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Diagnostic evidence for the presence of β-agonists using two consecutive derivatization procedures and gas chromatography–mass spectrometric analysis

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Abstract

A GC–MS procedure for the detection of different β-agonists in urine samples based on two consecutive derivatization steps is described. The derivatization procedure is based on the consecutive formation of cyclic methylboronate derivatives followed by a second derivatization step with MSTFA on the same extract, forming TMS derivatives. Injections in the GC–MS system may be carried out after each one of the derivatization steps, obtaining enough information for unambiguous identification. Limits of detection for the two derivatization steps ranged from 0.5 to 5 ng/ml. This procedure was tested with the β-agonists bambuterol, clenbuterol, fenoterol, formoterol, salbutamol, salmeterol, α-hydroxy-salmeterol and terbutaline.

Keywords: Derivatization, GC-MS; β-Agonists

1. Introduction

β-Agonists are synthetic molecules used for the treatment of pulmonary disorders [1] as well as tocolytic agents in human and veterinary medicine. Apart from their clinical use and therapeutic value, β-agonists are compounds with a high potential for misuse, both in sport and zootechnics, due to the stimulation on the central nervous system and promotion of certain anabolic effects when higher doses are administered [2]. For this reason, in sport, most of these compounds are prohibited by the International Olympic Committee [3]. Present rules authorise the administration of specific compounds (salbutamol, formoterol and salmeterol) for the treatment of asthma and exercise-induced asthma but only in inhaled form. For veterinary purposes, several restrictions have also been made with respect to their use.

β-Agonists, which are related to endogenous cathecolamines, are phenyl β-ethanolamines bearing different substituents on the aromatic ring and on the terminal amino group (Fig. 1). For some high efficacy drugs, therapeutic doses are normally low

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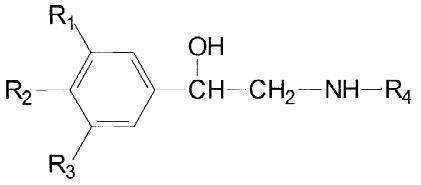

β-agonists	R_1	R_2	R_3	R_4
Salmeterol	$-CH_2OH$	-OH	-H	$-(CH_2)_6$-O-$(CH_2)_4$Ph
α-Hydroxi Salmeterol	$-CH_2OH$	-OH	-H	$-(CH_2)_6$-O-$(CH_2)_3$CH(OH)Ph
Fenoterol	-OH	-H	-OH	$-CH(CH_3)-CH_2$Ph-OH
Formoterol	-NH-CHO	-OH	-H	$-CH(CH_3)-CH_2$-Ph-OCH_3
Bambuterol	$-O-CO-N(CH_3)_2$	-H	$-O-CO-N(CH_3)_2$	$-C(CH_3)_3$
Clenbuterol	-Cl	$-NH_2$	-Cl	$-C(CH_3)_3$
Salbutamol	$-CH_2$-OH	-OH	-H	$-C(CH_3)_3$
Terbutaline	-OH	-H	-OH	$-C(CH_3)_3$

Fig. 1. Chemical structures of the different β-agonists.

and the expected concentrations in urine are in the order of ng/ml or even sub ng/ml levels. For this reason, identification of β-agonists in biological matrices is difficult and requires sensitive and selective extraction and detection methodologies. Efficient screening procedures using immunological [4] or chromatographic methods [5–13] have been reported to analyse β_2-agonists with *tert*-butyl, isopropyl or isopentyl lateral chains. Recently, a comprehensive screening procedure addressed to the detection of β_2-agonists with other N-substituents has been published [14]. The most common employed procedure is GC–MS.

Common criteria for identification of compounds using MS analysis have been defined by different organizations [15,16]. The minimal criteria to report the presence of compounds in biological matrices are based on the comparison of relative retention times and relative abundances of diagnostic ions of the compounds analyzed using MS detection.

Due to the presence of polar functional groups in the β-agonists molecules, a derivatization step is mandatory to convert the target analytes into compounds suitable for gas chromatography analysis. In addition, the derivatization reagent should allow the obtention of characteristic mass spectra, giving specific fragment ions, relevant for identification purposes. Recently, a systematic derivatization study evaluated different agents and catalysts for the analysis of β-agonists [17]. The results obtained showed that MSTFA is the best derivatisating agent for screening purposes, as derivatives with adequate diagnostic information were obtained and mainly one derivative is formed for each compound. Another strategy to increase detection sensitivity and selectivity is the formation of cyclic methylboronate derivatives using two functional groups of the β-agonists molecules whose spatial separation is suitable for ring formation [9,12,13,18]. Methylboronate derivatization is only effective for some of the compounds under study.

To improve the identification capacity of β-agonists in biological samples with more informative and selective mass spectral data, this work reports a derivatization method based on the consecutive formation of methylboronate and trimethylsilyl de-

rivatives. Both derivatization methods can be used on the same extract, yielding more diagnostic data for identification. This procedure was tested with the compounds bambuterol, clenbuterol, fenoterol, formoterol, salbutamol, salmeterol, α-hydroxy-salmeterol (salmeterol metabolite) and terbutaline.

2. Experimental

2.1. Chemicals and reagents

The β-agonists were supplied by the following pharmaceutical manufacturers: bambuterol hydrochloride (Astra Draco AB, Lund, Sweden); clenbuterol hydrochloride (Biomedica Foscama, Rome, Italy); fenoterol hydrobromide (Boehringer Ingelheim Pharma KG, Ingelheim/Rhein, Germany); formoterol fumarate (Novartis Pharma AG, Basel, Switzerland); salbutamol sulphate (Glaxo S.A., Madrid, Spain); salmeterol xinafoate (Alter S.A., Madrid, Spain); terbutaline sulphate (Laboratorios Astra España S.A., Esplugues del Llobregat, Spain) and α-hydroxy-salmeterol (Glaxo Wellcome, Hertfordshire, UK). Penbutolol sulphate (used as internal standard) was supplied by Hoechst Iberica (Barcelona, Spain). The pharmaceutical preparation of bambuterol hydrochloride (Bambec®, Astra España S.A., Esplugues de Llobregat, Spain) was used to perform an excretion study in healthy volunteers.

Methanol, 2-propanol, ethyl acetate (HPLC grade) and potassium carbonate, glacial acetic acid, 25% ammonia, ammonium chloride, sodium acetate trihydrate, potassium hydroxide (analytical grade) were purchased from Merck (Darmstadt, Germany). Acetone and chloroform (both HPLC grade) were supplied by Scharlau (Barcelona, Spain). Deionized water was obtained by a Milli-Q purification system (Millipore Ibérica, Barcelona, Spain).

N-Methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA), gas chromatographic grade, was obtained from Macherey-Nagel (Düren, Germany). Trimethylboroxine, the cyclic anhydride of methylboronic acid, was purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Trimethylboroxine (2 mg/ml) solution in ethyl acetate was prepared by adding 23 μl of trimethylboroxine (99% purity) to 10 ml of ethyl acetate maintained free of humidity with Na_2SO_4. This mixture was stored in a desiccator at room temperature until use.

Ammonium chloride buffer was prepared by dissolution of 28 g of ammonium chloride in 100 ml of deionized water and adjusting the pH to 9.5 with a solution of concentrated ammonia. Acetate buffer (pH 5.2) was prepared by adjusting the pH of a 1.1 mol/l sodium acetate solution to 5.2 with glacial acetic acid. Acetate buffer, 0.1 *M*, pH 4, was prepared by mixing adequate volumes of glacial acetic acid and a 10 *M* potassium hydroxide solution.

β-Glucuronidase containing substantial arylsulphatase activity from *Helix pomatia* type HP-2 (Sigma Chemicals, St Louis, MO, USA), was used for enzymatic hydrolysis. Bond-Elut Certify™ solid-phase extraction columns (130 mg/10 ml) were provided by Varian International (Harbor City, CA, USA). The vacuum manifold used for the solid-phase extraction (SPE) procedure was obtained from Biochem Diagnostics (Edgewood, NY, USA).

2.2. Standard solution

Separate stock standard solutions (1 mg/ml expressed as free bases) of β-agonists and penbutolol (used as internal standard) were prepared by dissolving the compounds in methanol. Separate working standard solutions were prepared by 1:10 and 1:100 dilutions of the stock standard solutions with methanol, in order to achieve concentrations of 100 and 10 μg/ml, respectively. All solutions were stored in the dark at −20 °C.

2.3. Sample preparation procedure

A hydrolysis and extraction procedure recently described [14] was used. Briefly, urines (2 ml) were adjusted to pH 5.2 with 1.1 mol/l acetate buffer (pH 5.2) and 50 μl of *Helix pomatia* (HP-2) were added. The samples were vortexed, heated to 55 °C for 2 h on a water bath, and later cooled to room temperature. The urines were adjusted to pH 9.5 with 100 μl of ammonium chloride buffer. After vortexing (10 s), the samples were centrifuged at 2500 rpm for 5 min. Bond Elut Certify™ columns were conditioned by washing with 2 ml of methanol and 2 ml of deionized water. The columns were prevented from

drying before applying specimens. Hydrolysed urines were applied to the pre-conditioned columns. The columns were washed consecutively with deionized water (2 ml), acetate buffer pH 4 (1 ml) and methanol (2 ml). After drying for 2 min, two consecutive elutions (2 ml each, joint collection) were carried out with a mixture of chloroform–isopropyl alcohol (80:20, v/v) containing 2% ammonium hydroxide. The extracts were then evaporated to dryness under a stream of nitrogen at 40 °C. The organic phase was kept in a desiccator containing di-phosphorous pentoxide and potassium hydroxide pellets, and maintained under vacuum for at least 30 min, before the derivatization procedures were applied.

2.4. Derivatization procedures

Two consecutive derivatization procedures were applied on the same extract:

2.4.1. Derivatization I: formation of cyclic methyl boronates

Fifty microliters of the trimethylboroxine solution were added to the dried residues, and they were incubated at 60 °C for 30 min. The derivatized extracts (I) were transferred into injection vials and analysed by GC–MS.

2.4.2. Derivatization II: trimethylsilylation

After the first GC–MS analysis, 50 μl of MSTFA were added to the same extracts and the samples (extracts II) were heated at 60 °C for 20 min and analysed again by GC–MS.

2.5. Instrumental analysis

Analyses were performed using a HP 5890 gas chromatograph equipped with a crosslinked HP methyl/siloxane fused-silica capillary column (17.5 m×0.2 mm I.D., 0.11 μm), coupled to a HP 5970 MSD. Injections were made in the splitless mode (0.5 min delay) using helium as carrier gas (0.7 ml/min). Injector and transfer line temperatures were set to 280 °C. Oven temperatures were programmed as follows: initial temperature 100 °C for 2 min, rise at 30 °C/min to 190 °C, rise at 20 °C/min to 300 °C hold for 4 min. Sample injection volume was 1 μl. The analyses were performed in the EI mode (ionization energy=70 eV) using scan acquisition (50–700 u), or SIM acquisition mode (monitoring three characteristic ions for each compound).

2.6. Validation of the procedure

The extraction recovery was calculated by analysis of four replicates of urines spiked with 50 ng/ml of the β-agonists bambuterol, clenbuterol, fenoterol, formoterol, salbutamol, salmeterol, salmeterol metabolite and terbutaline, as already reported [14]. Penbutolol was used as external standard and was added after solid-phase extraction (10 μl of a methanolic solution of 10 μg ml^{-1}). The ratio of the peak areas of the analytes and the external standard obtained from the extracted spiked samples were compared with the peak area ratios obtained when pure standards and external standard were added to a blank urine after extraction (representing the 100% of extraction recovery).

The limit of detection (LOD) was estimated with urine samples spiked with decreasing concentrations of the compounds: 10, 5, 2, 1 and 0.5 ng/ml. The samples were subjected in triplicate to the whole procedure including hydrolysis, extraction, derivatizations and GC–MS analysis. The LOD was estimated by establishing the minimum concentration at which the analytes could be detected with a signal-to-noise ratio of the diagnostic ions higher than three to one.

Repeatability was evaluated after analysis, on the same day, of three replicates of extracted spiked samples (50 ng/ml). The repeatability was measured by the relative standard deviation (RSD) between the ratio of the areas of the compounds and the ISTD in the three replicates, after each one of the derivatization steps (methyl boronate and TMS derivatization). Moreover, the intermediate precision was determined after analysis of extracted spiked samples (50 ng/ml) on three consecutive days. The precision was expressed as the relative standard deviation (RSD), calculated after analysis of each derivatization extracts (methyl boronate and TMS).

2.7. Identification criteria

Identification criteria according to IOC [15] were applied to the β-agonists bambuterol, salbutamol and terbutaline, after each derivatization step (methyl boronate and TMS derivatives formation). The evaluation was based on the relative retention time (RRT) and comparison of three diagnostic ions for each derivative. According to this criteria, the difference in RT (or RRT) of the analyte should be not higher than 1% compared with the same substance present in a positive control urine, and the relative abundances of the ions should not differ by more than 5% (absolute) or 20% (relative), whichever is greater, from that of the positive control urine sample.

2.8. Samples from excretion studies

Urine from a healthy volunteer was collected after oral administration of bambuterol hydrochloride (10 mg), according to a clinical trial approved by the local Ethical Committee (Barcelona, Spain). The urine was collected before (blank urine) and during 48 h after drug administration at the following collection periods: 0–8, 8–24, 24–32 and 32–48 h. Terbutaline was present as the main metabolite in the bambuterol positive urine. For salbutamol, the urine sample from an asthmatic volunteer was collected during an unknown period.

3. Results and discussion

3.1. Derivatization

A scheme of the consecutive derivatization proposed is presented in Fig. 2. The derivatives formed for each compound in each derivatization conditions are listed in Table 1, together with the absolute and relative retention times, the diagnostic ions, and the estimated LOD.

The formation of cyclic methylboronates (Derivatization I) was effective for bambuterol, clenbuterol, formoterol, salbutamol, salmeterol and penbutolol (ISTD). For fenoterol, α-hydroxy-salmeterol and terbutaline, although the formation of a cycle in the β-ethanolamine chain is possible, the resulting derivative does not have suitable gas chromatographic properties due to the presence of additional hydroxyl groups in the *meta* position of the phenyl ring whose spatial separation is inadequate for ring formation [17,19,20]. Only one derivative was observed for each compound derivatized (Table 1). Derivatives of bambuterol, clenbuterol and penbutolol (ISTD) resulted from the cyclization of the ethanolamine chain, while for formoterol, salbutamol and salmeterol, an additional cycle was formed with substituents on the aromatic ring. As previously described, selective mass spectra have been obtained [17].

Addition of MSTFA (Derivatization II) to the same extract produced silylation of the hydroxy, amino and other groups amenable to derivatization, and no cyclic derivatives were observed in the second GC–MS analysis. The derivatives formed for each compound are listed in Table 1. Diagnostic ions of each derivative are also listed in Table 1.

The formation of TMS derivatives after addition of MSTFA to the extract previously subjected to Derivatization I, suggested that cyclic methylboronates did not exist in solution, even after heating at 60 °C for 30 min. To study the formation of the methylboronate derivatives, some additional experiments were performed with salbutamol.

After Derivatization I, the derivatization reagent (solution of trimethylboroxine in ethyl acetate) was evaporated and the residues were reconstituted with ethyl acetate only. Under these conditions, where trimethylboroxine was not present during the injection process, the formation of salbutamol dimethylboronate was reduced to 1% compared to the results obtained when the chromatographic analysis was performed in the presence of trimethylboroxine. Additionally, results obtained with salbutamol and penbutolol showed that the incubation process (heating at 60 °C for 30 min) did not affect the formation of the cyclic derivatives as the same peak areas were obtained when the extracts were injected without previous incubation.

These results demonstrate that the formation of cyclic methylboronated derivatives occurs during the injection process. In these conditions, when MSTFA is added to the reaction mixture, TMS derivatives are formed, blocking all groups amenable to form cyclic derivatives, and only TMS derivatives are observed.

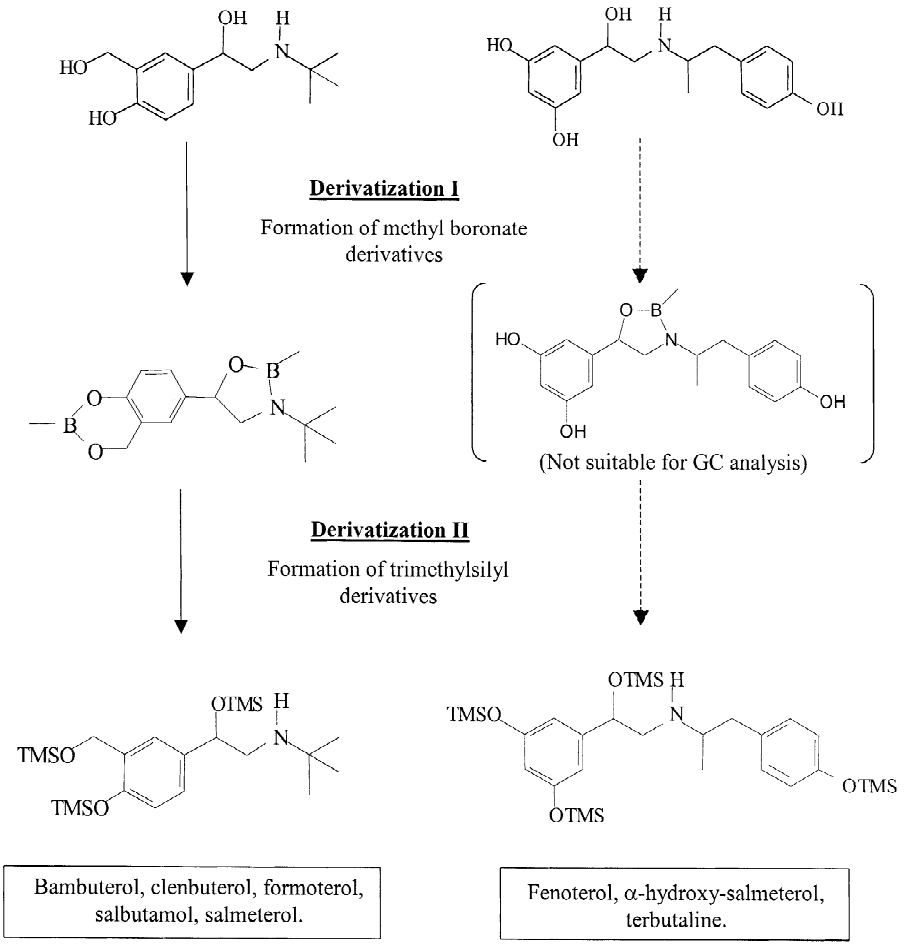

Fig. 2. Scheme of the consecutive derivatization procedure for salbutamol (left) and fenoterol (right). Compounds with the same derivatization behaviour are indicated.

3.2. Analysis of urine samples

Limits of detection (LOD) of the two derivatization steps were estimated using spiked urines are listed in Table 1. LOD ranged from 0.5 to 5 ng/ml, except for formoterol in Derivatization I. In general, better results were obtained using TMS derivatization. Taking into account the concentrations of the compounds of interest in urine after therapeutic doses [14], only the detection of formoterol and salmeterol could be difficult.

Extraction recoveries were calculated for the β-agonists bambuterol, clenbuterol, fenoterol, formoterol, salbutamol, salmeterol, salmeterol metabolite, and terbutaline, ranging from 68.1% for terbutaline to 103.7% to fenoterol, as previously reported [14].

Although the method proposed was aimed to the proper identification of β-agonists and not their quantitation, some factors as repeatability or intermediate precision were considered not of fundamental importance. However, the fact that one of the derivatization steps occurred in the injector makes it interesting to know the degree of consistence of the results in different analyses carried out the same day or in different days, the later receiving more impact from the variable instrument injector conditions. Table 2 gives the results for repeatability and

Table 1
Diagnostic data of different β-agonists as MB[a] (Derivatization I) or TMS[b] (Derivatization II) derivatives

Compound	Derivatization I						Derivatization II					
	Derivative	M[c]	*m/z* (SIM)[d]	RT[e] (min)	RRT[f]	LOD[g] (ng/ml)	Derivative	M	*m/z* (SIM)	RT (min)	RRT[h]	LOD (ng/ml)
Bambuterol	MB	391	72, 334, 376	9.56	1.32	5	O-TMS	439	72, 86, 354	8.70	1.25	2
Clenbuterol	MB	300	243, 285, 300	6.84	0.94	0.5	O-TMS	348	86, 243, 262	6.24	0.89	5
Fenoterol	No suitable derivative						tetrakis-O-TMS	591	236, 322, 412	9.34	1.34	5
Formoterol	di-MB	392	121, 229, 271	9.94	1.38	>10	bis-O-TMS	488	178, 277, 367	10.13	1.45	5
Salbutamol	di-MB	287	230, 272, 287	6.38	0.89	2	tris-O-TMS	455	86, 369, 440	6.54	0.94	2
Salmeterol	di-MB	463	202, 244, 421	11.83	1.63	5	tris-O-TMS	631	262, 369, 616	11.86	1.70	2
Salmeterol metabolite	No suitable derivative						tetrakis-O-TMS	719	260, 350, 369	12.46	1.79	2
Terbutaline	No suitable derivative						tris-O-TMS	441	86, 356, 426	6.22	0.89	5
Penbutolol (ISTD)	MB	315	84, 300, 315	7.23	1.00	–[i]	O-TMS	363	57, 86, 348,	6.98	1.00	–[i]

[a] Methylboronate.
[b] Trimethylsilyl.
[c] Molecular mass.
[d] *m/z* monitored in SIM acquisition mode.
[e] Retention time.
[f] Relative retention time to penbutolol-MB.
[g] Limit of detection.
[h] Relative retention time to penbutolol-*O*-TMS.
[i] Not calculated.

intermediate precision obtained for the β-agonists in study after each step of the derivatization process (methylboronate formation and trimethylsilylation). Repeatability values in the range of 0.5–23% were obtained in all cases. Intermediate precisions were lower than 30% for most of the compounds. In spite of the good repeatability obtained for salmeterol-diMB derivative, the intermediate precision was low

Table 2
Repeatability and intermediate precision for the β-agonists bambuterol and clenbuterol, measured after each one of the derivatization steps

Compound	Diagnostic ion	Repeatability						Intermediate precision	
		Day 1		Day 2		Day 3			
		n	(RSD, %)	*n*	(RSD, %)	*n*	(RSD, %)	*n*	(RSD, %)
Bambuterol MB	72	3	1.5	3	23.3	3	15.6	9	16.3
Bambuterol O-TMS	72	3	17.7	3	3.7	3	17.7	9	15.0
Clenbuterol MB	243	3	8.8	3	4.8	3	7.4	9	18.5
Clenbuterol O-TMS	86	3	1.3	3	2.2	3	0.5	9	8.5
Formoterol di-MB	271	3	18.0	–	–	–	–	–	–
Formoterol bis-*O*-TMS	178	3	12.5	–	–	–	–	–	–
Salbutamol di-MB	230	3	16.6	3	12.9	3	18.5	9	27.2
Salbutamol tris-*O*-TMS	369	3	4.2	3	1.7	3	7.5	9	15.6
Salmeterol di-MB	202	3	11.2	3	16.9	3	3.6	9	51.7
Salmeterol tris-*O*-TMS	262	3	5.1	3	3.1	3	13.3	9	24.6
Terbutaline tris-*O*-TMS	86	3	19.1	3	19.3	3	21.0	9	24.5
Fenoterol tetrakis-*O*-TMS	322	3	6.5	3	18.3	3	5.1	9	33.2
Salmeterol metabolite tetrakis-*O*-TMS	260	3	15.8	3	9.9	3	6.3	9	27.0

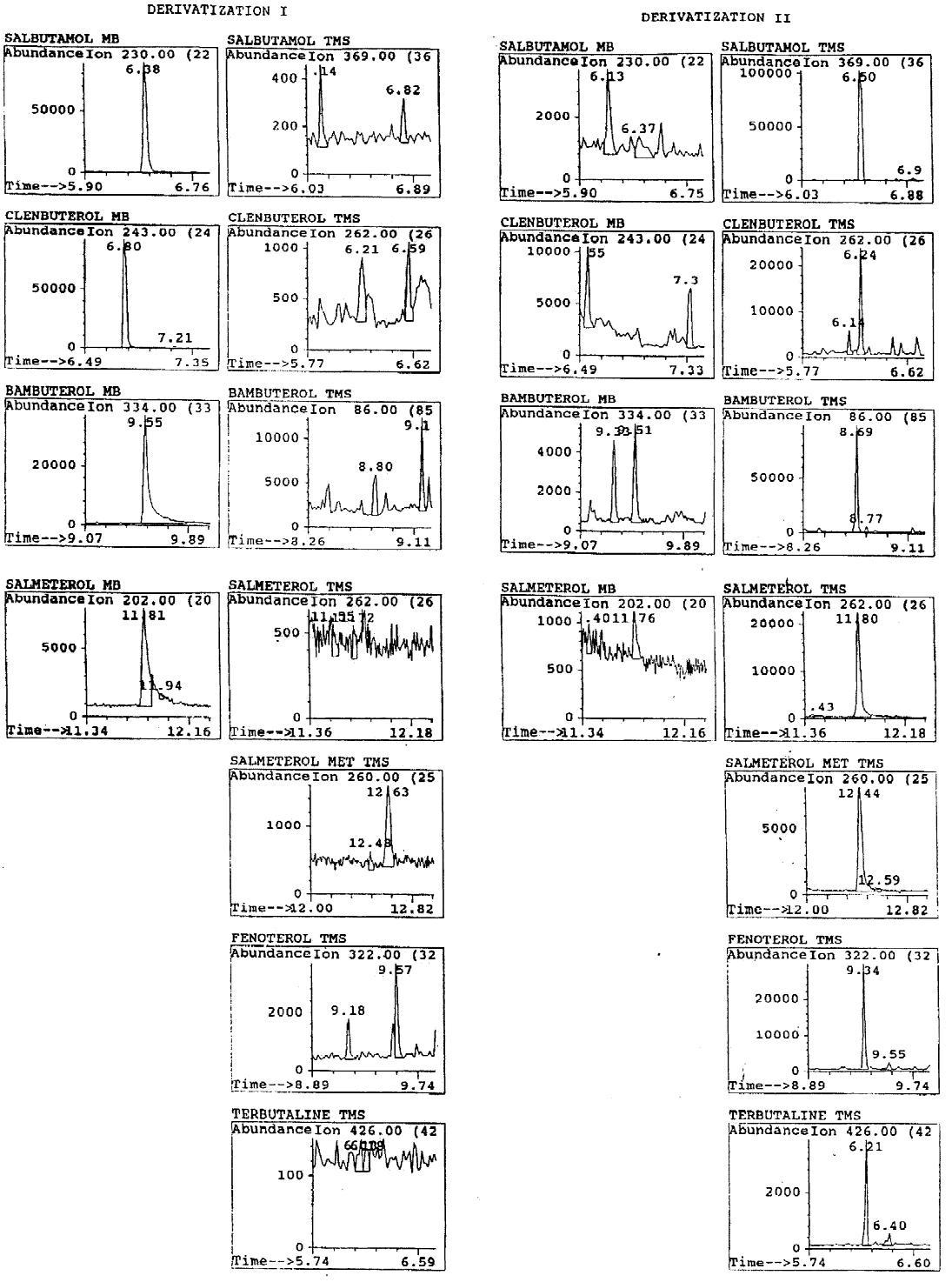

Fig. 3. Chromatograms obtained after analysis of a urine spiked with salbutamol, clenbuterol, bambuterol, salmeterol, salmeterol metabolite, fenoterol and terbutaline, by using Derivatization I (left) and adding Derivatization II (right).

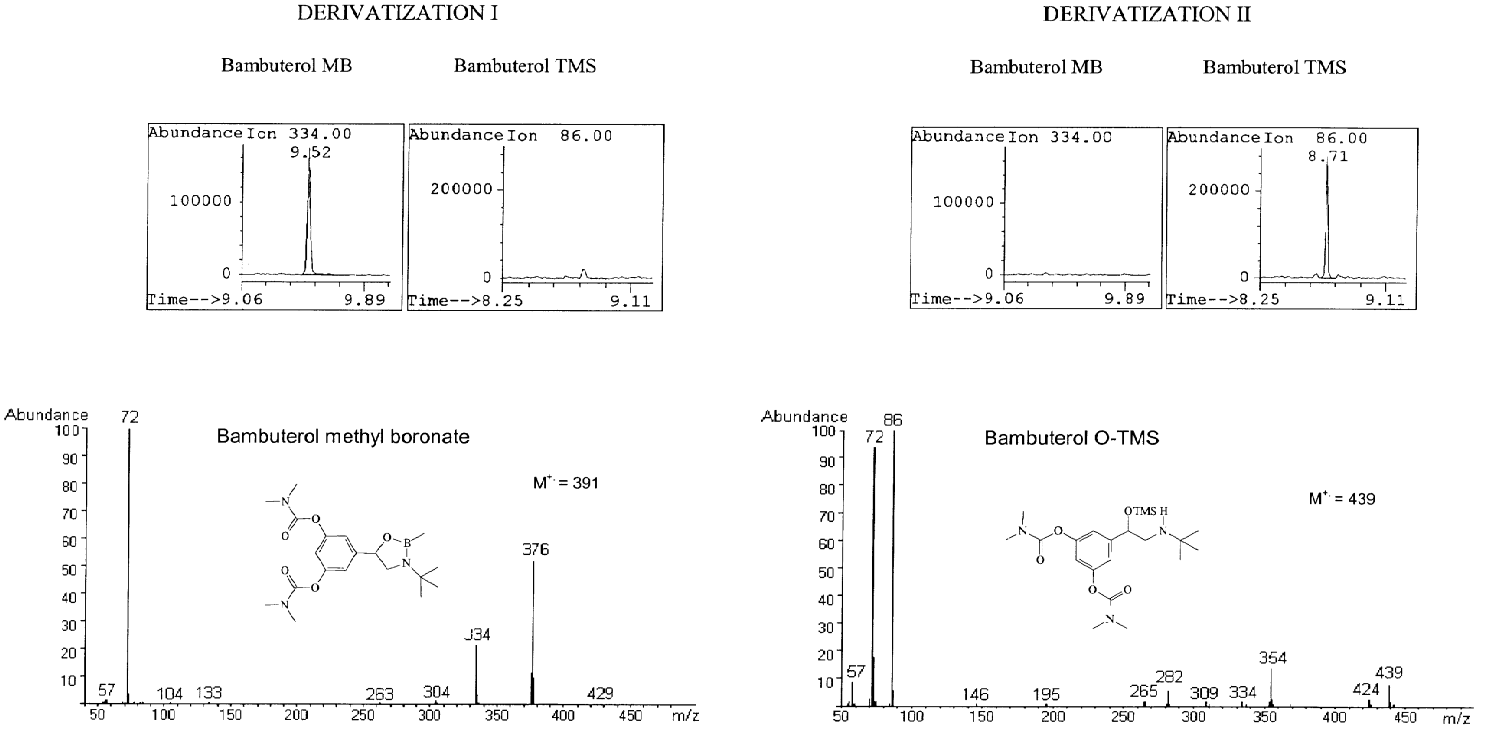

Fig. 4. Chromatograms and mass spectra obtained after analysis of a urine obtained from an excretion study of the β-agonist bambuterol (0–8 h) using the combined derivatization procedure.

(high RSD%). Higher RSD values of methylboronate derivatization compared to those of TMS formation were obtained for all of the compounds able to give both kinds of derivatives; it is understandable taking into account that methylboronate formation occurs in the injector of the chromatographic system and, the conditions of the injector are subjected to relatively high variability depending on the number and origin of the previous samples injected.

Chromatograms of spiked urines showing the disappearance of methylboronates and the appearance of TMS derivatives using the consecutive procedure are shown in Fig. 3. The combination of appearance and disappearance of chromatographic peaks, as well as changes in mass spectra and in retention times of the derivatives, affords additional evidence to confirm the presence of these β-agonists in urine samples. For compounds that cannot form cyclic derivatives, this method gives evidence for the presence of β-agonists without the need of a separate sample preparation to form the silyl derivatives.

Demonstration of the suitability of the proposed procedure for the analysis of the β-agonist bambuterol after a therapeutic dose is shown in Fig. 4. Differences in the retention times of the two derivatives as well as differences in the mass spectra of the derivatives obtained with the same extract, are shown in this figure.

The IOC recommended criteria for identification [15] were applied to real urine samples of bambuterol, salbutamol and terbutaline (as the main metabolite in the bambuterol positive urine) after each one of the derivatization procedures. Results of the comparison of RRT and relative abundances of the diagnostic ions are presented in Table 3. For the compounds evaluated, the differences in RRT relative to standards were lower than 1%, and the percent differences in the relative abundance of the diagnostic ions were less than 5% (absolute) or 20% (relative).

4. Conclusion

In summary, using this procedure, two derivatization methods can be used on the same extract, yielding complementary diagnostic data, in the case where both derivatives can be formed, affording

Table 3
Comparison between relative retention times (RRT) and relative abundances for the diagnostic ions of the β_2-agonists as their methyl boronate and TMS derivatives[a]

Compound	Diagnostic ions	Control urine			Real urine			Difference in RRT(%)	Difference in relative abundance (%)	
		Conc. (ng/ml)	RRT	Ratio (%)	Collection period	RRT	Ratio (%)		Absolute	Relative
Bambuterol MB	72	10	1.344	100	8–24 h	1.344	100	0.02	0	0
	376		1.346	45.57		1.345	47.90		2.33	4.86
	334		1.344	26.37		1.344	27.01		0.63	2.34
Bambuterol O-TMS	86	10	1.278	100	8–24 h	1.277	100	0.09	0	0
	72		1.278	80.05		1.277	80.52		0.47	0.59
	354		1.279	8.84		1.278	8.85		0.01	0.15
Salbutamol di-MB	230	10	0.876	100	0–2 h	0.876	100	0.04	0	0
	272		0.876	86.22		0.876	87.05		0.83	0.95
	287		0.876	3.97		0.876	4.09		0.12	2.92
Salbutamol tris-*O*-TMS	86	100	0.911	100	0–2 h	1.341	100	0.03	0	0
	369		0.913	70.90		1.341	70.86		0.04	0.06
	440		0.913	1.33		1.341	1.33		0	0.11
Terbutaline tris-*O*-TMS	86	100	0.860	100	8–24 h[b]	0.860	100	0.03	0	0
	356		0.862	47.58		0.862	58.90		11.32	19.21
	426		0.862	2.23		0.862	2.48		0.25	10.20

[a] Differences in relative abundance expressed as absolute and relative percent differences between the real sample and the positive control sample.

[b] Obtained as metabolite from a real urine of the β_2-agonist bambuterol.

enough information for unambiguous identification. For compounds that do not form cyclic derivatives, compound identification can be accomplished by formation of the trimethylsilyl derivative, with no need of a separate sample preparation. According to the IOC identification criteria, this procedure demonstrated to be useful for identification or confirmation of the β-agonists tested.

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References

[1] F.S. Virant, in: J.M. Weiler (Ed.), Allergic and Respiratory Disease in Sports Medicine, Marcel Dekker, New York, 1997, p. 65.
[2] L. Martineau, M.A. Horan, N.J. Rothwell, R.A. Little, Clin. Sci. 83 (1992) 615.
[3] International Olympic Committee, Prohibited classes of substances and prohibited methods, in: IOC Medical Code and Explanatory Document, IOC, Lausanne, Switzerland (2001).
[4] R. Ventura, G. González, M.T. Smeyers, R. de la Torre, J. Segura, J. Anal. Toxicol. 22 (1998) 127.
[5] M.C. Dumasia, E. Houghton, J. Chromatogr. 564 (1991) 503.
[6] L. Leyssens, C. Driessen, A. Jacobs, J. Czech, J. Raus, J. Chromatogr. 564 (1991) 515.
[7] M.-P. Montrade, B. Le Bizec, F. Monteau, B. Siliart, F. Andre, Anal. Chim. Acta 275 (1993) 253.
[8] A. Solans, M. Carnicero, R. de la Torre, J. Segura, J. Anal. Toxicol. 19 (1995) 104.
[9] G. Van Vyncht, S. Preece, P. Gaspar, G. Maghuin-Rogister, E. DePauw, J. Chromatogr. A 750 (1996) 43.
[10] F.J. Couper, O.H. Drummer, J. Chromatogr. B 685 (1996) 265.
[11] A. Polettini, M. Montagna, J. Segura, X. de la Torre, J. Mass Spectrom. 31 (1996) 47.
[12] F. Ramos, M.C. Castilho, M.I.N. Silveira, J. AOAC Int. 81 (1998) 544.
[13] F. Ramos, C. Santos, A. Silva, M.I.N. Silveira, J. Chromatogr. B 716 (1998) 366.
[14] R. Ventura, L. Damasceno, M. Farré, J. Cardoso, J. Segura, Anal. Chim. Acta 418 (2000) 79.
[15] International Olympic Committee, Analytical criteria for reporting low concentrations of anabolic steroids, Internal Communication, IOC, Lausanne, Switzerland, 1998.
[16] International Laboratory Accreditation Cooperation, Accreditation requirements and operating criteria for horseracing laboratories, ILAC-G7, 1996. http://www.ilac.org/
[17] L. Damasceno, R. Ventura, J. Ortuño, J. Segura, J. Mass Spectrom. 35 (2000) 1285.
[18] Y. Gaillard, A. Balland, F. Doucet, G. Pépin, J. Chromatogr. B 703 (1997) 85.
[19] A. Polettini, J. Chromatogr. B 687 (1996) 27.
[20] J. Segura, R. Ventura, C. Jurado, J. Chromatogr. B 713 (1998) 61.