LC–MS based analysis of endogenous steroid hormones in human hair

Article *in* The Journal of steroid biochemistry and molecular biology · December 2015

DOI: 10.1016/j.jsbmb.2015.12.022

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

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Review

LC–MS based analysis of endogenous steroid hormones in human hair

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 15 December 2015
Accepted 17 December 2015
Available online xxx

Keywords:
Steroid
Hormone
Hair
LC–MS
Human

ABSTRACT

The quantification of endogenous steroid hormone concentrations in hair is increasingly used as a method for obtaining retrospective information on long-term integrated hormone exposure. Several different analytical procedures have been employed for hair steroid analysis, with liquid chromatography–mass spectrometry (LC–MS) being recognized as a particularly powerful analytical tool. Several methodological aspects affect the performance of LC–MS systems for hair steroid analysis, including sample preparation and pretreatment, steroid extraction, post-incubation purification, LC methodology, ionization techniques and MS specifications. Here, we critically review the differential value of such protocol variants for hair steroid hormones analysis, focusing on both analytical quality and practical feasibility issues. Our results show that, when methodological challenges are adequately addressed, LC–MS protocols can not only yield excellent sensitivity and specificity but are also characterized by relatively simple sample processing and short run times. This makes LC–MS based hair steroid protocols particularly suitable as a high-quality option for routine application in research contexts requiring the processing of larger numbers of samples.

Contents

1. Introduction

The measurement of endogenous concentrations of steroid hormones constitutes an important aspect of research across a range of domains, including clinical, epidemiological and fundamental psychobiological inquiry (e.g., stress research). Besides potent and multiple biological functions, steroid hormones are of

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http://dx.doi.org/10.1016/j.jsbmb.2015.12.022
0960-0760/

particular interest due to their close mutual relationship with psychological functioning. This makes steroid hormones important potential mediators for linking psychological and health-related processes. A particularly relevant hormone in this context is the stress-reactive glucocorticoid cortisol, which is considered a crucial candidate for conveying the adverse effects of chronic stress on a range of diseases [1–3]. However, besides glucocorticoids, other steroid hormones are also of great interest for psychobiological inquiries, including testosterone [4–6], dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) [7,8], as well as progesterone [9,10].

Traditionally, the assessment of endogenous steroid hormone concentrations has been conducted from measurements in blood, saliva or urine samples. Although these are well-established methods, it is important to note that they reflect short-term hormone levels over periods ranging from minutes (plasma or saliva) to hours (urine). While making them well-suited for capturing dynamic aspects of endocrine activity (e.g., acute stress reactivity), these characteristics constitute a problem when aiming to derive information on *long-term* patterns of steroid hormone secretion. This is due to the fact that acutely circulating steroid hormone concentrations are strongly influenced by situational factors. For example, acute cortisol levels have been shown to be affected by factors including circadian rhythmicity and pulsatility [11–13], acute stress [14], smoking [15], alcohol consumption [16], food intake [17] or exercising [18]. Furthermore, issues of non-compliance regarding the accurate timing of saliva sampling further affect the results at ambulatory cortisol assessments [19,20]. Together, these influences can easily induce a state-dependent bias on the results of previous assessment methods. Hence, these methods are not well-suited for reflecting long-term secretory patterns.

A potential solution to these methodological caveats is provided by the analysis of steroid hormones in hair. While initially envisaged as a method for the detection of exogenous steroids, e.g., androgens in doping-related research [21,22], hair analyses for endogenous hormones in humans have received increasing attention over the past decade. By capitalizing on the continuous incorporation of lipophilic substances into the slowly growing hair matrix, hair analyses are assumed to provide an easily obtainable index of hormone levels integrated over extended periods of several months [23–25]. Over the past years, considerable evidence has supported the assumptions underlying the use of hair steroid analysis, particularly regarding glucocorticoids. Such research has generally supported hair cortisol analysis in terms of overall validity [26–29], reliability [30] and robustness to a range of confounding influences [28,31–33], although some open questions still remain to be solved (e.g., a potential role of locally produced hormones toward hair concentrations [23,34,35]). Besides validation efforts, increasing evidence also shows relevant associations between hair cortisol concentrations and stress-related psychological [36–38], psychiatric [39–41], and health-related constructs [42–44], thus leading to an increasing interest in the assessment of hair steroid concentrations.

Here we review different analytical strategies for quantifying endogenous steroid hormone levels in human hair. After providing a general overview of previous analytical methods, we will particularly focus on protocols employing liquid chromatography–mass spectrometry (LC-MS) for hair steroid analysis.

1.1. Overview of methods

Several analytical methods have been used for the measurement of steroid hormones in hair. Table 1 provides an overview of the advantages and disadvantages associated with these methods. Immunoassays with luminescence detection (LIA) or enzyme-linked immunosorbent assays (ELISA) are commonly employed methods for hair steroid analysis [45]. Given their specificity to only a single analyte, they have mostly been used in research focusing exclusively on cortisol [27,28,46]. Immunoassays are relatively sensitive and can be rapidly and easily performed. However, given that antibodies can be subject to cross-reactivity with other steroids, lipids and/or parts of the hair matrix, their specificity is likely to be low, thus leading to potential overestimation of the actual steroid content in hair [47]. Furthermore, immunoassays particularly suffer problems at low hormone concentrations (e.g., as in hair), due to low specificity of the antibody and poor method optimization over a large concentration range [48,49]. Finally, given their specificity to only a single analyte, different assays are needed in research contexts interested in studying a panel of steroid hormones in parallel [50].

Beyond immunoassay-based methods, high performance liquid chromatography with fluorescence detection (HPLC–FLU) has been used for the measurement of cortisol in human hair [51]. Although this method achieves superior specificity for cortisol than

Table 1
Advantages and disadvantages of methods employed for hair steroid analysis.

	Advantages	Disadvantages
Immunoassay	■ High throughput ■ High sensitivity ■ Easy to perform	■ Low specificity ■ Inadequate standardization ■ Limited dynamic range
HPLC-FLU	■ High specificity ■ Low cost	■ Limited sensitivity ■ Large amount of hair required ■ Intensive sample preparation ■ Only used for cortisol measurement
GC-MS	■ High specificity ■ Multiple steroids measurement	■ Limited sensitivity ■ Low throughput ■ Larger hair amounts required ■ Intensive sample preparation
LC-MS	■ High specificity ■ High sensitivity ■ Wide dynamic range ■ Less sample preparation ■ Multiple steroids measurement	■ High costs

Please cite this article in press as: W. Gao, et al., LC–MS based analysis of endogenous steroid hormones in human hair, J. Steroid Biochem. Mol. Biol. (2016), http://dx.doi.org/10.1016/j.jsbmb.2015.12.022

immunoassays, a clear downside of HPLC–FLU is that it requires relatively large amounts of hair matrix as well as extensive and time-consuming pretreatment procedures [51]. Furthermore, the protocol involves derivatization steps, which we have found to be adequate for cortisol but not for other steroid hormones (unpublished observation).

A similar situation as for HPLC–FLU pertains to the use of gas chromatography mass spectrometry (GC–MS) in the context of hair steroid analysis. GC–MS has been successfully used for the quantification of androgens, estrogens, progestins, corticoids and sterols in human hair with high specificity [52–55]. However, GC–MS protocols tend to be characterized by time-consuming work and derivatization steps as well as long run times. In addition, published GC-MS protocols have tended to work with rather large hair sample volumes (200 mg [52,53] and 30 mg [54]) compared to most LC–MS protocols (see Table 2). Hence, although HPLC–FLU and GC–MS enable accurate quantification of steroid hormone levels in hair, both methods show limited practical feasibility when larger sample numbers need to be processed.

Recently LC–MS has been reported as an accurate and convenient method for measuring small molecules [56], thus highlighting its potential suitability for steroid hormone quantification in hair. Besides excellent sensitivity and specificity, LC–MS based systems have the advantage over immunoassays and HPLC–FLU that they can measure a panel of steroid hormones simultaneously [57]. Furthermore, compared with GC–MS, no complex and time-consuming work up and derivatization of samples is necessary. LC–MS is thus highly suitable for conducting routine, high throughput analyses as are often required in the context of epidemiological or psychobiological research. Over the recent years, a number of hair steroid analytical protocols based on LC–MS have been reported. Below, we focus on different methodological aspects of these protocols.

2. Hair preparation

2.1. Collection

Hair collection procedures should follow consensus recommendations provided by the Society of Hair Testing [58]. Specifically, hair should be sampled from the *vertex posterior* region at the back of the head, which exhibits the most uniform hair growth rates [59]. Concordantly, the variability of cortisol concentrations has also been found to be smallest for hair sampled from this region [60]. Hair strands should be cut as close as possible from the scalp using fine scissors. The length of analyzed hair segments has differed between studies (range: 1–6 cm; see Table 2 for the presented methodological papers), with >80% of studies examining the scalp-near 2 or 3 cm segments (unpublished observation from an ongoing meta-analysis of hair cortisol research). Although more distal hair segments are also analyzed occasionally, the interpretability of respective findings is limited as cortisol levels have been found to decline from proximal to more distal hair segments ("wash out effect") in some studies [47,51,61], although reasons for this effect are not yet fully understood (see [23] for a more detailed discussion). This indicates that absolute hair cortisol concentrations should not be compared between different hair segments. Overall, it has been suggested that hair cortisol analyses should be restricted to the first scalp-near 6 cm [45]. The amount of hair used for analysis by LC–MS/(MS) protocols has varied considerably between methods (range: 10–100 mg; see Table 2). Besides analytical requirements, the decision about the amount of hair used for analyses further needs to include practical considerations, as participants may not always be willing to provide large amounts of hair. Importantly, the diameter of hair strands required for making up a certain amount of material depends on the length of analyzed hair segments. Hence, deciding to focus on longer hair segments (i.e., longer time periods are reflected) means that thinner hair strands would be sufficient, thus making the hair sampling more acceptable for participants.

Hair samples should be stored in a dry and dark environment at room temperature. Storage in a refrigerator or freezer is not recommended as this may induce swelling of hair which may lead to a potential loss of steroid content [58]. In addition, it has been recommended that hair samples that are wet on collection should be dried before storage and analysis [58].

2.2. Hair washing

Most protocols include one or more hair washing steps prior to analysis. These are used to (i) prevent interferences with the analysis from external sources, such as hair care products, sweat, sebum or other surface material (e.g., skin cells, head lice, body fluids), as well as (ii) to avoid potential contamination by non-blood-borne steroids coating the outer surface of hair strands. Several hair washing protocols have been employed using chloroform [21], methylene chloride [22], methanol [51,62] and isopropanol [63–65] as washing solutions. An important requirement for these solutions is that they effectively remove external contamination without actively extracting steroids from hair. This is best achieved by the use of organic solvents with low polarity whereas aqueous solutions or methanol may swell the hair, which leads to an increased risk of steroid extraction from within the hair matrix [58]. Hence, organic solvents (e.g., isopropanol, methylene chloride) are considered the method of choice for pre-analytical washing of hair samples.

There is also variability in the number and length of washing steps employed. Most protocols have used one [63–65] or two [21,22,51,62] washing steps, lasting between 2 and 10 min each. In the protocol employed by our laboratory as well as by other recent publications, hair strands are washed by shaking them in 2.5 mL isopropanol for 3 min at room temperature and subsequently allowed to dry under a fume hood for at least 12 h [63–65].

2.3. Milled hair versus whole hair

Hair analytical protocols often involve different preparation procedures prior to incubation, such as the milling [21,22,66] or mincing of hair [62,65,67] as compared to the use of whole hair [63,64]. Milling or mincing of hair is assumed to open up the hair matrix and to thus increase the efficiency of subsequent extraction [68]. However, recent evidence suggests that steroid extraction from whole/non-milled hair may also provide a valuable alternative. In one immunoassay-based study, we could show that the results of cortisol extraction from milled and non-milled hair were closely related and no differences in absolute concentrations were observed [30]. Similarly, in a more recent LC–MS/MS study, we found that the sizes of chromatographic peak areas for a panel of seven steroid hormones were in a comparable range for hair being milled or non-milled prior to incubation [63]. Although some indication was seen that steroid extraction from hair powder was slightly more efficient than extraction from whole hair, the size of this effect was minor compared to the overall signal strength and all analytes could be detected without problems using either method [63]. Given that the milling of hair involves manual sample handling steps and thus reduces throughput times, steroid extraction from non-pulverized hair seems a good choice, particularly if there is a need to process a larger number of samples. Another disadvantage of milling or mincing of hair is that it can result in an increased loss of material available for analysis compared to the use of whole hair sample.

Please cite this article in press as: W. Gao, et al., LC–MS based analysis of endogenous steroid hormones in human hair, J. Steroid Biochem. Mol. Biol. (2016), http://dx.doi.org/10.1016/j.jsbmb.2015.12.022

2.4. Steroid extraction

For steroid extraction, hair samples are incubated with the extraction solution plus added internal standards of all steroid hormones. Solutions that have been used for steroid extraction from hair include Soerensen buffer, hydrochloric acid, and methanol (see Table 2). Of these, methanol is considered the preferential extract [58] as it is able to dissolve neutral, hydrophilic and moderately lipophilic compounds and, given its hydrophilic nature, can penetrate into hair cells and produce swelling of the matrix, thus liberating the enclosed steroids. A downside of extraction by methanol is that it often incorporates interfering

Table 2
Parameters of LC-MS based methods for the measurement of steroid hormones in hair.

Analyte	Acquisition mode	Mass transition	Ionization	IS	LOQ (pg/mg)	Chromatographic column	Time (min)	Sample amount (mg)	Hair length (cm)	Incubation solution	Sample preparation	Reference
Cortisol	SIM	363	ESI+	Cortisol-d_3	40	C18,150 × 2.0 mm, 4 μm	10	50	N	Soerensen buffer	Offline SPE	[22]
	SIM	331/407	ESI-	Prazepam	100	C18,150 × 2.0 mm, 5 μm	26	50	N	Methanol	Offline SPE	[21]
	SIM	363	ESI+	Cortisol-d_3	5	C18,150 × 1.0 mm, 5 μm	17	30-100	2	Soerensen buffer	Offline SPE&LL	[66]
	MRM	363/121	APCI+	Cortisol-d_4	0.09	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
	MRM	407/331	ESI-	Cortisol-d_4	5	C18,150 × 4.6 mm, 5 μm	20	20	1	Methanol	Offline SPE	[62]
	MRM	363/121	ESI+	Cortisol-d_4	5	C18,100 × 2.1 mm, 1.7 μm	11.5	50	6	Methanol	Centrifugation	[67]
	MRM	363/121	ESI+	Cortisol-d_3	1.3	C18, 1.8 μm	10	10-30	1	Methanol	Offline SPE	[64]
	MS/MS/MS	361/331/297	ESI-	Cortisol-d_4	2	C18,50 × 4.6 mm, 2.7 μm	20	50	3	Methanol	Online SPE	[65]
Cortisone	SIM	361	ESI+	Cortisol-d_3	40	C18,150 × 2.0 mm, 4 μm	10	50	N	Soerensen buffer	Offline SPE	[22]
	SIM	329/405	ESI-	Prazepam	100	C18,150 × 2.0 mm, 5 μm	26	50	N	Methanol	Offline SPE	[21]
	SIM	361	ESI+	Cortisol-d_3	5	C18,150 × 1.0 mm, 5 μm	17	30-100	2	Soerensen buffer	Offline SPE&LL	[66]
	MRM	361/163	APCI+	Cortisone-d_7	0.07	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
	MRM	405/329	ESI-	Cortisol-d_4	5	C18,150 × 4.6 mm, 5 μm	20	20	1	Methanol	Offline SPE	[62]
	MRM	361/121	ESI+	Cortisol-d_4	5	C18,100 × 2.1 mm, 1.7 μm	11.5	50	6	Methanol	Centrifugation	[67]
	MRM	361/163	ESI+	Cortisone-d_8	9.3	C18, 1.8 μm	10	10-30	1	Methanol	Offline SPE	[64]
	MS/MS/MS	359/329/301	ESI-	Cortisone-d_7	2	C18,50 × 4.6 mm, 2.7 μm	20	50	3	Methanol	Online SPE	[65]
Testosterone	MRM	289/109	ESI+	Stanozolol-d_3	0.25	C18,150 × 2.1 mm, 1.8 μm	25	50	3	Sodium Hydroxide	LL	[82]
	MRM	289/109	APCI+	Testosterone-d_5	0.08	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
	MRM	289/97	ESI+	Testosterone-d_5	2.3	C18, 1.8 μm	10	10-30	1	Methanol	Offline SPE	[64]
Progesterone	MRM	315/109	APCI+	Progesterone-d_9	0.09	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
DHEA	MRM	271/213	APCI+	DHEA-d_4	0.9	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
DHEAS	MRM	367/97	ESI-	Cortisol-d_4	5	C18,150 × 4.6 mm, 5 μm	20	20	1	Methanol	Offline SPE	[62]
	MRM	367/97	ESI-	DHEAS-d_6	15.9	C18, 1.8 μm	10	10-30	1	Methanol	Offline SPE	[64]
	SIM	367	ESI-	DHEAS-d_2	10	C18,150 × 1.5 mm, 5 μm	22	30	N	NaOH	Online SPE	[83]
Corticosterone	MRM	347/120	APCI+	Corticosterone-d_8	0.08	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
Androstendione	MRM	287/97	APCI+	Androstendione-d_3	0.08	C18,73 × 3.0 mm, 2.2 μm	9	10	3	Methanol	Online SPE	[63]
	MRM	287/97	ESI+	Androstendione-d_5	1.3	C18, 1.8 μm	10	10-30	1	Methanol	Offline SPE	[64]
17-OH-pregnenolone	MRM	331/97	ESI+	17-OH-pregnenolone-d_8	1.87	C18, 1.8 μm	10	10-30	1	Methanol	Offline SPE	[64]
Cholesterol sulphates	SIM	465	ESI-	Cholesterol sulphates-d_7	5.0×10^4	C18,150 × 1.5 mm, 5 μm	22	30	N	NaOH	Online SPE	[83]

MRM: multiple reactions monitoring, SIM: single ion monitoring, ESI: electrospray ionization, APCI: atmospheric pressure chemical ionization, IS: internal standard, LOQ: limit of quantification, SPE: solid phase extraction, N: not mentioned.

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substances, so that the use of clean-up procedures (such as liquid/liquid or solid phase extraction) is recommended in routine practice. While aqueous acids and buffered solutions may yield cleaner extracts than methanol, they may induce hydrolysis thus leading to unwanted loss/decomposition of analytes [58,69].

Besides the solutions being used for extraction, protocols also differ in other aspects, such as extraction time, temperature and specific conditions (e.g., use of ultrasonic baths). However, systematic evaluation of data concerning these aspects is scarce. One study investigated the impact of different incubation times on cortisol and cortisone concentrations in hair [68]. Interestingly, the authors report two or more maxima for the extracted steroid levels following incubation in methanol between 5 min and 72 h. The authors interpret this as indication for a two- or multi-staged dissolution mechanism of hair cortisol and cortisone from the hair matrix which may be related to the thee-layered structure of hair [68]. However, additional future corroboration is required to confirm this notion. Based on current knowledge, it appears acceptable to employ a standardized routine using 12–18 h overnight extraction [63].

3. Analytical methodology

3.1. Solid phase extraction

The above-specified hair preparation procedures are unlikely to fully prevent the incorporation of interfering substances. Over time, this can induce blockade of the analytical column and contamination of the mass analyzer, leading to increased background noise, ion suppression or enhancement, and a more frequent need for instrument maintenance. Hence, post-incubation procedures for sample purification are required. While liquid/liquid extraction has been employed in one HPLC–FLU based protocol [51], the use of solid phase extraction (SPE) is far more common in hair analyses so that below we thus focus on this procedure.

SPE can be used to isolate analytes of interest from a wide variety of matrices, including urine, blood, and animal tissue [70–72]. Concerning the use in hair analyses, offline SPE has been employed by most laboratories [21,22,51,62,66]. However, offline SPE is relatively time-consuming, labor-intensive as well as costly, hence reducing productivity. In addition, there may be increased inter-batch variability in recovery due to the manual handling steps involved in offline SPE. Recently, online SPE techniques have been introduced to hair steroid analysis [63,65]. The respective protocols employ a column-switching technique for semi-automated hair preparation, using two pump systems and one switching valve system for online sample enrichment, purification and separation. Online SPE has been associated with high method sensitivity, shortened pretreatment and analytical times as well as a greater capacity for analyzing samples simultaneously. Furthermore, online SPE is associated with low carryover, high recovery and long column lifetime [72]. Given these advantages, the use of online SPE appears preferable to offline SPE procedures for routine hair steroid analyses, particularly when larger sample numbers need to be processed.

3.2. LC method

Chromatographic separation plays an important role in the performance of an LC–MS analytical system [73] and this particularly applies to methods working at low concentration ranges, such as with hair steroid analysis. LC separation of steroid hormones in hair has usually been performed using C_{18} reverse phase columns coupled with HPLC (see Table 2). An interesting addition in this context is the use of ultra-high pressure liquid chromatography (UPLC), a method that has been associated with superior characteristics in steroid analyses on other sample matrixes [74,75]. Specifically, narrower chromatographic peaks with higher resolution can be realized and chromatographic run times shortened. Recently, UPLC systems with sub-2 μm particle columns have first been introduced to hair steroid analysis [64,67]. The performance characteristics of these protocols have generally been superior to most HPLC based protocols (i.e., shorter run times and lower LOQs) [62,65,66], except for one protocol using a 2.2 μm particle column [63]. Still, based on the currently available data, UPLC appears to provide a promising future development for hair steroid analysis.

In all reviewed LC–MS protocols (see Table 2), the mobile phases were composed of organic and aqueous content, such as methanol, acetonitrile, water and volatile buffers (like ammonium acetate). In some protocols, the pH values of mobile phases are adjusted to acidic values between 2.6 and 4.5 [21,22,62,63,66,67]. The purpose of such mobile phase adjustment is to achieve higher sensitivity, resolution and stability as well as shorter run times.

4. Mass spectrometry

4.1. Ionization techniques

Two ‘soft’ (not-disintegrating) atmospheric pressure ionization techniques are commonly used for LC–MS based hair steroids analysis: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [50,71,72,76]. ESI is generally considered to yield better efficiency of ionization for steroid hormones than APCI. Conversely, APCI is less prone to ion suppression and matrix effects and more prone to selective ionization [77]. The low susceptibility of APCI to matrix effects should be considered a positive characteristic concerning its application in hair analyses, given that hair has been associated with strong matrix effects (see Section 5). However, as the majority of previous LC–MS based hair steroid protocols has worked with ESI (see Table 2), future evaluation is needed to confirm if APCI is indeed superior to ESI in this context.

Concerning the use of ESI and APCI, previous hair analytical protocols have used the positive mode, although for some steroids the use of the negative mode has also been reported (see Table 2). Although the structure of different steroids is similar, the ionization efficiency of individual hormones may still differ. It is thus important that the choices of ESI vs. APCI and of negative vs. positive mode during method development are made specifically for each hormone.

4.2. Mass spectrometric scan mode

All published protocols for quantitative analysis of steroid hormones in hair used triple-quadrupole mass spectrometers, which are characterized by high sensitivity and specificity as well as a large dynamic range. Acquisition has been achieved using single ion monitoring (SIM), multiple reactions monitoring (MRM) or multistage fragmentation (MS/MS/MS) modes (see Table 2). SIM mode with information of only the precursor ion has been used more commonly for hair steroids analysis. However, the limited data obtained in SIM mode make it more difficult to identify potential contaminants in the biological matrix, which may interfere with the determination of analytes (see section below). Hence, the more accurate method of LC–MS/MS with MRM mode has increasingly been employed, offering higher specificity and sensitivity even in complex matrices such as hair. The recent introduction of MS/MS/MS mode for the analysis of cortisol and cortisone in hair [65] is associated with further advantages. MS/MS/MS mode is considered to be more specific and sensitive as

Please cite this article in press as: W. Gao, et al., LC–MS based analysis of endogenous steroid hormones in human hair, J. Steroid Biochem. Mol. Biol. (2016), http://dx.doi.org/10.1016/j.jsbmb.2015.12.022

well as to yield a reduced chromatographic noise background. These characteristics of MS/MS/MS mode may be particularly advantageous when working with complex matrices such as hair.

5. Method validation

Validation procedures for the establishment of analytical LC–MS/MS protocols should include the evaluation of specificity, linearity, sensitivity, precision, accuracy, recovery, matrix effects, quality control and reference intervals [72]. Concerning specificity, the available data on hair steroid LC–MS/(MS) protocols suggests adequate performance, e.g., with one study showing that prednisolone and prednisone do not interfere with the identification of cortisol and cortisone in hair [67] and other protocols suggesting no relevant interfering peaks in the chromatograms [62,63,66].

A more critical methodological aspect concerning hair steroid analyses is the limit of quantification (LOQ). Adequacy of LOQs is frequently defined as accuracy and precision less than 15% and a corresponding signal/noise ratio greater than 10 [71,72]. Table 2 reveals that considerable variability in sensitivity estimates has been reported for hair steroid analytical protocols, with differences being particularly marked when comparing SIM mode against MRM mode protocols. Importantly, given the low concentrations of steroid hormones in hair, the LOQs of analytical protocols using SIM mode may be insufficient for capturing low endogenous concentrations. Concerning cortisol, the range of LOQs for SIM mode protocols has been between 5 and 100 pg/mg [21,22,66]. Given that reported references ranges for hair cortisol often fall considerably below 5 pg/mg [63: 1.62–7.06 pg/mg; 65: 2.18–7.22 pg/mg], protocols using SIM mode cannot be considered sensitive enough for covering the whole physiological range of steroid hormones in hair. By contrast, the LOQs for protocols using MRM mode are considerably lower, ranging between 0.09 and 5 pg/mg [62–65,67]. For most of these protocols this indicates sufficiently high sensitivity for hair steroid analysis. Still, sensitivity problems may occur even when using MRM mode. This was indicated by one published protocol using MRM mode, which reported an LOQ of 5 pg/mg. When this protocol was used to measure hair cortisol concentrations in a population of elementary school girls, the majority of samples (82.5%) could not be quantified [67]. Potential explanations for the latter situation could be that ESI was used in the positive mode, which is associated with heavy matrix effects, and that no pretreatment purification technique was used before analysis.

Precision and accuracy are closely related to sensitivity and are usually computed as the percentage coefficients of variation (%CV) and of relative error (%RE), with values below 15% generally being considered acceptable [71,78]. Concerning hair steroid analytical protocols, the reported data have tended to indicate suitable precision and accuracy of methods. Concerning hair cortisol, %CVs have ranged between 4.9 and 11% for protocols using SIM mode [21,22,66] and between 0.9 and 14.8% for protocols using MRM mode [62–64]. Likewise, published %RE ranges for protocols using MRM mode have been between 2.7 and 15.5% [63,65], while, unfortunately, no respective data has been reported for protocols using SIM mode. A similar pattern as for cortisol has also been found for other steroid hormones [62–65,67]. On balance, the available data thus suggest that both protocols based on SIM or MRM mode mostly yield satisfactory precision and accuracy.

An aspect of method validation with particularly high importance for hair steroid analysis is the investigation of matrix effects. In any LC–MS protocol, matrix constituents may enhance or suppress ionization of the analytes and thus negatively affect the quality of results. Concerning hair analyses, a common approach for the evaluation of matrix effects is the comparison of analyte signal intensity with and without the hair matrix [72,79]. Interestingly, considerable divergence of findings is seen in published protocols, with both reports of negative and positive matrix effects for steroid hormones in hair. Evidence for negative matrix effects (range: −9% to −50%) has been reported for a panel of six steroid hormones in an MRM protocol [45] and for cortisol and cortisone in an MS/MS/MS protocol [range: −8.0% to −31.5%; 65], suggesting severe suppression of ionization for these analytes and the internal standards. Conversely, indications of positive matrix effects, signaling ion enhancement effects, have been reported for MRM protocols examining progesterone [range: 12–18%; 63] as well as cortisol and cortisone [range: 29.3–49.0%; 67]. To deal with these problems, analyte-specific extraction (e.g., SPE) and the use of stable isotope labeled internal standards can be employed to minimize matrix effects [62]. Given the potentially strong adverse effects of hair matrix constituents on analytical results, the use of such techniques and regular monitoring/validation of matrix effects is crucial for ensuring the method accuracy.

6. Panels of steroids analysis

In many instances, researchers are interested in assessing a panel of different steroid hormones rather than only a single analyte. Initially, the steroid panels of LC–MS protocols used for hair analyses very much focused on exogenous substances, e.g., different corticosteroids used as illegal doping agents by athletes [21,22]. More recent approaches, however, have focused on the quantification of endogenous steroid hormones. To date, the respective panels have particularly included the assessment of glucocorticoids (cortisol, cortisone, corticosterone) as well as sexual hormones and their precursors (testosterone, progesterone, DHEA, DHEAS, androstenedione, 17-OH-pregnenolone, cholesterol sulphate; see Table 2).

While endogenous concentrations of these hormones have been shown to be reliably quantifiable in hair, it must be stated that general validation/proof-of-concept research is by far most advanced for the assessment of cortisol in hair [23,24]. Besides the availability of such fundamental data, cortisol is also the only analyte for which inter-method/inter-laboratory comparison data are available. In an earlier study, the parallel analysis of 195 hair samples by immunoassay and LC–MS/MS confirmed a high correlation between the respective results ($r^2 = 0.96$), although immunoassay data were linearly inflated by approximately 15% over LC–MS/MS data [47]. Furthermore, results of an inter-laboratory Round Robin have been reported for hair cortisol protocols, featuring blinded parallel analysis of 15 hair samples in the four internationally leading laboratories [45]. Results showed that two different LC–MS/MS methods yielded practically identical results, which, in turn, were also quite highly correlated to the findings obtained by four immunoassay protocols (r_s between 0.80 and 0.97; $ps < 0.0001$). However, again, immunoassay methods were found to yield substantially inflated hair cortisol levels, exceeding the respective LC–MS/MS data by between 2.5 and 20-fold [45].

7. Conclusion and future directions

Over the next years, the technological development of LC–MS/MS protocols for hair steroid analysis will be expected to yield further advancement in terms of more sensitive, easier to use and more robust systems. There are several areas that may further impact future developments of hair steroids analysis with LC–MS. First, the combination of triple-quadrupole and ion trap mass spectrometers within the same instrument (MS/MS/MS) has been associated with further improvements in terms of sensitivity and

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specificity [72,80], and may also be interesting for use in hair steroid analysis. Second, TOF, QTOF and Orbitrap systems (high-resolution MS), which provide accurate mass measurement capabilities as well as good sensitivity, have been recognized as a potential alternative to triple-quadrupole mass spectrometers [72,81]. Although the use of such techniques as part of hair steroid analytical protocols has some potential, further development is required. In addition, given the already excellent performance of available MS/MS/(MS) systems, the use of high-resolution MS systems, which are usually very expensive, may only be warranted for specialized applications but not for routine hair steroid analysis. Third, improvements of practicability and sample throughput, including automation of sample preparation protocols and separation science of hair steroids, may help to enhance the applicability of hair steroid analysis in clinical laboratories.

In summary, LC–MS based protocols for the analysis of endogenous steroid hormones in human hair provide highly sensitive, specific, and reliable quantification methods. Besides such analytical qualities, further characteristics that make LC–MS based protocols for hair steroid analysis particularly attractive to research and clinical laboratories include simplicity of sample processing and high sample throughput. Hence, this methodology represents a powerful and well-balanced analytical tool to be used in clinical, epidemiological, and psychobiological research.

References

[1] G.P. Chrousos, T. Kino, Glucocorticoid signaling in the cell: expanding clinical implications to complex human behavioral and somatic disorders, Ann. N. Y. Acad. Sci. 1179 (2009) 153–166, doi:http://dx.doi.org/10.1111/j.1749-6632.2009.04988.x.
[2] G.P. Chrousos, T. Kino, E. Charmandari, Evaluation of the hypothalamic-pituitary-adrenal axis function in childhood and adolescence, Neuroimmunomodulation 16 (2009) 272–283, doi:http://dx.doi.org/10.1159/000216185.
[3] G.P. Chrousos, Stress and disorders of the stress system, Nat. Rev. Endocrinol. 5 (2009) 374–381, doi:http://dx.doi.org/10.1038/nrendo.2009.106.
[4] E.R. Montoya, D. Terburg, P.A. Bos, J. van Honk, Testosterone, cortisol, and serotonin as key regulators of social aggression: a review and theoretical perspective, Motiv. Emot. 36 (2012) 65–73, doi:http://dx.doi.org/10.1007/s11031-011-9264-3.
[5] J. Archer, Testosterone and human aggression: an evaluation of the challenge hypothesis, Neurosci. Biobehav. Rev. 30 (2006) 319–345, doi:http://dx.doi.org/10.1016/j.neubiorev.2004.12.007.
[6] E. Ingudomnukul, S. Baron-Cohen, S. Wheelwright, R. Knickmeyer, Elevated rates of testosterone-related disorders in women with autism spectrum conditions, Horm. Behav. 51 (2007) 597–604, doi:http://dx.doi.org/10.1016/j.yhbeh.2007.02.001.
[7] B. Allolio, W. Arit, DHEA treatment: myth or reality? Trends Endocrinol. Metab. 13 (2002) 288–294, doi:http://dx.doi.org/10.1016/s1043-2760(02) 00617-3.
[8] O.M. Wolkowitz, V.I. Reus, E. Roberts, F. Manfredi, T. Chan, W.J. Raum, et al., Dehydroepiandrosterone (DHEA) treatment of depressiondroepiandrosterone (DHEA) treatment of depression, Biol. Psychiatry 41 (1997) 311–318 S0006322396000431.
[9] G.A. van Wingen, F. van Broekhoven, R.J. Verkes, K.M. Petersson, T. Bäckström, J.K. Buitelaar, et al., Progesterone selectively increases amygdala reactivity in women, Mol. Psychiatry 13 (2008) 325–333, doi:http://dx.doi.org/10.1038/sj.mp.4002030.
[10] A.C. Granger, M.R. Underwood, Review of the role of progesterone in the management of postnatal mood disorders, J. Psychosom. Obstet. Gynaecol. 22 (2001) 49–55, doi:http://dx.doi.org/10.3109/01674820109049950.
[11] E.A. Young, J. Abelson, S.L. Lightman, Cortisol pulsatility and its role in stress regulation and health, Front. Neuroendocrinol. 25 (2004) 69–76, doi:http://dx.doi.org/10.1016/j.yfrne.2004.07.001.
[12] F. Spiga, J.J. Walker, J.R. Terry, S.L. Lightman, HPA axis-rhythms, Compr. Physiol. 4 (2014) 1273–1298, doi:http://dx.doi.org/10.1002/cphy.c140003.
[13] S.L. Lightman, C.C. Wiles, H.C. Atkinson, D.E. Henley, G.M. Russell, J.A. Leendertz, et al., The significance of glucocorticoid pulsatility, Eur. J. Pharmacol. 583 (2008) 255–262, doi:http://dx.doi.org/10.1016/j.ejphar.2007.11.073.
[14] C. Kirschbaum, K.M. Pirke, D.H. Hellhammer, The Trier Social Stress Test—a tool for investigating psychobiological stress responses in a laboratory setting, Neuropsychobiology 28 (1993) 76–81 119004.
[15] A. Steptoe, M. Ussher, Smoking, cortisol and nicotine, Int. J. Psychophysiol. 59 (2006) 228–235, doi:http://dx.doi.org/10.1016/j.ijpsycho.2005.10.011.
[16] J.H. Mendelson, S. Stein, Serum cortisol levels in alcoholic and nonalcoholic subjects during experimentally induced ethanol intoxication, Psychosom. Med. 28 (1951) 616–626, doi:http://dx.doi.org/10.1097/00006842-196607000-00037.
[17] E.L. Gibson, S. Checkley, A. Papadopoulos, L. Poon, S. Daley, J. Wardle, Increased salivary cortisol reliably induced by a protein-rich midday meal, Psychosom. Med. 61 (1999) 214–224, doi:http://dx.doi.org/10.1097/00006842-199903000-00014.
[18] H. Galbo, Endocrinology and metabolism in exercise, Curr. Probl. Clin. Biochem. 11 (1982) 26–44, doi:http://dx.doi.org/10.1055/s-2008-1034610.
[19] B.M. Kudielka, J.E. Broderick, C. Kirschbaum, Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects, Psychosom. Med. 65 (2003) 313–319, doi:http://dx.doi.org/10.1097/01.psy.0000058374.50240.bf.
[20] J.E. Broderick, D. Arnold, B.M. Kudielka, C. Kirschbaum, Salivary cortisol sampling compliance: comparison of patients and healthy volunteers, Psychoneuroendocrinology 29 (2004) 636–650, doi:http://dx.doi.org/10.1016/s0306-4530(03) 00093-3.
[21] F. Bévalot, Y. Gaillard, M.A. Lhermitte, G. Pépin, Analysis of corticosteroids in hair by liquid chromatography–electrospray ionization mass spectrometry, J. Chromatogr. B Biomed. Sci. Appl. 740 (2000) 227–236, doi:http://dx.doi.org/10.1016/s0378-4347(00) 00085-2.
[22] V. Cirimele, P. Kintz, V. Dumestre, J.P. Goullé, B. Ludes, Identification of ten corticosteroids in human hair by liquid chromatography–ionspray mass spectrometry, Forensic Sci. Int. 107 (2000) 381–388, doi:http://dx.doi.org/10.1016/s0379-0738(99) 00180-2.
[23] T. Stalder, C. Kirschbaum, Analysis of cortisol in hair—state of the art and future directions, Brain. Behav. Immun. 26 (2012) 1019–1029, doi:http://dx.doi.org/10.1016/j.bbi.2012.02.002.
[24] E. Russell, G. Koren, M. Rieder, S.H.M. Van Uum, Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions, Psychoneuroendocrinology 37 (2012) 589–601, doi:http://dx.doi.org/10.1016/j.psyneuen.2011.09.009.
[25] S.M. Staufenbiel, B.W.J.H. Penninx, A.T. Spijker, B.M. Elzinga, E.F.C. van Rossum, Hair cortisol, stress exposure, and mental health in humans: a systematic review, Psychoneuroendocrinology 38 (2013) 1220–1235, doi:http://dx.doi.org/10.1016/j.psyneuen.2012.11.015.
[26] S. Thomson, G. Koren, L.A. Fraser, M. Rieder, T.C. Friedman, S.H.M. Van Uum, Hair analysis provides a historical record of cortisol levels in Cushing's syndrome, Exp. Clin. Endocrinol. Diabetes 118 (2010) 133–138, doi:http://dx.doi.org/10.1055/s-0029-1220771.
[27] T. Stalder, C. Kirschbaum, K. Heinze, S. Steudte, P. Foley, A. Tietze, et al., Use of hair cortisol analysis to detect hypercortisolism during active drinking phases in alcohol-dependent individuals, Biol. Psychol. 85 (2010) 357–360, doi:http://dx.doi.org/10.1016/j.biopsycho.2010.08.005.
[28] L. Manenschijn, J.W. Koper, S.W.J. Lamberts, E.F.C. Van Rossum, Evaluation of a method to measure long term cortisol levels, Steroids 76 (2011) 1032–1036, doi:http://dx.doi.org/10.1016/j.steroids.2011.04.005.
[29] K.L. D'Anna-Hernandez, R.G. Ross, C.L. Natvig, M.L. Laudenslager, Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol, Physiol. Behav. 104 (2011) 348–353, doi:http://dx.doi.org/10.1016/j.physbeh.2011.02.041.
[30] T. Stalder, S. Steudte, R. Miller, N. Skoluda, L. Dettenborn, C. Kirschbaum, Intraindividual stability of hair cortisol concentrations, Psychoneuroendocrinology 37 (2012) 602–610, doi:http://dx.doi.org/10.1016/j.psyneuen.2011.08.007.
[31] L. Dettenborn, A. Tietze, C. Kirschbaum, T. Stalder, The assessment of cortisol in human hair: associations with sociodemographic variables and potential confounders, Stress 15 (2012) 578–588, doi:http://dx.doi.org/10.3109/10253890.2012.654479.
[32] S. Feller, M. Vigl, M.M. Bergmann, H. Boeing, C. Kirschbaum, T. Stalder, Predictors of hair cortisol concentrations in older adults, Psychoneuroendocrinology 39 (2014) 132–140, doi:http://dx.doi.org/10.1016/j.psyneuen.2013.10.007.
[33] J. Grass, C. Kirschbaum, R. Miller, W. Gao, S. Steudte-Schmiedgen, T. Stalder, Sweat-inducing physiological challenges do not result in acute changes in hair cortisol concentrations, Psychoneuroendocrinology 53 (2015) 108–116, doi:http://dx.doi.org/10.1016/j.psyneuen.2014.12.023.
[34] A.T. Slominski, M.A. Zmijewski, C. Skobowiat, B. Zbytek, R.M. Slominski, J.D. Steketee, Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system, Adv. Anat. Embryol. Cell Biol. 212 (2012) 1–115, doi:http://dx.doi.org/10.1016/j.biotechadv.2011.08.021 v, vii, Secreted.
[35] C.F. Sharpley, J.R. McFarlane, A. Slominski, Stress-linked cortisol concentrations in hair: what we know and what we need to know, Rev. Neurosci. 23 (1) (2012) 11–121, doi:http://dx.doi.org/10.1515/rns.2011.058.
[36] S. Kalra, A. Einarson, T. Karaskov, S.H.M. Van Uum, G. Koren, The relationship between stress and hair cortisol in healthy pregnant women, Clin. Investig. Med. 30 (2007) E103–E107.
[37] L. Dettenborn, A. Tietze, F. Bruckner, C. Kirschbaum, Higher cortisol content in hair among long-term unemployed individuals compared to controls, Psychoneuroendocrinology 35 (2010) 1404–1409, doi:http://dx.doi.org/10.1016/j.psyneuen.2010.04.006.
[38] T. Stalder, A. Tietze, S. Steudte, N. Alexander, L. Dettenborn, C. Kirschbaum, Elevated hair cortisol levels in chronically stressed dementia caregivers, Psychoneuroendocrinology 47 (2014) 26–30, doi:http://dx.doi.org/10.1016/j.psyneuen.2014.04.021.
[39] L. Manenschijn, A.T. Spijker, J.W. Koper, A.M. Jetten, E.J. Giltay, J. Haffmans, et al., Long-term cortisol in bipolar disorder: associations with age of onset and

Please cite this article in press as: W. Gao, et al., LC–MS based analysis of endogenous steroid hormones in human hair, J. Steroid Biochem. Mol. Biol. (2016), http://dx.doi.org/10.1016/j.jsbmb.2015.12.022

psychiatric co-morbidity, Psychoneuroendocrinology 37 (2012) 1960–1968, doi:http://dx.doi.org/10.1016/j.psyneuen.2012.04.010.
[40] S. Steudte, T. Stalder, L. Dettenborn, E. Klumbies, P. Foley, K. Beesdo-Baum, et al., Decreased hair cortisol concentrations in generalised anxiety disorder, Psychiatry Res. 186 (2011) 310–314, doi:http://dx.doi.org/10.1016/j.psychres.2010.09.002.
[41] S. Steudte, C. Kirschbaum, W. Gao, N. Alexander, S. Schönfeld, J. Hoyer, et al., Hair cortisol as a biomarker of traumatization in healthy individuals and posttraumatic stress disorder patients, Biol. Psychiatry 74 (2013) 639–646, doi:http://dx.doi.org/10.1016/j.biopsych.2013.03.011.
[42] T. Stalder, C. Kirschbaum, N. Alexander, S.R. Bornstein, W. Gao, R. Miller, et al., Cortisol in hair and the metabolic syndrome, J. Clin. Endocrinol. Metab. 98 (2013) 2573–2580, doi:http://dx.doi.org/10.1210/jc.2013-1056.
[43] L. Manenschijn, L. Schaap, N.M. van Schoor, S. van der Pas, G.M.E.E. Peeters, P. Lips, et al., High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease, J. Clin. Endocrinol. Metab. 98 (2013) 2078–2083, doi:http://dx.doi.org/10.1210/jc.2012-3663.
[44] D. Pereg, R. Gow, M. Mosseri, M. Lishner, M. Rieder, S.H.M. Van Uum, et al., Hair cortisol and the risk for acute myocardial infarction in adult men, Stress 14 (2011) 73–81, doi:http://dx.doi.org/10.3109/10253890.2010.511352.
[45] E. Russell, C. Kirschbaum, M.L. Laudenslager, T. Stalder, Y. de Rijke, E.F.C. van Rossum, et al., Toward standardization of hair cortisol measurement: results of the first international interlaboratory round robin, Ther. Drug Monit. 37 (2015) 71–75, doi:http://dx.doi.org/10.1097/FTD.0000000000000148.
[46] S.H.M. Van Uum, B. Sauvé, L.A. Fraser, P. Morley-Forster, T.L. Paul, G. Koren, Elevated content of cortisol in hair of patients with severe chronic pain: a novel biomarker for stress, Stress 11 (2008) 483–488, doi:http://dx.doi.org/10.1080/10253890801887388.
[47] C. Kirschbaum, A. Tietze, N. Skoluda, L. Dettenborn, Hair as a retrospective calendar of cortisol production-Increased cortisol incorporation into hair in the third trimester of pregnancy, Psychoneuroendocrinology 34 (2009) 32–37, doi:http://dx.doi.org/10.1016/j.psyneuen.2008.08.024.
[48] V. Moal, E. Mathieu, P. Reynier, Y. Malthièry, Y. Gallois, Low serum testosterone assayed by liquid chromatography–tandem mass spectrometry. Comparison with five immunoassay techniques, Clin. Chim. Acta 386 (2007) 12–19, doi:http://dx.doi.org/10.1016/j.cca.2007.07.013.
[49] J. Taieb, C. Benattar, A.S. Birr, A. Lindenbaum, Limitations of steroid determination by direct immunoassay, Clin. Chem. 48 (2002) 583–585.
[50] M. Rauh, Steroid measurement with LC–MS/MS in pediatric endocrinology, Mol. Cell. Endocrinol. 301 (2009) 272–281, doi:http://dx.doi.org/10.1016/j.mce.2008.10.007.
[51] W. Gao, Q. Xie, J. Jin, T. Qiao, H. Wang, L. Chen, et al., HPLC-FLU detection of cortisol distribution in human hair, Clin. Biochem. 43 (2010) 677–682, doi:http://dx.doi.org/10.1016/j.clinbiochem.2010.01.014.
[52] M.H. Choi, B.C. Chung, GC–MS determination of steroids related to androgen biosynthesis in human hair with pentafluorophenyldimethylsilyl–trimethylsilyl derivatisation, Analyst 124 (1999) 1297–1300, doi:http://dx.doi.org/10.1039/a903912k.
[53] M.H. Choi, K.R. Kim, B.C. Chung, Determination of estrone and 17β-estradiol in human hair by gas chromatography–mass spectrometry, Analyst 125 (2000) 711–714, doi:http://dx.doi.org/10.1039/a909107f.
[54] H.J. Jung, S.J. Kim, W.Y. Lee, B.C. Chung, M.H. Choi, Gas chromatography/mass spectrometry based hair steroid profiling may reveal pathogenesis in hair follicles of the scalp, Rapid Commun. Mass Spectrom. 25 (2011) 1184–1192, doi:http://dx.doi.org/10.1002/rcm.4975.
[55] H.H. Son, D.Y. Lee, H.S. Seo, J. Jeong, J.Y. Moon, J.E. Lee, et al., Hair sterol signatures coupled to multivariate data analysis reveal an increased 7β-hydroxycholesterol production in cognitive impairment, J. Steroid Biochem. Mol. Biol. 155 (2016) 9–17, doi:http://dx.doi.org/10.1016/j.jsbmb.2015.09.024.
[56] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, Recent advances in high-throughput quantitative bioanalysis by LC–MS/MS, J. Pharm. Biomed. Anal. 44 (2007) 342–355, doi:http://dx.doi.org/10.1016/j.jpba.2007.02.006.
[57] J.P. Holst, S.J. Soldin, R.E. Tractenberg, T. Guo, P. Kundra, J.G. Verbalis, et al., Use of steroid profiles in determining the cause of adrenal insufficiency, Steroids 72 (2007) 71–84, doi:http://dx.doi.org/10.1016/j.steroids.2006.11.001.
[58] G.A.A. Cooper, R. Kronstrand, P. Kintz, Society of hair testing guidelines for drug testing in hair, Forensic Sci. Int. 218 (2012) 20–24, doi:http://dx.doi.org/10.1016/j.forsciint.2011.10.024.
[59] M.R. Harkey, Anatomy and physiology of hair, Forensic Sci. Int. 63 (1993) 9–18, doi:http://dx.doi.org/10.1016/0379-0738(93)90255-9.
[60] B. Sauvé, G. Koren, G. Walsh, S. Tokmakejian, S.H.M. Van Uum, Measurement of cortisol in human hair as a biomarker of systemic exposure, Clin. Investig. Med. 30 (2007) E183–E191.
[61] Q. Xie, W. Gao, J. Li, T. Qiao, J. Jin, H. Deng, et al., Correlation of cortisol in 1-cm hair segment with salivary cortisol in human: hair cortisol as an endogenous biomarker, Clin. Chem. Lab. Med. 49 (2011) 2013–2019, doi:http://dx.doi.org/10.1515/cclm.2011.706.
[62] Z. Chen, J. Li, J. Zhang, X. Xing, W. Gao, Z. Lu, et al., Simultaneous determination of hair cortisol, cortisone and DHEAS with liquid chromatography–electrospray ionization-tandem mass spectrometry in negative mode, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 929 (2013) 187–194, doi:http://dx.doi.org/10.1016/j.jchromb.2013.04.026.
[63] W. Gao, T. Stalder, P. Foley, M. Rauh, H. Deng, C. Kirschbaum, Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 928 (2013) 1–8, doi:http://dx.doi.org/10.1016/j.jchromb.2013.03.008.
[64] G. Noppe, Y.B. de Rijke, K. Dorst, E.L.T. van den Akker, E.F.C. van Rossum, LC–MS/MS-based method for long-term steroid profiling in human scalp hair, Clin. Endocrinol. (Oxf.) 83 (2015) 162–166, doi:http://dx.doi.org/10.1111/cen.12781.
[65] N. Quinete, J. Bertram, M. Reska, J. Lang, T. Kraus, Highly selective and automated online SPE LC-MS3 method for determination of cortisol and cortisone in human hair as biomarker for stress related diseases, Talanta 134 (2015) 310–316, doi:http://dx.doi.org/10.1016/j.talanta.2014.11.034.
[66] J.S. Raul, V. Cirimele, B. Ludes, P. Kintz, Detection of physiological concentrations of cortisol and cortisone in human hair, Clin. Biochem. 37 (2004) 1105–1111, doi:http://dx.doi.org/10.1016/j.clinbiochem.2004.02.010.
[67] B. Vanaelst, N. Rivet, I. Huybrechts, B. Ludes, S. De Henauw, J.S. Raul, Measurement of cortisol and cortisone in children's hair using ultra performance liquid chromatography and tandem mass spectrometry, Anal. Methods 5 (2013) 2074, doi:http://dx.doi.org/10.1039/c3ay26570f.
[68] X. Xing, Z. Chen, J. Li, J. Zhang, H. Deng, Z. Lu, Study on dissolution mechanism of cortisol and cortisone from hair matrix with liquid chromatography–tandem mass spectrometry, Clin. Chim. Acta 421 (2013) 62–72, doi:http://dx.doi.org/10.1016/j.cca.2013.02.035.
[69] United Nations International Drug Control Programme, Guidelines for Testing Drugs under International Control in Hair, Sweat and Saliva, (2014).
[70] M.C. Hennion, Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography, J. Chromatogr. A 856 (1999) 3–54, doi:http://dx.doi.org/10.1016/s0021-9673(99)00832-8.
[71] M.M. Kushnir, A.L. Rockwood, J. Bergquist, Liquid chromatography–tandem mass spectrometry applications in endocrinology, Mass Spectrom. Rev. 29 (2010) 480–502, doi:http://dx.doi.org/10.1002/mas.20264.
[72] M.M. Kushnir, A.L. Rockwood, W.L. Roberts, B. Yue, J. Bergquist, A.W. Meikle, Liquid chromatography tandem mass spectrometry for analysis of steroids in clinical laboratories, Clin. Biochem. 44 (2011) 77–88, doi:http://dx.doi.org/10.1016/j.clinbiochem.2010.07.008.
[73] T.M. Williams, A.J. Kind, E. Houghton, D.W. Hill, Electrospray collision-induced dissociation of testosterone and testosterone hydroxy analogs, J. Mass Spectrom. 34 (3) (1999) 206–216 10.1002/(SICI) 1096-9888(199903)34<206: AID-JMS785>3.0. CO;2-1.
[74] U. Ceglarek, L. Kortz, A. Leichtle, G.M. Fiedler, J. Kratzsch, J. Thiery, Rapid quantification of steroid patterns in human serum by on-line solid phase extraction combined with liquid chromatography-triple quadrupole linear ion trap mass spectrometry, Clin. Chim. Acta 401 (2009) 114–118, doi:http://dx.doi.org/10.1016/j.cca.2008.11.022.
[75] E. Schwarz, A. Liu, H. Randall, C. Haslip, F. Keune, M. Murray, et al., Use of steroid profiling by UPLC-MS/MS as a second tier test in newborn screening for congenital adrenal hyperplasia: the Utah experience, Pediatr. Res. 66 (2009) 230–235, doi:http://dx.doi.org/10.1203/pdr.0b013e3181aa3777.
[76] M. Vogeser, K.G. Parhofer, Liquid chromatography tandem-mass spectrometry (LC-MS/MS)-Technique and applications in endocrinology, Exp. Clin. Endocrinol. Diabetes 115 (2007) 559–570, doi:http://dx.doi.org/10.1055/s-2007-981458.
[77] A. Garcia-Ac, P.A. Segura, L. Viglino, C. Gagnon, S. Sauvé, Comparison of APPI, APCI and ESI for the LC-MS/MS analysis of bezafibrate, cyclophosphamide, enalapril, methotrexate and orlistat in municipal wastewater, J. Mass Spectrom. 46 (2011) 383–390, doi:http://dx.doi.org/10.1002/jms.1904.
[78] D. Caruso, S. Scurati, O. Maschi, L. De Angelis, I. Roglio, S. Giatti, et al., Evaluation of neuroactive steroid levels by liquid chromatography-tandem mass spectrometry in central and peripheral nervous system: effect of diabetes, Neurochem. Int. 52 (2008) 560–568, doi:http://dx.doi.org/10.1016/j.neuint.2007.06.004.
[79] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal. Chem. 75 (2003) 3019–3030, doi:http://dx.doi.org/10.1021/ac020361s.
[80] G. Hopfgartner, E. Varesio, V. Tschäppät, C. Grivet, E. Bourgogne, L.A. Leuthold, Triple quadrupole linear ion trap mass spectrometer for the analysis of small molecules and macromolecules, J. Mass Spectrom. 39 (2004) 845–855, doi:http://dx.doi.org/10.1002/jms.659.
[81] J.P. Danaceau, M.S. Morrison, M.H. Slawson, Quantitative confirmation of testosterone and epitestosterone in human urine by LC/Q-ToF mass spectrometry for doping control, J. Mass Spectrom. 43 (2008) 993–1000, doi:http://dx.doi.org/10.1002/jms.1443.
[82] N.I. Deshmukh, J. Barker, A. Petroczi, D.P. Naughton, Detection of testosterone and epitestosterone in human hair using liquid chromatography–tandem mass spectrometry, J. Pharm. Biomed. Anal. 67–68 (2012) 154–158, doi:http://dx.doi.org/10.1016/j.jpba.2012.04.011.
[83] S.H. Cho, M.H. Choi, W.Y. Sim, W.Y. Lee, B.C. Chung, Metabolic alterations of DHEA and cholesterol sulphates in the hair of patients with acne measured by liquid chromatography–mass spectrometry, Exp. Dermatol. 19 (2010) 694–696, doi:http://dx.doi.org/10.1111/j.1600-0625.2010.01094.x.

Please cite this article in press as: W. Gao, et al., LC–MS based analysis of endogenous steroid hormones in human hair, J. Steroid Biochem. Mol. Biol. (2016), http://dx.doi.org/10.1016/j.jsbmb.2015.12.022