17
10	Syg	$C_{15}H_{26}O_5Si_2$		342.13
11	Pth	$C_{14}H_{22}O_4Si_2$		310.11
12	Hyd	$C_{15}H_{26}O_5Si_2$		342.13

*[Note: table 2.5 contains structures of TMS derivative of target analytes, these structures are sketched according to proposed reaction and fragments produced from these proposed structures].

2.3.3 Selection of internal standard:

1-Phenyl dodecane (97%) was purchased from Acros Organic (Geel, Belgium). Internal standard was used to get consistent and reproducible results [32]. The derivatization reagent did not react with the internal standard. The peak area of the target analyte (A_a) was divided with the peak area of the internal standard (A_{is}) i.e. A_a/A_{is}. Peak areas were calculated from the detector response.

2.3.4 Drying procedure (solvent):

Drying of the solvent, containing the sample was carried through evaporation. Two methods were utilized for evaporation of the solvent till dryness. The first method was; to dry in oven at 80 °C, the second method was; to dry under a gentle stream of nitrogen at 30-35 °C. Both methods were applied and results were compared. The first method of drying was applied for the samples containing methanol and the second method was applied for the samples containing water. Samples after membrane extraction were neutralized by using 24 µl of 0.1 M HCl prior to evaporation (the same amount was used as acceptor). The drying procedure under nitrogen all solutions after extraction were evaporated till dryness by the same procedure.

2.4. Quantification of aerosol containing samples after UAE:

Unknown real samples of aerosols, containing monocarboxylic and dicarboxylic acids (C_3-C_{10}) were provided for analysis after UAE [35]. The solvent from the Ultra sonic extracted mixture (containing samples) was evaporated (section; 2.3.4) till dryness. The dried samples were derivatized (section; 2.3.1 2.3.3). Quantification of each aerosol sample (1-23) was done after derivatization.

2.5. Limit of detection (LOD):

The limit of detection of an analyte is defined as the signal from the minimum concentration of an analyte, which can be distinguished from the signals of the blank [23] or background signals [36]. LOD information is very important for trace analysis. LOD is calculated [23] from the standard deviation of the standard's response in the calibration curve and the slope of the curve (b) and is given in equation [36].

$$LOD = b + 3s_{y/x} \quad \text{..... 2.1}$$

In Eq. 2.1, "$s_{y/x}$" is the residual standard deviation of the regression line.

2.6. Limit of Quantification (LOQ):

Limit of quantification is the smallest concentration of an analyte that can be determined quantitatively with a certain degree of assurance [23]. It is calculated from the linear response from the analyte area/height. Eq. 2.2 illustrates the limit of quantification, which is calculated from the regression line [36].

$$LOQ = b + 10s_{y/x} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2.2$$

3. Results

3.1. Detection and quantification of aerosol samples after UAE:

3.1.1 – Detection by GC-MS:

All analytes, individually (100 µg/ml) were injected to the GC-MS system during the start of the project at absolute quantity 33,333 ng/ml (in flask) to confirm the presence of each analyte. Each analyte was run by the scan mode of the mass spectrometer. After derivatization, characteristic ions of the target analytes were determined along with their retention time. The retention time, the characteristic ions and the selected ions of each analyte are presented in Table 3.1.

Table 3.1- Set up for retention time and characteristic ion study for each analyte including internal standard in scan mode of MS.

Sr. No.	Analyte name	Retention time*min	Characteristic ion	Selected ion
1	Mal	13.89	147,73,233,75	233
2	Suc	18.70	147,73,148,75,247	147
3	Glu	22.85	147,73,261,75,158,	147
4	Ad	25.56	73,111,147,75	147
5	Pim	27.30	73,75,147,155,125,	147
6	Sub	28,65	73,75,187,217	187
7	Aze	29.87	73,75,201,129,147	201
8	Pin	25.84	73,171,75,83	171
9	Pnc	28.26	73,129,75,171,172	171
10	Syg	31.06	297,73,253,141	297
11	Pth	28.63	147 , 73 , 295	295
12	Hyd	27.61	267 , 223 , 193	223
13	IS	30.77	246	246

3.1.2 LOD, LOQ and linearity of standard injections:

The concentrations of multi-component standards, in the range of 16.7-666.7 ng/ml (absolute concentration) were injected into the GC-MS system after derivatization and the results are presented in Table 3.2. The total ion chromatogram (standard) show a minimum concentration of the target analytes i.e. 16.7 ng/ml and the aerosol sample "23" after UAE presented in Fig. 3.1. Calibration curves of the individual target analytes are presented in Fig. 3.2-3.3.

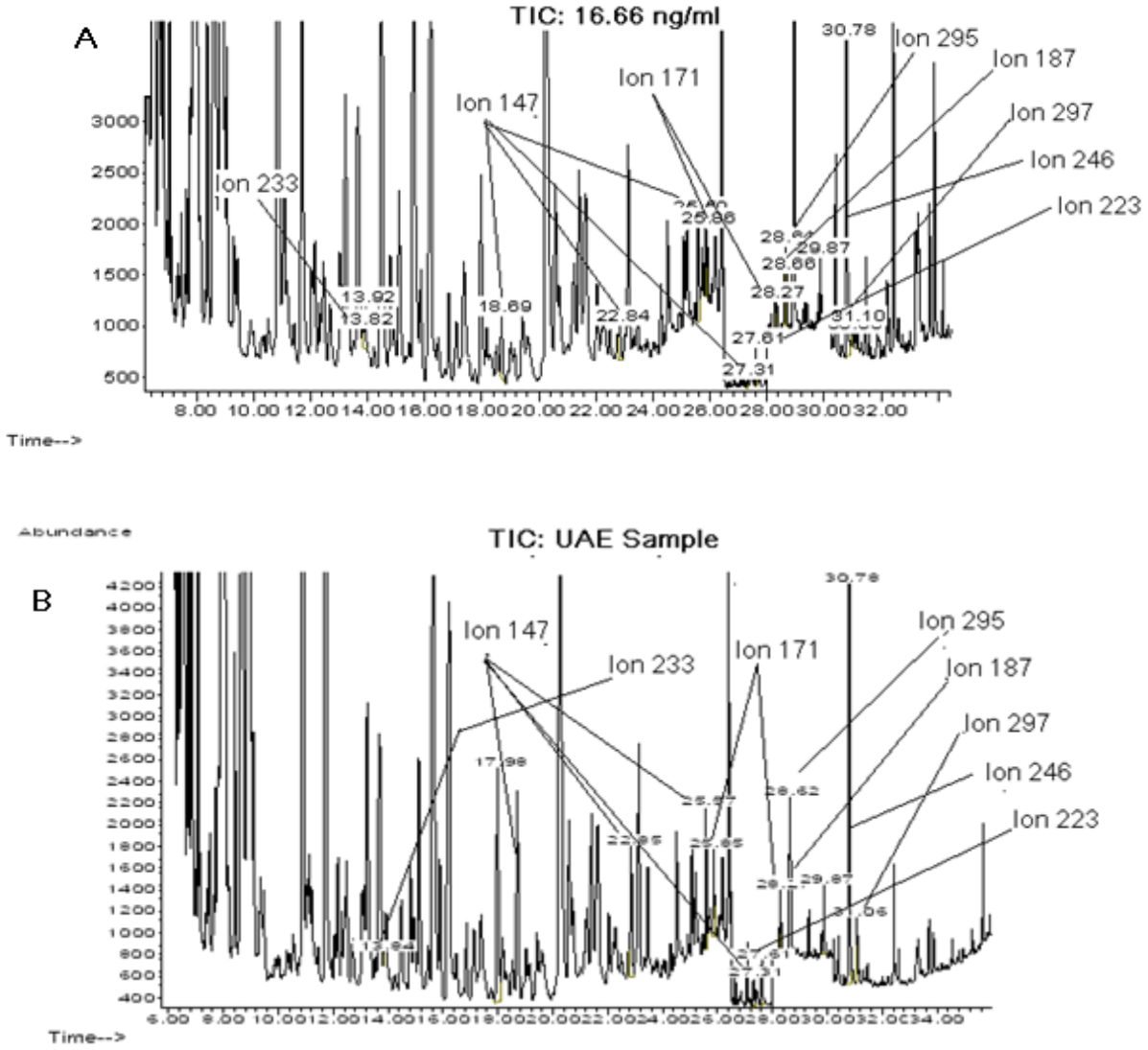


Fig. 3.1- TIC of the (**A)** multi-component standard (16.7ng/ml); **(B)** sample "23" after UAE

Fig. 3.2- 3.3 show the peaks of the selected ions. Chromatograms were superimposed through extracted ion chromatogram by MS computer window using standard and sample chromatograms for the comparative study of analytes in sample and standard solutions.

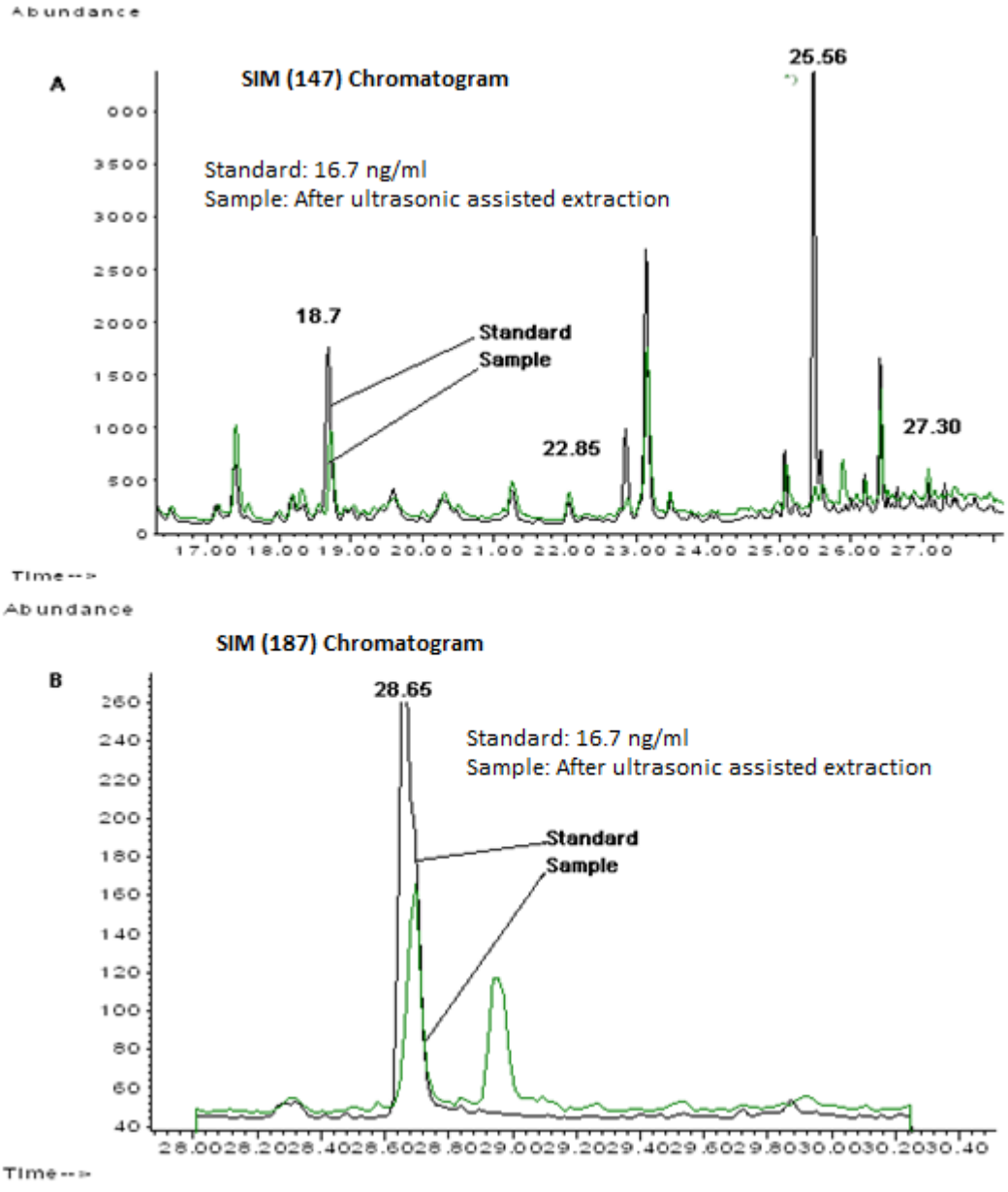


Fig. 3.2- SIM, GC-MS overlay chromatograms for the standard analysis (16.7 ng/ml), UAE sample (23). **(A)** SIM (147); (**B)** SIM (187)

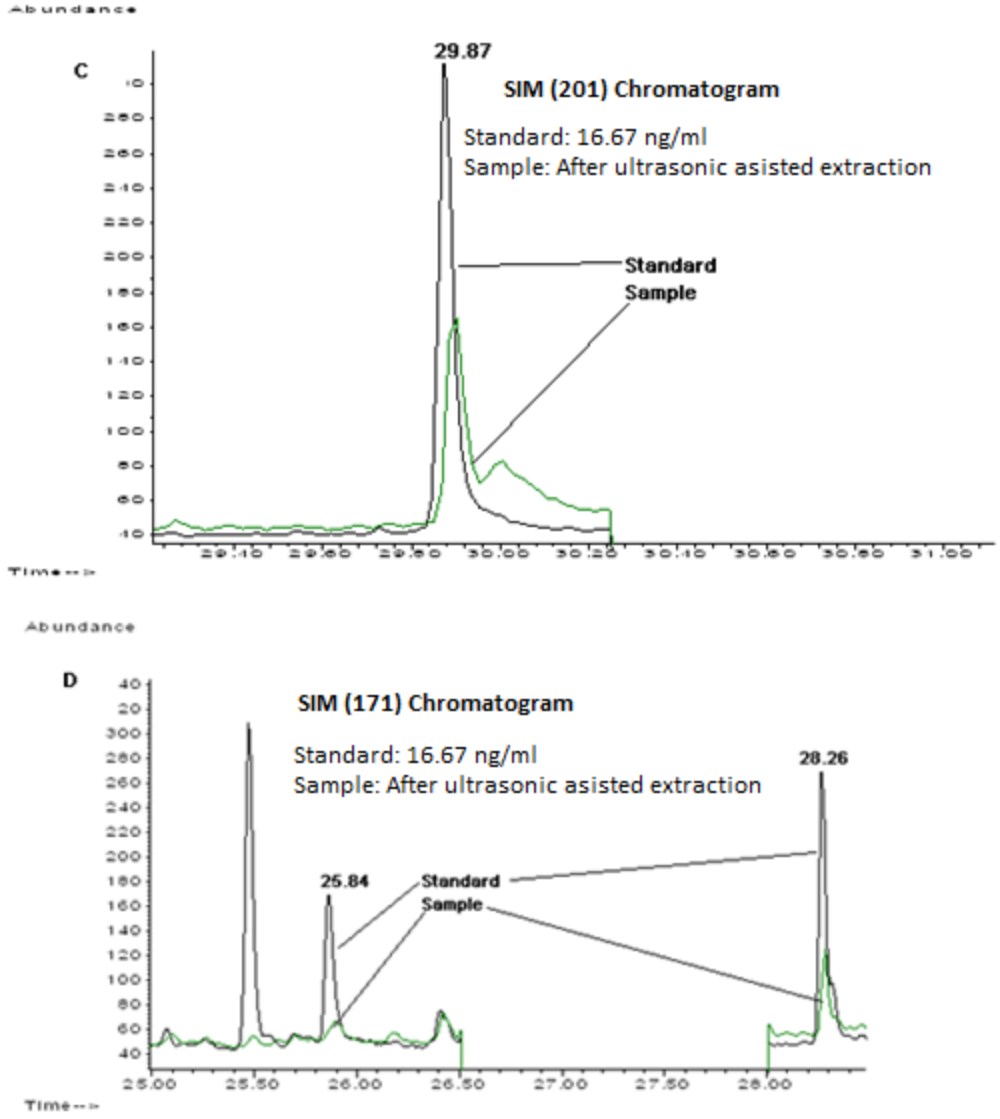


Fig. 3.3- SIM, GC-MS overlay chromatogram for the standard analysis (16.7 ng/ml), UAE sample (23). **(C)** SIM (201); **(D)** SIM (171)

The linear regression line (calibration curve) of each target analyte was obtained by computing six multi-component standard mixtures (for "Mal" through five points). Fig. 3.4 - 3.5 represent calibration curves (linear regression lines), calculated through the quantification from the characteristic ions of each target analyte (Section 2.3.1), through XIC (GC-MS).

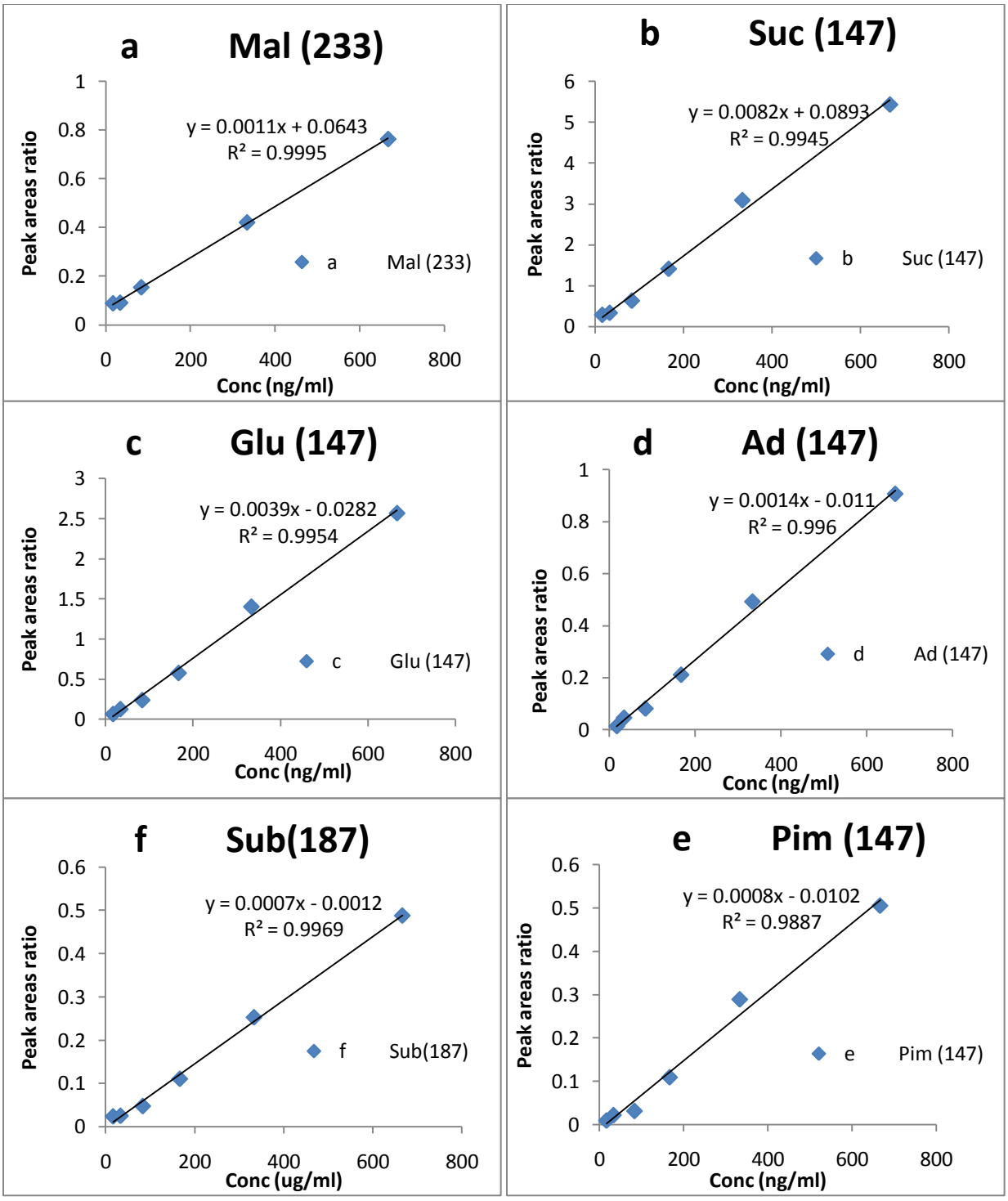


Fig. 3.4- Calibration curves (a-e) for the standards (16.7-666.7 ng/ml)

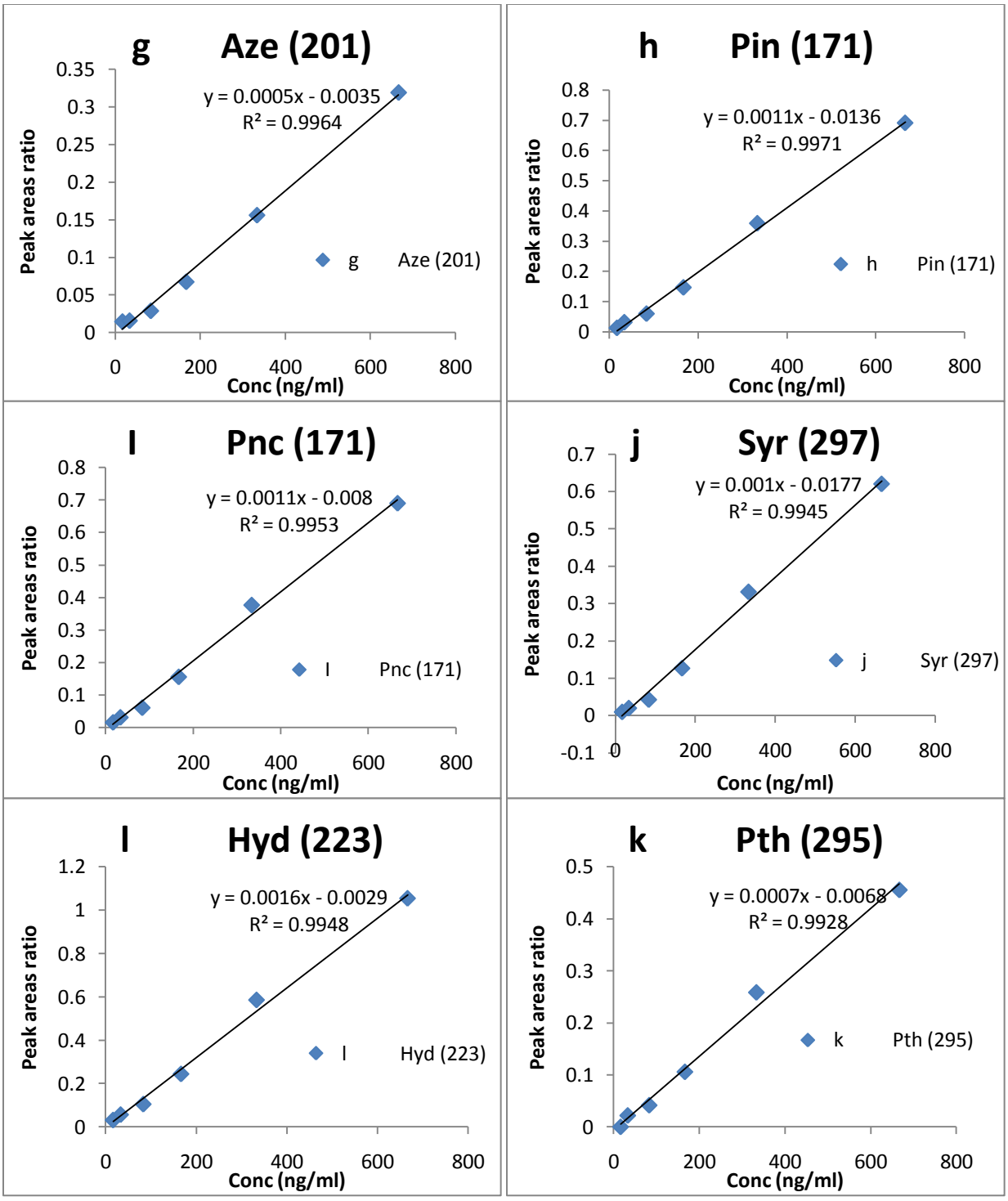


Fig. 3.5- Calibration curves (g-k) for the standards (16.7-666.7 ng/ml)

Eq. 2.1 -2.2 were used to calculate LOD and LOQ from parameters of the calibration curve.

Table 3.2- Slope and regression coefficients of the regression line, LOD and LOQ of individual analytes.

Sr. No.	Analytes	Conc. range (ng/ml)	Slope (m)	Regression coefficient	LOD (ng/ml)	LOQ (ng/ml)
1	Mal	16.7-666.7	0.0011	0.999	0.405	1.347
2	Suc	16.7-666.7	0.0082	0.9945	0.146	1.465
3	Glu	16.7-666.7	0.0039	0.995	0.236	0.776
4	Ad	16.7-666.7	0.0014	0.996	0.083	0.247
5	Pim	16.7-666.7	0.0008	0.99	0.079	0.238
6	Sub	16.7-666.7	0.0007	0.997	0.059	0.177
7	Aze	16.7-666.7	0.0005	0.996	0.040	0.132
8	Pin	16.7-666.7	0.0011	0.997	0.066	0.217
9	Pnc	16.7-666.7	0.0011	0.995	0.076	0.250
10	Syg	16.7-666.7	0.001	0.994	0.072	0.236
11	Pth	16.7-666.7	0.0007	0.993	0.060	0.200
12	Hyd	16.7-666.7	0.0016	0.995	0.099	0.328

3.1.3- Quantification of the analytes in aerosol samples:

The concentration of the target analytes were quantified by GC-MS after UAE. Each sample (1-23) was supposed to contain multiple analytes and was run in duplicate by the GC-MS system. The amount of each analyte (ng/ml) is presented in Table 3.3 as quantified by computing with the regression lines of the standard (table 3.2). Quantification of the aerosol samples was presented in Fig. 3.6- 3.8. Comparison, between the calculated amounts of the individual analytes is presented (Fig. 3.6- 3.8) graphically with respect to three other analytes to study the variations in the amount of the same analyte in the real samples (1-23).

Table 3.3- Amount (ng/ml) of each analyte in separate samples (1-23) after UAE.

Sample. No.	Mal	Suc	Glu	Ad	Pim	Sub	Aze	Pin	Pnc	Syg	Pth	Hyd
1	<LOQ	46	62	56	46	87	104	47	70	52	135	122
2	<LOQ	845	103	134	45	114	130	49	94	56	118	163
3	<LOQ	97	82	114	61	110	132	51	73	60	132	132
4	<LOQ	258	112	142	83	103	127	48	72	57	117	143
5	<LOQ	47	63	89	53	89	90	51	56	54	73	126
6	<LOQ	119	78	124	47	102	104	67	95	53	161	197
7	<LOQ	95	78	126	47	104	110	61	124	163	126	158
8	<LOQ	151	89	115	52	99	110	50	63	51	106	130
9	<LOQ	315	111	134	68	124	169	53	104	51	126	183
10	<LOQ	38	62	97	52	96	99	53	65	56	105	141
11	<LOQ	78	60	91	46	93	101	42	51	53	75	119
12	<LOQ	69	83	369	138	308	547	92	100	59	93	208
13	<LOQ	66	87	315	174	211	465	102	75	53	85	177
14	<LOQ	156	62	131	80	101	176	50	52	43	59	78
15	<LOQ	175	101	238	86	150	173	67	90	49	103	184
16	<LOQ	54	72	171	81	141	156	63	67	50	83	144
17	<LOQ	99	92	212	97	144	167	71	105	53	83	167
18	<LOQ	4	52	84	67	96	98	82	69	57	69	167
19	<LOQ	76	74	180	63	112	84	97	77	61	99	171
20	<LOQ	59	75	219	83	115	125	123	94	57	144	145
21	<LOQ	208	121	245	76	149	291	113	175	55	508	268
22	<LOQ	56	75	137	64	103	93	71	132	58	172	174
23	<LOQ	86	106	159	79	122	166	100	137	62	84	226

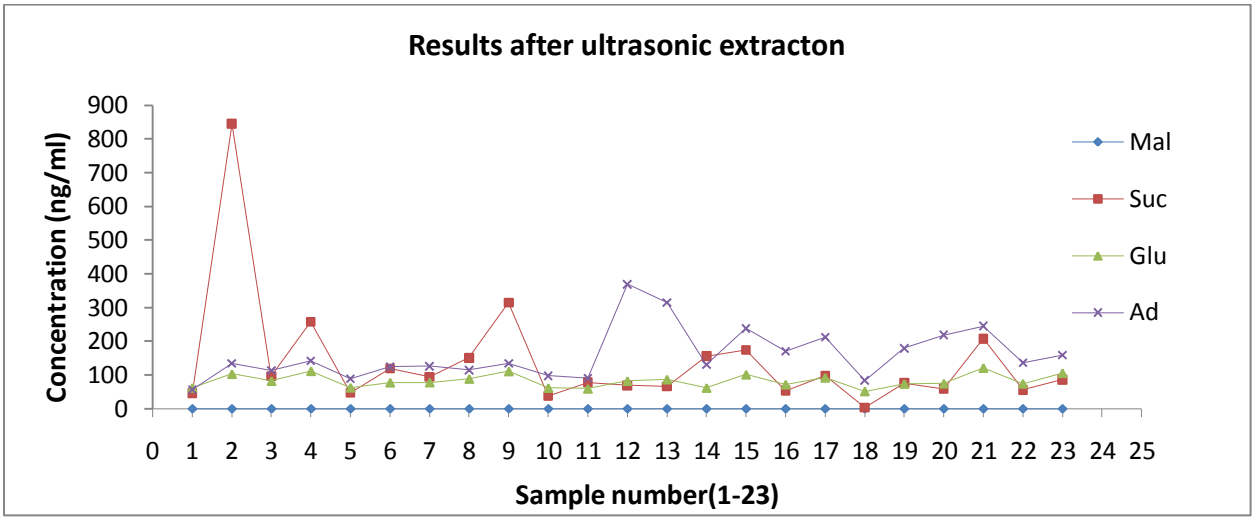


Fig. 3.6- A comparitive study of the amount of the individual target analyte found in aerosole samples (Mal, Suc, Glu and Ad) after UAE (samples 1-23).

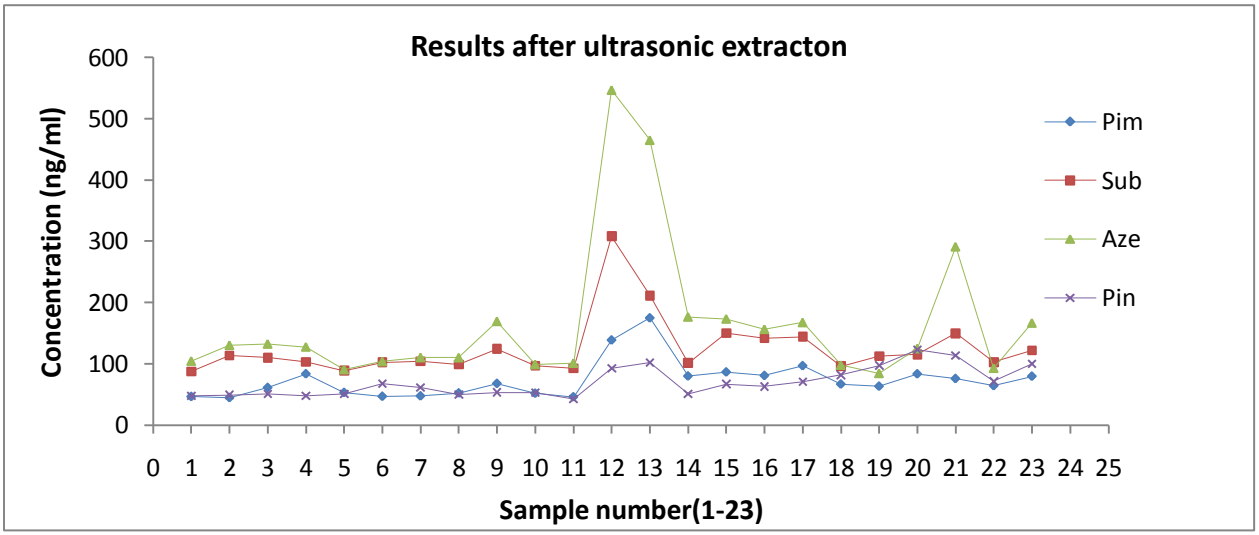


Fig. 3.7- A comparitive study of the amount of the individual target analyte found in aerosole samples (Pim, Sub, Aze and Pin) after UAE (samples 1-23).

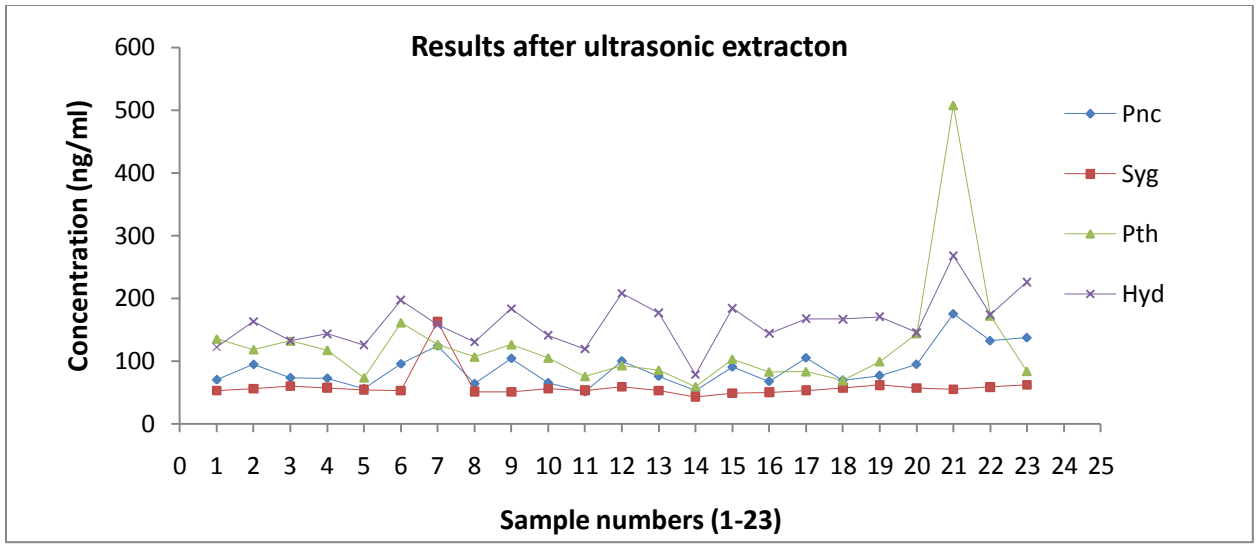


Fig. 3.8- A comparitive study of the amount of the individual target analyte found in aerosole samples (Pnc, Syg, Pth and Hyd) after UAE (samples 1-23).

The average amount of the individual target analyte, in all of the extracted aerosol samples (1-23) is plotted to compare with all other target analytes as found in the same aerosol samples quantified after UAE and presented in Fig. 3.9.

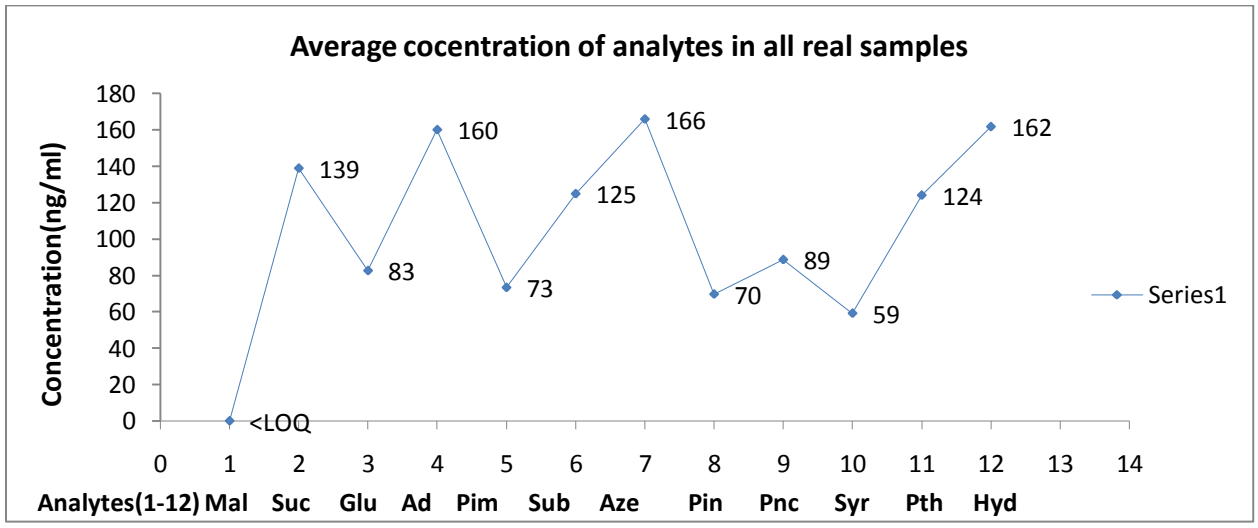


Fig. 3.9 Average concentration (ng/ml) of all of the target analytes as found in aerosol samples (1-23)

3.2. Membrane Extraction:

3.2.1 LOD, LOQ and linearity of Standard injections after column cutting:

After three months continuous work, the GC-MS system started to cause problems. Approximately 1.5 meter column was cut and the GC-MS instrument was tuned successfully again. New standards were run on the GC-MS and new regression lines were obtained and are presented in Table 3.4 with respect to the individual target analyte (section 2.3.1) and new LOD and LOQ were determined for the same analytes (Eq. 2.1-2.2).

Table 3.4- Slope and regression coefficients of the **r**egression line, LOD and LOQ of individual analytes (after column cutting and without retention gap).

Sr. No.	Analytes	Slope (b)	Regression coefficient	LOD (ng/ml)	LOQ (ng/ml)
1	Mal	0.0010	$R^2 = 0.998$	0.3	1.1
2	Suc	0.0145	$R^2 = 0.999$	0.4	1.4
3	Glu	0.0078	$R^2 = 0.998$	0.3	1.1
4	Ad	0.0029	$R^2 = 0.998$	0.2	0.6
5	Pim	0.0018	$R^2 = 0.998$	0.1	0.5
6	Sub	0.0013	$R^2 = 0.998$	2.7	9.0
7	Aze	0.0009	$R^2 = 0.999$	0.1	0.1
8	Pin	0.0021	$R^2 = 0.999$	0.1	0.2
9	Pnc	0.0019	$R^2 = 0.998$	1.2	4.1
10	Syg	0.0017	$R^2 = 0.997$	0.2	0.5
11	Pth	0.0011	$R^2 = 0.998$	0.1	0.3
12	Hyd	0.0032	$R^2 = 0.998$	0.2	0.7

3.2.2 Enrichment factor (E_e) of extracted analytes by three-phase HF-LPME:

The enrichment factor of the target analytes is presented in Table 3.5 after triplicate extractions and from duplicate injections. Average values were calculated for each organic phase separately. Results, from different organic phase solvents (0% - 19% TOPO in DHE) are presented in Table 3.5. Fig. 3.13 graphically represents the enrichment factor of each target analyte ('E_e' values, Table 3.5). Total ion chromatograms of the standard and sample are presented in Fig. 3.10.

Table 3.5- Enrich factor of each extracted analyte by three-phase HF-LPME with different %age of TOPO in DHE

Sr. No.	**Analytes (TMS)**	**0%** TOPO **(DHE)**	**1 %** TOPO	**5 %** TOPO	**10%** TOPO	**15%** TOPO	**19%** TOPO
1	Mal	42	34	137	205	401	35
2	Suc	223	240	559	541	419	805
3	Glu	169	165	230	235	305	464
4	Ad	408	314	576	520	842	1327
5	Pim	239	236	433	382	1042	940
6	Sub	431	401	987	1192	2100	1188
7	Aze	1126	1061	4699	4449	4607	1349
8	Pin	139	170	280	490	320	135
9	Pnc	177	180	320	660	1216	660
10	Syg	200	200	209	297	521	207
11	Pth	225	220	358	263	1345	2741
12	Hyd	307	542	1575	1591	543	195

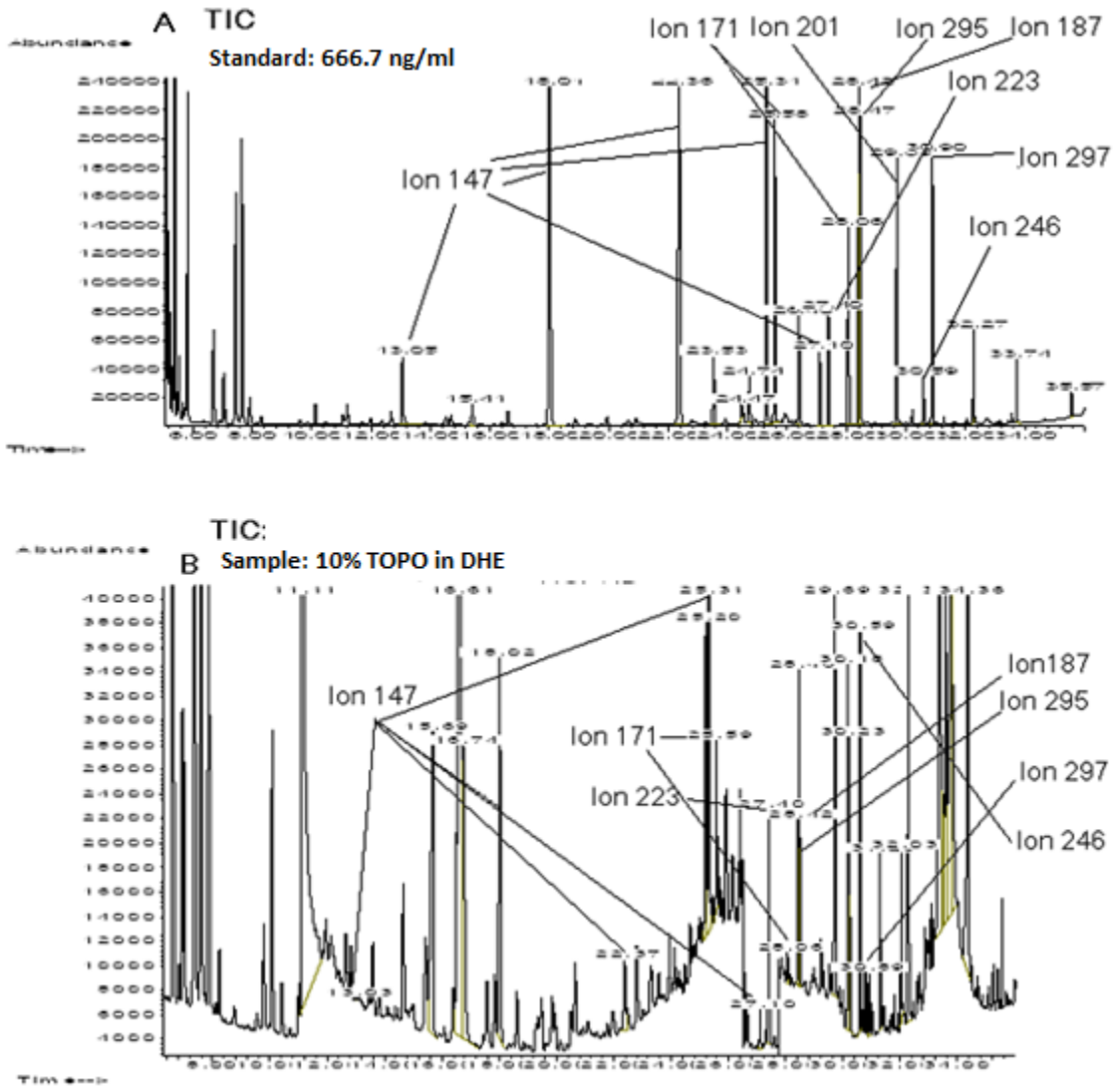


Fig. 3.10- TIC of the **(A)** standard analysis 666.7 ng/ml; **(B)** extracted sample (2000 ng/ml standard spiked,) after mixing 10% TOPO in DHE

Fig. 3.11-3.12 represent the selected ion chromatograms. The SIM chromatograms of the standard (multi-component) having a concentration of 166.6 ng/ml and extracted sample 10% TOPO in DHE were overlaid through XIC.

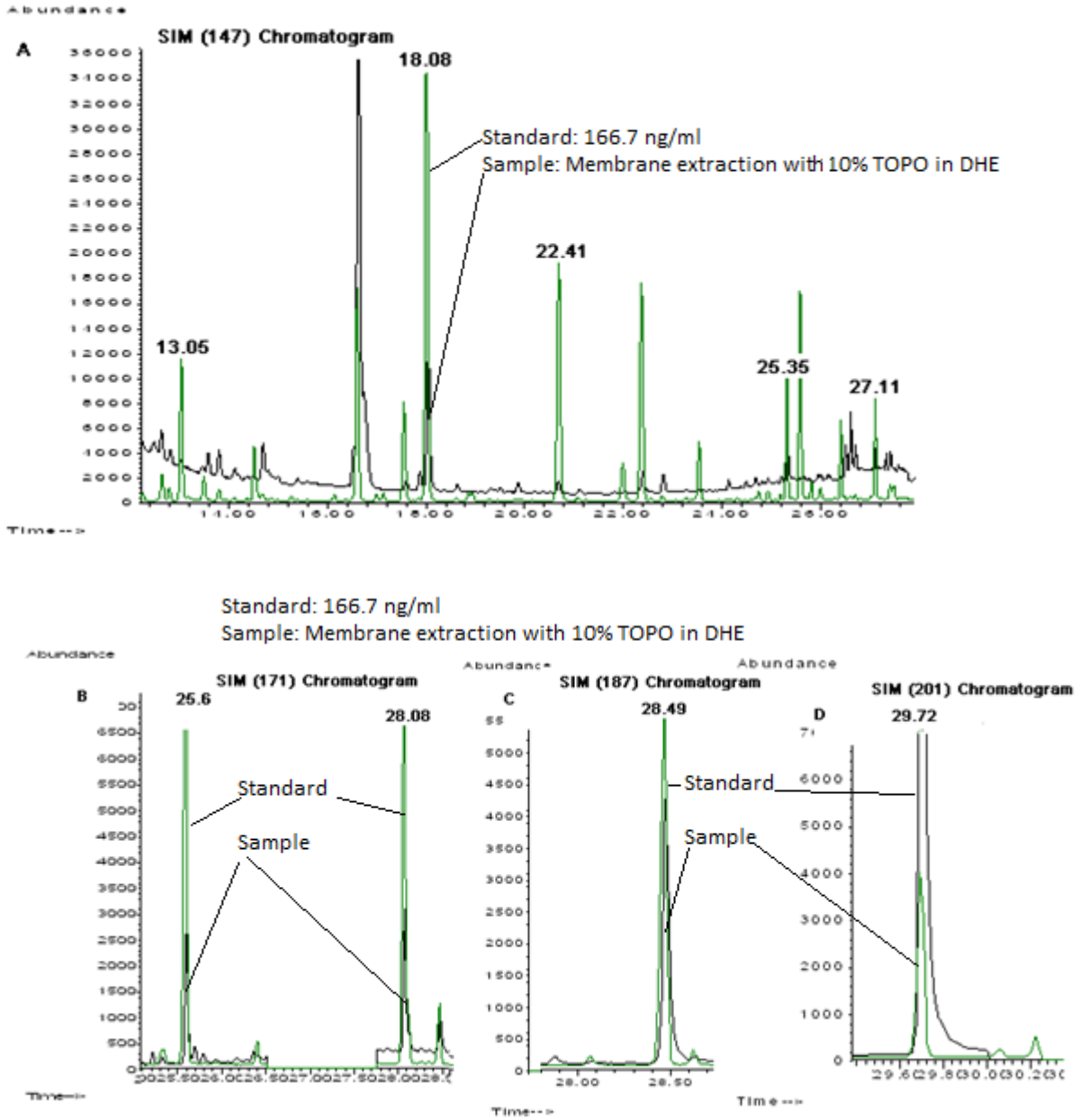


Fig. 3.11- SIM (GC-MS) Overlay chromatograms for the standard (1666.7 ng/ml) and the extracted sample (2000 ng/ml, standard spiked) by using 10% TOPO in DHE. **A.** m/z= 147; **B.** m/z= 171; **C.** m/z= 187; **D.** m/z= 201

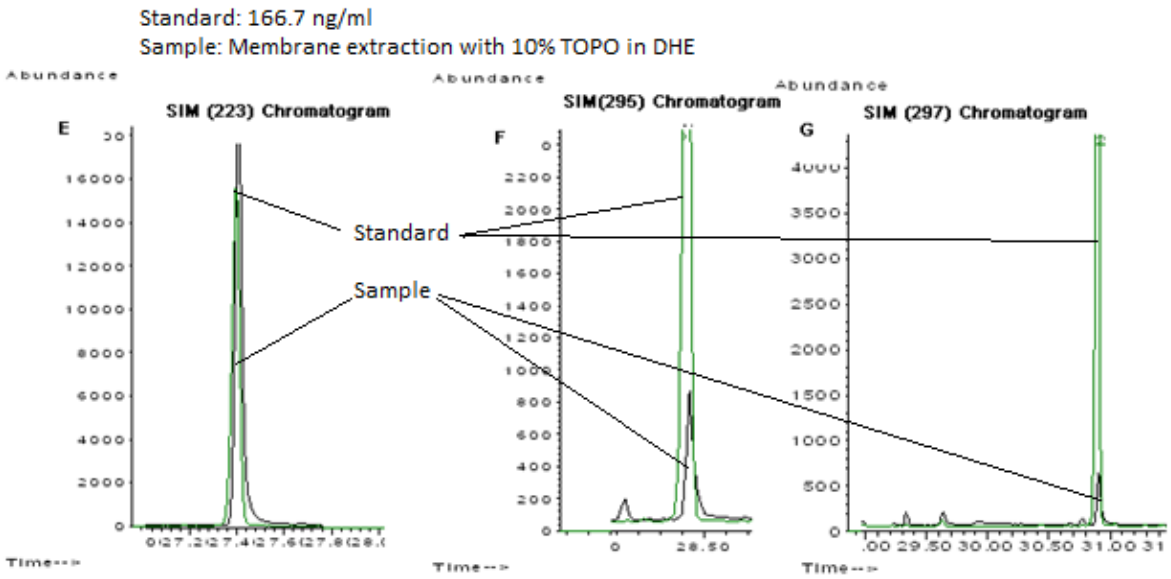


Fig. 3.12- SIM (GC-MS) overlay chromatograms for the standard (1666.7 ng/ml) and the extracted sample (2000 ng/ml, standard spiked) by using 10% TOPO in DHE. **E.** m/z= 223; **F.** m/z= 295; **G.** m/z= 297

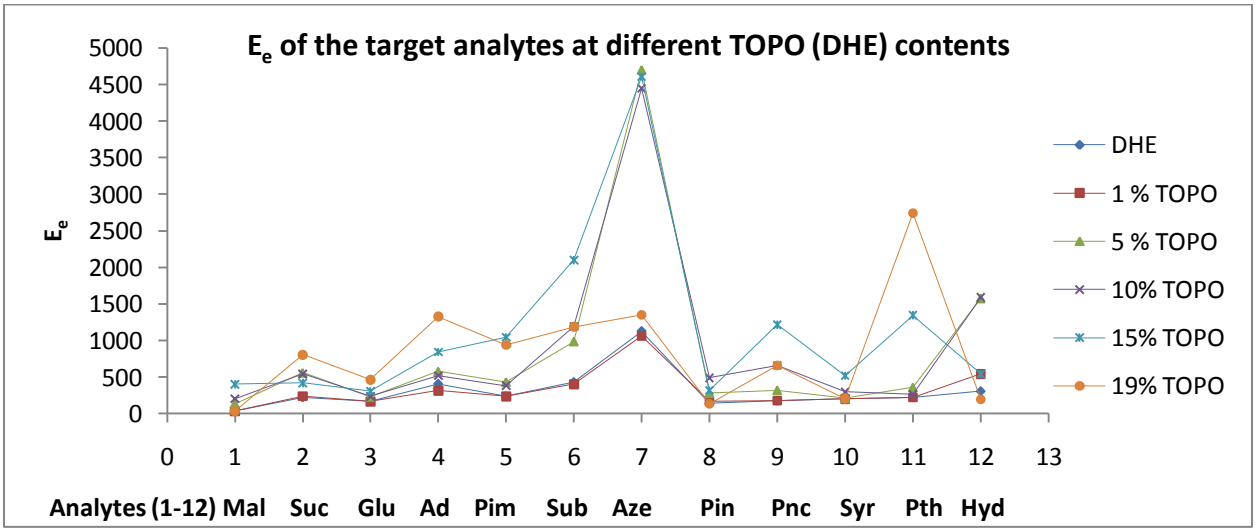


Fig. 3.13- Enrichment factor of the target analytes at different TOPO contents (mixture in DHE)

3.2.3- Enrich efficiency (E) of extracted analytes by three-phase HF-LPME:

Enrichment efficiency was calculated according to Eq. 1.4 and results are presented in Table 3.6. Calculated results of each analyte are presented with respect to different membrane organic phases (%age) (3.2.2). A corresponding graph in Fig. 3.14 is obtained from Table 3.6.

Table 3.6- Extraction efficiency of each extracted analyte by three-phase HF-LPME with different TOPO% in DHE

Sr. No.	**Analytes (TMS)**	**0%**TOPO **(DHE)**	**1 %** TOPO	**5 %** TOPO	**10%** TOPO	**15%** TOPO	**19%** TOPO
1	Mal	1	1	3	5	9	1
2	Suc	5	5	13	12	10	18
3	Glu	4	4	5	5	7	11
4	Ad	9	7	13	12	19	30
5	Pim	5	5	10	9	24	21
6	Sub	10	9	23	27	48	27
7	Aze	26	24	107	102	105	31
8	Pin	3	4	6	11	7	3
9	Pnc	4	4	7	15	28	15
10	Syg	5	5	5	7	12	5
11	Pth	5	5	8	6	31	63
12	Hyd	7	12	36	36	12	4

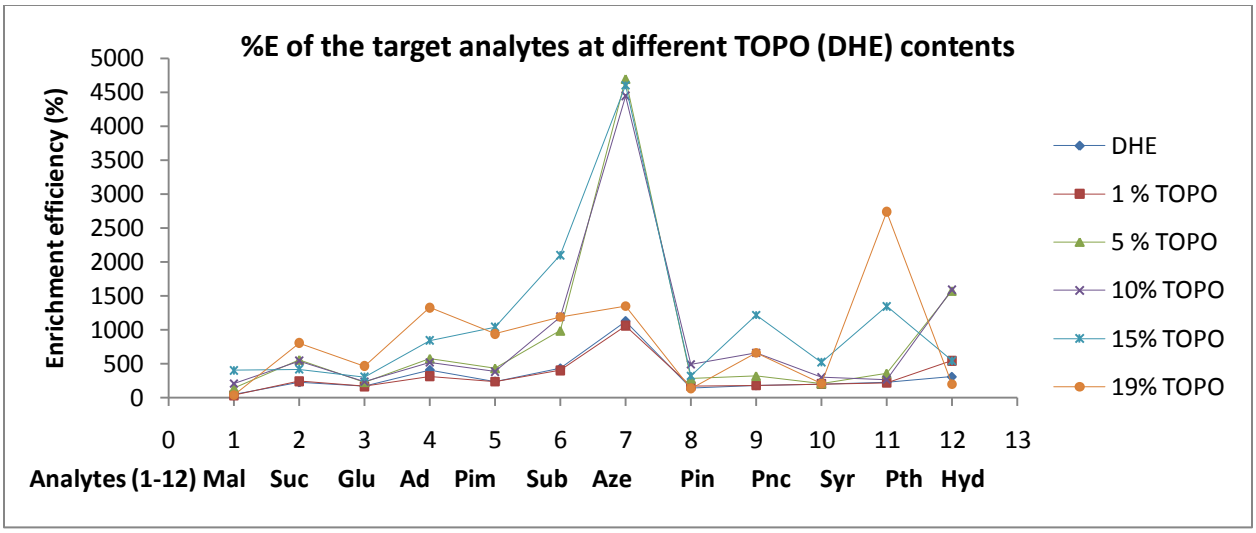


Fig 3.14-Enrichment efficiency (%) of target analytes at different TOPO contents

3.2.4 Repeatability between different membranes

The repeatability (%RSD) of each extracted analyte, as a result of triplicate extractions by three-phase HF-LPME is presented in Fig. 3.15 relative to each membrane phase (%TOPO in DHE).

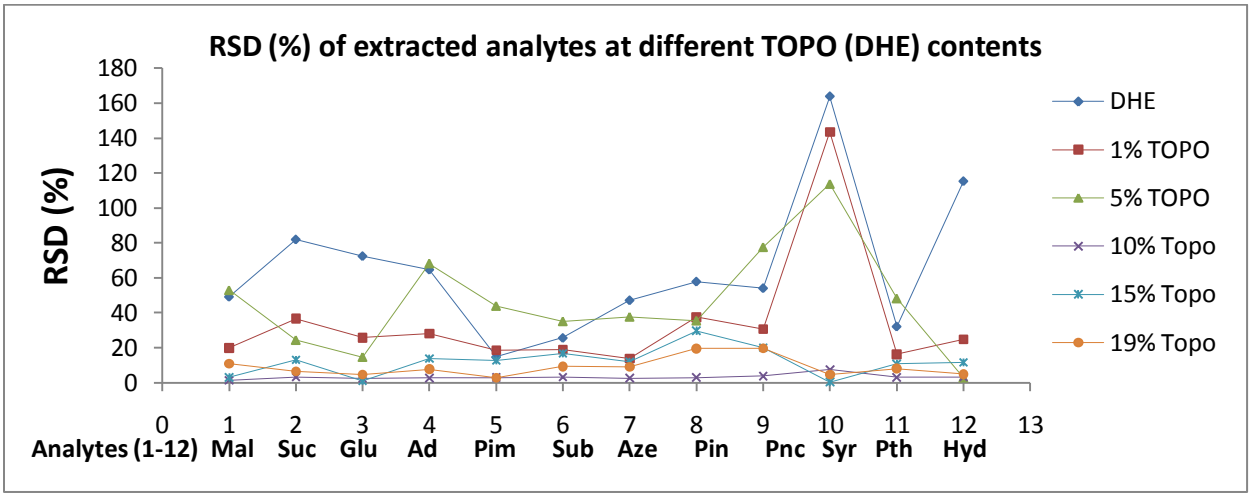


Fig 3.15- A comparison of repeatability between different membranes after triplicate extractions

A comparison of the total enrichment factor of the individual target analytes in the extracted mixture to the various organic phases (0-19% TOPO) is presented in Fig. 3.16. The enrichment factor is calculated as average value from triplicate extractions (Table 3.12).

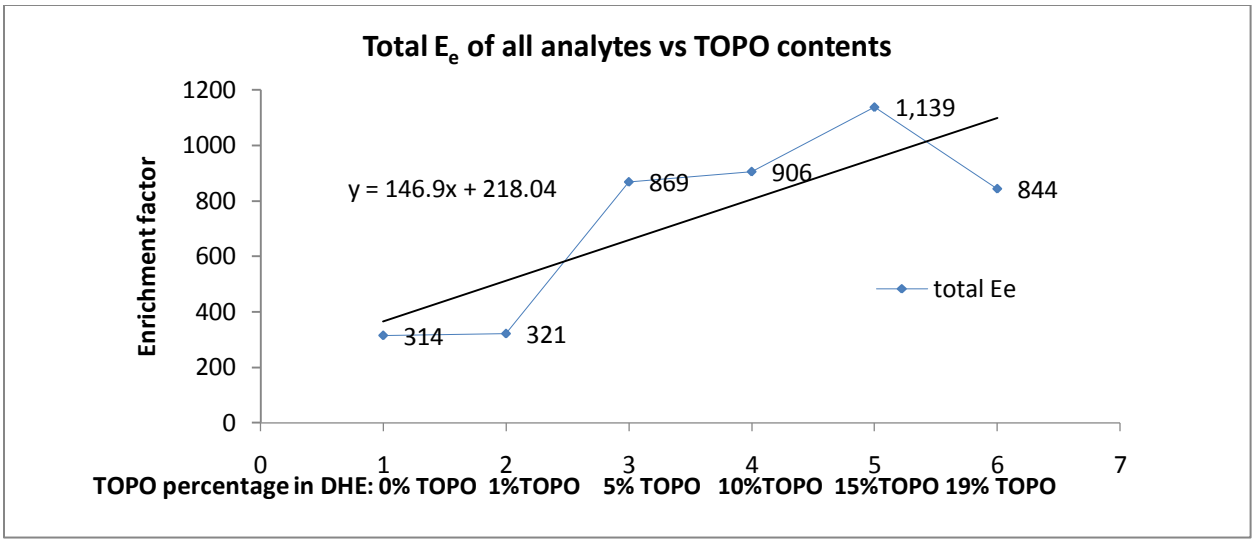


Fig. 3.16- A comparison between total enrichment of the target analytes with 0-19% of TOPO in DHE after triplicate extractions.

4- Discussion:

4.1-Chromatographic Analysis:

4.1.1 Selection of the derivatization reagent:

Three solvents; acetone, acetonitrile and n-hexane were used to inject TMS derivatives of the target analytes (Table 2.1) in chromatographic analysis during the start of this project. BSTFA and MSTFA (section; 2.3.3) were used as derivatizing reagents i.e. these reagents were compared for better selection and efficiency. Derivatization with MSTFA was found unsuitable for the current chromatographic analysis compared with BSTFA. BSTFA reagent provided excellent results (Fig. 2.1). The base-line of the chromatograms was also found satisfactory with Acetonitrile, which was found more effective then when BSTFA was used as derivatizing reagent compared with MSTFA. The chromatographic results were also acceptable with acetone, but more importantly 'derivatization' was taken place more effectively in acetonitrile. All analytes were derivatized with BSTFA in acetonitrile latter on for further analysis. BSTFA and acetonitrile (containing internal standard) were found to work more effectively with a ratio of 1:2 (Fig. 3.1).

4.1.2- Optimum drying procedure

Evaporation of the solvent (water, methanol) was very important because primary solutions were prepared in methanol and the derivatization reaction was taking place in acetonitrile. Similarly after extraction the acceptor was in aqueous medium. Derivatization is water sensitive [18]. Water was required to be removed dry prior to derivatization.

Samples after extraction were in basic solution so the analytes existed in their corresponding salt. Derivatization with BSTFA is difficult to perform with salt therefore the solutions containing the extracted sample were neutralized by adding low concentrated acid (0.1 M HCl) [4, 8, 32].

Standard solutions containing methanol were evaporated till dryness in oven at 80 °C and several standards (16.7 - 1666.7 ng/ml) were run on GC-MS to check the linearity after drying in the same scheme (2.3.4). Drying of standards (primary standards were in methanol) evaporated in the oven (80 °C) for 20 min, provided good linearity (Fig. 3.2-3.3).

Evaporation under a stream of nitrogen was performed to samples obtained after derivatization, to eliminate excess of BSTFA and to avoid unwanted peaks (side products) produced from BSTFA side reactions (Fig. 3.1, 1.6). TMS derivatives of the analytes were found

to escape quickly through drying. This step of evaporation was eliminated and the solution containing TMS derivatives of acids were injected directly containing BSTFA.

After membrane extractions, recovered samples (in acceptor) were evaporated to dryness under a gentle nitrogen stream prior to derivatization (Fig 3.10-3.11, 3.12-3.13).

4.1.3- Confirmation of TMS derivative of acids on their retention time on scan mode:

Primary multi-component standard solutions (33,333 ng/ml), containing Mal, Suc, Glu, Ad, PIm, Sub, Aze, Pin, Pnc, Syg, Pth and Hyd were run on GC-MS. Full scan detection mode was used on the GC-MS system and EI (scan) spectra were used to find fragmented ions and proper retention times of each analyte. The EI mass spectra of the analytes were very complex and many fragments were obtained in the chromatograms. These fragments are difficult to explain because many side products (Fig. 3.1, 3.10) were produced due to the use of BSTFA [27]. Detection of individual analytes at their characteristic ions is discussed below after the selected ion monitoring study (Fig. 3.2-3.3, 3.11-3.13).

When standard containing 'Mal' was injected (33,333 ng/ml), ion fragments at m/z =147, 73 and 233 were obtained (Table 3.1) in decreasing order of their intensity at 13.89 min (retention time). Base peak at m/z = 147 was produced due to the $[(CH_3)_2Si{=}Si\ (CH_3)_2]^+$ ion, which is also an abundant ion fragment for all analytes containing a dicarboxylic functional group. A third abundant fragment ion appeared at m/z =233 due to the $[C_8H_{17}Si_2O_4]^+$ molecular ion.

Suc, Glu, Ad and PIm exhibited retention time at 18.70 min, 22.85 min, 25.56 min and 27.30 min, respectively, and were confirmed by a minimum of three injections (each analyte was run separately). These four analytes show many ion fragments, intense ion fragments at respective m/z are listed in Table 3.1, in decreasing order of their intensity. The most important and common ion fragment was at m/z=147 (already described), so this molecular ion was selected as SIM for Suc, Glu, Ad and PIm.

Sub (analyte) retention time was found at 28.65 min (temperature programmed, (section 2.3). Ion fragment at m/z=187 was the third intensive ion peak, this molecular ion was selected to provide good separation from other interfering peaks.

Aze (analyte) retention time was at 29.87min and the selected ion was at m/z= 201.

For Pin and Pnc, their retention times were found at 25.84 min and 28.26 min, respectively. Pin and Pnc produced a common fragment ion at m/z=171 (Table 3.1), so this ion peak was selected for SIM mode.

For Syg the retention time was found at31.06 min and the ion at m/z= 297 was selected for SIM mode. This molecular ion was also a base peak for this analyte (Table 3.1).

For Pth the retention time was found at 28.63 min and the third intensive fragment ion was at m/z=295, selected for SIM. The analyte peaks Pth and Sub were eluted at almost the same retention time but since different ions were selected no interference was observed in-between them (Fig. 3.7).

For Hyd (33,333ng/ml) the main peak was eluted at 27.61 min. The ion fragment at m/z=223 was selected for SIM.

The peak from the internal standard (1-Phenyl dodecane) was eluted at 30.77 min and the molecular ion at m/z=246 was selected for SIM analysis. 1-Phenyl dodecane was unaffected by derivatization.

There were some unwanted peaks in the total ion chromatogram. Most of these unwanted peaks were eluted before 13.8 min, due to the BSTFA (more volatile) side products (Fig. 3.1, 3.10). There could be several reasons and sources for unwanted chromatographic peaks (beyond the scope of this project). The important source (investigated) was the peaks eluted from the column bleed.

There were some unwanted peaks eluted in between the analytes. Extra peaks were made ineffective (ignored) due to SIM mode i.e. all required peaks were well resolved from the unwanted peaks.

As a result of extraction (GC-MS analysis), the ghost peak was eluted continuously at (around) 12 min (Fig 3.8). This unwanted peak did not disturb the analyte 'Mal' (eluted at 13.05 min), but this peak disturbed the column performance. This ghost peak at 12 min (might be) was eluted due to TOPO or due to some impurity in DHE etc.

4.1.4- Optimum temperature and SIM method validity:

The temperature programmed was selected and validated, considering elution of every analyte and through a literature survey, after several chromatographic experiments by injecting standard solutions of known concentration [27]. All analytes (including internal standard) were eluted before 240 °C at 32 min before reaching to the column maximum temperature (325 °C). Each analyte was well separated by the selected temperature program (Table 2.2).

4.1.5- Calculation from XIC:

After selecting the SIM method the required ions were extracted through XIC from the data analysis window. All quantification of the analytes was done through this window.

4.1.6- Limit of detection:

The limit of detection (LOD) for this method containing the C_3-C_{10} acids (when most of the work was done with ultrasonic assisted samples) varied in the range of 0.040-0.405 ng/ml (Table 2.3, 3.2), after computing the calibration curves passing through six (at least five) multi-component standard solutions (16.7 - 666.7 ng/ml).

LOD, after the column cut was obtained from computing multi-component standard solutions containing C_3-C_{10} acids. LOD was found in the rage of 0.1-2.7 ng/ml (Table 2.3, 3.4). The sensitivity was not as good as it was reported elsewhere [8]. UAE and membrane extraction were found to enrich the analytes many times, so this LOD was acceptable and applicable with these enrichment techniques i.e. especially for the membrane extractions (Table 3.5).

4.1.7- Limit of quantification:

The limit of quantification (LOQ) was calculated from the LOD values (Eq. 2.2). The LOQ of the analytes (C_3-C_{10}) was found in the range of 0.1 -1.3 ng/ml (Table 3.2). LOQ for 'Mal' was higher than that for the other analytes. LOQ was found satisfactory for further analysis and calculations.

After column cut, LOQ was found in the range of 0.1 -9 ng/ml (Table 3.4).

4.1.8- Linearity:

The current method shows good linearity for all of the target analytes. Linearity was estimated through the square of regression coefficient (R^2) of the calibration curve from the standard mixtures [37]. Linearity ranges from 0.990-0.999 for all of the analytes (Table 2.3; Fig. 3.4-3.5).

4.1.9- Trouble shooting with GC-MS system:

The GC-MS equipment caused a lot of problems during the half time of this project and many experiments were aimed for this project could not be put into practice. Problems started due to the poor response from internal standard; improper results and continuous declining of the chromatographic peaks (peak height, area) of the internal standard were observed. After poor response from IS, small peaks of the target analytes started to disappear (especially 'Mal'). After six days, the peak response from IS reduced to less than 10 times (approximately).

The syringe of the GC auto sampler was replaced, but no improvement was observed to the quality of chromatographic peaks (It was suggested that the syringe did not inject the samples properly).

The retention gap coupled to the GC capillary column was cut, half a meter from the injector side. This treatment to the column did not work as well. New standards were prepared to confirm the presence of the target compounds.

The GC oven temperature was left at 300 °C, overnight to elute any impurity which might have been trapped inside the column. Another half meter retention gap was cut. The GC-MS system was pumped down, a new tuning was performed and also air water check was run again to check the possible leaks in the column. The liner from the injector was removed and washed with methanol and glass wool was inserted in the liner [34]. Problems were still not rectified by applying different techniques (normally used).

The problem with the GC-MS sustained for more than three and a half months. During this time different troubleshooting procedures were utilized (discussed above). Finally the retention gap was removed and 1.5 meter of the column was cut i.e. one meter from the injector side and half a meter from the detector side. The problems were rectified after the column cutting, the liner cleaning, the pumping down of instrument etc. The GC-MS system started to work again for 20 days only.

Calibration curves of standards (multi-component) were performed again. The chromatographic experiments (GC), those were practically possible after problem rectifications, are described in (section 4.3).

4.2- Quantification of the analytes in aerosols (real) samples after UAE:

Real samples were extracted by ultrasonic assisted extraction before detection (Table 3.3). Descriptions of individual detected analytes (after UAE) are presented in the next paragraphs of this section (4.2).

Malonic acid (Mal) was detected below LOQ, i.e. 'Mal' could not be quantified through this method even though the 'Mal' peak was visible and larger than the noise peak (S/N=3). The existence of malonic acid below the limit could be due to the reason that when samples were dried under a stream of nitrogen, malonic acid has strong affinity with moisture and this could cause absorption of water from the environment or from the less dried sample flask. The malonic acid could not be derivatized with BSTFA in acetonitrile because BSTFA can only derivatize when the analytes were dissolved in acetonitrile.

The concentrations of succinic acid, glutaric acid and adipic acid in aerosols samples were varied in the range of 4-845 ng/ml, 52-111 ng/ml and 56-369 ng/ml respectively. Malonic acid, glutaric acid, adipic acid and succinic acid concentrations are presented graphically in Fig. 3.6. These analytes have been found to display variations from one sample to the other. The glutaric acid concentration (Fig. 3.1) was steady throughout the samples (1-23). The concentrations of succinic and adipic acid were inconsistently varied (samples 1-23).

The concentrations of pimelic acid, suberic acid, azelic acid and cis-pinonic acid were varied in the range of 45-174 ng/ml, 87-211 ng/ml, 84-465 ng/ml and 48-113 ng/ml (Fig. 3.7) respectively in aerosol (real) samples after UAE (1-24). In samples 12-13, the concentrations of these analytes were high, typically in sample 12, especially for azelaic acid (45 ng/ml). The preence of azelaic acid was dominant compared with the other three members (Fig. 3.8). The concentrations of pimelic acid, suberic acid, azelic acid and cis-pinonic acid were consistent (except; sample 12, 13) and did not show many variations in the aerosol samples 1-23.

The concentration of the target analytes pinic acid, syringic acid, phthalic acid and 4-hydroxy benzoic acid were varied in the range of 51-175 ng/ml, 43-163 ng/ml, 59-508 ng/ml, 78-268 ng/ml respectively in aerosol samples (1-23) after UAE (Fig. 3.8). 4-Hydroxy benzoic acid was found dominant in almost all of the samples except sample '21'; phthalic acid was in higher concentration compared with the other analytes in the Fig. 3.3. Syringic acid was found in a lesser amount compared with the other three analytes. The calculated amounts of these analytes did not show many variations in between samples 1-23 (except; sample 7, 21). In sample '7' the amount of syringic acid was unexpectedly high.

From the above discussions, it was obvious that all analytes were present in the aerosols samples although malonic acid was detected below LOQ.

The average concentrations of the individual analytes in all of the aerosol samples 1-23 were calculated also (Fig. 3.9). The calculated amount for azelaic acid was 166 ng/ml, this amount was higher comparaed with the other analytes. The concentrations of 4-hydroxy benzoic acid (162 ng/ml) and adipic acid (160 ng/ml) were found close to that of azelaic acid. Malonic acid could not be quantified. The existence of syringic acid (59 ng/ml) was lowest of all the other analytes.

4.3. Membrane Extraction:

4.3.1- Selection for donor pH:

The donor pH was adjusted at 2.0. This pH value was found enough acidic to prevent dissociation of the analytes into their ions (Table 3.1). This pH value forced the analytes to remain in their original (non dissociative) state and as a result the membrane organic phase captured the target analytes.

4.3.2- Selection for acceptor pH and optimum Volume:

The acceptor pH was adjusted to basic pH for trapping the analytes in the acceptor to prevent back diffusion. For trapping of acidic analytes (C_3-C_{10}), the pH of the acceptor was selected at 12 (0.1 M NaOH). This pH (acceptor) was 3 pH units higher than the pK_a values of each analyte except for 4-hydroxy benzoic where the difference was about 2.7 pH units (Table 2.1).

Two techniques were applied to inject the acceptor in hollow fiber. The first method was to inject the acceptor via a micro syringe containing (Approx) 45 µl of the acceptor, the hollow fiber was flushed with some of the acceptor and the remaining acceptor inside the lumen of the fiber was then trapped by sealing one end of the fiber with aluminum strip while the other end of the fiber already contained the needle of the micro syringe.

The second technique was to calculate the actual acceptor volume i.e. the volume that the lumen of a hollow fiber could contain easily. This volume was calculated as the average with five fibers having the length of 7.5 cm each. A 24 µl volume of the acceptor was adjusted, which was also capable to hold the target analytes. The second technique (24 µl) was found to work efficiently, because this technique was easy to handle and the repeatability (%RSD) of the analytes (enrichment) was found best in this case.

4.3.3- Selection for hollow fiber length:

A length of 7.5 cm the hollow fiber was found sufficient, because it has enough capacity to hold 23-26 µl of the acceptor easily and this length can be inserted into a (100 ml) volumetric flask vertically via a syringe (Fig. 1.13) i.e. the membrane assembly for this experiment.

4.3.4- Stirring speed:

The stirring speed was fixed at 800 rpm. This speed of the magnetic stirrer was found reasonable for the membrane extraction to increase the mass transfer of the target analytes with three-phase HF-LPME.

4.3.5-Selection of organic phase:

Dihexyly ether was used as the organic phase solvent in the three-phase HF-LPME for the enrichment of acidic analytes (C_3-C_{10}) presented in Table 2.1. Every analyte was enriched when DHE was (Table 3.5) used. When TOPO was mixed with DHE, the polarity of the DHE as solvent was changed. The organic phase was found more capable to transfer the target analytes after mixing TOPO in DHE rather than DHE only [27].

A multi-component standard mixture (2000 ng/ml) was spiked to (105 ml) with the donor. Results were calculated, after triplicate extractions. The extracted analytes were quantified from computing the results with the standards regression lines obtained after GC-MS (Table 3.5, Fig. 3.13). It was obvious from the analysis of the target analytes that the organic phase has a major effect in the three-phase HF-LPME.

4.3.6- Enrichment factor (E_e) and efficiency (%):

The enrichment factor (E_e) was calculated from triplicate extraction experiments (Table 3.5). The enrichment factor was calculated with different organic phases (SLM), while the same amount of standard mixture was spiked in all extractions. The nnrichment factor (all the analytes) was found to vary in the range of 34-4699 times after 120 min of extraction. The minimum enrichment factor was obtained when pure DHE was used as the organic phase (Fig 3.13).

Malonic acid has the lowest enrichment factor compared with every other analyte. The reason of the lowest enrichment factor for malonic acid could be explained by considering three reasons. One reason could be, its low pK_a value compared with any other analyte i.e. it is more soluble in the donor phase. The second reason could be that it reacts with the acceptor and precipitates more than the others. The third reason could be its strong hydrogen bonding interactions with SLM (polar organic phase).

The maximum enrichment factor that was theoretically possible calculated according to Eq. 1.8 and it was 4375 times for any of the individual target analytes. The maximum enrichment factors of each analyte calculated experimentally were: malonic acid = 407 times (15% TOPO), succinic acid = 805 times (19% TOPO), glutaric acid = 464 times (19% TOPO), adipic acid = 1327 times (19% TOPO), pimelic acid = 1042 times (15% TOPO), suberic acid = 2100 times (15% TOPO), azelaic acid = 4699 times (5% TOPO), cis-pinonic acid = 490 times (10% TOPO), pinic acid = 1216 times (15% TOPO), syringic acid = 521 times (15% TOPO), pthalic acid = 2741 times (19% TOPO) and 4-hydroxy benzoic acid; 1591 times (10% TOPO) . Values in parenthesis represent the different organic phases (TOPO in DHE).

From this (enrichment) study it is apparent that a selective enrichment of each analyte is possible with different organic phases in the three-phase HF-LPME and this selective enrichment can lead to efficient detection of environmental samples.

According to Eq. 1.4, the enrichment factor and efficiency are interrelated. Fig. 3.14 graphically represents efficiency. The efficiency of extraction varied in the range of 1 -107% from malonic acid (minimum) to azelaic acid (maximum).

When the total enrichment factor of all of the target analytes was compared (Fig. 3.16), an interesting feature was found that the enrichment curve first started to increase from 0-1% TOPO (in DHE), suddenly increased from 1 to 15% TOPO (in DHE), a maximum at 15% TOPO (in DHE) and then this curve again started to decrease with the more concentration of TOPO (19% TOPO in DHE).

4.3.7- Repeatability:

Different organic phases were used in triplicate extraction experiments and the results were compared (0-19% TOPO in DHE). Results from the triplicate extractions were observed to show variations (bad repeatability) in between different extraction experiments even with the same reaction conditions and the same spiked standards for the same target analytes (Fig. 3.15). The repeatability was worse when only DHE (RSD%; 20-160%) was used as a membrane organic phase. The results were better when TOPO was mixed with DHE from 1-19% TOPO, remarkable difference was obtained from 5-19% TOPO in DHE as compared to the DHE only.

Sustainable results were obtained when 10% TOPO was used as SLM, the repeatability was found better (RSD% ≤ 10%) for all the analytes. The results were also acceptable with 19% TOPO (RSD% ≤ 20%).

10% TOPO in DHE was selected as the best organic phase composition, because this organic phase mixture provides excellent repeatability (RSD% ≤ 10%).

5. Conclusion:

This diploma work was focused on the detection and quantification of monocarboxylic and dicarboxylic acids (C_3- C_{10}). Quantification of the target compounds was done after enrichment through the membrane and ultrasonic extractions despite the GC-MS system was having some problems throughout this project. This method has provided good LOD (0.040-0.4 ng/ml). Good linearity ($R^2 \geq 0.99$) was obtained from the multi-component standard mixtures from the multiple and serial dilutions.

Most of the target analytes were enriched many times, which certainly reveals the importance of membrane extraction. The use of TOPO has been observed to cause a lot of improvement in the enrichment factor compared with the DHE (only). 10% TOPO in DHE was found excellent in terms of repeatability and enrichment. Enrichment was improved for selective analytes.

There were some unwanted peaks in the chromatograms during membrane extractions. These unwanted peaks were eluted at different retention times, mainly before the elution of the target analytes. The target peaks were well separated from the unwanted peaks.

The target analytes in aerosols samples after UAE were effectively quantified through this method (except for malonic acid). The use of BSTFA was found excellent to derivatize all of the target analytes.

Further studies are required to validate the method of the membrane extractions to achieve exhaustive end points and to get better enrichment from the same organic phase. Membrane extractions of the samples containing aerosol would be more interesting to analyze.

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