Mass-defect filtering of isotope signatures to reveal the source of chlorinated palm oil contaminants

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This paper reports new insights at the molecular level into the route of a worldwide problem of the food industry: the occurrence of monochloro-propanediol (MCPD) esters. The application of mass defect-driven workflows is described to generate a hypothesis on the identity and occurrence of those thermally labile, chlorinated contaminant precursors that may act as chlorine donors during the formation of MCPD esters. For the first time, holistic mass-defect filtering of isotope signatures is used to pinpoint completely unknown and unexpected chlorine-containing substances naturally present in various extracts of palm fruit and partially and fully refined oils. Supervised multivariate analysis showed the effective classification of samples from various stages of industrial processing, suggesting that these steps strongly impact a complex and dynamic pool of chlorinated substances. In-vitro experiments confirmed that several of these naturally occurring chlorinated plant constituents decompose upon heat treatment, thus potentially being a source of chlorine for further reactions with palm oil lipids in a subsequent chlorination cascade. It is hypothesised that during oil refining the organochlorines naturally present in palm fruits act as a ‘chlorine source’ for the generation MCPD diesters. This discovery implies that industrial efforts targeting the mitigation of chlorinated substances must intervene at the earliest possible production stage or preferably even prior to oil processing. Current performance and limitations of mass-defect filtering are discussed and future developments are outlined.

Keywords: oils and fats; 3-MCPD; environmental contaminants; process contaminants – 3-MCPD; LC/MS; isotope ratios

Introduction

Mass-defect filtering and isotope pattern matching are considerable improvements in the rationalisation of high-resolution mass spectrometric signals. Yet, the identification of substances with a completely unknown structure, but with a partially known atomic composition, still represents an unresolved issue. A typical problem for such holistic applications is that no reference masses are available that could be used as a basis for mass defect comparison and ultimately mass-defect filtering. Further, in such cases the applicability of conventional isotope pattern matching is compromised by the limited quantitative performance of instrumentation (Li et al. 2007) and more importantly by the undesired superimposition and interference of isotopes that originate from various elements (e.g. the characteristic isotope pattern of chlorine is masked by the abundant $^{13}$C isotope substitution). This latter phenomenon is magnified as the mass of analyte increases. The distortion of the isotope pattern can be further impacted if background subtraction is performed on the data.

The concept of mass defect (or fractional mass) was defined as the deviation of the exact mass of a particle from its nearest integer (nominal) mass (Stagliano et al. 2010). Several precedents were described and reviewed for the utility of mass defect, including filtering for metabolites that have a similar mass defect to that of the parent drug (Zhu et al. 2006, 2007; Zhang et al. 2007, 2008, 2009), various Kendrick scale interpretations, and van Krevelen projections (Wu et al. 2004). As an example, according to the definition of Kendrick mass scale, the exact mass of a CH$_2$ unit is set to 14.00000 Kendrick units (Hughey et al. 2001, 2002; Wu et al. 2004) in opposition to the IUPAC mass scale which sets CH$_2$ at 14.01565 Da. This type of exact mass conversion compensates for the natural skewness of the mass defect along the $m/z$ scale and has been particularly useful to characterise the aromatic/aliphatic nature of constituents in petroleum products (Hughey et al. 2001, 2002; Wu et al. 2004). Furthermore, lipids were also successfully classified in various biological fluids using variants of Kendrick interpretations (Lerno et al. 2010) since their...
constituents include a homologous series (e.g. triacylglycerols that differ only by integer multiples of CH₂ and thus have the same absolute mass defect).

A particularly popular application of mass-defect filtering is screening for reactive electrophilic drug metabolites. In these studies the metabolites are tagged using various nucleophile-trapping agents (e.g. glutathione, potassium cyanide, semicarbazide) that also serve the purpose of defining various mass defect windows (usually ±40 mDa) to remove non-drug-related interferences (Rousu et al. 2009; Zhang et al. 2009; Deng et al. 2010; Ruan and Zhu 2010). Since in general every additional element (except carbon) incorporated into a molecule has an impact on its overall mass defect (Pomerantz and McCloskey 1987; Toumi and Desaire 2010), the biotransformation knowledge and structure-based analyte prediction play a key role in the identification of the correct mass defect windows (Lim et al. 2008; Li et al. 2009; Mortishire-Smith et al. 2009). This latter scenario is not possible for the discovery of completely unknown structures with a partially known atomic composition, since no reference mass value is available to set the mass defect window for filtering. On the other hand, the exact mass difference caused by the substitution of various isotopes of a given element (e.g. 35Cl and 37Cl isotopes) is always constant, independent of the size of the molecule and, thus, it can be easily recognised along the whole m/z range. Taking advantage of this fact, it is possible to pinpoint completely unknown and unexpected chlorine- (i.e. or other halogen) containing substances merely by searching for the specific mass defect signatures within the isotope envelopes (isotopomers) of analytes.

The objective of the present paper is to assess and employ this mass defect-based filtering approach to discover the source of halogenated contaminants and their precursors in edible oils. Perhaps the most prominent examples of such contaminants are monochloro-propanediol (MCPD) fatty acid esters, which represent a worldwide problem for several decades (Crews et al. 2002; Hamlet et al. 2002; Seefelder et al. 2008; Weiβhaar 2008). These lipid-like contaminants are found in high abundance in refined palm oil (Weiβhaar 2011), which is used in a broad range of food categories from confectionary to dairy, from ice cream to infant formulas. Due to the potential toxicity of these contaminants and/or their hydrolysed counterparts (Lynch et al. 1998; Habermeyer et al. 2011), pre-emptive mitigation of concentration in food is recommended. To date, the effective mitigation of MCPD esters has not been achieved since the exact chemical pathways and most importantly the source of chlorine responsible for the formation of these compounds were unknown, until now.

Materials and methods

Chemicals and samples
ULC-grade ammonium-formate, methanol and isopropanol were obtained from Chemie Brunschwig AG (Basel, Switzerland). LC-grade acetone was purchased from Sigma-Aldrich (Buchs, Switzerland). 13C3-Sn1-palmitoyl-Sn2-stearyl-MCPD was obtained from Atlanchim Pharma (Nantes, France). Fresh palm fruits collected manually and then frozen were the generous gift of the Nestlé’s Research and Development Center in Singapore, Malaysia. Commercially available partially and fully refined palm oil samples were procured from a Nestlé laboratory (NQAC Weiding, Polling, Germany).

In-vitro heat treatment of crude palm oil samples
The heat treatment of crude palm oil samples at various temperatures was conducted in sealed glass ampoules under nitrogen for 2h at temperatures ranging from 100 to 280°C in an Agilent 6890 gas chromatograph oven (Agilent Technologies, Palo Alto, CA, USA). The glass ampoules were fabricated from glass Pasteur pipettes by flushing them with nitrogen and sealing them using a Bunsen gas burner. The permanent wearing of a laboratory coat, protection gloves and glasses is imperative when working with such ampoules since they develop overpressure due to the high temperature applied and may explode if sealed inappropriately.

Sample preparation for chlorine filtering
Samples were melted at 70°C to ensure homogeneity. A 200 µl aliquot from each sample was transferred into a glass vial along with 1200 µl n-hexane:methanol (3:1). The vial was shaken for 5 min in a VWR DVX 2500 vortexer at 2000 rpm. The sample was left to settle for 30 min and 100 µl aliquot from the methanolic phase was transferred into a new glass vial along with 900 µl methanol. A 10 µl aliquot from this solution was injected for analysis.

Sample preparation for MCPD analysis
Samples were melted at 70°C to ensure homogeneity. A total of 10 µl aliquot from each sample were transferred into a glass vial along with 980 µl acetone and 10 µl internal standard solution (100 µg ml⁻¹ 13C3-Sn1-palmitoyl-Sn2-stearyl-MCPD in methanol:acetone 1:4). The mixture was shaken manually to allow for dilution. Next, 100 µl of this solution were added to 900 µl acetone. Finally, 100 µl aliquot of this latter solution were transferred into new glass vial and 900 µl
methanol were added. A 25 µl aliquot was injected for analysis.

**Liquid chromatography**

An Accela 1250 liquid chromatograph (ThermoFisher Scientific, Bremen, Germany) equipped with a Waters Acquity HSS C18 column (1.8 µm particle size, 2.1 × 150 mm) was used for the separation of analytes. The applied gradient for chlorine filtering is shown in Table 1. Solvent A was methanol, whereas solvent B was isopropanol. The gradient applied for relative quantification of chlorine donors is shown in Table 2. Solvent A was methanol, whereas solvent B was isopropanol. MCPDs were quantified using the gradient shown in Table 1, but in this case solvent A was 1 mM ammonium-formate in methanol; solvent B was 100 µM ammonium-formate in isopropanol.

**Mass spectrometry**

Filtering and identification experiments were carried out using an LTQ-Orbitrap XL hybrid mass spectrometer (ThermoFisher Scientific). Negative electrospray ionisation with 3.5 kV capillary voltage was employed to form ions at 400°C nebuliser temperature. The nebuliser and auxiliary gases were nitrogen at 40 and 20 units, respectively. The tube lens was adjusted to 80 V. Other parameters were the typical values optimised during calibration. Relative quantification of chlorine donors and MCPD esters was carried out using a TSQ Quantum Access Max mass spectrometer (ThermoFisher Scientific). Negative electrospray ionisation with 3.5 kV capillary voltage was employed to form ions from chlorine donors at 400°C nebuliser temperature. Parameters of selected reaction monitoring (SRM) experiments are given in Table 3. Positive electrospray ionisation with 5 kV capillary voltage was utilised to form ions from MCPDs at 400°C nebuliser temperature. Parameters of SRM experiments are given in Table 4. The nebuliser and auxiliary gases were nitrogen at 40 and 20 units, respectively. For all transitions, tube lens values of 80 V, a dwell time of 150 ms and a span of 0.2 m/z were used. Other parameters were the typical values optimised during calibration.

**Data mining**

High-resolution Orbitrap data were interrogated for the presence of chlorine using MetWorks 1.4 software (ThermoFisher Scientific). A mass accuracy of 2 ppm was specified for the recognition of the 37Cl isotope; the intensity threshold was set to 50,000; the isotope pattern tolerance was set to 0.1. Chromatographic peak areas of the identified hits were extracted in 10 ppm m/z windows by Quanbrowser software. The peak areas were investigated with the Statistica 9.1 software package (StatSoft Inc., Tulsa, OK, USA) using principal component analysis (PCA), discriminant function analysis (DFA) and linear discriminant analysis (LDA) features.

**Results and discussion**

**Assessment of the applied filtering approach**

One intrinsic limitation of conventional isotope pattern filtering is that the isotopic contribution of various elements is superimposed, thus masking the contribution of individual atoms. This study shows that if the details of the isotope signature are resolved utilising sufficiently high mass resolution, then the recognition of an element with a specific mass defect pattern becomes unambiguous. This is because every distinct isotopic form of each chemical element has a different mass defect, which then together represent a unique mass defect signature for that element (Audi et al. 2003). Practically speaking this means that at sufficiently high mass resolution the 13C isotopes will not interfere with the detection of chlorine isotopes and so the detection of 37Cl,35Cl exact mass difference reflects...
the presence of chlorine for any observed ion. (Significantly higher resolving power is required for the resolution of $^{37}$Cl and $^{34}$S isotopes, and this is not the subject of the present paper.) This approach also can be considered as a particular type of mass-defect filtering that targets analytes which are naturally tagged by the specific isotopes of various elements.

The calculated theoretical resolution necessary to distinguish between the signals of $^{37}$Cl and $^{13}$C isotopes is shown in Figure 1. Calculations are based on resolving a mass difference of 9.66 milliamu (mamu), which corresponds to the change in mass defect if a $^{35}$Cl-$^{37}$Cl substitution is considered instead of two $^{13}$C substitutions. The current state of the art in terms of mass resolution is $4300,000$ (Kim et al. 2006), which resolves $^{13}$C and $^{37}$Cl isotopes until approximately $m/z$ 3000. Furthermore, in practice such measurements are straightforward since they correspond to the ideal case of analysis where the $m/z$ of the internal standard ($^{35}$Cl peak) is very close that of the analyte ($^{37}$Cl peak). Consequently, this novel and holistic approach should enable the discovery of those up-to-date completely unknown chlorinated substances which are responsible for the formation of worldwide occurring contaminants, such as MCPD esters.

**Statistical evaluation of chlorinated substances**

Palm oil samples obtained from various suppliers and processing stages were analysed as described above. The chlorine-specific mass-defect filtering process yielded approximately 300 variables (chemical compounds) that comprised a complete and merged set of hits from all samples. Most of these hits were found to be monoclorinated (data not shown). The unexpectedly high number of hits implies that the investigated samples contain a complex pool of chlorinated substances that have never been reported before.

The absolute peak areas belonging to these substances (based on retention time and accurate mass)
were evaluated using PCA. The results in Figure 2 show a remarkable trajectory of samples (see the arrow) along the process from crude to the endpoint of fully refined, bleached and deodorised palm oil samples (RBD). Since this pattern was obtained via the unsupervised factor analysis approach, it suggests that the abundance of these chlorinated substances is in strong correlation with the processing conditions, and thus this complex pool of chlorinated substances is strongly affected during the oil-processing chain. For instance, certain compounds disappear while other completely new constituents appear in the oil (for examples, see Figure 6).

To evidence further the correlation between the chlorinated substances and the processing stages, LDA was applied to the dataset. It was necessary first to reduce the number of variables (chlorinated hits) in order to avoid over-fitting of the model. This was achieved by dividing the variables into subsets of 50 and applying DFA. This data-mining technique assigned a parameter (partial Wilk’s lambda) to every variable reflecting its discriminative power. Based on this parameter, the variables were sorted and the 50 most discriminating ones were selected for further analysis. A final DFA step then sorted these last 50 variables according to the discriminative power. The five most discriminative variables were used for LDA without any manipulation. The resulting scores plot shown in Figure 3 confirms a strong correlation between the chlorinated substances found and processing conditions involved in palm oil production.
Nature of the chlorinated substances

The fact that many of the variables appeared in high abundance in the crude samples suggests that the chlorinated substances are present in the oil already at an early stage of industrial processing, or perhaps present in the palm fruit even before harvesting. To ascertain the latter, oil from handpicked palm fruits was extracted using isopropanol: n-hexane (2:1, v/v). Surprisingly, most chlorinated substances (such as those listed in Table 3) also appeared in the oil extracted from the fruits, implying that the chlorine sources are already present in the palm fruit well before any processing is applied to the oil or the fruits.

To ascertain the nature of some of these chlorinated substances, MS4 experiments were carried out. Isolation and fragmentation of the parent ions was performed in the linear trap, while the detection of the fragments at high resolution was carried out in the Orbitrap. An abundant chlorine donor similar in structure to phytosphingosines was discovered (Figure 4). For a detailed description of structural elucidation, see the Supplementary Material. Further analysis of the accurate mass data revealed four additional compounds with similar elemental composition differing only in the number of hydrogen and oxygen atoms (Figure 4). Based on this similarity, it can be speculated that these substances rather correspond to endogenous metabolites produced by the plant itself than to contaminants from the environment. This important discovery implies that industrial efforts targeting the mitigation of chlorinated substances must intervene at a very early point of processing.

Investigation of the few multichlorinated hits also led to highly unexpected observations. Interrogation of their isotope envelope and the unusually negative mass defects revealed that these substances correspond to inorganic compounds, concretely FeCl3, FeCl2, MgCl2 and CaCl2, amongst which FeCl3 and FeCl2 exhibited the most intense signals. As an example, the theoretical and measured isotopomer distribution of FeCl3 ion is shown in Figure 5. The presence of these substances in both industrial crude- and in-house-extracted oil samples suggests that in addition to the above reported phytosphingosine-like organic substances, inorganic compounds also may represent at least partly a source of chlorine that may ultimately lead to the formation of MCPDs.

Associations of chlorinated substances with the formation of MCPD esters

In the next step a subset of 20 hits was selected manually to confirm the hypothesis that these chlorinated substances could serve as the source of chlorine involved in the formation of MCPD contaminants. The subset was established by applying three criteria: (1) the compound’s intensity should be high enough to perform MSn experiments; (2) the compound should vanish upon deodorisation; and (3) the compound should vanish upon in-vitro heat treatment. The latter was achieved by experiments where 1 ml oil was incubated in sealed glass ampoules fabricated from Pasteur pipettes. Heating was performed at 235°C for 2 h.

In order to obtain the fragmentation information necessary for the SRM experiments, the chlorinated candidates were fragmented using the LTQ-Orbitrap. Both molecular ions of each candidates corresponding to the 35Cl and 37Cl isotope substitution were fragmented. Interestingly, several of the hypothetical chlorine-containing substances exhibited the loss of H35Cl or H37Cl in their fragmentation pattern. This observation further confirms the presence of chlorine
and implies that these candidates could indeed be susceptible to the loss of chlorine in the form of hydrogen chloride upon heat treatment. This latter point is particularly important since the objective of the present study was to elucidate the source(s) of chlorine that is/are involved in the formation of MCPD esters. In order to facilitate interpretation of the results, only those compounds were further investigated which did exhibit the loss of HCl as a first fragmentation step. A list of candidates following the described behaviour is given in Table 3 along with the corresponding SRM transitions, retention times and mass defect shifts previously observed using the Orbitrap instrument. These chlorinated compounds reported in Table 3 were found in both industrially produced crude oil samples and also in the oil samples extracted in our laboratory from palm fruits using solvents.

To test the hypothesis that these chlorinated candidates may indeed act as chlorine donors for MCPD ester formation, crude palm oil samples were subjected to thermal treatment at temperatures ranging from 100 to 280°C for 2 h in glass ampoules. The relative abundance of selected organochlorines (see above) and the levels of MCPD diesters formed during the reaction were monitored by ULC-MS/MS using SRM mode (Tables 3 and 4). The results confirmed that levels of MCPD diesters increase upon thermal treatment at high temperatures (Figure 6), which is in accordance with the literature (Weißhaar 2008). In parallel, we observed that the level of organochlorines decreased progressively upon heat treatment at temperatures above 120–130°C. This observation supports the hypothesis that the monitored organochlorines might act as ‘chlorine donors’ in the generation of MCPD diesters during conditions employed in edible oil refining. While most monitored chlorine donors show vanishing kinetics, some exhibit a curve peaking at approximately 180°C (Figure 7). This behaviour suggests that some chlorine donors are formed due to the heat treatment and ultimately decompose. Several other chlorine donors that show vanishing kinetics might also form during heating, but due to different rates of formation/decomposition they do not accumulate in the oil. This further confirms that the array of chlorinated substances detected in palm oil represents a highly complex and dynamic pool.
of chlorine donors which strongly interact upon heat treatment. Consequently, it can be hypothesised that the organochlorine degradation and MCPD diester formation is likely to happen via both direct routes as well as indirectly via intermediates. Further research is needed to address these latter points.

Conclusions and outlook
This paper assessed and employed a high-mass resolution-based mass-defect filtering approach to discover the source of chlorinated contaminants such as MCPD esters and their precursors in crude and partially/fully refined palm oils. It was shown that both organic and inorganic chlorinated substances are present in every stage of palm oil processing, as well as even in the palm fruits themselves before milling. The chlorinated substances found represent a complex and dynamic pool of compounds, and are affected strongly by the processing conditions, e.g. the high temperature during deodorisation. Many of these substances are also susceptible to the loss of chlorine during fragmentation, further implying their ability to act as chlorine donors in the MCPD formation reaction. In-vitro experiments confirmed that the level of organochlorines present in palm oil decreased progressively upon heat treatment, while the level of MCPD esters increased. Consequently, it is postulated that during oil refining these organochlorines naturally present in palm fruits act as a ‘chlorine source’ for the generation of MCPD diesters. Finally and most importantly, this discovery implies that industrial efforts targeting the mitigation of chlorinated substances in palm oil must intervene at the earliest possible point in the oil production chain, even prior to oil processing.

The presently described approach has the potential of pinpointing completely unknown and unexpected halogen-containing substances in complex matrices. It demonstrates that the usefulness of mass defect values is not only the classical removal of interferences outside a defined mass defect window, but in particular the detection of ions with a specific mass defect signature in their isotope distribution. This way, ions containing certain elements (e.g. chlorine) can be filtered and identified without any preliminary information on the absolute mass of the analytes. This latter aspect is a major difference compared with conventional mass-defect filtering approaches where a reference mass defect value is a prerequisite for filtering. The detection of chlorine in the mass defect signature of isotopomers requires relatively moderate (>50,000 FWHM) mass resolution and it is only the first example for this approach. Future applications with higher mass-resolving power will enable the distinction and recognition of other additional elements such as sulfur in the isotope pattern. This type of interpretation of mass defect values will open a new horizon for the assignment of elemental composition, in particular for large molecules where the number of elemental composition combinations is high.

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References
Audi G, Wapstra AH, Thibault C. 2003. The 2003 atomic mass evaluation: (II). Tables, graphs and references. Nucl Phys A. 729:337–676.
Crews C, Hough P, Breretton P, Harvey D, Macarthur R, Matthews W. 2002. Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999–2000. Food Addit Contam. 19:22–27.
Deng P, Zhong D, Nan F, Liu S, Li D, Yuan T, Chen X, Zheng J. 2010. Evidence for the bioactivation of 4-nonylphenol to quinone methide and ortho-benzoquinone metabolites in human liver microsomes. Chem Res Toxicol. 23:1617–1628.
Habermeyer M, Gruth S, Eisenbrand G. 2011. Identification of gaps in knowledge concerning toxicology of 3-MCPD and glycidol esters. Eur J Lipid Sci Technol. 113:314–318.
Hamlet CG, Sadd PA, Crews C, Velisek J, Baxter DE. 2002. Occurrence of 3-chloro-propane-1,2-diol (3-MCPD) and related compounds in foods: a review. Food Addit Contam. 19:619–631.
Hughes CA, Hendrickson CL, Rodgers RP, Marshall AG, Qian K. 2001. Kendrick mass defect spectrum: a compact visual analysis for ultrahigh-resolution broadband mass spectra. Anal Chem. 73:4676–4681.
Hughes CA, Rodgers RP, Marshall AG. 2002. Resolution of 11,000 compositionally distinct components in a single
electrospray ionization Fourier transform ion cyclotron resonance mass spectrum of crude oil. Anal Chem. 74:4145–4149.

Kim S, Rodgers RP, Marshall AG. 2006. Truly ‘exact’ mass: Elemental composition can be determined uniquely from molecular mass measurement at 0.1 mDa accuracy for molecules up to 500 Da. Int J Mass Spectrom. 251:260–265.

Lerno Jr LA, German JB, Lebrilla CB. 2010. Method for the identification of lipid classes based on referenced Kendrick mass analysis. Anal Chem. 82:4236–4245.

Li AC, Ding J, Jiang X, Denissen J. 2009. Two-injection workflow for a liquid chromatography/LTQ-Orbitrap system to complete in vivo biotransformation characterization: demonstration with buspirone metabolite identification. Rapid Commun Mass Spectrom. 23:3003–3012.

Li AC, Shou WZ, Mai TT, Jiang XY. 2007. Complete profiling and characterization of in vitro nefazodone metabolites using two different tandem mass spectrometric platforms. Rapid Commun Mass Spectrom. 21:4001–4008.

Lim HK, Chen J, Cook K, Sensenhauser C, Silva J, Evans DC. 2008. A generic method to detect electrophilic intermediates using isotopic pattern triggered data-dependent high-resolution accurate mass spectrometry. Rapid Commun Mass Spectrom. 22:1295–1311.

Lynch BS, Bryant DW, Hook GJ, Nestmann ER, Mundro IC. 1998. Carcinogenicity of monochloro-1,2-propanediol (a-chlorohydrin, 3-MCPD). Int J Toxicol. 17:47–76.

Mortishire-Smith RJ, Castro-Perez JM, Yu K, Shockcor JP, Goshawk J, Hartshorn MJ, Hill A. 2009. Generic dealkylation: a tool for increasing the hit-rate of metabolite rationalization, and automatic customization of mass-defect filters. Rapid Commun Mass Spectrom. 23:939–948.

Pomerantz SC, McCloskey JA. 1987. Fractional mass values of large molecules. Organic Mass Spectrom. 22:251–253.

Rousu T, Pelkonen O, Tolonen A. 2009. Rapid detection and characterization of reactive drug metabolites in vitro using several isotope-labeled trapping agents and ultra-performance liquid chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 23:843–855.

Ruan Q, Zhu M. 2010. Investigation of bioactivation of ticlopidine using linear ion trap/orbitrap mass spectrometry and an improved mass-defect filtering technique. Chem Res Toxicol. 23:909–917.

Seefelder W, Varga N, Studer A, Williamson G, Scanlan FP, Stadler RH. 2008. Esters of 3-chloro-1,2-propanediol (3-MCPD) in vegetable oils: significance in the formation of 3-MCPD. Food Addit Contam A Chem Anal Control Expo Risk Assess. 25:391–400.

Staglino MC, DeKeyser JG, Omiecinski CJ, Jones AD. 2010. Bioassay-directed fractionation for discovery of bioactive neutral lipids guided by relative mass-defect filtering and multiplexed collision-induced dissociation. Rapid Commun Mass Spectrom. 24:3578–3584.

Toumi ML, Desaire H. 2010. Improving mass-defect filters for human proteins. J Proteome Res. 9:5492–5495.

Weißhaar R. 2008. 3-MCPD-esters in edible fats and oils – a new and worldwide problem. Eur J Lipid Sci Technol. 110:671–672.

Weißhaar R. 2011. Fatty acid esters of 3-MCPD: overview of occurrence and exposure estimates. Eur J Lipid Sci Technol. 113:304–308.

Wu Z, Rodgers RP, Marshall AG. 2004. Two- and three-dimensional van krevelen diagrams: a graphical analysis complementary to the Kendrick mass plot for sorting elemental compositions of complex organic mixtures based on ultra-high-resolution broadband fourier transform ion cyclotron resonance mass measurements. Anal Chem. 76:2511–2516.

Zhang D, Wang L, Raghavan N, Zhang H, Li W, Cheng PT, Yao M, Zhang L, Zhu M, Bonacorsi S, et al. 2007. Comparative metabolism of radiolabeled muraflitazar in animals and humans by quantitative and qualitative metabolite profiling. Drug Metab Dispos. 35:150–167.

Zhang H, Zhang D, Ray K, Zhu M. 2009. Mass-defect filter technique and its applications to drug metabolite identification by high-resolution mass spectrometry. J Mass Spectrom. 44:999–1016.

Zhang H, Zhu M, Ray KL, Ma L, Zhang D. 2008. Mass defect profiles of biological matrices and the general applicability of mass-defect filtering for metabolite detection. Rapid Commun Mass Spectrom. 22:2082–2088.

Zhu M, Ma L, Zhang H, Humphreys WG. 2007. Detection and structural characterization of glutathione-trapped reactive metabolites using liquid chromatography/high-resolution mass spectrometry and mass-defect filtering. Anal Chem. 79:8333–8341.

Zhu M, Ma L, Zhang D, Ray K, Zhao W, Humphreys WG, Skiles G, Sanders M, Zhang H. 2006. Detection and characterization of metabolites in biological matrices using mass-defect filtering of liquid chromatography/high resolution mass spectrometry data. Drug Metab Dispos. 34:1722–1733.