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Quantification of terbutaline in urine by enzyme-linked immunosorbent assay and capillary electrophoresis after oral and inhaled administrations ☆

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Abstract

The International Olympic Committee and World AntiDoping Agency restricts the use of β_2-agonists and only the inhaled administration of terbutaline, salbutamol, formoterol and salmeterol is permitted for therapeutic reasons. The aim of this study was to develop a test for the quantitation of terbutaline in urine and evaluate different parameters to distinguish between oral and inhaled administration of the drug. Urine samples were collected from asthmatic and non-asthmatic recreational swimmers who had received repeated doses of oral (3×2.5 mg plus 1×5 mg during 24 h) and inhaled (12×0.5 mg in 24 h with half of it being in the last 4 h) racemic terbutaline, and single oral (5 mg) or single inhaled doses (1 mg). Total terbutaline concentrations (free+conjugated) were determined by enzyme-linked immunosorbent assay. Results showed that after oral administrations urinary terbutaline concentrations were higher than those detected after inhalation. For confirmation purposes, a chiral capillary electrophoretic procedure was established and validated. A solid-phase extraction with Bond-Elut Certify cartridges was undertaken, separation performed using a 50 m*M* phosphate buffer (pH 2.5) containing 10 m*M* of (2-hydroxypropyl)-β-cyclodextrin as running buffer and diode-array UV detection set at 204 nm. The proposed procedure is rapid, selective and sensitive allowing quantitation of free terbutaline enantiomers in urine. No statistical differences were found between total free terbutaline concentrations [*S*-(+)+*R*-(−)] in urine collected after oral and inhaled administrations of the drug. After oral doses enantiomeric [*S*-(+)]/[*R*-(−)] ratios lower than those obtained after inhalation were observed probably due to an enantioselective metabolism that take place in the intestine, but differences between both routes of administration were not statistically significant. Although different trends were observed after oral and inhaled doses in total terbutaline, total free terbutaline concentrations and in ratios between its enantiomers, differences observed were not sufficiently significant to establish cut-off values to clearly distinguish between both routes of administration.

Keywords: Terbutaline

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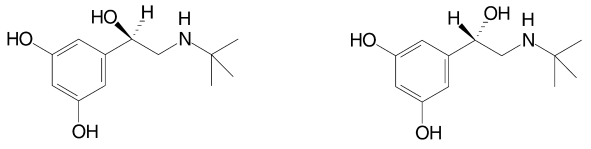

Fig. 1. Chemical structures of terbutaline enantiomers.

1. Introduction

Terbutaline (Fig. 1) is a selective β_2-adrenoreceptor agonist widely used in the treatment of asthma and exercise induced asthma [1]. The use of terbutaline and other β_2-agonists has been restricted by the International Olympic Committee (IOC) and World AntiDoping Agency (WADA) due to the stimulatory effect on the central nervous system and certain anabolic effects observed after oral administration in high doses [2,3]. The oral administration of β_2-agonists is prohibited but inhalation of terbutaline, salbutamol, formoterol and salmeterol is allowed for therapeutic reasons. Their use must be notified to the relevant medical authority before the competition [4]. The establishment of criteria to distinguish between the authorised use (inhaled) and the prohibited use (oral) of terbutaline is pending of the development of a quantitative test in urine.

Terbutaline has an asymmetric carbon atom and is administered as a mixture of the two enantiomers: *S*-(+)- and *R*-(−)-terbutaline (Fig. 1). The primary route of metabolism of terbutaline in man is conjugation with sulfate, appearing in urine as the unchanged drug and as the conjugated metabolite [5–7]. The proportion of conjugated metabolite heavily depends on the route of administration. After intravenous administration, terbutaline is largely excreted unchanged in urine whereas the oral drug is extensively conjugated before reaching the systemic circulation. Conjugation of terbutaline takes place in the intestine wall and in the liver but terbutaline sulfotransferases demonstrate higher activity in gut wall than in liver [7]. The oral bioavailability of *R*-(−)-terbutaline has been shown to be higher than that of *S*-(+)-terbutaline. This difference has been proposed to be due to enantioselectivity in absorption as well as in first-pass metabolism [8]. The rate of conjugation with sulfate has also been shown to be stereoselective [9].

For salbutamol, the different rate of sulfate conjugation depends on the route of administration, and the different rate of sulfation between *S*-(+)- and *R*-(−)-enantiomers has been the basis of the criterion recently proposed to distinguish between oral and inhaled administration of this substance [10,11]. A discriminant function based on the determination of total free salbutamol, *S*-(+)+*R*-(−), and the ratio between enantiomers, *S*-(+)/*R*-(−), has been proposed as a confirmation criterion. The overall recommended procedure was to apply this confirmation criteria to samples showing either total salbutamol concentrations higher than 1400 ng/ml or free salbutamol concentrations higher than 500 ng/ml measured in the conventional screening procedures by enzyme-linked immunosorbent assay (ELISA) or gas chromatography–mass spectrometry (GC–MS), respectively.

The aim of this study was to evaluate different parameters to distinguish between oral and inhaled administration of terbutaline. These parameters could be concentrations of total terbutaline (free+conjugated) obtained by ELISA, concentrations of *S*-(+)- and *R*-(−)-enantiomers obtained by capillary electrophoresis (CE) and the ratio between them. The method developed has been applied to the analysis of urine samples obtained from recreational swimmers after oral and inhaled administration of terbutaline racemate.

2. Experimental

2.1. Chemicals and reagents

Labsystems (Sant Just Desvern, Barcelona, Spain) supplied ELISA test Generic Broncodilators (ELISA Technologies, Neogen, Lexington, KY, USA).

Methanol, 2-propanol (all HPLC grade), 25% ammonia (analytical grade), phosphoric acid 85% (analytical grade) and sodium hydroxide pellets (analytical grade) were purchased from Merck (Darmstadt, Germany). Chloroform and glacial acetic acid (both HPLC grade) were supplied by Scharlau (Barcelona, Spain). The cyclodextrin (2-hydroxypropyl)-β-cyclodextrin (OHP-β-CD) was

purchased from Hewlett-Packard (Palo Alto, CA, USA). Deionised water was obtained by a Milli-Q system (Millipore Ibérica, Barcelona, Spain).

Acetate buffer was prepared adjusting the pH of a 1.1 mol/l sodium acetate solution to 5.2 with glacial acetic acid. Phosphate buffer at pH 2.5 was prepared by adding a 1 *M* sodium hydroxide solution to a 50 m*M* phosphoric acid solution until pH 2.5. The 10 m*M* 2-OHP-β-CD solution was prepared by dissolving 138.2 mg of OHP-β-CD in 10 ml of phosphate buffer at pH 2.5.

R-(−)-Terbutaline and *S*-(+)-terbutaline (hydrobromide form) were donated by Astra Draco (Pharmaceutical & Analytical R&D, Lund, Sweden) and codeine was supplied by Radian (100 μg/ml in methanol, in free base form). Stock standard solutions (1 mg/ml, in free base form) of *R*-(−)-terbutaline and *S*-(+)-terbutaline were prepared by dissolving 13.5 mg of the hydrobromide form in 10 ml of methanol. Working solutions of 100 and 10 μg/ml were prepared by 1:10 and 1:100 dilutions of the 1 mg/ml stock solution with methanol. Working solutions of 10 μg/ml codeine were prepared by dilution of the 100 μg/ml solution with methanol. All solutions were stored at −20 °C.

Bond Elut Certify (130 mg/10 ml) columns were provided by Varian (Harbor City, CA, USA). The extraction was performed on a Vac-Elut Vacuum manifold (Supelco, Bellefonte, PA, USA). Organic phases were evaporated to dryness under nitrogen stream with a Turbo-Vap LV evaporator from Zymark (Hopkinton, MA, USA).

2.2. ELISA analyses

Samples were analysed by ELISA using the Generic Bronchodilators test under the conditions described in a previous study [12]. A calibration curve of racemic terbutaline was analysed in duplicate with each batch of samples. The following calibration levels were used in duplicate: 0, 0.1, 0.3, 1, 3, 10, 30 and 100 ng/ml. Calibration curves of terbutaline were calculated using a sigmoidal equation (GRAFIT program, R.J. Leatherbarrow). A blank urine and a positive control of 10 ng/ml of terbutaline were analysed in each strip of wells with the problem samples. Samples were diluted 1:10 and 1:100 with dilution buffer to obtain a response in the range of the calibration curve.

2.3. Urine analyses of terbutaline enantiomers

Bond Elut Certify cartridges were conditioned with 2 ml of methanol and 2 ml of deionised water. Codeine was used as internal standard (50 μl of a 10 μg/ml solution). Urine samples (4 ml) were adjusted to pH 5.2 with 1 ml of 1.1 *M* sodium acetate buffer and were centrifuged at 2300 *g* for 5 min before being applied to the preconditioned cartridges. The columns were washed with 2 ml of deionised water, 1 ml of 1 *M* acetic acid, 2 ml of methanol and dried for 5 min under full vacuum. Two consecutive elutions (2 ml each) were carried out with a mixture of chloroform–2-propanol (80:20, v/v) containing 4% ammonia. The combined eluates were evaporated to dryness under a stream of nitrogen in a 40 °C water bath, reconstituted and analysed.

A CE system (^{3D}CE, Hewlett-Packard) equipped with a diode-array UV detector was used. Separation was performed in an untreated fused-silica capillary of 48.5 cm [40 cm effective length (from the injector port to detector window)]×50 μm I.D., and a standard 50 μm optical path length cell (Hewlett-Packard). The working voltage was set at 30 kV and the cartridge temperature was maintained at 15 °C. The working wavelength was set at 204 nm. A 50 m*M* phosphate buffer (pH 2.5) containing 10 m*M* of 2-OHP-β-CD was used as running buffer for chiral separation. All solutions were passed through a membrane of reduced cellulose and a housing of polypropylene filter of 0.45 μm pore size (Hewlett-Packard) and degassed by sonication prior to use. At the beginning of each experiment (daily), the capillary was conditioned with a 50 m*M* phosphoric acid solution for 5 min at 6 bar, water for 2 min at 6 bar and running buffer for 20 min at 6 bar. Before each injection, the capillary was rinsed with a 50 m*M* phosphoric acid for 2 min and with the running buffer for 1 min. Running buffers were prepared freshly each experimental day. For CE analyses, the dried urine extracts were reconstituted with 50 μl of 50 m*M* phosphate buffer (pH 2.5), vortex mixed for 5 min and centrifuged at 2300 *g* for 5 min. The injection was done by applying external pressure of 50 mbar for 2 s.

Calibration curves for both enantiomers were prepared daily. Quantification was performed by using ratios between the corrected peak areas of terbutaline enantiomers and that of the internal standard. Corrected areas (peak area divided by its migration time) were used to correct variations produced when molecules migrate with different velocities giving different detector response and different peak areas [13,14].

2.4. Validation of quantitative CE

The following parameters were evaluated in the method validation: selectivity, linearity, intra-assay precision and accuracy, inter-assay precision and accuracy, recovery, limits of detection and limits of quantitation.

The selectivity of the method was verified by analysing different blank urines ($n=5$) and checking for the presence of interfering substances at the migration time of the compounds of interest, terbutaline enantiomers and codeine.

For the study of linearity a calibration graph for both enantiomers of terbutaline was prepared covering the whole expected concentration range. Spiked urine samples with concentrations of 40, 100, 200, 400 and 600 ng/ml of each terbutaline enantiomer were prepared daily by adding appropriate volumes of the stock solutions to 4-ml aliquots of blank urine. These samples were prepared and analysed in duplicate. Corrected peak area ratios between each enantiomer and the internal standard were subjected to a proportional weighted least-squares regression analysis.

Extraction recoveries of *S*-(+)-terbutaline and *R*-(−)-terbutaline from urine were calculated by comparison of the corrected peak areas obtained after analysis of spiked urines with the corrected peak areas obtained when the standards were added to a blank urine after extraction (representing 100% of recovery). Three concentration levels were studied for each enantiomer: 40, 200 and 600 ng/ml ($n=4$).

The standard deviation (SD) of the estimated concentration values of the lowest calibration point (40 ng/ml) was used as a measure of the noise. The limits of detection and quantitation were defined as 3 and 10 times the value of noise, respectively.

Precision is expressed as the relative standard deviation (RSD) of the concentration values obtained for low, medium and high control samples after repeated analysis. Accuracy is the difference between the estimated and the real concentrations and it is expressed as a relative error. The intra- and inter-assay accuracies are expressed as the mean of the absolute values of the relative errors of the estimated concentration for the control samples. Control samples with 50, 250 and 500 ng/ml of each enantiomer were used to evaluate precision and accuracy. Three replicates of each concentration were analysed the same day for intra-assay experiments ($n=3$), and 3 different days for inter-assay values ($n=9$).

2.5. Samples from volunteers: study design

The study was designed to examine urinary levels of terbutaline after oral and inhaled administrations after different doses. The study protocol was approved by the Committee for Human Rights at the University of Western Australia (Perth, Australia).

Four asthmatics (three males and one female) and 17 non-asthmatics (10 males and seven females) recreational swimmers were included in this study. All subjects were at least 18 years old. The first day of the study, each subject signed a written consent form and was asked to provide a baseline urine sample.

Terbutaline was administered as a racemic mixture, orally as Bricanyl tablets (Astra Pharmaceuticals, manufactured in Sweden) or by inhalation Bricanyl Turbuhaler.

All subjects received three treatments: treatment A (repeated oral doses), treatment B (repeated inhaled doses) and treatments C (single oral dose) or D (single inhaled dose). The order of treatments A and B was assigned by random order. Subjects were asked to self-administer terbutaline medications according to described protocols. A blank period of 72 h was kept between treatments.

Treatment A consisted of the administration of terbutaline orally at the following doses: one half-tablet (2.5 mg) three times on day 1 and one tablet (5 mg) twice on day 2. A urine sample was collected 3 h after the last tablet.

In treatment B, terbutaline was administered by inhalation: 12 inhalations (0.5 mg) with a Turbuhaler

over a 24 h period including six inhalations in the last 4 h. A urine sample was collected 1 h after the last inhalation.

Treatment C consisted of administration of one tablet (5 mg) 3 h prior to providing a urine sample; and treatment D consisted of the administration of two puffs (0.5 mg each) 1 h prior to providing a urine sample. Treatments C and D were assigned by random order between swimmers.

3. Results and discussion

Regarding sample collection, the conditions of routine doping control were simulated as closely as possible. Estimation of total terbutaline excreted in urine (free+conjugated) was measured by ELISA based on specificity of the antibodies of the test applied [12]. Concentrations of free terbutaline enantiomers were measured by capillary electrophoretic techniques.

3.1. ELISA results

Prior to the application of the ELISA test, urine samples were diluted with dilution buffer to obtain a response in the range of the calibration curve. No signal was observed in urine samples collected prior to the administration of terbutaline (baseline samples). Distribution of the concentrations of terbutaline obtained after oral and inhaled administration of the compounds are shown in Fig. 2.

Oral doses administered in treatment A (repeated doses) were higher than those administered in treatment C (single dose). However, as can be observed in Fig. 2, the same range of concentrations of total terbutaline were obtained after both treatments. No statistical differences were detected in terbutaline urinary concentrations after repeated and single oral doses. Regarding inhaled administrations, doses in treatment B (repeated doses) were higher than those in treatment D (single dose) and concentrations of terbutaline measured in urine 1 h after both treatments were also higher in treatment B. Statistical differences were obtained between these two population values.

Concentrations after oral administrations (treatments A and C) ranged from 150 to 900 ng/ml and

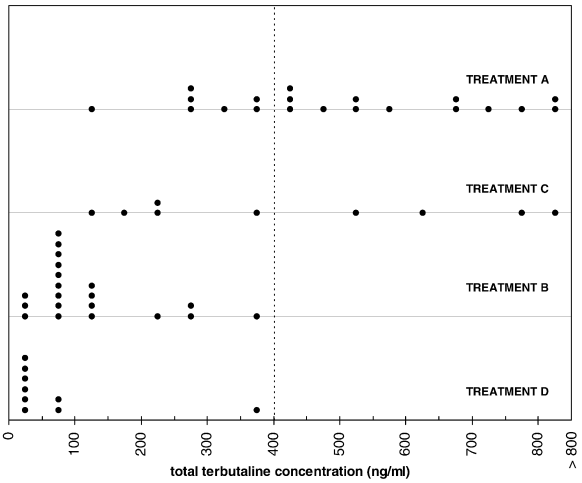

Fig. 2. Distribution of total terbutaline concentrations obtained by ELISA in urine samples collected after the different treatments. Dotted line: presumptive cut-off at 400 ng/ml (see text).

were statistically higher than those detected after inhalation (treatments B and D) which ranged from 20 to 400 ng/ml. There was an overlapping between the distributions of concentrations after oral and inhaled doses but the use of a cut-off concentration value to select suspicious samples of oral intake appeared possible.

A presumptive cut-off could be established at 400 ng/ml (see Fig. 2). No urine samples with total terbutaline concentration higher than this value were observed after inhalation and thus a specificity of 100% (0% of false positive results) is achieved. However, the selection of that cut-off value results in a sensitivity of only a 59% (41% of false negative results). The discriminatory capacity of total terbutaline concentrations determined by ELISA techniques to fully differentiate between oral and inhaled ingestion of the drug is limited since a high percentage of false negatives is obtained.

3.2. CE analyses

A previously described extraction procedure for salbutamol enantiomers [15] was modified for extracting terbutaline enantiomers from urine. The sensitivity was improved by increasing the amount of urine sample extracted to 4 ml. Solid-phase extraction using Bond-Elut Certify cartridges, contain-

ing a mixed phase with both hydrophobic and ion-exchange properties, provided successful results. Two dissociation constant values have been described for terbutaline [1]: 8.7, that corresponds to the deprotonation of the secondary amine group, and 10.1, that probably corresponds to the ionisation equilibrium of one of the phenolic groups (Fig. 1). Therefore, working at an acidic pH allows hydrophobic interactions and cationic exchange simultaneously between the sorbent and the analyte. The buffered urine sample (pH 5.2) is loaded on the preconditioned column and after washing with water, an acetic acid solution and methanol, two consecutive elutions (2 ml each) are carried out with a mixture of chloroform–2-propanol (80:20, v/v) containing ammonia. An increase from 2 to 4% of the percentage of ammonia in the final eluent improves the recovery of the more polar β_2-agonists [16], such as terbutaline.

A good enantiomeric separation has been described for some β-agonists at pH 2.5 using different cyclodextrins [17], in similar conditions to that initially described for another β-adrenergic drug (3,4-methylenedioxymethamphetamine enantiomers, MDMA) [18]. CE enantioselective separation of terbutaline in urine samples was accomplished using an untreated fused-silica capillary with an acidic running buffer and a cyclodextrin as chiral selector. The running buffer consisted of 50 m*M* phosphate buffer (pH 2.5) with 10 m*M* 2-OHP-β-CD, and the separation of terbutaline enantiomers was obtained within about 10 min. The dried urine extracts were reconstituted in phosphate buffer, pH 2.5. Electropherograms of extracts of blank urine are free from interferences allowing determination and quantitation of terbutaline enantiomers. The procedure provides the sensitivity required and offers great reliability for the assay of large number of urine samples.

The elution order of terbutaline enantiomers was determined by electrophoretic analysis of the individual enantiomers. Codeine was chosen as internal standard because of its electrophoretic behaviour, eluting at a migration time close to those of terbutaline enantiomers and presenting good response in the ultraviolet detector at the selected wavelength. A great variability from run to run in the migration times of terbutaline enantiomers and internal standard was observed producing variation in peak areas. The effect of changing analyte mobility can be compensated by the use of corrected peak area (peak area divided by the migration time) [13,14]. Furthermore, this experimental variability make the identification of the analytes difficult when analysing large number of sample. This problem was avoided by analysing a standards mixture every five samples.

Only free terbutaline enantiomers are determined with the procedure proposed. Urine samples are buffered at a pH 5.2 before the extraction. This is a usual pH for urine samples, and the stability of terbutaline sulfate enantiomers should be the same as in urine. Moreover, in the case that the sulfates could be extracted with procedure applied, their migration times would be different from those of free terbutaline enantiomers not interfering in their determination.

3.3. CE quantitative validation

After the analysis of different blank urines, no interferences were detected at the retention times of terbutaline enantiomers and codeine, used as internal standard. In Fig. 3, the electropherogram obtained after analysis of a blank urine was compared with those obtained after analysis of a spiked urine (250 ng/ml of each enantiomer) and a sample obtained from a volunteer after high inhaled doses of terbutaline.

Extraction recoveries for *R*-(−)- and *S*-(+)-terbutaline were 45.1% and 49.1%, respectively. The detection and quantitation limits were estimated at 7.3 and 22.2 ng/ml for *R*-(−)-terbutaline, and 4.9 and 15.0 ng/ml for *S*-(+)-terbutaline, respectively.

The method was found to be linear ($r>0.98$ in all cases) over the range of 40 to 600 ng/ml for both *R*-(−)- and *S*-(+)-terbutaline. Typical calibration curves for *R*-(−) and *S*-(+)-terbutaline were: $y=0.0051x+0.0264$, and $y=0.0048x+0.0174$, respectively, where y is the area ratio between the enantiomer and the internal standard and x is the concentration (ng/ml).

Results obtained for intra-assay and inter-assay accuracy and precision studies are described in Tables 1 and 2. The intra-assay precision and accuracy ranged from 4.7 to 7.8% and 3.2 to 5.6%, respectively, for both enantiomers. Higher RSDs

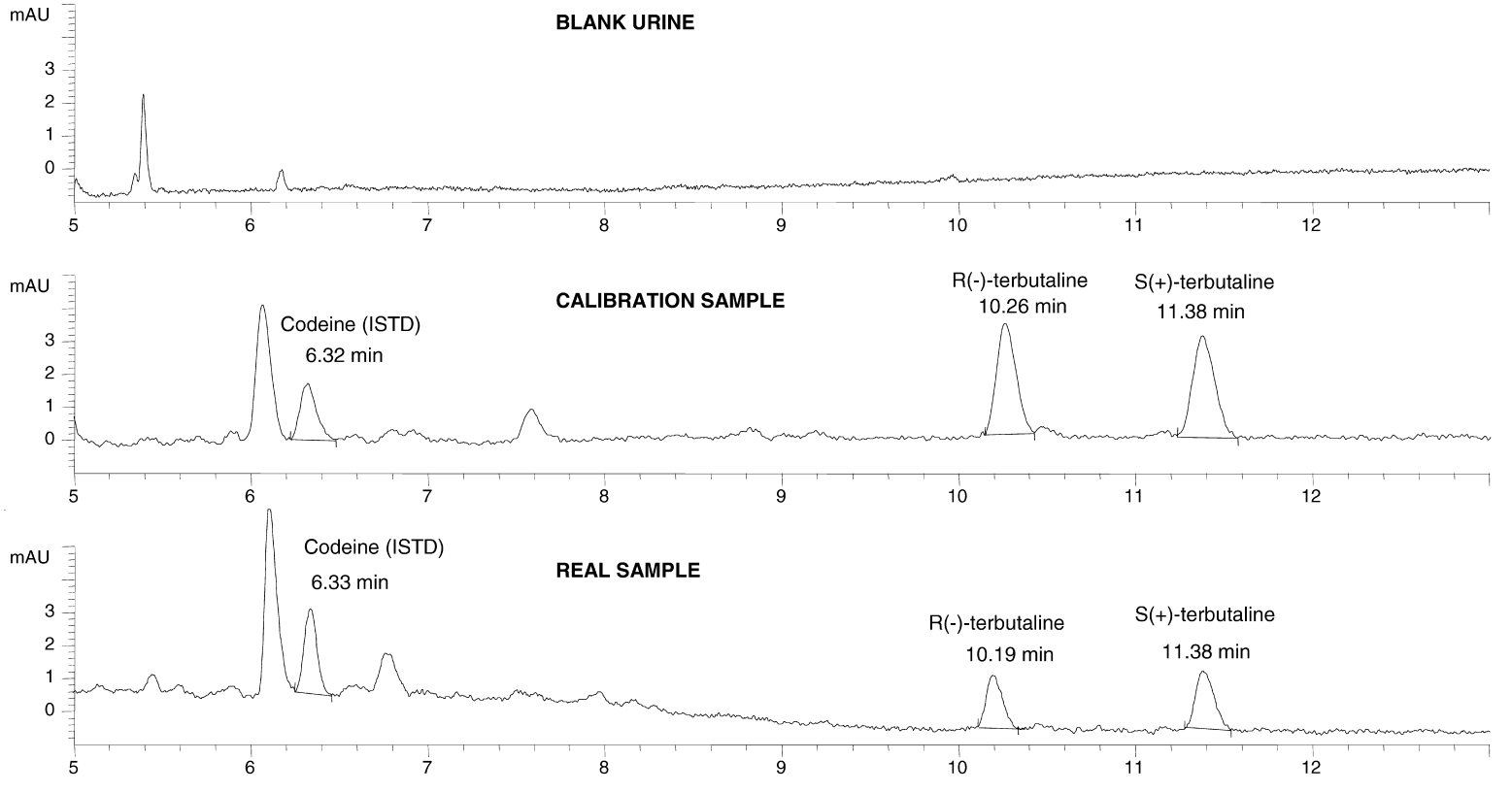

Fig. 3. Electropherograms obtained after analysis of a blank urine (top), a calibration sample containing 250 ng/ml of each terbutaline enantiomer (middle), and a real sample containing 120 ng/ml *R*-(−)-terbutaline and 145 ng/ml *S*-(+)-terbutaline obtained after repeated inhaled administration of terbutaline racemate (bottom).

Table 1
Intra-assay precision and accuracy obtained for quantification of *R*-(−)-terbutaline and *S*-(+)-terbutaline added to urine

Compound	Concentration (ng/ml)	Number of observations	Estimated concentration (mean±SD) (ng/ml)	Precision (RSD, %)	Accuracy (relative error, %)
R-(−)-Terbutaline	50	3	49.8±3.7	7.4	5.6
	250	3	243.7±19.1	7.8	5.0
	500	3	488.1±23.2	4.7	3.2
S-(+)-Terbutaline	50	3	48.5±2.3	4.7	3.8
	250	3	255.4±17.7	6.9	4.7
	500	3	482.2±26.8	5.6	5.1

Table 2
Inter-assay precision and accuracy obtained for quantification of *R*-(−)-terbutaline and *S*-(+)-terbutaline added to urine

Compound	Concentration (ng/ml)	Number of observations	Estimated concentration (mean±SD) (ng/ml)	Precision (RSD, %)	Accuracy (relative error, %)
R-(−)-Terbutaline	50	9	52.0±9.1	17.5	14.5
	250	9	255.5±31.1	12.2	8.4
	500	9	482.6±41.1	8.5	5.9
S-(+)-Terbutaline	50	9	51.0±6.6	12.9	9.7
	250	9	256.7±24.9	9.7	7.2
	500	9	488.5±34.5	7.1	6.1

were obtained in inter-assay experiments (range 7.1–17.5%), while inter-assay accuracy ranged from 5.9 to 14.5% for both enantiomers.

3.4. Study results: quantitation by CE

All samples obtained from the volunteers study were analysed using the enantioselective CE method developed. Concentrations of *S*-(+)- and *R*-(−)-terbutaline excreted in urine after oral and inhaled doses of the racemic drug were determined.

No peaks were detected at the migration times of terbutaline enantiomers after analysing urine samples collected before the administration of terbutaline (baseline samples) either in asthmatic or in non-asthmatic volunteers. The distribution of the concentrations of total free terbutaline (addition of both enantiomers) obtained in urine from all subjects participating in the study is presented in Fig. 4. The results indicate that, in general, terbutaline concen-

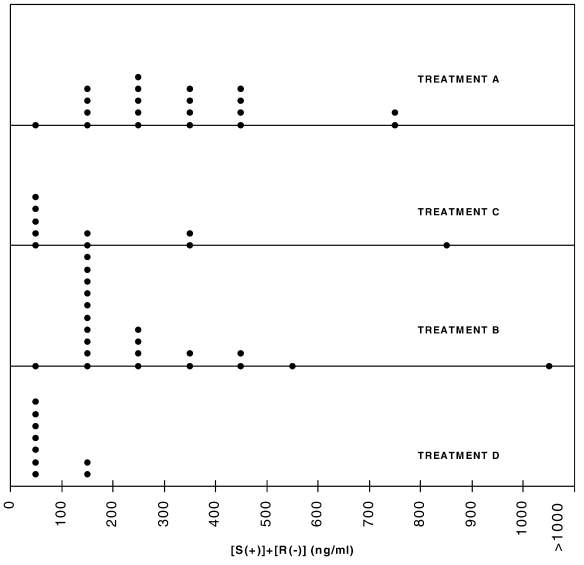

Fig. 4. Distribution of the concentrations of total free terbutaline [*R*-(−)-terbutaline plus *S*-(+)-terbutaline] determined by CE in urine samples collected after the different treatments.

trations up to 200 ng/ml were detected in urine after the administration of single inhaled doses (treatment D). After repeated inhaled doses (treatment B) concentrations of free terbutaline lower than 600 ng/ml were generally obtained. After the administration of single and repeated oral doses (treatments C and A, respectively) concentrations up to 900 ng/ml were measured.

Statistical differences were not found between concentrations obtained 3 h after repeated oral doses (treatment A) and 1 h after single oral doses (treatment C), or between concentrations obtained 1 h after repeated (treatment B) and single (treatment D) inhaled doses. There were great differences between terbutaline doses administered orally in treatment A (17.5 mg) and by inhalation in treatment D (1 mg) and, therefore, differences of terbutaline concentrations in urine samples obtained after those treatments were expected. However, statistical differences were not found between distribution of concentrations after oral and inhaled doses, probably due to the high degree of sulfate conjugation after oral administration. This results in low concentrations of free terbutaline after oral doses in spite of the high doses administered [1,5,7].

Terbutaline is metabolised by sulfate conjugation in the human intestine and liver. As indicated, the ratio of conjugated to non-conjugated drug depends on the route of administration and it increases after oral ingestion because of significant presystemic conjugation. The oral bioavailability of the *R*-(−) enantiomer was statistically higher than that of *S*-(+)-terbutaline [9], reflecting differences in absorption and also in the conjugation with sulfate. *S*-(+)-terbutaline is sulfated in a higher rate than *R*-(−)-terbutaline [10]. Therefore, after oral intake free *R*-(−)-enantiomer should be excreted in a greater proportion than free *S*-(+)-terbutaline, and comparing with inhaled administration, lower ratios of *S*-(+)/*R*-(−) should be obtained. Distributions of the ratios *S*-(+)/*R*-(−) obtained in urine collected from all subjects participating in the study after different doses and administration routes are presented in Fig. 5. In accordance with the enantioselective metabolism, after repeated oral doses of racemic terbutaline (treatments A) *S*-(+)/*R*-(−) ratios slightly lower than those obtained after inhaled administration (treatments B and D) have been determined. However, the differences between both administration routes were not statistically significant and a cut-off ratio has been difficult to establish.

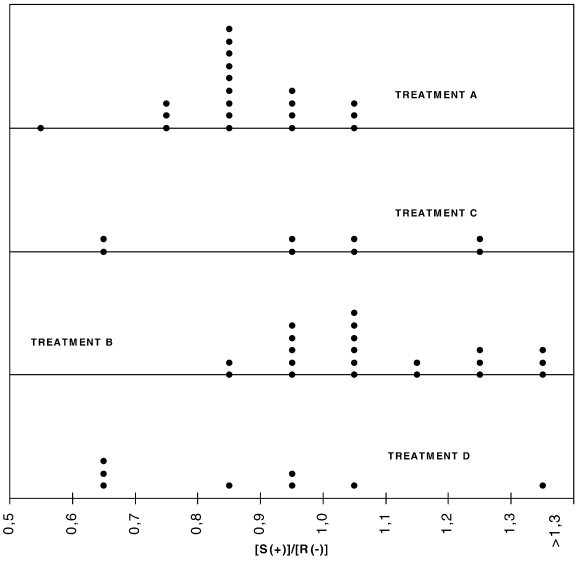

Fig. 5. Distribution of the *S*-(+) to *R*-(−) enantiomeric ratio determined by CE in urine samples collected after the different treatments.

4. Conclusions

Different parameters to distinguish between oral and inhaled administration of terbutaline have been evaluated. Total terbutaline has been measured using ELISA test. A chiral analytical methodology to quantify free terbutaline enantiomers excreted in urine has been developed. This procedure can be used to perform enantioselective pharmacokinetic studies of terbutaline after different routes of administration that is important in the study of the pharmacological activity of racemic mixtures.

Although some different trends have been observed between oral and inhaled administrations in total terbutaline in urine and in *R*-(+)+*S*-(−) and *S*-(+)/*R*-(−) ratios, reflecting the differences in metabolism depending on the administration route and on the enantiomer, the differences observed were not sufficiently significant to establish cut-off values to clear distinguish between oral and inhaled routes of administration. The possibility of using the ratios

of parent to metabolite enantiomers for detection of the route of administration should be a topic of discussion. Thus further studies are pending on the synthesis of the conjugated sulfated standards.

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