Journal of Steroid Biochemistry & Molecular Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Review

Chemical derivatization for enhancing sensitivity during LC/ESI–MS/MS quantification of steroids in biological samples: a review

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ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 29 September 2015
Accepted 2 October 2015
Available online xxx

Keywords:
Chemical derivatization
LC/ESI–MS/MS
Endogenous steroid
Biological sample
Sensitivity
Stable isotope-coded derivatization

ABSTRACT

Sensitive and specific methods for the detection, characterization and quantification of endogenous steroids in body fluids or tissues are necessary for the diagnosis, pathological analysis and treatment of many diseases. Recently, liquid chromatography/electrospray ionization–tandem mass spectrometry (LC/ESI–MS/MS) has been widely used for these purposes due to its specificity and versatility. However, the ESI efficiency and fragmentation behavior of some steroids are poor, which lead to a low sensitivity. Chemical derivatization is one of the most effective methods to improve the detection characteristics of steroids in ESI–MS/MS. Based on this background, this article reviews the recent advances in chemical derivatization for the trace quantification of steroids in biological samples by LC/ESI–MS/MS. The derivatization in ESI–MS/MS is based on tagging a proton-affinitive or permanently charged moiety on the target steroid. Introduction/formation of a fragmentable moiety suitable for the selected reaction monitoring by the derivatization also enhances the sensitivity. The stable isotope-coded derivatization procedures for the steroid analysis are also described.

Contents

Abbreviations: 1,25(OH)$_2$D$_2$, 1α,25-dihydroxyvitamin D$_2$; 1,25(OH)$_2$D$_3$, 1α,25-dihydroxyvitamin D$_3$; 17(OH)P$_4$, 17α-hydroxyprogesterone; 17(OH)P$_5$, 17α-hydroxypregnenolone; 24,25(OH)$_2$D$_3$, 24*R*,25-dihydroxyvitamin D$_3$; 25(OH)D$_2$, 25-hydroxyvitamin D$_2$; 25(OH)D$_3$, 25-hydroxyvitamin D$_3$; 3α,5α-Adiol, 3α,5α-androstanediol; 4,25(OH)$_2$D$_3$, 4β,25-dihydroxyvitamin D$_3$; AD, androstenedione; AP, allopregnanolone; APA, aldosterone-producing adenoma; APCI, atmospheric pressure chemical ionization; BPH, benign prostate hyperplasia; CAH, congenital adrenal hyperplasia; Ch, cholesterol; CID, collision induced dissociation; CSF, cerebrospinal fluid; C-triol, cholestane-3β,5α,6α-triol; CTX, cerebrotendious xanthomatosis; DAPTAD, 4-(4′-dimethylaminophenyl)-1,2,4-triazoline-3,5-dione; DBS, dried blood spot; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; DMBA, 4-dimetylaminobenzoic acid; DMABI, dimethylaminobutyryl imidazolide; DNSCl, dansyl chloride; E$_1$, estrone; E$_2$, estradiol; E$_3$, estriol; EAD, enzyme-assisted derivatization; ESI, electrospray ionization; FA, fusaric acid; FMP, 2-fluoro-1-methylpyridinium p-toluenesulfonate; GP, Girard reagent P; GT, Girard reagent T; HMP, 2-hydrazino-1-methylpyridine; HP, 2-hydrozinopyridine; HTP, 2-hydrazino-4-trifluoromethylpyrimidine; IAC, immunoaffinity chromatography; ICD, stable isotope-coded derivatization; IHA, idiopathic hyperaldosteronism; INA, isonicotinoyl azide; IS, internal standard; LC, liquid chromatography; LLOQ, lower limit of quantification; LOD, limit of detection; LOH, late onset hypogonadism; MNAHS, *N*-methyl-nicotinic acid *N*-hydroxysuccinimide ester; MS/MS, tandem mass spectrometry; NPC, Niemann–Pick type C; NS, neuroactive steroid; O7AHD, oxysterol 7α-hydroxylase deficiency; P$_4$, progesterone; P$_5$, pregnenolone; PA, picolinic acid; PPZ, 1-(2,4-dinitro-5-fluorophenyl)-4-methylpiperazine; PTAD, 4-phenyl-1,2,4-triazoline-3,5-dione; PSCl, pyridine-3-sulfonyl chloride; QqQ, triple quadrupole; SLOS, Smith–Lemli–Opitz syndrome; SRM, selected reaction monitoring; T, testosterone; TMAPHA, *O*-(3-trimethylammoniumpropyl)hydroxylamine; UPLC, ultra high-performance liquid chromatography.

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http://dx.doi.org/10.1016/j.jsbmb.2015.10.003
0960-0760/

1. Introduction

Steroids exert strong biological activities at very low (nanomolar and even picomolar) concentrations mostly via their specific intracellular/nuclear receptors in the target organs. Sensitive and specific methods for the detection, characterization and quantification of the steroids, not only the active forms, but also their precursors and metabolites, in body fluids or tissues are necessary to elucidate the nature of the many endocrine disease processes and thus be useful for diagnosis and treatment. Because of the close structural similarity, the metabolic versatility and their occurrence at low concentrations in the body fluids and tissues, the development of reliable analytical methods for the steroids is a challenging subject for analytical chemists. Numerous methods have been proposed for the quantification of the steroids, but every one of these methods has both advantages and disadvantages. For example, immunoassay is now the mainstream method for the quantification of the steroids in the clinical field because it has a high sample throughput, but its specificity and accuracy are sometimes poor due to interference from other endogenous compounds including structurally-similar steroids.

At present, liquid chromatography (LC) coupled with electrospray ionization (ESI)–tandem mass spectrometry (MS/MS) is the most promising technique for the steroid analysis due to its specificity, versatility and simultaneous multi-analyte quantification (steroid profiling) capability. ESI has been more widely used for the steroid analysis than other atmospheric pressure ionization methods, such as atmospheric pressure chemical ionization (APCI). However, the ESI efficiency is poor for some steroids and low-energy collision induced dissociation (CID), which is employed in the triple quadrupole (QqQ) mass spectrometer (the most widely used instrument for the steroid quantification), provides only a nonspecific and/or low-intensity product (fragment) ion for most steroids. Therefore, insufficient sensitivity sometimes becomes a major problem in the trace analysis of steroids. Current LC/ESI–MS/MS instruments are extremely sensitive, but their absolute sensitivity is still analyte-dependent. Chemical derivatization is one of the most effective methods to overcome the sensitivity problem; it can enhance the ESI efficiency of the steroids and assist fragmentation suitable for the selected reaction monitoring (SRM) mode. The chromatographic behavior of the analyte also changes after derivatization, which will be good for the separation from interfering substances and for decreasing the ionization suppression (matrix effect) related to the co-elution of the biomatrix components. Furthermore, in our experience, chemical derivatization is useful for reducing carry-over for some highly hydrophobic steroids, such as sterols.

In this article, the authors present an overview of the chemical derivatization of steroids in biological sample analyses by LC/ESI–MS/MS, which was published during last decade (from January, 2005 to May, 2015); one of the authors has already presented a review of the earlier chemical derivatization for steroid analysis by LC/MS in 2004 [1]. Steroids are categorized according to their structures and functions [estrogens, androgens, progestogens, corticoids, neuroactive steroids, sterols (including intermediates in bile acid biosynthesis), and vitamin D metabolites (secosteroids)] and their derivatization procedures (reagents and reaction schemes) are reviewed. The stable isotope-coded derivatization (ICD) procedures used for the steroid analysis are also described. This review provides references on only endogenous steroids, while no attention has been given to steroid hormones used as growth promoters of animals (steroids in meat and dairy products) and used in sports doping. The review articles on LC/ESI–MS/MS assays for estrogens [2], neuroactive steroids [3,4], sterols [5–7] and vitamin D metabolites [8,9], which include fairly-detailed descriptions of the chemical derivatization for the respective steroids, have been published since 2005. Several review articles focusing on the chemical derivatization for LC/MS/MS assays of low molecular weight compounds including steroids have also been published [10–16].

2. Requirements for derivatization reagents used in ESI–MS/MS

Because the ESI process occurs in the liquid phase, the best detectability using ESI–MS has been achieved for the analysis of compounds that are either ionic or can be readily ionized in solution. Therefore, the basic idea for enhancing the detection sensitivity in the positive ESI–MS is the introduction of a proton-affinitive or permanently charged moiety to the target steroid. The proton-affinitive derivatization mostly involves the introduction of basic nitrogens, such as amino groups. The representative permanently charged moieties are the quaternary ammonium and pyridinium salts. Although the introduction of acidic moieties, which are easily deprotonated, to the analyte seems to increase the sensitivity in the negative ESI–MS, there are few practical derivatization procedures suitable for the negative ion mode [17]. It was also reported that non-amidated and glycine-conjugated bile acids were more sensitively analyzed in the positive-ion mode after converting them to the pyridine-containing derivatives than in the negative-ion mode as the intact forms, though the bile acids have a carboxyl group [18,19].

The large shift in the mass of the analyte to a higher mass range by derivatization is not always preferable for the sensitivity when using a QqQ instrument; a derivatization reagent with a higher molecular weight is not suitable [19]. When the hydrophobicity of the analytes is increased by derivatization, the mobile phase with the higher organic solvent content can be used for the reversed-phase chromatographic separation. The higher organic solvent content is suitable for the generation of charged droplets by electrospray and thus gives a higher ESI–MS response [12]. The ions of the hydrophobic compounds also reside on the droplet surface; these ions enter the gas phase more readily than those in the droplet interior and show a higher ESI–MS response. The formation or introduction of a fragmentable moiety by chemical derivatization enhances the assay sensitivity in the SRM detection, by which background noise derived from the matrices is significantly reduced. In general, ester, aromatic sulfonyl compound, urea and hydrozone are easily cleaved by the low-energy CID [12].

When taken together, the derivatization reagent for ESI–MS/MS is required to have the following properties: (1) the reagent has a proton-affinitive moiety or permanently charged moiety, (2) the

Please cite this article in press as: T. Higashi, S. Ogawa, Chemical derivatization for enhancing sensitivity during LC/ESI–MS/MS quantification of steroids in biological samples: a review, J. Steroid Biochem. Mol. Biol. (2015), http://dx.doi.org/10.1016/j.jsbmb.2015.10.003