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QUANTITATIVE ANALYSIS OF TERBUTALINE IN SERUM AND URINE AT THERAPEUTIC LEVELS USING GAS CHROMATOGRAPHY—MASS SPECTROMETRY

J.G. LEFERINK*, I. WAGEMAKER-ENGELS and R.A.A. MAES

Centre for Human Toxicology, State University, Vondellaan 14, Utrecht 2503 (The Netherlands)

and

H. LAMONT, R. PAUWELS and M. VAN DER STRAETEN

Academic Hospital, Department of Pneumology, De Pintelaan 135, 9000 Gent (Belgium)

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SUMMARY

A simple and sensitive method for the determination of terbutaline in serum and urine has been developed. A mass spectrometer in the multiple ion detection mode was used as a gas chromatographic detector. Levels were monitored after oral and subcutaneous administration of the drug. The sensitivity is 1 ng/ml using 1 ml of serum.

INTRODUCTION

Terbutaline [1-(3′,5′-dihydroxyphenyl)-2-(*tert.*-butylamino)ethanol] is a β_2-receptor stimulator and is widely used in the treatment of asthmatics to relieve bronchoconstriction [1]. Terbutaline is inactivated in humans mainly by conjugation to a sulphate and, to a minor extent, to a glucuronide. The presence of the conjugates was confirmed in tritium labelling studies by Nilsson et al. [2]. Owing to its strong hydrophilic character and its chemical instability at ele-

* To whom correspondence should be addressed.

vated pH, the drug requires special extraction procedures. Clean-up procedures for tritiated terbutaline with subsequent liquid scintillation counting have been performed [2]. Extraction for subsequent chromatographic analysis of this and similar drugs, as proposed by Modin and Johansson [3] and Evans et al. [4], could not be used owing to the low therapeutic levels present in serum.

Recently we developed a semiquantitative method for terbutaline using ion-pair extraction [5]. In this paper we present a fully quantitative method, which has been checked on a large number of biological samples and proved to be reliable and accurate.

EXPERIMENTAL

Materials

Pure terbutaline sulphate and deuterium-labelled d_6-terbutaline were obtained from Astra Chemicals and Pharmaceuticals (Lund, Sweden). Ethyl acetate was of analytical-reagent grade and used without further purification. Bis(2-ethylhexyl) hydrogen phosphate (DEHP) was a synthetic-grade reagent from Merck (Darmstadt, G.F.R.). The silylating reagent BSTFA was a Pierce (Rockford, Ill., U.S.A.) product. Gas chromatography (GC) was performed at 165° on a 120 × 0.3 cm I.D. glass column packed with 3% OV-1 on 80—100-mesh Gas-Chrom Q. The carrier gas was either helium or 99.95% pure methane (Matheson, Oevel, Belgium), both flow-regulated at 24 ml/min with a Brooks digital flow controller.

Mass spectrometry (MS) was performed on a Finnigan 1015 D electron impact (EI) or 3200 F chemical ionization (CI) instrument, both connected to a Finnigan 6000 computer system. In mass fragmentography at high sensitivity levels, a four-channel peak selector was used.

The temperature of the CI ion source was digitally controlled at 85 ± 1° by a CRL temperature controller/meter (Control and Readout Ltd., Worthing, Great Britain). The temperature stated was measured at the elution time of terbutaline. The mass spectrometer was tuned for optimal sensitivity at the selected ions.

Extraction

To 1 ml of a serum sample at a pH of 7.2—7.5, 20 ng of an aqueous solution of d_6-terbutaline were added. After an equilibration period of 15 min, 8 ml of a 0.015% (w/v) solution of DEHP in ethyl acetate were added. After thorough mixing on a Vortex mixer for 5 min, and subsequent centrifugation at 1500 *g* for 5 min, the organic phase was transferred into a Reacti-vial (Pierce) and evaporated to dryness under a stream of nitrogen at 55°. BSTFA (20 μl) was added and silylation was completed after 15 min at 80°. A 1-μl sample was injected into the GC—MS system.

The procedure for the determination of the free drug in urine is slightly different owing to the high levels of terbutaline involved. With 1 ml of a phosphate buffer, 0.1 ml of the urine was adjusted to a pH of 7.3, then 100 ng of the internal standard were added. The extraction was then continued as described for the serum.

RESULTS AND DISCUSSION

GC—MS of terbutaline when amounts of more than 100 ng are injected can easily be performed by EI mass spectrometry even in the presence of impurities from biological samples. The EI spectrum shows an intensive fragmentation. No molecular ion is observed.

The only intense ions are at *m/e* 86 and 356. In serum samples spiked with concentrations of 20 ng/ml or less, these ions proved to be inadequate for mass fragmentography. The ion at *m/e* 356 was always present as a background ion with varying intensity from numerous types of columns, and the ion at *m/e* 86 was present as a fragment ion from biological contaminants. CI mass spectrometry with methane as reactant gas yielded a more useful spectrum (Fig. 1). Although the ion at *m/e* 86 is the most abundant, the quasi-molecular ion $(M + H)^+$ at *m/e* 442 and the fragment ion $(M - CH_3)^+$ at *m/e* 426 are intense and proved to be very useful for quantitation at low levels in serum or urine samples. An even more intense quasi-molecular ion is obtained when isobutane is used as the reactant gas. However, the overall sensitivity for the GC—MS method is higher when methane is used as both a carrier and reactant gas. In this instance no separator is needed and the entire sample enters the mass spectrometer.

The use of the $(M + H)^+$ and the $(M - CH_3)^+$ ions in mass fragmentography placed no restrictions on the site of the deuterium label in the internal standard. The preparation of a standard with six deuterium atoms, present in the *tert.*-butyl group at a high purity level (99.8%), was performed at the Astra Laboratories. The presence of terbutaline in a sample was identified by three parameters: the retention time; the ions at *m/e* 426 and 442; and the ratio of the intensities of *m/e* 442 and 446, which should remain constant at all levels. For an accurate quantification, the ratio of the deuterium-labelled ions (*m/e* 448 and 432) should also remain constant.

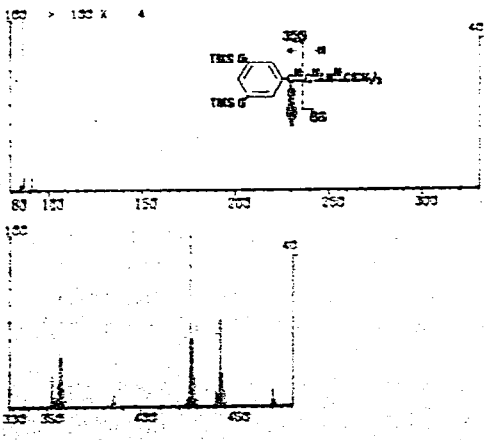


Fig. 1. The CI mass spectrum of tri-trimethylsilyl (TMS) terbutaline. The $(M - 15)^+$ ion is very abundant (25%); the abundance of the quasi-molecular ion is 12%.

TABLE I

CALIBRATION DATA FROM SPIKED SERUM SAMPLES

Correlation coefficient for the line = 0.998.

Sample	$(I_{442} + I_{426})/(I_{448} + I_{432})$				
	Concentration of terbutaline in serum (ng/ml)				
	1	2	5	10	20
1	0.045	0.095	0.27	0.55	0.96
2	0.053	0.098	0.25	0.48	1.01
3	0.055	0.095	0.24	0.52	0.99

Quantification could now be performed via a calibration equation calculated from spiked serum samples. To blank serum, 1, 2, 5, 10 and 20 ng of terbutaline were added together with a fixed amount of 20 ng of d_6-terbutaline. Each analysis was performed in triplicate and the results are given in Table I. The terbutaline concentration in an unknown serum sample is calculated from the equation

$$\text{Terbutaline concentration (ng/ml)} = 20.135 \times \frac{I_{442} + I_{426}}{I_{448} + I_{432}} - 0.066$$

where I_{442}, I_{448}, I_{426} and I_{432} represent the peak heights of the respective peaks in the fragmentogram. In urine analysis the concentration should be multiplied by 50. Frequent controls were carried through the procedure for optimal accuracy.

The overall accuracy of the extraction procedure was calculated from an actual sample divided into nine equal portions. Each portion was treated as described previously. The sample contained 2.5 ng/ml of terbutaline. The coefficient of variation was 8%.

The extraction efficiency was calculated from measurements on spiked serum samples. To each sample 2 ng of terbutaline were added and the sample was extracted without prior addition of d_6-terbutaline. Before the final step (the addition of BSTFA) 20 ng of d_6-terbutaline were added. The results from these samples were compared with those obtained from standards containing 2 ng of terbutaline and 20 ng of d_6-terbutaline in 20 μl of BSTFA. Six extractions were performed and the recovery was 80 ± 6%.

In the final part of this pilot study, we applied the method to two groups of patients. One group of seven individuals received 5 mg of Bricanyl® (equivalent to 4.1 mg of terbutaline base) on an empty stomach and the same breakfast was given to all subjects after 30 min, samples subsequently being collected after 0, 1, 2, 3 and 5 h. The serum levels are given in Table II and the mean and standard deviation are plotted in Fig. 2.

The second group of six individuals received 250 μg of Bricanyl subcutaneously and samples were collected after 0, 15, 30, 60, 120 and 180 min. The results are given in Table III and the mean and standard deviation are plotted in Fig. 3. Fig. 4 shows the recordings of the four multiple ion detection (MID)

TABLE II

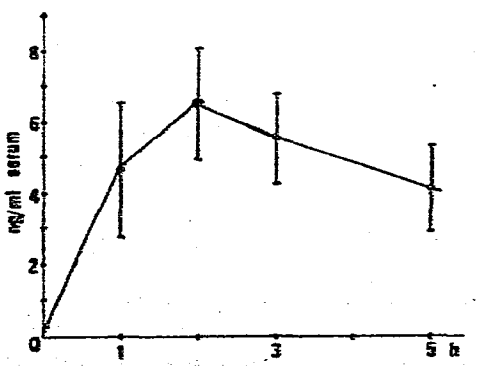
SERUM CONCENTRATIONS AFTER A SINGLE ORAL DOSE OF 5 mg OF BRICANYL

Patient	Concentration (ng/ml) after			
	1 h	2 h	3 h	5 h
B.E.	5.2	9.4	4.7	No sample
M.C.	1.0	5.1	5.3	3.9
D.C.	3.5	5.7	6.2	3.2
H.v.G.	4.4	5.3	3.9	3.9
L.R.	5.4	7.4	7.8	6.3
M.G.	6.8	5.5	4.7	2.7
M.M.	5.6	7.0	6.2	4.4

TABLE III

SERUM CONCENTRATION AFTER A SUBCUTANEOUS INJECTION OF 250 μg of BRICANYL

Patient	Concentration (ng/ml) after				
	15 min	30 min	60 min	120 min	180 min
B.R.	4.9	4.7	3.2	1.7	1.4
W.J.	5.8	4.7	4.1	2.7	1.7
S.J.	3.3	4.7	3.2	2.7	1.2
M.v.P.	3.1	4.7	4.4	2.7	1.9
M.v.S.	4.1	5.6	3.7	2.4	1.6
R.d.B.	3.4	3.7	3.6	2.7	2.5



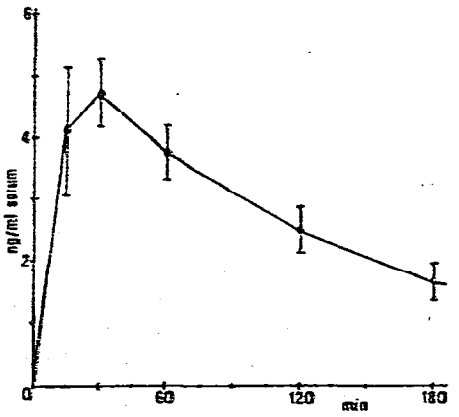


Fig. 2. Serum concentration versus time after an oral dose of 5 mg of Bricanyl. The mean and standard deviation are plotted.

Fig. 3. Serum concentration versus time after a subcutaneous dose of 250 μg of Bricanyl.

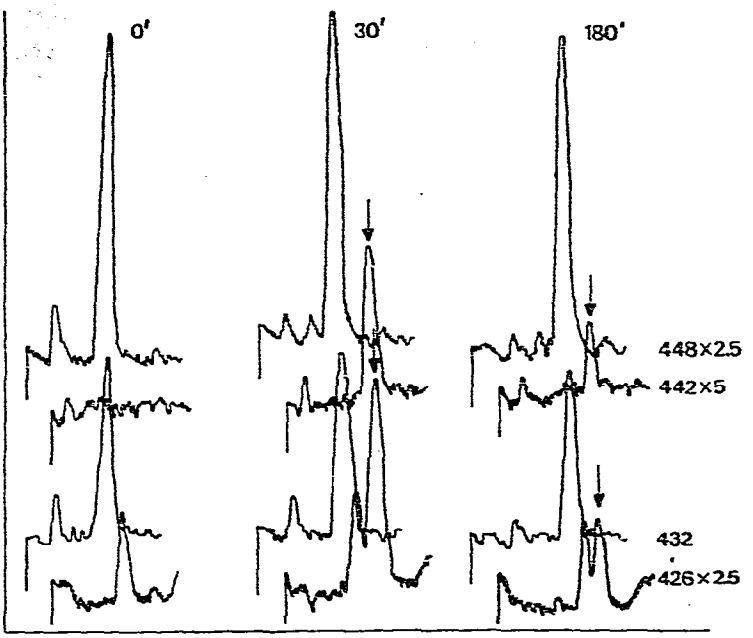


Fig. 4. Mass fragmentogram traces of serum samples from one patient (M.v.P.) at time 0, 30 and 180 min. The arrows indicate the elution time of terbutaline (5 min 11 sec). To all samples 20 ng/ml of d_6-terbutaline were added. The elution time of the internal standard was 5 min 7 sec. The traces represent the ion intensities at the *m/e* 448, 442, 432 and 426.

traces of three serum samples of a patient at zero time, at the maximum concentration after 30 min and at the minimum level after 180 min. The MID trace at *m/e* 426 contains a slightly interfering substance. Such interferences are patient related. On some occasions a clear trace is observed at all *m/e* values; sometimes the MID trace at *m/e* 448 is contaminated. If, however, the ratios *m/e* 442 : 446 and *m/e* 448 : 432 remain constant there is virtually no chance that biological compounds are contributing to the respective traces at the retention time of terbutaline.

Other β-receptor stimulating drugs, such as salbutamol, orciprenaline, isoetharine and fenoterol, do not interfere chemically, although they are extracted equally well, because of their different mass spectra and different retention times.

From the patients in the second group, urine samples were collected at the intervals 0—3 h, 3—6 h, 6—12 h and 12—14 h and analyzed. Large differences between the individuals occurred (Table IV).

All of the samples were analyzed without prior knowledge of either the patient or the time of sample collection.

From these results it can be concluded that the method described is sensitive and specific for studying serum levels in patients receiving terbutaline either parenterally or orally. Further pharmacokinetic and clinical studies are in progress.

TABLE IV

CONCENTRATION OF FREE TERBUTALINE EXCRETED IN URINE AFTER SUBCUTANEOUS INJECTION OF 250 μg OF BRICANYL

The number in parentheses are the total amounts of urine excreted in the measured intervals.

patient	Concentration (ng/ml)			
	0—3 h	3—6 h	6—12 h	12—24 h
B.R.	560 (175)	216 (25)	21 (200)	8.0 (740)
W.J.	1020 (25)	290 (75)	No sample	31 (270)
S.J.	480 (100)	190 (75)	30 (310)	8.2 (550)
M.v.P.	76 (200)	79 (240)	12 (170)	11 (550)
M.v.S.	820 (70)	218 (70)	20 (10)	24 (300)
R.d.B.	194 (80)	230 (460)	18 (700)	No sample

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