Review

Utility of isotope-coded derivatization in gas chromatographic-mass spectrometric analyses with special emphasis on metabolomics

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Abstract Gas chromatography (GC) is widely used in various fields. Among GC detectors, mass spectrometry detection (MS) provides highest selectivity and sensitivity, thus it is the most used detector especially in metabolomics studies. In GC-MS analysis, derivatization is mostly unavoidable for most of the analytes in order to increase volatility, thermal stability, and detectability of the analytes. Nevertheless, chemical derivatization has some limitations including uncertainty in quantitation, differences in derivatization kinetics, and the stability for variable analytes. Thus, the GC-MS analysis strongly requires standardizing protocols. One of the most used protocols for standardization is isotope dilution strategy. However, available isotope-internal standards are limited in number and expensive. A cost-effective and simpler alternative is isotope-coded derivatization (ICD), in which the standard or the control sample is labeled with a heavy isotope-coded moiety of the reagent and used as internal standards. On the other hand, the sample is labeled with the light form of the reagent. Then, both mixed and analyzed by GC-MS(/MS) permitting the absolute quantification of analytes and identification of unknown ones. Due to the high importance of ICD, there are many reviews summarized its use in LC-MS analysis. However, there is no single review dedicated to summarizing its use in GC-MS. Until now, few types of reagents were designed for ICD for GC-MS applications. These reagents can be classified into silylation, chloroformates esterification, transmethylation, and aminooxyethyl propionates oximation ICD reagents. In this review, we will discuss every class of them and their reported applications with special emphasis on metabolomics.

Key words: GC-MS, isotope-coded derivatization, standardization protocols, silylation, metabolite profiling

Introduction

Gas chromatography (GC) is a separation technique that uses gases as mobile phases and liquid or solids as stationary phases. It is capable of resolving very complex mixtures of analytes based upon the differences in their boiling point/ vapor pressure and polarity[1)]. GC is coupled with different types of detectors and every one of them is selective for a special type of analyte. Among them, the most widely used detector is flame ionization detector due to its universal nature and acceptable sensitivity[2)].

Nowadays, mass spectrometry (MS) detection is being used widely in particular when combined with chromatographic analysis. MS is naturally compatible with the GC analysis as there is no need for the sample vaporization interface, which is needed in the case of LC-MS. In addi-

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tion, MS detectors offer great sensitivity and selectivity compared with all other GC detectors. Besides, MS detectors not only record the presence of analytes, but also provides additional structural information regarding its chemical composition and molecular weight and chemical structure[1]. All these factors lead to the wide use of GC-MS analysis[3,4]. GC-MS is used for the analysis of many low molecular weight compounds[5,6]. GC-MS is widely used in different fields including food analysis[7,8], plant bioactive compounds analysis[9,10], and most importantly; biological samples metabolite profiling[6,11].

GC analysis mostly needs chemical derivatization for analytes prior to their determination. The derivatization is needed in order to increase the volatility and thermal stability of the analytes, improve their chromatographic behavior and suppress tailing by decreasing their polarity, improve the recovery of a compound from complex matrices, and finally increase their detection sensitivity and selectivity. Nevertheless, chemical derivatization has some drawbacks and limitations. For instance, the derivatization reagent and procedure could cause potential uncertainty in quantitation and could introduce interfering by-products. In addition, derivatization adds an extra stair in the sample treatment[12–15]. Furthermore, most of the derivatization reagents are hazardous and could harm the environment and the analyst which is not coping with recent green trends in analytical chemistry[16–19]. Thus, it is better to avoid them if possible. However, the use of derivatization reagents is unavoidable in the case of nonvolatile compounds that are inherently not suitable for GC analysis[15]. In this regard, many reviews concerned with GC analytical derivatization have been published[12–15,20–23]. The optimal derivatizing reagent for GC analysis should be highly reactive towards a specific group of analytes and give only one thermally stable and sufficiently volatile product per each analyte. In addition, if the derivatization reaction leads to the formation of any by-products, there should be an easy way to remove them from the reaction mixture prior to GC analysis[12,15].

The most commonly used derivatization reaction in GC analysis is silylation as thermally stable and volatile silylated derivatives can be produced easily[14,24]. Several reagents have been designed for this purpose including: *N*, *O*-bis(trimethylsilyl)acetamide (BSA)[25,26], bis(trimethylsilyl) trifluoroacetamide (BSTFA)[27,28], *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA)[29,30] or *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA)[31,32].

In addition to silylation, there are various reactions used prior to GC analysis such as alkylation, esterification or acylation reactions; where an alkyl or acyl group replaces a reactive proton[33]. Based on the used reagents, these reactions may be performed in aqueous or organic medium. However, *in situ* aqueous derivatizations have several advantages such as avoiding extraction step before derivatization and unnecessity of excess reagents removal due to their water solubility. *In situ* aqueous derivatization included many reactions such as alkylation, acylation, esterification, oxime formation, hydrazine formation, and Schiff base formation. These derivatization reactions were used for profiling of many types of metabolites including alcohols, carbonyls, carboxylic acids, and amines[15,17,18,34]. However, *in situ* aqueous derivatizations requires an additional step for sample introduction from the aqueous phase to the GC injectors including liquid−liquid extraction (LLE), dispersive liquid−liquid microextraction (DLLME)[35,36], liquid-phase microextraction (LPME)[37], salting-out assisted liquid−liquid extraction (SALLE)[38], solid-phase extraction (SPE)[39], solid-phase microextraction (SPME)[40], and headspace sampling (HS)[41].

Furthermore, other alternatives to the conventional and *in situ* aqueous derivatizations, have been offered. One of the alternative derivatization methods is the extractive derivatization, in which extraction and derivatization are combined into one step where the analytes are transmitted from an aqueous phase to an organic one containing the derivatization reagent (reactive organic phase) through pH control or ion-pairing[42,43]. Another alternative derivatization method is solid-phase analytical derivatization (SPAD) in which the analytes are derivatized while adsorbed on the SPE sorbent. Furthermore, derivatization in an SPME fiber coating was also reported based on the same idea of SPAD[44]. Lastly, derivatization in the GC injector port was also performed, where the analytes and derivatization reagents are injected into the injector port at the same time. The derivatization step and sample volatilization occur simultaneously through thermal decomposition in the GC heated injector [45,46].

From the above we can conclude that the derivatization reactions can occur in various ways; either in organic solvents media, *in situ* aqueous derivatizations, injector port *in situ* derivatization, or combined in one-step with the extraction procedure[15]. In principle, derivatization adds an

extra processing step to the sample treatment introducing extra analytical error possibilities. As derivatization is often unavoidable for GC-MS analysis of most analytes, a procedure called isotope dilution is often used to overcome the errors introduced in the derivatization step and overcome matrix effect[47]. Isotope dilution is based on the fact that many elements have two or more stable isotopes that have a constant natural proportion. Examples of the isotope atoms pairs are H/d, $^{12}C/^{13}C$, $^{16}O/^{18}O$, and $^{14}N/^{15}N$ [48]. A known amount of a stable isotope (d, ^{13}C, ^{18}O, or ^{15}N) labeled standard is added to a sample and the ratio between the isotopes can then be measured by GC-MS. Isotope dilution could provide very accurate and precise results, in addition to its use as an essential tool for the validation of analytical procedures and reference materials. Recently, many isotope dilution GC-MS methods were reported[49–55] owing to their advantageous reduction of total analysis time and providing high-quality analytical data[47,56]. However, isotope dilution requires isotope-labeled standards that are either very expensive or unavailable[48,57]. In addition, Choi and Jung reported that the isotope dilution did not correct the matrix effect of cola and beer during the analysis of methyl-imidazoles[58]. Cola and beer have less complicated matrix than other biological samples, thus, this finding is very important and applies to other fields.

To overcome the limitations of isotope dilution, isotope-coded derivatization (ICD) was recently designed and introduced for overcoming problems of stable isotope dilution. For GC-MS analysis, ICD is available for the general isotope-labeled reagents such as deuterium (d), ^{13}C, ^{18}O and/or ^{15}N-substitutions, and reacted with analytes for preparing internal standard (IS). ICD could be used for differential analysis, absolute quantification, and metabolic profiling through derivatization of a standard material or control sample with the heavy isotope-labeled reagent and derivatization of the targeted samples with the light isotope (H, ^{12}C, ^{16}O, or ^{14}N) labeled reagent[48,59,60]. The limiting factor of ICD is the commercial availability and the price of the ICD reagent and when it is unavailable, how complex would be its chemical synthesis. As ICD is a very important tool to produce high-quality analytical data, there are many reviews summarized its use in LC-MS analysis[48,59–62]. However, there is no single review dedicated to summarizing its use in GC-MS.

ICD Methods in the Field of GC-MS Analysis as Standardization Protocol with Special Emphasis on Metabolomics

As mentioned previously, in GC-MS analysis, derivatization is mostly unavoidable for most analytes. However, artifacts can be introduced in crude samples exclusively without being formed in pure standard mixtures causing matrix effect[63]. In addition, analytes with different functional groups react with different derivatization kinetics and the stability of the formed derivatives can vary significantly[64]. Thus, the GC-MS analysis strongly requires standardizing protocols[64,65]. One of the most used protocols for standardization is isotope dilution strategy as mentioned previously[47,56]. The introduction of isotopically labeled internal standards at different steps of sample preparation and using quality control samples for evaluation of instrument performance is for sure a good practice for protocols standardization and identification of derivatization biases sources[64,66]. A cost-effective and simpler alternative to isotope dilution strategy for protocol standardization is ICD. Until now few types of reagents were designed for ICD for GC-MS applications and most of them were applied to metabolomics or have the potential for that. These reagents can be classified into silylation, chloroformates esterification, transmethylation, and aminooxyethyl propionates oximation ICD reagents. In this review, we will discuss every class of them and their reported and potential applications in the field of metabolomics. A summary of the ICD method applied in GC-MS analysis is presented in Table 1.

Silylation reagents

Silylation reaction is one of the most used derivatization approaches due to its universal reactivity to different functional groups including alcohol, amines, and carboxylic acids (Fig. 1). Thus, it was logic that the first designed ICD reagent was the silylation reagent, d_9-*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (d_9-MSTFA). This reagent was developed by Herebian et al.[67], where they used trimethyl-d_9-chlorosilane to synthesize d_9-MSTFA following the procedure reported by Donike[68]. Herebian et al. used d_9-MSTFA not to form heavy labelled internal standards, but for the identification of unknown peaks in the chromatogram. In silylation reactions, alkoxyamines are added in case of analytes possessing the enolizable carbonyl groups to avoid the formation of multiple derivatives upon silylation, as they form stable oximes with them. Thus, in

Table 1. Summary of the GC-MS ICD reagents and their applications

Reagent	Analytes	Matrix	Application	Reference
d_0/d_9-MSTFA+d_0/d_9-MHA	Amines, amino acids, fatty acids, alcohol	Bacterial extract	Metabolite profiling of Corynebacterium glutamicum	67)
d_0/d_9-MSTFA	Sugars, amino acids, and organic acids	Urine and serum	Absolute quantification of metabolites	69)
d_0/d_6-MTBSTFA	Amino acids, fatty acids, and organic acid	Serum	Relative quantification and differential metabolomics	70)
d_0/d_3-MCF in d_0/d_3-methanol	Amino acids and organic acids	Urine and serum	Absolute quantification of metabolites	74,75)
NaOH in d_0/d_3-methanol	Fatty acids	Plant oil and yeast cell lysates	Quantitative comparison of the fatty acid composition	76)
	Fatty acids	Yeast extracts	Identification and classification of microorganisms based on their fatty acids composition	77)
	S-fatty acylated proteins	Zebrafish liver	Monitoring changes of S-fatty acid acylation of the liver proteins upon exposure to the DDT	78)
	Fatty acids	Zebrafish liver	Monitoring aberrant hepatic lipid composition upon exposure to DDT	79)
$^{13}C_0$-d_3-AEP, $^{13}C_1$-d_3-AEP, $^{13}C_2$-d_3-AEP	Aldehydes and ketones	Turmeric root	Triplex ICD with ZMI mass tagging for carbonyl profiling and absolute quantifications of two methyl ketones	80)
$^{13}C_0$-d_3-MAP, $^{13}C_1$-d_3-MAP, $^{13}C_2$-d_3-MAP	Aldehydes and ketones	Laboratory prepared mixtures	Upgrading aminooxyethyl propionates through improving reagent selectivity and adduct stability and aided EI-induced ZMI reporter ion generation	81)

addition to d_0/d_9-MSTFA, Herebian et al. used d_0/d_3-o-methylhydroxylamine (d_0/d_9-MHA) for methoximation and identification of metabolites possessing carbonyl groups. It was found that d_0/d_9-MHA labeled metabolite pairs showed only a small shift in retention time with appropriate changes in the mass spectra. While metabolites pairs labeled with d_0/d_9-MSTFA shown very similar retention times. In addition, d_0-MSTFA and d_9-MSTFA were found to have very similar reactivity as proved by the similar numbers of reacted metabolites to each one of them. Using d_0/d_3-MHA and d_0/d_9-MSTFA, Herebian et al. were able to elucidate many derivatization groups and molecular weights of various unknown metabolites. They could identify about 31 metabolites in Corynebacterium glutamicum hydrophilic extracts for the first time[67]. Silylation and methoximation reactions schemes are presented in Fig. 1a and 1b, respectively. Afterward, Lien et al. used also d_9-MSTFA, but this time for synthesizing internal standards[69]. They found that the normalization of data by ICD significantly increased the precision when compared with traditional derivatization strategies even when tested on serum and urine samples. However, it was found that d_9-MSTFA derivatives had varying responses when spiked as IS in MSTFA derivatized samples. This fact pointed to a major challenge of MSTFA derivatization, which is the requirement of optimizing derivatization condition to obtain complete reaction with stable products[69]. Furthermore, Huang and Regnier introduced another silylation ICD reagent, d_0/d_6-*N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (d_0/d_6-MTBSTFA) (Fig. 1c). They used d_0/d_6-MTBSTFA as an ICD reagent in combination with GC-MS (time-of-flight; TOF) analysis for relative quantification and differential metabolomics study[70]. They used d_0/d_6-MTBSTFA due to the greater hydrolytic stability of its derivatization products than those obtained from MSTFA derivatization, thus, preventing the scrambling of isotopomers in the course of GC-MS analysis. Using differential coding with d_0/d_6-MTBSTFA, the authors were able to compare the concentration of amino and fatty acids between two different samples simultaneously. The authors confirmed that the derivatization efficiency and sta-

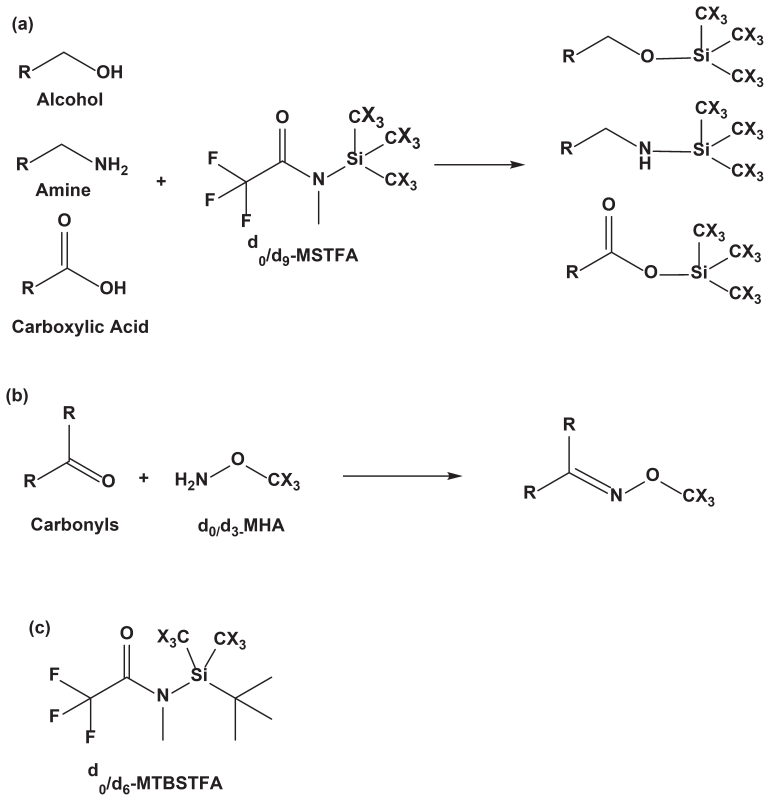

Fig. 1. Silylation and combinatorial methoximation reagents and their reaction schemes where (a) silylation reaction of d_0/d_9-MSTFA with different functional groups, (b) reaction scheme of d_0/d_3-MHA with carbonyls and (c) the structure of d_0/d_6-MTBSTFA. The isotope-coded parts of the reagents are referred to as "X".

bility of the product are identical for the derivatization reagent and its deuterium-labeled analog[70]. Thus, d_0/d_6-MTBSTFA are perfect candidate ICD reagent pair for metabolomics studies. However, for absolute quantification, spiking of internal standards in all samples and the calibration curves standard series is required[60,71]. In conclusion, silylation ICD GC-MS analysis is among the most frequently used methods in the field of metabolomics, however, there are some drawbacks inherited in this technique. For instance, the instability of MSTFA products and the scrambling caused by this. Thus, this ICD approach is more suited for target metabolite profiling and not for non-target approaches. In addition, the high price and limited availability of isotopically labeled silylation reagents is also another obstacle. Thus, more development and further research is strongly needed in this area.

Esterification using methyl chloroformate

Esterification with chloroformates is a general derivatization approach for the carboxylic functional group in GC-MS analysis due to the rapid nature of the esterification reactions in aqueous media forming stable derivatives[72]. The method was modified and optimized for the determination of both amino and non-amino organic acids including di- and tri-carboxylic acids, through modification of reaction medium and conditions[73]. Hence, esterification with chloroformate, including methyl chloroformate (MCF), is very useful in metabolomics studies concerned with amino and organic acids metabolites. In addition, when they are compared with MSTFA derivatization, the reaction products of MCF derivatization are more stable and reproducible[24]. Also, esterification reaction could be performed using *in situ* aqueous derivatization, contrast for the silylation reactions that necessitate the use of hazardous organic solvent. Thus, esterification with chloroformate ICD reagents seems to be a better alternative to the ICD strategy done with silylation. The formed derivatives are more stable and carboxylic and amino functional groups are labeled differently with the alcohol solvent (usually methanol) and the chloroformate (usually MCF), respectively (Fig. 2). Kvitvang et al. established an absolute quantitative GC coupled with tandem MS (GC-MS/MS) for analysis of about seventy metabolites upon ICD with d_0/d_3-MCF in d_0/d_3-methanol, where the heavy labeled standards are spiked

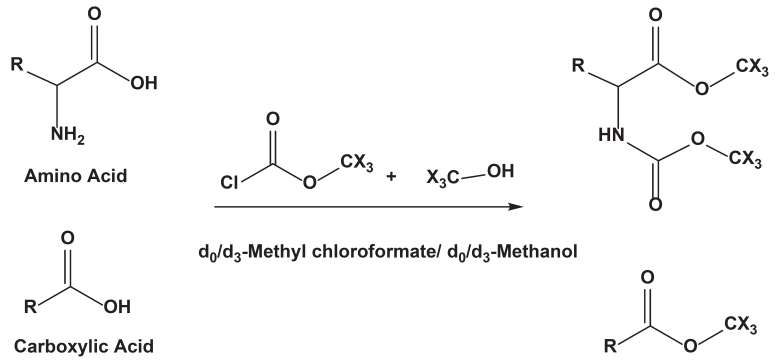

Fig. 2. Reaction scheme for esterification reactions with d_0/d_3-MCF where the isotope-coded parts of the reagents are referred to as "X".

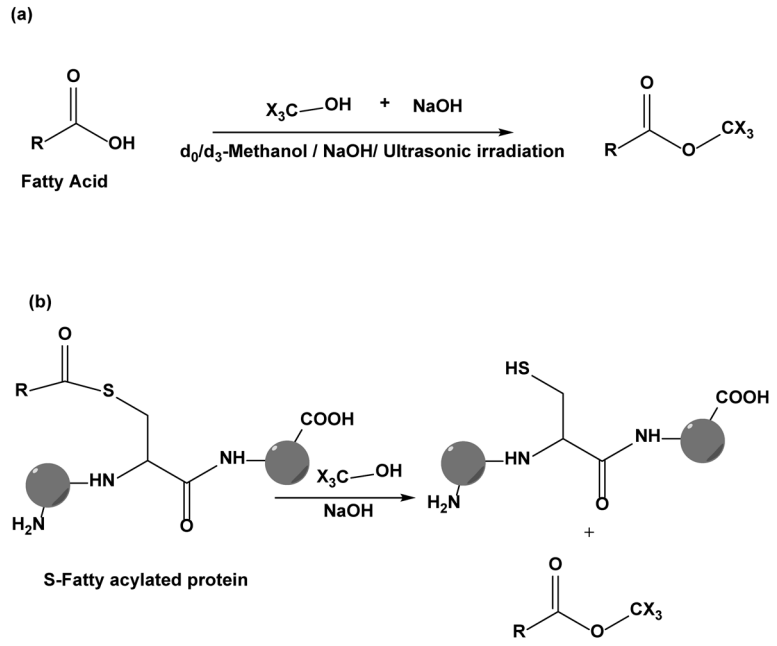

Fig. 3. Reaction scheme for IFAT derivatization where (a) is the general IFAT for free fatty acids and (b) IFAT for S-fatty acylated proteins.
The isotope-coded parts of the reagents are referred to as "X".

into light-labeled samples. Moreover, they used positive chemical ionization (PCI) instead of the traditional electron impact ionization. PCI has the privilege of preserving the molecular ion even for high molecular weight compounds, thus, enabling selective and unique selected reaction monitoring (SRM) in the tandem mass mode even between co-eluting metabolites[74]. However, d_3-MCF is expensive to buy and even its synthesis is not cost-effective as the used substrates are also expensive. An alternative way is to use a combination of d_0-MCF and d_0/d_3-methanol. This will not perform ICD correction for analytes lacking carboxylic groups; however, it is still feasible to use this strategy for creating internal standards for amino acids as their carboxylic groups will be labeled with the d_3-methanol[60]. Furthermore, to overcome shipment restriction for MCF, d_0/d_3-methanol was used in combination with ethyl chloroformate. These methods yielded lower sensitivity than the original d_0/d_3-MCF method, due to the unavoidable use of less intensity SRM transitions to maintain selectivity. However, the analytical precision was much better than those obtained using external standards only[75].

Isotope-coded fatty acid transmethylation (IFAT)

Recently, Li et al. reported the isotope-coded fatty acid transmethylation (IFAT) as a novel ICD approach[76]. IFAT integrates extraction, transmethylation, and isotopic labeling in one-step using ultrasonic irradiation (Fig. 3a). In this technique, the samples were mixed simultaneously with

sodium hydroxide in d_0/d_3-methanol for ICD transmethylation and n-hexane solution for extraction. The wave shocks and cavitation produced by the ultrasonic irradiation speeded up the alkaline-catalyzed transmethylation and facilitated the mass transfer of the produced fatty acid methyl esters into the top n-hexane layer, which was then separated and injected into the GC-MS system. The obtained sensitivity were at the picogram level with this approach. This technique has many advantages including carrying out heterogeneous reactions efficiently where solid samples can be easily derivatized and analyzed with the aid of ultrasonic irradiation. In addition, it provides accurate quantification of fatty acids where ICD can be used for providing the internal standards for a perfect comparison of the composition of fatty acids in different samples. Furthermore, the IFAT strategy volatilizes the labeled analytes, thus, it isolates them from the background matrices and minimizes the interferences from the biological components. This approach was applied successfully for quantitative determination of the fatty acid composition of plant oil and budding yeast cell lysates[76]. The same research group used the IFAT approach for quantitative analysis of fatty acids in different microorganisms aiming at classifying and identifying unknown microorganisms based on their fatty acids identity and quantity. This method is best suited for typing fungal species due to their distinguished lipid biosynthetic pathways compared with bacteria and animals[77]. Furthermore, IFAT was applied for qualitative analysis of S-fatty acylation of proteins. Cellular proteins were extracted from different cell states and then separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein bands are excised followed by in-gel IFAT. Acyl moieties can be directly released from S-fatty acylated proteins by IFAT alkaline-catalyzed transmethylation (Fig. 3b). The resulted fatty acid methyl esters after the ICD with d_0/d_3-methanol were identified by GC-MS. This approach was successfully applied to monitor the changes of S-fatty acid acylation of zebrafish liver protein upon its exposure to the insecticide, dichlorodiphenyltrichloroethane (DDT). This approach was found to be simpler, more effective, and by far less hazardous than the commonly used radioactive fatty acid analogs metabolic incorporation approach[78]. Furthermore, the IFAT approach was used again for monitoring changes that occur to zebrafish metabolomics upon exposure to DDT, but this time through monitoring of the aberrant hepatic lipid composition. Polyunsaturated fatty acids were found to decrease in response to DDT exposure. On the other hand, saturated long-chain fatty acids were found to be increased in response to DDT in a concentration-dependent way. From all these reported methods, we can conclude the outstanding performance of IFAT in the field of lipidomic and even in proteomics[79].

Oximes formation using aminooxyethyl propionates

Recent reports showed that the first multiplex ICD reagent was synthesized based on three isotopic 2-aminooxyethyl propionates (AEP) and applied to chemo-selective compounds for the determination of aldehydes and ketones using GC-MS[80]. The three derivatives were as follow: $^{13}C_0$-d_3-AEP, $^{13}C_1$-d_3-AEP, $^{13}C_2$-d_3-AEP. Upon reaction of the aforementioned three AEPs with carbonyl compounds, the formed oximes yielded three mass products ions (*m/z* 32, 33, and 34), upon their electron ionization (EI) induced cleavage (Fig. 4a). The reagents were designed specifically to produce these reporter ions as they lie in the zone of minimal interference (ZMI; 31−37 *m/z*) explored by the authors. Through this triplex ICD strategy, simultaneous GC-MS analysis of multiple samples that allows identification and quantification of multiple analytes became possible with accuracy reached about 95%. It is noteworthy that this type of oximes formation reactions is performed

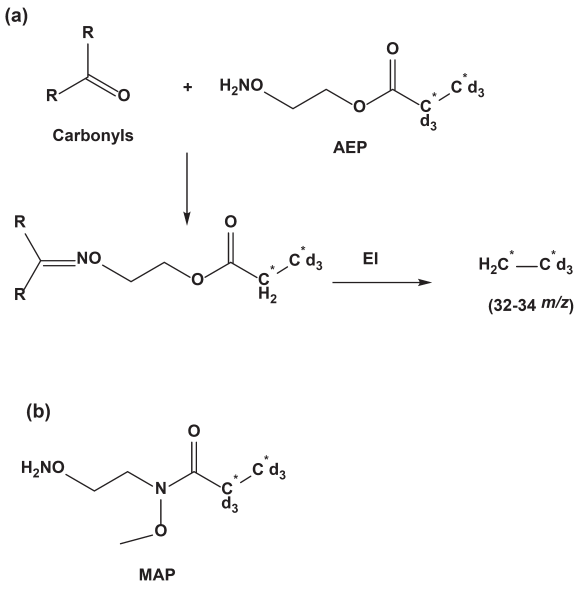

Fig. 4. Aminooxyethyl propionates reagents and their reaction scheme where (a) the reaction scheme of AEP with carbonyls and (b) the structure of MAP. The isotope-coded parts of the reagents are marked with asterisks (*).

through *in situ* aqueous derivatization, thus, there is no need to remove excess reagents as discussed previously in the introduction part. This simultaneously multiple samples analysis approach can significantly increase the analytical throughput cost-effectively and improve the accuracy of the quantification at the same time. The method was applied successfully for the absolute quantifications of the two methyl ketones namely 2-nonanone and 2-undecanone in the turmeric root extract[80].

Despite the success of AEP triplex ICD, there is still a concern about the susceptibility of the ester linkage toward hydrolytic cleavage, in addition to the possibility of transacylation reactions with nucleophiles, by either the AEP itself or its by-products. These reactions could perform ICD for metabolites that are not originally targeted by the aminooxy functionality. Furthermore, the EI induced cleavage process of AEP derivatization products needs to be enhanced in order to improve that mass spectral tag detection in the ZMI through minimizing uninformative fragmentations. Thus, the same research group of Nantz and coworkers pursued the synthesis and evaluation of an alternative *N*-methoxy amide analog, *N*-methoxy-*N*-(2-aminooxyethyl)-propionate (MAP). In MAP, the ester group of AEP is replaced with an *N*-methoxy amide group (Fig. 4b). This design for the reagent increased the its stability towards hydrolysis and increased adduct stability towards uninformative fragmentations, endorsing selective EI-induced generation of the ZMI reporter ion. Moreover, the *N*-methoxy amide functionality was proved to be strongly resistant to untargeted reactions with amines or bases even at elevated temperatures. Hence, MAP is considered as a better alternative to AEP for multiplexed GC-MS analyses of aldehydes and ketones, especially at complex samples mixtures[81]. At last, it is noteworthy that the triplex ICD with AEP or MAP is not applied yet in metabolomics. However, they have a very strong potential for that owing to their ability for multiple sample analysis and their targeting to interesting group of metabolites, aldehydes and ketones.

Conclusion

In this review, the developed ICD in the field of GC-MS analyses were discussed and summarized highlighting their potential applications to metabolomics. Until now, few types of reagents were designed for ICD for GC-MS applications. These reagents can be classified into silylation, chloroformates esterification, transmethylation, and aminooxyethyl propionates oximation ICD reagents. The reported ICD GC-MS methods showed great success in metabolic profiling and sometimes in absolute quantification, however, the reported methods are not so much. The reasons for the non-wide use of ICD in GC-MS analysis can be the high price and limited commercial availability of ICD reagents. Moreover, GC-MS methods targeted for metabolomics are mostly performed in scan mode, leading to the generation of very complex chromatograms, with many fragment ions connected to each analyte. Therefore, the introduction of isotope-coded internal standards can significantly further increase the chromatogram and mass spectra complexity, compromising the data analysis process. Thus, cost-effective ICD such as IFAT was introduced to overcome such a problem. Moreover, new techniques such as ZMI reporter ICD were introduced and improved recently. With this emerging technique, we expect great future development in the ICD-GC-MS field either through the design of new reagents which is either commercially available or can be easily synthesized to satisfy the increasing needs for such reagents in different analytical areas, especially in the metabolomics field.

Declarations of Interest Statement

All the authors declare that there is no conflict of interest.

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