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MASS SPECTROMETRY OF FATTY ALDEHYDES

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Abstract

Fatty aldehydes are important components of the cellular lipidome. Significant interest has been developed towards the analysis of the short chain α,β -unsaturated and hydroxylated aldehydes formed as a result of oxidation of polyunsaturated fatty acids. Multiple gas chromatography-mass spectrometry (GC/MS) and subsequently liquid chromatography-mass spectrometry (LC/MS) approaches have been developed to identify and quantify short-chain as well as long-chain fatty aldehydes. Due to the ability to non-enzymatically form Schiff bases with amino groups of proteins, lipids, and with DNA guanidine, free aldehydes are viewed as a marker or metric of fatty acid oxidation and not the part of intracellular signaling pathways which has significantly limited the overall attention this group of molecules have received. This review provides an overview of current GC/MS and LC/MS approaches of fatty aldehyde analysis as well as discusses technical challenges standing in the way of free fatty aldehyde quantitation.

Keywords

Aldehyde mass spectrometry; Fatty aldehydes; Plasmalogens; 4-Hydroxynonenal; (2E)-Hexadecenal

1. INTRODUCTION

Fatty aldehydes do not generally belong to a group of molecules that immediately attract the attention of investigators studying lipids. When searching the Web of Sciences database using “fatty aldehyde” as a keyword combination, it returns only 881 publications. This number pales in comparison to about 100,000 citations found when searching for the phrase “fatty acids” within just a few years. This huge discrepancy in “interest” is in part the consequence of geological and biochemical evolution. As it is seen currently [[1]], the first fatty aldehyde-containing polar lipids, or plasmalogens, present in anaerobic bacteria inhabiting early anaerobic Earth’s oceans were eliminated along with the “invention” of photosynthesis and the oxygenation of prehistoric oceans and atmosphere that facilitated vinyl bond breakdown through the oxygen attack. Then, plasmalogens were “re-invented” in the animal kingdom with a specialized but a limited function as a protective tool from the excess of reactive oxygen species (ROS)¹ [[1],[2]]. The level of plasmalogens varies dramatically between phospholipid classes, tissues, and within animal phyla. In some cases, phosphatidylethanolamine, the main fatty-aldehyde containing phospholipid in all organisms, may be comprised almost entirely by plasmalogen molecules [[3]–[5]]. Due to

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the lack of proven direct involvement of glycerolipid-derived long-chain fatty aldehydes in cell signaling and challenges associated with the ability of aldehydes to form Schiff-base products, the overall interest to fatty aldehydes was minimal and the methodology of fatty aldehyde analysis was not well known within lipidologists. This situation is currently changing, in part due to the heightened interest in the short-chain α,β -unsaturated and hydroxylated aldehydes derived from oxidized polyunsaturated fatty acids and the development of new LC/MS-based approaches, which allows simultaneous analysis of free aldehydes as well as aldehyde adducts and the entire classes of plasmalogen phospholipids. Methodologically, mass spectrometric analysis of the long-chain fatty aldehydes has many similarities with the analysis of the short-chain and hydroxylated aldehydes. This review will summarize methodological approaches in mass spectrometric analysis of aldehydes and discuss challenges related to aldehyde chemical reactivity and endogenous metabolism.

2. PLASMALOGENS

Virtually all long chain fatty aldehydes present in biological systems are found within plasmalogens – 1-O-alkenyl-2-O-acyl-*sn*-glycero-3-phospho(ethanolamine:choline:serine) (Figure 1). The plasmalogen vinyl ether bond is sensitive to acidic pH; hence, non-acidic conditions are required to preserve their integrity during lipid extraction. On the other hand, the same lability of the vinyl ether bond can be used to break apart fatty aldehydes from plasmalogens and subject them to further analyses as well as to determine relative proportion of plasmalogens within each class of phospholipids. The so called reaction chromatography is an old but helpful tool to determine relative percentages of plasmalogens [[6]]. In this approach, total lipid extract is applied to the corner of a thin layer chromatography (TLC) plate and the plate is developed in a basic mixture of organic solvents. The plate is then covered with a protective glass cover leaving exposed only the area with resolved lipids, which is further sprayed with 2N HCl in methanol until full saturation. After complete drying, the TLC plate is once again developed in the second direction using the same solvent system. The use of the same solvent system in both directions positions non-hydrolyzed diacyl and alkylacyl phospholipids on a diagonal line while plasmalogens are degraded by 2N HCl in methanol and form corresponding lysophospholipids clearly separated from non-hydrolyzed counterparts. The analysis of lipid phosphorus content in those spots provides the relative percentage of plasmalogens in each phospholipid class while acid-liberated aldehydes can be isolated and subjected to further analysis.

3. FATTY ALDEHYDE ANALYSIS BY GC/MS

3.1. GC/MS analysis of the long-chain fatty aldehydes

One of the earliest detection techniques for fatty aldehydes was their analysis by gas chromatography (GC) as dimethylacetal (DMA) derivatives. Aldehyde DMAs are well resolved from the analogous fatty acid methyl esters (FA-Me) on both polar and non-polar

¹ABBREVIATIONS: 4-APC, 4-(2-(trimethyl ammonio)ethoxy)benzenaminium halide; 4-APEBA, 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide; APCI, atmospheric pressure chemical ionization; BSTFA, bis-trimethylsilyltrifluoroacetamide; CHD, cyclohexanedione; DMA, dimethylacetals; DNPH, 2,4-dinitrophenylhydrazones; ESI, electrospray ionization; FA-Me, fatty acid methyl esters; GC, gas chromatography; GC-ECD, GC with electron-capture detection; GC/MS, gas chromatography mass spectrometry; 4-HNE, 4-hydroxynonenal; 4-HHE, 4-hydroxyhexenal; HTMOB, 4-hydrazino-N,N,N-trimethyl-4-oxobutaninium iodide; LC/MS, liquid chromatography mass spectrometry; LOD, limit of detection; MDA, malondialdehyde; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; NICI-GC/MS, negative ion chemical ionization gas chromatography mass spectrometry; PFBO, pentafluorobenzyl oxime; PFNHA, O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride; PUFA, polyunsaturated fatty acids; RCS, reactive chlorinated species; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SIM, selected ion monitoring; SPC, sphingosylphosphorylcholine; TLC, thin layer chromatography; TMS, trimethylsilyl.

columns. Aldehyde DMA and FA-Me can be also separated on silica TLC plates developed in toluene that facilitates compound identification during GC.

Electron Ionization (EI) mass spectrometry of DMA yields characteristic ions at $[M-31]^+$ (due to the loss of a methoxy group) and at m/z 75 (Figure 2) [[7],[8]]. Together with the retention time parameter of DMA during GC separation, this provides enough information to properly identify straight chain and branched isomers of DMA (the latter are particularly abundant in marine organisms) [[9]].

Identification of free long-chain fatty aldehydes within biological matrices is a challenging task. Due to reactive properties of aldehydes, they are usually rapidly metabolized or react with nucleophilic targets (see below). However, in rare occasions, free long-chain fatty aldehydes are important constituents of the biological milieu. Sex pheromones of *Lepidoptera* species contain substantial amounts of free C10–C18 fatty aldehydes, in many cases with two or three conjugated double bonds. There are currently 75 identified free fatty aldehydes as components of *Lepidoptera* sex pheromones (as listed at <http://www-pherolist.slu.se/pherolist.php>). Within the methods of pheromone fatty aldehyde analysis, the direct GC/MS analysis of hexadienyl compounds with a conjugated diene system provides an interesting example of the behavior of non-derivatized aldehydes with conjugated diene bonds during electron ionization (EI)-induced dissociation [[10]]. In particular, 4,6-hexadecenal and 5,7-hexadecenal generate prominent heterocyclic product ions with m/z 84 and m/z 98, correspondingly (Figure 3). The 6,8-hexadecenal also forms a homologous ion at m/z 112; however, its intensity is minimal. Most of the tested C16-aldehydes with conjugated double bonds were detected with the base peak at m/z 67 (6,8-, 7,9-, 8,10-, 9,11-, 10,12-, and 13,15-dienes) while 5,7-diene had the base peak at m/z 80, and 4,6-diene had it at the m/z 84 [[10]].

While the GC/MS analysis of non-derivatized fatty aldehydes provides sometimes interesting structural insight and is required for pheromone analysis when coupled to electroantennographic detector [[11]], this type of analysis is the least sensitive. Derivatization of carbonyls with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) with the formation of oximes is considered a reference approach in the analysis of aldehydes by GC with electron-capture detection (GC-ECD) or by GC/MS [[12]–[17]]. When using GC/MS in Selected Ion Monitoring (SIM) mode, the aldehyde pentafluorobenzyl oxime (PFBO) derivatives are clearly identified by their $[M-181]^+$ ion (m/z 254, 268, 282, 280 for hexadecanal-, heptadecanal-, octadecanal-, and octadecenal-PFBO derivatives, respectively), and by the presence of m/z 181 and m/z 239 ions characteristic to all straight-chain saturated aliphatic aldehydes (Figure 4 and [[13]]). Usually, two stereo isomers (Syn- and Anti-) are formed during aldehyde derivatization (Figure 5) [12], which are often resolved by gas chromatography. However, the formation of the two isomers complicates GC profiling and subsequent data processing.

Derivatization of fatty aldehydes with (pentafluorobenzyl)hydroxylamine hydrochloride is accomplished in mild buffered conditions (50 mM PFBHA, HCl in 50 mM Tris-HCl, pH 7.4). Hence, this derivatization allows the analysis of free aldehydes and aldehydes derived from plasmalogens in the same tissue sample when one portion of the homogenate is processed with acidification of lipids. This approach allows quantitation of both free and plasmalogen-associated aliphatic aldehydes in several brain regions [[16],[18]] and in cells [[19]]. PFBO of α,β -unsaturated 2-alkenals have an additional characteristic ion with m/z 250, which corresponds to the cleavage between the β and γ carbon atoms [[13]] (Figure 6). If an additional conjugated double bond is present at C4, such as in the case of 2,4-decadienal, the mass spectra of dienal PFBOs still have the ion at m/z 181 but its intensity is usually decreased, and the ion with m/z 276 becomes the most abundant [[13]].

Derivatization of aldehydes with 2,4-dinitrophenyl hydrazine is another frequently employed approach. In addition to the strong molecular ion, the EI mass spectra of aliphatic 2,4-dinitrophenylhydrazones (DNPH) show prominent ions at m/z 224 and m/z 206, which are derived due to the hydrazone McLafferty rearrangement and the subsequent loss of water [[13]]. Chemical Ionization (CI) mass spectrometry in negative ion mode with methane employed as ionizing gas provides two prominent ions from DNPH derivatives – the base peak at m/z 182 and the molecular ion while ammonia chemical ionization in positive ion mode provides more complex but structure-specific spectrum [[20]].

It is common for the endogenous free aldehydes to bear a hydroxy group. This hydroxy group originates from the oxidative cleavage of plasmalogen vinyl bond or from oxidative degradation of polyunsaturated fatty acids (PUFA) and the formation of short- and medium-chain aldehydes like 4-hydroxynonenal (see below). To be analyzed by GC or GC/MS, the hydroxy group is usually modified with *bis*-trimethylsilyltrifluoroacetamide (BSTFA) [[13]]. Hence, the GC/MS analysis of hydroxyalkenals is performed as the PFBO-TMS derivatives. Those derivatives have a prominent characteristic $[M-15]^+$ ion and the common ions for all PFBO-TMS derivatives at m/z 326 and m/z 181 (Figure 7). Using this approach, it was demonstrated that the degradation of endogenous plasmalogens by UV irradiation generates predominantly 15:0-, 17:0-, and 17:1-aldehydes, while the Fe^{2+} /ascorbate-induced oxidation of plasmalogens results in the formation of 16:0-OH and 18:0-OH aldehydes [[16],[21],[22]]. The authors also investigated the effect of trapping the aldehydes during UV- and Fe^{2+} /ascorbate-induced plasmalogen degradation. To trap aldehydes, PFBHA.HCl was added to tissue homogenate before the beginning of the stress [[21]]. It was found that aldehyde trapping improves the recovery of aldehydes formed. Interestingly, the effect of trapping was more pronounced in the case of UV-induced formation of odd-chain aldehydes (15:0, 17:0, 17:1) than during the Fe^{2+} /ascorbate-initiated generation of hydroxylated even-chain aldehydes (16:0-OH and 18:0-OH).

Aldehyde stability in biological matrices is a serious challenge. In addition to the ability of aldehydes to form Schiff base products with amino groups of proteins and lipids [[23]–[26]], free fatty aldehydes can be further metabolized to fatty acids and fatty alcohols [[27]]. In addition, aldehyde stability/reactivity depends on its structure and varies as a function of the aldehyde chain length, the presence of double bonds and their position, and the presence and position of hydroxy group. Hence, if no precautions are taken, the measured levels of aldehydes in biological matrices represent a snap-shot image of the system, which contains continuously metabolized and reacting aldehydes. Trapping aldehydes with derivatizing reagent during non-enzymatic aldehyde generation [[21]] is ideal to provide the most correct estimation of the rate of aldehyde formation. However, such an approach is difficult to execute during enzymatic formation of aldehydes [[28]] due to the inactivation of enzyme(s) by the trapping reagent. As a solution, the estimation of the disappearance of aldehyde standards at the exact experimental conditions can be used to correct for aldehyde bio- and chemical transformations (see chapter 6).

3.2. GC/MS analysis of 4-hydroxynonenal

In contrast to the long-chain fatty aldehydes, short-chain aldehydes are actively studied due to their direct relation to the oxidative breakdown of polyunsaturated fatty acids. Within this broad group of aldehydes, 4-hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE) as the end-products of ω -6 and ω -3 fatty acid oxidation, respectively, receive the most attention due to their reactive properties and the ability to react with proteins, lipids, DNA, and other nucleophiles [[29]]. In fact, this immediate connection to the oxidation of PUFA is an important factor promoting the development of the analysis of free aldehydes. Since the discovery of 4-HNE in 1980 [[30],[31]], several GC/MS approaches were developed and are now actively used for 4-HNE and related aldehyde analysis. To preserve the hydroxyl group

in 4-HNE from temperature-induced dehydration it must be protected and this is usually achieved through silylation. If *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) is used for silylation, both 4-trimethylsilyloxy-2-nonenal and its corresponding MSTFA adduct are present, especially with prolonged silylation times [[30]]. This is a good example of an one-step reaction, which can derivatize both carbonyl and hydroxy groups. The mass spectrum of 4-trimethylsilyloxy-2-nonenal is characterized by an intense fragment ion at m/z 157 due to the α -cleavage of 4-HNE TMS derivative (Figure 8). The ion at m/z 157 can further decompose to the ion with m/z 129 after the loss of CO group. The mass spectrum of the MSTFA adduct of 4-trimethylsilyloxy-2-nonenal is dominated by the ion with m/z 356, which is produced by the α -cleavage of the derivative. It is also characterized by the presence of the pair of $[M-126]^+$ and $[M-127]^+$ ions at m/z 301 and m/z 300, which correspond to the loss of $CH_3N^+-COCF_3$ and $CH_3-N=C(OH)-CF_3$, respectively [[13]].

PFBO derivatives of 4-trimethylsilyloxy-2-alkenals are examples of derivatives obtained in a two-step derivatization process. In this case, the carbonyl group is derivatized first then the hydroxyl group is protected [[13]]. The mass spectrum of the PFBO derivative of 4-trimethylsilyloxy-2-nonenal has prominent characteristic fragments with m/z 352 $[M-71]^+$, m/z 242 $[M-181]^+$, m/z 226 $[M-197]^+$, and m/z 408 $[M-15]^+$ [[13],[22]]. The scheme of 4-trimethylsilyloxy-2-nonenal PFBO derivative MS/MS fragmentation is presented in Figure 9.

While PFBO-TMS derivatives are most commonly used for GC/MS analysis of 4-HNE and related aldehydes, other derivatives are also employed, especially when partial hydrolysis of trimethylsilylethers during TLC purification of the derivatives is of concern. For example, *tert*-butyldimethylsilyl-PFBO derivatives are stable during TLC separation; their mass spectra are dominated by a prominent $[M-57 ((CH_3)_3C^*)]^+$ peak [[32]].

Oxidation of PUFA generates a vast number of short-chain aldehydes with varying numbers and position of double bonds and hydroxyl groups within the aldehyde molecules. While GC/MS analysis of all aldehydes follows same basic principles, the stability of derivatives and the structure-specific choice of derivatizing reagent sometimes have to be considered. This topic has been extensively addressed in the excellent review by Spiteller et al. [[13]], which provides exhaustive information on the analysis of aldehydes by the GC/MS.

4. FATTY ALDEHYDE ANALYSIS BY LC/MS

4.1. LC/MS analysis of 4-hydroxynonenal and related short-chain aldehydes

The development of the LC/MS methodology opened a new era in the analysis of all lipids including fatty aldehydes and aldehydes derived from PUFA oxidation. The LC/MS approach resolves many challenges intrinsic to GC/MS analysis of aldehydes like the lability of hydroxy-aldehydes at high temperatures used during GC separation but also brings some other challenges like the constant struggle with background signal reduction and the need for highly purified derivatizing reagents. As is the case for 4-HNE GC/MS analysis, 4-HNE and its analogs can be analyzed by LC/MS without derivatization. In fact, the analysis of non-derivatized short-chain aldehydes by LC/MS was one of the first approaches tried during the development of the LC/MS interface [[33]]. This interface, in combination with methane-facilitated chemical ionization, allowed the detection of free hexanal as the $[M+H-H_2O]^+$ ion but much higher ion intensity was obtained after conversion of hexanal into its methoxime derivative and its detection as $[M+H]^+$ ion at m/z 130. Sensitivity of this interface was enough to detect 10 pmol of hexanal injected but it still could not provide enough ionization for such aldehydes as malondialdehyde (MDA) and 4-HNE [[33]]. Modern LC/MS interfaces allow detection of 4-HNE without derivatization, and direct analysis of 4-HNE is sporadically employed [[34]–[36]]. In an acidified methanolic solution, 4-HNE forms a derivative with methanol, which produces an ion at m/z 171. The collision

spectra of the m/z 171 ion demonstrate ion formation at m/z 139, m/z 71, and m/z 69, with the ion at m/z 69 being the most abundant [[35]].

The LC/MS analysis of 4-HNE and other aldehydes as 2,4-dinitrophenylhydrazone (DNPH) derivatives is the most commonly used approach [[37]–[42]] (Figure 10). 2,4-Dinitrophenyl-hydrazine was used long before the development of LC/MS methodology as DNPH derivatives possess characteristic absorption spectrum that can be readily identified by HPLC with UV detection with a sensitivity of about 1 pmol on column [[43]]. A thorough examination of mass spectrometric properties of DNPH derivatives of thirty carbonyl compounds was performed by Kolliker et al. [[38]]. Compounds were analyzed using a quadrupole ion trap system with atmospheric pressure chemical ionization (APCI) in the negative ion mode. Electrospray ionization (ESI) resulted in strong derivative fragmentation and insufficient sensitivity. In APCI negative ion mode, all DNPH derivatives showed molecular $[M-H]^-$ ion. In MS/MS experiments, all aldehyde DNPHs generated a strong m/z 163 ion, which can be used as a selection criteria for their identification. Then, unsaturated aldehydes with a double bond in α -position are further distinguished from saturated aldehydes by the presence of $[M-H-47]^-$ and the absence of $[M-45/46]^-$ and $[M-H-30]^-$ ions while saturated aldehydes have prominent $[M-H-30]^-$ and $[M-45/46]^-$ ions. DNPHs of aromatic aldehydes also have $[M-H-47]^-$ fragment ion but aldehydes with a double bond at α -position can be further distinguished by the $[M-167]^-$ ion, which is absent in DNPHs of aromatic aldehydes. The DNPH derivatives of 4-HNE and 4-HHE produce an intense m/z 167 ion. The pathways for the formation of several characteristic ions from aldehyde DNPH derivatives analyzed using negative ion MS/MS (Figure 11) and the sequence of structure-characteristic ion pathways are shown below (Figure 12).

While APCI was demonstrated to be somehow more sensitive for the analysis of DNPH derivatives of aldehydes, satisfactory results were also obtained with ESI-MS/MS [[42]]. In these studies, ESI-LC/MS/MS in negative ion mode was employed to analyze carbonyls in air samples. Same transitions from $[M-H]^-$ ions to m/z 163 and m/z 152 were used for quantitation. This work is interesting by the comment about the need for triple crystallization of 2,4-DNPH and special handling of air sample collection cartridges that reflects the need for special precautions to achieve low background noise and to limit the number of impurity peaks.

As a variation of the most commonly used aldehyde derivatization with 2,4-dinitrophenylhydrazine, the analysis of aliphatic aldehydes as 2-nitrophenylhydrazones by APCI-LC/MS/MS in negative ion mode has been described [[44]]. These derivatives give a strong signal for the radical ion and produce very stable fragments at m/z 199 and m/z 137 (Figure 13). It was concluded that the m/z 199 ion is formed by the intramolecular rearrangement while the ion at the m/z 137 can be explained from the fragmentation of 2-nitrophenylhydrazone derivative.

4.2. Dimedone and cyclohexanedione derivatives of aldehydes

In addition to frequently employed DNPH derivatives, there is an array of alternative derivatizing reagents applicable for the LC/MS/MS identification and quantitation of aldehydes. The Hantzsch reaction is used to obtain heterocyclic dimedone derivatives suitable for UV- and APCI- or ESI-LC/MS/MS analysis of aldehydes. In this reaction, an aldehyde, two β -carbonyl compounds, and ammonia combine to form a heterocyclic system (Figure 14). Zurek and Karst [[45]] first described this type of derivative for the LC/MS/MS analysis of short-chain aldehydes. In *positive ion mode*, the dimedone derivatives give a strong $[M+H]^+$ ion with both APCI-MS and ESI-MS. The separation of formaldehyde, acetaldehyde, propanal, and butanal dimedone derivatives was achieved within 15 minutes using acetonitrile-water gradient elution system and a LiChroSorb RP-18ec column.

Williams et al. [[46],[47]] extended the spectrum of aldehydes analyzed using this approach, with up to C8-aldehydes and 4-HNE being resolved within 46 minutes by the LC. In the MS/MS experiment, the major bond cleavage was between the R group and the heterocyclic ring. However, the fragmentation pattern of 4-HNE derivative differed from that of aliphatic aldehydes by the preferential loss of 18 Da (H₂O) and 43 Da (C₃H₇) and the formation of the product ions with m/z 339 and m/z 382 as the predominant ones. Of note, after derivatization for 1 h at 60°C, aldehyde dimedone derivatives require purification on C18 SPE columns that further extends the total duration of the assay.

O'Brien-Coker et al. [[48]] characterized the fragmentation pattern of different cyclohexanedione (CHD) derivatives, which included the derivatives of 4-HNE, 4-HHE, and α,β -unsaturated aldehydes. It was found that in positive ions, all aldehyde CHD derivatives form a prominent product ion at m/z 216 due to the neutral loss of the aliphatic portion of the molecule (Figure 15). The LC separation of CHD derivatives of aldehydes (up to C10) was accomplished within 22 minutes using a water-acetonitrile gradient solvent system. Derivatization with CHD as well as with 5,5-dimethylcyclohexanedione is selective for aldehydes as other carbonyl compounds do not react with both reagents [[45]–[47]]. The limit of detection for aldehyde CHD derivatives depends upon their chain length, unsaturation, and the presence of hydroxy group. Thus, the limit of detection (LOD) for hexanal, hex-2-enal, and 4-HHE CHD derivatives were 10, 20, and 40 pg on column, but the LOD for decanal CHD was found to be at 100 pg on column [[48]].

4.3. (S)-Carbidopa derivatives of 4-hydroxynonanal

4-HNE has one chiral atom (C4); hence two stereoisomers of 4-HNE do exist. To separate and quantify (*R*)- and (*S*)- enantiomers of *trans*-4-hydroxy-2-nonenal, their (*S*)-carbidopa derivatives have been prepared [[49],[50]]. Derivatization was performed in phosphate-buffered saline (pH 5.0) for 30 min at room temperature with a 100-fold excess of (*S*)-carbidopa (Figure 16). When using D₁₁-4-HNE as the internal standard, both transitions from m/z 363 to m/z 241 or m/z 319 (from m/z 374 to m/z 252 and m/z 330 in case of deuterated 4-HNE) can be used for quantitation. The limit of detection was determined to be 12 fmol on column with methanol-ammonium acetate buffer and Beta Basic 18 RP column suitable for the separation of enantiomer derivatives.

4.4. HTMOB, 4-APC, and 4-APEBA aldehyde derivatives

A modified Girard's reagent 4-hydrazino-*N,N,N*-trimethyl-4-oxobutaninium iodide (HTMOB) was proposed for the analysis of long-chain fatty aldehydes [[51]]. The HTMOB derivatives provide strong molecular ion signal during positive ESI-MS/MS and have in common, for all tested aldehyde derivatives, a neutral loss of 59 Da (Figure 17). This method was applied to the analysis of long-chain aldehydes by the infusion after plasmalogen hydrolysis with 1.2N HCl. 19:0-Aldehyde was used as the internal standard. Aldehydes from C15:0 to 24:1 were identified in the human plasma.

Eggink et al. [[52]] have developed a 4-APC (4-(2-(trimethyl ammonio)ethoxy)benzenaminium halide) reagent for quantitation of aldehydes in biological matrices. This reagent, in combination with NaBH₃CN, provides a convenient 30 minute one-pot derivatization at a mild pH (5.7) and temperature (10°C). The aliphatic aldehyde derivatives form a strong molecular ion during positive ESI-MS and produce two prominent ions due to the loss of 59 and 87 Da as outlined in the Figure 18. 4-HHE and 4-HNE derivatives have the additional loss of 18 Da due to the loss of water, and the most characteristic product ions for those aldehydes are formed as a result of the loss of 77 Da and 105 Da [[53]]. This aniline-based reagent approach was further developed by the creation of 4-APEBA (4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium

dibromide) reagent [[54]]. This new reagent has additional beneficial characteristics. 4-APEBA has bromophenethyl group (Figure 19), which provides a $^{79}\text{Br}/^{81}\text{Br}$ (100:98) isotopic signature convenient for confirmation of the derivative structure. Derivatization is complete within 30 minutes and derivatives have common fragmentation patterns during MS/MS experiments. The M^+ precursor ions show characteristic neutral loss of 4-bromophenethyl dimethylamine and both 4-bromophenethyl dimethylamine and ethen (see Figure 19). The 4-APEBA derivatives of aldehydes up to C10 are well resolved chromatographically within 15 minutes on Waters Atlantis dC18 (150mm $\times$ 2.1mm, 3 μm) reverse phase column and a water-methanol gradient system run at 45°C.

4.5. Analysis of (2E)-hexadecenal as a semicarbazone derivative

The end-point of sphingolipid metabolism is the degradation of sphingosine-1-phosphate (S1P) by S1P lyase with the formation of (2E)-hexadecenal. As S1P lyase is a critical enzyme regulating the entire sphingolipid metabolism [27], we have recently developed an ESI-LC/MS/MS assay for (2E)-hexadecenal quantitation as a semicarbazone derivative [[28]]. The assay consists of derivatization of aldehydes with semicarbazide in methanol at acidic conditions and 40°C for 2 hours (Figure 20), and the reaction products are directly infused or used for the LC/MS/MS experiments.

When designing the assay, we intended to achieve full chromatographic separation of (2E)-hexadecenal from hexadecanal and other natural hexadecenals with differently-positioned double bond as the derivatization procedure performed at acidic conditions may liberate aldehydes from plasmalogens. Also, if not chromatographically resolved, palmitaldehyde could interfere (by overlapping from its $[\text{M}+3]$ natural isotopic analog) with the signal from the stable isotope-labeled internal standard (D5-(2E)-hexadecenal). To study chromatographic and MS/MS behavior of (2E)-hexadecenal we compared it to hexadecanal and *cis*-11-hexadecenal. In positive ions, aldehyde semicarbazone derivatives produced strong molecular ions (hexadecenals, m/z 296, and hexadecanal, m/z 298). The MS/MS experiments revealed a substantial difference between all three aldehyde semicarbazones in their fragmentation pattern (Figure 21A–C). Thus, both (2E)-hexadecenal and *cis*-11-hexadecenal derivatives produced major product ions at m/z 279, 253, 251, and 97. However, (2E)-hexadecenal semicarbazone produced ions at m/z 279, 253, and 97 with nearly equal output when collision energies were optimized for their maximum yield while *cis*-11-hexadecenal semicarbazone produced minimal amount of the ion at m/z 97. Hexadecanal semicarbazone had the only predominant product ion at m/z 281 and did not form the ion at m/z 97. The precursor-product relationship studies of (2E)-hexadecenal semicarbazone revealed a direct relationship between ions at m/z 97 and m/z 279. The m/z 279 ion also produced the ion at m/z 251 while the ion at m/z 253 was directly formed from the molecular ion. Figure 21D shows the suggested structure of ions and their relationships.

The efficiency of aldehyde derivatization in methanol was pH-dependent and was complete within 2 hours in the presence of 5% formic acid. (2E)-Hexadecenal semicarbazone was well resolved within 6 minutes from the derivatives of hexadecanal and *cis*-11-hexadecenal on the Supelco Discovery C8 (50mm $\times$ 2.1mm, 5 μm) column in the gradient from methanol:water:formic acid (60:40:0.5, v/v) to acetonitrile:chloroform:water:formic acid (90:10:0.5:0.5, v/v) (both system contained 5 mM ammonium formate). Interestingly, (2E)-hexadecenal semicarbazone had a small shoulder peak (Figure 22), which disappeared if (2E)-hexadecenal was hydrogenated before derivatization. This indicates at potential isomer formation during the reaction, which may be characteristic to α,β -unsaturated aldehydes.

5. CHLORINATED ALDEHYDES AND THEIR DERIVATIVES

The vinyl bond in plasmalogens is not only acid-labile but is also vulnerable to reactive oxygen species (ROS) attack. In 2001, the Ford's group identified the formation of a 16-carbon fatty aldehyde with one chlorine atom from 1-O-hexadec-1-enyl-*sn*-glycero-3-phosphocholine by a myeloperoxidase reactive chlorinated species generating system *in vitro* [[57]]. TLC isolation and subsequent NICI-GC/MS analysis of the neutral product as a PFBO derivative identified it as a 2-chloro-hexadecenal. A chlorine-specific mass signature in the molecular ion and its m/z 288/290 product ion confirmed the presence of chlorine atom in the structure of aldehyde (Figure 23). Later, both 2-chloro-hexadecenal and 2-chloro-octadecenal were identified in human atherosclerotic lesions by NICI-GC/MS of aldehyde PFBO derivatives [[58]]. The same NICI-GC/MS method was used to confirm the formation of (2*E*)-hexadecenal from sphingosylphosphorylcholine (SPC) and S1P as a result of reactive chlorinated species (RCS) attack [[59]] (Figure 24). In all experiments, 2-[15,15,16,16,16-D5]-hexadecenal was used as the internal standard.

Free aldehydes are very reactive molecules and they easily form Schiff bases with amino groups of proteins and lipids. Hence, it was of no surprise to identify 2-chlorohexadecenal derivatives of ethanolamine glycerophospholipids [[60]]. The Schiff base formation was confirmed by the ESI-MS as well as by the NICI-GC/MS analysis of 2-chlorohexadecenal-*N*-modified ethanolamine as pentafluorobenzoyl derivative after degradation of modified phosphatidylethanolamine with phospholipase D (Figure 25). When *N*-2-chlorohexadecenal-phosphatidylethanolamine was reduced with NaCNBH₃ before hydrolysis with phospholipase D (PLD), the molecule no longer had a chlorine atom in the structure and did not show a characteristic chlorine mass signature (Figure 25). 2-Chlorohexadecenal formed Schiff bases not only with ethanolamine-containing phospholipids but also with the amino group of modified lysine *in vitro* [[60]].

6. CHALLENGES ON THE WAY OF QUANTIFYING ENDOGENOUS LEVELS OF FREE ALDEHYDES

Free aldehydes are quickly metabolized through a variety of pathways. Due to its high cellular toxicity and direct link to PUFA oxidation, the metabolic transformations of 4-HNE are the most studied. Phase I and Phase II pathways are responsible for the majority of 4-HNE metabolism [[61]–[63]] (Figure 26). However, some portion of 4-HNE is able to form Schiff base aldehyde derivatives with amino groups of proteins and lipids [[63],[64]], and antibodies to 4-HNE-modified proteins are widely used for an overall visualization of 4-HNE formation in tissues [[29]]. Similar to 4-HNE, the same general metabolic rules apply to all aldehydes. Hence, when measuring free aldehyde content in biological systems one detects only non-metabolized and non-reacted portion of the aldehyde formed in the system at any given moment. Thus, the trapping of aldehydes during their formation [[21],[56]] may become a solution. However, the investigation of the ability of several carbonyl trapping reagents (hydrazine, aminoguanidine, α -histidine hydrazide, camazine, methozylamine, pyridoxamine, and taurine) to affect protein carbonylation in rat brain slices subjected to glutathione depletion failed to demonstrate their effectiveness [[65]]. On the other hand, the use of trapping reagents during aldehyde enzymatic formation may result in the enzyme inhibition/modulation of its activity. This was the case when we attempted to trap S1P lyase-formed (2*E*)-hexadecenal with semicarbazide during enzymatic reaction that completely abolished the enzymatic activity [[28]]. We suggested to correct for (2*E*)-hexadecenal disappearance during the reaction as a result of its Phase I metabolism (Figure 27) by measuring the disappearance rate with a standard (2*E*)-hexadecenal added at the beginning of the reaction with all components present except for S1P [[28]].

7. SUMMARY AND FUTURE DIRECTIONS

There is no doubt that current technological achievements in mass spectrometry of lipid molecules in both GC/MS and LC/MS platforms ensure identification and quantitation of all short-chain and long-chain aldehydes at picomolar to femtomolar levels. The availability of choice between derivatives to use and methodology to be employed provides flexibility to accommodate differential access to either of the mass spectrometric platforms. Constant improvement in the availability of the appropriate standards further extends analytical capabilities. What is more challenging is to properly address the rate of free aldehyde generation and distinguish between forms that are free and bound to different nucleophilic targets. This question becomes of special importance due to recent discovery of 4-HNE and 4-hydroxy-2E,6Z-dodecadial direct signaling through the binding and activation of PPAR δ receptor which does not involve covalent modification of the protein [[66]–[68]]. This finding opens a new direction in fatty aldehyde research and demonstrates a novel functional role for this unique group of lipids thought to be just the scavengers (in the form of plasmalogens) of ROS, the end products of fatty acid oxidation, or the toxic molecules without any particular signaling function.

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Literature cited

1. Goldfine H. The appearance, disappearance and reappearance of plasmalogens in evolution. *Prog Lipid Res.* 2010; 49:493–498. [PubMed: 20637230]
2. Lessig J, Fuchs B. Plasmalogens in biological systems: their role in oxidative processes in biological membranes, their contribution to pathological processes and aging and plasmalogen analysis. *Curr Med Chem.* 2009; 16:2021–2041. [PubMed: 19519379]
3. Dembitsky VM, Vaskovsky VE. A quantitative distribution of plasmalogens in different phospholipid classes of marine invertebrates. *Biol Morya-VLAD.* 1976; 5:68–72.
4. Dembitsky VM. Plasmalogens in phospholipids of marine invertebrates. *Biol Morya-VLAD.* 1979; 5:86–90.
5. Kostetskii EY, Sergeyuk NN. The influence of seasonal factors on the content of phospholipids and their plasmalogen forms in muscles of marine invertebrates. *J Evol Biochem Phys.* 1986; 22:132–142.
6. Vaskovsky VE, Dembitsky VM. Determination of plasmalogen contents of phospholipid classes by reaction micro-thin-layer chromatography. *J Chromatogr.* 1975; 115:645–647. [PubMed: 1206073]
7. Ohshima T, Wada S, Koizumi C. 1-O-alk-1'-enyl-2-acyl and 1-O-alkyl-2-acyl glycerophospholipids in white muscle of bonito *Euthynnus-pelamis* (Linnaeus). *Lipids.* 1989; 24:363–370.
8. Medina I, Aubourg SP, Martin RIP. Analysis of 1-O-alk-1-enylglycerophospholipids of Albacore tuna (*Thunnus-alalunga*) and their alterations during thermal-processing. *J Agric Food Chem.* 1993; 41:2395–2399.
9. Misra KK, Shkrob I, Rakshit S, Dembitsky VM. Variability in fatty acids and fatty aldehydes in different organs of two prosobranch gastropod mollusks. *Biochem, Syst Ecol.* 2002; 30:749–761.
10. Nishida T, Vang VL, Yamazawa H, Yoshida R, Naka H, Tsuchida K, Ando T. Synthesis and characterization of hexadecadienyl compounds with a conjugated diene system, sex pheromone of the persimmon fruit moth and related compounds. *Biosci Biotechnol Biochem.* 2003; 67:822–829. [PubMed: 12784624]
11. Arn H, Stadler E, Rauscher S. Electroantennographic detector - selective and sensitive tool in gas-chromatographic analysis of insect pheromones. *Z Naturforsch C – J Biosci.* 1975; 30:722.

12. Hsu FF, Hazen SL, Giblin D, Turk J, Heinecke JW, Gross ML. Mass spectrometric analysis of pentafluorobenzyl oxime derivatives of reactive biological aldehydes. *Int J Mass Spectrom.* 1999; 187:795–812.
13. Spiteller G, Kern W, Spiteller P. Investigation of aldehydic lipid peroxidation products by gas chromatography-mass spectrometry. *J Chromatogr A.* 1999; 843:29–98.
14. Requena JR, Fu MX, Ahmed MU, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR. Quantification of malondialdehyde and 4-hydroxynonenal adducts to lysine residues in native and oxidized human low-density lipoprotein. *Biochem J.* 1997; 322:317–325. [PubMed: 9078279]
15. Esterbauer H, Ertl A, Scholz N. Reaction of cysteine with alpha,beta-unsaturated aldehydes. *Tetrahedron.* 1976; 32:285–289.
16. Stadelmann-Ingrand S, Wahl A, Favrelière S, Barbot F, Tallineau C. Quantification of long-chain aldehydes by gas chromatography coupled to mass spectrometry as a tool for simultaneous measurement of plasmalogens and their aldehydic breakdown products. *Anal Biochem.* 2000; 280:65–72. [PubMed: 10805522]
17. Ferreira V, Culleré L, Loscos N, Cacho J. Critical aspects of the determination of pentafluorobenzyl derivatives of aldehydes by gas chromatography with electron-capture or mass spectrometric detection: Validation of an optimized strategy for the determination of oxygen-related odor-active aldehydes in wine. *J Chromatogr A.* 2006; 1122:255–265. [PubMed: 16709416]
18. Andre A, Cabaret S, Berdeaux O, Juaneda P, Sebedio JL, Chardigny JM. Bioequivalence of docosahexaenoic acid and alpha-linolenic acid supplementations on plasmalogen, long-chain aldehyde, and docosahexaenoic acid levels in the brain of very old rats. *Nutr Res.* 2006; 26:214–220.
19. Fauconneau B, Stadelmann-Ingrand S, Favrelière S, Baudouin J, Renaud L, Piriou A, Tallineau C. Evidence against a major role of plasmalogens in the resistance of astrocytes in lactic acid-induced oxidative stress in vitro. *Arch Toxicol.* 2001; 74:695–701. [PubMed: 11218046]
20. Thomas MJ, Robison TW, Samuel M, Forman HJ. Detecting and identifying volatile aldehydes as dinitrophenylhydrazones using gas chromatography mass spectrometry. *Free Radic Biol Med.* 1995; 18:553–557. [PubMed: 9101246]
21. Stadelmann-Ingrand S, Favrelière S, Fauconneau B, Mauco G, Tallineau C. Plasmalogen degradation by oxidative stress: production and disappearance of specific fatty aldehydes and fatty alpha-hydroxyaldehydes. *Free Radic Biol Med.* 2001; 31:1263–1271. [PubMed: 11705705]
22. Loidl-Stahlhofen A, Spiteller G. α -Hydroxyaldehydes, products of lipid peroxidation. *Biochim Biophys Acta.* 1994; 1211:156–160. [PubMed: 8117742]
23. Guichardant M, Taibi-Tronche P, Fay LB, Lagarde M. Covalent modifications of aminophospholipids by 4-hydroxynonenal. *Free Radic Biol Med.* 1998; 25:1049–1056. [PubMed: 9870558]
24. Bacot S, Bernoud-Hubac N, Baddas N, Chantegrel B, Deshayes C, Doutheau A, Lagarde M, Guichardant M. Covalent binding of hydroxy-alkenals 4-HDDE, 4-HHE, and 4-HNE to ethanolamine phospholipid subclasses. *J Lipid Res.* 2003; 44:917–926. [PubMed: 12588949]
25. Bacot S, Bernoud-Hubac N, Chantegrel B, Deshayes C, Doutheau A, Ponsin G, Lagarde M, Guichardant M. Evidence for in situ ethanolamine phospholipid adducts with hydroxy-alkenals. *J Lipid Res.* 2007; 48:816–825. [PubMed: 17220481]
26. Grasse LD, Lamé MW, Segall HJ. In vivo covalent binding of trans-4-hydroxy-2-hexenal to rat liver macromolecules. *Toxicol Lett.* 1985; 29:43–49. [PubMed: 2417386]
27. Van Veldhoven PP, Mannaerts GP. Subcellular localization and membrane topology of sphingosine-1-phosphate lyase in rat liver. *J Biol Chem.* 1991; 266:12502–12507. [PubMed: 2061324]
28. Berdyshev EV, Goya J, Gorshkova I, Prestwich GD, Byun HS, Bittman R, Natarajan V. Characterization of sphingosine-1-phosphate lyase activity by electrospray ionization-liquid chromatography/tandem mass spectrometry quantitation of (2E)-hexadecenal. *Anal Biochem.* 2011; 408:12–18. [PubMed: 20804717]
29. Long EK, Picklo MJ Sr. Trans-4-hydroxy-2-hexenal, a product of n-3 fatty acid peroxidation: make some room HNE.... *Free Radic Biol Med.* 2010; 49:1–8. [PubMed: 20353821]

30. Benedetti A, Comporti M, Esterbauer H. Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. *Biochim Biophys Acta*. 1980; 620:281–296. [PubMed: 6254573]
31. Ferrali M, Fulceri R, Benedetti A, Comporti M. Effects of carbonyl compounds (4-hydroxyalkenals) originating from the peroxidation of liver microsomal lipids on various microsomal enzyme activities of the liver. *Res Commun Chem Pathol Pharmacol*. 1980; 30:99–112. [PubMed: 6254122]
32. Loidl-Stahlhofen A, Hannemann K, Spiteller G. Detection of short-chain alpha-hydroxyaldehydic compounds as pentafluorbenzyloxime derivatives in bovine liver. *Chem Phys Lipids*. 1995; 77:113–119. [PubMed: 7586088]
33. St-Germain F, Vachon B, Montgomery J, Des Rosiers C. Instantaneous analysis of aldehydes in biological fluids using a spray interface coupled to a mass spectrometer. *Free Radic Biol Med*. 1997; 23:166–172. [PubMed: 9165310]
34. Gioacchini AM, Calonghi N, Boga C, Cappadone C, Masotti L, Roda A, Traldi P. Determination of 4-hydroxy-2-nonenal at cellular levels by means of electrospray mass spectrometry. *Rapid Commun Mass Spectrom*. 1999; 13:1573–1579. [PubMed: 10421899]
35. Zanardi E, Jagersma CG, Ghidini S, Chizzolini R. Solid phase extraction and liquid chromatography-tandem mass spectrometry for the evaluation of 4-hydroxy-2-nonenal in pork products. *J Agric Food Chem*. 2002; 50:5268–5272. [PubMed: 12207460]
36. Alghazeer R, Howell NK. Formation of 4-hydroxynonenal (4-HNE) in frozen mackerel (*Scomber scombrus*) in the presence and absence of green tea. *J Sci Food Agric*. 2008; 88:1128–1134.
37. Deighton N, Magill WJ, Bremner DH, Benson EE. Malondialdehyde and 4-hydroxy-2-nonenal in plant tissue cultures: LC-MS determination of 2,4-dinitrophenylhydrazone derivatives. *Free Radic Res*. 1997; 27:255–265. [PubMed: 9350430]
38. Kolliker S, Oehme M, Dye C. Structure elucidation of 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds in ambient air by HPLC/MS and multiple MS/MS using atmospheric chemical ionization in the negative ion mode. *Anal Chem*. 1998; 70:1979–1985. [PubMed: 21651289]
39. Zwiener C, Glauner T, Frimmel FH. Method optimization for the determination of carbonyl compounds in disinfected water by DNPH derivatization and LC-ESI-MS-MS. *Anal Bioanal Chem*. 2002; 372:615–621. [PubMed: 11941429]
40. Andreoli R, Manini P, Corradi M, Mutti A, Niessen WM. Determination of patterns of biologically relevant aldehydes in exhaled breath condensate of healthy subjects by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2003; 17:637–645. [PubMed: 12661015]
41. Nagy K, Pollreis F, Takáts Z, Vékey K. Atmospheric pressure chemical ionization mass spectrometry of aldehydes in biological matrices. *Rapid Commun Mass Spectrom*. 2004; 18:2473–2478. [PubMed: 15384130]
42. Chi Y, Feng Y, Wen S, Lü H, Yu Z, Zhang W, Sheng G, Fu J. Determination of carbonyl compounds in the atmosphere by DNPH derivatization and LC-ESI-MS/MS detection. *Talanta*. 2007; 72:539–545. [PubMed: 19071652]
43. Esterbauer H, Zollner H. Methods for determination of aldehydic lipid peroxidation products. *Free Radic Biol Med*. 1989; 7:197–203. [PubMed: 2680787]
44. Peters R, Hellenbrand J, Mengerink Y, Van der Wal S. On-line determination of carboxylic acids, aldehydes and ketones by high-performance liquid chromatography-diode array detection-atmospheric pressure chemical ionisation mass spectrometry after derivatization with 2-nitrophenylhydrazine. *J Chromatogr A*. 2004; 1031:35–50. [PubMed: 15058566]
45. Zurek G, Karst U. Liquid chromatography-mass spectrometry method for the determination of aldehydes derivatized by the Hantzsch reaction. *J Chromatogr A*. 1999; 864:191–197. [PubMed: 10669286]
46. Williams TI, Lovell MA, Lynn BC. Analysis of derivatized biogenic aldehydes by LC tandem mass spectrometry. *Anal Chem*. 2005; 77:3383–3389. [PubMed: 15889933]

47. Williams TI, Lynn BC, Markesbery WR, Lovell MA. Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease. *Neurobiol Aging*. 2006; 27:1094–1099. [PubMed: 15993986]
48. O'Brien-Coker IC, Perkins G, Mallet AI. Aldehyde analysis by high performance liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2001; 15:920–928. [PubMed: 11400197]
49. Honzatko A, Brichac J, Murphy TC, Reberg A, Kubátová A, Smoliakova IP, Picklo MJ Sr. Enantioselective metabolism of trans-4-hydroxy-2-nonenal by brain mitochondria. *Free Radic Biol Med*. 2005; 39:913–924. [PubMed: 16140211]
50. Honzatko A, Brichac J, Picklo MJ. Quantification of trans-4-hydroxy-2-nonenal enantiomers and metabolites by LC-ESI-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007; 857:115–122.
51. Johnson DW. A modified Girard derivatizing reagent for universal profiling and trace analysis of aldehydes and ketones by electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2007; 21:2926–2932. [PubMed: 17676710]
52. Eggink M, Wijtmans M, Ekkebus R, Lingeman H, de Esch IJ, Kool J, Niessen WM, Irth H. Development of a selective ESI-MS derivatization reagent: synthesis and optimization for the analysis of aldehydes in biological mixtures. *Anal Chem*. 2008; 80:9042–9051. [PubMed: 19551932]
53. Eggink M, Charret S, Wijtmans M, Lingeman H, Kool J, Niessen WM, Irth H. Development of an on-line weak-cation exchange liquid chromatography-tandem mass spectrometric method for screening aldehyde products in biological matrices. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009; 877:3937–3945.
54. Eggink M, Wijtmans M, Kretschmer A, Kool J, Lingeman H, de Esch IJ, Niessen WM, Irth H. Targeted LC-MS derivatization for aldehydes and carboxylic acids with a new derivatization agent 4-APEBA. *Anal Bioanal Chem*. 2010; 397:665–675. [PubMed: 20238107]
55. Serra M, Saba JD. Sphingosine 1-phosphate lyase, a key regulator of sphingosine 1-phosphate signaling and function. *Adv Enzyme Regul*. 2010; 50:349–362. [PubMed: 19914275]
56. Zhu Q, Zheng ZP, Cheng KW, Wu JJ, Zhang S, Tang YS, Sze KH, Chen J, Chen F, Wang M. Natural polyphenols as direct trapping agents of lipid peroxidation-derived acrolein and 4-hydroxy-trans-2-nonenal. *Chem Res Toxicol*. 2009; 22:1721–1727. [PubMed: 19743801]
57. Albert CJ, Crowley JR, Hsu FF, Thukkani AK, Ford DA. Reactive chlorinating species produced by myeloperoxidase target the vinyl ether bond of plasmalogens: identification of 2-chlorohexadecanal. *J Biol Chem*. 2001; 276:23733–23741. [PubMed: 11301330]
58. Thukkani AK, McHowat J, Hsu FF, Brennan ML, Hazen SL, Ford DA. Identification of alpha-chloro fatty aldehydes and unsaturated lysophosphatidylcholine molecular species in human atherosclerotic lesions. *Circulation*. 2003; 108:3128–3133. [PubMed: 14638540]
59. Brahmbhatt VV, Hsu FF, Kao JL, Frank EC, Ford DA. Novel carbonyl and nitrile products from reactive chlorinating species attack of lysosphingolipid. *Chem Phys Lipids*. 2007; 145:72–84. [PubMed: 17126823]
60. Wildsmith RR, Albert CJ, Hsu FF, Kao JL, Ford DA. Myeloperoxidase-derived 2-chlorohexadecanal forms Schiff bases with primary amines of ethanolamine glycerophospholipids and lysine. *Chem Phys Lipids*. 2006; 139:157–170. [PubMed: 16417904]
61. Laurent A, Perdu-Durand E, Alary J, Debrauwer L, Cravedi JP. Metabolism of 4-hydroxynonenal, a cytotoxic product of lipid peroxidation, in rat precision-cut liver slices. *Toxicol Lett*. 2000; 114:203–214. [PubMed: 10713486]
62. Srivastava S, Conklin DJ, Liu SQ, Prakash N, Boor PJ, Srivastava SK, Bhatnagar A. Identification of biochemical pathways for the metabolism of oxidized low-density lipoprotein derived aldehyde-4-hydroxy trans-2-nonenal in vascular smooth muscle cells. *Atherosclerosis*. 2001; 158:339–350. [PubMed: 11583712]
63. Meyer MJ, Mosely DE, Amarnath V, Picklo MJ Sr. Metabolism of 4-hydroxy-trans-2-nonenal by central nervous system mitochondria is dependent on age and NAD⁺ availability. *Chem Res Toxicol*. 2004; 17:1272–1279. [PubMed: 15377162]

64. Doorn JA, Petersen DR. Covalent adduction of nucleophilic amino acids by 4-hydroxynonenal and 4-oxononenal. *Chem Biol Interact.* 2003; 143–144:93–100.
65. Zheng J, Bizzozero OA. Traditional reactive carbonyl scavengers do not prevent the carbonylation of brain proteins induced by acute glutathione depletion. *Free Radic Res.* 2010; 44:258–266. [PubMed: 20001647]
66. Coleman JD, Prabhu KS, Thompson JT, Reddy PS, Peters JM, Peterson BR, Reddy CC, Vanden Heuvel JP. The oxidative stress mediator 4-hydroxynonenal is an intracellular agonist of the nuclear receptor peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta). *Free Radic Biol Med.* 2007; 42:1155–1164. [PubMed: 17382197]
67. Riahi Y, Sin-Malia Y, Cohen G, Alpert E, Gruzman A, Eckel J, Staels B, Guichardant M, Sasson S. The natural protective mechanism against hyperglycemia in vascular endothelial cells: roles of the lipid peroxidation product 4-hydroxydodecadienal and peroxisome proliferator-activated receptor delta. *Diabetes.* 2010; 59:808–818. [PubMed: 20107107]
68. Riahi Y, Cohen G, Shamni O, Sasson S. Signaling and cytotoxic functions of 4-hydroxyalkenals. *Am J Physiol Endocrinol Metab.* 2010; 299:E879–E886. [PubMed: 20858748]

Highlights

- Fatty aldehyde analysis by gas chromatography mass spectrometry
- Fatty aldehyde analysis by liquid chromatography mass spectrometry
- Challenges during the analysis of free fatty aldehydes by mass spectrometry

Figure 1. General structure of plasmalogens

X – ethanolamine, choline, serine; R – C13–C23 aliphatic radical with 0–6 methylene-interrupted double bonds.

Figure 2. MS/MS fragmentation of aldehyde dimethylacetal derivatives during electron ionization (EI) GC/MS analysis.

Figure 3. Proposed fragmentation pathways for generation of fragment ions with m/z 84 and m/z 98 during EI-MS analysis of 4,6- and 5,7-diene aldehydes (adapted from [[10]])

Figure 4. MS/MS fragmentation of hexadecanal-PFBO (A) and the formation of the m/z 239 ion from PFBO derivatives of saturated aldehydes through McLafferty rearrangement.

Figure 5. Formation of Syn- and Anti- PFBO isomers during aldehyde derivatization with PFBHA,HCl.

Figure 6.

Generation of the ion with m/z 250 derived from PFBO derivative of 2-alkenals according to A. Loidl-Stahlhofen (adapted from [[13]]).

Figure 7.
MS/MS fragmentation of PFBO-TMS ether derivative of α -hydroxy-hexadecanal.

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Figure 9.
MS/MS fragmentation of PFBO-TMS derivative of 4-hydroxy-nonenal.

Figure 10.
2,4-Dinitrophenylhydrazone derivatives of selected aldehydes.

Figure 11.

Selected fragmentation sequences of aldehyde 2,4-dinitrophenylhydrazones identified by APCI(−) MS/MS (adopted from [[38],[39]]).

Figure 12.
Structure elucidation scheme for selected aldehyde 2,4-dinitrophenylhydrazone derivatives by negative mode APCI.

EDC - 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

Figure 13.

Generation of aliphatic aldehyde 2-nitrophenylhydrazone derivatives and the formation of ions with m/z 137 and m/z 199.

Figure 14.
Formation of aldehyde dimedone derivatives.

Figure 15.

(A) Formation of aldehyde cyclohexanedione derivatives. (B) Suggested mechanism for the formation of the $m/z\ 216$ ion from aldehyde cyclohexanedione derivatives.

Figure 16. (A) Formation of (S)- and (R)-4-hydroxy-nonenal (S)-carbidopa derivatives. (B) Suggested scheme of 4-HNE-(S)-carbidopa fragmentation during ESI(-) MS/MS analysis.

Figure 17.

Formation and MS/MS fragmentation of HTMOB aldehyde derivatives.

Figure 18.
Formation and characteristic fragmentation pattern of aldehyde 4-APC derivatives.

Figure 19.
Formation and MS/MS fragmentation of aldehyde 4-APEBA derivatives.

Figure 20.

Synthesis of aldehyde semicarbazone derivatives (reproduced from [[28]] with permission from Elsevier).

Figure 21.

Positive product ion mass spectra of fatty aldehyde semicarbazone derivatives. (A) (2E)-Hexadecenal semicarbazone product ions. (B) *cis*-11-Hexadecenal semicarbazone product ions. (C) Hexadecanal semicarbazone product ions. (D) Proposed pathways for product ion formation during (2E)-hexadecenal semicarbazone CID. cps, counts per second (reproduced from [[28]] with permission from Elsevier).

Figure 22.

Positive ion ESI-LC/MS/MS analysis of semicarbazone derivatives of cis-11-hexadecenal, (2E)-hexadecenal, (2E)-D5-hexadecenal, and hexadecanal. The MRM based on the $[M-17]^+$ transition is shown. The MRM profile for (2E)-D5-hexadecenal semicarbazone is shown as a dotted line (reproduced from [[28]] with permission from Elsevier).

Figure 23.
MS/MS fragmentation of PFBO derivative of 2-chloro-hexadecanal.

Figure 24.

Formation of (2E)-hexadecenal as a result of RCS attack on sphingosylphosphorylcholine. Similar mechanism is involved in the formation of (2E)-hexadecenal from sphingosine-1-phosphate.

Figure 25. 2-Chlorohexadecanal Schiff base formation with phosphatidylethanolamine and the route to identify the presence of 2-chlorohexadecanal-linked ethanolamine moiety.

Figure 26.
4-HNE biotransformation pathways.

Figure 27.

Disappearance of (2E)-hexadecenal during enzymatic reaction as a function of time and protein concentration. (2E)-Hexadecenal (20 pmol) was incubated with the indicated amounts of mouse liver microsomal protein in the reaction buffer. At the indicated time points, proteins were denatured by the addition of methanol and chloroform (2:1, v/v). (2E)-D5-Hexadecenal (20 pmol) was added as the internal standard before lipid extraction and phase separation. Total lipid extracts were derivatized with 5 mM semicarbazide in 5% formic acid in methanol. Data represent the averages $\pm$ standard errors of three different experiments (reproduced from [[28]] with permission from Elsevier).