

Reprint from

RECENT ADVANCES
IN DOPING ANALYSIS
(12)

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Sport und Buch Strauß, Köln, 2004

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping
analysis (12). Sport und Buch Strauß, Köln (2004) 37-44

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Excretion Studies with Corticosteroids

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1. Introduction

The therapeutical and doping use of corticosteroids is widespread since several decades.

Unfortunately their detection in biological matrices has been problematic for a long time. The existing analytical techniques including GC/MS, ELISA or HPLC/UV show poor sensitivity or selectivity.

However since the introduction of liquid chromatography coupled to mass spectrometry (LC/MS) this problem could be solved. LC/MS shows very good sensitivity and selectivity for corticosteroids and as a consequence different papers on their detection in doping analysis by LC/MS have been published (Fluri et al., 2001; Poletini et al., 1998; Popot et al., 1997; Bevalot et al., 2000).

Nine commonly used and abused corticosteroids were selected to be included in our screening method (Deventer et al., 2003). The major metabolite of budesonide, 16 α -hydroxyprednisolone (Edsbäcker et al, 1987), was also included in the screening method.

In order to study the detectability of corticosteroids after administration, the validated screening method was applied for betamethasone, triamcinolone acetonide, beclomethasone, budesonide and methylprednisolone following different administration routes.

2. Experimental

2.1. Excretion studies

Oral administration

One tablet of the following commercially available drugs was orally taken by one healthy female and one healthy male volunteer, aged 57 and 26 years respectively: Medrol® containing 32 mg of methylprednisolone, Pharmacia (Diegem, Belgium) and Celestone® containing 0.5 mg betamethasone, Schering-Plough (Brussels, Belgium).

Urine samples were collected at 0h and after 1, 2, 3, 6, 9, 12, 24, 48 and 72h.

Dermatological application

A single dose of 0.5 g of following commercially available ointments was applied topically between the buttocks by two male volunteers, aged 26 and 34 years respectively: Diprosone® 0.05% containing 0.643 mg betamethasone dipropionate in each gram of ointment (equivalent to 0.5 mg of betamethasone), Schering Plough (Brussels, Belgium) and Delphi® containing 0.1% triamcinolone acetonide, Lederle (Louvain-la-Neuve, Belgium).

Urine samples were collected at 0h and after 1, 2, 3, 6, 9, 12, 24 and 48h.

Inhalation

Two formulations were tested: A single dose of Pulmicort®, Astra-Zeneca (Brussels), containing 0.2 mg of budesonide was administered by inhalation using a turbohaler by a male volunteer aged 23.

In another experiment one dose of Becotide®, GlaxoSmithKline (Genval, Belgium), containing 0.25 mg of beclomethasone dipropionate was administered by inhaler.

Urine samples were collected at 0h and after 1, 2, 3, 6, 9, 12, 24h.

Parenteral administration

Diprophos®, Schering-Plough (Brussels), containing dihydrogenphosphate-disodium and betamethasone dipropionat, (equivalent to 7 mg betamethasone) was administered intramuscularly to one male patient aged 34 and intra-articularly to 2 male patients aged 55 and 36 respectively.

Diprophos® (equivalent of 14 mg betamethasone) was administered intramuscular to a male patient aged 37.

Kenacort®, Bristol-Myers (Braine l'Alleud, Belgium) containing 40 mg triamcinolone acetonide was injected intrarticularly to a male volunteer.

Urine samples were collected at 0h and after 1, 2,3, 6, 9, 12 and 24h and then on a daily basis for 20 days.

2.2. Sample preparation

The internal standard solution (50 µl desoximethasone, 1 µg/ml) was added to 5 ml of urine, followed by addition of 1 g of potassium carbonate. Five ml diethylether were added and the sample was extracted by rolling for 20 minutes. After centrifugation the organic layer was transferred into a new tube and evaporated to dryness under oxygen free nitrogen (OFN) at 40 °C. The residue was dissolved in 200 µl mobile phase.

A Thermo Separation Products (TSP) Model P4000 quaternary pump, equipped with a TSP Model AS 3000 autosampler with 100 µl sample-loop and connected to a Finnigan MAT LCQ-Deca® mass spectrometer was used. Separation was performed on a 10 cm Nucleosil 5 octadecylsilyl column (id 3 mm, particle size 5 µm) (Chrompack; Antwerp Belgium). The mobile phase consisted of 1% acetic acid (solution A) and acetonitrile. Gradient elution at a flow rate of 0.3 ml/min was used.

Mass spectrometry was performed on a LCQ DECA instrument in ESI negative ionisation mode for all compounds except 16α-hydroxyprednisolone, which was screened in positive mode. From the MS/MS spectrum two abundant ions were selected for monitoring the presence of a corticosteroid. The diagnostic ions are presented in Table 1.

Substance	Precursor ion	Diagnostic ions	Detection limit (ng/ml)
16 α –hydroxyprednisolone	377	341, 323	4
Hydrocortisone	421	361, 331	Endogenous
Cortisone	419	359, 329	Endogenous
Methylprednisolone	433	343, 373	2
Betamethasone	451	361, 391	2
Dexamethasone	451	361, 391	1
Beclomethasone	467	377, 407	0.5
Triamcinolone acetonide	493	375, 413	0.5
Desoximethasone (ISTD)	435	375, 355	-
Budesonide	489	357, 339	4

Table 1: diagnostic ions

3. Results and discussion

3.1. Oral administration

Already one hour after administration methylprednisolone and betamethasone were detected in the urine samples. Methylprednisolone could be detected up to 48h after intake while betamethasone could be detected up to 72 h post administration.

3.2. Dermatological application

Betamethasone could not be detected in the urine samples. The administration was repeated by applying a double dose (i.e. 1 g) of ointment but betamethasone could still not be detected. The dermatological application of Delphi® ointment resulted in the detection of triamcinolone acetonide from 2h until 24h after administration.

3.3. Inhalation

Inhaled corticosteroids are mostly used for the treatment of asthma. Budesonide, beclomethasone, fluticasone and flunisolide are most commonly used.

Recently the detection of non conjugated beclomethasone in equine urine and plasma was reported (Guan et al., 2003). In this study beclomethasone could not be detected after administration of a single dose of beclomethasone dipropionate. A probable reason of non detectability is the low administered dose and the fact that the drug and its metabolites are excreted mainly in faeces (Martin et al., 1974).

As shown in figure 1 (22R)-budesonide is metabolised to 16 α -hydroxyprednisolone by cleavage of the 16 α , 17 α -acetal group of (22R)-budesonide. The reaction requires oxidative and hydrolytic enzyme activity (Edsbäcker et al., 1987).

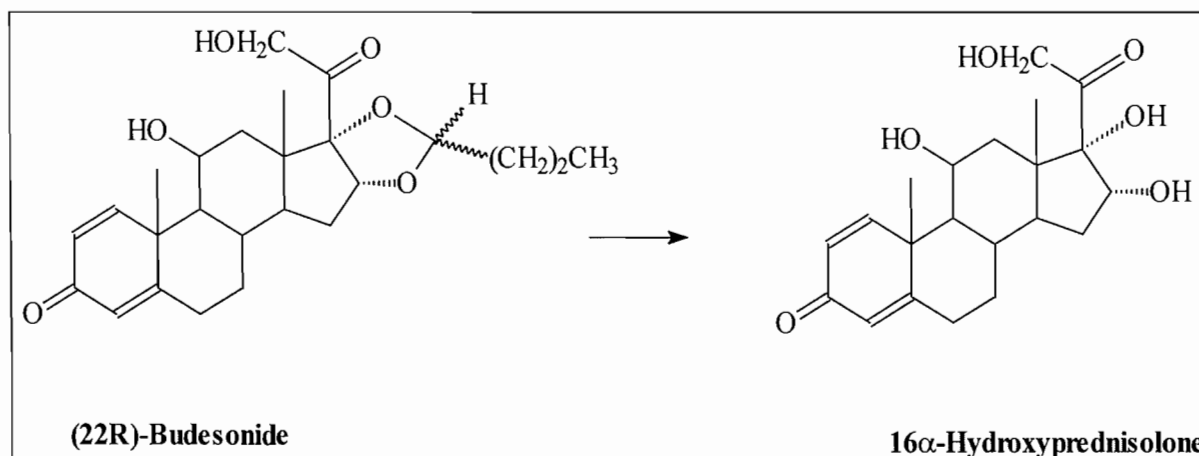


Figure 1: Metabolic pathway of budesonide

In the urine samples budesonide could be detected up to 1h while 16 α -hydroxyprednisolone remains detectable up to 9 hours after administration. Ion chromatograms and mass spectra for budesonide and 16 α -hydroxyprednisolone after the inhalation of budesonide are presented in (Figure 2).

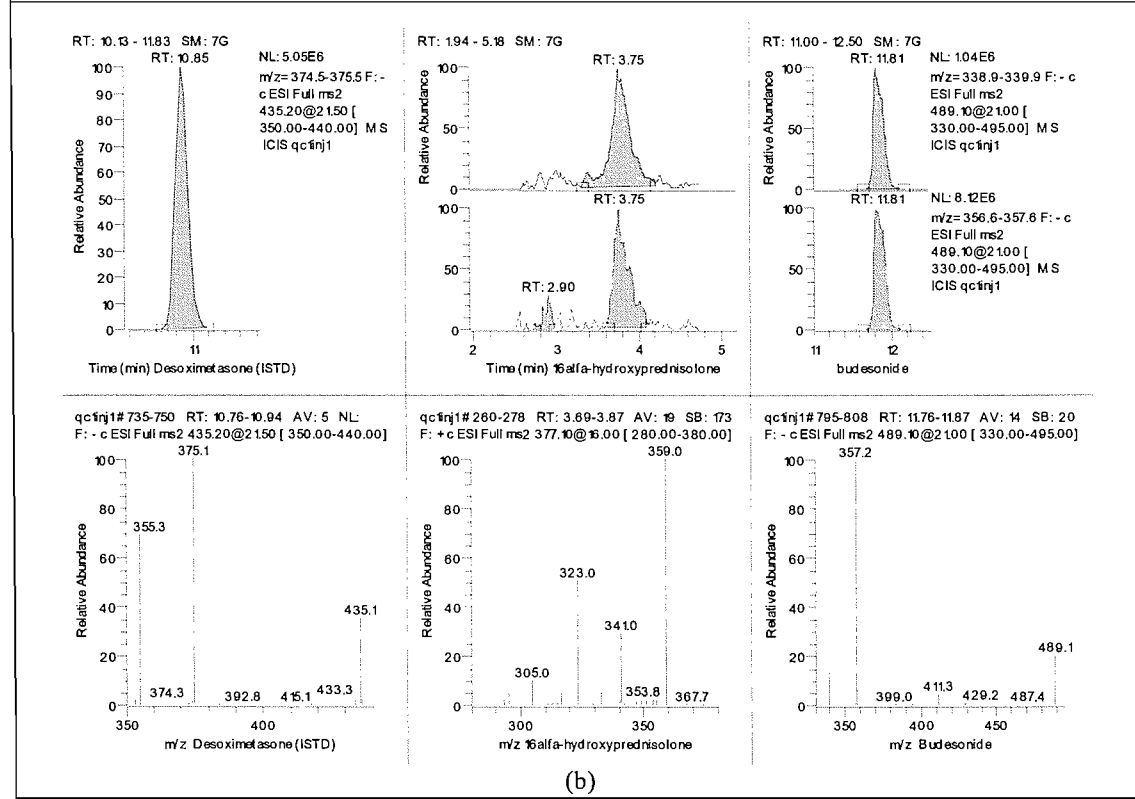
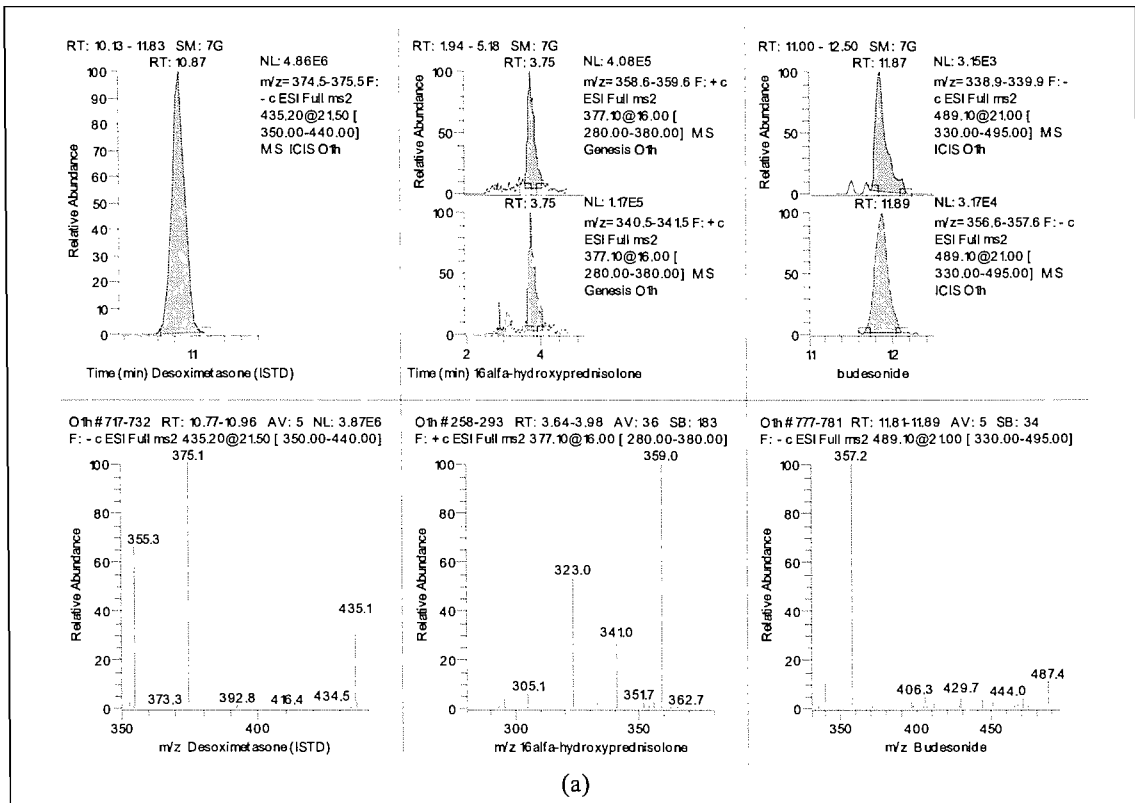


Figure 2: (a) urine sample 1h after inhalation of 200 µg of budesonide, (b) quality control urine spiked at 10 ng/ml with 16α-hydroxyprednisolone and budesonide

3.4. Parenteral Administration

Corticosteroids can be detected for a long period after injection (Table 2).

After intra-articular injection of Kenacort®, triamcinolone acetonide could be detected up to 12 days. The intra-articular injected betamethasone could be detected from 1h until 14 days post administration. For one excretion study with intramuscular injected betamethasone the detection time was underestimated (urine collection was stopped after 20 days while betamethasone was still detectable).

Commercial name	Active Compound	Amount	Injection	Detection time
Diprophos®	Betamethasone	7 mg	IM	12 days
Kenacort®	Triamcinolone acetonide	40 mg	IA	12 days
Diprophos®	Betamethasone	14 mg	IM	+20 days*
Diprophos®	Betamethasone	7 mg	IA	14 days
Diprophos®	Betamethasone	7 mg	IA	14 days

*urine collection was stopped after 20 days

IM: intramuscular, IA: intra-articular

Table 2: Detection times of some corticosteroids after parenteral administration

4. Conclusions

The major goal of this study was to establish urinary detection times for several corticosteroids after different administration routes.

The results demonstrated that the validated method is highly sensitive.

For triamcinolone acetonide and budesonide a single dose of 0.5 mg (topically) or 0.2 mg (inhaled) could be detected up to 24 hours respectively 9 hours after administration.

Longest detection times were observed for the injected corticosteroids, up to 20 days for betamethasone.

Including the 16 α -hydroxyprednisolone metabolite of budesonide in the screening method seemed to be a successful approach.

Despite the good results with the aforementioned administration routes, inhaled beclomethasone and topical applied betamethasone could not be detected unconjugated in the urine samples. Athletes should be aware of the possibility of testing positive when a corticosteroid containing ointment is used.

5. Acknowledgment

The authors are grateful to the Belgian National Lottery for the purchase of the LCQ-DECA[®] instrument and the Ministry of the Flemish Community for a grant. (KD).

The help of D. Bruggeman, T. Teulingkx and G. Gillis for the collection of urine samples is greatly acknowledged.

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