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PAPER IN FOREFRONT

Direct quantitation and characterization of fatty acids in salmon tissue by condensed phase membrane introduction mass spectrometry (CP-MIMS) using a modified donor phase

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Abstract

Existing mass spectrometric methods for the analysis of fatty acids often require derivatization, chromatographic separations, and/or extensive sample preparation. Direct mass spectrometry strategies can avoid these requirements, but may also suffer from poor quantitation and/or lack of sensitivity. Condensed phase-membrane introduction mass spectrometry (CP-MIMS) provides direct quantitative measurements of analytes in complex samples with little or no sample preparation. CP-MIMS uses a semi-permeable membrane to transfer neutral, hydrophobic compounds from real-world samples to a mass spectrometer. The results presented utilize aqueous/organic sample solvent (donor) mixtures to allow for the sensitive (pptr) detection of a range of fatty acids. The relative sensitivity across a homologous series of fatty acids is observed to change, favoring short- or long-chain fatty acids, depending on the amount of miscible co-solvent added to the donor phase. Further, lithium acetate added online via the acceptor phase was used in tandem mass spectrometry experiments to determine the location of double bonds in polyunsaturated fatty acids (PUFAs). The method was applied to direct measurements and structural determinations for selected PUFAs in salmon tissue samples. Standard addition was employed to quantify the amount of PUFAs in a variety of salmon samples, yielding 0.27–0.42 and 0.40–0.84 *w/w* % for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively, for Sockeye and Chinook salmon, in good agreement with the literature. This work presents, to our knowledge, the first use of CP-MIMS for the direct analysis of fatty acids in oily foodstuff samples.

Keywords Long-chain fatty acids · Membrane introduction mass spectrometry · Salmon · Direct mass spectrometry · Omega-3 fatty acids · Fatty acid aggregation

Introduction

Fatty acid (FA) measurements with mass spectrometry have been widely used for a range of applications, including food security and authentication [1, 2], bacterial identification [3, 4], a bioindicator of chemical stresses in marine organisms [5], and clinical assessments in humans [6–8]. The measurement of omega-3 polyunsaturated essential FAs is of increasing relevance today due to reported health benefits [9, 10]. In particular, the aquaculture industry is struggling to meet the increased global feedstock demand for eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids derived from marine products for farmed salmon [11]. Traditional marine ingredients for farmed salmon diets (e.g., fishmeal, fish oil) are limited and have begun to be replaced by more abundant terrestrially sourced ingredients (e.g., vegetable oil). A study tracking the FA composition of over 3000 farmed Atlantic salmon from

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2006 to 2015 observed a decrease in FAs of marine origin (e.g., EPA and DHA) accompanied by an increase in FAs of terrestrial origin (e.g., octadecadienoic acid) [11]. It is also known that livestock diets can drastically affect the FA composition of dairy products [12], in fact, purposefully modified diets are increasingly being utilized to select for specific FA compositions [13, 14]. Traditional aquaculture and livestock feedstocks are being replaced as industries struggle to adapt to increased global demand for their products, or to purposefully change feed to manipulate nutritional composition. This highlights the need for simple, direct, and cost-effective methods of FA profiling in complex biological samples as the scope of this practice expands.

Modern day analysts have a breadth of new and innovative mass spectrometric techniques to choose from when analyzing lipids. Gas chromatography mass spectrometry (GC-MS) and other chromatographic methods remain the most used and reliable methods for the analysis of FAs to date [15]. Conventionally, gas chromatography (GC) coupled with electron ionization (EI) mass spectrometry (MS) has been used to address many of the challenges encountered with lipidomics throughout its development [16, 17]. However, because a GC separation is employed, it requires volatile, thermally stable analytes. FAs exhibit low volatilities, requiring derivatization to increase their vapor pressure prior to GC resolution. A popular method of FA analysis is the derivatization of FAs into fatty acid methyl esters (FAME) [18]. MS methods for the analysis of lipids have also been developed for high-performance and ultra-high-performance liquid chromatography HPLC/UHPLC-MS [8, 19–22] and supercritical fluid chromatography SFC-MS [2]. Many of these methods utilize tandem mass spectrometric techniques (MS/MS) to enhance both sensitivity and selectivity as well as increase the amount of structural information that can be obtained during analysis [15]. While these methods are very effective, they involve chromatographic separations and/or chemical derivatization, and require extensive sample preparation for solid samples, increasing the analysis time and cost while simultaneously decreasing sample throughput.

A variety of direct mass spectrometry methods have also been developed for FA analysis to overcome some of the limitations associated with chromatography (i.e., highly manual workflows, complex operation and maintenance, sample throughput limitations, high costs, as well as the need for extensive sample preparation to achieve sufficient detection sensitivity and specificity) [23]. Popular options for direct lipid analysis in the literature include “shotgun lipidomics” [24, 25], direct analysis in real time (DART) MS [1, 4], desorption electrospray ionization (DESI) imaging MS [6], and matrix-assisted laser desorption ionization (MALDI) MS [3, 26]. Although all are rapid analytical strategies, direct techniques based upon ESI can potentially suffer from matrix effects, resulting in lower sensitivity [27], and quantitative calibrations for methods such as DART, DESI and MALDI are still challenging [28–30]. This paper presents an alternative direct FA measurement approach that offers rapid and sensitive characterization and detection in complex biological samples without the need for sample handling, cleanup, derivatization, or chromatography: condensed phase - membrane introduction mass spectrometry (CP-MIMS) [31–40].

CP-MIMS is a simple and direct, online technique that allows for the continuous analysis of analytes in complex mixtures in situ [31, 32, 40]. Frequently, a semipermeable hollow fiber membrane (HFM) is used to separate and pre-concentrate neutral compounds from complex sample matrices and transfer them to an electrospray ionization (ESI) mass spectrometer via a condensed (liquid) acceptor phase that is continuously flowed through the HFM lumen. CP-MIMS does not employ a chromatographic separation step, and therefore must resolve analytes with the MS, either by MS/MS, accurate mass and/or selective ionization [40]. Unfortunately, for underivatized FAs, negative ion ESI MS/MS fragmentations do not yield usable structural information, and cationization prior to MS/MS is one strategy to generate useful structural information [15, 41]. Additionally, FAs have very low water solubilities [42] and high surface activities which can drive aggregation phenomena such as dimer and lamellar aggregate formation well below the critical micelle concentration [43, 44], potentially inhibiting their detection using CP-MIMS in aqueous samples.

For the analysis of FAs in complex samples by CP-MIMS, polydimethylsiloxane (PDMS) is a good membrane material choice. PDMS is a robust and inexpensive membrane substrate that is permselective for small (< 500 Da), neutral, hydrophobic molecules, but impermeable to ionized matrix components and particulate matter. This presents a key advantage when analyzing FAs in complex biological sample matrices. In the case of carboxylic acids such as FAs, it is the neutral form that is membrane permeable; therefore, the pH of the sample is adjusted to < 4, prior to analysis. This is well below their pKa values (Table 1), yielding protonated (neutral) carboxylic acids for CP-MIMS detection, consistent with other published results [47]. In a typical CP-MIMS measurement, an aqueous sample/suspension (the donor phase) is exposed to the PDMS HFM, and mass transport across the membrane is driven by a concentration gradient, maintained by continuously sweeping permeating analytes away from the backside of the membrane in the flowing acceptor phase. This process occurs in a series of concerted steps: 1) adsorption of the analyte(s) on the outer surface (sample side) of the membrane, 2) diffusion of the analyte(s) through the membrane, and 3) solvation from the inner membrane surface into the flowing acceptor phase [31, 40].

In a typical CP-MIMS measurement, aqueous samples are commonly paired with a methanolic acceptor phase to facilitate partitioning into and out of the HFM for hydrophobic analytes. However, different solvents have been shown to interact with the PDMS membrane and significantly affect

Table 1 Physicochemical properties for the target FA analytes

Target analyte	MW/g mol^{-1} [a]	Vapor pressure/Pa @ 25 °C [a]	Estimated log K_{ow} [a]	pK_a [b]
(12:0) Dodecanoic acid	200.318	1.88×10^{-1}	5.00	4.95
(14:0) Tetradecanoic acid	228.371	3.47×10^{-2}	5.98	4.95
(16:0) Hexadecanoic acid	256.424	7.36×10^{-3}	6.96	4.95
(18:0) Octadecanoic acid	284.477	1.11×10^{-3}	7.94	4.95
(20:5) Eicosapentaenoic acid	302.451	1.69×10^{-5}	7.85	4.82
(20:0) Eicosanoic acid	312.530	1.93×10^{-2}	8.93	4.95
(22:6) Docosahexaenoic acid	328.488	2.44×10^{-6}	8.62	4.89
(22:0) Docosanoic acid	340.584	6.52×10^{-5}	9.91	4.95

[a] ChemSpider [45]

[b] Human Metabolomics Database [46]

membrane transport characteristics [37, 48, 49]. In early, unpublished work by our group, we observed that CP-MIMS was satisfactory for the qualitative and quantitative determination of short to medium chain length FAs (i.e., C6-C12), but exhibited poor sensitivity for longer chain FAs (i.e., > C14). This greatly limited the applicability of the technique for direct measurement of long chain FAs, many of which are both biologically and nutritionally relevant. The work presented here demonstrates that simple modifications of the sample solution through the addition of a miscible co-solvent such as methanol, greatly improves the performance characteristics for longer chain FAs (> C14).

To our knowledge, this manuscript reports the first use of methanol modified samples (donor phases) with CP-MIMS for the direct detection and quantitation of FAs. Included is a systematic investigation of the phenomenon for a homologous series of saturated FAs (satFAs), and the direct quantitation and structural verification via online cationization of two essential polyunsaturated FAs (PUFAs), eicosapentaenoic acid (20:5, EPA) and docosahexaenoic acid (22:6, DHA), in base saponified salmon tissue samples.

Materials and methods

Reagents

Certified FA reference standards of dodecanoic acid (12:0), tetradecanoic acid (14:0), hexadecanoic acid (16:0), octadecanoic acid (18:0), eicosanoic acid (20:0), docosanoic acid (22:0), and 2,2-dodecanoic acid-d_2 were obtained from Sigma-Aldrich (Oakville, ON, CAN). Eicosapentaenoic acid (20:5) (EPA) and docosahexaenoic acid (22:6) (DHA) were obtained from Cayman Chemical (Ann Arbor, MI, USA). Lithium acetate, barium acetate, sodium hydroxide and hydrochloric acid (ACS grade) were purchased from Fisher Scientific (Ottawa, ON, CAN) and HPLC grade methanol was obtained from VWR International (Mississauga, ON, CAN). Deionized (DI) water (18 MΩ·cm) was prepared using a water purification system (model MQ Synthesis A10, Millipore Corp., Billerica, MA, USA).

Preparation of standard stock solutions and samples

Standard stock solutions were prepared gravimetrically in methanol either as a combined suite (satFAs), or individually in the case of EPA and DHA, storing them in sealed 40-mL glass vials (EPA/VOA Type, Scientific Specialties Inc., Hanover, MD, USA) at −4 °C in the dark. For all experiments, a *ca* 5 ppb solution of 2,2-dodecanoic acid-d_2 in methanol, was used as the CP-MIMS acceptor phase. The online addition of labeled dodecanoic acid present in the acceptor phase allows for direct observation of any deviations in ionization efficiency and instrument signal drift, and analyte signals are corrected by normalizing against the labeled dodecanoic acid to extend the linear dynamic range [39]. Table 1 outlines the physicochemical properties of the targeted FAs.

Aqueous standard solutions were gravimetrically prepared from methanolic stocks using DI water in 40-mL glass vials, ensuring that the final methanol concentration in aqueous samples was kept below 0.25% by mass. A five decimal place analytical balance (Model AS 60/220/C/2, Radwag Balances and Scales, Radom, Poland) was used for all mass determinations. For MeOH:H_2O donor phase standards and samples, methanol and DI water composition was prepared volumetrically. All samples were thoroughly mixed and stored at 4 °C in the dark prior to measurement. All concentrations are expressed as mass ratios (μg/kg, ppb), with the exception of the satFA solution prepared at equimolar concentrations (160 μM).

Fish tissue samples (Vancouver Island, BC, CAN) were prepared from seafood products purchased from local supermarkets (Sockeye salmon, *Oncorhynchus nerka*, and Steelhead trout, *Oncorhynchus mykiss irideus*) or caught during sport fishing by one of the authors (Chinook salmon, *Oncorhynchus tshawytscha*). Microwave-assisted, base saponified salmon tissue samples were prepared in 40-mL glass vials by adding the sample

(*ca* 1 to 10 mg) to 20 mL of water, followed by a drop of 6 M aqueous NaOH such that the pH was > 10, verified using universal indicator pH strips (McolorpHast, Millipore Corporation, Billerica, MA, USA). The alkaline sample was then heated using a conventional microwave oven (1350 W, Model: SLMW921, Osram Sylvania Inc., Mississauga, ON, CAN), heating the loosely capped 40 mL glass vials for 4.5 min at 10% power to aid in saponification of the lipids. Following this, 20 mL of methanol was then added to the sample to make a 50:50 MeOH:H_2O (*%v/v*) sample (donor) solution. Prior to CP-MIMS measurements, samples were pH adjusted to < 4 with 6 M aqueous HCl to ensure the FAs were present as protonated (neutral) carboxylic acids, facilitating their permeation through the PDMS membrane.

CP-MIMS interface

A syringe pump (Fusion 100, Chemyx Inc., Stafford, TX, USA) equipped with a 10-mL gastight syringe (Hamilton Corporation, Reno, NV, USA) was used to deliver the methanolic acceptor phase at 75 μL/min, passing it through a 20-μm inline filter (Upchurch Scientific A-313, Oak Harbor, WA), then through the interior of a PDMS HFM with a wall thickness of 0.17 mm (0.30 mm I.D., 0.64 mm O.D., 2.2 cm length, Silastic® tubing, Dow Corning, Midland, MI, USA) mounted on a CP-MIMS immersion probe. The membrane mounting and design of the CP-MIMS probe has been discussed in detail previously [38, 39]. Polyether ether ketone (PEEK) tubing (O.D. 1/16″) was purchased from Chromatographic Specialties (Brockville, ON, CAN) and was used to couple the flowing methanolic acceptor phase to the ESI source. Blue PEEK tubing (I.D. 0.010″) was used between the syringe, filter, and probe. Yellow PEEK tubing (I.D. 0.007″) was used between the probe and grounding connection of the mass spectrometer. Red PEEK tubing (I.D. 0.005″) was connected between the grounding connection on the MS and the ESI source.

Mass spectrometry

The mass spectrometer used for all experiments was a triple quadrupole ESI system (Q-Sight 220 LC/MS/MS, PerkinElmer, Boston, MA, USA). Nitrogen gas (UHP grade, Praxair, Nanaimo, BC, CAN) was used as the drying, nebulizer, and collision gas. All experiments used the following conditions: Hot Source Induced Desolvation (HSID) at 320 °C, unit mass resolution, drying gas at 60 psi, nebulizer gas at 90 psi.

All full scan mass spectrometry experiments used negative ion ESI, a mass range of 190–350 *m/z*, scan step size of 0.5 *m/z*, total scan time of 1.61 s. Selected ion monitoring (SIM) detection also utilized negative ion ESI with a total scan time of 1.00 s. Positive ion ESI-MS/MS and standard addition experiments for the structural verification and quantification of EPA and DHA were conducted by adding 5 mM or lithium acetate or 0.1 mM barium acetate and 5 ppb dodecanoic acid-d_2 to the methanolic acceptor phase with 2.5% H_2O (*%v/v*). The optimized MS scan parameters are given in Table 2 and in the Electronic Supplementary Material (ESM).

Data analysis

All data was background subtracted, and six-point moving boxcar smoothing was applied to all ion signal chronograms. Analyte signals were corrected for any ionization suppression and instrument drift by calculating their relative response to the continuously infused labeled dodecanoic acid using Eq. (1):

$$\text{Relative Response} = \frac{(\text{Analyte Signal})}{(\text{Dodecanoic Acid}{-}_{d2}\text{Signal} \div \text{Dodecanoic}{-}_{d2}\text{Concentration})} \quad (1)$$

All mass spectra, calibration curves, and SIM experiments were averaged from a minimum of 100 steady state and background scans. Measurements were recorded in triplicate, and uncertainties are reported as the standard deviation.

Results and discussion

Membrane transport studies

In a CP-MIMS measurement, Fick's law can be used to describe the steady-state mass transfer of an analyte through an ideal one-dimensional membrane barrier [50]:

$$J = \frac{C_s K_{m-s} D_m}{l} \quad (2)$$

where J is the analyte flux, C_s represents the free (non-aggregated) concentration of the analyte in the sample, $K_{m\text{-}s}$ the relative solubility of the analyte between the membrane and the sample, D_m is the diffusivity of the analyte in the membrane, and l is the membrane thickness. Membrane permeability is the product of $K_{m\text{-}s}$ and D_m.

The signal intensity for a permeating analyte in a CP-MIMS experiment increases with time until it reaches a

Table 2 Target FA analyte MS scan parameters

Fatty acids and MS scans used	*m/z*	Capillary voltage/V	Entrance voltage/V	Collision energy/eV
(12:0) SIM + Full Scan	199	− 4000	− 20	–
(14:0) SIM + Full Scan	227	− 4000	− 20	–
(16:0) SIM + Full Scan	255	− 4000	− 20	–
(18:0) SIM + Full Scan	283	− 4000	− 20	–
(20:0) SIM + Full Scan	311	− 4000	− 20	–
(22:0) SIM + Full Scan	339	− 4000	− 20	
(20:5) SIM + Full Scan	301	− 4000	− 20	–
(22:6) SIM + Full Scan	327	− 4000	− 20	–
(20:5) MS/MS + Product Scan w/ 5 mM Lithium Acetate	315 ➔ 179 315 ➔ 72	+ 3500	87	− 33
(22:6) MS/MS + Product Scan w/ 5 mM Lithium Acetate	341 ➔ 165 341 ➔ 72	+ 3500	87	− 35
(12:0) Dodecanoic Acid-d_2 MS/MS + Product Scan w/ 5 mM Lithium Acetate	215 ➔ 73	+ 3500	87	− 31

steady-state (equilibrium) signal. The signal intensity at steady state (S_{ss}) also depends on the ionization efficiency (*IE*) in the ESI source, therefore at steady state [37]:

$$S_{ss} \propto C_s\, K_{m-s}\, D_m\, IE \tag{3}$$

where the proportionality constant is related to geometric features of the capillary hollow fiber membrane [51].

The time required for the signal to reach steady-state depends on the square of the membrane thickness (*l*) and is inversely related to the diffusivity of the permeant. Therefore, the time required for the signal to increase from 10% to 90% of the steady-state signal, $t_{10–90\%}$, is given by [51]:

$$t_{10–90\%} \propto \frac{l^2}{D_m} \tag{4}$$

Early attempts to measure a wide range of FAs in aqueous samples with CP-MIMS proved unsuccessful for larger FAs (> C14). Despite strong signals for a satFA test suite (C12–C22) measured by direct infusion in methanol (Fig. 1a), we observed little or no signal for FAs larger than C14 present in water samples analyzed by CP-MIMS (Fig. 1b). To illustrate the initial FA CP-MIMS analysis problem, an aqueous sample of the target satFA suite was prepared at low concentrations (*ca* 65 ppb each) and analyzed by CP-MIMS in full scan mode. Figure 1b clearly shows that the relative signal intensities obtained for the CP-MIMS measurement of the homologous satFA series drops off significantly for satFAs larger than tetradecanoic acid (14:0) in an aqueous solution. Comparing this result with the target satFA suite prepared at the same bulk concentration in a 50:50 MeOH:H_2O (*%v/v*) sample mixture (Fig. 1c), illustrates a very different result with the response to shorter chain FAs dropping off dramatically relative to longer chain FAs, such as octadecanoic acid (18:0). These results demonstrate that the addition of a methanol co-solvent to the sample has dramatically improved the sensitivity of CP-MIMS for a wider range of FAs.

We initially attributed the poor analytical performance of larger FAs measured in aqueous samples by CP-MIMS to reduced permeability through the membrane, or a reduced free FA solution phase concentration in the sample due to some molecular aggregation phenomena, which would be expected to be more pronounced for longer chain (more hydrophobic) FAs [44]. For example, if the larger FAs were forming micelles in aqueous solution, the free concentration in solution (C_s) would be significantly lower than that predicted by the bulk mixing ratios and could thus explain the lack of detection in CP-MIMS experiments in aqueous samples. Micelle formation in aqueous samples at the pH and concentration levels examined in this work was not supported by pyrene spectrofluorimetry studies (ESM Fig. S1) [52]. Although we were not able to be probe other pre-micellar aggregation assemblies such as lamellar, or FA crystal micro-phases noted by others [43, 44], these aggregation phenomena may still be important.

It is clear that altering the donor solution composition by adding methanol yields improved analytical performance of CP-MIMS for longer chain FAs. If the poor performance in water samples was due to reduced membrane permeability, the explanation would come down to a decrease in either or both of K_{m-s} and D_m across the saturated C12-C22 FA series. While we can reasonably expect larger FAs to have a lower diffusivity due to a greater molar volume, we also would expect that they will have a greater driving force to partition into PDMS membrane due to their greater hydrophobicity (log K_{ow} values). In support of this assertion, we do observe a modest increase (~ twofold) in the $t_{10–90\%}$ signal rise times from C12

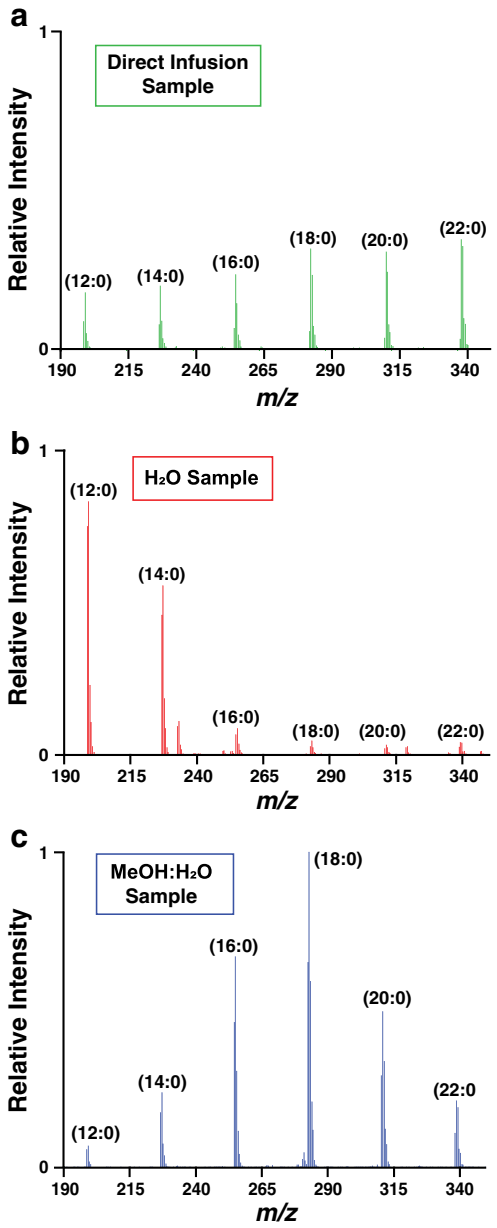

Fig. 1 Full scan, background subtracted mass spectra for **a** direct infusion measurement of the satFA suite in methanol (no membrane used); **b** CP-MIMS measurement of an aqueous sample of the satFA suite; **c** CP-MIMS measurement of a 50:50 MeOH:H_2O (*%v/v*) sample (donor) composition. All signal intensities were normalized relative to the (18:0) peak in the 50:50 MeOH:H_2O (*%v/v*) sample ($n = 3$)

to C22 FAs (ESM Fig. S2). Adding a methanol co-solvent to the sample decreases the rise times for all FAs, consistent with the greater amount of MeOH dissolved in the PDMS, making it more permeable, leading to greater diffusivities. At the same time, increasing the organic character of the sample will decrease the activity of hydrophobic analytes and thus be expected to decrease partitioning (lower $K_{m\text{-}s}$).

Using experimental data for the steady-state signal intensity and the signal rise times, we compare performance characteristics for the series of satFAs in 50:50 MeOH:H_2O to that in

Table 3 Comparison of satFA measurements in 50:50 MeOH:H_2O and in H_2O donor (sample) solvents

Analyte	$S_{ss}^{50:50}$ / S_{ss} H_2O	$D_m^{50:50}$ / D_m H_2O	$(C_s{*}K_{m\text{-}s})^{50:50}$ / $(C_s{*}K_{m\text{-}s})$ H_2O a
(12:0)	0.068	2.93	0.020
(14:0)	0.50	2.49	0.20
(16:0)	10	1.98	5.0
(18:0)	54	1.64	33
(20:0)	23	1.52	15
(22:0)	8.4	1.25	7.0

[a] $C_s{*}K_{m\text{-}s}$ ratio calculated from S_{ss} / D_m using Eq. (3)

100% H_2O in Table 3. It is evident that adding 50% MeOH decreases S_{ss} intensity for C12 and C14 FAs while substantially increasing it for the longer chain FAs. The $t_{10\text{–}90\%}$ rise times for the satFAs in these two solvent systems are included in Fig. S2 (see ESM). Since the rise time is inversely proportional to the diffusivity (Eq. 4), we can use the relative rise times to determine how much of the signal intensity improvement is due to changes in D_m. While adding MeOH to the sample clearly increases the diffusivity, the data shows that this effect is more pronounced for the smaller FAs and does not explain the marked improvements in signal observed for the longer chain FAs. Thus, the increase in S_{ss} must be due to increases in *IE*, C_s or $K_{m\text{-}s}$. It is reasonable to assume that the *IE* between the two sample types is constant given that the analyte is ionized from a methanol acceptor phase in either case. Therefore, the increases in S_{ss} in the 50:50 MeOH:H_2O (*%v*/v) samples relative to aqueous samples must be due to increases in the product of C_s x $K_{m\text{-}s}$. Considering that the activity of FAs (and therefore the $K_{m\text{-}s}$) is higher in 100% water samples compared to 50:50 MeOH:H_2O (*%v/v*) samples, we conclude that the substantial increases in S_{ss} for the longer chain FAs are due primarily to a significant increase in free solution phase concentration of FAs (C_s) with minor contributions from increased D_m.

To further examine the influence of the methanol co-solvent in CP-MIMS upon FA measurements, a series of satFA standards were prepared as a suite at equimolar concentrations (*ca* 160 μM) in various MeOH:H_2O sample solvent compositions, analyzing each by CP-MIMS. The results of this parametric investigation are summarized in Fig. 2, which presents the relative response for each FA for the various MeOH:H_2O sample solvent compositions, normalized to the maximum FA signal observed in each case. The results suggest that each FA chain length has an optimum donor solvent composition, with shorter chain FAs favoring higher water content samples, and longer chain FAs demonstrating optimal measurement at increasing methanol compositions. This systematic investigation is consistent with the expected trend in the critical aggregation threshold concentrations. We propose therefore, that the sample composition can be adjusted depending upon the target MW range desired, or an intermediate methanol sample composition can be used to detect a range of

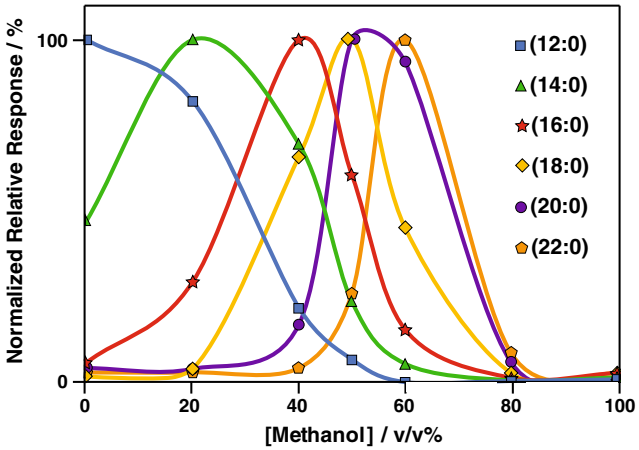

Fig. 2 Normalized relative responses for the CP-MIMS measurement of the satFA suite at equimolar concentrations in various MeOH:H_2O sample solvent compositions

FAs, provided that analyte calibrations are done in the same solution, or directly in the sample by standard additions.

Literature on the phase behavior of FAs in water demonstrates that micelle formation generally occurs only at higher pH values, while low pH values (such as the levels employed in the presented CP-MIMS measurements) tend to favor the distribution of FAs into two phases once the critical aggregation concentration is reached: FAs in the aqueous phase and FA crystal aggregates [43, 44]. Although the concentration levels analyzed in these studies are in the low ppb range, water solubilities of satFAs are incredibly low, and decrease markedly as chain length increases. The results from Fig. 2 support the presumption of FA aggregation in aqueous samples. As methanol is added to the aqueous sample, these aggregations are broken down, yielding free FAs in solution. Uniquely, the C12 satFA signal is highest in water, and decreases upon the addition of methanol: it is presumed that this FA is below its critical aggregation concentration and exists solely as FAs in solution. Therefore, adding methanol to the system lowers the activity of the analyte and thus the overall membrane transport, since $K_{m\text{-}s}$ is decreasing with increasing methanol. Critical aggregation concentrations available in the literature are conflicting, but Fig. 2 suggests that the longer chain FAs (C > 12) are forming aggregates in aqueous samples that are broken down to yield free FAs upon the addition of methanol. Higher methanol concentrations are required to break up the aggregates of longer chain FAs. Increasing the amount of methanol will act to increase analyte signal by breaking up these aggregates and increasing the free FA concentration in solution, up to a certain maximum. After the maximum is reached the reductions to $K_{m\text{-}s}$ outweigh the increases in free FA concentration (C_s) gained and the signal starts to decrease. Based upon these results, a 50:50 MeOH:H_2O (%*v*/*v*) sample composition was used for all subsequent work in order best measure a wide range of FAs with CP-MIMS.

Table 4 Summary of CP-MIMS direct calibration results obtained for satFAs and PUFAs prepared in 50:50 MeOH:H_2O (%*v*/v) donor (sample) solvent

Fatty acid	Equation	R^2	Detection Limit [a] / pptr
(12:0)	y = 0.00924(x) + 0.0158	0.997	1700
(14:0)	y = 0.0293(x) + 0.0945	0.996	330
(16:0)	y = 0.0628(x) + 0.725	0.997	230
(18:0)	y = 0.0698(x) + 0.490	0.998	130
(20:0)	y = 0.0678(x) – 0.00936	1.000	570
(22:0)	y = 0.0431(x) + 0.0976	0.995	420
(20:5)	y = 0.172(x) + 0.225	1.000	330
(22:6)	y = 0.208(x) + 0.197	0.996	240

[a] S/*N* = 3

Direct FA quantitation

To evaluate the direct, quantitative measurement capabilities of CP-MIMS utilizing a modified donor phase, a series of linear calibration curves were generated for all target analytes

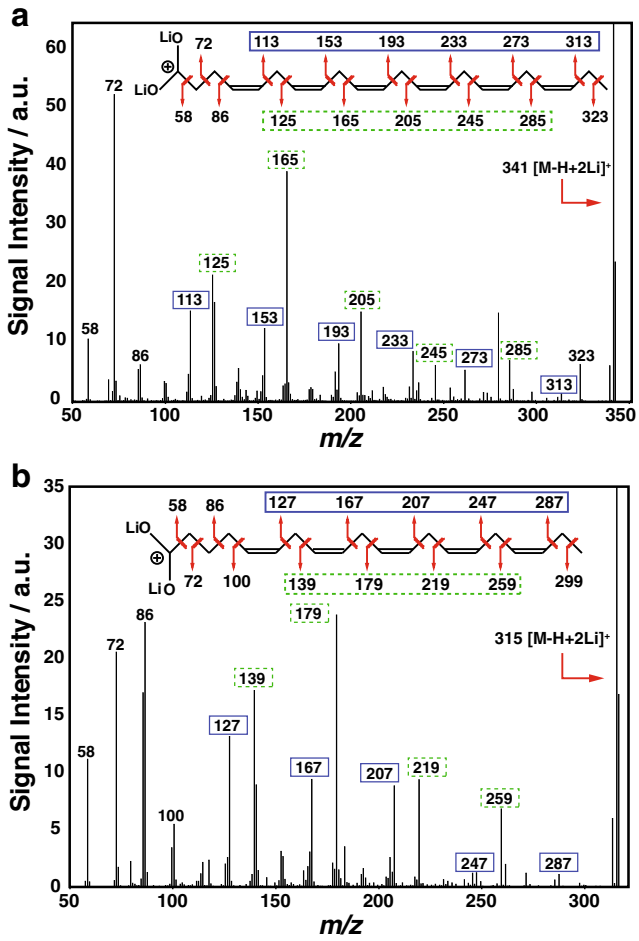

Fig. 3 Positive ion ESI MS/MS spectra obtained for dilithiated **a** DHA and **b** EPA standards, obtained by the direct infusion of 200 ppb methanol standards with 5 mM lithium acetate

from 500 pptr to 100 ppb (5 data points, 3 replicates), including the satFAs as well as EPA and DHA. All calibration standards were prepared in 50:50 MeOH:H_2O (*%v/v*) sample solvent, and all signals obtained were normalized against the signal obtained for the continuously infused dodecanoic acid-d_2 present in the acceptor phase. Good linearity was achieved in all cases ($R^2 \geq 0.995$) with detection limits in the parts per trillion range for all FAs except C12 (Table 4). Representative calibration curves are given in the ESM (Fig. S3). Direct calibration curves for EPA and DHA in 50:50 MeOH:H_2O (*%v/v*) using lithium cationization and MS/MS (ESM Fig. S4) were generated using gravimetrically prepared standards, and the same MS/MS conditions used in both the standard addition and product scan experiments.

Cationization and tandem mass spectrometry for PUFA structural verification

FAs in biological samples generally exist as the deprotonated carboxylate anion or are esterified to various lipid types, wax esters, as well as cholesterol esters among others [53]. Analysis of FAs using ESI can measure free FA content, which can be used as a method to determine the degree of food spoilage, or for total FA content by using saponification to liberate the FAs [53]. Determination of double bond location in FAs is important in order to differentiate and characterize different lipid profiles, especially important when using CP-MIMS because of the absence of any chromatographic separation.

The collision-induced dissociation (CID) fragmentation of underivatized FAs using negative ion ESI-MS/MS generates both charge-remote and charge-mediated fragmentations, leading to confounding mass spectra (and a lack of interpretable structural information) [41, 54, 55]. One strategy to increase the amount of information given in FA MS/MS spectra is to encourage charge-remote fragmentation (CRF) while inhibiting charge-mediated fragmentation (CMF), which can be accomplished through cationization [41, 54, 55]. Metal ion cationization is frequently employed for lipid structural analysis using positive ion ESI-MS/MS because CRF of FAs occurs without carbon skeletal rearrangements. This produces cleaner and more detailed product ion spectra, allowing for improved structural characterization. Barium acetate cationization has previously been demonstrated using CP-MIMS to be an effective, online cationization agent for the selective determination of naphthenic acids in aqueous samples by CP-MIMS [56]. For the presented work, we investigated the use of both Ba^{2+} and Li^+ for FA measurement and structural determinations. We observed that for the structural determinations of EPA and DHA, Li^+ cationization gave superior performance, with cleaner product ion spectra (Fig. 3). For comparison, the results for Ba^{2+} cationization are given in the ESM (Fig. S5).

A proposed pericyclic mechanism for the MS/MS fragmentation pattern for dilithiated PUFAs using low energy

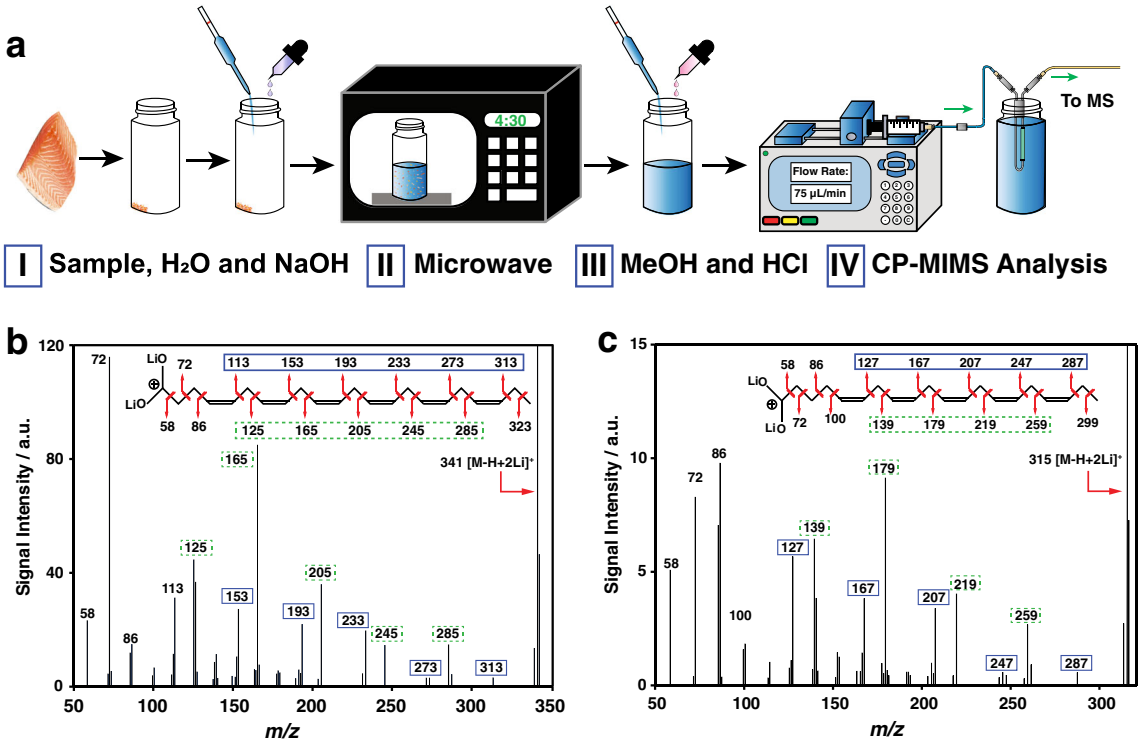

Fig. 4 **a** Workflow for the direct CP-MIMS measurement and identification of DHA and EPA in salmon tissue samples prepared in 50:50 MeOH:H_2O (*%v/v*). Product ion MS/MS spectra for a saponified 4-mg Chinook salmon sample directly analyzed by CP-MIMS using a 5 mM lithium acetate methanolic acceptor phase; **b** docosahexaenoic acid (DHA); **c** eicosapentaenoic acid (EPA)

CID has been described in detail by Gross et al. [41, 55, 57]. Figure 3a gives the MS/MS product ion spectrum of dilithiated DHA, obtained for the direct infusion of a 200 ppb methanol standard (without the CP-MIMS membrane). The *m/z* values outlined in the lower dashed line box (125, 165, 205, 245, and 285 *m/z*) can be described by the distal allyl-vinyl bond cleavage mechanism proposed in the literature [41, 55, 57] and the *m/z* values outlined in the upper solid box (113, 153, 193, 233, 273, and 313 *m/z*) can be described by the proximal allyl-vinyl bond cleavage mechanism. Figure 3b shows a similar MS/MS spectrum for dilithiated EPA (direct infusion, 200 ppb), again with ions resulting from distal allyl-vinyl bond cleavages outlined with a dashed line box (139, 179, 219, 259 *m/z*) and ions from proximal allyl-vinyl bond cleavages outlined with a solid line (127, 167, 207, 247, 287 *m/z*). Proximal allyl-vinyl bond cleavages are separated from distal allyl-vinyl bond cleavages by 12 Da, and ions that fragment according to the same mechanism are separated by 40 Da, peaks separated by 28 Da indicate the position of the double bond and thus allow for the structural determination of EPA and DHA. It should be noted that the parent peak $[M\text{-}H + 2Li]^+$ is off the scale given on Fig. 3 (and Fig. 4). Although the presented method can determine double bond location, it is unable to distinguish between geometric isomers. A recently published ESI method has reported the use of online derivatization coupled with UV irradiation for this purpose [58]. If needed, our presented method would also be amenable to this approach.

Direct PUFA characterization in complex samples: base saponified salmon tissue

A significant advantage of CP-MIMS is the ability to make direct measurements in complex, heterogenous samples. This is because the membrane interface rejects ionized matrix components and particulate matter that may otherwise lead to significant ionization suppression (or fouling) in the ESI source [31]. To demonstrate the potential use of CP-MIMS for the direct structural confirmation and quantitative measurements of PUFAs in complex, real samples, freshly saponified Chinook salmon tissue samples were evaluated. Samples can be analyzed without digestion to evaluate unbound, free FA content, or saponified before analysis to probe total FA content. For these studies, salmon samples were prepared using microwave assisted base saponification, with the workflow represented graphically in Fig. 4a. The salmon digest is a highly complex sample, full of cellular debris, very high salt levels (especially following acid/base addition) and other matrix components that would significantly suppress ESI signals, yet it can be directly analyzed by CP-MIMS. In order to generate MS/MS spectra for PUFAs present in the salmon sample, 5 mM of lithium acetate was added to the CP-MIMS methanolic acceptor phase to facilitate online dilithiation, without adding the cationization reagent to the sample. Positive ion CP-MIMS MS/MS spectra of EPA (20:5) and DHA (22:6) present in a *ca* 4 mg saponified salmon sample are given in Fig. 4, confirming their structures via double bond position in an analogous manner to the results present in Fig. 3.

Quantitation of PUFAs in salmon samples

To demonstrate the potential for direct, quantitative PUFA measurements in complex samples, freshly saponified salmon flesh samples were analyzed by the method of standard additions (four points) using CP-MIMS with online lithium cationization and MS/MS to quantify the amount of EPA and DHA present. Figure 5 illustrates the signal chronogram observed for the successive addition of 7.5 μL spikes of EPA (spike level = 696 ppb) to a freshly prepared 8.85 mg (wet mass) saponified Chinook salmon sample, as well as the corresponding standard additions plot. Signal response was relative to that obtained for the online dodecanoic acid-d_2 (5 ppb) present in the acceptor phase. Using this data, the Chinook

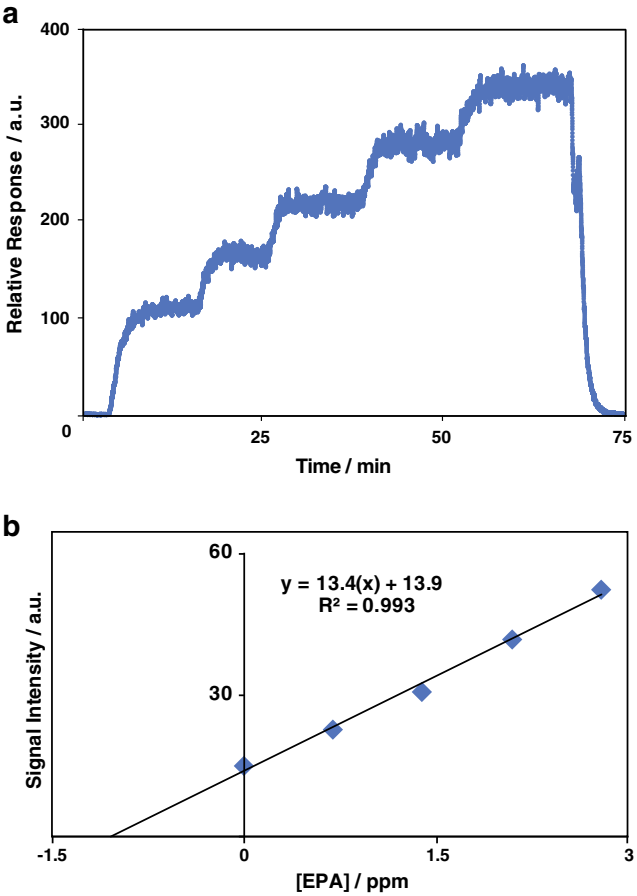

Fig. 5 **a** Signal chronogram for EPA standard additions measurement in saponified Chinook salmon sample prepared in 50:50 MeOH:H_2O (*%v/v*) analyzed by CP-MIMS MS/MS using a methanolic acceptor phase with 5 mM lithium acetate cationization reagent and 5 ppb dodecanoic acid-d_2; **b** Standard additions plot generated from (**a**)

salmon sample was determined to have a final solution concentration of EPA of 1.04 ± 0.05 ppm (relative standard error determined using eq. 5 of the ESM), representing 0.42 ± 0.02% EPA in the original tissue sample by mass (5.0% relative uncertainty in a single determination). The 7.5 μL standard addition spikes also contained DHA (spike level = 677 ppb) yielding 0.73 ± 0.03% DHA by mass (3.5% relative uncertainty) in the Chinook salmon flesh. Using the same approach, the amount of EPA and DHA was quantified for a variety of different salmon samples, summarized in Table 5. The results are in fair agreement with (though not directly comparable to) typical EPA/DHA values reported by the US Department of Agriculture food composition database [60] for each type of salmon with the exception of the canned sockeye salmon, illustrating that the presented methodology holds promise for the direct analysis of PUFAs in a wide variety of complex biological samples. Furthermore, the results compared favorably with work by Santerre et al. using GC-FID [61]. They report a *w/w* % of EPA and DHA in wild Chinook salmon samples ($n = 5$) as 0.496 and 0.610%, respectively. Compared to the results for our CP-MIMS measurements of wild, fresh Chinook salmon (*w/w* % EPA = 0.42, *w/w* % DHA = 0.71), there is a percent difference of 16% for EPA and 15% for DHA. To further validate the PUFA measurement results, seven replicates of a *ca* 90 ppb EPA/DHA combined standard were prepared in 50:50 MeOH:H_2O (%*v*/v) sample solvent, and analyzed by CP-MIMS with MS/MS. The quantifier/qualifier ion ratios for the standards were found to be 1.00 and 0.628 for EPA and DHA, respectively. These were compared with those obtained in the salmon tissue standard addition experiments, and the % difference is summarized in Table 5. The results indicate reasonable DHA and EPA ion ratio agreement (< 20% difference in all cases) between the saponified salmon samples and clean analytical standards. Although a simple demonstration and not a full, validated assay study, the presented method exhibits significant promise as a future direct PUFA measurement strategy.

Conclusions

CP-MIMS offers an alternative to established methods for the direct measurement of fatty acids (FAs) in complex biological samples without the need for sample cleanup, derivatization or chromatography. The direct quantitative measurement (pptr levels) as well as structural confirmation of FAs in complex saponified salmon tissue samples has been demonstrated. The use of MeOH:H_2O mixtures as a sample (donor) solvent greatly increased longer chain FA sensitivity, allowing for the simultaneous detection of the entire suite of FAs tested. Systematic investigation of the donor phase composition for a homologous series of saturated FAs demonstrated that adjusting the MeOH:H_2O sample solvent ratio can selectively optimize sensitivity for lower or higher molecular weight FAs. The use of donor phase co-solvents for CP-MIMS shows promise for the analysis of a number of other compound classes with low water solubilities, and may potentially be used to probe for aggregation behavior in solution. The percent composition and structural confirmation of two important PUFAs in salmon samples, EPA and DHA, was determined using CP-MIMS with lithium acetate as an online cationization agent added to the acceptor phase. To our knowledge, this is the first

Table 5 Quantitative results for the measurement of EPA and DHA in a variety of British Columbian salmon samples prepared in 50:50 MeOH:H_2O (%*v*/v) obtained using CP-MIMS with MS/MS and the method of standard additions

Sample	Analyte	*w/w* % [a]	Quantifier/ qualifier ion ratio [b]	% Difference [c]	Relative standard error [d] / %
Sockeye Salmon (Fresh)	EPA	0.32	0.857	15.3	2.5
	DHA	0.84	0.567	10.1	1.9
Sockeye Salmon (Frozen)	EPA	0.27	0.900	10.5	1.1
	DHA	0.40	0.563	10.9	1.7
Sockeye Salmon (Canned)	EPA	0.048	0.864	14.6	15
	DHA	0.14	0.518	19.2	6.3
Chinook Salmon (Fresh)	EPA	0.42	1.08	7.99	5.0
	DHA	0.71	0.658	4.73	3.5
Steelhead Trout (Fresh)	EPA	0.031	0.880	12.8	17
	DHA	0.12	0.636	1.28	2.1

[a] Wet weight of salmon flesh

[b] EPA quantifier ion: 315 ➔ 179, EPA qualifier ion: 315 ➔ 72; DHA quantifier ion: 341 ➔ 165, DHA qualifier ion: 341 ➔ 72

[c] Percent difference between the ion ratio from the salmon standard addition experiments to the EPA / DHA (1.00 / 0.628, respectively) standards prepared in 50:50 MeOH:H_2O (%v/v) ($n = 7$)

[d] Relative standard error for the determination of a single tissue sample based on the variability in the standard addition curve. (see ESM) [59]

demonstration of the use of methanol modified donor phases with CP-MIMS for the direct, quantitative measurement of FAs. Future work includes the development, application, and validation of the presented technique for food authentication and nutritional analyses, with a longer-term goal of on-site operation with compact MS systems.

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Compliance with ethical standards

All fish samples used for this research were obtained as "food" items, and not specifically raised or collected for the purposes of this research. This includes the sport caught Chinook salmon, which was legally obtained as food by fly fishing using a BC Non-Tidal Angling Licence with the appropriate Non-Tidal Salmon Fishing Stamp.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Hrbek V, Vaclavik L, Elich O, Hajslova J. Authentication of milk and milk-based foods by direct analysis in real time ionization–high resolution mass spectrometry (DART–HRMS) technique: a critical assessment. Food Control. 2014;36(1):138–45.
2. Qu S, Du Z, Zhang Y. Direct detection of free fatty acids in edible oils using supercritical fluid chromatography coupled with mass spectrometry. Food Chem. 2015;170:463–9.
3. Voorhees KJ, Jensen KR, McAlpin CR, Rees JC, Cody R, Ubukata M, et al. Modified MALDI MS fatty acid profiling for bacterial identification. J Mass Spectrom. 2013;48(7):850–5.
4. Cody RB, McAlpin CR, Cox CR, Jensen KR, Voorhees KJ. Identification of bacteria by fatty acid profiling with direct analysis in real time mass spectrometry. Rapid Commun Mass Spectrom. 2015;29(21):2007–12.
5. Filimonova V, Gonçalves F, Marques JC, De Troch M, Gonçalves AMM. Fatty acid profiling as bioindicator of chemical stress in marine organisms: a review. Ecol Indic. 2016;67(Suppl. C):657–72.
6. Calligaris D, Caragacianu D, Liu X, Norton I, Thompson CJ, Richardson AL, et al. Application of desorption electrospray ionization mass spectrometry imaging in breast cancer margin analysis. Proc Natl Acad Sci. 2014;111(42):15184–9.
7. Valianpour F, Selhorst JJ, van Lint LE, van Gennip AH, Wanders RJ, Kemp S. Analysis of very long-chain fatty acids using electrospray ionization mass spectrometry. Mol Genet Metab. 2003;79(3):189–96.
8. Trufelli H, Famiglini G, Termopoli V, Cappiello A. Profiling of non-esterified fatty acids in human plasma using liquid chromatography-electron ionization mass spectrometry. Anal Bioanal Chem. 2011;400(9):2933–41.
9. Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv Nutr. 2012;3(1):1–7.
10. Dyall SC. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci. 2015;7:52.
11. Sprague M, Dick JR, Tocher DR. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. Sci Rep. 2016;6:21892.
12. Chilliard Y, Ferlay A, Doreau M. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest Prod Sci. 2001;70(1–2):31–48.
13. Vahmani P, Mapiye C, Prieto N, Rolland DC, McAllister TA, Aalhus JL, et al. The scope for manipulating the polyunsaturated fatty acid content of beef: a review. J Anim Sci Biotechnol. 2015;6(1):29.
14. Nudda A, Battacone G, Boaventura Neto O, Cannas A, Francesconi AHD, Atzori AS, et al. Feeding strategies to design the fatty acid profile of sheep milk and cheese. R Bras Zootec. 2014;43(8):445–56.
15. Griffiths WJ. Tandem mass spectrometry in the study of fatty acids, bile acids, and steroids. Mass Spectrom Rev. 2003;22(2):81–152.
16. Niehaus WG, Ryhage R. Determination of double bond positions in polyunsaturated fatty acids by combination gas chromatography-mass spectrometry. Anal Chem. 1968;40(12):1840–7.
17. Tomer KB, Crow FW, Gross ML. Location of double-bond position in unsaturated fatty acids by negative ion MS/MS. J Am Chem Soc. 1983;105(16):5487–8.
18. Eder K. Gas chromatographic analysis of fatty acid methyl esters. J Chromatogr B. 1995;671(1–2):113–31.
19. Zehethofer N, Pinto DM, Volmer DA. Plasma free fatty acid profiling in a fish oil human intervention study using ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2008;22(13):2125–33.
20. Kortz L, Dorow J, Ceglarek U. Liquid chromatography–tandem mass spectrometry for the analysis of eicosanoids and related lipids in human biological matrices: a review. J Chromatogr B. 2014;964(Suppl. C):1–11.
21. Cajka T, Fiehn O. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. Trends Anal Chem. 2014;61(Suppl. C):192–206.
22. Soliman LC, Andrucson EM, Donkor KK, Church JS, Cinel B. Determination of fatty acids in beef by liquid chromatography-electrospray ionization tandem mass spectrometry. Food Anal Methods. 2016;9(3):630–7.
23. Grebe SK, Singh RJ. LC-MS/MS in the clinical laboratory–where to from here? Clin Biochem Rev. 2011;32(1):5.
24. Han X, Gross RW. Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev. 2005;24(3):367–412.
25. Han X, Yang K, Gross RW. Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses. Mass Spectrom Rev. 2012;31(1):134–78.
26. Schiller J, Süß R, Arnhold J, Fuchs B, Lessig J, Müller M, et al. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research. Prog Lipid Res. 2004;43(5):449–88.
27. King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C, Olah T. Mechanistic investigation of ionization suppression in electrospray ionization. J Am Soc Mass Spectrom. 2000;11(11):942–50.
28. Chen H, Gamez G, Zenobi R. What can we learn from ambient ionization techniques? J Am Soc Mass Spectrom. 2009;20(11):1947–63.

29. Ifa DR, Wu C, Ouyang Z, Cooks RG. Desorption electrospray ionization and other ambient ionization methods: current progress and preview. Analyst. 2010;135(4):669–81.
30. Awad H, Khamis MM, El-Aneed A. Mass spectrometry, review of the basics: ionization. Appl Spectrosc Rev. 2015;50(2):158–75.
31. Krogh ET, Gill CG. Membrane introduction mass spectrometry (MIMS): a versatile tool for direct, real-time chemical measurements. J Mass Spectrom. 2014;49(12):1205–13.
32. Davey NG, Krogh ET, Gill CG. Membrane-introduction mass spectrometry (MIMS). TrAC Trends Anal Chem. 2011;30(9):1477–85.
33. Johnson RC, Cooks RG, Allen TM, Cisper ME, Hemberger PH. Membrane introduction mass spectrometry: trends and applications. Mass Spectrom Rev. 2000;19(1):1–37.
34. Duncan K, McCauley E, Krogh E, Gill C. Characterization of a condensed-phase membrane introduction mass spectrometry (CP-MIMS) interface using a methanol acceptor phase coupled with electrospray ionization for the continuous on-line quantitation of polar, low-volatility analytes at trace levels in complex aqueous samples. Rapid Commun Mass Spectrom. 2011;25(9):1141–51.
35. Duncan KD, Willis MD, Krogh ET, Gill CG. A miniature condensed-phase membrane introduction mass spectrometry (CP-MIMS) probe for direct and on-line measurements of pharmaceuticals and contaminants in small, complex samples. Rapid Commun Mass Spectrom. 2013;27(11):1213–21.
36. Srinivasan N, Johnson RC, Kasthurikrishnan N, Wong P, Cooks RG. Membrane introduction mass spectrometry. Anal Chim Acta. 1997;350(3):257–71.
37. Vandergrift GW, Krogh ET, Gill CG. Polymer inclusion membranes with condensed phase membrane introduction mass spectrometry (CP-MIMS): improved analytical response time and sensitivity. Anal Chem. 2017;89(10):5629–36.
38. Willis MD, Duncan K, Krogh ET, Gill CG. Delicate polydimethylsiloxane hollow fibre membrane interfaces for condensed phase membrane introduction mass spectrometry (CP-MIMS). Rapid Commun Mass Spectrom. 2014;28(7):671–81.
39. Duncan KD, Vandergrift GW, Krogh ET, Gill CG. Ionization suppression effects with condensed phase membrane introduction mass spectrometry: methods to increase the linear dynamic range and sensitivity. J Mass Spectrom. 2015;50(3):437–43.
40. Krogh ET, Gill CG. Condensed phase membrane introduction mass spectrometry-continuous, direct and online measurements in complex samples. In: Cappiello A, Palma P, editors. Advances in the use of liquid chromatography mass spectrometry (LC-MS)-instrumentation developments and applications. Comprehensive Analytical Chemistry,vol 79. Elsevier; 2018. p. 173–203.
41. Cheng C, Gross ML. Applications and mechanisms of charge-remote fragmentation. Mass Spectrom Rev. 2000;19(6):398–420.
42. Yalkowsky SH, He Y, Jain P. Handbook of aqueous solubility data: CRC press; 2016.
43. Cistola DP, Hamilton JA, Jackson D, Small DM. Ionization and phase behavior of fatty acids in water: application of the Gibbs phase rule. Biochemistry. 1988;27(6):1881–8.
44. Wang Y, Ma J, Cheon HS, Kishi Y. Aggregation behavior of tetraenoic fatty acids in aqueous solution. Angew Chem. 2007;119(8):1355–8.
45. Royal Society of Chemistry. Chemspider. London: Royal Society of Chemistry; 2015. [cited July 21, 2018]. Available from: http://www.chemspider.com/
46. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, et al. HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res. 2017;46(D1):D608–17.
47. Duncan KD, Letourneau DR, Vandergrift GW, Jobst K, Reiner E, Gill CG, et al. A semi-quantitative approach for the rapid screening and mass profiling of naphthenic acids directly in contaminated aqueous samples. J Mass Spectrom. 2016;51(1):44–52.
48. Gelotte KM, Lostritto RT. Solvent interaction with polydimethylsiloxane membranes and its effects on benzocaine solubility and diffusion. Pharm Res. 1990;7(5):523–9.
49. Twist JN, Zatz JL. A model for alcohol-enhanced permeation through polydimethylsiloxane membranes. J Pharm Sci. 1990;79(1):28–31.
50. Cussler EL. Diffusion: mass transfer in fluid systems. Cambridge University Press; 2009.
51. LaPack MA, Tou JC, Enke CG. Membrane mass spectrometry for the direct trace analysis of volatile organic compounds in air and water. Anal Chem. 1990;62(13):1265–71.
52. Domínguez A, Fernández A, González N, Iglesias E, Montenegro L. Determination of critical micelle concentration of some surfactants by three techniques. J Chem Educ. 1997;74(10):1227–31.
53. Murphy RC. Tandem mass spectrometry of lipids: molecular analysis of complex lipids. R Soc Chem. 2014.
54. Adams J, Gross ML. Tandem mass spectrometry for collisional activation of alkali metal-cationized fatty acids: a method for determining double bond location. Anal Chem. 1987;59(11):1576–82.
55. Jensen NJ, Tomer KB, Gross ML. Gas-phase ion decomposition occurring remote to a charge site. J Am Chem Soc. 1985;107(7):1863–8.
56. Duncan KD, Volmer DA, Gill CG, Krogh ET. Rapid screening of carboxylic acids from waste and surface waters by ESI-MS/MS using barium ion chemistry and on-line membrane sampling. J Am Soc Mass Spectrom. 2016;27(3):443–50.
57. Crockett JS, Gross ML, Christie WW, Holman RT. Collisional activation of a series of homoconjugated octadecadienoic acids with fast atom bombardment and tandem mass spectrometry. J Am Soc Mass Spectrom. 1990;1(2):183–91.
58. Ma X, Chong L, Tian R, Shi R, Hu TY, Ouyang Z, et al. Identification and quantitation of lipid C= C location isomers: a shotgun lipidomics approach enabled by photochemical reaction. Proc Natl Acad Sci. 2016;113(10):2573–8.
59. Bruce GR, Gill PS. Estimates of precision in a standard additions analysis. J Chem Educ. 1999;76(6):805–7.
60. US Department of Agriculture. USDA Food Composition Databases. 2018 [cited July 21, 2018]. Available from: https://ndb.nal.usda.gov/ndb/.
61. Cladis DP, Kleiner AC, Freiser HH, Santerre CR. Fatty acid profiles of commercially available finfish fillets in the United States. Lipids. 2014;49(10):1005–18.

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