Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (6)

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Sport und Buch Strauß, Köln, 1999

M. MACHNIK, H. GEYER, S. HORNING, A. BREIDBACH, P. DELAHAUT, W. SCHÄNZER:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (6). Sport und Buch Strauß, Köln, (1999) 31-35

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Long-term Detection of Clenbuterol in Human Scalp Hair by Means of Gaschromatography/High Resolution Mass Spectrometry (GC/HRMS)

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Introduction

A number of publications are meanwhile available which report on the analysis of clenbuterol using hair as matrix. The main reason for the use of hair as biological specimen is due to its prolonged time window in which the drug can be detected. In comparison to urine analysis which allows the retrospective up to some weeks under optimal conditions [1,2] hair analysis enables the detectability of clenbuterol up to at least a couple of months and can provide contemplementary information about the point of time and duration of drug intake. In most of these studies a liquid-liquid extraction step follows after the digestion of the hair material. The low concentration of clenbuterol in hair requires very sensitive detection techniques. Often used techniques in this low ng/ml range are immuno chemical methods (e.g. Enzyme Linked Immuno Sorbent Assays = ELISA) or GC-MS-techniques combined with a preceding purification step via High Performance Liquid Chromatography (HPLC) or Immuno Affinity Chromatography (IAC) [3,4].

Here we present the analysis of an chemical digested hair extract, which is directly derivatized with MSTFA/NH_4I and injected into the GC-HRMS instrument. No extra purification was neccessary when using a resolution of 3000. The method specified under experimental allows the detection of 4ng clenbuterol/g hair in the selected ion monitored mode (SIM). Further purification was neccessary to improve the detection limit. Therefore the normal sample preparation method was modified and an additional purification via IAC was integrated.

Hair was collected from four pregnant women who were therapeutically treated with Spiropent ® (clenbuterol HCl) and from the infant of one female patient. Hair samples were taken during the therapy and 2 to 6 months after cessation of clenbuterol administration. A complete article of this issue has been published in the Journal of Chromatography [5].

Profile of patients

Table I lists the data of the four females and the time schedule of hair sample collection. Information regarding doses and duration of therapy is given in the table, too.

Sample preparation and GC-HRMS analysis

The complete procedure can be taken from [5].
In brief: Hair samples were collected in full length bundles from the surface of the skin.
The hair strants were cut into 20 mm segments, hydrolysed with 2 ml 1 M KOH at 70 °C for two hours, diluted with 3 ml of H_2O and extracted with 5 ml of TBME.
The dried residue was either derivatized with 50 µl MSTFA/NH_4I and 2 µl were injected onto the GC-HRMS system or used for further purification via immuno affinity chromatography.
Ions 300.1006, 334.0617, 335.0695, 336.0587, 337.0666 and 338.0558 were monitored, because they provided signals with low background noise and high intensity. For GC-HRMS conditions refer to [5].

Results

The HRMS analysis of spiked hair extracts shows linear behaviour within the concentration range from 4 to 200 ng clenbuterol/g hair. Extraction recoveries were approximately 90%. The hydolysis under the alkaline conditions did not degradate the analyte. Detection limit was estimated to a concentration of 4 ng clenbuterol/g hair.
The IAC purified extracts showed reduced biological background interference and an improved limit of detection (0,8 ng/g) of spiked hair samples. The protocol of the IAC handling is also described in [5].

Table II summarises the results of clenbuterol determination in the hair of the tested females.

References

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[3] A. Gleixner, H. Sauerwein and H.H.D. Meyer, Clin. Chem., 42 (1996) 1869 - 1871.

[4] W.A. Baumgartner and V.A. Hill, Forensic Sci. Int., 63 (1993) 157 - 160.

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Patient:	A	B	C	D
Race:	Caucasian	Caucasian	Caucasian	Caucasian
Age:	31 years	40 years	24 years	33 years
Body weight:	53 kg	68 kg (50 kg)*	66 kg	71,5 kg
Coloration of hair:	dark brown	brown (synthetically bleached)	dark blond	black
Duration of application:	5 weeks	3 months	12 days	3 months
Dosage:	3 x 20 µg/d	3 x 20 µg/d	3 x 20 µg/d	3 x 20 µg/d
Hair collection:	2 and 6 months after ending of clenbuterol application	3 months after beginning and 5½ months after ending of clenbuterol application	5 months after ending of clenbuterol application	2½ months after ending of clenbuterol application

Tab. I: Profile of patients who used clenbuterol therapeutically.

* Weight measured 5½ months after delivery

Female A				Female B				Female C		Female D	
Hair sampling 2 months after completion of administration		Hair sampling 6 months after completion of administration		Hair sampling 3 months after beginning of administration		Hair sampling 5½ months after completion of administration		Hair sampling 5 months after completion of administration		Hair sampling 2½ months after completion of administration	
Hair segment (mm)	concen-tration (ng/g)	Hair segment (mm)	concen-tration (ng/g)	Hair segment (mm)	concen-tration (ng/g)	Hair segment (mm)	concen-tration (ng/g)	Hair segment (mm)	concen-tration (ng/g)	Hair segment (mm)	concen-tration (ng/g)
10 - 30	28, 41	10 - 30	0, 0	10 - 30	236,212	10 - 30	0, 0	10 - 30	0, -	10 - 30	24, 44
30 - 50	100, 90	30 - 50	0, 0	30 - 50	30, 32	30 - 50	7, 6	30 - 50	b. LOQ	30 - 50	139,139
50 - 70	4, 4	50 - 70	b. LOQ	50 - 70	4, 6	50 - 70	31, 47	50 - 70	3, -	50 - 70	29, -
70 - 90	0, 0	70 - 90	16, 17	70 - 90	4, 5	70 - 90	10, 5	70 - 90	2, -		
90- 110	0, 0	90- 110	17, 13					90- 110	b. LOQ		
110-130	0, 0	110-130	b. LOQ					110-130	b. LOQ		
		Tips of hairs	0, 0					130-150	b. LOQ		
								Tips of hairs	b. LOQ		

Tab. II: Concentration of clenbuterol in the hair segments of the tested females.
Two values for each hair segment are given except for those of female C. b. LOQ: below limit of quantitation.