Journal of Chromatography B, 879 (2011) 1159–1165

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

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

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ARTICLE INFO

Article history:
Received 2 November 2010
Accepted 7 February 2011
Available online 13 February 2011

Keywords:
Mass spectrometry
Ionization enhancement
Derivatization
Electrospray ionization
Atmospheric pressure chemical ionization

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.

☆ This paper is part of the special issue "Enhancement of Analysis by Analytical Derivatization", Jack Rosenfeld (Guest Editor).

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doi:10.1016/j.jchromb.2011.02.008

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.

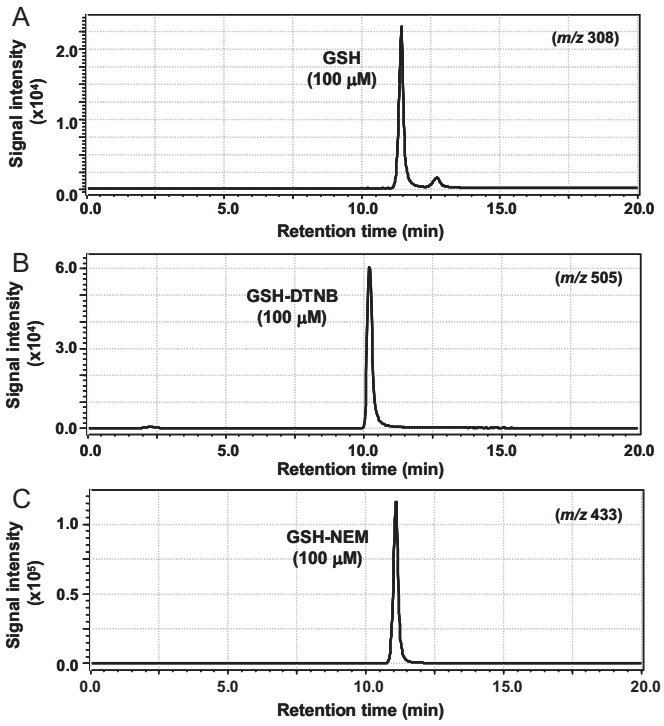

Fig. 1. Comparison of chromatograms by derivatization with DTNB or NEM. (A) glutathione, (B) glutathione-DTNB, and (C) glutathione-NEM. Analytical separation was performed on Atlantis™ HILIC silica column. The elution profile of chromatogram (B) was as follows: 0–20 min 90–70% (B). Mobile phase (A) was 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