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Determination Of Aldehydes In Particulate Matter Using Gas Chromatography-Mass Spectrometry

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DETERMINATION OF ALDEHYDES IN PARTICULATE MATTER USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

by

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Manikyala R Chintapalli

08/07/2015

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ABBREVIATIONS

Abbreviation	Full meaning
ACN	Acetonitrile
BSTFA	*N, O*-Bis(trimethylsilyl)trifluoroacetamide
DCM	Dichloromethane
DMDCS	Dimethyldichlorosilane
DNPH	2, 4-Dinitrophenyl Hydrazine
EI	Electron Ionization
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
ITMS	Ion Trap Mass Spectrometry
IS	Internal Standard
LC	Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
MG	Methylglyoxal
MS	Mass Spectrometry
MW	Molecular Weight
NCI	Negative Ion Chemical Ionization

PAH	Polycyclic Aromatic Hydrocarbon
PFBHA	O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride
PT	Purge & Trap
PTFE	Polytetrafluoroethylene
PM	Particulate Matter
RS	Recovery Standard
SIM	Selected Ion Monitoring
SOA	Secondary Organic Aerosol
TIC	Total Ion Current
US EPA	United States Environmental Protection Agency

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ABSTRACT

A gas chromatographic-mass spectrometric (GC-MS) method has been developed for the determination of a broad range of aldehydes (including, hydroxylated and aromatic aldehydes) in particulate matter (PM). In this method, the aldehydes are derivatized with O-2, 3, 4, 5-pentafluorobenzyl hydroxylamine hydrochloride (PFBHA) using various solvents for extraction and derivatization including acetonitrile, water, methanol, and acetonitrile/dichloromethane/methanol. An ACN/DCM/MeOH mixture with sonication was shown to be optimal as it increased the derivatization efficiency in addition to efficient extraction for all tested aldehydes. The optimal derivatization conditions were determined and then tested on a variety of oxy-PAHs which resulted in a complete derivatization of carboxaldehydes but only incomplete derivatization of quinonic species. Application of the extraction protocol to wood smoke (WS) PM and comparison to the traditional EPA buffer method resulted in higher recoveries (up to 150%) of several aldehydes. This artifact was shown to be due to the presence of organics like syringol and levoglucosan in WS PM at higher concentrations and not be caused by which were not due to completeness/incompleteness of the analyte derivatization. This effect was attributed to a matrix-assisted response enhancement, i.e., loss of analytes upon injection due to its adsorption, which may be masked by organics as blocking agents. Further application of the optimized method to particulate matter in GC-MS EI and NCI methods resulted in identification of up to 30 compounds. The NCI method proved to be more sensitive, with up to 28 compound detected with LODs in a range of 0.06 µg – 1.47 µg and comparable quantities (mean ± SD) of

aldehydes in all concentrations of WS PM used. By contrast, the EI method was shown to be less sensitive, with only 16 compounds being detected.

CHAPTER I

1. INTRODUCTION

1.1. Particulate Matter

Particulate matter (PM) is defined as the mixture of microscopically small solid and liquid particles present in the atmosphere.[1] PM with diameter less than 2.5 μm ($PM_{2.5}$) has a significant impact on human health (causing asthma, cardio-respiratory decease and lung cancer) and climate (scattering of radiation and formation of cloud-condensation nuclei).[1-5] Consequently $PM_{2.5}$ is regulated by the United States Environmental Protection Agency (EPA) at concentration of 12 $\mu g/m^3$.[6] PM consists of both inorganic and organic species, where organic matter may represent a significant portion (10–70%) of PM.[2] However, the role of organics in climate and health studies is not fully understood due to complexity of their characterization and large portion being uncharacterized.[2]

Various oxidation processes of organic matter lead to formation of Secondary Organic Aerosols (SOA) in the atmosphere affecting overall concentrations of PM.[1,3] Among the most challenging classes of compounds belonging to SOAs are, due to their reactivity and instability, aldehydes and carbonyl compounds such as oxy-PAHs (products of PAHs oxidation).

1.2. Aldehydes in Particulate Matter

Aldehydes are volatile, polar and reactive organic species, which are ubiquitous components of PM.[7-11] Aldehydes are released into the atmosphere by both anthropogenic

(combustion of organic matter) and natural (emission from plants) sources and are also formed as a result of various reactions such as ozonolysis of olefins, and photochemical reactions of hydrocarbons.[7-11] A wide variety of the aldehydes is associated with adverse health and climate effects. For instance, some of the lower molecular weight aldehydes such as formaldehyde, acetaldehyde and acrolein cause eye and lung irritation.[11] By contrast, dialdehydes (e.g., methylglyoxal and glyoxal) received much attention as potential SOA precursors undergoing acid catalyzed heterogeneous reactions (aldol condensation, hemiacetal and acetal formation, polycondensation of carboxylic and hydroxy-carboxylic acids and alcohols, and hydration of aldehydes) in the aerosol phase which result in the formation of higher molecular weight compounds.[12,13]

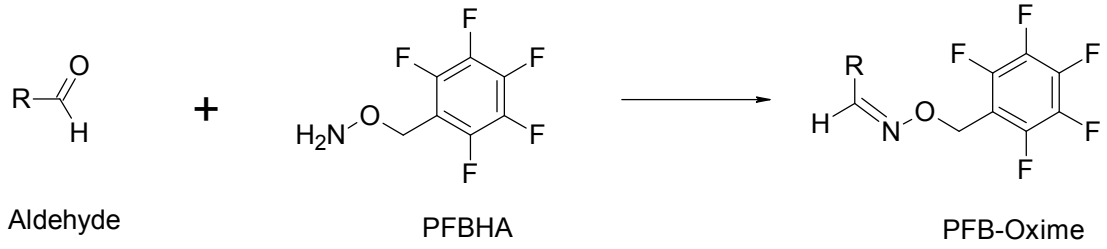
1.3. Approaches used for Determination of Aldehydes in PM

Direct determination of aldehydes in complex matrices is complicated due to their occurrence in trace concentrations, volatility and high reactivity.[8,9] Reactions of aldehydes can occur during sample collection or solvent extraction procedures, which result in deviations of the determined aldehyde concentration from the actual concentrations.[13] Hastings et.al, reported that formation of oligomers from aldehydes may occur during their ionization, an essential step of mass spectrometric (MS) analysis.[14] These facts indicate the importance of understanding the chemical behavior of aldehydes to ensure high accuracy and precision of both sample preparation and analysis.

1.3.1. Derivatization of Aldehydes

To ensure high sensitivity and stability of analytes, the preferred methods of aldehyde analysis involve derivatization.[7,15] Two most commonly used derivatization agents are 2,4-

dinitrophenyl hydrazine (2,4-DNPH) and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA).[5,7,15] 2,4-DNPH derivatization products are typically analyzed by HPLC.[16] Although 2,4-DNPH derivatives may be analyzed using GC, PFBHA derivatization with GC-MS analysis is often preferred due to a better resolution and ability to derivatize aldehydes with various functionalities, such as hydroxy carbonyls and dicarbonyls, when characterizing atmospheric samples.[7,16] Derivatization of aldehydes with PFBHA provides thermally stable and volatile oximes (Scheme 1), which have specific mass spectra and high sensitivity in different detection systems, such as electron ionization mass spectrometry (EI-MS) and negative-ion chemical ionization mass spectrometry (NICI-MS).[8]



Scheme 1. Schematic representation of PFBHA derivatization of aldehydes[15]

The solvent system used for sample preparation in aldehyde analysis may have an effect on derivatization efficiency of aldehydes. Temime et al. compared the PFBHA derivatization efficiency of aldehydes of several solvent systems including MeOH, water/ACN (9.875:0.125 v/v), and ACN/DCM/MeOH (1:8.5:0.5 v/v/v) and reported comparable derivatization yields in three solvent systems.[17] In case of derivatization of multifunctional carbonyls involving a two-step derivatization (PFBHA followed by N, O-bis (trimethylsilyl)-trifluoroacetamide (BSTFA), the presence of water or methanol should be avoided, considering their reactivity with BSTFA.[17]

1.3.2. Extraction of Aldehydes from PM

Although various protocols for the derivatization and analysis of aldehydes were evaluated, no considerable attention was given to the methods for extraction (sample preparation) of aldehydes from PM and other matrices. The extraction procedure for solid samples suggested by Environmental Protection Agency (method 8315A) proposes 18 h extraction on a rotary shaker with acetate-based buffered aqueous solution (EPA Buffer). By contrast, the atmospheric studies involving characterization of aldehydes from PM employed various solvent systems as well as using different extraction techniques.[17-26] The extraction of multifunctional aldehydes from $PM_{2.5}$ was shown to be significantly more efficient in polar solvents (providing 50–124% recovery in water) and at lower temperatures (up to -8 ºC) compared to DCM and toluene/isopropanol (2:1 v/v).[23] However, the use of polar protic solvents for extraction of aldehydes from PM was shown to catalyze aldol condensation reactions.[25] On the other hand, non-polar solvents cause depolymerization of oligomers, which may potentially overestimate the amounts of free aldehydes in PM.[26] These findings support the importance of application of an appropriate solvent system and extraction conditions. Besides the consideration of reactivity, it is difficult to predict if the solvent of choice is able to disrupt analyte-matrix interactions.

A comprehensive summary of these extraction protocols (using PFBHA for derivatization) employed in atmospheric studies is provided in Table 1. In most of the reported methods, sampling of aldehydes was done using XAD resin coated denuders (for gas-phase) and quartz fiber filters (for PM), which were typically coated with PFBHA dissolved in either MeOH or water (Table 1).[17-23] This sampling method was proven to be efficient in providing reproducible measurements with relative standard deviations ranging from 2.2 % to 12.2 %.[17] Extraction of the PM was conducted using various polar and non-polar solvent systems including (ACN/DCM/MeOH,

ACN/DCM, hexane/DCM, DCM, water and toluene/isopropanol) and employing different techniques such as sonication, rotary-shaking and soxhlet extraction.[17-24] The most common extraction/derivatization solvent system consisted of ACN/DCM/MeOH and was applied with/without PFBHA pretreatment.[17,18] Temime et al. reported that ACN/DCM/MeOH is better than MeOH and water/ACN as this protocol is water-free and minimizes the use of MeOH.[17] On the other hand, Healy et al. reported the use of ACN/DCM/MeOH for monitoring carbonyl compounds in the photoxidation of isoprene.[18]

Table 1. Comprehensive overview of the protocols used for extraction of aldehydes from PM with PFBHA derivatization

Source of PM & sampling procedure	Aldehydes analyzed	Sample preparation		Use of IS/RS	Analysis Method	Reference
		Methods	Optimized parameters			
Chamber experiments Fluorocarbon coated glass fiber filters (47 mm): a) Filter directly collecting PM b) Filter doped with 3 mg PFBHA placed after denuder	Carbonyls, dicarbonyls, aromatic aldehydes, carbonyl- containing furans and benzoquinones	Filters extracted/derivatized with PFBHA 3 mg in 10 mL MeOH for 72 h, concentrated under N_2 to near dryness	Comparable derivatization yields in three solvent systems. ACN/DCM/MeOH was selected as it is water free and minimizes the use of MeOH.	NR[a]	GC-EI-MS	17
Chamber experiments Glass fiber filter (47 mm) precoated with PFBHA (2 mg/410 µL 14% H_2O in MeOH)	Methacrolein, 2-methylbut-3-enal, glycolaldehyde, glyoxal, methylglyoxal , oxopropanedial, hydroxymethylglyoxal, and 2,3-dioxobutanal	Filters extracted in 10 mL of the mixture of ACN/DCM/MeOH (1:8.5:0.5 v/v/v) overnight in dark, evaporated to ~3 mL by rotary evaporation and filtered using a PTFE membrane	NR	NR	GC-EI-MS	18
Suburban ambient air a) Teflon filter pack with three quartz fiber filters (47 mm), impregnated with PFBHA b) Denuder coated with 40 mM PFBHA	glyoxal, methylglyoxal, glycolaldehydehyde and some bifunctional carbonyls	Filters sonicated with 5 mL ACN/DCM (1:1 v/v) in an ice bath for 15 min Derivatization with 30 µL of 40 mM PFBHA for 24 h at room temperature, evaporated to dryness and derivatized with BSTFA (50 µL)	NR	IS 1-phenyldodecane	GC-EI-MS	19
Diesel emissions a) Quartz fiber filters (47 mm) b) XAD4-coated annular denuder for gas-phase	Aliphatic and aromatic carbonyls	Filters sonicated (15 min) with 3x10 mL hexane/DCM (1:1 v/v) followed by 3x extraction with 10 mL MeOH. Combined extracts evaporated to 50 µL under N_2, transferred to 200 µL ACN/DCM (9:1 v/v) and derivatized with 50 mg/mL PFBHA in MeOH at room temperature for 24 h	NR	2-fluoro-9-fluorenone $^{13}C_6$ 4-hydroxybenzaldehyde and d_4 1, 4-benzoquinone	HPLC-ITMS	20

Table 1 contd.

Ambient $PM_{2.5}$ a) 47-mm Zefluor filters b) XAD4-coated annular denuder for gas-phase	Ketolimononaldehyde, C_4-C_6 linear dicarboxylic acids and limonic acid	Filters soxhlet extracted with ACN/DCM (1:1 v/v) for 24 h. Solutions evaporated to dryness. Derivatization with 1) PFBHA, 2) BF3-methanol-PFBHA-BSTFA, 3) PFBHA-BSTFA and 4) BF3-methanol-BSTFA	NR	*trans-p*-menth-6-ene-2, 8-diol, bornyl acetate, cis-ketopinic acid and d_{50}-tetracosane	GC-ITMS	3
Gasoline and diesel motor emissions. 47 mm quartz fiber filters and polyurethane foam substrates for PM	C_6-C_{16} quinones	Filters extracted 3x by sonication into 10 mL DCM/hexane (1:1 v/v) followed by 3x extraction with 10 mL MeOH Extracts evaporated to <50 µL and transferred to 150 µL ACN/DCM (9:1 v/v) followed by addition of a 200 mM solution of PFBHA in MeOH. Left at room temperature for 24 h	NR	4-fluorobenzaldehyde, benzaldehyde-d_6, 2,2'-difluorobiphenyl, 6-fluoro-4-chromanone and 2-fluoro-9-fluorenone	GC-ITMS and HPLC-APCI-ITMS	21
$PM_{2.5}$. Teflon-impregnated glass fiber filters (128-mm)	Carbonyls and hydroxy carbonyls	Filters soxhlet extracted in ACN /DCM (1:1 v/v) for 24 h. Extracts spiked with 350 µL of 160 µM/mL PFBHA in water. Evaporated to dryness and redissolved in 800 µL of DCM/hexane (1:2 v/v) followed by addition of 50 µL BSTFA and 100 µL pyridine. Left overnight at room temperature and filtered	NR	Benzaldehyde-d_5	GC-ITMS	22
$PM_{2.5}$ and model compounds. Two samplers in parallel 1) 47 mm Teflon filter 2) 47 mm quartz fiber filter.	Carbonyls and multifunctional carbonyls	Filters sonicated (2 min) with water, DCM or toluene/isopropanol (1:1 v/v). Derivatization with 0.1 mM solution of PFBHA in water at room temperature for 24 h. LLE into methyl tert-butyl ether Evaporated under N_2, dissolved in 200 µL of BSTFA and allowed to react at 42 °C for 12 h	Recovery of multifunctional carbonyls followed solvent polarity. (Water> DCM> toluene/isopropanol). Lowering the sonication bath temperature improved the % recoveries.	NR	GC/CI-ITMS	23

Table 1 contd.

PM_1 150 mm quartz fiber filter	Glyoxal and methylglyoxal	25 % of the filter is extracted into water. Derivatization with 10000 fold excess PFBHA LLE into hexane Dried over sodium sulfate.	NR	2-Trifluormethylbenzaldehyde	GCMS	24

[a]NR denotes Not reported

Evaluation of several solvent systems (not including ACN/DCM/MeOH) was reported by Rao et al.[26] However, no comprehensive comparison of the extraction solvent systems combined with PFBHA derivatization was performed.

1.3.3. Composition of Wood Smoke PM and Effect on Derivatization of Aldehydes

The possible species which may affect the extraction or derivatization of aldehydes are co-extracted aldehydes or simply the most abundant organics in PM potentially altering the medium polarity and other parameters. Table 2 gives brief evaluation of composition of organics in wood smoke PM. The major contribution to PM is from syringol derivatives (47.1 – 144.0 mg/g of PM), guaiacol derivatives (5.4 − 57.0 mg/g of PM), and levoglucosan (204 mg/g of PM).[32-38] Other phenolics in wood smoke PM have concentrations in a range of 6421 – 47800 μg/g of PM.[32-39] Overall, levoglucosan in wood smoke PM has a significantly higher concentration while other phenols like syringol and guiacol derivatives have moderate concentrations.[32-38]

Table 2. Previously reported concentrations of syringol, phenols and levoglucosan in wood smoke PM

Source of PM	**Extraction technique/solvent**	**Concentration of syringol derivatives** (μg/g)	**Concentration of guaiacol derivatives** (μg/g)	**Conc. of other phenols** (μg/g)	**Conc. of Levoglucosan** (μg/g)	**Reference**
Wood smoke PM collected from a chimney, burning mix of hardwoods	Hot pressurized water extraction	19000	3400	3800 μg/g	-	32
Wood smoke PM_{10}, beech wood and pine wood combustion experiments	Extracted with toluene for 30 min by sonication	260	2.572	-	22868	33
$PM_{2.5}$	-	Acetonyl syringol - 62.7 Propionyl syringol - 5.25		-	365	34
Hardwood smoke burning	Extraction by sonication for 2 h with 60 mL of acetone	181400	105900	74600	-	35
Wood smoke (Wood combustion experiments)	Extraction with hexane (2x) and with benzene/2-propanol (2:1 mixture, 3x) by sonication.	-	-	-	673 - 1490	36
Residential wood combustion	Extraction with hexane (2x) and with benzene/2-propanol (2:1 mixture, 3x) by sonication.	47150	5375	6421	204210	37
Residential wood combustion	-	144000	56500	47800	-	38
Residential wood combustion	Extraction with ethyl acetate containing 3.6 mM trimethylamine by sonication.	-	-	-	1980-3700	39

1.4. Accurate Quantification of Aldehydes in PM

Aldehydes are reactive and volatile, thus it is essential to employ for their quantification appropriate internal standards (IS) and recovery standards (RS). The purpose of the internal standard (IS) is to correct for any volume errors that occurred during sample preparation. By contrast, the RS are added prior to the sample preparation in order to evaluate matrix effects and sample preparation losses. Usually yields of a recovery standard are reported based on 4-5 point calibration curves. Two important requirements for RS are 1) the behavior of the RS should mimic that of the analytes (chemically identical nature) and 2) if many analytes of differing functionalities/properties are involved, one would have to consider using more than one RS.[18] In the case of aldehyde analysis in PM, deuterated aldehydes such as 4-hydroxybenzaldehyde-d_4 and benzaldehyde-d_6 (one standard for all analytes) were previously used as RSs.[20,22] However, since a typical PM analysis involves a variety of aldehydes with multi-functional groups, the method requires the use of more than one RS which resemble the chemical nature of different groups of aldehydes.

1.5. Derivatization of Oxy-PAHs in Particulate Matter

Another important class of compounds formed during the oxidation process of organic PM matter are oxy-PAHs, which are primarily the oxidation products of PAHs. Previously, the PFBHA derivatization of quinones and carboxaldehydes was achieved using different solvent systems including MeOH, ACN/DCM and ACN/DCM/MeOH,[17,20,21] but no attempts were made to optimize the solvent systems and conditions to achieve complete derivatization of oxy-PAHs. A

possible reason for this lack of optimization is that carbonyl PAHs can be observed without derivatization.[21] Nevertheless assessing the effectiveness of derivatization and identification is essential when performing the derivatization of carbonyl species in general.

1.6. Goals & Objectives

At present, the extraction of aldehydes from PM of different origin is accomplished by a variety of different solvent systems (Table 1).[17-23] However, to the best of our knowledge, no studies providing a comprehensive comparison of solvent effects on the efficiency of aldehyde extraction/derivatization from PM have been reported. For this reason, the specific goal of this research project was to evaluate derivatization/ extraction efficiencies of a wide range of aldehydes in PM using various solvent systems including the buffer used in the EPA method, deionized water, ACN, MeOH, and mixture of ACN/DCM/MeOH (1:8.5:0.5 v/v/v). Two different extraction techniques (sonication and rotary-shaking) and the impact of PFBHA pre-treatment prior to the extraction were evaluated. In this work, we have also used labeled standards as recovery standards to fully understand the extraction process and account for possible artifacts during extraction/derivatization as well as to ensure accurate quantification in ambient PM samples.

CHAPTER II

2. EXPERIMENTAL

2.1.Materials

Solvents including MeOH (99.9% Purge & Trap grade), MeOH (LC-MS Optima grade), ACN (99.9%, LC-MS Optima grade), DCM (99.9%, LC-MS grade) and acetone (99.9%, LC-MS grade) were purchased from Fisher Scientific (Waltham, MA, USA). EPA buffer (64.3 mL 1M NaOH + 5.7 mL glacial acetic acid in 900 mL water with final pH of 4.9±0.2) was prepared prior to the experiment in the laboratory.[28] Deionized water was obtained using a Direct-Q3 water purification system with an incorporated dual wavelength UV lamp (Millipore, Billerica, MA, USA) for low total organic carbon content (the manufacturer's claimed purity is less than 5 ng/g). The derivatization agent, PFBHA (>99%) and the reagent grade selenium dioxide (SeO_2) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The detailed information on a broad range of aldehydes (i.e., linear, hydroxylated and aromatic aldehydes), recovery standards (deuterated aldehydes) and internal standards employed in this study including supplier names, retentions times, quantification (*m/z*), and confirmation ions used in the GC-NCI-MS (Negative Chemical Ionization) and GC-EI-MS (Electron Ionization) analysis is provided in Table 3. Oxy-PAHs employed in this study including supplier names, retention times and confirmation ions of both derivatized and underivatized oxy-PAHs are listed in Table 4.

Table 3. List of aldehydes studied including target ions and confirmation ions of their derivatives used for GC-MS analysis and data processing.

				NCI-MS		EI-MS		
Aldehydes	**Supplier**	**MW (g/mol)**	**MW Derivatized (g/mol)**	**Q ion[a] (*m/z*)**	**Confirmation Ions (*m/z*)**	**Q Ion (m/z)**	**Confirmation Ions (m/z)**	**RS**
Formaldehyde	Fisher Scientific[b]	30	225	225	205	181	195, 225	Formaldehyde-^{13}C-d_2
Acetaldehyde	Sigma Aldrich[c]	44	239	239	218	181	209, 239	Acetaldehyde-d_4
Propanal	Sigma-Aldrich	58	253	253	233	181	223, 236	Propanal-d_2
Acrolein	Sigma-Aldrich	56	251	231	201	181	221, 251	Propanal-d_2
Isobutanal	Sigma Aldrich	72	267	178	247, 267	181	250	Propanal-d_2
Butanal	Fluka[c]	72	267	247	267	239	226	Butanal-d_2
Crotonal	Chem Service[d]	70	265	245	215	181	195, 250	Butanal-d_2
Pentanal	Sigma Aldrich	86	281	178	261, 231	181	207, 239	Butanal-d_2
Hexanal	Sigma Aldrich	100	295	178	248, 275	181	239, 295	Butanal-d_2
Furaldehyde	Sigma Aldrich	96	271	241	271	291	248	Furaldehyde-d_4
***trans*-2-Hexenal**	Sigma Aldrich	98	293	273	243	181	250, 293	Furaldehyde-d_4
Heptanal	Sigma Aldrich	114	309	178	289, 262	181	207, 239	Furaldehyde-d_4
Octanal	Sigma Aldrich	128	323	178	276, 303	181	239, 323	Octanal-d_{16}
Benzaldehyde	Sigma Aldrich	106	301	281	251	301	271	Benzaldehyde-d_6
Phenylacetaldehyde	Sigma Aldrich	120	315	178	295, 267	181	91, 315	Benzaldehyde-d_6
Nonanal	Fluka	142	337	178	317	181	239	Benzaldehyde-d_6
***m*-Tolualdehyde**	Sigma Aldrich	120	315	295	265, 167	181	91, 315	Benzaldehyde-d_6
***o*-Tolualdehyde**	Sigma Aldrich	120	315	295	265, 167	181	91, 315	Benzaldehyde-d_6
Hydrocinnamaldehyde	Sigma Aldrich	132	329	178	309	181	271, 329	Benzaldehyde-d_6
***trans*-2-Nonenal**	Sigma Aldrich	140	335	315	285	181	250, 335	Benzaldehyde-d_6
2-Hydroxy benzaldehyde	Chem Service	122	317	136	280	181	300, 317	Benzaldehyde-d_6
Decanal	Sigma Aldrich	156	351	178	331	181	239, 351	Benzaldehyde-d_6
2,5-Dimethylbenzaldehyde	Sigma Aldrich	134	329	309	279	181	286, 329	Benzaldehyde-d_6
5-Hydroxymethyl furfural	Sigma Aldrich	126	321	271	285, 301	181	291, 321	Benzaldehyde-d_6
2,4-Nonadienal	Sigma Aldrich	138	333	283	167	181	276, 333	Benzaldehyde-d_6
Glyoxal	Sigma Aldrich	58	448	267	167	181	418, 448	Benzaldehyde-d_6

Table 3 contd.

Undecanal	Sigma Aldrich	170	365	345	318	239	181, 345	Benzaldehyde-d_6
Anisaldehyde	Chem Service	136	331	311	281	331	181, 288	*p*-Anisaldehyde-d_3
Methylglyoxal	Sigma Aldrich	72	462	281	167, 392	181	432, 462	*p*-Anisaldehyde-d_3
4-Hydroxybenzaldehyde	Chem Service	122	317	297	267	181	274, 317	4-Hydroxybenzaldehyde-d_4
Dodecanal	Sigma Aldrich	184	379	178	332, 359	181	239	4-Hydroxybenzaldehyde-d_4
Glutaraldehyde	Sigma Aldrich	100	490	178	450	181	293, 490	4-Hydroxybenzaldehyde-d_4
Syringaldehyde	Chem Service	182	377	357	327	377	181	4-Hydroxybenzaldehyde-d_4
Recovery standards (RS)								
Formaldehyde-^{13}C-d_2	Isotech[e]	32	227	228	208	181	198	–
Acetaldehyde-d_4	CDN Isotopes[f]	48	243	243	222	181	213	–
Propanal-d_2	CDN Isotopes	60	255	255	235	181	238, 225	–
Butanal-d_2	CDN Isotopes	74	269	269	221	181	241	–
Furaldehyde-d_4	CDN Isotopes	100	275	275	245	181	295, 251	–
Octanal-d_{16}	CDN Isotopes	134	329	319	289	181	243, 339	–
Benzaldehyde-d_6	CDN Isotopes	112	307	287	257	181	307, 277	–
***p*-Anisaldehyde-d_3**	CDN Isotopes	139	334	314	284	181	334	–
Methylglyoxal – d_4	Synthesized	76	271	-	-	181	466, 436	–
4-Hydroxybenzaldehyde-d_4	CDN Isotopes	126	321	301	271	181	321	–
Internal standards (IS) tested								
2-Nitroaniline	Acros	–		138	–	–	–	–
1,3-Dinitrobenzene-d_4	Sigma Aldrich	–		172	–	–	–	–
Octafluoronaphthalene	Sigma Aldrich	–		272	–	272	242	–

[a]"Q"denotes quantification ion; [b](Pittsburgh, PA,USA); [c](St. Louis, MO, USA); [d](West Chester, PA, USA); [e](Champaign, IL, USA); [f](Pointe-Claire, Quebec, Canada)

For calibration, a defined mixture of aldehydes was prepared with concentrations ranging from 0.02 μg/mL to 7.5 μg/mL. Two oxy-PAH mixtures were prepared at ~1 μg/mL and ~10 μg/mL per compound.

To evaluate the applicability of the method, a wood smoke PM collected from a chimney that vented an airtight wood stove burning a mix of hardwoods was used for extraction and determination of aldehydes.[28] Silica particles and carbon particles used in this study were purchased from Sigma Aldrich (St. Louis, MO, USA).

Table 4. List of oxy-PAHs studied including GC-MS retention times (t_R) and MS quantification and confirmation ions of derivatized and underivatized species.

Oxy-PAHs	Supplier	Underivatized			PFBHA Derivatized		
		MW (g/mol)	t_R (min)	EI MS ions (*m/z*)[a]	MW (g/mol)	t_R (min)	EI MS ions (m/z)[a]
2-Chloranthraquinone	Sigma Aldrich	242	16.74	**242**,214,186	437	NA[c]	-
1,4-Naphthaquinone	Alfa Aesar	158	9.42	**158**,130,102	353	17.21	181,**353**
Anthraquinone	Sigma Aldrich	208	15.21	**208**,180,152	403	-	-
Phenanthraquinone	Alfa Aesar	208	17.11	**208**,180,152	403	-	-
Pentacenequinone	Sigma Aldrich	308	27.59	**308**,280,252	503	-	-
1, 8-Dihydroxyanthraquinone	Sigma Aldrich	240	17.30	**240**,212,184	435	-	-
2-Methylanthraquinone	Sigma Aldrich	222	16.46	**222**,194,165	417	-	-
Bianthrone	Sigma Aldrich	384	29.93	**384**,355, 207	579	-	-
Acetophenone	Chem Service	120	6.13	**120**,105,77	315	12.48	181,**315**
1-Indanone	Sigma Aldrich	132	8.08	**132**,104,78	327	14.68	181,**327**,116
9-Fluorenone	Sigma Aldrich	180	12.86	**180**,152,126	375	19.07	181,345,**375**
Benzophenone	Fisher	182	11.65	**182**,105,77	377	16.47	181,**377**,196
3-Methoxyacetophenone	Sigma Aldrich	150	8.22	**150**,135,107	345	14.69	181,**345**,328
Phenoxy-2-propanone	Sigma Aldrich	150	7.39	**150**,107,77	345	13.62	181,**345**,328
3-Methyl-2-cyclohexen-1-one	Sigma Aldrich	110	ND[b]	-	305	11.87	181,**305**,288
Xanthone	Sigma Aldrich	196	14.11	**196**,168,139	391	-	-
1, 3-Indandione	Alfa Aesar	146	8.82	**146**,118,104	341	15.95	181,**341**,324
9-Phenanthrenecarboxaldehyde	Sigma	206	16.63	**206**,178,151	401	22.15	181,**401**,358
Anthrone	Alfa Aesar	194	15.00	**194**,165,139	389	-	-
1-Naphthalaldehyde	Sigma Aldrich	156	11.45	**156**,128	351	17.29	181,**351**,308
Anthracene-9-carboxaldehyde	Fluka	206	16.86	**206**,178,151	401	20.30	181,**401**,220
1,4-Benzoquinone	Sigma Aldrich	108	-	-	303	12.64	181,**303**
Methyl-1,4-benzoquinone	Sigma Aldrich	122	-	-	317	13.32	181,**317**
1,2-Naphthaquinone	Sigma Aldrich	158	11.43	**158**,130,102	353	18.35	181,336,**353**
Perinaphthenone	Sigma Aldrich	180	14.54	**180**,152	375	-	-
2-Methyl-1,4-naphthaquinone	Sigma Aldrich	172	10.49	**172**,115,104	367	-	-
1-Pyrene carboxaldehyde	Sigma Aldrich	230	19.88	**230**,201,100	425	25.11	181,**425**,244
Biphenyl-4-carboxaldehyde	Sigma Aldrich	182	12.55	**182**,181,152	377	19.26	181,**377**,334
Dicinnamalacetone	Sigma Aldrich	286	-	-	481	-	-
Pyrene-4,5-dione	Sigma Aldrich	232	20.16	204, **232**, 176	427	-	-

[a] Ions in bold denote quantification ions and the other ions were used for confirmation. [b] ND denotes not detected. [c] NA denotes not applicable as these species were not possible to be derivatized

2.2. Sample Preparation

Individual stock solutions of aldehydes were prepared in methanol at a concentration of 20 mg/mL and stored at -18 °C. Extraction and derivatization of aldehydes was evaluated in several solvent systems including EPA buffer, deionized water, ACN, MeOH, and mixture of ACN/DCM/MeOH (1:8.5:0.5 v/v/v). In our initial work, we first evaluated the effectiveness of derivatization method. This was followed by comparison of several protocols employing simultaneous or sequential extraction and derivatization. The detectable concentration of underivatized aldehydes was determined by serial dilutions in a range from 7.5 µg/mL to 250 µg/mL per compound.

2.2.1. PFBHA Derivatization in Aqueous Media

The protocol for derivatization in aqueous media employed either purified water or EPA buffer. The derivatization method was first evaluated using a mixture of aldehydes (listed in Table 2) spiked into 10 mL of either water or buffer resulting in a final total concentration of 0.1–1.5 mg/L. The mixture of recovery standards (RS) listed in Table 2 (15 µL of 0.2–0.5 mg/mL per RS) was added to this solution. PFBHA solutions (15 mg/mL) were prepared freshly in purified water prior to the derivatization. The final concentration of PFBHA in 10 mL solution was 1.5 mg/mL thus resulting in a minimum 10-fold excess of PFBHA compared to the molar concentration of carbonyls. The solutions consisting of target analytes and PFBHA were placed at room temperature in the dark overnight under either rotary shaking (for derivatization in EPA buffer) or sonication (for derivatization in water) to ensure the complete derivatization of the aldehydes. After the reaction, a few drops of concentrated sulfuric acid (1:3 v/v) were added to prevent the potential interference from excess PFBHA during the GC analysis. Liquid-liquid extraction (LLE) of PFBHA-aldehydes into 3 times 1.0 mL of DCM (each time shaking for 1 min) was employed.

DCM was used as it was proven to be an efficient solvent for extraction of PFBHA derivatives of carbonyls,[29] then by EPA method recommended hexane. The DCM fractions were combined and filtered through anhydrous Na_2SO_4 layered on top of purified glass wool, and filtered over a 0.2 μm Teflon syringe filter to remove any water residues and solid particles from the extract, respectively. Internal standards (5 μL of 10.5 mg/mL) were added prior to the GC analysis.

Evaluation of purity of solvent systems was performed by adding 15 mg of PFBHA to 10 mL of pure solvents of different grades and derivatization for 18 hours (no aldehydes were added). LLE was performed three times into DCM and the extracts were analyzed using GC-MS.

2.2.2. PFBHA Derivatization in Organic Media

The protocol for derivatization in organic media employed MeOH, ACN, or the ACN/DCM/MeOH (1:8.5:0.5 v/v/v) mixture. For the optimization of derivatization conditions, a mixture of aldehydes (listed in Table 3) was spiked into the solvent resulting in a final concentration of 0.1–1.5 μg/mL per analyte. The mixture of recovery standards (RS) listed in Table 2 (15 μL of 0.2–0.5 mg/mL per RS) was added to this solution. PFBHA solution (15 mg/mL) was prepared freshly in methanol prior to the derivatization. A final concentration of PFBHA in 10 mL of the derivatizing solutions was 1.5 mg/mL. These solutions were sonicated overnight to ensure complete derivatization of the aldehydes. No further processing for extraction of derivatized aldehydes was needed since the derivatization was done in an organic solvent. The solutions were filtered with 0.2 μm Teflon filter to remove any solid particles in the samples. Internal standards (5 μL of 10.5 mg/mL) were added prior to the GC analysis.

2.2.3. Derivatization of oxy-PAHs

The protocol for derivatization of oxy-PAHs (individually at 10 µg/mL per analyte) involved the use of ACN/DCM/MeOH (1:8.5:0.5 v/v/v) and MeOH. The PFBHA solution was prepared freshly in MeOH. Final concentration of each oxy-PAH in 1 mL of the solution was 10 µg/mL with 200 µg/mL of PFBHA. The solutions were left at room temperature in the dark providing sonication overnight. To the analytes derivatized in MeOH, the ACN/DCM mixture was added later prior to the analysis. The solutions were then analyzed on GC-EI-MS.

2.2.4. Extraction of Aldehydes from PM

The extraction of aldehydes from PM was performed using 15 mg of wood smoke PM. The extraction efficiencies were compared in two solvent systems consisting of ACN/DCM/MeOH and EPA buffer. Extraction using ACN/DCM mixture was performed employing sonication for 24 h and followed the protocol for derivatization in organic media. The extraction using EPA buffer was carried out using rotary-shaking for 24 h and followed the protocol for derivatization in aqueous media (described in section 2.2.1).

2.3. Evaluation of Extraction Protocol

In order to evaluate the extraction/derivatization protocol several experiments were designed.

1) To evaluate the recovery of aldehydes (RS), an experiment was performed by spiking a standard solution (15 µL) of deuterated RS aldehydes (listed in Table 1) to 5 mL of the ACN/DCM/MeOH solvent system containing 15 mg of WS PM. The analytes were derivatized overnight using sonication and analyzed on GC-EI-MS. Further, to compare the recoveries of labeled aldehydes in

ACN/DCM/MeOH, another experiment was done using EPA buffer as a solvent. The derivatized aldehydes were extracted into DCM by LLE and analyzed on GC-EI-MS.

2) In order to evaluate completeness of derivatization, the sensitivity and limits of detection for the analysis of selected aldehydes in underivatized form were determined. Table 5 shows the list of aldehydes analyzed including the quantification ions and LODs (ranging between 2 to 5 ppm) of aldehydes (the protocol for determination of LODs is in Section 2.6).

Table 5. GC-MS analysis of aldehydes in underivatized form and determination of LODs

Compound name	t_R (min)[a]	Q Ion[b] (m/z)	Slope	Intercept	R^2	Sy	LOD (ppm)
Butanal	4.00	72	0.2509	1.3522	0.9926	0.3955	5
Benzaldehyde	9.17	106	0.6286	2.7049	0.9958	0.5769	3
Octanal	9.77	84	0.1438	0.6688	0.9958	0.1935	4
Anisaldehyde	13.86	135	0.9465	2.3823	0.9983	1.0828	4
4-Hydroxybenzaldehyde	15.21	121	0.6651	0.1183	0.9997	0.4924	2
9-Fluorenone	20.18	180	1.1936	3.9151	0.9977	1.3163	4

[a]t_R denotes retention time [b]Q Ion denotes quantification ion

3) To evaluate possible impact of glassware inertness, the glassware was silanized with 15% (v/v) dimethyldichlorosilane (DMDCS) in toluene overnight, washed with MeOH and DCM, and dried. The derivatization of selected aldehydes was performed both individually and as a mixture and analyzed by GC-EI-MS.

4) To investigate the effect of PM particles (matrix-analyte interaction) on recoveries of aldehydes, the recovery standards (deuterated aldehydes) were derivatized in ACN/DCM/MeOH in presence of four different types of particles including carbon, silica and wood smoke particles. The analysis was performed on GC-EI-MS within 24 h of derivatization.

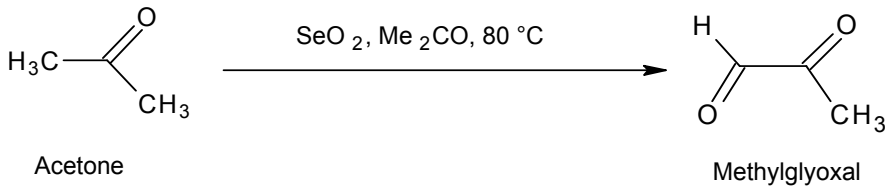
5) To compare the matrix effect of co-extracted organic matter, the derivatization of RS aldehydes was performed in presence of 15 mg of PM, 3.0 mL of extracted organic matter and the remaining PM particles without organic matter and compared to those derivatized without PM (controls). To extract organic matter from PM, 15 mg of wood smoke PM was dissolved in 5 mL of deionized water and the organic matter in PM was extracted into 3 mL of DCM by LLE. The remaining aqueous solution of PM was centrifuged and the aqueous part was decanted. The remaining solid particles were dried in the oven. The analysis was done by GC-EI-MS.

6) For sequential extraction/derivatization of aldehydes and carboxylic acids from WS PM three steps were employed. In step 1, 2 mg of WS PM was derivatized in 10 mL of MeOH under sonication. Second step involves extraction from un-extracted PM residue in step 1 using 10 mL of ACN/DCM/MeOH under sonication. Final step is soxhlet extraction from un-extracted PM residue in step 2 for 18 h with 90 mL of MeOH. The extracts were filtered on a filteration paper between each extraction step to obtain the un-extracted PM residue. The analysis was done by GC-EI-MS.

7) For quantification of aldehydes from WS PM the analysis was done in both GC-EI-MS and GC-NCI-MS. WS PM concentration of 1 mg, 3 mg and 15 mg were used in EI method and concentrations of 3 mg and 7 mg were used in NCI method. The WS PM was dissolved in 5 mL of ACN/DCM/MeOH containing the RS and derivatized overnight under sonication. The solid particles were removed and the extracts were concentrated to 250 μL and analyzed in EI and NCI methods.

2.4. Selection of IS & Synthesis of Methylglyoxal-d_4 as an RS for Dicarbonyls

The application of different internal standards (listed in Table 2) to control for volumetric changes during the GC injection was evaluated. In addition a series of stable isotope labeled aldehydes (listed in Table 2) was employed as recovery standards. However, no labeled dicarbonyl was found to be commercially available. Thus, in order to develop a method for synthesis of methylglyoxal-d_4, a preliminary experiment was done to conduct the oxidation of acetone with SeO_2 to yield the non-labeled methylglyoxal (MG) (Goswami et al.).[29] A schematic representation of the synthesis is given in Scheme 2. Briefly, 5 g of SeO_2 and 25 mL of acetone were heated at reflux for 10–12 h. The yellow liquid product was decanted and the black residue was washed with acetone. The whole liquid was fractionally distilled and the distillate up to 80 °C was collected. The residual liquid was again fractionally distilled under reduced pressure. The higher boiling fraction which condensed as a bright yellow liquid contained a greater portion of MG.



Scheme 2. Schematic diagram of synthesis of methylglyoxal.[32]

The identity of synthesized MG was confirmed by the GC-MS analysis after derivatization with PFBHA. The ion with m/z 462 which is the result of a derivatization of both carbonyl groups of MG confirmed the identity of MG (Figure 1a). Following the successful synthesis of MG, deuterium labeled MG (methylglyoxal-d_4) was also synthesized using the same protocol but starting with acetone-d_6. The identity of synthesized methylglyoxal-d_4 was confirmed by

derivatization with PFBHA and analysis on GC-EI-MS. The ion with *m/z* 466 confirms the identity of MG-d_4 (Figure 1 b).

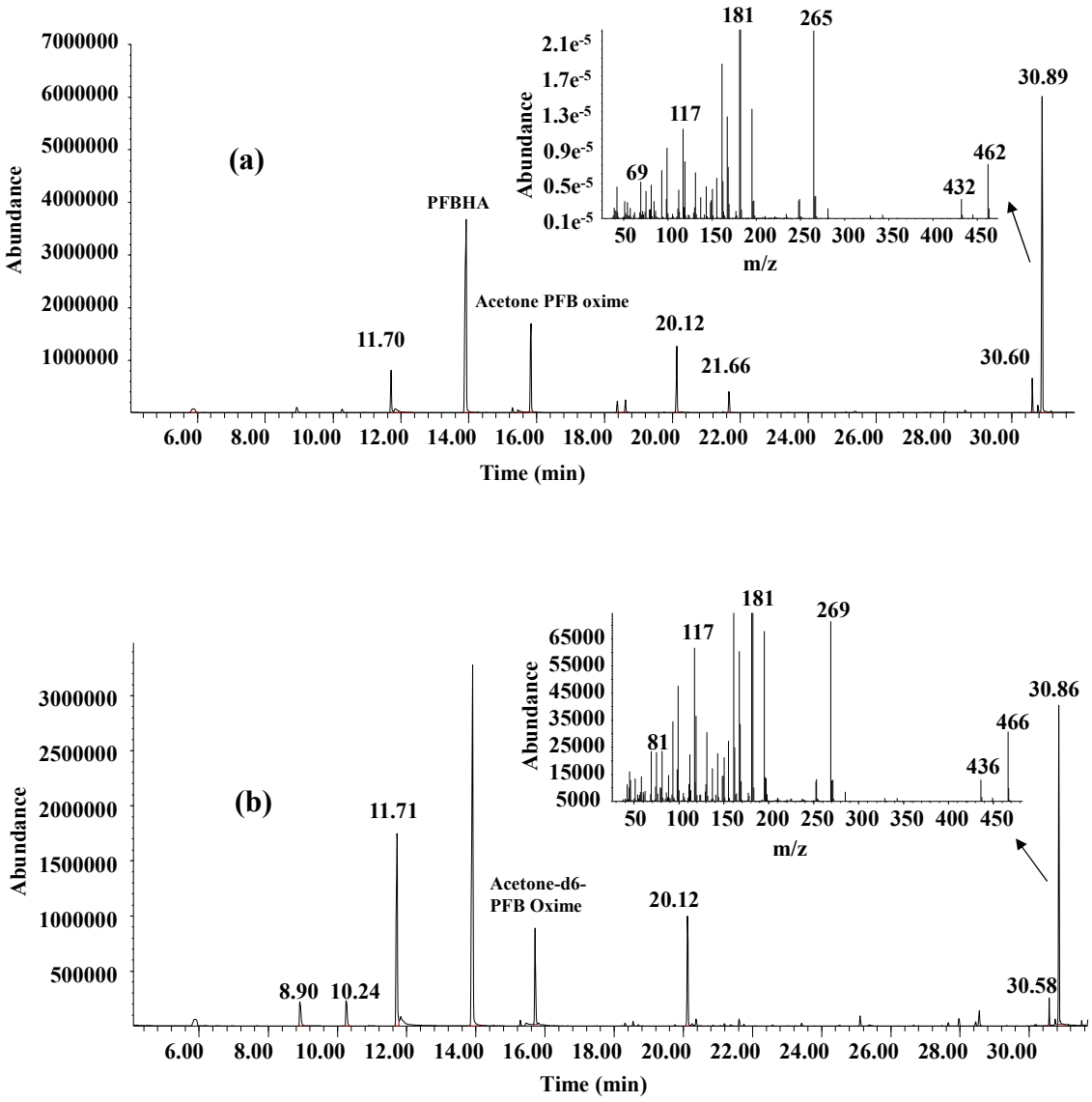


Figure 1. GC-MS chromatogram and mass spectra showing and confirming the identity of synthesized and PFBHA derivatized a) nonlabeled methylglyoxal (MW = 462) and b) deuterated methylglyoxal-d_4(MW = 466).

2.5. Instrumentation

GC-MS analyses were performed using an Agilent 6890N GC with 5975C inert XL EI/CI MSD (Agilent Technologies, Inc., Wilmington, DE, USA) and Gerstel MPS2 autosampler (Gerstel, Baltimore, MD, USA). Separations were accomplished using a 30 m long DB-5 MS column ((5% phenyl) dimethyl polysiloxane) with 0.25 mm i.d and, 0.25 μm film thicknesses (J&W Scientific, Inc., Folsom, CA). The carrier gas was ultra-pure helium (99.999%) with a constant flow rate of 1.0 mL/min. Injections were performed in a splitless mode with a splitless time of 0.5 min. The injector temperature was held at 250 °C. The chromatographic program started with hold at 50 °C for 2 min, then increased at 6 °C/min to 210 °C, then 30 °C/min to 320 °C and hold for 10 min. The temperatures of MS-NCI source, mass analyzer, and the transfer line were 155 °C, 150 °C and 280 °C respectively. The reagent gas used for NCI ionization was methane with a flow rate of 3 mL/min and ionization energy of 230 eV. The MS analysis was performed in selected ion-total ion (SITI) mode. The ions used for selected ion monitoring (SIM, within SITI) are specified in Tables 3 and 4, the total ion current (TIC) mass range was 50–500 amu.

2.6. Data Processing

All the experiments were performed in triplicate. MSD Chemstation E.01.00.237 software was employed for the GC-MS data acquisition and processing. Quantification of aldehydes was performed using the internal standard calibration method. The recovery standards were used to study the behavior of deuterated (unaffected by matrix) aldehydes in PM samples independently of native aldehydes and/or to correct recoveries of native aldehydes extracted from PM. Thus a least square calibration curve was obtained for each recovery standard using Microsoft Office Excel to obtain calibration curve parameters. The LOD were calculated using eq 1.

$$LOD = \frac{3.3\ X\ Sy}{k} \quad (1)$$

where k is the slope of the calibration curve and S_y is the standard deviation of the linear regression residuals which is obtained as a square root of the sums of residual mean square representing the unbiased estimate of a calibration curve variance within one order of magnitude of LODs.[10] The LLOQ were calculated using eq 2.

$$LLOQ = \frac{5\ X\ Sy}{k} \quad (2)$$

The final concentrations of aldehydes in PM were reported as those corrected by RS responses. The list of the RS used for corresponding aldehydes is provided in Table 3.

CHAPTER III

3. RESULTS AND DISCUSSION

3.1.Optimization of Derivatization Conditions

3.1.1. Solvent Purity

Occurrence of aldehydes in reagents and solvents results in interferences and has thus a negative impact on their trace analysis. Therefore, in an initial derivatization experiment, the purity of two different grades of methanol solvents (purge & trap grade and LC-MS grade) and deionized water was evaluated. Figure 2 shows for methanol (LC-MS grade) a high formaldehyde peak which is unacceptable for determination of this compound. This peak has been attributed to the oxidation of methanol during manufacturing. The methanol of a purge & trap grade demonstrated the comparable and lower formaldehyde content as in deionized water. These levels were considered to be acceptable for further work, however should be addressed by control experiments.

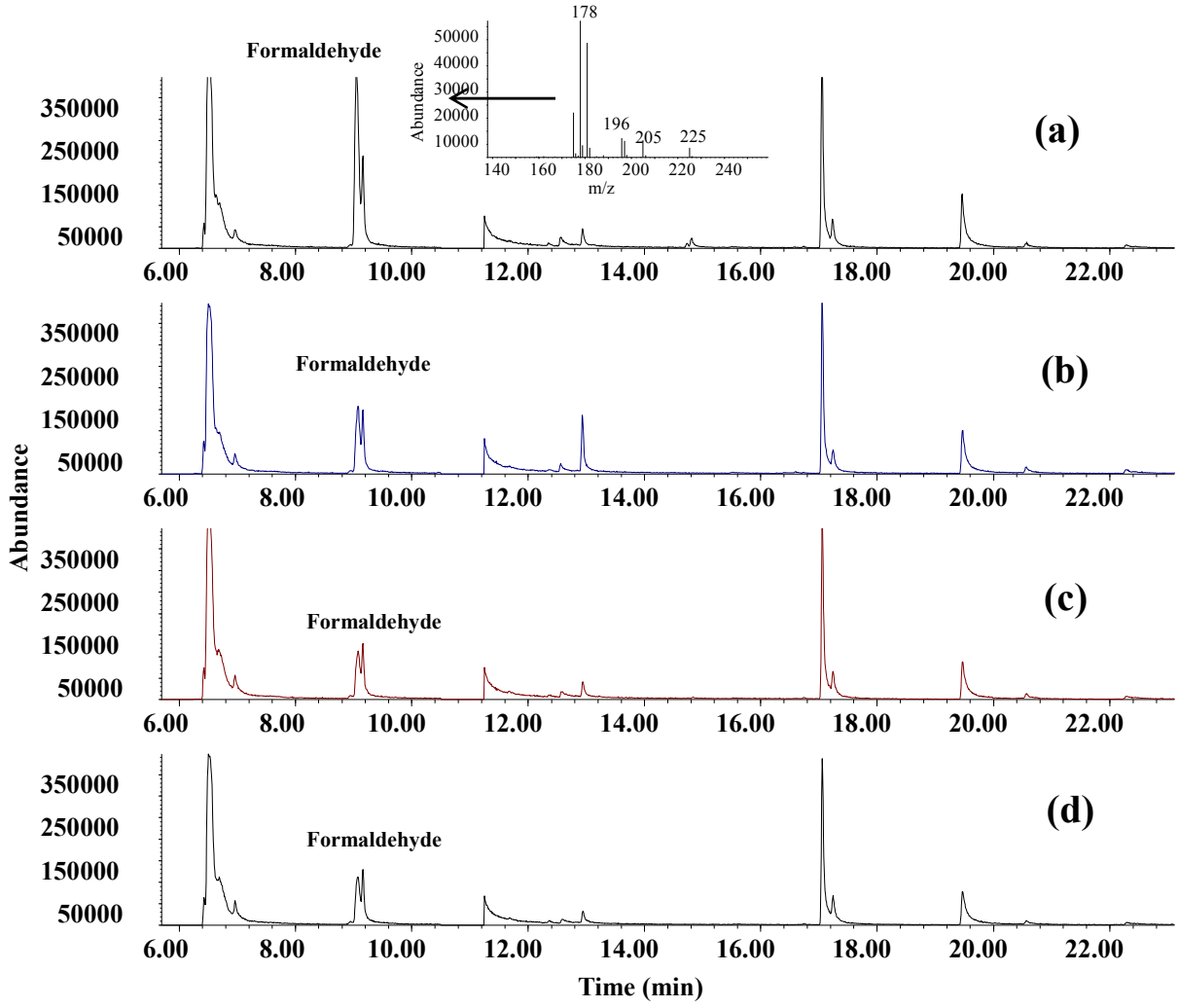


Figure 2. GC-MS-TIC chromatogram showing the content of PFBHA derivatized formaldehyde in a) MeOH LC-MS grade b) MeOH purge & trap (Sigma-Aldrich) c) MeOH purge & trap (Fisher Scientific) and d) Water LC-MS grade

3.1.2. Aldehyde Derivatization Optimization

3.1.2.1. Initial Screening of Derivatization Solvent System

To our knowledge, only one study reported a comparison of derivatization solvents; specifically evaluating MeOH, ACN/water and ACN/DCM/MeOH and showing the last solvent system as optimal (Table 1).[17] However, no comparison was performed with respect to the EPA method (using aqueous buffer system) for solid samples or with exception to MeOH for derivatization in individual solvents. Another concern was that the solvent system previously reported as optimal[17] and used in a number of studies consisting of ACN/DCM/MeOH (1:8.5:0.5 v/v/v) caused the precipitation of PFBHA (due to its insolubility in DCM).

In this study, the derivatization efficiency of aldehydes in different solvent systems including ACN, water, EPA buffer, MeOH (Purge & Trap grade) and the mixture of ACN/DCM/MeOH was evaluated. The detailed protocols applied for derivatization in organic and aqueous media are provided in the sample preparation section 2.2. To ensure the complete derivatization of aldehydes, the reaction time was set to 18 h. The analytes derivatized in EPA buffer and water were then extracted using LLE into DCM. No further extraction was required for those analytes derivatized in ACN, MeOH and ACN/DCM/MeOH mixture. Our results corroborate the previously reported higher responses for the majority of aldehydes derivatized in the mixture of ACN/DCM/MeOH (Figure 3) indicating that water and EPA buffer protocols are unsuitable for derivatization of aldehydes. The insolubility of PFBHA in DCM probably favored the (ACN/DCM/MeOH) protocol removing an extra purification step of removing the excess of PFBHA. The comparison of organic solvents showed higher recoveries for ACN and ACN/DCM/MeOH than for MeOH. In the following sections the possible incomplete

derivatization as well as suitability of different solvents for direct GC injection are evaluated and whether these factors affect the results.

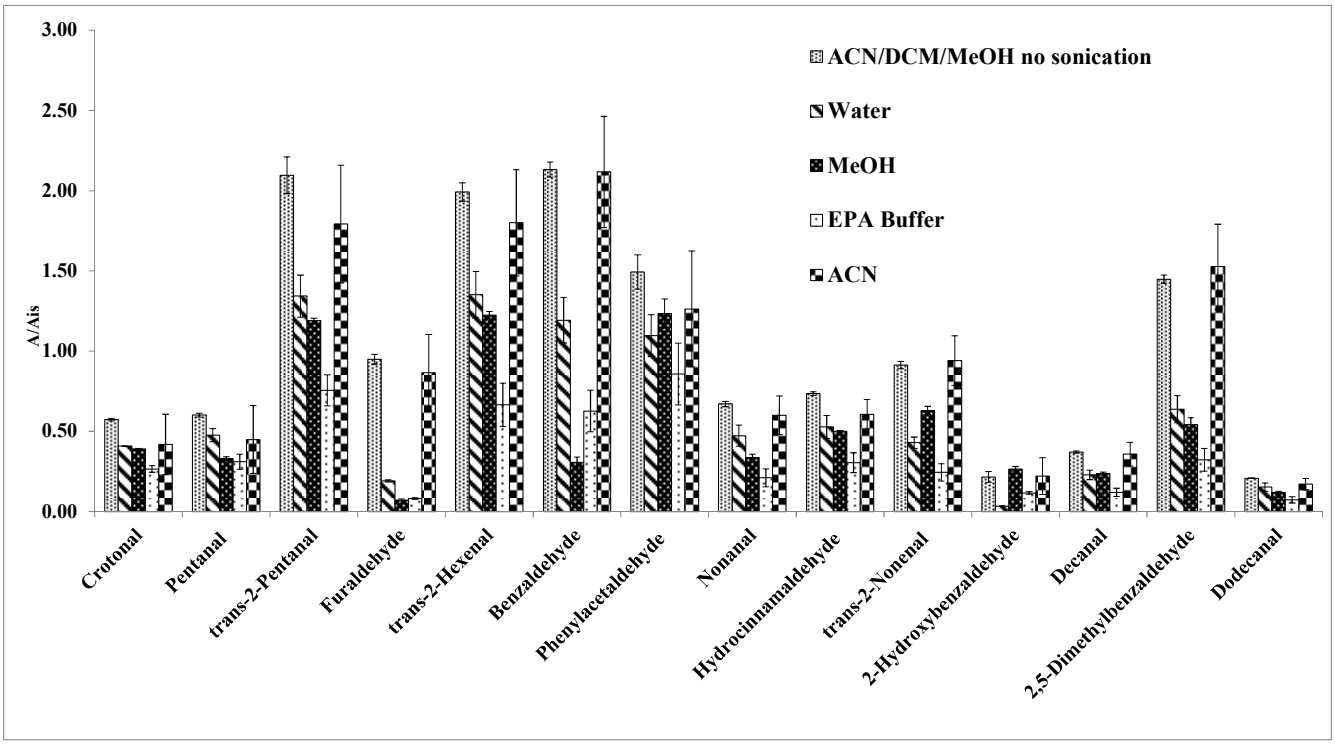


Figure 3. Comparison of PFBHA derivatization efficiencies (18 h in dark, no sonication) of aldehydes using different solvent systems reported as mean and standard deviation of triplicate experiments. The derivatization in deionized water and EPA buffer was followed by the LLE into DCM, while the systems ACN/DCM/MeOH and MeOH and ACN enabled direct analysis.

3.1.2.2. Factors Affecting Response of Derivatized Aldehydes

The lower recoveries in MeOH, EPA Buffer and ACN (Fig. 3) may be a result of either incomplete derivatization or extra purification step (compared to ACN/DCM/MeOH). The verification of completeness of derivatization was performed on the selected set of analytes (listed in Table 5) in the concentration range enabling to detect 10 times of LODs of underivatized aldehyde. The three different solvent systems, MeOH, ACN, and ACN/DCM/MeOH were employed, and the derivatization was followed by the direct analysis, eliminating the extra

purification step. With the exception of 9-fluorenone, which was observed only below LOD, no underivatized aldehydes were observed.

This experiment enabled a comparison of the response in different solvent systems under sonication. Similarly as for derivatization without sonication, ACN/DCM/MeOH and ACN provided similar responses to each other and higher than that of MeOH (Fig. 4). The lower responses (areas) of derivatized aldehydes and the internal standard in MeOH (PT) and ACN suggested that the response is affected by the solvent in which the analytes are analyzed and not due to the completeness/incompleteness of derivatization. To confirm this assumption, the derivatized aldehydes were evaporated to dryness, redissolved in two different solvents, DCM and ACN, and analyzed on GC-EI-MS. This was confirmed by the high responses for the target analytes dissolved in DCM and ACN, respectively (Figure 5). This comparison confirms that differences in responses of derivatized aldehydes were due to the solvent system used for analysis.

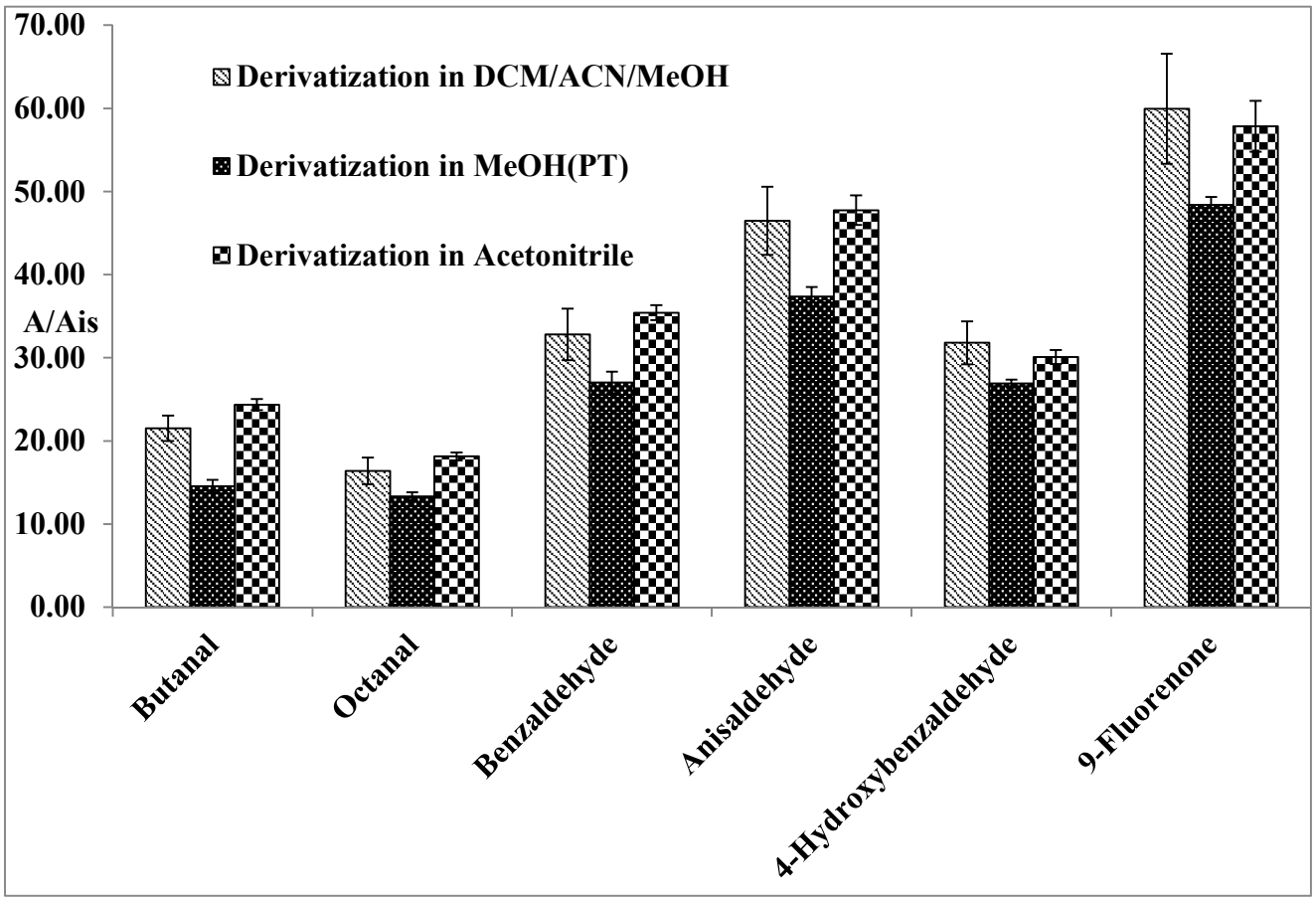


Figure 4. Derivatization /sonication of selected aldehydes in various solvent systems with concentrations 10 times the LOD of underivatized aldehydes

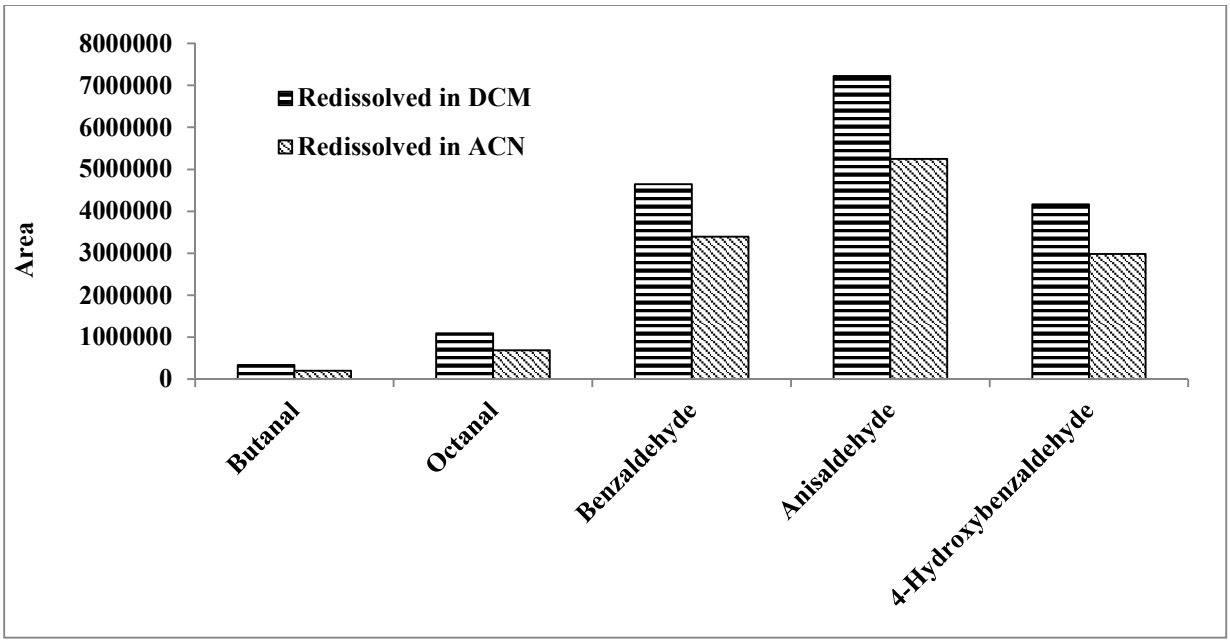


Figure 5. Responses of derivatized aldehydes redissolved (after evaporation to dryness) in two solvent systems: DCM and ACN.

3.1.2.3. Impact of Sonication in ACN/DCM/MeOH

The extraction protocols for PM characterization typically employ sonication or shaking. Often this part of the protocol is used simultaneously with derivatization. Thus the effect of sonication on the derivatization was also tested. The results indicate that the derivatization was slightly improved for linear aldehydes upon sonication overnight (Figure 6). No statistically significant difference was observed for derivatization with or without sonication for the other aldehydes (Figure 6). However, since the sonication process is associated with the generation of heat, it is also possible that the derivatization of aldehydes was enhanced by temperature rather than sonication.

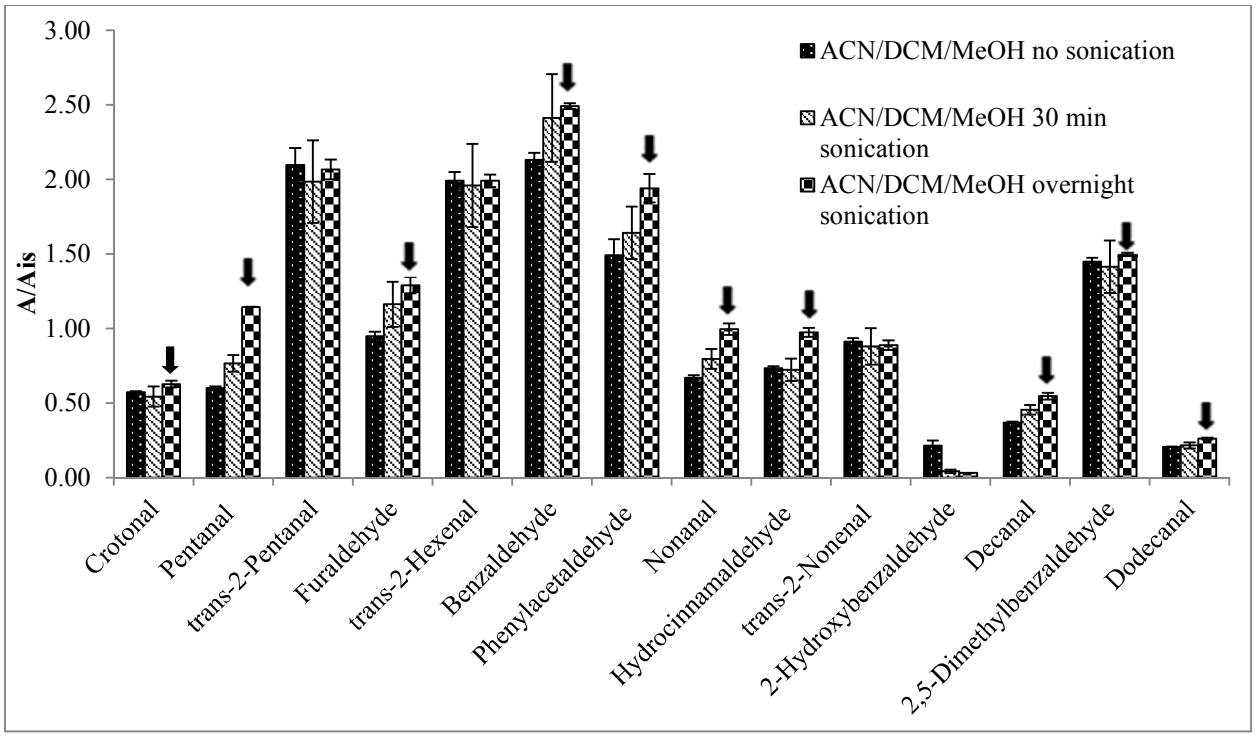


Figure 6. Effect of sonication on derivatization of aldehydes with ACN/DCM/MeOH (1:8.5:0.5 v/v/v). The arrow denotes statistically significant difference between no sonication and overnight sonication (t-test at 95 % confidence level).

In summary, ACN/DCM/MeOH was the optimal solvent system for derivatization/extraction of PFBHA derivatives of aldehydes and enhanced further with the sonication (or possibly by temperature).

3.1.2.4.Effect of Active Sites of Glassware on Recoveries of Derivatized Aldehydes

To ensure consistent recoveries, the possible impact of the active surface of glassware was evaluated. To investigate this effect, an experiment was conducted to compare the derivatization of selected aldehydes in silanized and non-silanized glassware. Recoveries of selected aldehydes hexanal, nonanal, anisaldehyde and 4-hydroxybenzaldehyde were tested in silanized and non-silanized glassware employing ACN/DCM/MeOH as the solvent system.

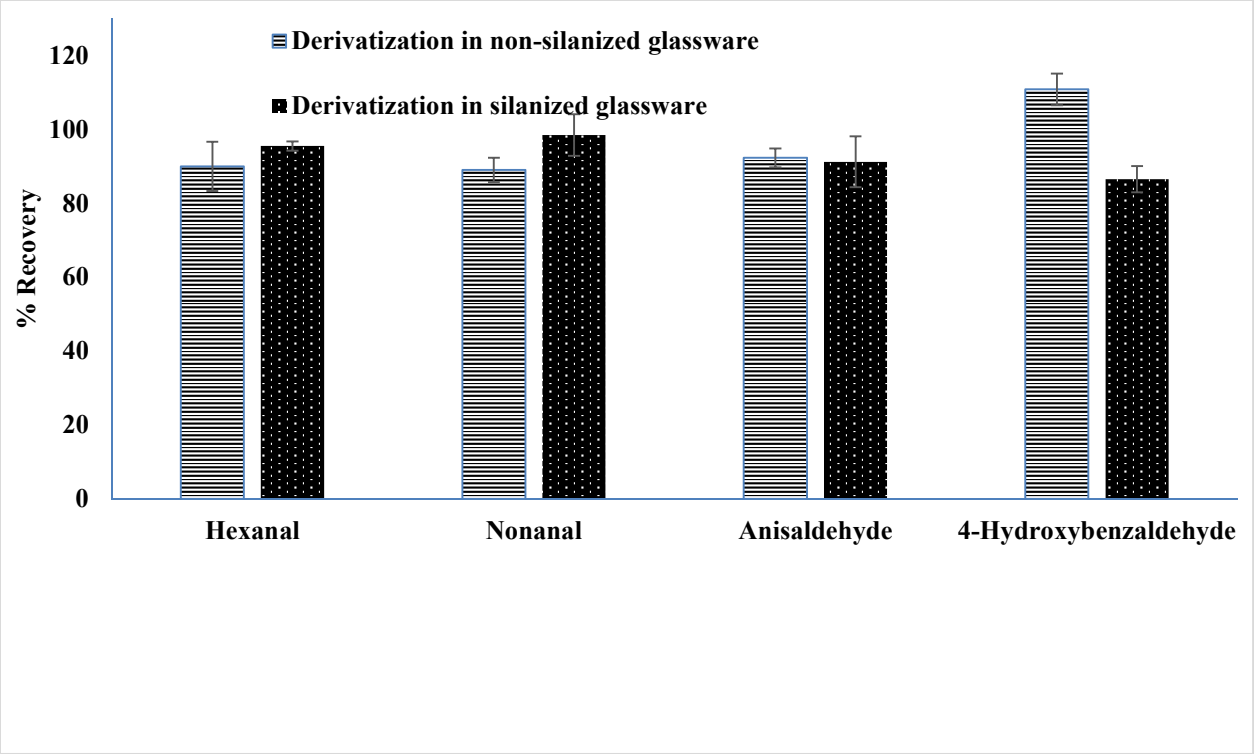


Figure 7. % Recoveries of aldehydes derivatized in non-silanized and silanized glassware

No significant difference was observed in the recoveries of aldehydes in silanized and non-silanized glassware (Figure 7). Thus, the glassware appears to cause no effect on aldehyde derivatization.

3.2. Derivatization of Oxy-PAHs

Previously the PFBHA derivatization of quinones and carboxaldehydes has been achieved using various solvent systems including ACN/DCM and ACN/DCM/MeOH.[17,20,21] No attempts were made to optimize the conditions to achieve complete derivatization of quinonic carbonyls. To gain further insight into the derivatization of carbonyls in the optimized solvent system, the derivatization was performed on a variety of oxy-PAHs (listed in Table 4). Derivatization of

selected oxy-PAHs was compared in two different solvent systems, ACN/DCM/MeOH and MeOH, with and without sonication overnight in the dark (Table 6).

Sonication of the solvent system during derivatization improved the derivatization efficiency. Compounds such as pyrene-4,5-dione, methyl-1,4-benzoquinone, 1-pyrene carboxaldehyde, perinaphthenone, dicinnamalacetone, 1,3-indandione, 1-indanone, biphenyl-4-carboxaldehyde have shown partial or complete increase in derivatization efficiency with sonication overnight at room temperature. One possible reason for the improved derivatization could be an increase of temperature during sonication.

The solvent system used for the derivatization makes a considerable impact on derivatization efficiency. Most of the compounds showed a considerably improved derivatization in MeOH with sonication and adding ACN/DCM after derivatization in comparison to derivatization in ACN/DCM/MeOH solvent system with sonication (Table 6).

Compounds with a carbonyl group not attached to the aromatic ring such as acetophenone, 1-naphthaldehyde, and phenanthrene-9-carboxaldehyde were completely derivatized under all conditions (Fig. 8 shows the chromatograms and mass spectra of representative compounds. acetophenone is shown in App. Fig 1). Some of Oxy-PAHs such as pyrene-4,5-dione (App. Fig 2) and methyl -1,4-benzoquinone (App. Fig 3) showed incomplete derivatization in ACN/DCM/MeOH but were completely derivatized in the same solvent system under sonication. 1-indanone (App. Fig 4), 9-fluorenone showed incomplete derivatization in ACN/DCM/MeOH, and required the change of solvent system to MeOH for complete derivatization under sonication. The example chromatograms showing derivatization of 9-fluorenone in two solvent systems are given in Figure 9. Other compounds such as bianthrone (App. Fig 5) and perinaphtheneone (App. Fig 6) were not derivatized at all in ACN/DCM/MeOH, but were completely derivatized in MeOH

under sonication. Four compounds including anthraquinone, 2-methyl-anthraquinone, xanthone and anthrone (App. Fig 7-10) were only partially derivatized and only in MeOH. Only pentacenequine could not be derivatized at all. This is possibly due to sterically hindered carbonyl group in pentacenequinone. The results indicate that sonication and MeOH solvent conditions enhance the derivatization efficiency of oxy-PAHs. However, some compounds are still incompletely derivatized thus caution must be applied when quantifying these compounds.

Table 6. List of oxy-PAHs and their efficiency of PFBHA (10 µg/mL) derivatization in different solvent systems ACN/DCM/MeOH and MeOH and sonication conditions.

Oxy-PAH	M. Wt	M.Wt(mono oxime)	M.Wt (dioxime)	EI ions (monooxime)	EI ions (dioxime)	PFBHA Derivatization without sonication	PFBHA Derivatization with sonication	
						ACN/DCM/MeOH	ACN/DCM/MeOH	MeOH
Pyrene 4,5 dione	232.24	427	622	427, 281, 246		Not derivatized	Complete	ND[a]
Methyl -1,4-benzoquinone	122.12	317	512	181, 317	512, 331, 181	Incomplete	Complete	ND
1-Pyrene carboxaldehyde	230.26	425		425, 224, 227, 181		Incomplete	Complete	Complete
9-Fluorenone	180.21	375		375, 345		Incomplete	Incomplete	Complete
Anthraquinone	208.21	403	598	403, 222, 181	598, 417, 181	Not derivatized	Not derivatized	Incomplete
2-Methyl-anthraquinone	222.24	417	612	417, 236, 181	612, 431, 181	Not derivatized	Not derivatized	Incomplete
Xanthone	196.19	391		391, 181		Not derivatized	Not derivatized	Incomplete
Pentacenequinone	308.33	503		-		Not derivatized	Not derivatized	Not derivatized
Bianthrone	384.43	579	774	549, 475, 181		Not derivatized	Not derivatized	Complete
Perinaphthenone	180.2	375		375, 281, 181		Not derivatized	Incomplete	Complete
Dicinnamalacetone	286.37	481		481, 404, 181		Incomplete	Complete	Complete
Menadione	172.18	367	562	367, 181		Incomplete	Incomplete	Complete
1,3-Indandione	146.14	341	536	341, 324, 181		Incomplete	Complete	ND
Anthrone	194.23	389		389, 181		Not derivatized	Not derivatized	Incomplete
1,4-Benzoquinone	108.1	303	498	498, 317, 181		Complete	Complete	Complete
1,4-Naphthaquinone	158.15	353	548	353, 181		Incomplete	Incomplete	Complete
1-Indanone	132.16	327		327, 181		Incomplete	Complete	Complete
Benzophenone	182.22	377		377, 196, 181		Incomplete	Incomplete	Complete
3-Methoxy-acetophenone	150.12	345		345, 328, 181		Incomplete	Complete	Complete
Biphenyl-4-carboxaldehyde	182.22	377		377, 334, 196, 181		Incomplete	Complete	Complete
Acetophenone	120.15	315		181, 315, 298		Complete	Complete	Complete

Table 6 contd.

3-Methyl-2-cyclohexen-1-one	110.15	305		181, 305, 288, 275	Complete	Complete	Complete
1-Naphthalaldehyde	156.18	351		181, 351, 334, 308	Complete	Complete	Complete
Phenoxy-2-propanone	150.18	345		181, 345, 252	Complete	Complete	Complete
Phenanthrene-9-carboxaldehyde	206.24	401		181, 401, 358, 203	Complete	Complete	Complete
Anthracene-9-carboxaldehyde	206.24	401		401, 220, 203, 181	Complete	Complete	Complete
1,2-Naphthaquinone	158.15	353	548	353, 336, 181	Complete	Complete	Complete

[a]ND denotes not detected

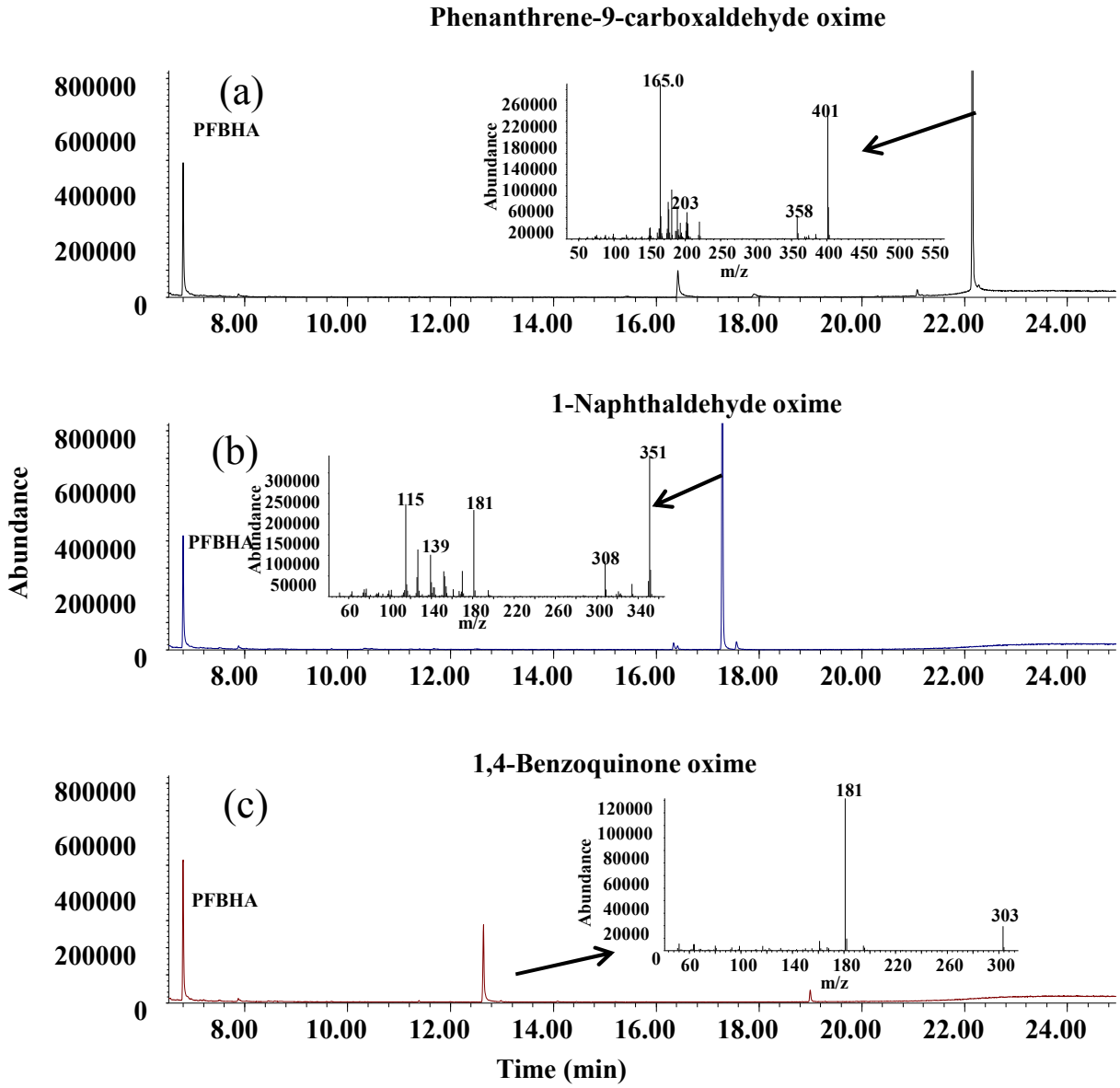


Figure 8. GC-EI-MS (TIC) chromatograms showing completely derivatized a) phenanthrene-9-carboxaldehyde, 1-naphthaldehyde, and 1,4-benzoquinone in ACN/DCM/MeOH

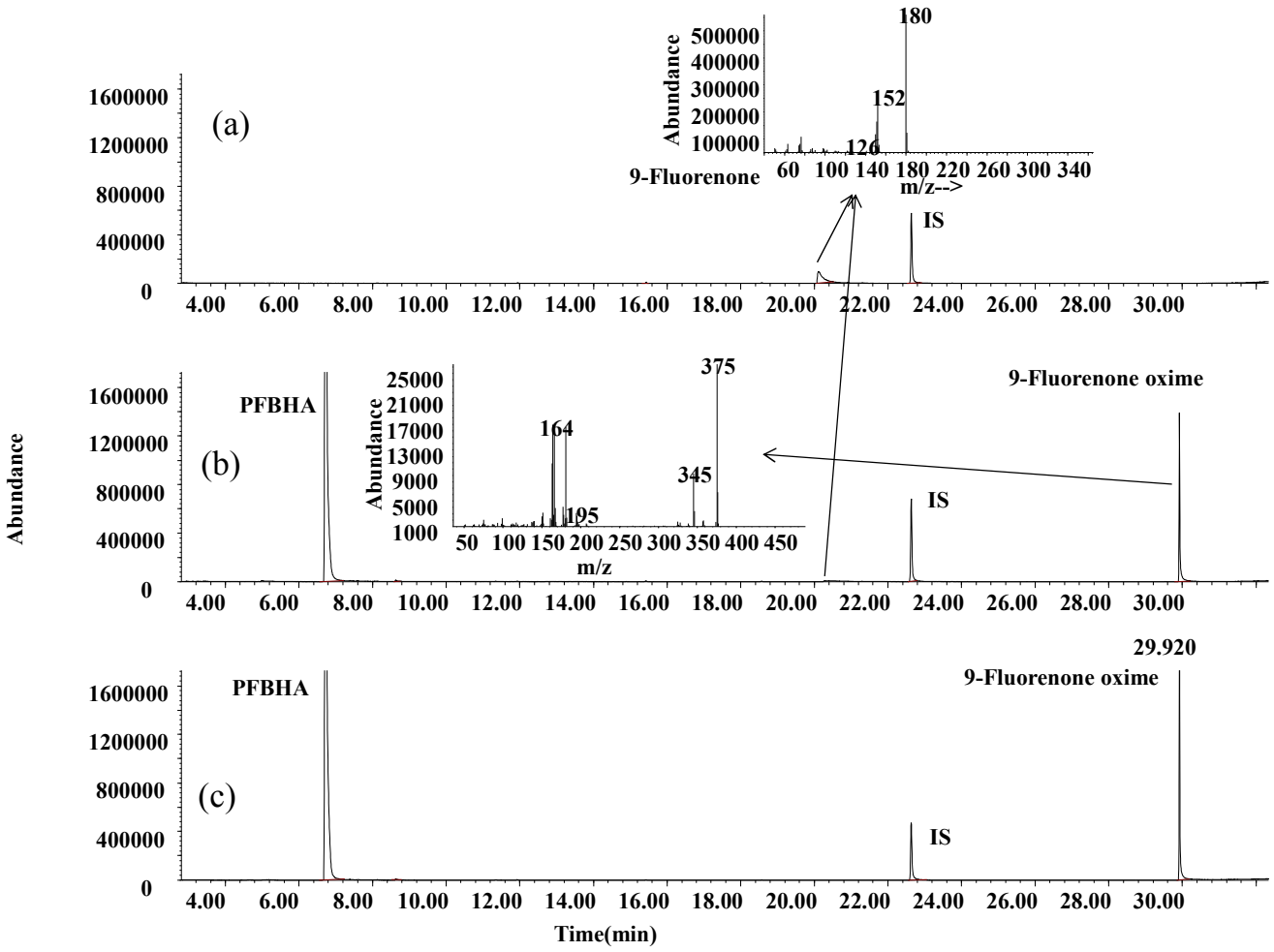


Figure 9. GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of 9-fluorenone a) underivatized 9- fluorenone, b) partial derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.

3.3. PM Matrix Effect on Recoveries of Isotopically Labelled Aldehydes from PM

A mixture of isotopically labelled (deuterated) aldehydes (used later on as RS) was employed to investigate the extraction efficiency and impacts of solvent and matrix on recoveries from PM. First, the recoveries of deuterated aldehydes from PM were evaluated in two common solvent systems, ACN/DCM/MeOH under sonication and EPA buffer under rotary shaking. This comparison showed a significant impact of the PM matrix on the RS. As expected for controls (no

PM) the recoveries of all deuterated species were ~ 100%. However, the derivatization of aldehydes in presence of PM was selectively affected by both solvents system. For a less polar organic solvent system (ACN/DCM/MeOH), we observed preconcentration of the deuterated aldehydes of higher molecular weight. This effect was not observed for water where increased recoveries were observed only for butanal-d_2. The mean recovery values (n = 3) from controls and from wood smoke PM using ACN/DCM/MeOH and EPA buffer are shown in Figures 10a and 10b, respectively. It was observed that the recoveries of butanal-d_2, furaldehyde-d_4, octanal-d_{16}, benzaldehyde-d_6, *p*-anisaldehyde-d_3 and 4-hydroxybenzaldehyde-d_4 were below 100 % in controls whereas they had highly enhanced recoveries (close to 150 %) in wood smoke PM samples. Especially, 4-hydroxybenzaldehyde which was not detected in controls showed an abnormally high recovery in presence of wood smoke PM. This behavior is comparable in both ACN/DCM/MeOH and EPA buffer solvent systems.

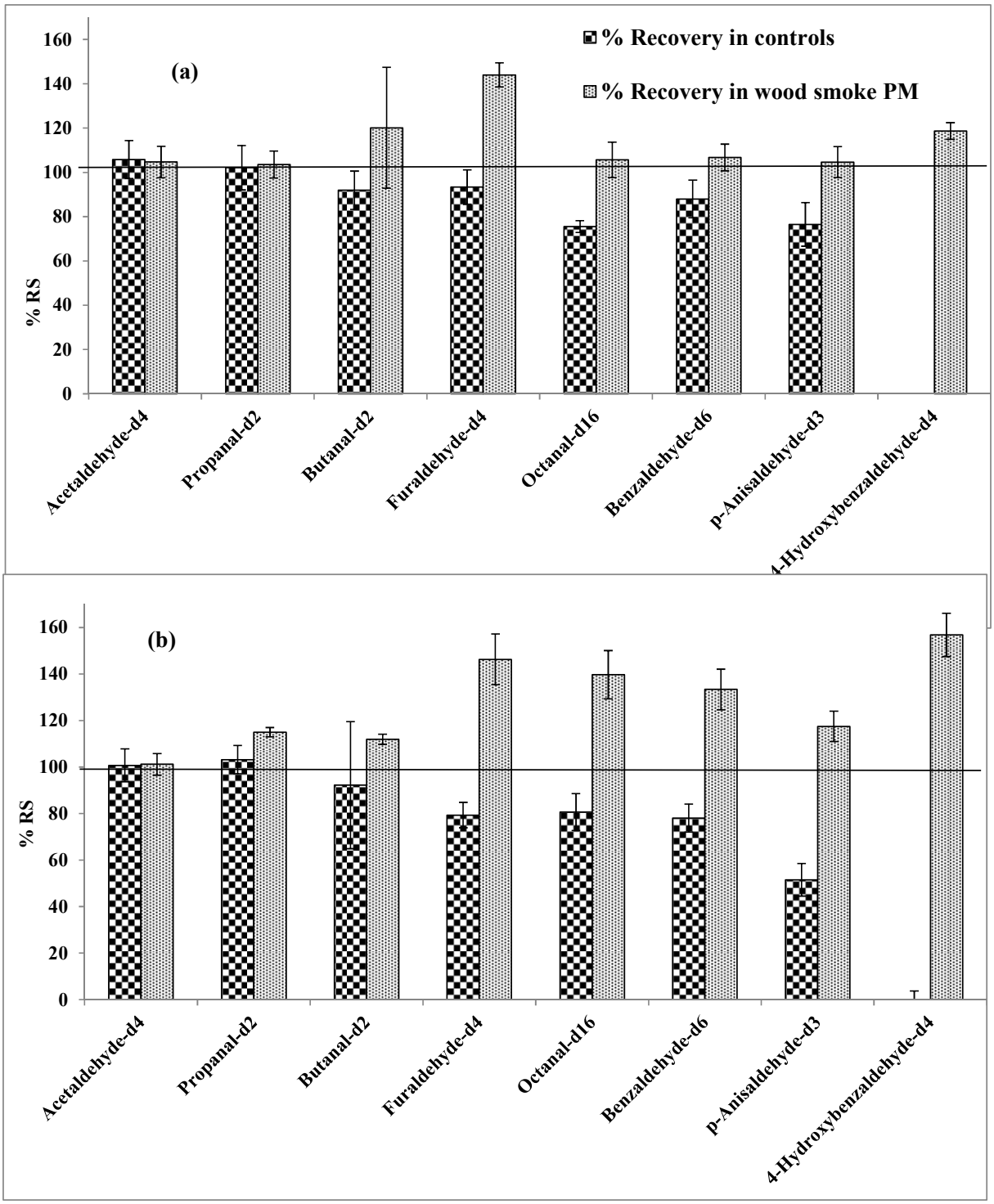


Figure 10. % Recoveries of RS (deuterated aldehydes) from controls (no PM) and wood smoke PM using a) ACN/DCM/MeOH (1:8.5:0.5 v/v/v) under sonication and b) EPA buffer under rotary shaking[28] reported as mean and one standard deviation of triplicate experiments.

Overall, the calculated recoveries of analytes in wood smoke samples are far above 100% especially for octanal, benzaldehyde, anisaldehyde, hydroxybenzaldehyde and furaldehyde.

The results suggest that the matrix has a strong impact on recoveries of aldehydes, which results in an overestimation of aldehydes concentrations in PM if RSs are not used. Therefore these RSs should be used to correct for the extraction/derivatization efficiency. It is important to note that previously reported studies did not use or were limited to 1-2 recovery standards.[19,20] Still the excessively high recoveries of RS raise a concern and require explanation to prevent analytical artifacts. Thus possible reasons for enhanced analyte recoveries were investigated in the following sections (3.3.1 – 3.3.4). This has been achieved by studying various factors affecting the recoveries of aldehydes in PM. In the sections above we have addressed:

- Completeness of derivatization (investigated in 3.1.2.2)
- Use of silanized glassware (investigated in section 3.1.2.4)

The results suggest that glassware did not affecte the results. Similarly we have shown that the derivatization under the conditions employed should be complete. Thus the next step was to evaluate the matrix effects including PM matrix i.e., particles (3.3.1), co-extracted organic matter (3.3.2), and coextracted individual compounds (3.3.3).

3.3.1. Effect of Particles on Recoveries of Derivatized Aldehydes

The effect of wood smoke particles on derivatization of aldehydes may be another reason for the response enhancement of derivatized aldehydes in PM. The assumption is that the PM particles might be acting as a catalyst for the aldehyde-PFBHA reaction further improving

derivatization. Figure 11 shows the effect of different types of particles on responses of derivatized aldehydes. The results indicate that the responses are significantly higher in presence of PM particles and the carbon (graphite) particles showed a similar trend although not for all the tested aldehydes. By contrast, silica particles did not cause any significant change in responses of derivatized aldehydes. This observation suggests that the response enhancement is not due to the catalytic behavior of PM particles.

The PFBHA derivatized samples are considered to be stable for long time.[30] To verify the stability of derivatized species and further clarify the sources of the observed artifact, we studied the impact of time following the sample derivatization after 24 h, 3 days, 9 days, and 18 days. In this study, samples were left in the derivatization media (as is typical for the derivatization process) taking a fresh aliquot from the sample containing particles (Fig. 12). The responses of linear aldehydes slightly decreased whereas the responses of other aldehydes, e.g., furaldehyde-d_4, benzaldehyde-d_{16}, *p*-anisaldehyde-d_3 and 4-hydroxybenzaldehyde-d_4, have slightly increased over the studied time period. These trends were significant, particularly when comparing the immediate analysis with the analysis after 18 days. This difference suggests that the effect of PM on responses of derivatized aldehydes is kinetic.

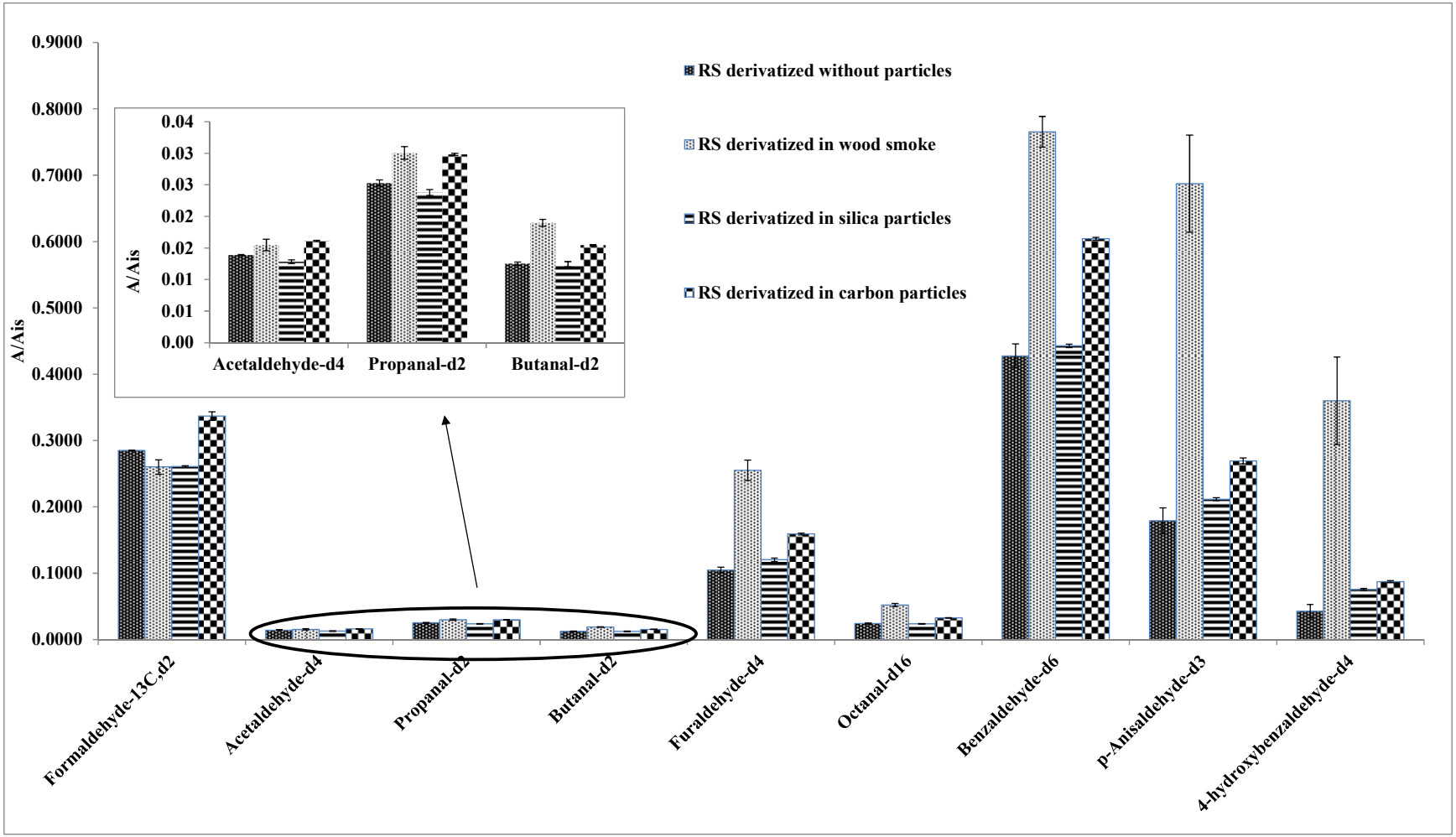


Figure 11. Comparison of effect of particles on derivatization of RS (deuterated aldehydes) derivatized in ACN/DCM/MeOH (1:8.5:0.5 v/v/v) under sonication reported as mean and one standard deviation of triplicate experiments

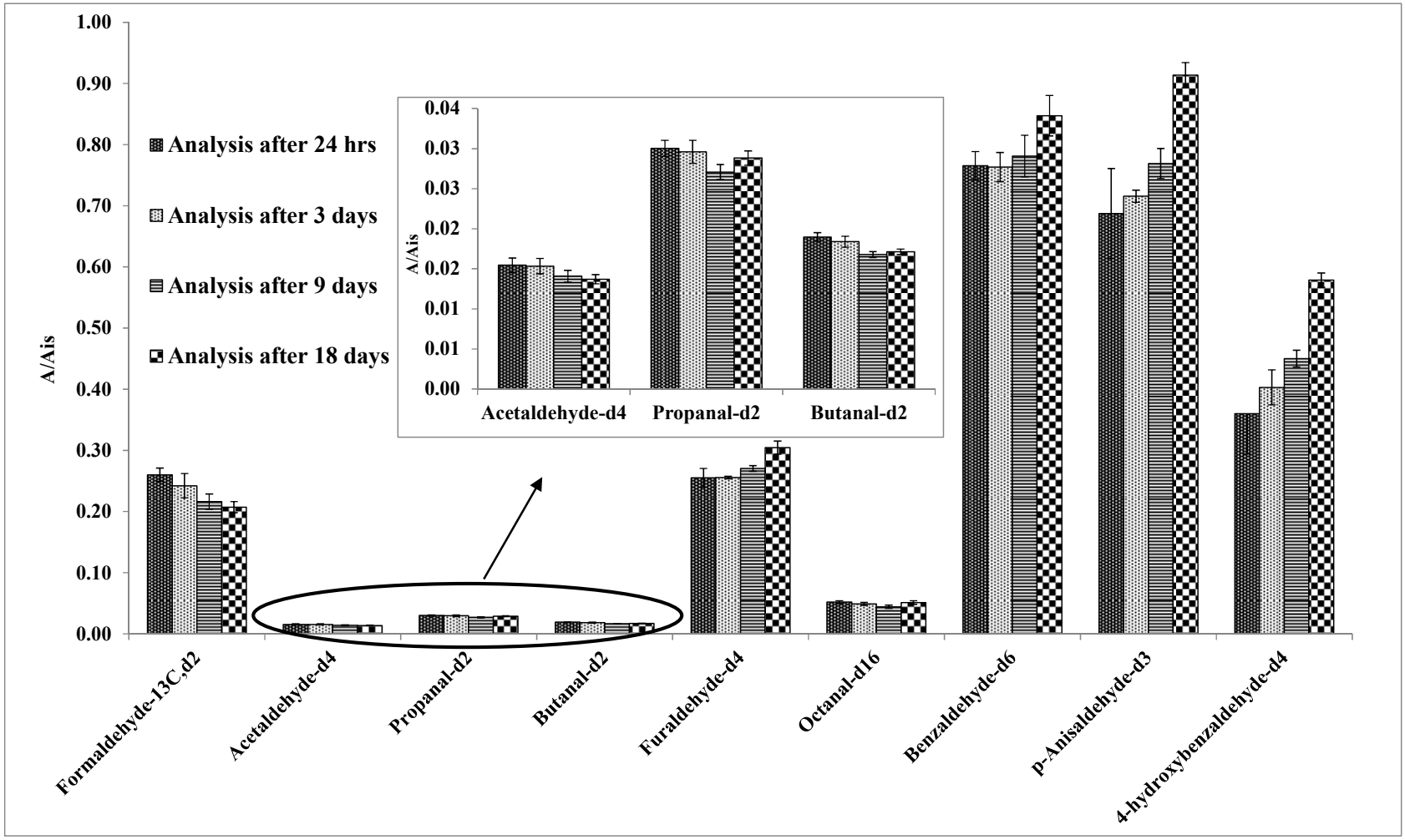


Figure 12. GC-MS analysis of RS derivatized in DCM/ACN/MeOH (1:8.5:0.5 v/v/v) under sonication in presence of wood smoke particles analyzed at different time periods following the sample preparation (derivatization) reported as mean and one standard deviation of triplicate experiments

3.3.2. Effect of Co-Extracted Organic Matter from PM on Responses of Aldehydes

The experiment in presence of various particles showed that the physical presence of particles such as silica (as catalysts) and carbon particles does not fully address the trends in recoveries observed with wood smoke PM. This observation suggests that the chemistry of particles (e.g., organic matter) may be the reason for higher responses of aldehydes. To test this hypothesis, we evaluated the impact of extracted organic matter PM, and residue after extraction and unextracted PM on responses of derivatized aldehydes (Fig. 13). The results confirm the hypothesis showing comparable responses of aldehydes derivatized in extracted organic matter to those in presence of PM. On the other hand, the responses were significantly lower in presence of residual PM particles (extracted) without organic matter. These results indicate that the response enhancement of aldehydes is due to the co-extracted organic species in the PM according the effect of major species known to be present in WS PM was investigated in the following section.

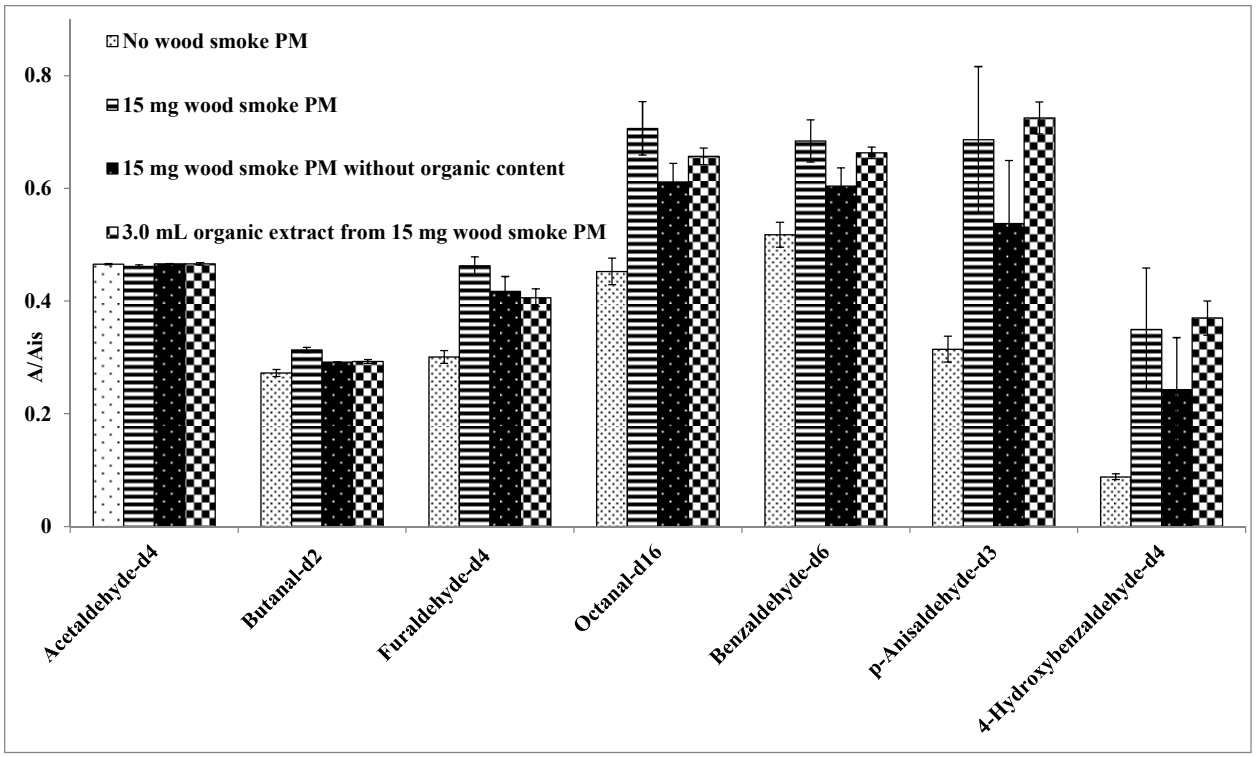


Figure 13. Comparison of recoveries of derivatized aldehydes (RS) derivatized in DCM/ACN/MeOH (1:8.5:0.5 v/v/v) under sonication upon derivatization in presence of wood smoke PM, wood smoke PM without organic content, and organic extract from wood smoke PM.

3.3.3. Effect of Co-extracted Species on Recoveries of Derivatized Aldehydes

We have demonstrated that organic extract matter contributes to the enhanced recoveries of aldehydes. Another possible reason for increased responses of derivatized labeled aldehydes could be from the co-extracted aldehydes present in higher concentrations in PM. In our previous experiments it was observed that the PM has higher abundance of syringaldehyde compared to other aldehydes. In order to verify the effect of syringaldehyde on responses of derivatized aldehydes, a mixture of aldehydes resembling the recovery standards was derivatized in ACN/DCM/MeOH with/without syringaldehyde testing also impact of sonication. Figure 14 shows that the responses of derivatized aldehydes were comparable in samples with and without

syringaldehyde. This result indicates that the presence of co-extracted aldehydes in PM in higher concentrations does not have any effect on responses of derivatized aldehydes.

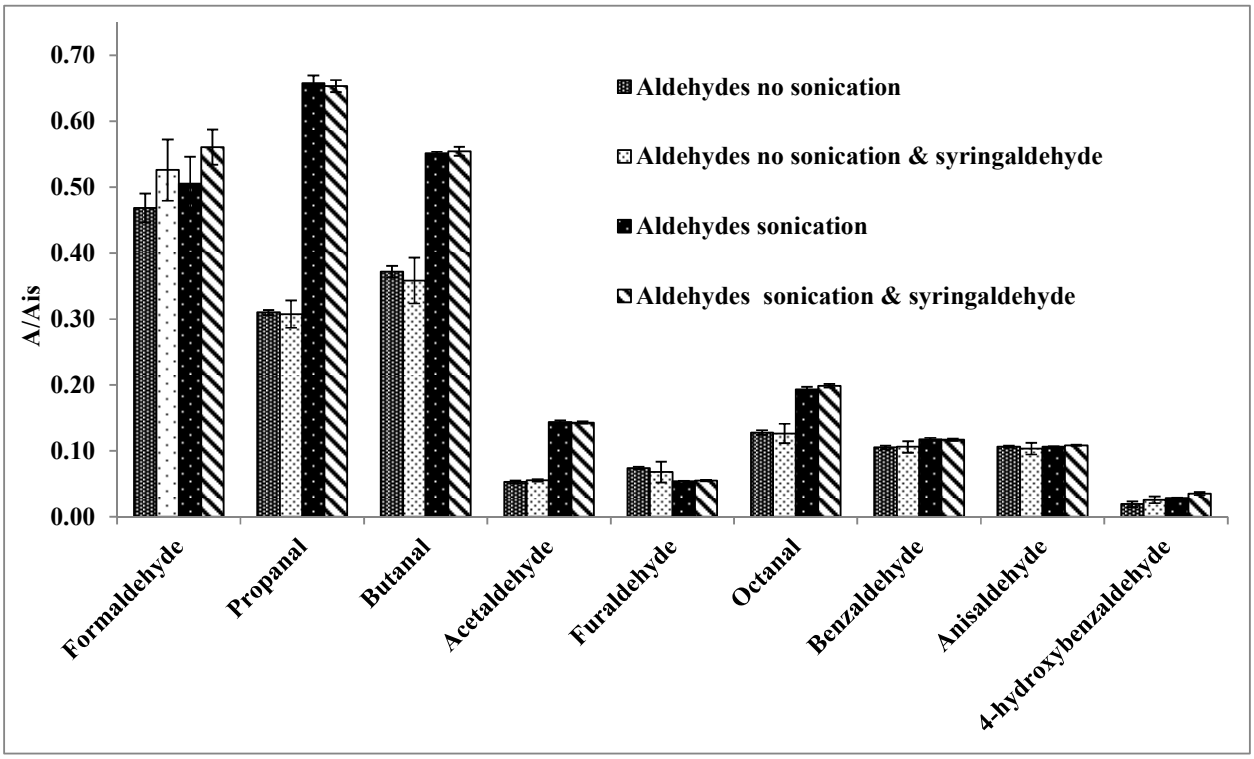


Figure 14. Comparison of responses of derivatized aldehydes (resembling RS) in presence of syringaldehyde derivatized in DCM/ACN/MeOH (1:8.5:0.5 v/v/v) under sonication

Since levoglucosan and syringol have higher abundance in PM, we attempted to test the effect of these organics on responses of derivatized aldehydes. Syringol was taken as a representative of phenolic compounds in PM. Derivatization of aldehydes (RS) was done in presence of syringol and levoglucosan with two different concentrations (100 μg and 1 mg) each. The derivatized aldehydes were analyzed on GC-EI-MS within 24 h of preparation. Figure 15 shows the effect of syringol and levoglucosan on responses of derivatized aldehydes (RS). It was observed that syringol and levoglucosan enhanced the responses of hydroxylated aldehydes, i.e.,

furaldehyde-d_4, benzaldehyde-d_6, p-anisaldehyde-d_3 and 4-hydroxybenzaldehyde-d_4. Also, the responses increased with increasing concentration of syringol and levoglucosan. These results confirm that the response enhancement is at least partially caused by the organics (especially levoglucosan and syringol) present in the PM.

One possible explanation for the higher responses of PFBHA-aldehydes could be matrix induced response enhancement due to a splitless injection.[40,41] This was first observed in the analysis of pesticides and polyflurorinated alkyl substance using splitless injection in gas chromatography-mass spectrometry.[40] Briefly, the problem consists of the presence of various active sites in the injection port (liner) that can be responsible for irreversible adsorption and/or catalytic decomposition of analytes.[38] Molecules of impurities from the matrix (*e.g.,* syringol and levoglucosan), which are most abundant in trace analysis, will effectively compete with the analytes for active sites there by reducing interaction of analyte molecules with the liner. Overall, the matrix increases the transfer of analytes from a hot injector to the column by reducing the thermal stress for labile compounds and by masking the active sites in the injector liner.[40,41] These results in enhancement of responses of derivatized aldehydes, which can lead to overestimation of the measured concentration. A possible approach to eliminate this effect is using a cold on-column injection, and using matrix matched calibration standards or analyte protectants which equalize the response enhancement for calibration standards and sample extract.[41]

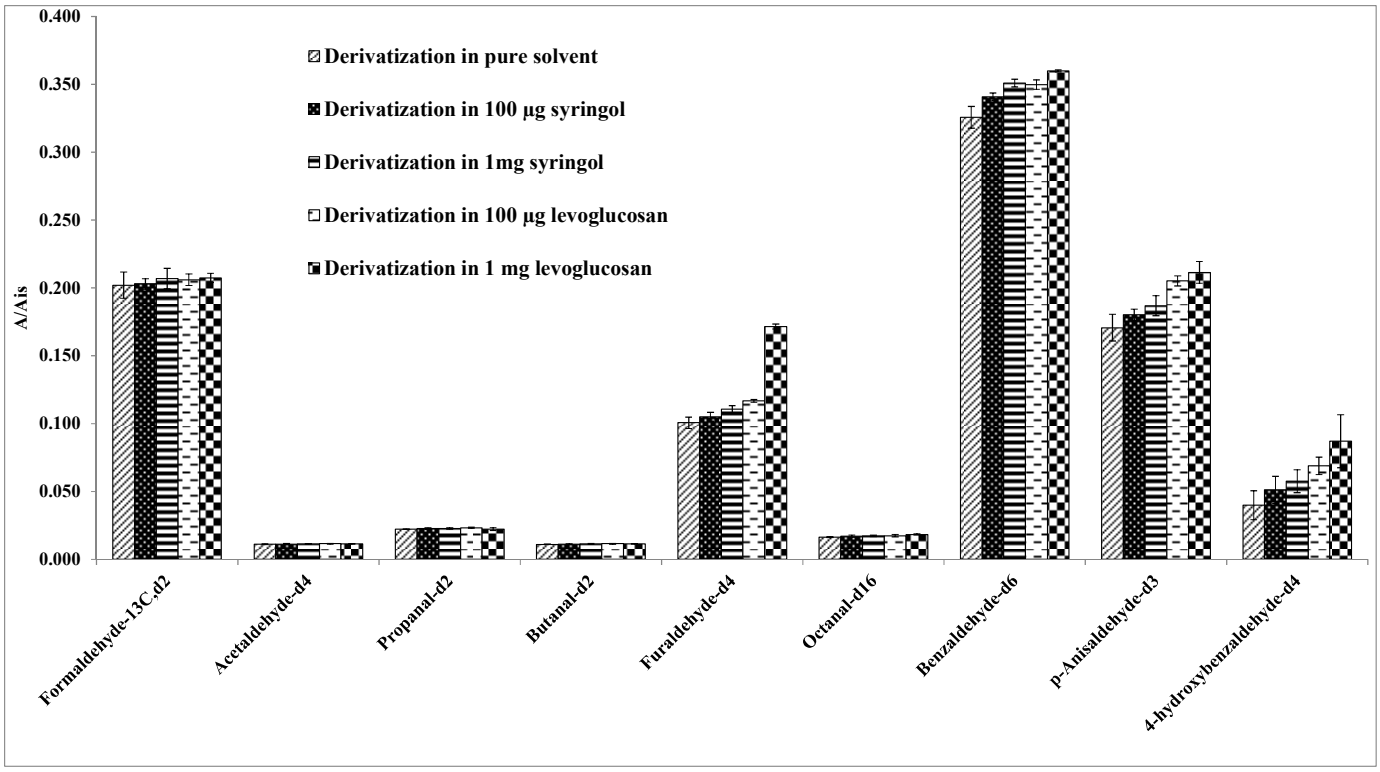


Figure 15. Comparison of responses of derivatized aldehydes (RS) in presence of syringol and levoglucosan at different concentrations under sonication

3.4. Sequential Extraction/Derivatization of aldehydes from PM

To further investigate the applicability of the optimized protocol a sequential extraction/derivatization of aldehydes from WSPM was performed using selected isotopically labelled aldehydes as recovery standards. The first step of the sequential extraction was sonication in MeOH in the presence of PFBHA ensuring derivatization of reactive carbonyls, this step was followed by the sonication with ACN/DCM/MeOH and the final step using soxhlet extraction with MeOH.

The chromatograms corresponding to the sequential extraction/derivatization of aldehydes from WSPM are shown in Fig 16. The results indicate that most of the aldehydes were extracted completely within the first extraction step using MeOH under sonication (Fig. 16 a).

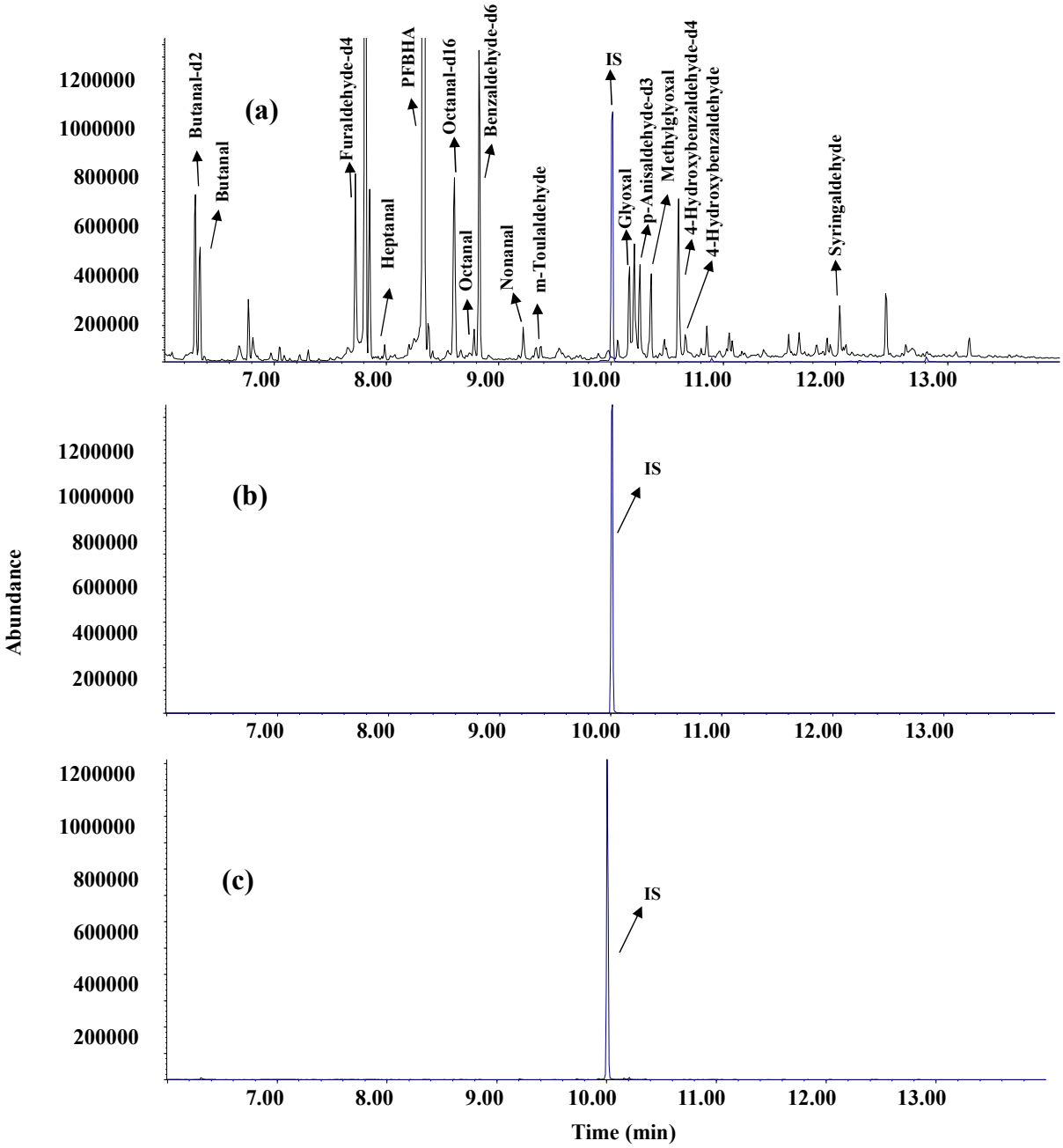


Figure 16. GC-EI-MS chromatograms showing composition of extracts obtained by sequential extraction of aldehydes from WS PM (2 mg) a) Sonication with MeOH in the presence of 2 mg WS PM under sonication b) Extraction from ACN/DCM/MeOH under sonication c) Soxhlet extraction for 18h.

3.5. Applications – Determination of Carbonyl Compounds in WS PM

The optimized method was applied to WS PM. Since the recoveries of RS seemed to be affected by the presence of extractable organics, the performance of the method was evaluated by extracting different amounts of WS PM. In order to evaluate and ensure the sensitivity of the method, the GC-MS analyses were performed in both EI and NCI modes. The calibration parameters including instrumental LODs and LLOQs obtained by a least square linear regression analysis are shown in Table 7. As expected NCI mode provided lower LODs within range of 0.02 μg – 0.12 μg in comparison to EI method with LODs within range of 0.05 μg – 0.16 μg.

The amounts of aldehydes in wood smoke PM reported as mean ± SD quantified are shown in Table 8. The results demonstrated significant concentrations of aldehydes in WS PM detected from all amounts of wood smoke PM were similar.

As expected, the repeatability of GC injection of the same sample was improved (RSD within range of 1 % – 43 %) compared to repeatability of the sample preparation (RSD within range of 2 % – 63 %). The high values of RSDs were characteristic for low concentration of aldehydes. For majority of the linear aldehydes such as propanal, pentanal, octanal and nonanal some discrepancies were identified between GC repeatability and repeatability of sample preparation. These discrepancies were attributed to interference from background.

For majority of aldehydes (highlighted in gray) similar results were obtained from both amounts of PM. However yields for some aldehydes were higher (20-100%) for extraction from 3 mg compared to 7 mg of wood smoke PM suggesting that the derivatization reagent was all consumed. Considering the molar content of PFBHA (provide value) in comparison to overall amount of aldehydes in 7 mg WS PM (provide molar value) this cannot be confirmed. However

there is a possibility that there additional carbonyls on large MW compounds within wood smoke PM which may use up the PFBHA reagent. The recoveries of RSs (Table 9) corresponded to our previous findings (shown above) suggesting that discrepancy between data is not due difference in extraction efficiencies.

Table 7: Calibration parameters of aldehydes by GC-MS in EI and NCI mode obtained by a least square linear regression analysis

	EI						NCI					
Aldehyde	**Slope**	**Intercept**	**R^2**	**Sy**	**LOD (µg)**	**LLOQ (µg)**	**Slope**	**Intercept**	**R^2**	**Sy**	**LOD (µg)**	**LLOQ (µg)**
Formaldehyde	NA	NA	NA	NA	NA	NA	0.0023	0.002300	0.5434	0.000708	1.0152	1.5382
Acetaldehyde	0.0071	0.000859	0.9543	0.000482	0.2248	0.3407	0.0009	0.000862	0.9740	0.000041	0.1562	0.2367
Propanal	0.0060	0.000114	0.9736	0.000276	0.1528	0.2314	0.0006	0.000574	0.9889	0.000009	0.0513	0.0778
Acrolein	0.0771	0.016258	0.4193	0.026374	1.1285	1.7098	0.0090	0.009010	0.9777	0.000218	0.0800	0.1212
Isobutanal	0.0118	0.000071	0.9741	0.000637	0.1781	0.2699	0.0020	0.001989	0.9926	0.000025	0.0410	0.0622
Butanal	0.0135	0.000652	0.9600	0.000818	0.1998	0.3028	0.0012	0.001230	0.9918	0.000019	0.0498	0.0754
Crotonal	0.0032	0.000038	0.9502	0.000200	0.2076	0.3145	0.0014	0.001376	0.9854	0.000018	0.0433	0.0657
Pentanal	0.0032	0.000038	0.9502	0.000200	0.2076	0.3145	0.0015	0.001529	0.9956	0.000010	0.0224	0.0339
trans-2-Pentenal	0.0085	0.000126	0.8353	0.001078	0.4208	0.6376	0.0008	0.000822	0.9898	0.000014	0.0565	0.0856
Hexanal	0.0051	-0.000008	0.9623	0.000293	0.1889	0.2862	0.0010	0.000962	0.9950	0.000005	0.0176	0.0266
Furaldehyde	0.0054	-0.000028	0.9662	0.000249	0.1510	0.2287	0.0180	0.018041	0.9798	0.000368	0.0673	0.1020
trans-2-Hexenal	0.0099	-0.000130	0.9641	0.000486	0.1626	0.2464	0.0194	0.019378	0.9914	0.000191	0.0326	0.0493
Heptanal	0.0032	-0.000011	0.9585	0.000185	0.1877	0.2843	0.0008	0.000770	0.9967	0.000004	0.0163	0.0247
Octanal	0.0051	-0.000102	0.9675	0.000281	0.1836	0.2782	0.0010	0.000970	0.9973	0.000005	0.0155	0.0234
Benzaldehyde	0.0101	-0.000241	0.9866	0.000352	0.1146	0.1737	0.0164	0.016431	0.9922	0.000245	0.0492	0.0745
Phenylacetaldehyde	0.0115	-0.000235	0.9899	0.000312	0.0893	0.1353	0.0055	0.005526	0.9926	0.000080	0.0480	0.0728
Nonanal	0.0075	-0.000107	0.9765	0.000316	0.1395	0.2114	0.0019	0.001859	0.9962	0.000007	0.0130	0.0196
m-Tolualdehyde	0.0069	-0.000265	0.9713	0.000329	0.1567	0.2374	0.0154	0.015417	0.9814	0.000298	0.0638	0.0966
Hydrocinnamaldehyde	0.0013	-0.000044	0.9756	0.000063	0.1649	0.2499	0.0014	0.001418	0.9902	0.000016	0.0370	0.0560
trans-2-Nonenal	0.0017	-0.000029	0.9837	0.000065	0.1231	0.1866	0.0008	0.000846	0.9935	0.000013	0.0520	0.0788
2-Hydroxybenzaldehyde	0.0038	-0.000138	0.9779	0.000167	0.1442	0.2184	0.0130	0.013014	0.9771	0.000307	0.0778	0.1179
Decanal	0.0035	-0.000063	0.9781	0.000152	0.1419	0.2150	0.0008	0.000850	0.9949	0.000006	0.0229	0.0347
2,5-Dimethylbenzaldehyde	0.0058	-0.000212	0.9806	0.000244	0.1400	0.2122	0.0076	0.007592	0.9948	0.000061	0.0265	0.0401
2,4-Nonadienal	0.0006	-0.000008	0.9746	0.000028	0.1605	0.2432	0.0002	0.000171	0.9904	0.000004	0.0855	0.1295
Glyoxal	0.0025	-0.000090	0.9750	0.000119	0.1562	0.2367	0.0176	0.017622	0.9846	0.000380	0.0711	0.1077

Table 7 contd.

Anisaldehyde	0.0135	-0.000859	0.9894	0.000449	0.1095	0.1659	0.0116	0.011580	0.9860	0.000160	0.0455	0.0689
Methylglyoxal	0.0042	-0.000253	0.9821	0.000176	0.1393	0.2111	0.0762	0.076244	0.9928	0.001215	0.0526	0.0797
4-Hydroxybenzaldehyde	0.0012	-0.000141	0.8868	0.000130	0.3492	0.5292	0.0025	0.002459	0.9693	0.000076	0.1014	0.1536
Dodecanal	0.0029	-0.000055	0.9887	0.000087	0.1002	0.1518	0.0038	0.003809	0.9801	0.000175	0.1519	0.2301
Syringaldehyde	0.0015	-0.000001	0.9479	0.000110	0.2400	0.3636	0.0010	0.001050	0.8731	0.000027	0.0835	0.1264

Table 8. Evaluation of the extraction method using different quantities of wood smoke PM reporting concentrations (µg/g) of aldehydes determined in WS PM GC-MS-NCI mode corrected for RS, the concentrations lower LOQ are reported as not detected (ND).

	Concentration of aldehydes in WS PM (µg/g)			
	3 mg		7 mg	
Aldehyde	Replicate (Mean ± SD)	GC Repeatability (Mean ± SD)	Replicate (Mean ± SD)	GC Repeatability (Mean ± SD)
Formaldehyde	62917 ± 4384	64027 ± 4690	58352 ± 2881	53409 ± 9118
Acetaldehyde	3590 ± 815	3787 ± 149	2596 ± 404	3017 ± 79
Acrolein	138 ± 46	153 ± 15	106 ± 21	116 ± 3
Propanal	526 ± 233	700 ± 9	328 ± 94	429 ± 4
Isobutanal	34 ± 8	47 ± 15	35 ± 9	33 ± 6
Butanal	201 ± 99	297 ± 11	103 ± 49	152 ± 5
Crotonal	234 ± 32	282 ± 11	67 ± 16	74 ± 8
Pentanal	28 ± 7	41 ± 14	25 ± 9	27 ± 6
Hexanal	83 ± 30	102 ± 16	65 ± 24	73 ± 12
Furaldehyde	249 ± 10	253 ± 4	230 ± 14	217 ± 4
Heptanal	32 ± 10	44 ± 4	31 ± 7	26 ± 1
Octanal	82 ± 51	145 ± 17	49 ± 24	64 ± 7
Benzaldehyde	102 ± 2	108 ± 7	97 ± 5	88 ± 4
Phenylacetaldehyde	101 ± 5	101 ± 6	124 ± 13	105 ± 4
Nonanal	326 ± 232	577 ± 20	153 ± 40	172 ± 5
m-Tolualdehyde	33 ± 3	38 ± 10	30 ± 7	29 ± 5
trans-2-Nonenal	31 ± 4	38 ± 12	33 ± 6	27 ± 7
Hydrocinnamaldehyde	188 ± 23	178 ± 16	188 ± 14	168 ± 6
2-Hydroxybenzaldehyde	271 ± 16	262 ± 10	232 ± 10	209 ± 12
Decanal	104 ± 24	146 ± 15	73 ± 9	72 ± 10
2,5-Dimethylbenzaldehyde	17 ± 3	23 ± 10	19 ± 6	17 ± 4
2,4-Nonadienal	357 ± 76	287 ± 74	367 ± 43	344 ± 10
Glyoxal	556 ± 69	480 ± 20	366 ± 46	316 ± 6
Anisaldehyde	45 ± 4	50 ± 6	24 ± 3	23 ± 2
Methylglyoxal	347 ± 34	324 ± 1	172 ± 19	149 ± 3
4-Hydroxybenzaldehyde	809 ± 68	770 ± 4	635 ± 29	603 ± 3
Dodecanal	286 ± 50	264 ± 32	275 ± 49	230 ± 13
Syringaldehyde	2810 ± 61	3079 ± 229	2721 ± 43	2769 ± 32

[a]NR not reported [b]ND not detected, Aldehydes not reported shown in Table 3 are those which were below LLOQ.

Table 9. % Recoveries of RS from different quantities of wood smoke PM in GC-MS-NCI mode

	% Recovery of RS			
	3 mg		7 mg	
Aldehyde	**Replicate (Mean ± SD)**	**GC Repeatablity (Mean ± SD)**	**Replicate (Mean ± SD)**	**GC Repeatablity (Mean ± SD)**
Formaldehyde-$^{13}C,d_2$	21 ± 3	20 ± 2	17 ± 1	13 ± 2
Acetaldehyde-d_4	61 ± 17	41 ± 3	82 ± 32	49 ± 3
Propanal-d_2	48 ± 14	34 ± 2	62 ± 21	38 ± 1
Butanal-d_2	79 ± 16	60 ± 4	90 ± 23	68 ± 4
Furaldehyde-d_4	126 ± 7	110 ± 10	123 ± 8	124 ± 8
Octanal-d_{16}	91 ± 21	65 ± 6	99 ± 33	75 ± 5
Benzaldehyde-d_6	121 ± 7	105 ± 10	115 ± 8	120 ± 9
p-Anisaldehyde-d_3	164 ± 13	141 ± 12	228 ± 14	233 ± 12
4-Hydroxybenzaldehyde-d_4	208 ± 16	178 ± 14	201 ± 10	201 ± 5

3.6. Conclusions

Following the selection of solvent purity, the derivatization efficiency of a variety of aldehydes was tested in different solvent systems. Employing the ACN/DCM/MeOH mixture increased the derivatization efficiency. Application of sonication to the derivatization protocol slightly increased the derivatization of n-aliphatic aldehydes. On the other hand, the derivatization of oxy-PAHs using the same solvent system resulted in complete derivatization of carboxaldehydes but only incomplete derivatization of quinonic species. Further investigation of conditions is required in order to achieve complete derivatization of oxy-PAH. The solvent system employed for the derivatization caused higher than expected responses of aldehydes which were not due to completeness/incompleteness of the derivatization. The developed protocol was applied for the extraction of aldehydes from PM and compared to the traditional method using the EPA buffer. The recoveries of isotope labeled aldehydes (RS) in wood smoke were higher than 100%

(up to 150%). A possible reasons for the high recoveries is that organics like syringol and levoglucosan, which are present in PM at higher concentrations, are causing the higher recoveries of derivatized aldehydes. This has been attributed to the matrix-induced response enhancement due to the splitless injection in the analysis. Application of the optimized method to particulate matter in EI and NCI methods resulted in identification up to 28 compounds. NCI mode was more sensitive providing LODs within range of 0.02 μg – 0.12 μg. in comparison to EI method with LODs within range of 0.05 μg – 0.16 μg. 28 compound were detected at low concentration of WS PM (3 mg) in NCI method indicating the higher sensitivity of the method. The amounts of aldehydes are comparable in all concentrations of WS PM used indicating the sensitivity of the method.

4. APPENDIX

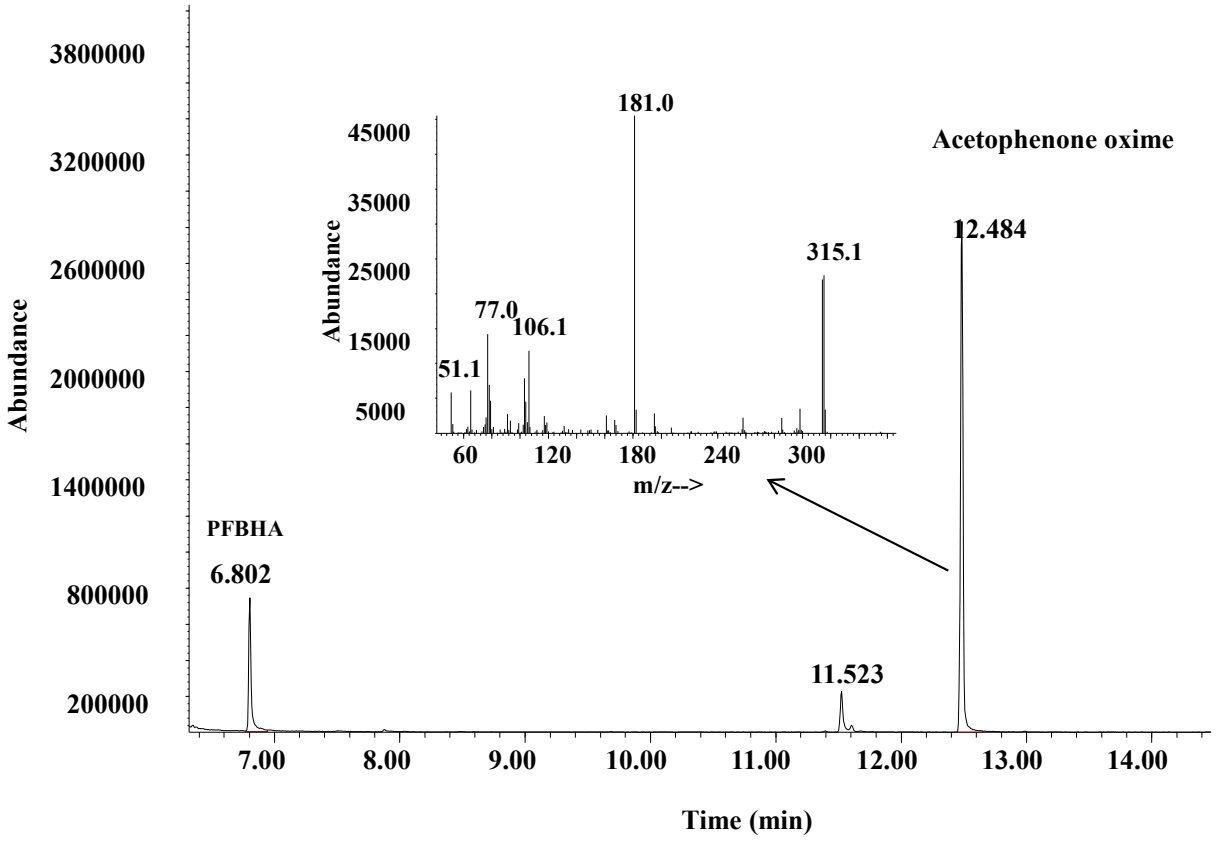
APPENDIX I: SUPPORTING INFORMATION

App. Table 1. List of targeted aldehydes and the corresponding RS used for quantification.

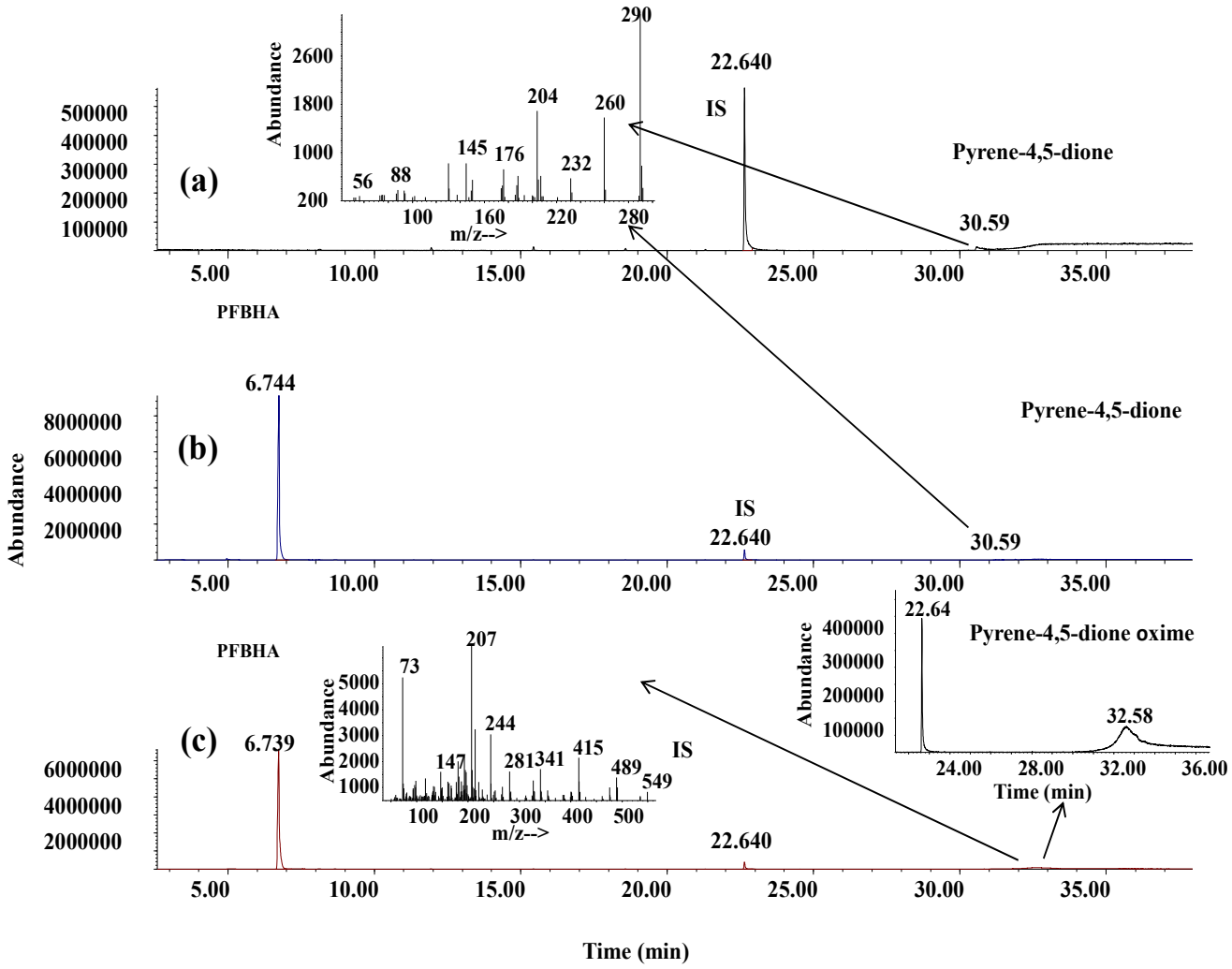
Aldehydes	RS	MW (g/mol)	MW Derivatized (g/mol)	NCI-MS		EI-MS	
				Q ion[a] (*m/z*)	Confirmation Ions (*m/z*)	Q Ion (m/z)	Confirmation Ions (m/z)
Formaldehyde	**Formaldehyde-^{13}C-d$_2$**	30	225	225	205	181	195, 225
Acetaldehyde	**Acetaldehyde-d$_4$**	44	239	239	218	181	209, 239
Propanal	**Propanal-d$_2$**	58	253	253	233	181	223, 236
Acrolein	**Propanal-d$_2$**	56	251	231	201	181	221, 251
Isobutanal	**Propanal-d$_2$**	72	267	178	247, 267	181	250
Butanal	**Butanal-d$_2$**	72	267	247	267	239	226
Crotonal	**Butanal-d$_2$**	70	265	245	215	181	195, 250
Pentanal	**Butanal-d$_2$**	86	281	178	261, 231	181	207, 239
Hexanal	**Butanal-d$_2$**	100	295	178	248, 275	181	239, 295
Furaldehyde	**Furaldehyde-d$_4$**	96	271	241	271	291	248
***trans*-2-Hexenal**	**Furaldehyde-d$_4$**	98	293	273	243	181	250, 293
Heptanal	**Furaldehyde-d$_4$**	114	309	178	289, 262	181	207, 239
Octanal	**Octanal-d$_{16}$**	128	323	178	276, 303	181	239, 323
Benzaldehyde	**Benzaldehyde-d$_6$**	106	301	281	251	301	271
Phenylacetaldehyde	**Benzaldehyde-d$_6$**	120	315	178	295, 267	181	91, 315
Nonanal	**Benzaldehyde-d$_6$**	142	337	178	317	181	239
***m*-Tolualdehyde**	**Benzaldehyde-d$_6$**	120	315	295	265, 167	181	91, 315
***o*-Tolualdehyde**	**Benzaldehyde-d$_6$**	120	315	295	265, 167	181	91, 315
Hydrocinnamaldehyde	**Benzaldehyde-d$_6$**	132	329	178	309	181	271, 329
***trans*-2-Nonenal**	**Benzaldehyde-d$_6$**	140	335	315	285	181	250, 335
2-Hydroxy benzaldehyde	**Benzaldehyde-d$_6$**	122	317	136	280	181	300, 317
Decanal	**Benzaldehyde-d$_6$**	156	351	178	331	181	239, 351
2,5-Dimethylbenzaldehyde	**Benzaldehyde-d$_6$**	134	329	309	279	181	286, 329
5-Hydroxymethyl furfural	**Benzaldehyde-d$_6$**	126	321	271	285, 301	181	291, 321
2,4-Nonadienal	**Benzaldehyde-d$_6$**	138	333	283	167	181	276, 333
Glyoxal	**Benzaldehyde-d$_6$**	58	448	267	167	181	418, 448
Undecanal	**Benzaldehyde-d$_6$**	170	365	345	318	239	181, 345
Anisaldehyde	***p*-Anisaldehyde-d$_3$**	136	331	311	281	331	181, 288

App. Table 1 contd.

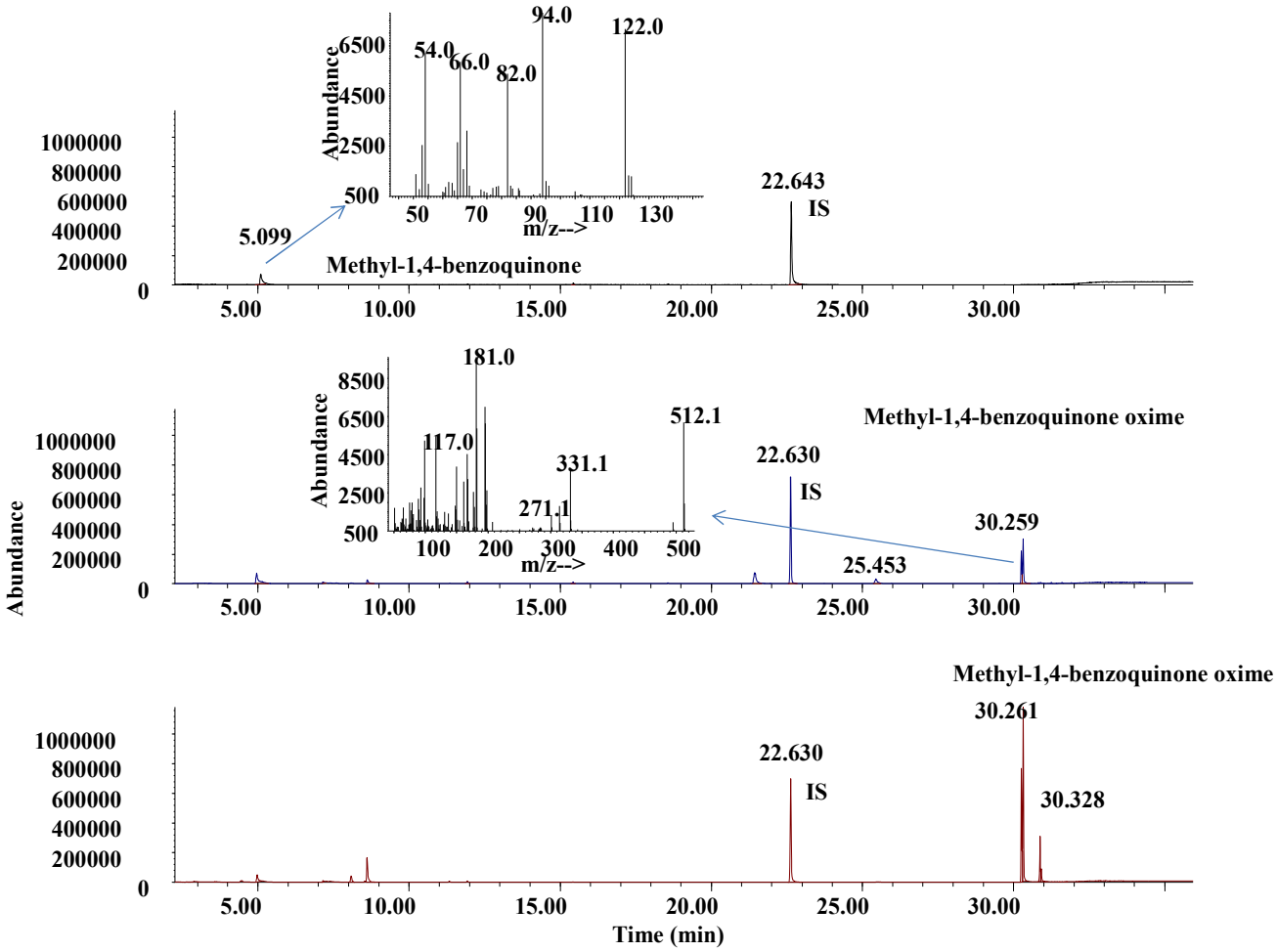
Methylglyoxal	***p*-Anisaldehyde-d_3**	72	462	281	167, 392	181	432, 462
4-Hydroxybenzaldehyde	**4-Hydroxybenzaldehyde-d_4**	122	317	297	267	181	274, 317
Dodecanal	**4-Hydroxybenzaldehyde-d_4**	184	379	178	332, 359	181	239
Glutaraldehyde	**4-Hydroxybenzaldehyde-d_4**	100	490	178	450	181	293, 490
Syringaldehyde	**4-Hydroxybenzaldehyde-d_4**	182	377	357	327	377	181



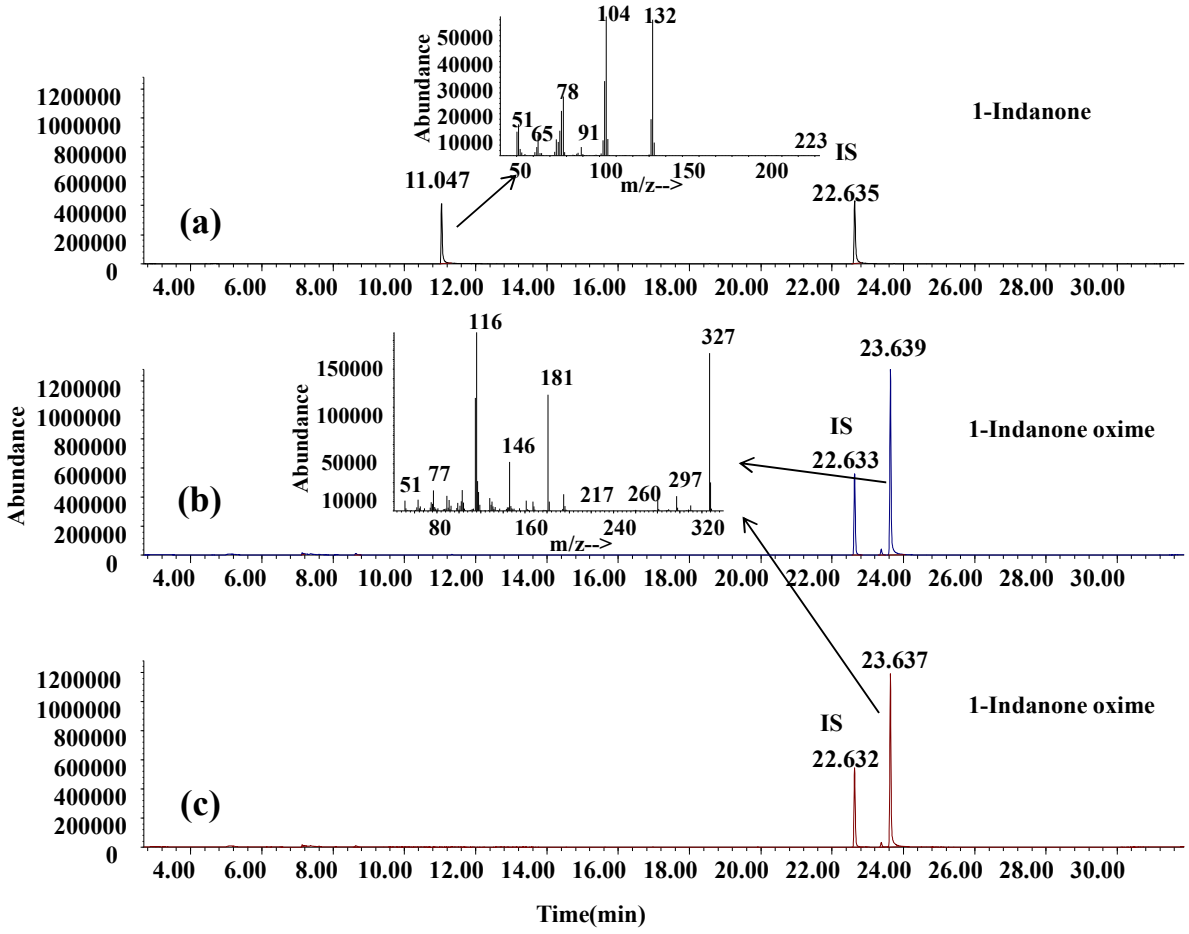
App. Figure 1: GC-EI-MS (TIC) chromatograms showing completely derivatized acetophenone in ACN/DCM/MeOH



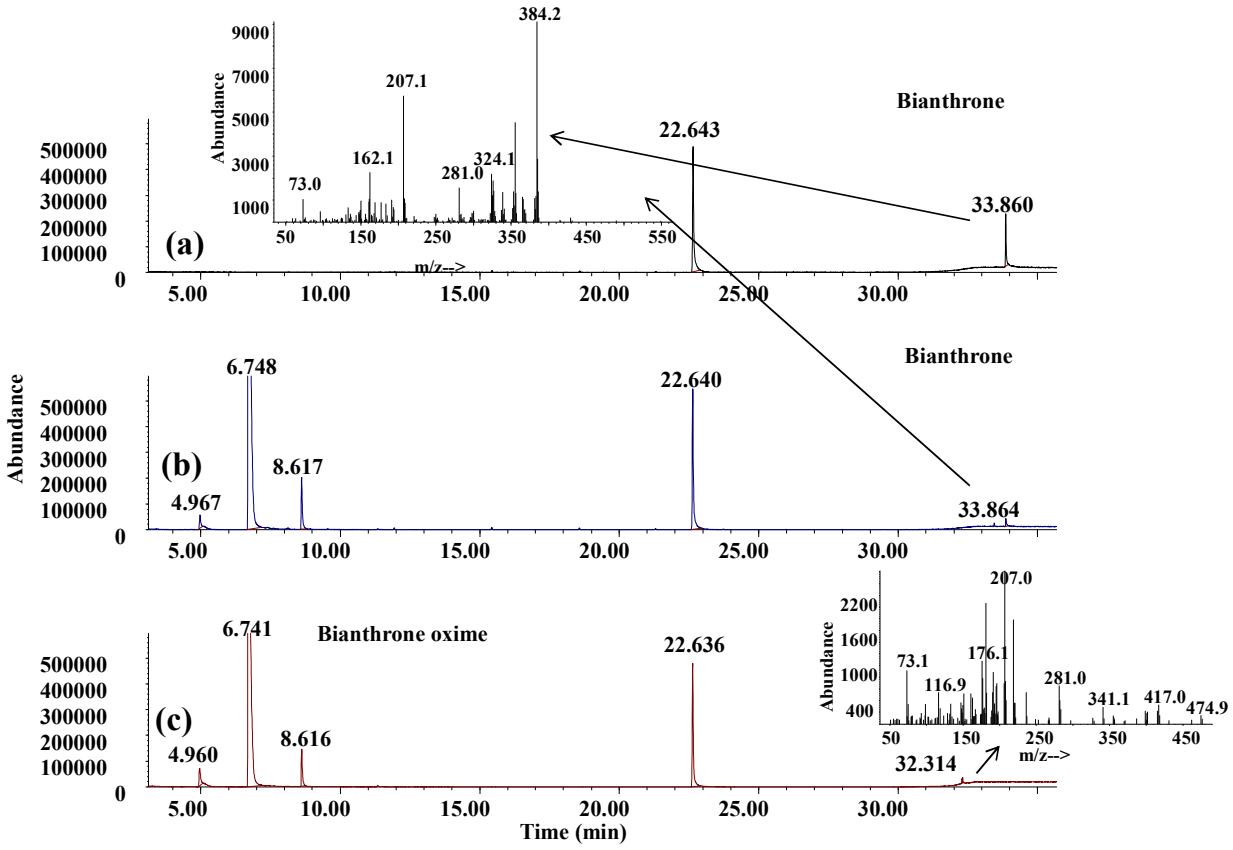
App. Figure 2: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of pyrene-4,5-dione a) underivatized pyrene-4,5-dione, b) Complete derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.



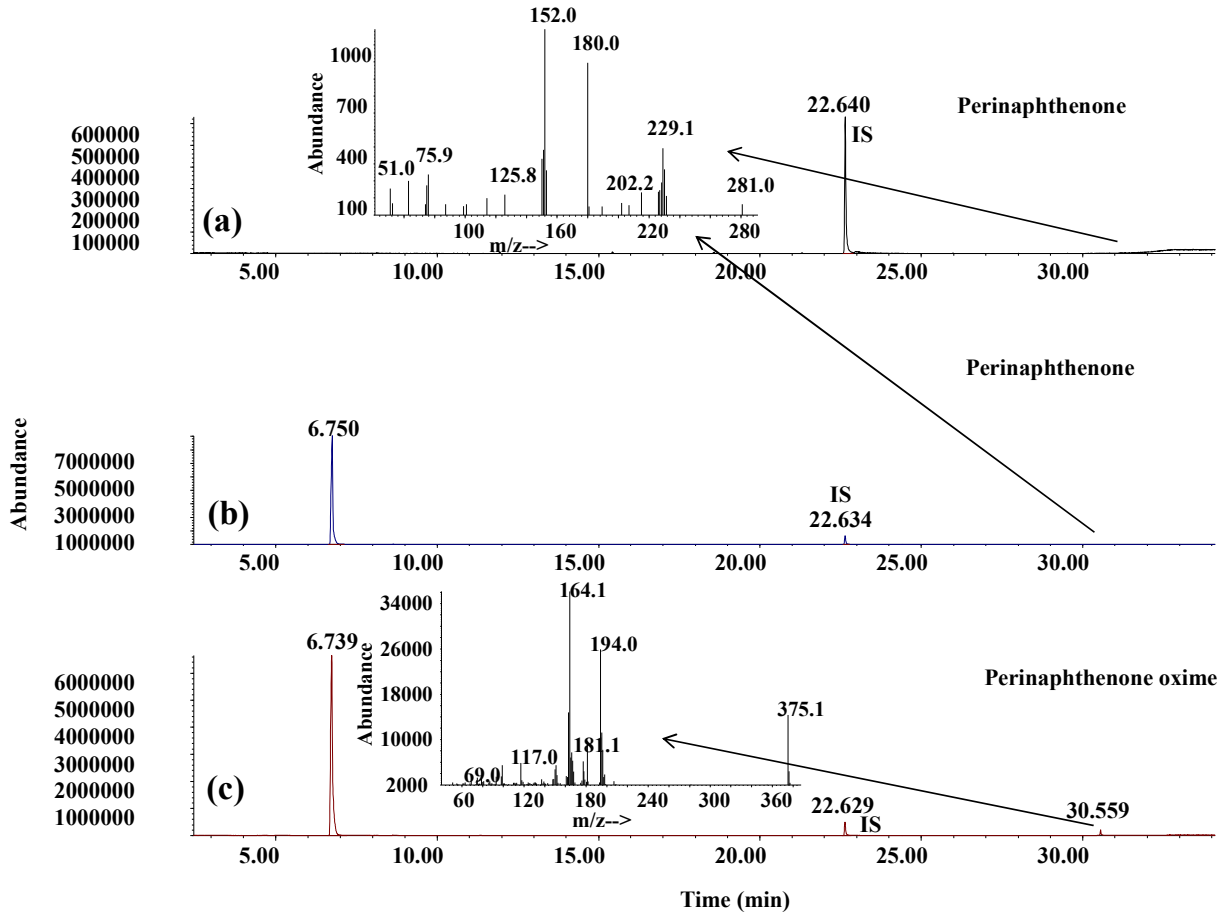
App. Figure 3: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of methyl-1,4-benzoquinone a) underivatized methyl-1,4-benzoquinone, b) Complete derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.



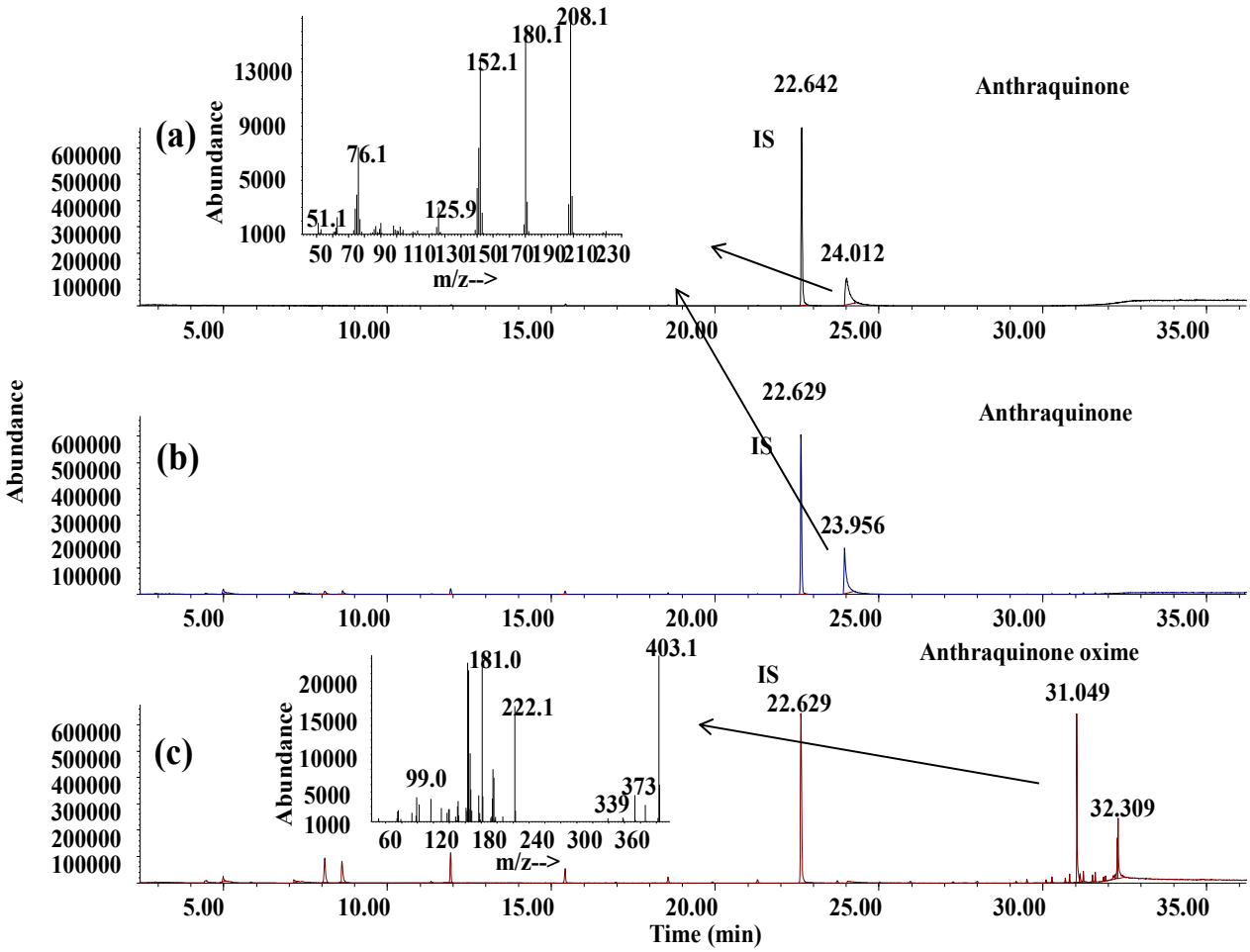
App. Figure 4: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of 1-Indanone a) underivatized 1-Indanone, b) Complete derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.



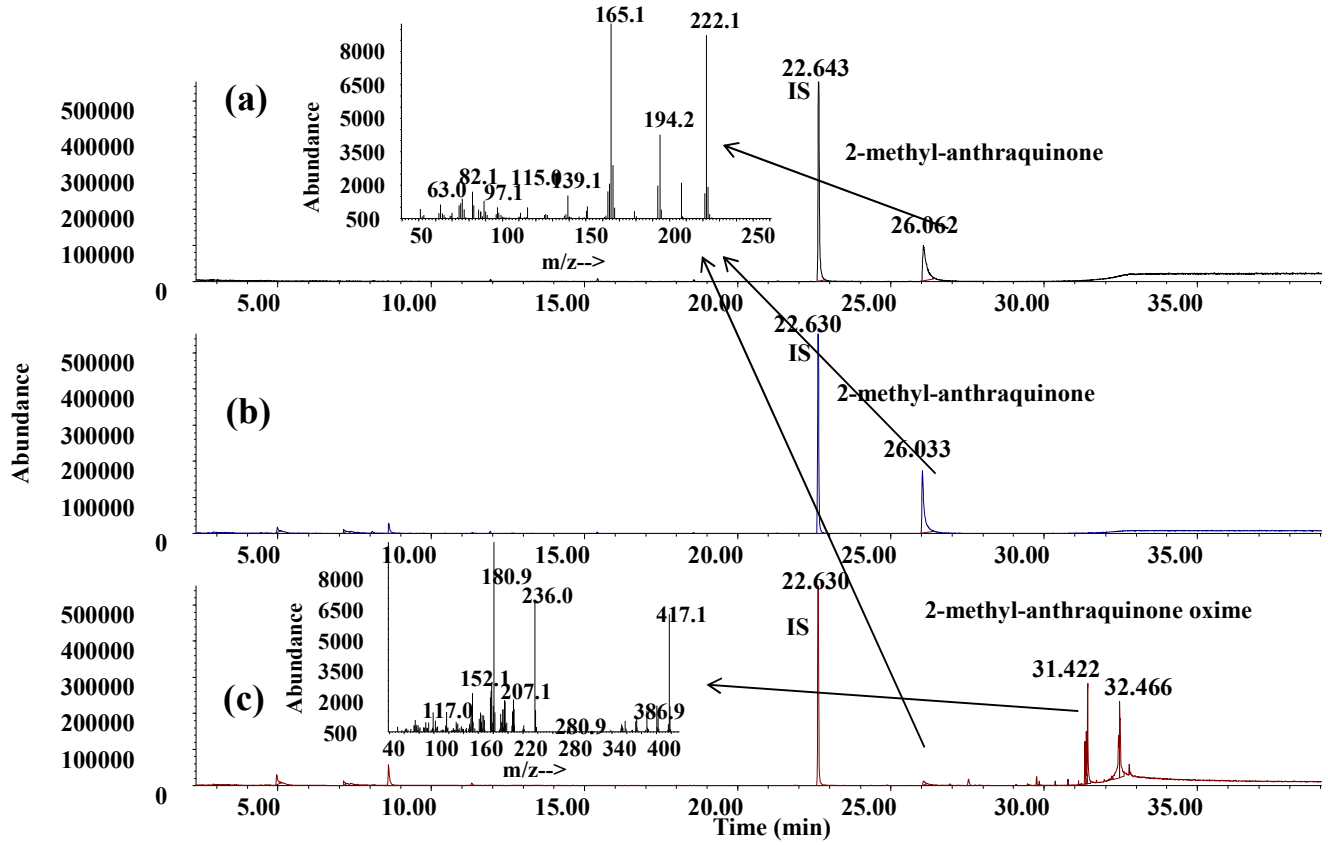
App. Figure 5: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of bianthrone a) underivatized bianthrone, b) No derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.



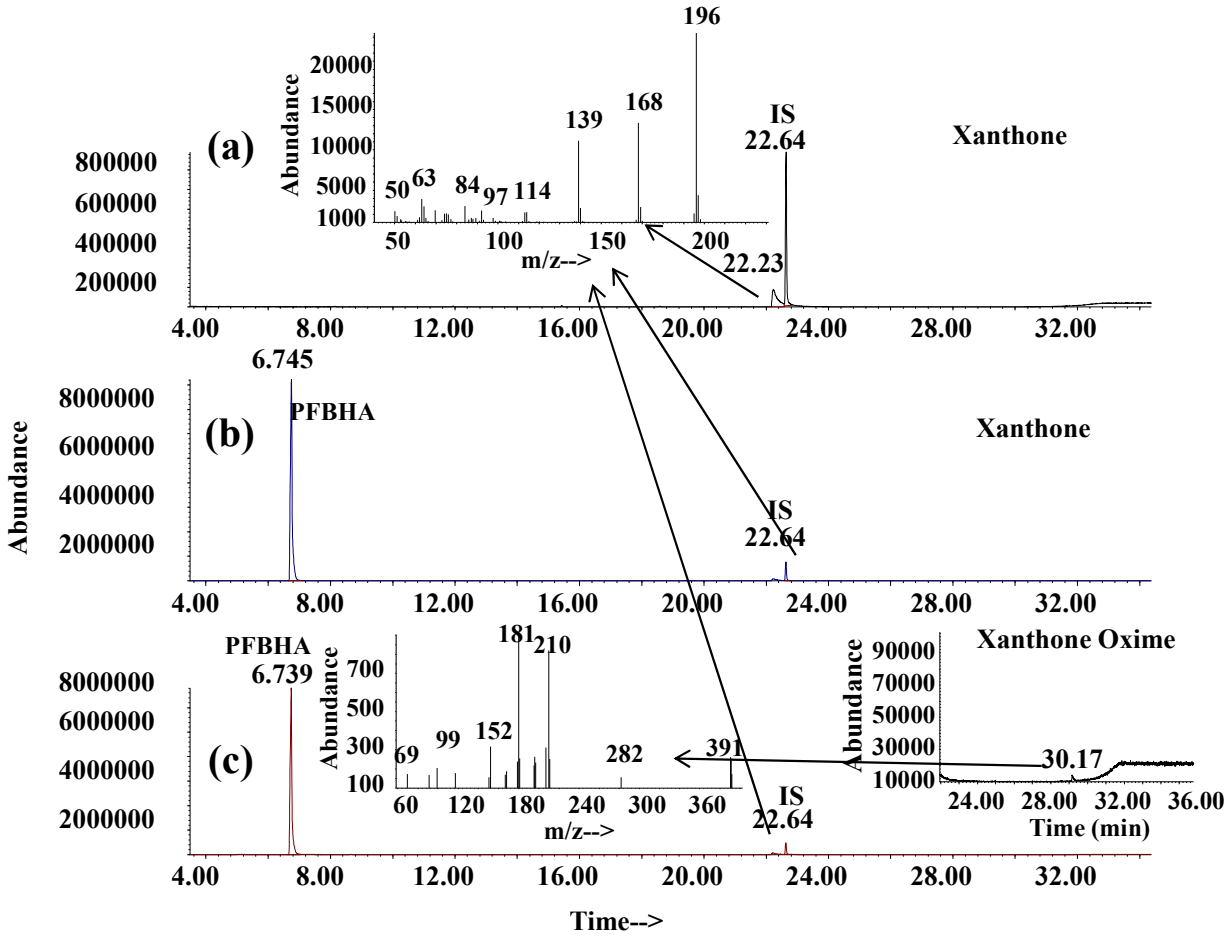
App. Figure 6: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of perinaphthenone a) underivatized perinaphthenone, b) No derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.



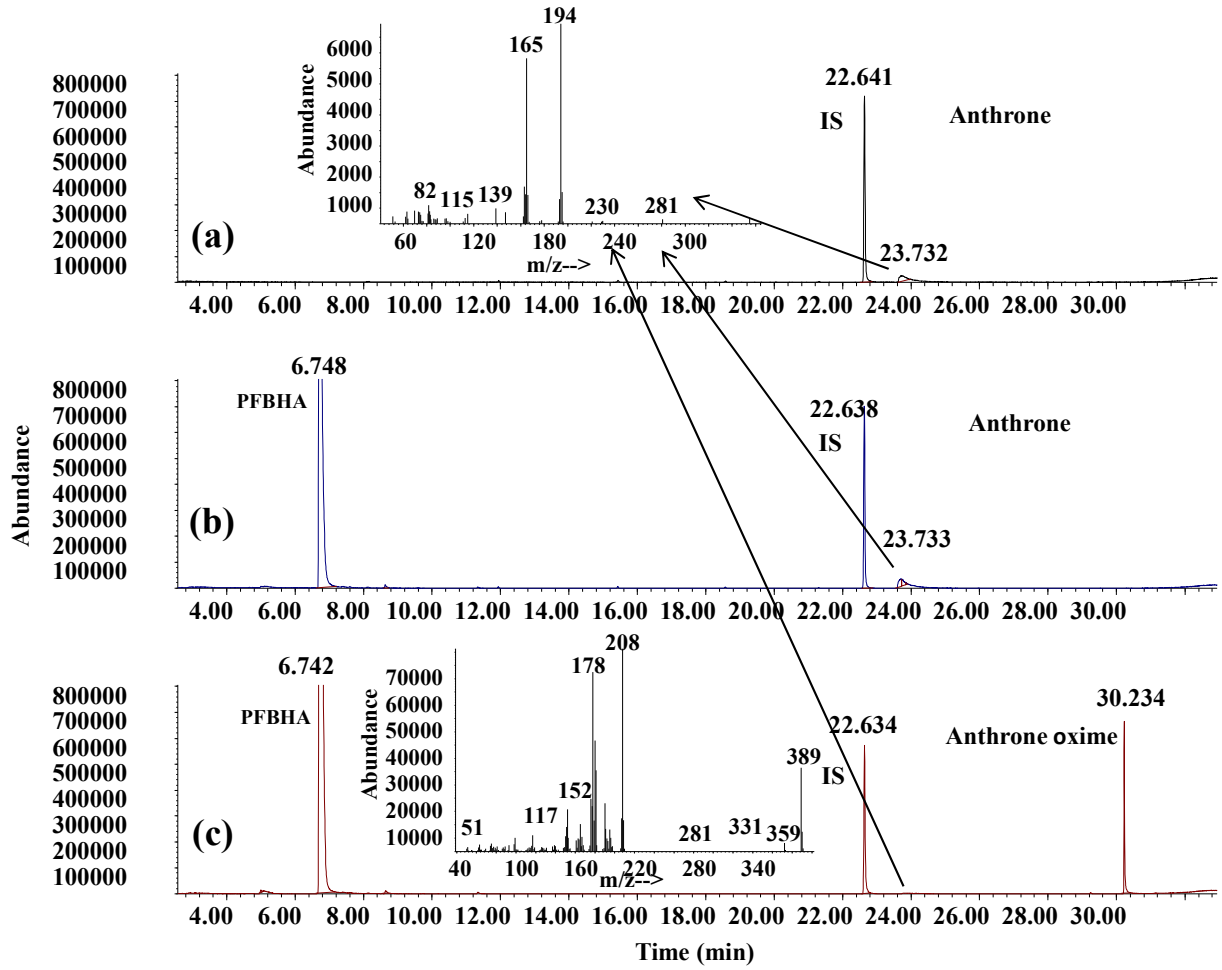
App. Figure 7: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of anthraquinone a) underivatized anthraquinone, b) No derivatization in ACN/DCM/MeOH c) partial derivatization in MeOH.



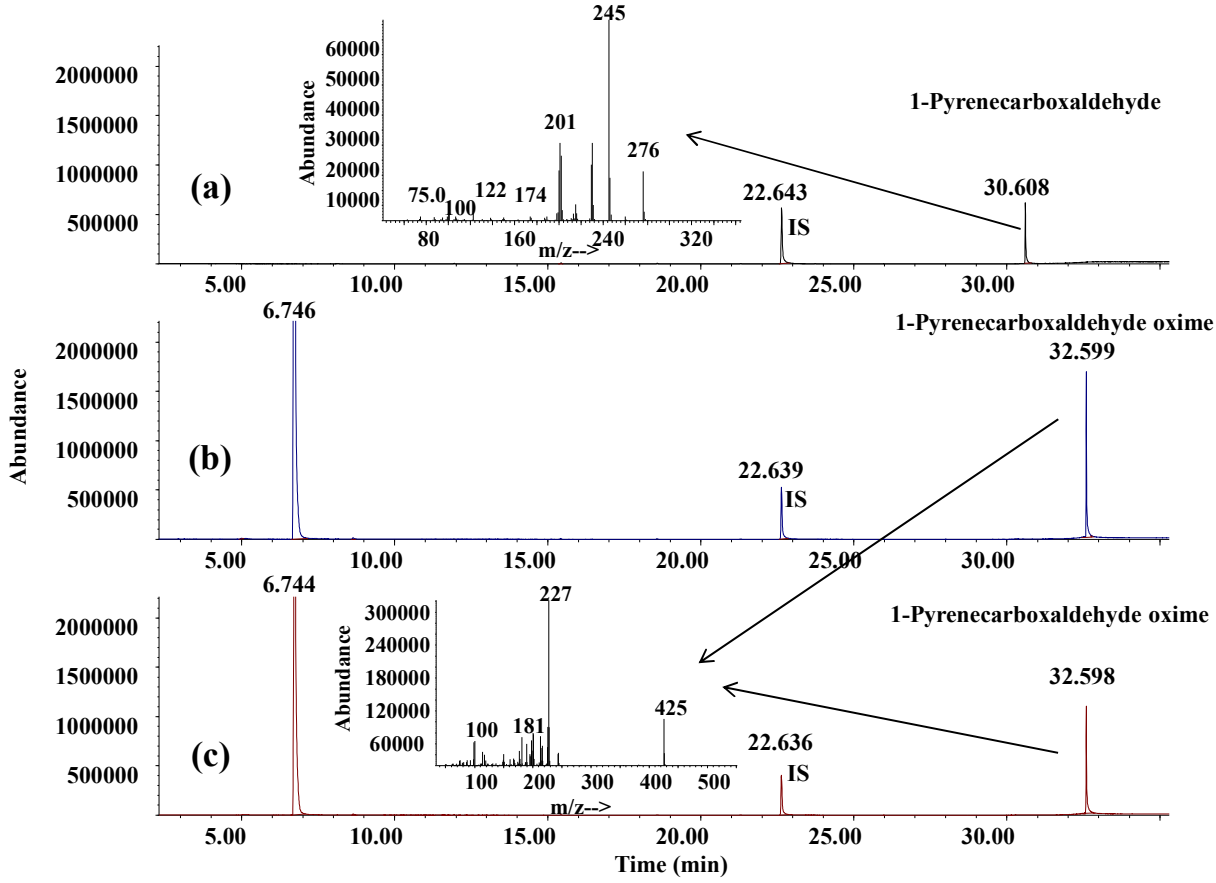
App. Figure 8: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of 2-methylanthraquinone a) underivatized 2-methylanthraquinone, b) No derivatization in ACN/DCM/MeOH c) partial derivatization in MeOH.



App. Figure 9: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of xanthone a) underivatized xanthone, b) No derivatization in ACN/DCM/MeOH c) partial derivatization in MeOH.



App. Figure 10: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of anthrone a) underivatized Anthrone, b) No derivatization in ACN/DCM/MeOH c) partial derivatization in MeOH.



App. Figure 11: GC-EI-MS total ion current chromatograms showing effectiveness of derivatization of 1-pyrene carboxaldehyde a) underivatized 1-pyrene carboxaldehyde, b) Complete derivatization in ACN/DCM/MeOH c) complete derivatization in MeOH.

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