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RECENT ADVANCES IN DOPING ANALYSIS (7)

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Identification of 10 corticosteroids in human hair by liquid chromatography ionspray mass spectrometry

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I. Introduction

Because of their anti-inflamatory properties, the naturally occurring and synthetic corticosteroids are widely used in medicine to treat many diseases. When administered systemically, they influence the natural production of corticosteroids by the body. Corticosteroids may produce mood changes including euphoria and other side effects such that their medical use demands medical control. Corticosteroids are included in the latest 1995 International Olympic Commitee (IOC) list of restricted classes of substances. In sport, the use of corticosteroids is banned except: (1) for topical use (aural, dermatological and ophthalmological) but not rectal, (2) by inhalation and (3) by intra-articular or local injection. A medical prescription must be produced to document the treatment.

The standard in drug testing for doping control is gas chromatography/mass spectrometry conducted on an urine sample. A wide range of methodologies have been investigated for the determination of corticosteroids in biological fluids, including radioimmunoassay (1), high-performance liquid chromatography with spectrophotometric detection (2), gas chromatography (3), as well as liquid or gas chromatography coupled to mass spectrometry (4,5). Blood or urine are usually employed to demonstrate drug consumption, however the elimination of such drugs occurs within a few days.

For the past twenty years, hair analysis has been proposed for identifying chronic drug abusers in forensic science. Hair is known to allow a drug administration to be tracked back for months, and thus offers the possibility of determinating long-term drug exposure (6).

In the case of corticosteroids, this may be of interest to demonstrate long-term abuse, as it can be observed in cycling races. As one objective in doping analysis is to increase the length of a detection window of particular doping agent, hair analysis may be also of interest.

At this time, no method was available for the determination of corticosteroids in hair samples. This paper describes a screening procedure based upon high-performance liquid chromatography-ionspray mass spectrometry (HPLC-IS-MS) for the identification of the 10 following corticosteroids in human hair: triamcinolone, prednisolone, prednisone, methylprednisolone, cortisone, cortisol, beta- and dexa-methasone, flumethasone and beclomethasone.

II. Material and methods

1. Specimen collection

Hair samples were obtained from a forensic case (38-year old male treated by Cortancyl® for sarcoidosis) and from living persons treated with prednisone after kidney grafts, or with beclomethasone for asthma. Athletes' specimens were not available to our laboratory. Hair strands were cut as close as possible to the skin with scissors in the vertex posterior region and stored in dry tubes at room temperature.

2. Extraction procedure

Hair strands (100 mg) were washed twice in 5 ml methylene chloride at room temperature (2 min each) then pulverized in a ball mill.

Fifty mg of the powdered hair were incubated in 1 ml Soerensen buffer, pH 7.6 for 16 h at 40 °C, in presence of 50 ng cortisol-d3 used as internal standard.

For further purification, SPE C18 Isolute extraction columns were used. Activation was operated with 3 ml methanol (MeOH) followed by 3 ml deionized water. The incubation medium was centrifugated and the supernatant was removed and deposited on the activated column, then rinsed with 1 ml deionized water followed by 1 ml deionized water/MeOH (90:10, v/v). Columns were dried for 30 min and the corticoids eluted with 3 successive volumes of MeOH (0.5 ml each). The eluates were evaporated to dryness and resuspended in 30 µl MeOH.

3. Analytical procedure

A 2-µl volume of the extract was injected onto the column (4-µm Novapak C18 Waters, 150 x 2.0 mm, i.d.). Each 10-min chromatographic run was carried out with a binary mobile phase of acetonitrile - 2 mM NH_4COOH, pH 3.0 buffer, using a gradient (acetonitrile 30 to 70 %) generated by a 20-ml dual-syringe HPLC pump (Applied Biosystems Model 140B). The flow rate was 200 µl/min with a post-column split of 1:3 (flow rate infused into the ionspray: 50 µl/min). Detection was carried out by a Perkin Elmer Sciex API-100 mass spectrometer. The instrument was operated in the positive or negative ionization mode with a voltage of +/- 4500 V applied to the sprayer. Ions generated in the ion source were sampled into the mass analyzer by passing through a 25-µm orifice held at +190 V for positive detection and -75 V for negative detection. MS data were recorded in the single ion monitoring mode. The following table shows the ions (*m/z*) monitored in positive and negative mode of detection for each corticosteroid.

The underlined ions (base peak) were used for quantification (M: molecular ion). Quantitative results were obtained in SIM mode after determination of the response factor of each corticosteroid against cortisol-d3.

	Positive detection	Negative detection
Triamcinolone	395(M) 375 357	345 393(M) 325
Prednisolone	361(M) 343 325	329 359(M)
Prednisone	359(M) 341	327 357(M) 285
Cortisol	363(M) 309 327	331 297 361(M)
Cortisone	361(M)	329
Methylprednisolone	375(M) 357 339	343 374(M) 309
Beta-, dexa-methasone	393(M) 373 355	361 391(M) 307
Flumethasone	411(M) 253 121	379 409(M) 305
Beclomethasone	391 409(M) 279	297 407(M) 341

4. Validation parameters

The absence of calibrated material lead us to spiked hair samples for use in the optimization of the reliability of corticosteroids assay method and for assessing the performance of the established procedure.

Standard calibration curves were obtained by adding 5 µl (1.0 mg/l), 5, 10, 25 and 50 µl (10 mg/l) of each corticosteroid to 50 mg of pulverized blank control hair (corticosteroids free) and 50 ng cortisol-d_3, resulting in final concentrations of 0.1, 1, 2, 5 and 10 ng/mg of hair.

Extraction recovery was determined by adding 20 µl of each corticosteroid (10 mg/l) to 50 mg of powdered blank control hair (corticosteroids free) and 50 ng of cortisol-d_3 (n = 3), corresponding to a final concentration of 4 ng/mg.

Repeatability was determined by adding 20 µl of each corticosteroid (10 mg/l) to 50 mg of powdered blank control hair (corticosteroids free) and 50 ng of cortisol-d_3 (n = 8), corresponding to a final concentration of 4 ng/mg.

The limits of detection were determined by decreasing the corticosteroid concentrations in order to obtained a signal to noise ratio (S/N) of 2.

All these validation parameters were determined after extraction of the spiked blank control hair by the established procedure, HPLC separation of the analytes and IS-MS detection.

III. Results

The fig. 1 shows the chromatographic profile obtained after separation of the 10 corticosteroids. Under the determined analytical conditions, no interference with corticosteroids was observed by any extractable endogenous materials present in hair.

The correlation coefficient of the calibration curve ranged from 0.939 to 0.997, showing linearity between 0.1 and 10 ng/mg. Extraction recovery ranged from 43.2 to 85.7%. Repetability (CV values) ranged from 6.1 to 17.5%. The limits of detection (LOD) ranged from 0.03 to 0.17 ng/mg. These parameters were found satisfactory for a screening procedure. The results of the validation procedure are summerized in the following table.

	Linearity	Recovery	CV	LOD
Triamcinolone	0.997	43.2%	14.2%	0.08 ng/mg
Prednisolone	0.994	73.1%	17.5%	0.03 ng/mg
Prednisone	0.968	55.4%	6.5%	0.03 ng/mg
Cortisol	0.939	67.9%	6.1%	0.04 ng/mg
Cortisone	0.994	56.7%	11.5%	0.04 ng/mg
Methylprednisolone	0.991	78.3%	6.1%	0.04 ng/mg
Beta-, dexa-methasone	0.983	85.7%	11.9%	0.05 ng/mg
Flumethasone	0.993	77.9%	13.0%	0.09 ng/mg
Beclomethasone	0.981	58.6%	12.2%	0.17 ng/mg

IV. Applications

A hair specimen, obtained from a 38-year old male treated for years by Cortancyl® (prednisone, 5-mg tablets, Roussel Labs.) for sarcoidosis, tested positive for prednisone with a measured concentration of 1.28 ng/mg. The active metabolite prednisolone was not detected. To the best of our knowledge, it was the first time that prednisone was identified in human hair (7).

For the hair sample obtained from a living person treated with prednisone after kidney grafts, prednisone concentration in a 2-cm long hair strand was 0.14 ng/mg. For the patient treated with beclomethasone for asthma, the determined concentration of beclomethasone was 0.23 ng/mg.

V. Conclusion and perspectives

This report describe a procedure to detect and quantify 10 corticosteroids in human hair: triamcinolone, prednisolone, prednisone, methylprednisolone, cortisone, cortisol, beta- and dexa-methasone, flumethasone and beclomethasone by HPLC-IS-MS. In hair samples from three individuals taking corticosteroids, the compounds were detected, indicating incorporation into human hair. The established method seems accurate but in the future, further investigations are necessary to verify if its sensitivity is sufficient in all cases of corticosteroids abuse, to enhance the number of positive cases and to validate the established method as a screening procedure for corticosteroids in human hair.

According to the consensus of The Society of Hair Testing on hair testing for doping agents, hair analysis can essentially contribute to doping analysis in special cases, in addition to urine (8). In case of negative urine result, the positive hair result demonstrates drug exposure during the period prior to sample collection.

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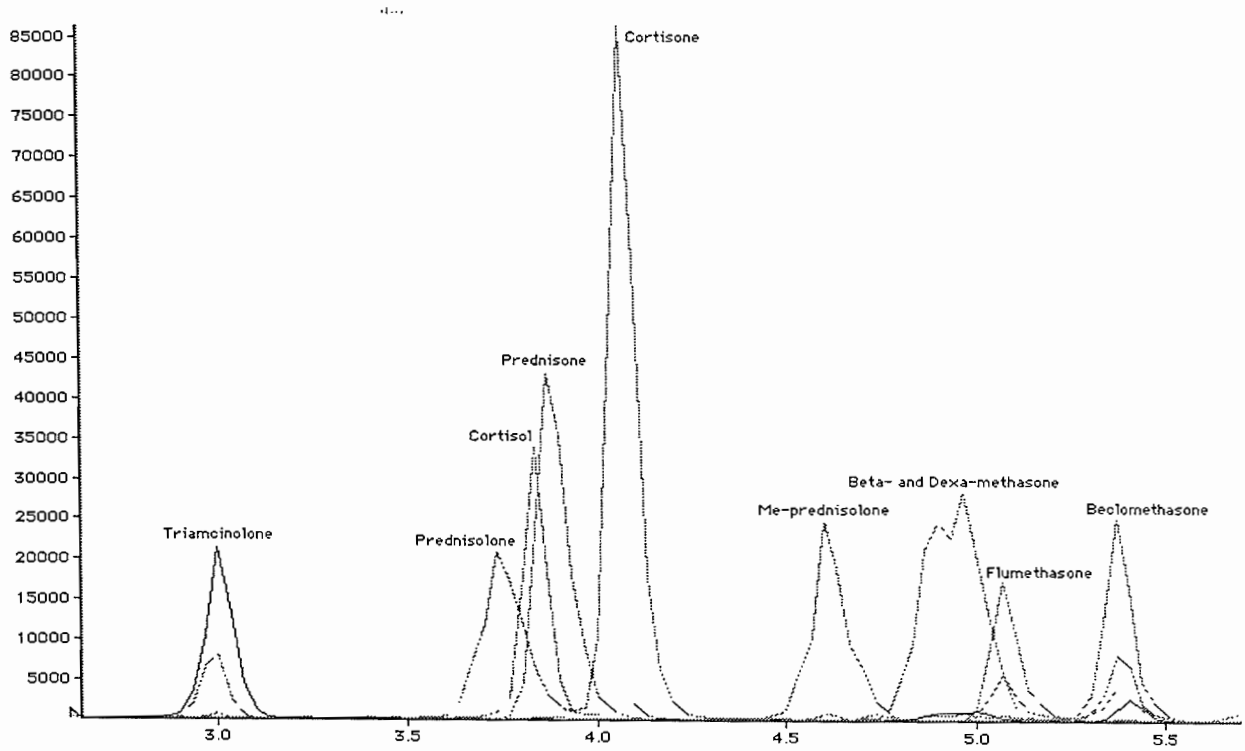

fig. 1: Chromatographic profile of the 10 corticosteroids separated by HPLC and detected with a IS-MS