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Review

A new strategy for ionization enhancement by derivatization for mass spectrometry☆

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ABSTRACT

Liquid chromatography–mass spectrometry (LC–MS) using atmospheric pressure ionization is drastically different from hitherto available analytical methods used to detect polar analytes. The electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources of MS have contributed to the advancement of LC–MS and LC–MS/MS techniques for the analysis of biological samples. However, one major obstacle is the weak ionization of some analytes in the ESI and APCI techniques. In this review, we introduce high-sensitivity methods using several derivatization reagents for ionization enhancement. We also present an overview of chemical derivatization methods that have been applied to small molecules, such as amino acids and steroids, in biological samples.

Contents

1. Introduction

Mass spectrometry (MS) is highly popular because of its high sensitivity and specificity compared to other analytical techniques [1,2]. The hyphenation of gas chromatography to MS (GC–MS) was achieved in the 1950s and such instruments became commercially available in the 1970s. Relatively inexpensive and reliable GC–MS systems are an indispensable fixture in many clinical biochemistry laboratories. Numerous methods that employ GC–MS and tandem mass spectrometry (MS/MS) have been developed as well [3–6]. The hyphenation of MS to liquid chromatography (LC–MS) is an obvious extension and several interfaces have been developed. Atmospheric pressure chemical ionization (APCI) was introduced and combined with MS analysis in the early 1970s [7–9]. Furthermore, this trend accelerated with the development of the electrospray ion source by Fenn et al. in the 1980s [10]. Manufacturers rapidly developed instruments equipped with electrospray ion sources, and this move had a great impact on protein and peptide biochemistry. In recent years, the number of publications opting for the use of LC–MS and LC–MS/MS techniques has increased. The ionization sources of MS contributed to the advancement of LC–MS

Abbreviations: APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; GC–MS, gas chromatography–mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; LC–MS/MS, liquid chromatography tandem mass spectrometry.

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and LC–MS/MS techniques for the analysis of not only biological samples [11–13] but also environmental samples [14–16].

Disease biomarkers are important because accurate diagnosis and treatment monitoring are the foundation for successful outcomes. Genomic, proteomic and metabolomic technologies are being used to search for novel disease biomarkers. Disease biomarkers met early diagnostic needs by acting as indicators of disease severity, response to treatment, disease recurrence, or patient's prognosis [17]. However, major obstacles for the determination are very low concentrations in human samples, and the weak ionization of analytes such as hormones in the electrospray ionization (ESI) and APCI techniques of LC–MS and LC–MS/MS, which leads to low inherent sensitivity and/or matrix effects. These reasons, the limit of detection (LOD) is required too high for application to disease biomarkers and related metabolites present at very low concentrations in biological samples. To overcome these drawbacks, several means to remove other matrix components and concentrate the sample have been proposed. One of them, solid-phase extraction (SPE), is commonly used for the removal of proteins and other matrix components from biological samples [18–20]. If we want to concentrate a sample to measure analytes present at low concentrations, the sample should have a large volume. Of course, long sample preparation times are obviously a disadvantage and multi-step procedures are prone to the loss of analytes. The adsorption of analytes on the walls of extraction devices may occur and trace impurities in the extraction solvent can simultaneously become concentrated. It is difficult to concentrate a biological sample, particularly if that sample has a small volume. A low LOD and a high sensitivity would allow for the reduction of the sample volume required for the analysis, and consequently the reduction of the volume of blood drawn from patients.

ESI is considered to be useful for compounds that form ionic species in solution, while APCI is useful for low to medium polarity compounds having high proton-affinity atoms, such as oxygen and nitrogen. The chemical and physical properties of an analyte are perhaps the most critical parameters for realizing superior sensitivity in various ionization modes. Ionization state and surface activity that are directly related to the properties of the analyte determine the ionization efficiency is expected to improve detection sensitivity [21]. Chemical derivatization should be performed for ionization state and surface activity in a target functional group before analysis by MS for enhancement of ionization. Derivatization changes the structure of an analyte and as a result, its physical and chemical properties are changed as well to yield high ionization efficiency. The chromatographic retention of the analyte will also be changed after derivatization and therefore, the decrease in ionization suppression caused by the co-elution of matrix components may be realized.

In this review, we introduce high-sensitivity methods that use several derivatization reagents for ionization enhancement. We also present an overview of chemical derivatization methods that have been applied to small molecules in biological samples.

2. Ionization enhancement by derivatization reagents

2.1. Aldehydes and ketones

It has been reported that carbonyl compounds, including aldehydes and ketones, could react with 2,4-dinitrophenylhydrazine (DNPH) [22]. Formaldehyde, acetaldehyde, benzaldehyde, acrolein, and C3–C6 n-alkanals were also determined as 2,4-dinitro-3,5,6-trideuterophenylhydrazones in air samples using LC–APCI-MS [23–25]. To this day, however, the use of MS to detect these DNPH derivatives in biological matrices is rare [26–28] and thus, the feasibility of this approach for the detection of compounds with multiple carbonyl moieties requires further investigation. Andreoli et al. reported the enhancement of both chromatographic separation and ionization efficiency of DNPH derivatives using LC–APCI-MS/MS [26]. Compared with ESI, APCI had a wide linear dynamic range of up to five orders of magnitude and an approximately 10-fold lower LOD. The LODs were in the 0.3–1.0 nM range for malondialdehyde, acrolein, 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal, and several alkanals in APCI. Lord et al. reported the derivatization with dansyl hydrazine reagent for ionization enhancement [29] and succeeded in increasing the detection responses of malonyldialdehyde from human plasma. Barry et al. developed a highly sensitive charged precolumn derivatization reagent (4-hydrazino-4-oxobutyl) [tris (2,4,6-trimethoxyphenyl)] phosphonium bromide (TMPP-PrG), to derivatize aldehydes and ketones and facilitate their detection by LC–ESI-MS [30]. The increase in molecular mass with the formation of the derivative allows for the easy discrimination from background interferences and the chemical noise of the mass spectrum. The derivatization reagents for aldehyde and ketones are summarized in Table 1.

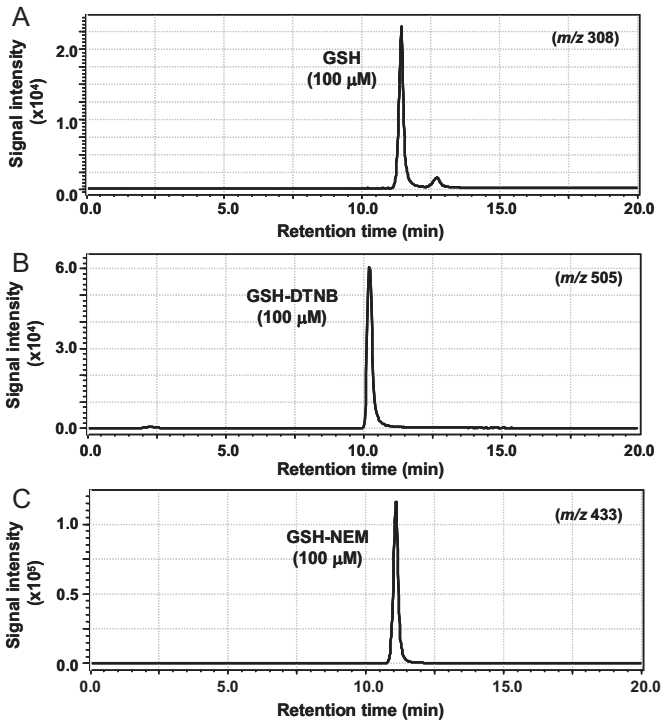

Fig. 1. Comparison of chromatograms by derivatization with DTNB or NEM. (A) glutathione, (B) glutathione-DTNB, and (C) glutathione-NEM. Analytical separation was performed on Atlantis™ HILIC silica column. The elution profile of chromatogram (B) was as follows: 0–20 min 90–70% (B). Mobile phase (A) was 0.5 mM ammonium formate buffer (pH 4.0) and (B) acetonitrile.
Source: Reproduced from Fig. 1 in Ref. [40].

2.2. Thiols

Reduced thiols are auto-oxidized by dissolved oxygen. Therefore, it is necessary to protect the thiol group. The derivatization reagents for thiols are summarized in Table 2. Reduced glutathione (GSH) could be determined by MS measurements after derivatization with 5,5′-dithio-(bis-2-nitrobenzoic) acid (DTNB) [37], iodoacetic acid [38], and *p*-(hydroxymercuri)benzoate [39]. Maleimide derivatization reagents react rapidly and are useful to protect the thiol group [40]. The chromatograms of GSH and derivatizaed with DTNB and NEM reagents were shown in Fig. 1. Zabet-Moghaddam et al. reported that peptides derivatized with

Table 1
Derivatization reagents for aldehydes and ketones.

Ion source	Derivatization reagent	Analyte	Sensitivity	Sample	Reference
APCI	DNPH	Malondialdehyde	1.0 nM (LOD)	Exhaled breath condensate	[26]
		Acrolein	1.0 nM (LOD)		
		4-Hydroxyhexenal	0.3 nM (LOD)		
		4-Hydroxynonenal	0.6 nM (LOD)		
		n-Hexanal	1.0 nM (LOD)		
		n-Heptanal	1.0 nM (LOD)		
		n-Nonanal	1.0 nM (LOD)		
ESI	DNPH	Succinic semialdehyde	10 nM (LOD)	Urine Cerebrospinal fluid	[27]
ESI	DNPH	β-Dicarbonyl compound (houttuynin)	1.0 ng/mL (LOQ)	Human plasma	[28]
ESI	Dansyl hydrazine	Malondialdehyde	0.14 μg/mL (LOQ)	Human plasma	[29]
ESI	TMPP-PrG	Aldehyde/ketone (butyraldehyde etc.)	N.D.	Standard	[30]
ESI	Girard reagent P	17-Hydroxyprogesterone	10 ng/mL (LOD)	Dried blood spots	[31]
ESI	Girard reagent T	5-Formyl-2′-deoxyuridine	4.3 fmol (LOD)	Hela-S3 cells	[32]
ESI	1-Phenyl-3-methyl-5-pyrazolone	Oligosaccharides	N.D.	Human urine	[33]
ESI	DNPH	Malondialdehyde 4-Hydroxy-2-nonenal	N.D.	Exhaled breath condensate	[34]
ESI	*o*-Phenylenediamine	Estrone-2,3-quinones	5 ng/mL (LOQ)	Rat liver microsome	[35]
		Estradiol-2,3-quinones	5 ng/mL (LOQ)		
		Estrone-3,4-quinones	5 ng/mL (LOQ)		
		Estradiol-3,4-quinones	5 ng/mL (LOQ)		
ESI	DNPH	Acetaldehyde	40 ng/L (LOD)	Human urine	[36]
		Acrolein	15 ng/L (LOD)		
		Propionaldehyde	30 ng/L (LOD)		
		Crotonaldehyde	65 ng/L (LOD)		
		Butyraldehyde	35 ng/L (LOD)		
		Valeraldehyde	35 ng/L (LOD)		
		Hexaldehyde	60 ng/L (LOD)		

N.D., not described; DNPH, 2,4-dinitrophenylhydrazine; TMPP-PrG, (4-hydrazino-4-oxobutyl) [tris(2,4,6-trimethoxyphenyl)phosphonium bromide; Girard reagent P, 1-(carboxymethyl)pyridinium chloride hydrazide.

NEM or iodoacetanilide showed significant enhancement in ionization efficiencies [41].

2.3. Phenols and alcohols

The accurate measurement of 17β-estradiol (E2) in human serum is important for the precise evaluation of ovarian function, fertility, menopausal status, and cancer risk [42,43]. Numerous methods employing GC–MS and MS/MS have been developed for steroid analysis [44–46]. A major obstacle in steroid analysis by LC–MS and LC–MS/MS is that many steroids are weakly ionizable by the ESI and APCI techniques of LC–MS and LC–MS/MS, leading to low inherent sensitivity. Over the years, chemical derivatization techniques were developed to enhance the ionization of various analytes in LC–MS or MS/MS [47]. One analyte, ethinylestradiol, was dansylated [48].

Xu and Spink compared dansyl chloride, 1,2-dimethylimidazole-4-sulfonyl (DMIS) chloride, pyridine-3-sulfonyl (PS) chloride, and 4-(1H-pyrazol-1-yl) benzenesulfonyl (PBS) chloride as derivatization reagents for sensitivity enhancement in the analysis of estrogens by LC–ESI-MS/MS [1]. As those reagents offer different hydrophobicities of the resultant estrogen derivatives, the analyst can choose the shorter retention times in reversed-phase HPLC by selecting one of the less hydrophobic derivatives. The product ion spectra of the dansyl and DMIS derivatives were dominated by ions representing derivatization reagent moieties. In contrast, the product ion spectrum of the PS derivative of E2 shows a series of intense ions that appear to have originated from the E2 moiety.

Higashi et al. examined the derivatization with isonicotinoyl azide reagent for ionization enhancement [49] and succeeded in increasing the detection responses of weakly ionizable hydroxys-

Table 2
Derivatization reagents for thiols.

Ion source	Derivatization reagent	Analyte	Sensitivity	Sample	Reference
ESI	5,5′-Dithio-(bis-2-nitrobenzoic) acid	Glutathione	3.3 pmol (LOD)	Rat brain, plasma, heart, lung, liver, erythrocytes and kidney	[37]
		Cysteine	16.5 pmol (LOD)		
		Homocysteine	29.6 pmol (LOD)		
ESI	Iodoacetic acid	Glutathione	100 ng/mL (LOD)	Mouse liver	[38]
		Cysteine	100 ng/mL (LOD)		
		Homocysteine	100 ng/mL (LOD)		
		γ-Glutamyl-cysteine	100 ng/mL (LOD)		
		Cysteinyl-glycine	100 ng/mL (LOD)		
ESI	*p*-(Hydroxymercuri)benzoate	Glutathione	5 nmol/g (LOD)	Yeast	[39]
		Cysteine	4 nmol/g (LOD)		
		Homocysteine	5 nmol/g (LOD)		
		γ-Glutamyl-cysteine	5 nmol/g (LOD)		
		Cysteinyl-glycine	4 nmol/g (LOD)		
ESI	*N*-Ethylmaleimide	Glutathione	30 nM (LOD)	Human saliva	[40]

Table 3
Derivatization reagents for phenols and alcohols.

Ion source	Derivatization reagent	Analyte	Sensitivity	Sample	Reference
ESI	Dansyl chloride	Ethinylestradiol	5 pg/mL (LOQ)	Rhesus monkey plasma	[48]
ESI	Pyridine-3-sulfonyl chloride	17β-Estradiol	10 pg/mL (LOQ)	Fetal bovine serum	[1]
ESI	Isonicotinoyl azide and methyl iodide	Estrone	3.0 fmol (LOD)	Standard	[49]
		Androsterone	2.1 fmol (LOD)		
ESI	*p*-Toluenesulfonyl isocyanate	3-Hydroxy-7-methyl-norethynodrel	100 pg/mL (LOQ)	Human plasma	[50]
ESI	Dansyl chloride	Testosterone	2.9 pg (LOD)	Human liver	[51]
		Cholesterol	3.9 pg (LOD)		
		Retinol (vitamin A)	8.6 pg (LOD)		
		Cholecalciferol (vitamin D3)	12 pg (LOD)		
		12-OH dodecanoic acid	22 pg (LOD)		
		3-OH palmitic acid	27 pg (LOD)		
		6β-OH testosterone	3.0 pg (LOD)		
		Deoxycholic acid	20 pg (LOD)		
		Hydrocortisone	109 pg (LOD)		
ESI	PPMP	Monosaccharides		SPPB-1 polysaccharide from *Spirulina platensis*	[52]
		D-Glucose	29.54 pmol (LOD)		
		D-Galactose	29.50 pmol (LOD)		
		D-Mannose	9.72 pmol (LOD)		
		D-Glucosamine	16.67 pmol (LOD)		
		D-Galactosamine	14.86 pmol (LOD)		
		D-Xylose	82.08 pmol (LOD)		
		D-Arabinose	117.7 pmol (LOD)		
		D-Fucose	154.0 pmol (LOD)		
		L-Rhamnose	29.58 pmol (LOD)		
ESI	Dansyl chloride	Estrogen metabolites	N.D.	Human serum and urine	[53]
ESI	C1-NANHS	γ-Tocopherol and its metabolites	N.D.	Human A549 cell	[54]
ESI	Dansyl chloride	Estrogens	2–4 pg/g (LOQ)	Human plasma	[55]
		Bisphenol A	5 pg/g (LOQ)		
		OH-PBDEs	3–30 pg/g (LOQ)		
		Bromophenols	3–60 pg/g (LOQ)		
ESI	2-Sulfobenzoic anhydride	Fatty alcohol ethoxylates	N.D.	Standard	[56]
ESI	Fusaric acid and 2-methyl-6-nitrobenzoic anhydride	Dehydroepiandrosterone	N.D.	Standard	[57]
		Testosterone			
		Pregnenolone			
		17α-Hydroxypregnenolone			
ESI	Dansyl chloride	17β-Estradiol	2.5 pg/mL (LOQ)	Rat serum	[58]

N.D., not described; PMPP, 1-(4-lsopropyl) phenyl-3-methyl-5-pyrazolone; C1-NANHS, N-methyl-nicotinic acid N-hydroxysuccinimide ester.

teroids. They concluded that charged derivatization is very useful for the detection of trace amounts of steroids having only one utilizable functional group. The derivatization reagents for phenols are summarized in Table 3.

2.4. Amines

Several derivatization methods are available for the determination of amino compounds. One of the derivatization reagents, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), has excellent features with regard to sensitivity, stability, and solubility in water [59,60]. Song et al. reported that NBD-F was used in positive ESI detection [61]. The method involves precolumn derivatization of the amine with NBD-F. The precolumn derivatization enhances the sensitivity of LC–MS/MS. They referred to the pre-column derivatization enhances the LC–MS/MS determination in three aspects: (1) allowing on-column enrichment of the highly water-soluble amines, (2) facilitating the separation on reversed-phase columns, and (3) improving the MS detection. Kurihara et al. compared the ion intensities of eight fluorescent reagents, 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F), NBD-F, dansyl chloride, 2,3-naphthalenedialdehyde (NDA), 1-pyrenesulfonyl chloride (PSC), fluorescein-5-isothiocyanate (FITC), 9-fluorenylmethyl chloroformate (Fmoc-Cl), and 3-chlorocarbonyl-6,7-dimethoxy-1-methyl-2(*1H*)-quinoxalinone (DMEQ-COCl) [62]. On the other hand, tris (trimethoxyphenyl) phosphonium promoted the reaction of carboxylic acids with primary amine groups in the 1-chloro-4-methylpyridinium iodide and triethylamine (CMPI/TEA) coupling reaction to form stable amide linkages with the corresponding amine. A simple method for the derivatization of primary amines and carboxylic acids with TMPP reagents to enhance their detection by ESI-MS was developed as well [63]. Recently, Inagaki et al. developed a highly sensitive and positively charged precolumn derivatization reagent (5-*N*-succinimidoxy-5-oxopentyl) triphenylphosphonium bromide (SPTPP), for the analysis of amines and amino acids by LC–ESI-MS/MS [64]. Using the selected reaction monitoring (SRM) mode, highly sensitive detection was possible as the SPTPP-derivatized amines and amino acids formed regular and intense product ions. Amines are easily derivatized with dansyl chloride. Timperio et al. performed the quantitative determination of glutamic acid and the ionization efficiency was significantly

Table 4
Derivatization reagents for amines.

Ion source	Derivatization reagent	Analyte	Sensitivity	Sample	Reference
ESI	NBD-F	Agmatine	0.6 ng/mL (LOD)	Rat brain, stomach and intestine	[61]
ESI	PSC	Asparaginyl-oligosaccharide	58 fmol (LOD)	Ovalbumin	[62]
	Fmoc-CI		61 fmol (LOD)		
	Dansyl chloride		164 fmol (LOD)		
	2,3-Naphthalenedialdehyde		304 fmol (LOD)		
	DMEQ-COCI		315 fmol (LOD)		
	Fluorescein-5-isothiocyanate		739 fmol (LOD)		
	NBD-F		746 fmol (LOD)		
	DBD-F		1950 fmol (LOD)		
ESI	2-Chloro-1-methylpyridinium and phosphonium compounds	*n*-Butylamine	N.D.	Standard	[63]
		Morpholine			
		Sarcosine methyl ester			
		Benzylamine			
ESI	(5-*N*-succinimidoxy-5-oxopentyl)	Phenylalanine	0.20 fmol (LOD)	Rat serum	[64]
	triphenylphosphonium bromide	Tyrosine	0.34 fmol (LOD)		
		Tryptophan	0.23 fmol (LOD)		
ESI	Dansyl chloride	Glutamic acid	0.5 pg (LOD)	Retina diffusion medium	[65]
ESI	Diethyl ethoxymethylenemalonate	23 Amino acids	0.05–0.72 mg/kg (LOQ) (exception of proline)	Honey	[66]
ESI	Dansyl chloride	Piperazine phosphate	0.01 μg/mL (LOD)	Human plasma	[67]
ESI	2,4-Dinitrofluorobenzene	α-Fluoro-β-alanine	1 μg/L (LOQ)	Human urine	[68]
ESI	3-Pyridyl isothiocyanate	Octylamine	N.D.	Standard	[69]
	DMAP-NCS	1-Aminodecane			
	m-Nitrophenyl isothiocyanate	*n*-Dodecylamine			
		n-Tetradecylamine			
		Hexadecylamine			
ESI	APDS	23 Amino acids	0.04–2.3 nmol/mL (LOD)	Human plasma	[70]

N.D., not described; NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole; PSC, 1-pyrenesulfonyl chloride; DMEQ-CI, 3-chlorocarbonyl-6,7-dimethoxy-1-methyl-2(*1H*)-quinoxalinone; DBD-F, 4-(*N*,*N*-Dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole; DMAP-NCS, *p*-(dimethylamino)phenyl isothiocyanate; APDS, 3-aminopyridyl-*N*-hydroxysuccinimidyl carbamate.

increased by the derivatization [65]. The derivatization reagents for amines are summarized in Table 4.

2.5. Carboxylic acids

Carboxylic acids are ionized by ESI-MS operating in the negative ion mode, using a basic mobile phase in which carboxyl groups are ionized. However, negative ESI-MS/MS sometimes lacks the sensitivity required for the trace analysis of carboxylic acids. Many pharmaceuticals contain carboxylic acids as either the main constituent or an impurity at low levels. MS is well suited for the detection because of its high sensitivity and selectivity. However, carboxylic acids tend to be poorly ionized by the ionization sources used in MS. To enhance the detection of carboxylic acids in ESI-MS/MS, several chemical derivatization procedures suitable for the positive ion detection have been developed [71–76]. For example, Cartwright et al. reported that tris(2,4,6-trimethoxyphenyl)phosphonium propylamine (TMPP) bromide was used as the derivatization reagent for the LC–ESI-MS/MS analysis of carboxylic acids in pharmaceutical products [75]. The derivatization reagents for carboxylic acids are summarized in Table 5.

2.6. Other

The analysis of vitamin D and its metabolites is very difficult because they exist at very low levels in the circulation. Their thermal instability and low polarity also impede the direct measurement with LC–MS or GC–MS. Aronov et al. demonstrated an LC–MS/MS method for the quantification of an array of the most biologically important vitamin D metabolites after Diels-Alder derivatization with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) [77,78]. The derivatization markedly improved the ionization efficiency of the analytes and stabilized the dihydroxyl metabolites. Duan et al. developed a highly sensitive, accurate, and selective method that permits the robust quantification of four key vitamin D metabolites in human serum [79].

3. Mechanism of ionization enhancement

Fenn et al. developed ESI into a robust ion source capable of interfacing to LC and demonstrated its application to a number of important biological molecules [10]. ESI works well with moderately polar molecules and is thus well suited for the analysis of many biological compounds. Dissolved samples are pumped through a metal capillary and nebulized at the tip of the capillary to form a fine spray of charged droplets. As a result, the liquid forms a cone jet, the so-called Taylor cone [80,81], in which positive ions drift towards the surface of the liquid jet. The droplets are rapidly evaporated by the application of heat and dry nitrogen gas, and the residual charges with electrical charge are transferred to the analytes [82]. At a certain radius, called the Rayleigh limit [83], the charge density at the surface becomes so high that the repulsion forces on the surface exceed the surface tension of the droplet. The ionized analytes are then transferred into the high vacuum of the MS via a series of small apertures and focusing voltages. The ion source and the subsequent ion optics can be operated to detect positive or negative ions, and switching between these two modes within an analytical run can be performed. ESI revolutionized biochemical research by offering a highly sensitive and specific method for the analysis of large biomolecules. For this reason, ESI has been widely used also for small polar organic molecules and is the most commonly used atmospheric pressure ionization technique.

Table 5
Derivatization reagents for carboxylic acids.

Ion source	Derivatization reagent	Analyte	Sensitivity	Sample	Reference
ESI	1-Chloro-4-methylpyridinium iodide and triethylamine	Levulinic acid	N.D.	Standard	[71]
		Benzoic acid			
		Phenylacetic acid			
		Pimelic acid etc.			
ESI	Heptadecafluoroundecylamine	N-Acetylneuraminic acid	60 amol (LOD)	Human urine	[72]
		N-Glycolylneuraminic acid	250 amol (LOD)		
		2-Keto-3-deoxy-D-glycero-D-galactonononic acid	750 amol (LOD)		
ESI	AMPP	Eicosanoids		Mouse serum Bronchial epithelial cells	[73]
		6-keto-$PGF_{2\alpha}$	0.3 pg (LOQ)		
		TxB_2	0.2 pg (LOQ)		
		PGE_2	0.3 pg (LOQ)		
		PGD_2	0.6 pg (LOQ)		
		$PGF_{2\alpha}$	0.5 pg (LOQ)		
		LTB_4	0.5 pg (LOQ)		
		5(S)-HETE	0.4 pg (LOQ)		
		8(S)-HETE	0.2 pg (LOQ)		
		11(S)-HETE	0.3 pg (LOQ)		
		12(S)-HETE	0.9 pg (LOQ)		
		15(S)-HETE	0.4 pg (LOQ)		
		Arachidonic acid	1 pg (LOQ)		
ESI	2-Hydrazinopyridine (HP) 2-Picolylamine (PA)	Chenodeoxycholic acid	5.1 fmol (HP) (LOD) 1.5 fmol (PA) (LOD)	Human saliva	[74]
		Glycochenodeoxycholic acid	6.7 fmol (HP) (LOD) 5.6 fmol (PA) (LOD)		
		α-Lipoic acid	2.9 fmol (HP) (LOD) 1.5 fmol (PA) (LOD)		
		PGE_2	5.7 fmol (HP) (LOD) 2.8 fmol (PA) (LOD)		
		5-Hydroxyindole-3-acetic acid	10 fmol (HP) (LOD) 2.6 fmol (PA) (LOD)		
		Homovanillic acid	11 fmol (HP) (LOD) 2.2 fmol (PA) (LOD)		
		2-(β-Carboxyethyl)-6-hydroxy-2,7,8-trimethylchroman	4.5 fmol (HP) (LOD) 1.9 fmol (PA) (LOD)		
ESI	2-Chloro-1-methylpyridinium and TMPP	Fumaric acid	2 fmol (LOD)	Tablets	[75]
		Sorbic acid	4 fmol (LOD)		
		Maleic acid	0.4 fmol (LOD)		
		Salicylic acid	540 fmol (LOD)		
ESI	4-APEBA	Aliphatic acid (C5–C9)	N.D.	Human urine and plasma	[76]

N.D., not described; AMPP, N-(4-aminomethylphenyl)pyridinium; TMPP, tris(2,4,6-trimethoxyphenyl) phosphonium propylamine bromide; 4-APEBA, 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide.

Mora et al. noted that the typical ESI source is a two-electrode system that consists of a capillary with a high applied voltage and an atmospheric sampling aperture plate. They proposed that an electrolytic process must be present in the ESI source for successful ionization according to the law of charge conservation [84]. Consequently, a series of derivatization methods to enhance the sensitivity of ESI-MS based on electron transfer reactions were developed by Van Berkel's group [85,86]. The introduction of an ionizable moiety can also be achieved by reacting with derivatization reagents possessing nitrogen atoms, particularly those possessing an amine group. In addition to the derivatization approaches introduced above, esterification has proven to be another useful tool for improving the detection of small polar compounds possessing hydroxyl groups [21].

In a growing number of publications, the analytes are derivatized even though LC–MS is used for analysis. Different intentions are associated with this approach [87]. The first is that the detection sensitivity can be increased for compounds that are difficult to ionize by introducing an ionic functional group. The second is that a compound class can be labeled by uniform fragmentation of the group introduced by derivatization in MS/MS analyses. This labeling can increase sensitivity and may also be suited for the screening for unknown members of a predefined class of compounds by precursor ion scanning. Finally, several derivatizations have also been proposed for some very polar compounds. Here, not ionization but chromatographic retention (and extractability) should be improved by derivatization.

4. Conclusions

MS coupled to a chromatographic detector has always been desirable due to its high sensitivity and specificity. Derivatization techniques are expected to improve the detectability of weakly ionizable analytes. Derivatization increases the molecular mass of a precursor ion and improves ionization efficiency. Thereby, there is a possibility to be able to detect weakly ionizable analytes because of eliminating interference and changing the polarity of the detection to improve fragmentation patterns and reduce matrix effects. LC–MS techniques will probably be most beneficial in clinical biochemistry when used in multiplexed and screening-type assays.

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