Steroids 85 (2014) 18–29

Contents lists available at ScienceDirect

Steroids

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

Simultaneous determination of 18 tetrahydrocorticosteroid sulfates in human urine by liquid chromatography/electrospray ionization-tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form 24 March 2014
Accepted 27 March 2014
Available online 8 April 2014

Keywords:
Collision-induced dissociation
Selected reaction monitoring
Urine
Cushing's syndrome

ABSTRACT

A liquid chromatography (LC)/electrospray ionization (ESI)-mass spectrometry (MS) method for the direct determination of eighteen tetrahydrocorticosteroid sulfates in human urine has been developed. The analytes were 3- and 21-monosulfates and 3,21-disulfates of tetrahydrocortisol (THF), tetrahydrocortisone (THE), tetrahydro-11-deoxycortisol (THS), and their corresponding 5α-H stereoisomers. The mass spectrometric behavior of these sulfates in negative-ion ESI-MS/MS revealed the production of intense structure specific product ions within the same group of sulfates and permitted distinction between regioisomeric sulfates by collision-induced fragmentation with the MS/MS technique using a linear ion-trap instrument. For the quantitative analysis, selected reaction monitoring analysis in the negative-ion detection mode using triple-stage quadrupole mass spectrometer was performed by monitoring transitions from $[M-H]^-$ to the most abundant product ion of each tetrahydrocorticosteroid sulfate. After addition of 3- and 21-monosulfates of [2,2,3β,4,4-d_5]-THF, -THE, and -THS as internal standards, urine sample was applied to a solid phase extraction using a lipophilic-weak anion exchange cartridge column, and then analyzed by LC/ESI-MS/MS. The method had satisfactory performance in terms of intra- and inter-assay precision (less than 9.7% and 9.6%, respectively), and accuracy (91.2–108.2%). The limit of quantification was lower than 2.5 ng/mL for all sulfates examined. We applied this method to determine the concentration of eighteen tetrahydrocorticosteroid sulfates in the urine of healthy subjects. Thus, we have developed a sensitive, precise and accurate assay for urinary tetrahydrocorticosteroid sulfates that should be useful for clinical and biological studies.

1. Introduction

Profiling of urinary steroids is an established non-invasive diagnostic procedure of great value in assessing defects in the biosynthesis and metabolism of adrenal steroids. The adrenal cortex is divided into 3 anatomic zones: the zona glomerulosa, the zona fasciculata and the zona reticularis. The zona glomerulosa produces the mineralocorticoids such as corticosterone and aldosterone, whereas the zona fasciculate and zona reticularis produce glucocorticoids (cortisol and its precursor 11-deoxycortisol) and adrenal androgens (dehydroepiandrosterone and its sulfate), respectively [1]. While cortisol as such is secreted by the adrenal gland, cortisone is mainly produced using 11β-hydroxysteroid dehydrogenase isozymes, which convert cortisol to hormonally inactive cortisone [1]. Cortisol, cortisone, and 11-deoxycortisol (a biosynthetic precursor of cortisol) are extensively metabolized to tetrahydro-reduced derivatives: tetrahydrocortisol (THF), tetrahydrocortisone (THE), tetrahydro-11-deoxycortisol (THS), and their 5α-H stereoisomers (allo-THF, allo-THE, and allo-THS) [1,2–4]. Metabolism decreases the biological activity of hormones and converts them to hydrophilic compounds that are less protein bound and are excreted into urine. Unmetabolized cortisol and cortisone comprise only ~0.1% of the total urinary cortisol metabolites; at least 90% of the tetrahydro-derivatives of cortisol and cortisone metabolites are excreted into the urine as sulfates or glucuronide conjugates [5–8].

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http://dx.doi.org/10.1016/j.steroids.2014.03.015
0039-128X/

In order to define the biochemistry, physiology, and pathophysiology of endocrine disorders, it is desirable to quantify all of the corticosteroid metabolites found in urine. At present, analytical methods for the detection of conjugated tetrahydrocorticosteroids are based on gas chromatography/mass spectrometry (MS) [9–13] and liquid chromatography (LC)/MS [14] after enzymatic or acidic hydrolysis of conjugates followed by chemical derivatization of the liberated steroids. Although these methods are robust and sensitive, sample preparation in this indirect method is time consuming, and the sample throughput is relatively low. In addition, these methodological approaches provide no information about the type and site of conjugation. Thus the development of more straightforward methods based on the direct analysis of steroid conjugates without the required deconjugation and derivatization process is of great interest.

The application of separation by LC interfaced with soft ionization techniques, such as electrospray ionization (ESI) with MS, offers an effective analytical approach for the direct monitoring of the urinary conjugates of tetrahydrocorticosteroids [15,16]. Recently, we reported a highly sensitive and specific method using LC/ESI-linear ion-trap MS for the direct measurement of the glucuronide conjugates of THF, THE, THS, and their 5α-stereoisomers in human urine [17].

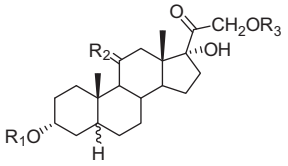
In this study, we describe a direct analytical method using LC/ESI-tandem MS (MS/MS) suitable for the simultaneous determination of 3- and 21-monosulfates and 3,21-disulfates of tetrahydrocorticosteroids (Fig. 1). These compounds represent structurally related tetrahydrocorticosteroid sulfates having slight differences between each other in the location of substituents, at C-3 and/or C-21, and the A/B ring juncture (5α-H and 5β-H steroid).

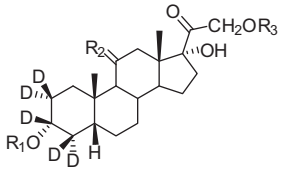
The object of this study was to elucidate the production of structure specific ions and thereby to distinguish isomeric pairs of monosulfate conjugates by collision-induced dissociation (CID) with the MS/MS technique using a linear ion-trap instrument. The formation of major product ions of selected tetrahydrocorticosteroid sulfates under negative-ion ESI-MS/MS are interpreted, and fragmentation pathways, which have not been previously reported, are proposed for these sulfates. Finally, an analytical method based on solid phase extraction combined with the use of chemically synthesized multiply deuterated tetrahydrocorticosteroid sulfates, 3-, and 21-sulfates of [2,2,3β,4,4-d_5]-THF, -THE, and -THS, as internal standards (ISs) was developed, permitting the separation and subsequent quantification of eighteen tetrahydrocorticosteroid sulfates in human urine.

2. Experimental

2.1. Chemicals and materials

All reference standard tetrahydrocorticosteroid sulfates and multi-deuterated IS were chemically synthesized in our laboratories [18,19] and their structures are shown in Fig. 1. Trivial and systematic names of the compounds are listed in Table 1. An Oasis® WAX cartridge (30 mg solid phase) was provided by Waters Co. Ltd. (Milford, MA) and conditioned by successively washings with





5β-H

THF-3S: R_1=SO_3H, R_2=β-OH, H, R_3=H
THS-3S: R_1=SO_3H, R=H_2, R_3=H
THE-3S: R_1=SO_3H, R_2=O, R_3=H
THF-21S: R_1=H, R_2=β-OH, H, R_3=SO_3H
THS-21S: R_1=H, R=H_2, R_3=SO_3H
THE-21S: R_1=H, R_2=O, R_3=SO_3H
THF-3,21diS: R_1=R_3=SO_3H, R_2=β-OH, H
THS-3,21diS: R_1=R_3=SO_3H, R=H_2
THE-3,21diS: R_1=R_3=SO_3H, R_2=O

5α-H

allo-THF-3S: R_1=SO_3H, R_2=β-OH, H, R_3=H
allo-THS-3S: R_1=SO_3H, R=H_2, R_3=H
allo-THE-3S: R_1=SO_3H, R_2=O, R_3=H
allo-THF-21S: R_1=H, R_2=β-OH, H, R_3=SO_3H
allo-THS-21S: R_1=H, R=H_2, R_3=SO_3H
allo-THE-21S: R_1=H, R_2=O, R_3=SO_3H
allo-THF-3,21diS: R_1=R_3=SO_3H, R_2=β-OH, H
allo-THS-3,21diS: R_1=R_3=SO_3H, R=H_2
allo-THE-3,21diS: R_1=R_3=SO_3H, R_2=O

ISs

d_5-THF-3S: R_1=SO_3H, R_2=β-OH, H, R_3=H
d_5-THS-3S: R_1=SO_3H, R=H_2, R_3=H
d_5-THE-3S: R_1=SO_3H, R_2=O, R_3=H
d_5-THF-21S: R_1=H, R_2=β-OH, H, R_3=SO_3H
d_5-THS-21S: R_1=H, R=H_2, R_3=SO_3H
d_5-THE-21S: R_1=H, R_2=O, R_3=SO_3H

Fig. 1. Chemical structures of 3-, 21-monosulfates, 3,21-disulfates of tetrahydrocorticosteroids, and deuterated internal standards.

Table 1
Trivial and systematic name of tetrahydrocorticosteroid sulfates.

Abbreviation	Trivial name	Systematic name
THF-3S	Tetrahydrocortisol 3-sulfate	11β,17α,21-Trihydroxy-3α-slufooxy-5β-pregnan-20-one
THF-21S	Tetrahydrocortisol 21-sulfate	3α,11β,17α-Trihydroxy-21-sulfooxy-5β-pregnan-20-one
THF-3,21diS	Tetrahydrocortisol 3,21-disulfate	11β,17α-Dihydroxy-3α,21-disulfooxy-5β-pregnan-20-one
THE-3S	Tetrahydrocortisone 3-sulfate	17α,21-Dihydroxy-3α-sulfooxy-5β-pregnane-11,20-dione
THE-21S	Tetrahydrocortisone 21-sulfate	3α,17α-Dihydroxy-21-sulfooxy-5β-pregnane-11,20-dione
THE-3,21diS	Tetrahydrocortisone 3,21-disulfate	17α-Hydroxy-3α,21-disulfooxy-5β-pregnane-11,20-dione
THS-3S	Tetrahydro-11-deoxycortisol 3-sulfate	17α,21-Dihydroxy-3α-sulfooxy-5β-pregnan-20-one
THS-21S	Tetrahydro-11-deoxycortisol 21-sulfate	3α,17α-Dihydroxy-21-sulfooxy-5β-pregnan-20-one
THS-3,21diS	Tetrahydro-11-deoxycortisol 3,21-disulfate	17α-Hydroxy-3α,21-disulfooxy-5β-pregnan-20-one
Allo-THF-3S	Allo-tetrahydrocortisol 3-sulfate	11β,17α,21-Trihydroxy-3α-slufooxy-5α-pregnan-20-one
Allo-THF-21S	Allo-tetrahydrocortisol 21-sulfate	3α,11β,17α-Trihydroxy-21-sulfooxy-5α-pregnan-20-one
Allo-THF-3,21diS	Allo-tetrahydrocortisol 3,21-disulfate	11β,17α-Dihydroxy-3α,21-disulfooxy-5α-pregnan-20-one
Allo-THE-3S	Allo-tetrahydrocortisone 3-sulfate	17α,21-Dihydroxy-3α-sulfooxy-5α-pregnane-11,20-dione
Allo-THE-21S	Allo-tetrahydrocortisone 21-sulfate	3α,17α-Dihydroxy-21-sulfooxy-5α-pregnane-11,20-dione
Allo-THE-3,21diS	Allo-tetrahydrocortisone 3,21-disulfate	17α-Hydroxy-3α,21-disulfooxy-5α-pregnane-11,20-dione
Allo-THS-3S	Allo-tetrahydro-11-deoxycortisol 3-sulfate	17α,21-Dihydroxy-3α-sulfooxy-5α-pregnan-20-one
Allo-THS-21S	Allo-tetrahydro-11-deoxycortisol 21-sulfate	3α,17α-Dihydroxy-21-sulfooxy-5α-pregnan-20-one
Allo-THS-3,21diS	Allo-tetrahydro-11-deoxycortisol 3,21-disulfate	17α-Hydroxy-3α,21-disulfooxy-5α-pregnan-20-one