Review

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Derivatization in liquid chromatography for mass spectrometric detection

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ABSTRACT: Liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) has been frequently utilized for the sensitive and selective determination of the trace level compounds in biological samples. In LC/ESI-MS/MS, chemical derivatization is sometimes used to enhance the detection sensitivity of the analytes. This review presents an overview of the derivatization reagents in LC/ESI-MS/MS that have been applied to the low molecular weight compounds in recent five years (2008-2012).

Keywords: Liquid chromatography, electrospray, tandem mass spectrometry, derivatization

1. Introduction

Liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) has been frequently utilized for the sensitive and selective determination of the trace level compounds in biological samples. In particular, LC/MS/MS equipped with electrospray ionization (ESI) as the ion source is most often used method, since ESI can ionize wide range of the compounds including the polar compounds or large molecular weight compounds. Furthermore, ESI requires lower temperature for ionization compared with other ionization methods such as atmospheric pressure chemical ionization (APCI), and thus it can be used for the thermally unstable compounds.

In LC/ESI-MS/MS, the analytes having the following properties can be sensitively detected. Firstly, the analytes must be ionic or ionizable forms in the solution phase, since, in the ESI, gas phase ions are mainly generated by transferring the ions in solution into gas phase in the presence of a strong electrical field. Secondly, it is preferable for the analytes to have the appropriate hydrophobic structures, since (*i*) the hydrophobic ions prefer to reside at the droplet surface generated by electrospray and these ions enter the gas phase more readily than those in the droplet interior and show the higher signal intensities. (*ii*) The hydrophobic compounds can be well separated on the reversed phase column from salts and interfering compounds possessing suppression effects on ESI. (*iii*) The hydrophobic compounds are eluted by the mobile phase with the higher organic solvent content. The higher organic solvent content is suitable for the stable generation of charged droplets by electrospray and thus gives the higher signal intensities. Thirdly, it is desired for the analytes to have the suitable structure for MS/MS detection (selected reaction monitoring (SRM)), *i.e.*, to fragment efficiently upon collision induced dissociation (CID) and generate an intense and particular product ion. However, not all the compounds can be favorably analyzed by LC/ESI-MS/MS. Thus, chemical derivatization of the analyte is often used to enhance the detection sensitivity.

So far, a number of derivatization reagents for LC/MS/MS have been reported and they are summarized in several review papers (*1-7*). This review presents an overview of the derivatization reagents for LC/ESI-MS/MS that have been applied to low molecular weight compounds in biological samples in recent five years (2008-2012).

2. Derivatization reagents and their application

It is preferable for the derivatization reagents in LC/ESI-MS/MS to have the following properties. (*i*) The reagent reacts with the analytes under the mild conditions and produces the derivatives in high yield. Usually the reagent having a small molecular weight is favorable for this purpose. The reagent generates the derivatives having (*ii*) the ionic or ionizable moieties, (*iii*) the appropriate hydrophobicity, and (*iv*) the suitable structures for SRM. (*v*) The reagent is commercially available or can be easily synthesized.

In the early stage, the derivatization reagents were used mainly for the improvement of the chargeability. Quirke *et al.* reported the derivatization of alkyl halides, alcohols, phenols, thiols, and amines using a number of

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the reagents to achieve the enhancement of the signal intensities. A typical example is the derivatization of alcohol using 2-fluoro-1-methylpiridinium *p*-toluensulfonate. Cholesterol was converted to its *N*-methylpiridyl ether, and an intense [M^+] ion of the derivative was observed in positive ESI-MS (*8*). To introduce a permanently charged moiety is sometimes called "charged derivatization" and to introduce an ionizable moiety is called "ionizable derivatization". Usually, charged derivatization gives the stronger signal intensity in ESI-MS, however, the compounds having permanently charged moieties were not always suitable for the separation on the reversed phase column. To improve the hydrophobicity is also effective to enhance the detectability, since the analytes' affinity for the droplet surface (surface activity) strongly affects the ESI response. Nordstrom *et al.* used propionyl and benzoyl anhydride for the derivatization of bases, cytokinins, ribosides, and intact nucleotides such as AMP, ADP, and ATP (*9*). The ESI response was enhanced by the formation of hydrophobic derivatives and the retention on a reversed phase column was greatly increased. Tandem mass spectrometry (MS/MS) enables sensitive detection of the analytes, since MS/MS detection decreases the noise level and improves the signal-to-noise ratios comparing with MS detection. Therefore, the transformation of the analyte to the structure suitable for SRM is effective to enhance the sensitivity. Usually, esters, hydrazones, urea and thiourea, aromatic sulfonyl compounds, amides, and alkyl quaternary ammonium compounds are efficiently fragmented upon CID and generate an intense and particular product ion. Recently, commercially available reagents are favorably utilized for this purpose.

2.1. *Alcohols and phenols*

Alcohols and phenols are neutral compounds and they are sometimes too hydrophilic. Therefore, deivatization is used to enhance the chargeability or hydrophobicity of these compounds. One of the most widely used reagents is dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride; Dns-Cl). Dns-Cl has a tertiary amino group as an ionization moiety and an aromatic ring as a hydrophobic moiety. And Dns-Cl generates the aromatic sulfonyl compounds which are suitable for SRM. Chang *et al.* used Dns-Cl for simultaneous quantification of multiple classes of 30 phenolic compounds, including estron, α-estradiol, β-estradiol, ethinylestradiol, and bisphenol A, in blood (*10*). After extraction, the phenolic compounds were reacted with Dns-Cl at 60ºC for 5 min in acetone and sodium bicarbonate buffer (pH 10.5). The generated derivatives were separated on the reversed phase column and detected upon ESI-MS/MS. As expected, protonated molecules ([M + H]$^+$) of their dansyl derivatives were observed in the mass spectra, and the common major product ions at *m/z* 171 and 156 were observed in the product ion spectra. The ion at *m/z* 171 originated from a cleavage of a C-S bond in dansyl portion of the molecule, and the ion at *m/z* 156 was produced by loss of the methyl group from the ion at *m/z* 171. The transitions from protonated molecules to *m/z* 171 and *m/z* 156 were monitored and these derivatives were sensitively detected. The attained detection limit of bisphenol A was 180-fold higher compared with the direct LC/MS analysis of the intact compound. Dns-Cl was also used for the derivatization of the alcohols, including testosterone, cholesterol, hydrocortisone, *etc*. Dns-Cl reacted these compounds at 60ºC for 1 h in the presence of 4-(dimethylamino)-pyridine and *N*,*N*-diisopropylethylamine. The generated derivatives were sensitively analyzed by LC/ESI-MS/MS (*11*). Dns-Cl is widely used for the analysis of the compounds having hydroxyl group (*10-19*). Similarly, picolinic acid (*20-26*), fusaric acid (*27*), dimethylglycine (*28*), isonicotinoyl azide (*29,30*), and *N*-methyl-nicotinic acid *N*-hydroxysuccinimide ester (C1-NA-NHS) (*31*) were used for the derivatization of alcohols and phenols. These reagents have an ionization moiety and a hydrophobic moiety, and the generated derivatives were efficiently fragmented upon CID and were suitable for SRM. The derivatization reagents for alcohols and phenols are summarized in Table 1.

2.2. *Aldehydes and ketones*

Aldehydes and ketones are neutral compounds and the ionization efficiencies in ESI of these compounds are sometimes low. Therefore a chargeable moiety is introduced to enhance the ionization efficiency. In the early stage, hydroxylamine, 2,4-dinitrophenylhydrazine (DNPH), 1-(carboxymethyl)pyridium chloride hydrazide (Girard's P), and (carboxymethyl)trimethylammon ium chloride hydrazide (Girard's T) were used for derivatization of carbonyl compounds. These reagents enhanced the ionization efficiency of aldehydes and ketones. However, usually, several product ions were observed in the product ion spectra of the generated derivatives. Therefore they were not always suitable for the sensitive detection by SRM, though these product ions were structurally informative. The promising reagents for carbonyl compounds are 2-hydrazino-1-methylpyridine (HMP) and 2-hydrazinopyridine (HP). HMP has a quaternary ammonium group as an ionization moiety and a hydrophobic aromatic structure. Shibayama *et al.* used HMP for the analysis of oxo-steroids such as testosterone (T) and dehydroepiandrosterone (DHEA) in saliva (*36*). HMP reacted with these compounds at 60ºC within 1 h in ethanol containing 0.5% trifluoroacetic acid (TFA). The derivatives provided intense [M^+] ions in MS analysis. And in the MS/MS analysis, the product ion at *m/z* 108 (T, [*N*-methylpyridine + NH^+]) or *m/z* 109 (DHEA, [*N*-methylpyridine + NH_2^+]) were observed, which

were formed by the cleavage of the N-N bond of the hydrazone. The HMP-oxo-steroids were separated on the reversed phase column and detected by ESI-MS/MS. The sensitivity of DHEA was 2,000 times higher compared with the analysis without derivatization. However, HMP is not effective for increasing the detection responses of di-oxo-steroids, since small molecules with a multi-charge are unstable in the gas phase and provided multiple ions. To overcome these problems, HP was used for di-oxo-steroids such as 17α-hydroxyprogesterone (17OHP) (*38*). HP does not have a permanently charged moiety and thus the generated derivative does not form a multiply charged compound. HP is usable for both mono- and di-oxo-steroids (*39,40*). Dansyl hydrazine (*41*), 4-(2-(trimethylam monio)ethoxy)benzenaminium dibromide (4-APC) (*49*), 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benze naminium dibromide (4-APEBA) (*50*), were also used for the derivatization of aldehydes and ketones. These reagents have an ionization moiety and a hydrophobic moiety, and produced the derivatives having the suitable structure for SRM. The derivatization reagents for aldehydes and ketones are summarized in Table 2.

Table 1. Derivatization reagents for alcohols and phenols

Derivatization reagent	Analyte (sample) (*reference*)
Dns-Cl	Multiple class of 30 phenols (blood) (*10*); hydroxysterols, retinol, cholecalciferol, 12-OH dodecanoic acid, 3-OH palmitic acid, *etc*) (tissue extracts) (*11*); 17β-estradiol (brain) (*12*); estrone, estradiol (serum) (*13*); estrone, estradiol, estriol, and their metabolites (commercial milk products) (*14*); eight steroids (androgen, oestrogen, progestogen) (food production) (*15*); 4-dimethylaminophenol (blood) (*16*); morphine (urine) (*17*); estradiol (serum) (*18*); estrogen, estrogen metabolites (cell culture) (*19*).
Picolinic acid	19 Cholesterol precursors, 11 available sterols (*20*); 4β-hydroxycholesterol, 7α-hydroxycholesterol, 22R-hydroxycholesterol, 24S-hydroxycholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, 24S,25-epoxycholesterol (serum, rat liver microsomes) (*21*); aldosterone (standard) (*22*), aldosterone, dehydrocorticosterone, corticosterone, cortexolone (serum) (*23*); tetrahydrocortisol, allotetrahydrocortisol, tetrahydrocortisone (urine) (*24*); 2-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestrone, 4-hydroxyestradiol (incubation mixture) (*25*); testosterone, dihydrotestosterone (serum, prostate tissue) (*26*).
Fusaric acid	Dehydroepiandrosterone, testosterone, pregnenolone, 17α-OH-pregnenolone (standard) (*27*).
Dimethylglycine	Cholesterol oxidation products (plasma) (*28*).
Isonicotinoyl azide	Androgens, androsterone, 5α-androstane-3α,17β-diol (brain and serum) (*29*); 5α-androstane-3α,17β-diol (brain, serum) (*30*).
C1-NA-NHS	16 Estrogens (serum) (*31*).
FMP	Dihydrotestosterone (prostatic tissue) (*32*); propofol (blood, serum) (*33*); dihydrotestosterone, androstanediol, androstanediol-glucronide (prostate tissue) (*34*).
3-Nitrophtalic anhydride	Isoprenols (farnesol, geranylgeraniol) (tissue) (*35*).

Dns-Cl, 5-dimethylamino-1-naphthalenesulfonyl chloride; FMP, 2-fluoro-1-methylpyridinium *p*-toluenesulfonate; C1-NA-NHS, *N*-methyl-nicotinic acid *N*-hydroxysuccinimide ester.

Table 2. Derivatization reagents for aldehydes and ketones

Derivatization reagent	Analyte (sample) (*reference*)
HMP	Testosterone (saliva) (*36*); allopregnanolone (brain) (*37*).
HP	17α-Hydroxyprogesterone (saliva) (*38*); 17α-hydroxypregnenolone, progesterone, 11-ketotestosterone, 11-deoxycortisol, 17α, 20β-dihydroxypregnenone (plasma) (*39*); 17α-hydroxypregnenolone and 17α-hydroxyprogesterone (blood spots) (*40*).
Dns-Hz	Malondialdehydes (plasma) (*41*).
Girard's P	3-Oxosterols (profiling) (blood) (*42*).
Girard's T	Oxysterols (24-hydroxycholesterol, 25-hydroxycholesterol, 22-hydroxycholesterol) (cell lysate) (*43*).
DNPH	Malonedialdehyde, 4-hydroxynonenal (exhaled breath condensate) (*44*); malonedialdehyde (urine) (*45*).
Hydroxylamine	17α-Hydroxypregnenolone, 17α-hydroxyprogesterone, androstenedione, dehydroepiandrosterone, testosterone, pregnenolone, progesterone (serum) (*46*); androstenodione, dehydroepiandrosterone, testosterone (serum) (*47*).
DAABD-MHz	Aliphatic aldehydes (standard) (*48*).
4-APC	Aldehydes (malondialdehydes, aliphatic aldehydes (C5-C10)), ketones (urine) (*49*).
4-APEBA	Aliphatic aldehydes (C5-C10) (urine and plasma) (*50*).
D-Cysteine	Aliphatic aldehydes (C1-C8) (beverages) (*51*).
HTP	Testosterone, dihydrotestosterone (tissue) (*52*).

HMP, 2-hydrazino-1-methylpyridine; HP, 2-hydrazinopyridine; Dns-Hz, 5-dimethylaminonaphthalene-1-sulfonyl hydrazine; Girard's P, 1-(carbo xymethyl)pyridium chloride hydrazide; Girard's T, (carboxymethyl)trimethylammonium chloride hydrazide; DNPH, 2,4-dinitrophenylhydrazine; DAABD-MHz, 4-[2-(*N*,*N*-dimethylamino)ethylaminosulfonyl]-7-*N*-methylhydrazino-2,1,3-benzoxadiazole; 4-APC, 4-(2-(trimethylammonio)-ethoxy)benzenaminium halide; 4-APEBA, 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide; HTP, 2-hydrazino-4-(trifluoromethyl)-pyrimidine.

2.3. *Carboxylic acids*

Carboxylic acids are detectable in the negative ESI-MS. However their sensitivities are rather poor. In addition the mobile phases for the carboxylic acids separation are not always compatible with ESI-MS. Therefore carboxylic acids are sometimes transformed to the hydrophobic and ionizable structures. Higashi *et al.* reported the simple and practical method for the analysis of carboxylic acids using 2-hydrazinopyridine (HP) (*53*) and 2-picolylamine (PA) (*54*). Several biological important compounds having carboxylic acids, such as chenodeoxycholic acid, were derivatized with these reagents at 60ºC for 10 min in the presence of the condensation reagents. The resulting HP- and PA-derivatives were highly responsive in ESI-MS. And in MS/MS analysis, the derivatives of HP gave the strong product ion at *m/z* 110, derived from the protonated 2-hydrazinopyridine moiety, and those of PA gave the strong product ion at *m/z* 109, derived from 2-picolylamine moiety. The transitions to *m/z* 109 from the $[M + H]^+$ of the PA-derivatives were used for SRM. The PA-derivatization was successfully applied to a biological sample analysis. The derivatization followed by LC/ESI-MS/MS enabled the detection of trace amount of the clinically important carboxylic acids such as bile acids, homovanillic acid in human saliva. The detection responses of PA-derivatives were increased by 9-158 folds over the intact carboxylic acids (*54*). Similarly, 3-picolylamine, 3-picolylcarbinol (*55*), 3-hydroxy-1-methyl-piperidine (*56*), 3-(hydroxymethyl)-pyridine (*57*), 4-APEBA (*50*,*58*), 4-diazomethylpyridine (*59*), and 4-[2-(*N*,*N*-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DAABD-AE) (*60*,*61*) were used for the derivatization of carboxylic acids. These reagents have an amino group as ionization moiety and a hydrophobic moiety. The formed derivatives, such as esters or amides, were efficiently fragmented upon CID and were suitable for SRM. Butanol-HCl was sometimes used for the derivatization of carboxylic acids (*62*-*65*). The generated butyl esters were rather hydrophobic and often gave the characteristic product ions. Butanol-HCl derivatization is routinely used for the simultaneous analysis of amino acids and acylcarnitines in dried blood spots for newborn screening. Amino acids and acylcarnitines themselves have the ionizable amino groups. Therefore, the improvement of the hydrophobicity and formation of the suitable structure for MS/MS detection are required to enhance the detectability. A simple butanol-HCl derivatization is suitable for this purpose. Benzyl alcohol (*66*) and 9-fluorenylmethyl chlorformate (FMOC-Cl) (*67*) also improved hydrophobicity of the analytes and produced the suitable structures for SRM. The derivatization reagents for carboxylic acids are summarized in Table 3.

2.4. *Amines*

The compounds having amino group are easily protonated under acidic conditions and suitable for ESI-MS. However, they are sometimes highly polar and too hydrophilic for the separation on the reversed

Table 3. Derivatization reagents for carboxylic acids

Derivatization reagent	Analyte (sample) (*reference*)
2-Hydrazinopyridine (HP)	Chenodeoxycholic acid and glycochenodeoxycholic acid (saliva) (*53*).
2-Picolylamine (PA)	Chenodeoxycholic acid, glycochenodeoxycholic acid, prostaglandin E_2, 2-(β-carboxyethyl)-6-hydroxy-2,7,8-trimethylchroman, α-lipoic acid, homovanillic acid, 5-hydroxyindole-3-acetic acid) (saliva) (*54*).
3-Picolylamine, 3-picolylcarbinol	Fatty acids (C16-C28) (red blood cell) (*55*).
3-Hydroxy-1-methyl-piperidine	Malonic acid (serum) (*56*).
3-(Hydroxymethyl)-pyridine	Aliphatic molecules containing carboxylic acids such as jasmonic acid and salicylic acid (plant extracts) (*57*).
4-APEBA	Aliphatic acid (C5-C9) (urine, plasma) (*50*); hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, hippuric acid, benzoic acid, hydrochloric acid, prostaglandins PGE1, PGE2, PGF2α, ibuprofen, ketoprofen, naprofen, *etc*. (urine) (*58*).
4-Diazomethylpyridine	Prostaglandin E2 (brain) (*59*).
DAABD-AE	Very long chain fatty acids (C19, C20, C22, C24, C26), phytanic acid, pristanicacid (plasma) (*60*); glutaric acid, 3-hydroxyglutaric acid (dried urine spots) (*61*).
Butanol + HCl	Succinylacetone (dried blood spots) (*62*); 3-aminolevulinic acid and porphobilinogen (urine, plasma) (*63*); guanidinoacetate (plasma) (*64*); 22 amino acids (plasma, serum) (*65*).
Benzyl alcohol + HCl	Low molecular organic acids (citric acid, fumaric acid, malic acid, malonic acid, oxalic acid, succinic acid, acotinic acid) (root exudation) (*66*).
FMOC-Cl	Valproic acid (serum) (*67*).
N-Methyl-2-phenylethanamide	Di- and tri-carboxylic acids (tricarboxylic acid cycle intermediates) (heart tissue) (*68*).
p-Dimethylaminophenacyl bromide	Profiling carboxylic acid containing metabolites (urine) (*69*).

4-APEBA, 4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide; DAABD-AE, 4-[2-(*N*,*N*-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3- benzoxadiazole; FMOC-Cl, 9-fluorenylmethyl chlorformate.

phase column. These compounds are transformed to more hydrophobic structures by derivatization. Furthermore, the increase in the molecular weight decreases in the background noise from the matrix, since the background is generally lower in the higher mass range. *p*-*N*,*N*,*N*-Trimethylammonioanilyl-*N*'-hydroxysuccidimidyl carbamate iodide (THAS) (*70*) and 3-aminopyridyl-*N*-hydroxysuccimidyl carbamate (APDS) (*71*) were the reagents designed for LC/ESI-MS/MS. THAS has a quaternary ammonium group as an ionization moiety and a hydrophobic aromatic structure. It reacted with amino acids at 60ºC for 10 min in borate buffer (pH 8.8) to form urea compounds. The urea bond of the derivatives was efficiently cleaved upon CID, and produced the particular product ion at *m/z* 177, derived from the reagent moiety. Amino acids were analyzed with the detection limits of atto-mole level (*70*). APDS has a more hydrophobic structure compared with THAS, and thus APDS derivatives are suitable for the separation on the reversed phase column. APDS was used for the analysis of amino acids (*72*,*73*) and more than 100 compounds having amino group in biological fluid. The transition of all the protonated molecules to the common product ion at *m/z* 121 was monitored (*71*). As described above, urea and thiourea moieties are efficiently fragmented upon CID and they are suitable for SRM. 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC), the commonly used fluorescence derivatization reagent for amines, also generated the derivatives having urea structure and thus suitable for SRM. It was applied to the analysis of amino acids (*74*) and peptides (*75*,*76*). The derivatives gave the characteristic product ions at *m/z* 145 and *m/z* 171 derived from the AQC moiety (*75*). Dns-Cl (*77*,*79*), 3-pyridyl isothiocyanate (*80*), 4-methylpiperazinebutyl succimide (MPBS) (*81*), and dimethylaminobutyl succimide (DMABS) (*81*) have an ionization moiety and a hydrophobic moiety, and generated the suitable structures for SRM. Phenyl isothiocyanate (*83*), propyl chloroformate (*84*), 9-fluorenylmethyl chloroformate (FMOC) (*84*,*85*), and isobutyl chloroformate (*86*) have a hydrophobic moiety and generated suitable structure for SRM, though these reagents do not have the strong ionization moiety such as amino group. The derivatization reagents for amines are summarized in Table 4.

3. Conclusion

The derivatization reagents in LC/ESI-MS/MS applied to the low molecular weight compounds are summarized. These reagents enhanced the chargeability and hydrophobicity of the analyte to improve the ionization efficiency. These reagents also transformed the analytes to the structures suitable for SRM. So far, a number of compounds were used as the derivatization reagents in LC/ESI-MS/MS. It is important to utilize the suitable reagent for the target analytes. Furthermore,

Table 4. Derivatization reagents for amines

Derivatization reagent	Analyte (sample) (*reference*)
THAS	Amino acids (plasma) (*70*).
APDS	100 Compounds with amino group (plasma) (*71*); amino acids (plasma) (*72*,*73*).
AQC	Amino acid profiles (tear fluids) (*74*); γ-Glu-Val-Gly (fish sauces) (*75*); β-*N*-methylamino-L-alanine and 2,3-diaminobutylic acid (various biological samples) (*76*).
Dns-Cl	α-Fluoro-β-alanine, 5-fluorourasil, capecitabine (plasma) (77); gemcitabine and 2,2-difluoro-2-deoxyuridine (plasma) (*78*); 672 metabolites containing primary amine, secondary amine, phenolic hydroxyl group (urine) (*79*).
3-Pyridyl isothiocyanate	Aliphatic amines (C8-C16) (standard) (*80*).
MPBS, DMABS	Amino acids (plasma, blood spots) (*81*).
iTRAQ reagent	Amino acids (urine) (*82*).
Phenyl isothiocyanate	Glycine (cerebrospinal fluid) (*83*).
Propyl chloroformate	Amino acids (microdialysis sample) (*84*).
FMOC-Cl	Amino acids (standard) (*84*); glyphosate and its aminomethylphosphoric acid (cereals) (*85*).
Isobutyl chloroformate	L-prolyl-4-L-hydroxyproline (urine) (*86*).
SPTPP	Amines and amino acids (serum) (*87*).
o-Phthaldialdehyde + thiol	Arginases inhibitor, arginine (plasma) (*88*); glucosamine (urine) (*89*); dimethylarginines (plasma) (*90*).
DBD-F	Nine polyamines (nail) (*91*).
p-Bromophenacyl bromide	Anticancer chemicals (tegafur, 5-fluorouracil, gimeracil) (urine, plasma) (*92*).
Ethyl bromoacetate	Trimethylamine (urine) (*93*).

THAS, *p*-*N*,*N*,*N*-trimethylammonioanilyl-*N*'-hydroxysuccidimidyl carbamate iodide; APDS, 3-aminopyridyl-*N*-hydroxysuccimidyl carbamate; AQC, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; Dns-Cl, 5-dimethylamino-1-naphthalenesulfonyl chloride; iTRAQ, isobaric tags for relative and absolute quantification; MPBS, 4-methylpiperazinebutyl succimide; DMABS, dimethylaminobutyl succimide; SPTPP, (5-*N*-succinimidoxy-5-oxopententyl)triphenylphosphonium bromide; DBD-F, 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole; FMOC-Cl, 9-fluorenylmethyl chloroformate.

it might be also helpful to adopt the methods such as mobile phase modification, hydrophilic interaction chromatography, or nano-electrospray to enhance the detectability of the various kinds of the analytes in the fields of biomedical analysis.

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