Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (7)

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Sport und Buch Strauß, Köln, 1999

M.K. HENZE, G. OPFERMANN, W. SCHÄNZER:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (7). Sport und Buch Strauß, Köln, (1999) 335-339

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Liquid-Liquid Extraction-pH-Profiles of selected Beta-2-Sympathomimetic Agonists

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Abstract

The influence of the pH-value on the extraction of selected beta-2-sympathomimetic agonists with t-butylmethylether/t-butanol/NaCl(saturation) was investigated.
There are five groups showing a different behaviour.

The extraction pH-profiles and the GC/MS-data of the TMS-derivatives are presented.

Introduction

In general beta-2-sympathomimetic agonists are prohibited in sports due to their anabolic and stimulating side effects.
Exceptionally the administration of salbutamol, salmeterol and terbutaline are permitted by inhalation when prescribed for therapeutic purposes and when prior clearance has been given to the relevant medical authority of the federation.

In order to analyse urine samples by GC/MS the influence of the pH-value on the liquid-liquid extraction of selected beta-2-agonists was investigated.

Experimental

2.5 ml buffer (pH 5 to 13) + beta-2-agonist standard solution containing
5 µg of cimaterol, cimbuterol, clenbuterol, clenpenterol, fenoterol, formoterol, isoetharine, isoprenaline, isoxsuprine, mabuterol, orciprenaline, procaterol, protokylol, ractopamine, reproterol, ritodrine, salbutamol, salmeterol, terbutaline, tulobuterol each, 10 µg of a metabolite of reproterol[1], 1 µg of brombuterol, mapenterol each

⇓

+ 5 µg 5β-androstane3α,17β-diol as internal standard

⇓

+ 5 ml of t.-butylmethylether, 1ml t.-butanol and NaCl (saturation)

⇓

shake for 20 min and centrifuge at 2600 rpm for 5 min

⇓

transfer the organic layer and evaporate to dryness in vacuo

⇓

add 100 µl MSTFA/NH_4I/ethanethiole 1000:2:6 (v:w:v)

heat at 60°C for 15 min

⇓

inject 3 µl into GC/MS

Figure 1: Sample preparation

Instrument:	GC hp 5880 / MSD hp 5971A		
Column:	hp ultra1: 16.8m OV1, 0.22mm I.D., 0.11µm film thickness		
Flow parameters:	carrier: He	head pressure:9psi	split flow: 11ml/min
Injection parameters:	mode: split 1:10	volume: 3µl	Temperature: 300°C
Oven temp. prog.:	init. temp.:140°C	init. time:0	
	rate: 20°/min	final temp: 320°	final time: 3 min
MS parameters:	ionisation mode: EI	acq. mode: SIM	Interface temp: 325°C

Tab. 1: Analytical parameters

		retention time [min]	methylene units	M	m/z
1.	Tulobuterol O-TMS	1,897	710	299	86, 194, 228
2.	Mabuterol N',O-bis-TMS	3,257	970	454	86, 369, 371
3.	Isoprenaline O,O',O''-tris-TMS	3,325	982	427	355, 356, 73
4.	Orciprenaline O,O',O''-tris-TMS	3,388	993	427	356, 73, 412
5.	Terbutaline O,O',O''-tris-TMS	3,487	1012	441	356, 86, 426
6.	Isoetharine O,O',O''-tris-TMS	3,589	1031	455	100, 355, 251
7.	Mapenterol N',O-bis-TMS	3,707	1053	468	100, 369, 349
8.	Salbutamol O,O',O''-tris TMS	3,819	1073	455	369, 86, 147
9.	Cimaterol N',O-bis TMS	3,960	1100	363	291, 73, 292
10.	Cimbuterol N',O-bis-TMS	4,090	1124	377	86, 291, 292
11.	Clenbuterol N',O-bis-TMS	4,151	1135	420	335, 86, 337
12.	Clenpenterol N',O-bis-TMS	4,608	1221	434	100, 335, 337
13.	Brombuterol N',O-bis-TMS	4,776	1253	508	86, 425, 424
14.	Procaterol N',O,O'-tris-TMS	5,306	1355	506	407, 408, 100
15.	Isoxsuprine O,O'-bis-TMS	5,718	1438	445	178, 267, 107
ISTD	5β-Androstane-3α,17β-diol bis-TMS	6,117	1520	436	241, 346, 256
16.	Ritodrine O,O',O''-tris-TMS	6,434	1587	503	236, 193, 267
17.	Fenoterol O,O',O'',O'''-tetrakis-TMS	6,809	1670	591	322, 236, 356
18.	Ractopamine O,O',O''-tris-TMS	6,886	1687	517	267, 250, 179
19.	Protokylol O,O',O''-tris-TMS	6,952	1702	547	355, 322, 192
20.	Formoterol O,O'-bis-TMS	7,579	1849	488	277, 178, 149, 367
21.	Salmeterol O, O', O''-tris-TMS	9,037	2223	631	262, 369, 112
22.	Reproterol O, O', O''-tris-TMS	9,133	2250	605	250, 356, 221
23.	Metabolite of Reproterol[1] O, O', O''-tris-TMS	9,637	2392	617	368, 527, 396

Tab. 2: beta-2-agonists, retention times and methylene units
N' means the nitrogen of a 4-amino group at the aromatic ring or, in case of procaterol, at the 3-imino group.
If the N-alkyl group is not t-butyl, often the pertrimethylsilylated compounds are detected to a minor extent in concentrated standards, but not in urinary samples. Persilylation of isoxsuprine and reproterol failed completely.

Results

There are five groups of beta-2-agonists which show a different behaviour:

	optimal pH-value	remarks
1. terbutaline, orciprenaline, reproterol, metabolite of reproterol, salbutamol, procaterol	9 to 11 (figure 2)	at lower pH-values improved hydrophilic character (⇒ less good extraction) by protonation of the amino-group, at high pH-values improved hydrophilic character (⇒ less good extraction) by deprotonation of the phenolic OH-groups
2. fenoterol, formoterol, ractopamine, ritodrine	5 to 12 (10) (figure 3)	long hydrophobic chain at N: good extraction at lower pH-values
3. cimaterol, cimbuterol	9 to 13 (figure 4)	no phenolic OH-group: good extraction at high pH-values
4. clenbuterol, clenpenterol, brombuterol, isoxsuprine, mabuterol, mapenterol, salmeterol, tulobuterol	5 to 13 (figure 5)	hydrophobic character dominates improved hydrophilic character by protonation of the amino-group or deprotonation of the phenolic OH-group
5. protokylol, isoetharine, isoprenaline	≤ 9 (figure 6)	catecholamines decompose at higher pH-values

Reference

[1] G. Niebch, K.H. Klingler, G. Eikelmann, N. Kucharczyk: Untersuchungen zur Biotransformation von Reproterol - Strukturaufklärung des Hauptmetaboliten, Arzneim.-Forsch./Drug Res. 28(I), 1978

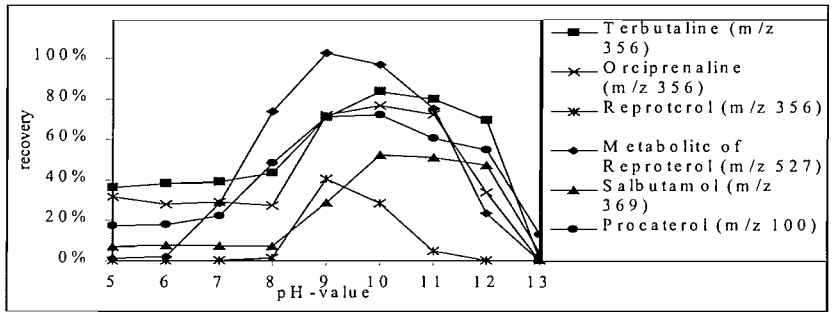

Figure 2

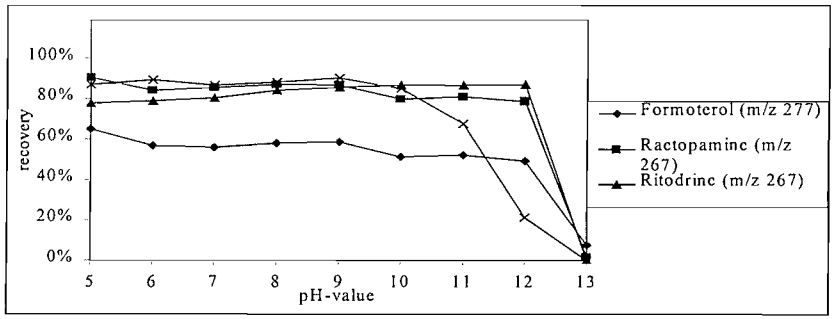

Figure 3

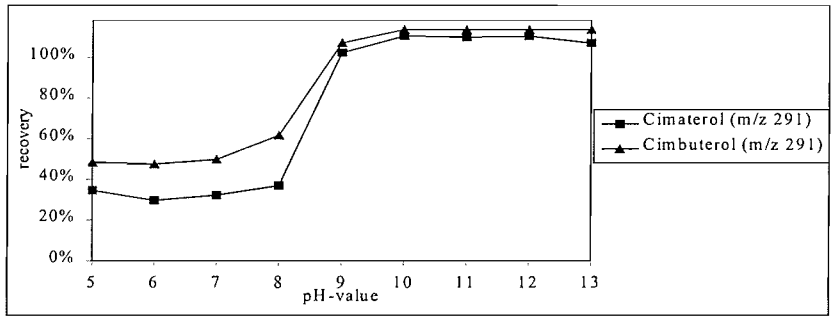

Figure 4

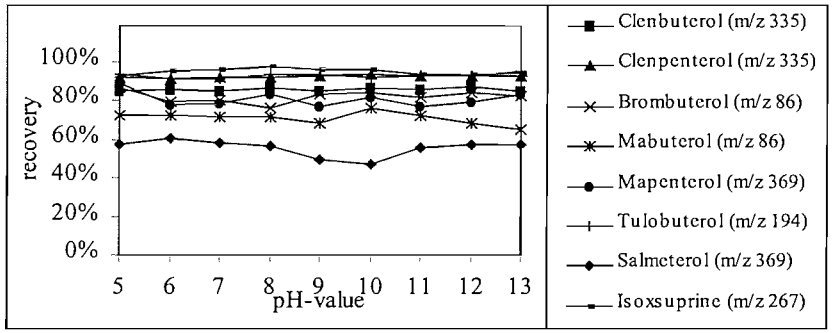

Figure 5

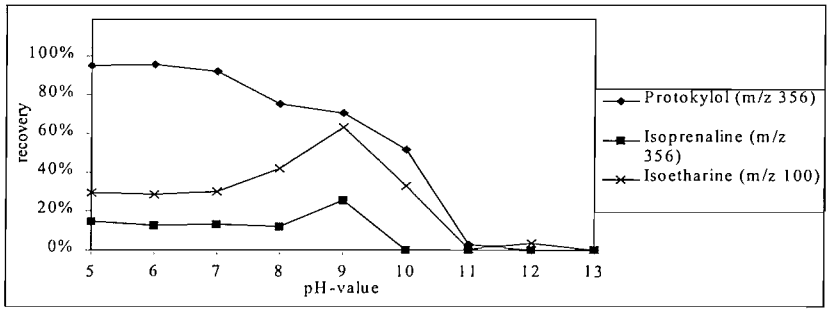

Figure 6

Acknowledgements

The Bundesinstitut für Sportwissenschaften, Köln, is acknowledged for the financial support, Asta Medica AWD, Frankfurt a.M., Boehringer Ingelheim, Glaxo Wellcome, Bad Oldesloe, Lindopharm GmbH, Hilden, Novartis Pharma GmbH, Nürnberg and Solvay Arzneimittel, Hannover for supplying the reference compounds. Gratefulness has to be given to Prof. H. Span-Langguth, Martin-Luther-Universität Halle-Wittenberg, Germany.