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Screening of β-2 agonists and confirmation of fenoterol, orciprenaline, reproterol and terbutaline with gas chromatography–mass spectrometry as tetrahydroisoquinoline derivatives

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Abstract

A new method for a comprehensive screening and confirmation of β-2 agonists in human urine is presented based on gas chromatography–low-resolution mass spectrometry (GC–MS) using electron impact ionisation (EI). After hydrolysis of the conjugates with β-glucuronidase/arylsulfatase a derivatisation step with formaldehyde converts fenoterol, orciprenaline, reproterol and terbutaline to one derivative, a tetrahydroisoquinoline, while the other β-2 agonists remain unchanged. Liquid–liquid extraction and trimethylsilylation follow. The tetrahydroisoquinoline derivatives show good gas chromatographic and mass spectrometric behaviour. The detection limit of these four β-2 agonists in the screening using low-resolution mass spectrometry is 10 ng/ml of urine. The other β-2 agonists are detected as parent compounds with the same recovery after sample preparation with and without formaldehyde. The EI mass spectra of the tetrahydroisoquinoline derivatives are presented.

Keywords: Derivatisation, GC; β-2 Agonists; Fenoterol; Orciprenaline; Reproterol; Terbutaline; Tetrahydroisoquinoline

1. Introduction

β-2-Sympathomimetic agonists are prohibited in sports due to their anabolic and stimulating side effects, but 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 [1].

For fenoterol (1-(3,5-dihydroxyphenyl)-2-{[1-(4-hydroxyphenyl)-2-propyl)amino}ethanol), orciprenaline [1-(3,5-dihydroxyphenyl)-2-isopropylaminoethanol], reproterol (1,3-dimethyl-7-{3-[(β,3,5-trihydroxyphenethyl)amino]propyl}-2,6(1H,3H)-purindion) and terbutaline (1-(3,5-dihydroxyphenyl)-2-*tert*.-butylaminoethanol) studies on biotransformation have been performed: fenoterol is excreted in human urine as its sulfate, conjugated with glucuronic acid and unconjugated [2–5], orciprenaline unconjugated, as sulfoconjugate and as 2-isopropyl-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline [4] and reproterol as free and conjugated 2-[3-theophyllinyl(7)-propyl]-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline [6–

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8]. Terbutaline passes unchanged and as sulfoconjugate [2,4,9].

Especially for the detection of β-2-sympathomimetic agonists fenoterol [10], orciprenaline [10,11], reproterol [12], and terbutaline [10,11,13], in urine samples, substance-specific methods are described.

The potential misuse as anabolic agents demands a method for screening analysis of many β-2 agonists in human urine.

As the tetrahydroisoquinoline derivatives are described as phase I metabolites, a derivatisation with formaldehyde seems to be a suitable method for the detection of these compounds because the obtained derivatives are identical to the described metabolites. Only one product of each compound has to be monitored. Hydrolysis of the sulfates and glucuronides by the β-glucuronidase/arylsulfatase enzyme is included due to the reported phase II metabolites.

2. Experimental

2.1. Chemicals

Reproterol hydrochloride was a kind gift of ASTA Medica (Frankfurt, Germany), ritodrine hydrochloride of Solvay Arzneimittel (Hannover, Germany), tulobuterol of Abbott (Wiesbaden, Germany), orciprenaline hemisulfate and Alupent tablets of Boehringer Ingelheim (Ingelheim, Germany) and terbutaline hemisulfate and Contimit tablets of Lindopharm (Hilden, Germany). Fenoterol hydrobromide, procaterol hydrochloride and salbutamol hemisulfate were obtained from Sigma (Deisenhofen, Germany), isoxsuprine hydrochloride from Sigma (St. Louis, MO, USA), cimaterol, clenbuterol hydrochloride, mabuterol and Berotec tablets from Boehringer Ingelheim, cimbuterol, clenpenterol from BGVV (Berlin, Germany) and Bronchospasmin tablets from ASTA Medica.

N - Methyl - *N* - trimethylsilyl - trifluoroacetamide (MSTFA) was purchased from Chem. Fabrik Karl Bucher (Waldstetten, Germany), β-glucuronidase/arylsulfatase from *Helix pomatia*, EC 3.2.1.31, EC 3.1.6.1 from Boehringer Mannheim (Mannheim, Germany), and Amberlite XAD-2 from Serva Feinbiochemica (Heidelberg, Germany). Other reagents and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Instrumentation

Gas chromatography–mass spectrometry (GC–MS) analyses were performed on a Hewlett-Packard (HP) 5890 gas chromatograph coupled to a Hewlett-Packard 5971 A mass-selective detector with the following parameters: injection parameters: volume: 3 μl, temperature: 300°C. Column: HP Ultra1 (OV1): 16.8 m×0.22 mm I.D., 0.11 μm film thickness. Carrier gas: helium, split flow: 11 ml/min, head pressure: 9 p.s.i., split 1:10 (1 p.s.i.=6894.76 Pa). Oven temperature: 140°C with 20°C/min to 320°C final time 3 min. Ionisation: 70 eV electron impact ionisation (EI).

When the mass-selective detector was operated in the selected ion monitoring (SIM) mode the following ions were monitored: group 1: 1.40–4.43 min, *m/z* 438, 424, 369, 368, 356, 355, 335, 296, 291, 194, 100, 86 and 72, dwell time of 10 ms, group 2: 4.43–6.07 min, *m/z* 438, 407, 368, 346, 337, 335, 267, 241, 183, 178, 100 and 86, dwell time of 10 ms, group 3: 6.07–8.30 min, *m/z* 455, 424, 356, 355, 346, 322, 308, 277, 267, 250, 241, 236, 193 and 178, dwell time of 10 ms and group 4: 8.30–12.00 min, *m/z* 527, 369, 368, 356, 262, 250, dwell time of 24 ms. Scan mode: 40–650 u, threshold 100, scan rate 2×1.

2.3. Sample preparation

2.3.1. Method A (without formaldehyde)

To 5 ml of urine 0.5 ml of sodium acetate buffer (4 *M*, pH 5.2) is added and the pH is adjusted to 5.0–5.5 with acetic acid. A 50-μl volume of β-glucuronidase/arylsulfatase from *Helix pomatia* is added followed by hydrolysis for 16 h at 37°C. A 0.25-ml volume of KOH solution (5 *M*) and 10 μl of 5β-androstane-3α,17β-diol (200 ng/μl in MeOH) as internal standard are added. The pH is adjusted to 9.6 by adding solid K_2CO_3–$NaHCO_3$ (1:2, saturation). The samples are extracted with a mixture of 5 ml of *tert*.-butylmethyl ether (TBME) and 1 ml of *tert*.-butanol (*tert*.-BuOH) after addition of NaCl (saturation). After centrifugation (750 *g* for 5 min) the organic layer is transferred and evaporated to dryness

in vacuo. A 100-μl volume of MSTFA–NH_4I–ethanethiol-TMS (1000:2:6, v/w/v) is added and the sample is heated at 60°C for 15 min. The mixture is injected into the GC–MS system.

2.3.2. Method B (with formaldehyde reaction)

The sample preparation is carried out as described in method A, but after the hydrolysis at the same pH a reaction of the mixture with 15 μl of formaldehyde (0.37% in water) at 80°C for 3 h is inserted.

2.4. Reference urine

A 5-ml volume of a blank urine is spiked with cimaterol, cimbuterol, clenbuterol, clenpenterol, fenoterol, isoxsuprine, mabuterol, orciprenaline, procaterol, reproterol, ritodrine, salbutamol, terbutaline and tulobuterol (500 ng/ml each) and analysed according to method A.

2.5. Reaction of β-2 agonists with formaldehyde

A 10-μg amount of cimaterol, cimbuterol, clenbuterol, clenpenterol, fenoterol, isoxsuprine, mabuterol, orciprenaline, procaterol, reproterol, ritodrine, salbutamol, terbutaline or tulobuterol each were put into 2.5 ml of sodium acetate buffer (4 *M*, pH 5.2). After addition of 15 μl of formaldehyde (0.37% in water) to each sample the reaction was carried out at 80°C for 3 h. An Amberlite XAD-2 column (pasteur pipette closed with a glass pearl, bed height 2 cm) was loaded with the mixture, washed twice with 2 ml of water and eluted with 2 ml of methanol. The eluate was evaporated to dryness, the residue derivatised with 100 μl of MSTFA–NH_4I–ethanethiol-TMS (1000:2:6, v/w/v) and injected into the chromatograph as described above. The mass-selective detector was operated in the scan mode.

2.6. Synthesis of the tetrahydroisoquinoline derivatives

2-[3-Theophyllinyl(7)-propyl]-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline, I, (C,N-methylene-reproterol) is prepared from 445 mg of reproterol hydrochloride according to the method described by Niebch et al. [8].

2-[1-(4-Hydroxyphenyl)-2-propyl]-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline, II, (C,N-methylene-fenoterol), 2-isopropyl-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline, III (C,N-methylene-orciprenaline) and 2-*tert*.-butyl-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline, IV, (C,N-methylene-terbutaline) are prepared in the following way:

Amounts of 1.1 mmol fenoterol hydrobromide (423 mg), 2.0 mmol terbutaline hemisulfate (537 mg) or 2.0 mmol orciprenaline hemisulfate (526 mg) are solved in 86 ml of water, then 5.4 mmol of formaldehyde (37% in water, 400 μl) is added. After stirring the mixture is kept at room temperature for 8 days. Then the water is evaporated in vacuo and the product is recrystallised from isopropanol (in the case of C,N-methylene-fenoterol from isopropanol–benzene).

The following products are obtained: C,N-methylene-reproterol (I): 271.0 mg (yield 59%) of light brown crystals, GC–MS characterisation as tris-TMS product, $M^+=617$ (Fig. 1). C,N-methylene-fenoterol (II): 72.2 mg (yield 10%) of light brown crystals, GC–MS characterisation as tetrakis-TMS product, $M^+=603$ (Fig. 2). C,N-methylene-orciprenaline (III): 155.1 mg (yield 52%) of light beige crystals, GC–MS characterisation as tris-TMS product, $M^+=439$ (Fig. 3). C,N-methylene-terbutaline (IV): 231.4 mg (yield 40%) of colourless crystals, GC–MS characterisation as tris-TMS product, $M^+=453$ (Fig. 4).

2.7. pH profiles of the liquid–liquid extraction

A 5-μg amount of reproterol (1), fenoterol (2), orciprenaline (3), terbutaline (4) each, 5 μg of 5β-androstane-3α,17β-diol as internal standard and NaCl (saturation) are added to 2.5 ml of the buffers (pH 5 to 13). The samples are extracted with a mixture of 5 ml of TBME and 1 ml of *tert*.-BuOH. After centrifugation (750 *g* for 5 min) the organic layer is transferred and evaporated to dryness in vacuo. A 100-μl volume of MSTFA–NH_4I–ethanethiol-TMS (1000:2:6, v/w/v) is added and the samples are heated at 60°C for 15 min. The mixture is transferred to an autosampler vial and an aliquot is injected into the GC–MS system [14]. The mass-selective detector is operated in the SIM mode as described above.

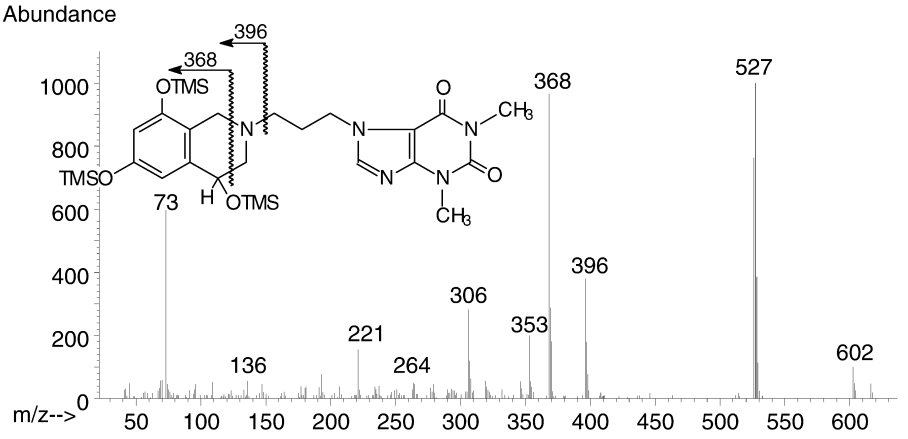

Fig. 1. Mass spectrum of C,N-methylene-reproterol tris-TMS, $M^+=617$, $t_R=9.20$ min.

The extraction yields are calculated relative to a 100% calibration standard obtained by adding 5 μg of reproterol, orciprenaline, terbutaline, fenoterol and 5β-androstane-3α,17β-diol to a mixture of 5 ml of TBME and 1 ml of *tert*.-BuOH. This organic mixture is derivatised as described above.

The extraction yields of the prepared tetrahydroisoquinoline derivatives (I–IV) are determined in the same way.

2.8. Analysis of spiked urines

A 5-ml volume of urine from three different persons are spiked each with cimaterol, cimbuterol, clenbuterol, clenpenterol, isoxsuprine, mabuterol, procaterol, ritodrine, salbutamol and tulobuterol (500 ng/ml each) and analysed six times each according to methods A and B; additionally cimaterol is added to 10 different urines in the same amount and analysed in the same way.

2.9. Calibration curve and limit of detection

A 5-ml volume of urine spiked with 10–400 ng/ml of fenoterol, orciprenaline, reproterol and terbutaline is analysed according to method B.

A calibration curve is regressed through the data points representing the peak area ratios of the tetrahydroisoquinoline derivatives (with *m*/*z* 368 for C,N-methylene-orciprenaline, C,N-methylene-re-

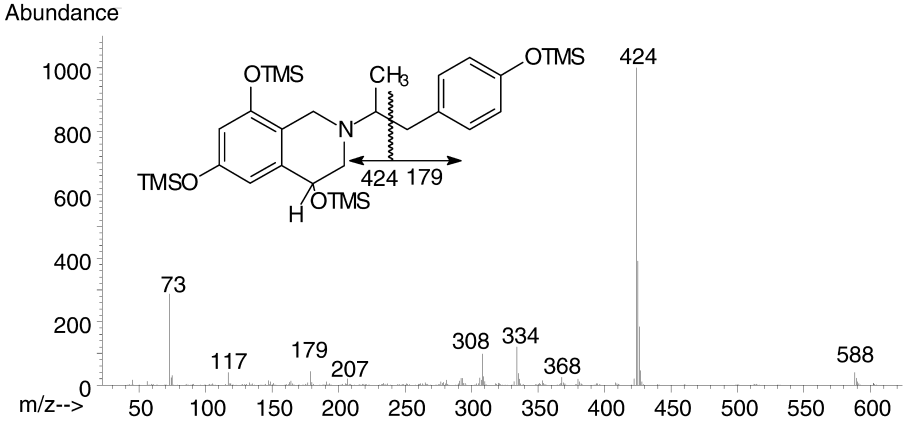

Fig. 2. Mass spectrum of C,N-methylene-fenoterol tetrakis-TMS, $M^+=603$, $t_R=7.22$ min.

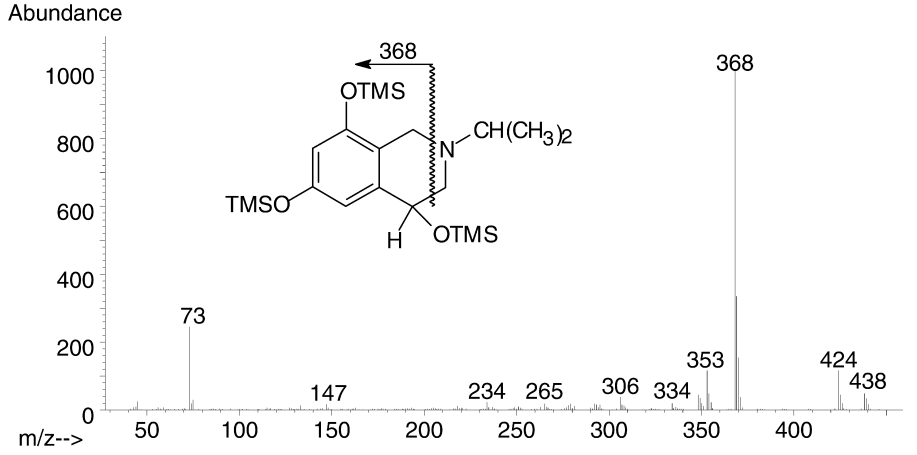

Fig. 3. Mass spectrum of C,N-methylene-orciprenaline tris-TMS, $M^+=439$, $t_R=4.00$ min.

proterol and C,N-methylene-terbutaline, and m/z 424 for C,N-methylene-fenoterol) relative to the peak area of the internal standard androstanediol (m/z 241).

To obtain a clear separation of the C,N-methylene-fenoterol derivative from coelution with an unidentified substance a modified temperature program was used: 140°C, 3°C/min to 229°C, 20°C/min to 320°C, 3 min.

For screening the detection limit is defined as the concentration yielding a signal-to-noise ratio (S/N) of 3:1 of two diagnostic ions. Additionally the retention time of the analyte should not differ by more than 1% from that of the standard solution.

For confirmational analysis at least three diagnostic ions have to be monitored (SIM mode): m/z 424, 334 and 308 for C,N-methylene-fenoterol, m/z 424, 368 and 353 for C,N-methylene-orciprenaline, m/z 527, 368 and 306 for C,N-methylene-reproterol and m/z 438, 368 and 353 for C,N-methylene-terbutaline. The internal standard is registered with m/z 241 and 346.

The signal-to-noise ratio of the diagnostic ions should be higher than 3:1. The relative abundances of these ions shall not differ by more than 5% (absolute) or 20% (relative), whichever is greater, from that of a reference.

The recoveries of fenoterol, orciprenaline, re-

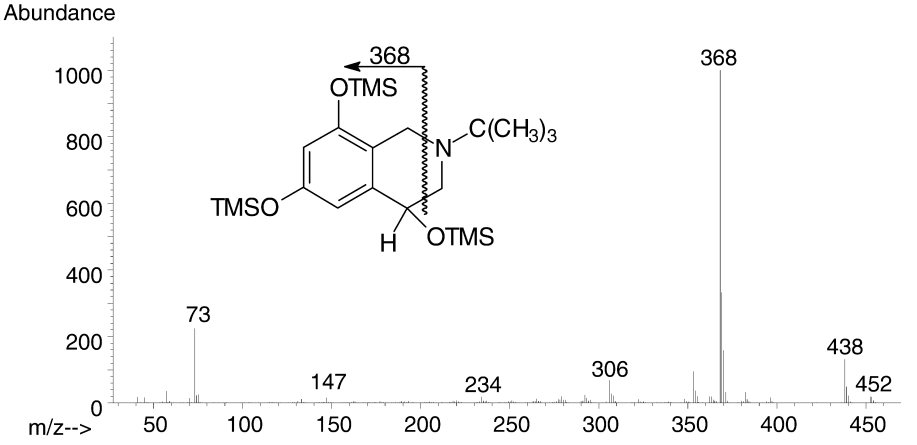

Fig. 4. Mass spectrum of C,N-methylene-terbutaline tris-TMS, $M^+=453$, $t_R=4.20$ min.

proterol and terbutaline are determined by analysing 5 ml of a spiked urine (200 ng/ml each) six times according to method B. The internal standard is added just before the derivatisation step. The 100% calibration standard was obtained by preparing 5 ml of blank urine according to method B. A 10.3-μg amount of C,N-methylene-fenoterol (10 μg fenoterol), 10.6 μg C,N-methylene-orciprenaline (10 μg orciprenaline), 10.3 μg C,N-methylene-reproterol (10 μg reproterol) and 10.4 μg C,N-methylene-terbutaline (10 μg terbutaline) and the internal standard are added just before derivatisation.

2.10. Excretion study

Fenoterol hydrobromide [Berotec, one tablet, 2.5 mg, subject A (male, 78 kg) and B (female, 67 kg)], orciprenaline hemisulfate [Alupent, one tablet, 20 mg, subject A (male, 70 kg) and B (female, 58 kg)], reproterol [Bronchospasmin, one tablet 20 mg, subject A (male, 70 kg) and B (female, 62 kg)] and terbutaline hemisulfate [Contimit, one tablet, 2.5 mg, subject A (female, 63 kg) and B (female, 61 kg)] were orally taken by volunteers.

The urine samples were collected in 2-h intervals for 24 h and stored at 4°C.

The samples were prepared according to method A.

3. Results and discussion

3.1. Analysis of a spiked urine

The analysis of the β-2 agonists presented in Fig. 5 is investigated.

The GC–MS properties of the TMS derivatives of those compounds obtained by analysing the reference urine are shown in Table 1.

Fig. 6 shows a chromatogram (SIM, *m/z* 368, 424) of a urine spiked with fenoterol, orciprenaline, reproterol and terbutaline, 200 ng/ml each, obtained after sample preparation according to method B. Only the TMS derivatives of the C,N-methylene products have to be monitored for these compounds.

After sample preparation of this urine according to method A two derivatives each (parent compound and C,N-methylene compound each as TMS derivative) have to be monitored as the C,N-methylene compounds are obtained in small amounts during sample preparation according to the assumption that formaldehyde may be present in urine in traces.

The main fragment ion of C,N-methylene-reproterol, C,N-methylene-orciprenaline and C,N-methylene-terbutaline, *m/z* 368, is generated via retro-Diels–Alder rearrangement of the tetrahydroisoquinoline ring (Fig. 7). A very stable radical with a conjugated π-system is obtained. The main fragment ion of C,N-methylene-fenoterol is obtained by α-cleavage of the bond between C-1 and C-2 in the side chain which is in the α-position to the nitrogen atom as well as to the phenyl ring. The obtained fragment includes the tetrahydroisoquinoline system with a C_2H_4 at the nitrogen. The loss of TMS-OH forms *m/z* 334 and the loss of ethanol-TMS *m/z* 308. Also after α-cleavage of the above mentioned bond *m/z* 179 is obtained. It can be assigned to the methylenphenol-*O*-TMS cation (Fig. 8). In the case of C,N-methylene-reproterol a strong *m/z* 527 is obtained via neutral loss of trimethylsilanol (TMS-OH). It is followed by a loss of the side chain from the nitrogen as a radical obtaining *m/z* 306. MS–MS experiments also confirm that the sequence of these two reactions can be inverse (Fig. 7).

Additionally $M^+ - 15$ ($-CH_3$) can be observed in all these spectra.

The proposed fragmentation schemes are confirmed by using the technique of MS–MS studies of the unlabelled as well as deuterated derivatives.

3.2. Reaction of β-2 agonists with formaldehyde

For fenoterol, orciprenaline, reproterol and terbutaline one derivative each (the 2-*R*-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline), identified by its mass fragmentation pattern (Figs. 1–4), is obtained via the Pictet–Spengler reaction [16] (reaction scheme in Fig. 9).

The chromatographic properties and response of the TMS-derivatives of these C,N-methylene products are improved compared to those of the β-2 agonists themselves (exceptionally shown for reproterol in Fig. 10).

For cimaterol, cimbuterol, clenbuterol, clenpenterol, isoxsuprine, mabuterol, procaterol, ritodrine,

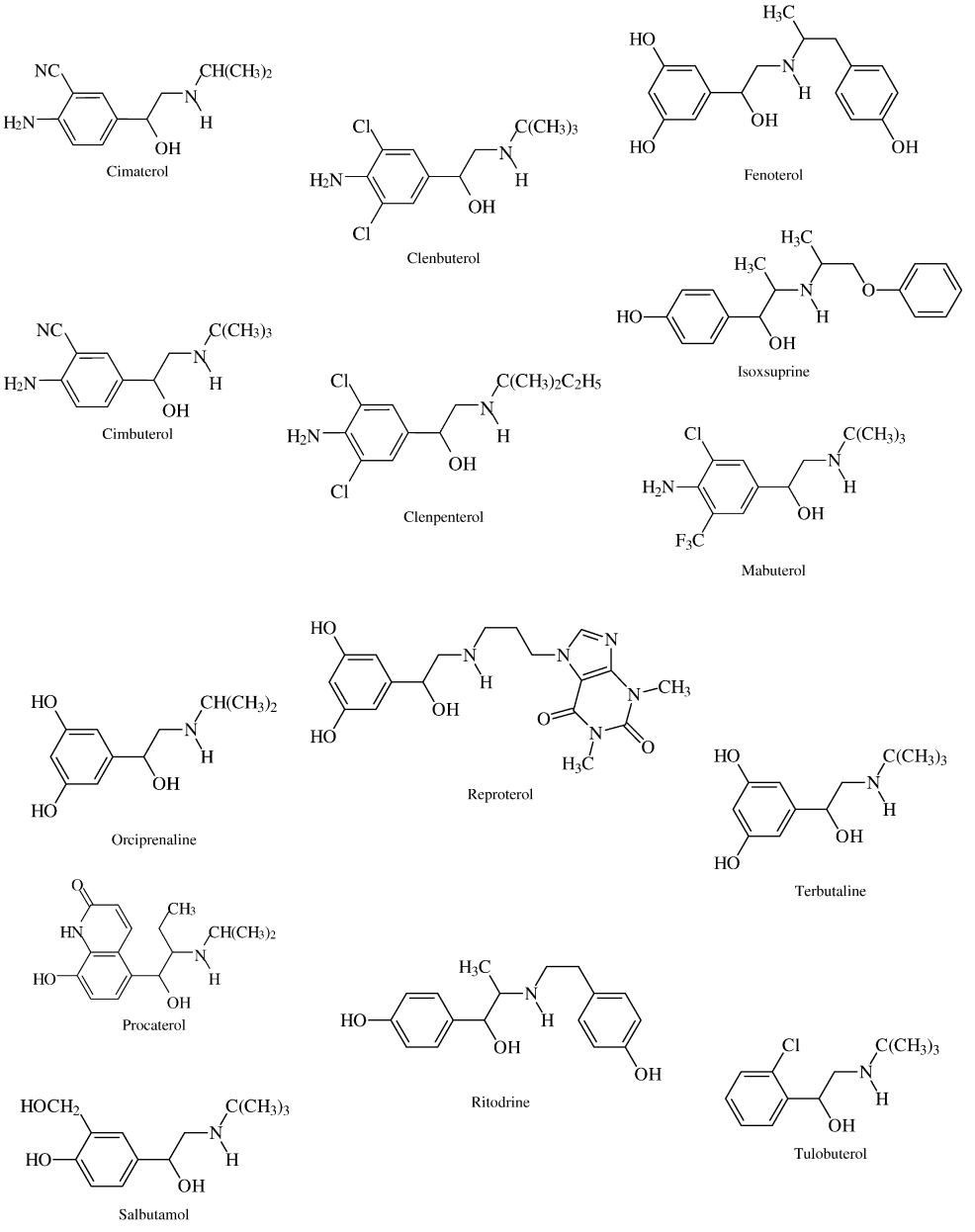

Fig. 5. Structures of the investigated β-2 agonists.

salbutamol and tulobuterol no reaction products with formaldehyde are obtained.

Under the conditions of the reaction (formaldehyde 0.5 mmol/ml of urine, pH 5.2, 80°C) an activating substituent in the positions 3 or 5 of the phenyl group is necessary to make the S_E-reaction possible (activation of the positions 2 or 6). In the case of fenoterol, orciprenaline, reproterol and terbutaline the two hydroxy groups in positions 3 and 5 show a strong activating effect (+M-effect). The phenyl groups of the other investigated β-2 agonists are not activated.

Table 1
DIPA retention index [15] and dominant fragment ions of the TMS-derivatives of the investigated β-2 agonists

	DIPA index	M^+	*m/z*
Tulobuterol, *O*-TMS	1022	299	86, 194, 228
Mabuterol, *N*,*O*-bis-TMS	1325	454	86, 369, 371
Orciprenaline, *O*,*O*′,*O*,″-tris-TMS	1350	427	356, 73, 412
Terbutaline, *O*,*O*′,*O*″-tris-TMS	1377	441	356, 86, 426
Salbutamol, *O*,*O*′,*O*″-tris-TMS	1451	455	369, 86, 147
Cimaterol, *N*,*O*-bis-TMS	1482	363	291, 73, 292
Cimbuterol, *N*,*O*-bis-TMS	1503	377	86, 291, 292
Clenbuterol, *N*,*O*-bis-TMS	1517	420	335, 86, 337
Clenpenterol, *N*,*O*-bis-TMS	1617	434	100, 335, 337
Procaterol, *N*,*O*,*O*′-tris-TMS	1772	506	407, 408, 100
Isoxsuprine, *O*,*O*′-bis-TMS	1869	445	178, 267, 107
Ritodrine, *O*,*O*′,*O*″-tris-TMS	2043	503	236, 193, 267
Fenoterol, *O*,*O*′,*O*″,*O*‴-tetrakis-TMS	2141	591	322, 236, 356
Reproterol, *O*,*O*′,*O*″-tris-TMS	2838	605	250, 356, 221

3.3. pH profiles of the liquid–liquid extraction

The influence of the pH value on the liquid–liquid extraction of the four β-2 agonists (1–4) and their reaction products with formaldehyde (I–IV) are compared and presented in Figs. 11 and 12.

The pH profiles of the liquid–liquid extraction of cimaterol, cimbuterol, clenbuterol, clenpenterol, isoxsuprine, mabuterol, procaterol, ritodrine, salbutamol and tulobuterol have been presented elsewhere [14].

The optimal pH value for the liquid–liquid extraction for all substances is between 9 and 10.

For the tetrahydroisoquinoline derivatives I and II the extraction yields are higher than for the parent compounds reproterol (1) and fenoterol (2). For orciprenaline and terbutaline similar extraction yields

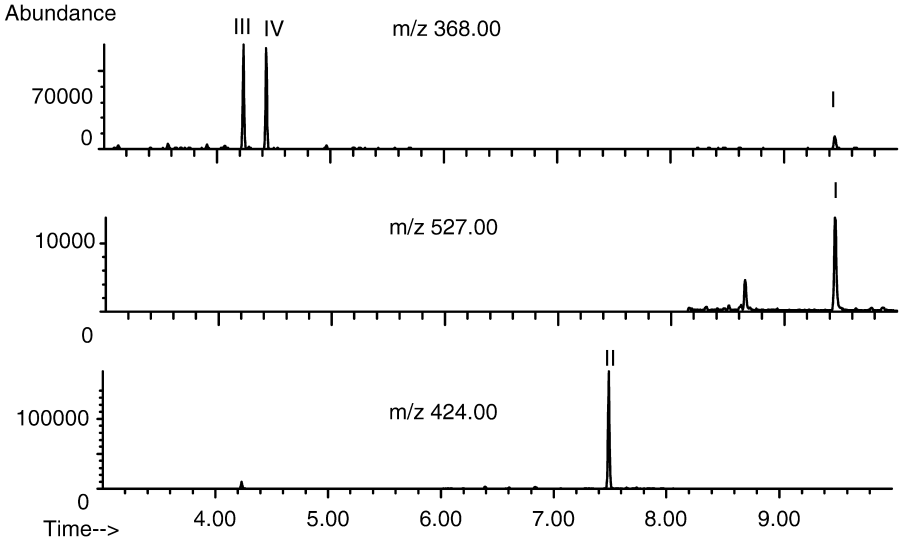

Fig. 6. Chromatogram (SIM, *m/z* 368, 424) of a spiked urine, 200 ng/ml after sample preparation (method B). Tetrahydroisoquinoline derivatives of reproterol (I), fenoterol (II), orciprenaline (III), terbutaline (IV).

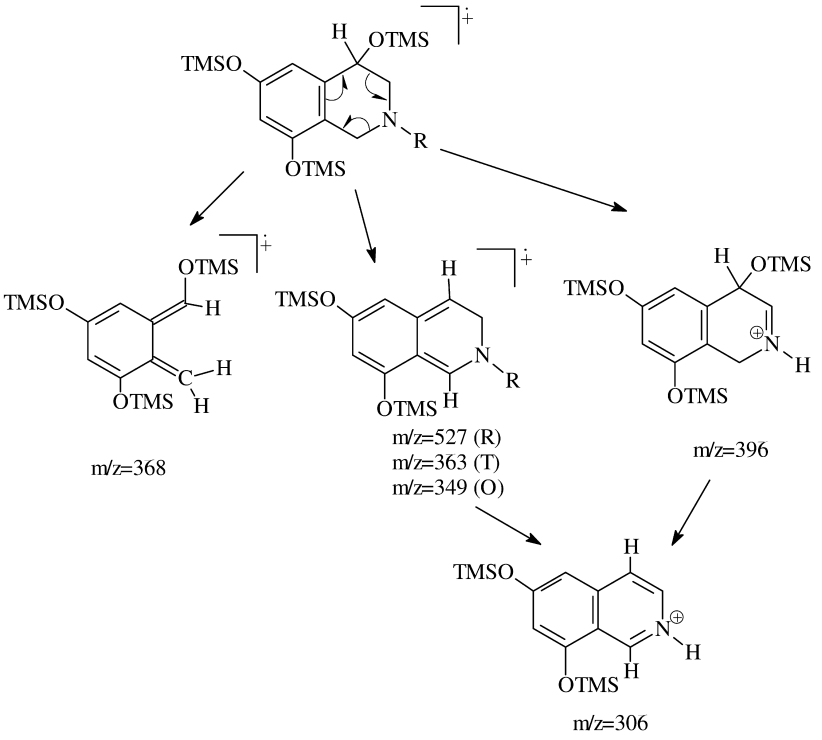

Fig. 7. Proposed ways of fragmentation of C,N-methylene-terbutaline tris-TMS, C,N-methylene-orciprenaline tris-TMS and C,N-methylene-reproterol tris-TMS.

for parent compounds (3, 4) and cyclisation products (III, IV) are obtained at the optimal pH value.

3.4. Behaviour of β-2 agonists which do not react with formaldehyde

The spiked urines were analysed according to method A (without formaldehyde) as well as by method B (with formaldehyde). The peak areas of cimaterol (m/z 291), cimbuterol (m/z 291), clenbuterol (m/z 86), clenpenterol (m/z 100), isoxsuprine (m/z 178), mabuterol (m/z 86), procaterol (m/z 407), ritodrine (m/z 193), salbutamol (m/z 369) and tulobuterol (m/z 86) were calculated relative to the peak area of the internal standard (m/z 241).

The two methods were performed for each substance and then compared using statistical analysis in block design. The *P*-values of the analysis of variance (ANOVA) are shown in Table 2.

No significant differences (*P*-values$<$0.05) are detected for the investigated substances except cimaterol which showed a *P*-value of 0.052. Therefore the comparison of the two methods (A and B) for cimaterol was further investigated.

Ten different spiked urines were analysed according to method A and method B each: The *f*-test for variances showed a *P*-value of 0.6755 and the paired *t*-test a *P*-value of 0.906 confirming that there was no significant difference between the two methods for cimaterol. From these results it can be concluded that for cimaterol, cimbuterol, clenbuterol, clenpenterol, isoxsuprine, mabuterol, procaterol, ritodrine, salbutamol and tulobuterol both methods are equivalent.

3.5. Calibration curve and limit of detection

The analysis of the samples according to method B shows linear behaviour within the analysed concentration range (10–400 ng/ml). The peak areas of the diagnostic ions were calculated relative to the peak area of the internal standard (m/z 241). The

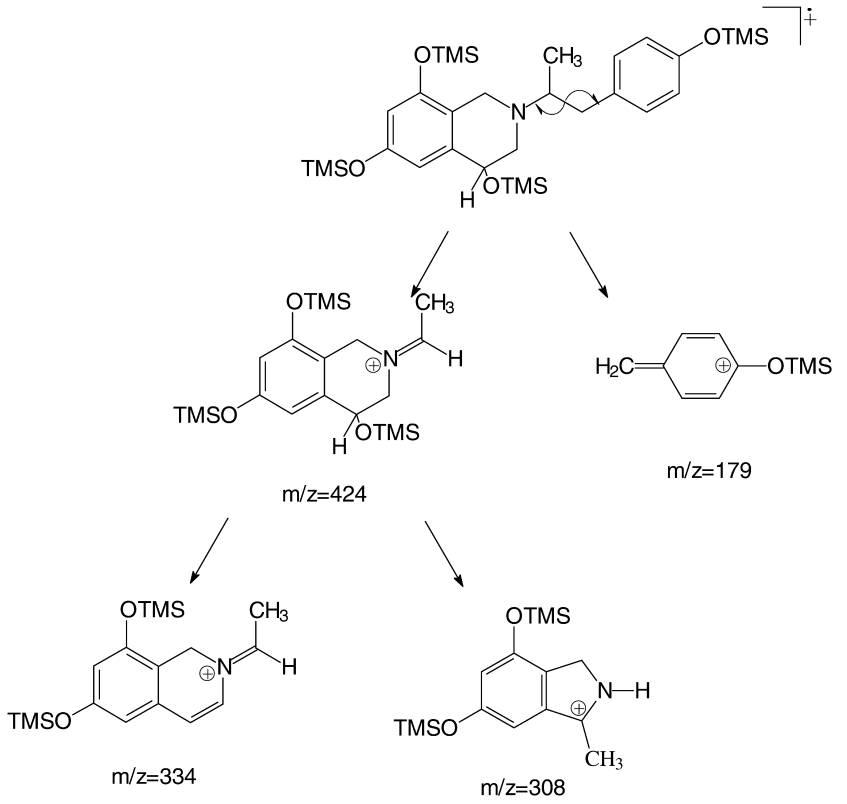

Fig. 8. Proposed ways of fragmentation of C,N-methylene-fenoterol tetrakis-TMS.

obtained values are multiplied by 1000 (result=y). The calibration graph data are shown in Table 3.

The lower level of detection for screening analysis according to method B (two diagnostic ions with a S/N ratio yielding 3:1) using low-resolution MS is determined at 10 ng/ml for fenoterol, orciprenaline, reproterol and terbutaline.

For confirmational analysis [three diagnostic ions with a S/N ratio higher than 3:1, relative abundances of these ions shall not differ by more than 5% (absolute) or 20% (relative) from that of a reference] minimum weights of 10 ng/ml for C,N-methylene-fenoterol, 30 ng/ml for C,N-methylene-orciprenaline, 50 ng/ml for C,N-methylene-reproterol and 30 ng/ml for C,N-methylene-terbutaline are required. The recovery for fenoterol is 109±13%,

Fenoterol R = 1-(4-hydroxyphenyl)-2-propyl, Orciprenaline R = isopropyl,
Reproterol R = 3-theopyllinyl(7)-propyl, Terbutaline R = t-butyl

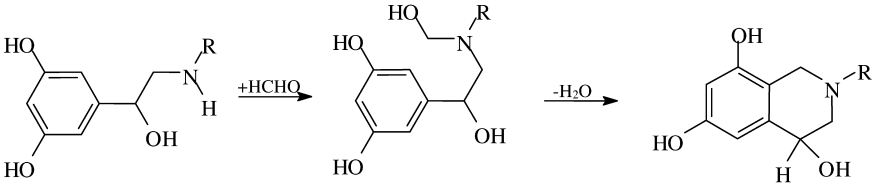

Fig. 9. Reaction scheme: reaction of the 3,5-dihydroxyphenyl-substituted β-2 agonists with formaldehyde.

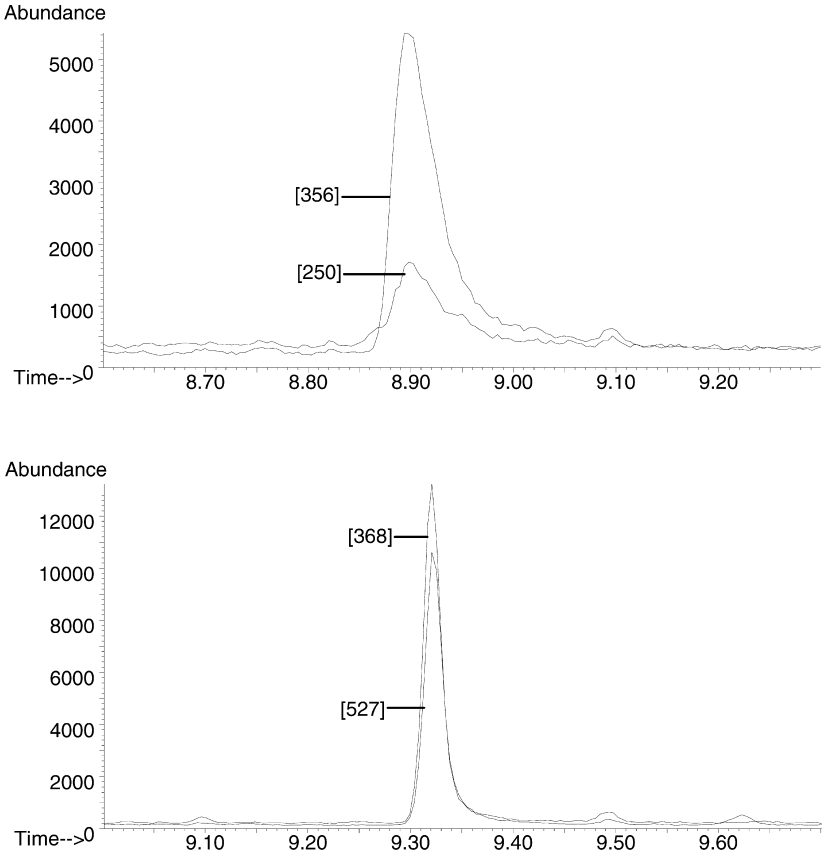

Fig. 10. EIC of reproterol tris-TMS (upper) and of C,N-methylene-reproterol tris-TMS (lower), 80 ng/ml urine each.

orciprenaline 72±9%, reproterol 53±5% and terbutaline 66±7%.

For confirmation purposes and also for screening more sensitive instruments such as a high-resolution mass spectrometer can be used. This was not object of this study and is addressed to further projects.

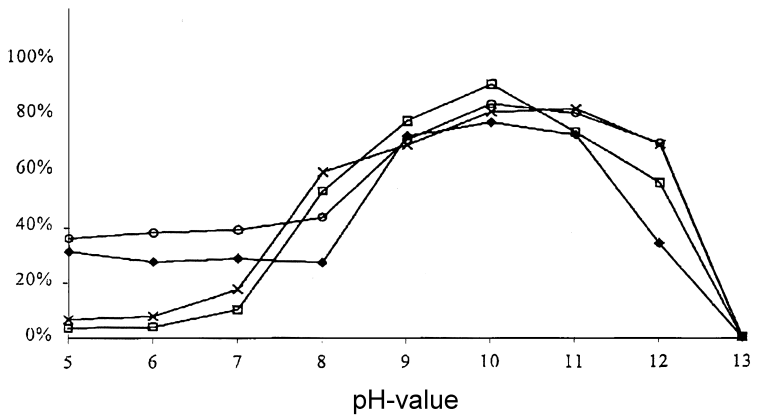

Fig. 11. Liquid–liquid extraction pH profiles of orciprenaline, C,N-methylene-orciprenaline, terbutaline, C,N-methylene-terbutaline.

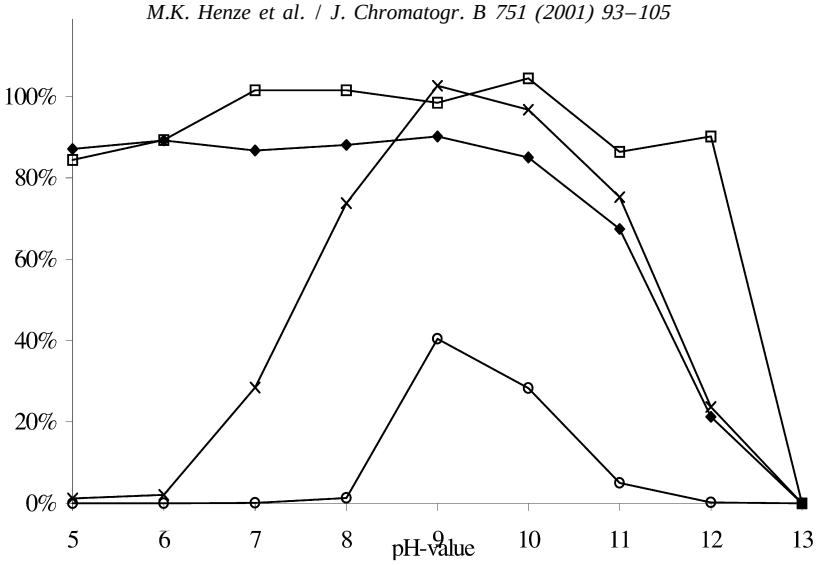

Fig. 12. Liquid–liquid extraction pH profiles of fenoterol, C,N-methylene-fenoterol, reproterol, C,N-methylene-reproterol.

Table 2
Results of comparison between analysis according to method A and B (*P*-values of ANOVA)

Substance	*P*-value
Cimaterol	0.052
Cimbuterol	0.292
Clenbuterol	0.120
Clenpenterol	0.189
Isoxsuprine	0.196
Mabuterol	0.458
Procaterol	0.622
Ritodrine	0.687
Salbutamol	0.959
Tulobuterol	0.947

3.6. Excretion study

In the samples after reproterol administration only C,N-methylene-reproterol is detected after analysis according to method A. In the samples after fenoterol, orciprenaline and terbutaline dosage the C,N-methylene products as well as the parent compounds are detected.

As described before also after analysis of spiked urines according to method A both, parent compound and C,N-methylene product, can be detected. The question, if the tetrahydroisoquinolines of the above mentioned β-2 agonists are metabolites or artefacts, needs further investigation. It is assumed that formaldehyde may be present in urine in traces.

After analysis according to method B only the tetrahydroisoquinolines are detected. Further results of the excretion studies will be presented elsewhere.

4. Conclusion

The presented method B (including a reaction step with formaldehyde) is suitable for screening and with minor modifications (monitoring of at least three diagnostic ions per compound, in case of fenoterol

Table 3
Calibration graph data for fenoterol, orciprenaline, reproterol and terbutaline, analysis according to method B

		Correlation coefficient (R^2)	Diagnostic ion (m/z)
Fenoterol	$y=15.50x+152.9$	0.997	424
Orciprenaline	$y=13.11x+59.43$	0.995	368
Reproterol	$y=1.945x-19.10$	0.993	368
Terbutaline	$y=9.07x+40.08$	0.995	368

usage of the modified temperature program) also for confirmation of fenoterol, orciprenaline, reproterol and terbutaline.

A tetrahydroisoquinoline derivative is obtained via the Pictet–Spengler reaction that is the only derivative per compound which has to be monitored.

The chromatographic properties and response of the TMS-derivatives of the C,N-methylene products of fenoterol, orciprenaline, reproterol and terbutaline are improved compared to those of the β-2 agonists themselves.

Even for screening of cimaterol, cimbuterol, clenbuterol, clenpenterol, isoxsuprine, mabuterol, procaterol, ritodrine, salbutamol and tulobuterol the method is suitable since no significant differences between method A and B can be detected because treatment with formaldehyde has no influence on these β-2 agonists.

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