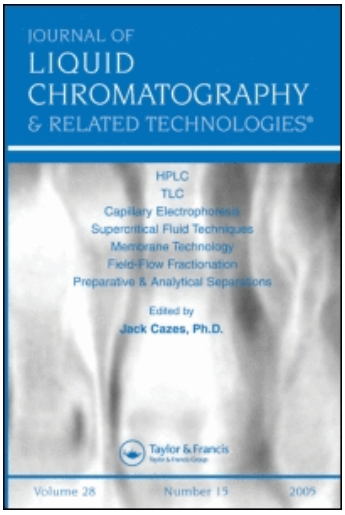

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Analysis of Terbutaline (Bricanyl®) in Human Plasma with Liquid Chromatography and Electrochemical Detection Using On-Line Enrichment

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To cite this Article Bergquist, S. and Edholm, L. -E.(1983) 'Quantitative Analysis of Terbutaline (Bricanyl®) in Human Plasma with Liquid Chromatography and Electrochemical Detection Using On-Line Enrichment', Journal of Liquid Chromatography & Related Technologies, 6: 3, 559 — 574
To link to this Article: DOI: 10.1080/01483918308076068
URL: http://dx.doi.org/10.1080/01483918308076068

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(3), 559-574 (1983)

QUANTITATIVE ANALYSIS OF TERBUTALINE (BRICANYL®) IN HUMAN PLASMA WITH LIQUID CHROMATOGRAPHY AND ELECTROCHEMICAL DETECTION USING ON-LINE ENRICHMENT

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ABSTRACT

A liquid chromatographic method with electrochemical detection (LC-EC) has been developed for the quantitative analysis of terbutaline in the range 5-50 pmole ml^{-1} of human plasma. Terbutaline is isolated from 2 ml of plasma on an ion-exchange column and the eluate is concentrated on a hydrophobic precolumn on-line in the chromatographic system. The precolumn is then back-flushed for further separation onto a hydrophobic analytical column. The mobile phase is a methanol-aqueous buffer to which sodium perchlorate is added to achieve resolution from interfering peaks. A glassy carbon electrode is used for detection. Comparison has been made with gas chromatography-mass spectrometry (GC-MS) to examine the accuracy of the method.

INTRODUCTION

Terbutaline (Fig.1) is a selective β_2-receptor agonist, widely used in the treatment of asthma (1). In man, the drug is inactivated by conjugation, mainly of the corresponding sulphate conjugate (2). Plasma concentrations of unchanged drug, that have been associated with effective therapy, are in the range of 10-30 pmole ml^{-1}. Because of the low concentrations and the complexity of the sample, sensitive and selective detection is necessary for the assay of terbutaline levels in plasma. Up to now only methods based on gas chromatograhy-mass spectrometry (GC-MS) have met these requirements (3,4,5).

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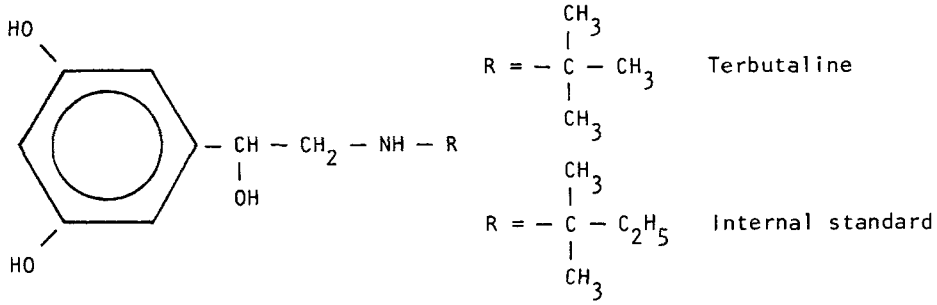

FIGURE 1

Structural formulas of terbutaline and internal standard.

The complex biological matrix also necessitates a selective clean-up procedure. Terbutaline can be extracted to organic solvents, either as zwitterion (4), as an ion-pair (3,6) or isolated by ion-exchange chromatography as described by Jacobsson et al. (5)

The objective of the present work has been to develop a less expensive and more generally accessible method than GC-MS for the analysis of terbutaline in concentrations normally found in the therapeutic situation. The combination of an electrochemical detector with liquid chromatography (LC-EC) is a well established and powerful technique for sensitive and selective analysis of phenolic compounds. For phenols with a low oxidation potential, like catecholamines, high selectivity and detectability down to less than one pmole injected amount has been obtained (7,8,9). LC-EC may also be favourable for the analysis of many other phenols, but often higher electrode potentials have to be used at the expense of selectivity to obtain pmole sensitivity (10). Terbutaline is a resorcinol derivative with the two hydroxylgroups substituted metha to each other. The formation of a quinone structure is impossible and thus terbutaline oxidation differ mechanistically from catecholamine oxidation. Another problem to take into consideration in the development of an analytical method for terbutaline is its liability to react with traces of aldehydes present in organic solvents (11), which makes a careful selection and handling of the solvents used for its extraction necessary. For the isolation of terbutaline, the ion-exchange procedure, described by Jacobsson et al. (5),

was utilized in this work. To concentrate the eluate from the ion--exchanger, we used a precolumn mounted in the loop-position of a valve--injector.

EXPERIMENTAL

Apparatus

Fig.2 is a schematic representation of the LC system used. A Waters Model M45 (Water Assoc.Milford, Mass. U.S.A.) solvent delivery system, equipped with a Glenco (Glenco Scientific Inc., Houston, Texas, U.S.A.) pulse dampener, was used. Samples were injected with a Valco (Valco Instruments Co., Houston, Texas, U.S.A.) CV-6-UHpa-N60 injector. The thin-layer amperometric detector (Bioanalytical Systems, West Lafayette, Ind., U.S.A.) comprised the following parts: a Model LC-4 potentiostat, a TL-4 or TL-5 glassy carbon electrode and an Ag/AgCl (3 M NaCl) reference electrode. The working potential was + 0.9 V. The detector was housed in a draught free Faraday cage. For centrifugation of plasma, an Eppendorf centrifuge Model 5412 (Eppendorf, G.F.R.) was used.

Columns

For enrichment a Waters guard column (23 x 3.9 mm I.D.) mounted in the loop position of a Valco injector, was dry-packed with C_{18} packing material obtained from SepPak™ C_{18} cartridges (Waters Assoc.).

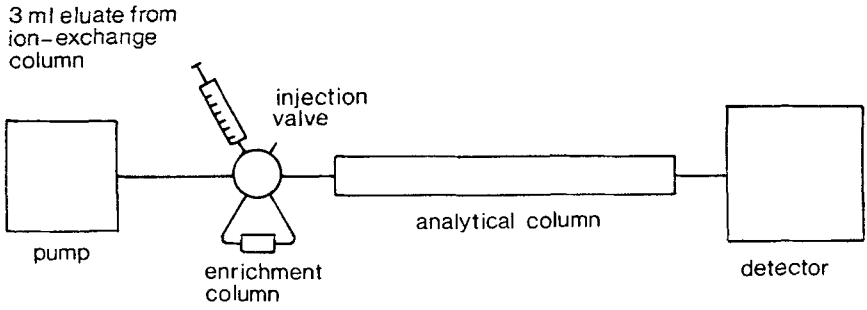

FIGURE 2

A schematic representation of the equipment used. 3 ml eluate from the ion-exchange column is manually injected onto the enrichment column and subsequently backflushed onto the analytical column.

The analytical column (200 x 5 mm I.D.) was packed with 10 µm particles of Nucleosil C_{18} (Macherey-Nagel & Co., Düren, G.F.R.) according to the procedure described by Edholm et al. (9).

Chemicals

Terbutaline [1-(3,5-dihydroxyphenyl)-2-(t-butylamino)ethanol] and the internal standard [1-(3,5-dihydroxyphenyl)-2-(1,1-dimethylpropyl amino)ethanol] were supplied by AB Draco (AB Draco, Lund, Sweden). The cation-exchanger was Biorad AG 50W-X2, 100-200 mesh (Biorad Lab., Richmond, Calif., U.S.A.), in the H^+ form. Sodium hydroxide p.a. was from EKA (EKA AB, Surte, Sweden). Methanol was HPLC grade (Rathburn Chemicals, Walkerburn, Great Britain). All water used was purified with a Milli Q system (Millipore Corp., Bedford, Mass., U.S.A.

All other chemicals were of analytical grade from Merck (E.Merck, Darmstadt, G.F.R.).

Buffers

Sodium phosphate buffer (pH 7.50). Sodium dihydrogen phosphate monohydrate (345 mg) and disodium hydrogen phosphate dihydrate (445 mg) were dissolved in 500 ml of water and the pH was adjusted to 7.50 ± 0.01 with 1 M sodium hydroxide.

Sodium carbonate buffer (pH 11). Sodium carbonate decahydrate (14.3 g) and sodium chloride (58.4 g) were dissolved in 1000 ml of water.

Mobile phase buffer (pH 6.00). Citric acid monohydrate (26.3 g) and orthophosphoric acid (14.5 g) were dissolved in 5000 ml of water and the pH was adjusted to 6.00 ± 0.01 with 5 M sodium hydroxide.

Mobile phase

The composition was 12:88 (v/v) methanol-buffer to which anhydrous perchlorate (50 mM) was added to enhance resolution. The mobile phase was degassed for five minutes under vacuum and continuous stirring.

Standard solutions and calibration curve

Stock solutions for terbutaline and internal standard were prepared in water, giving concentrations of 183 and 165 µmole l^{-1} respectively. Working standards were prepared by diluting stock solutions with water.

Calibration curves were prepared by adding working standards to 2.00 ml of plasma, giving concentrations in the range 3.67-36.7 pmole ml^{-1} at seven different levels for terbutaline and 33.0 pmole ml^{-1} for the internal standard. Each calibration point was run in duplicate.

Preparation of ion-exchanger

A 60-ml volume of resin (H^+ form) was added to 200 ml of water and allowed to swell over night. The slurry was packed in a glass column, washed with 150 ml of 1.0 M sodium hydroxide to get the ion-exchanger in sodium form and then washed back to neutral with water. The ion--exchange resin was packed to a height of 50 mm in glass columns (100 x 5 mm I.D.), fitted with 35 ml reservoirs at the top and a glass pearl in the bottom to hold the resin. Immediately before use, the resin was washed twice with 3 ml of the pH 11 buffer and subsequently back to neutral pH by washing three times with 3 ml of water.

Analytical procedure

The plasma was centrifuged for 60 seconds at 15000 rpm (10000 g) to get rid of protein residues. A 2.00-ml volume of plasma, 0.5 ml of the pH 7.50 buffer and 100 µl internal standard were mixed in a 10 ml glass tube. The mixture was transferred to the ion-exchanger with a pasteur pipette. The glass tube was washed three times with 3 ml portions of water which were subsequently added to the ion-exchanger. After this washing procedure, the column was eluted three times with 1 ml of the pH 11 buffer into glass tubes containing 100 µl of 2 M hydrochlorid acid for the adjustment of pH to 6. The total acidified ion-exchange eluate was injected with a syringe into the enrichment column. The enrichment column was thereafter switched into the HPLC eluent stream and backflushed onto the analytical column.

Quantitation

For quantitation, the peak height ratio between terbutaline and the internal standard was calculated and plotted versus concentration. Least square linear regression of the data was used to establish the calibration curve.

Recovery

The total absolute recovery of the method was tested on spiked plasma samples, that were taken through the entire analytical procedure. Comparison was made with directly injected aqueous samples.

The recovery in the enrichment step was tested by re-analysis, at the highest sensitivity possible, of the solution going to waste in the loading of the sample onto the enrichment column.

RESULTS AND DISCUSSION

Isolation procedure

Terbutaline is a hydrophilic compound, containing both weak acidic and basic functional groups:
pka_1 (phenol) = 8.8, pka_2 (amine) = 10.1, pka_3 (phenol) = 11.2 (5). These properties restict its isolation from an aqueous phase by solvent extraction. Due to the risk for unwanted side-reactions, poor selectivity and poor recovery of solvent extraction of terbutaline, we preferred to use a cation exchanger for its isolation. This has also proven to be very useful for the analysis of terbutaline in plasma using GC-MS (5). At pH 7.5 the amino group of terbutaline is ionized A strong cation exchanger will therefore strongly retard terbutaline at this pH. At pH 11 the phenolic groups will be ionized, while the amino group is neutral, thus allowing the substance to be eluted. The eluate volume from the ion-exchanger is quite large (3 ml) and i contains high concentrations of salt (1M NaCl), which is used to further enhance the elution of terbutaline with the pH 11 buffer. To concentrate the eluate, a trace enrichment technique was used (12).

To allow manual syringe injection of sample onto the enrichment column, minicolumns (3-5 mm) filled with 10 µm particles as used by Van Vliet et al (13) were tried. They were found unsatisfactory for reliable work due to clogging of capillaries and injector channels. Instead longer (23 mm) commercially available precolumns were used. In this case larger particles had to be used to make manual injection possible. It was found that extra band broadening due to these larger particles is not of any importance which is shown in Fig. 3.

This was tested using aqueous standards with the same amount of terbutaline and internal standard added. Several lots of SepPak C_{18} have been tested without giving any marked differences in recovery and performance. Other combinations of reversed phase materials might be used if the following requirements are met: the enrichment column should give complete recovery and good permeability to allow for manual injection. As analytical column, a C_{18} or C_8 type or even an

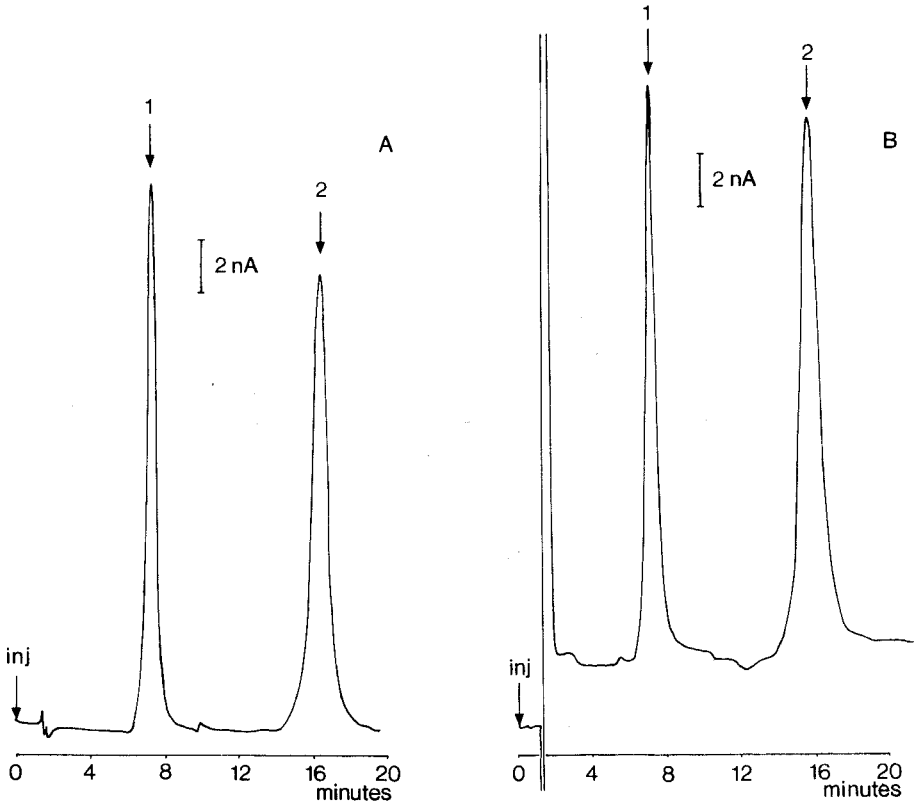

FIGURE 3

Comparison of 20 µl loop injection of aqueous standard (A) and enrichment of 3 ml aqueous standard (1 M NaCl, pH 6) to simulate the properties of the eluate from the ion-exchange column (B). Conditions: Precolumn (23 x 3.9 mm I.D.) with SepPak C_{18}. Analytical column (200 x 5 mm I.D.) with Nucleosil C_{18} (10 µm). Eluent 12/88 (v/v) MeOH/pH 6 buffer, 50 mM sodium perchlorate. Flow rate 2 ml min^{-1}. Electrochemical detector + 0.9 V vs Ag/AgCl. 1 = terbutaline, 2 = internal standard.

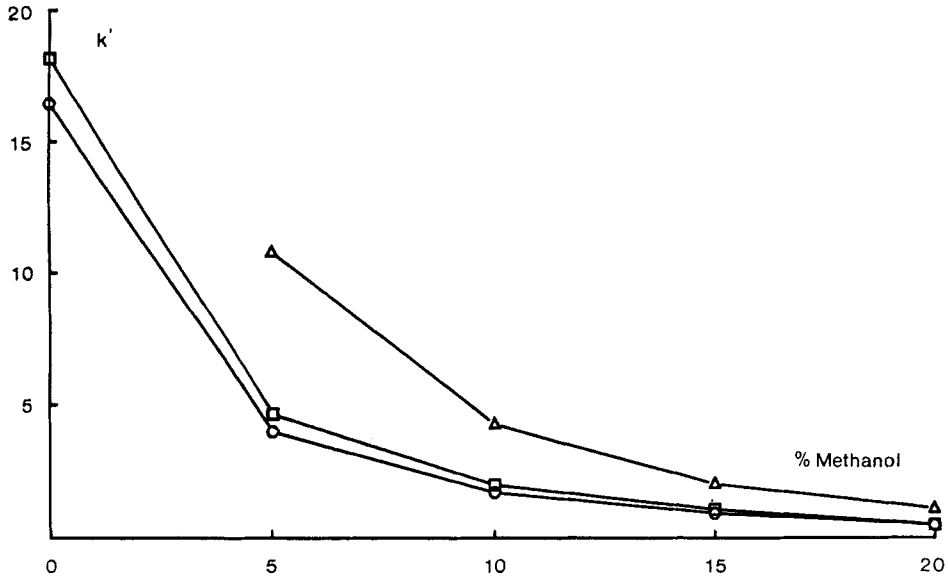

FIGURE 4

Relationship between capacity factors, k', for terbutaline (○), internal standard (△), a terbutaline analogue (R- = sec.butyl) (□) and methanol content of the eluent.

ion-exchange column (15) could be used. In the configuration shown here, the precolumn has little effect on the total capacity factor.

The advantages of the precolumn technique, compared to enrichment directly on the top of the analytical column, are among other things: analytical column life time is enhanced; most of the salt from the injection medium is not passed through the analytical column and the detector. The latter fact is important, since it gives shorter analysis time and less disturbance of the detection.

Chromatography

The retention of terbutaline and internal standard can be controlled by varying the methanol content in the mobile phase (Fig. 4). Addition of sodium perchlorate was found to be necessary, in order to remove an otherwise interfering peak observed in blank plasma. Table 1 shows how the separation of the terbutaline peak and the in-

TABLE 1

Effect on separation factor between terbutaline and an unknown compound

Sodiumperchlorate concentration mM	Separation factor (α)
0	*unresolved*
50	1.24
100	1.42
200	1.62

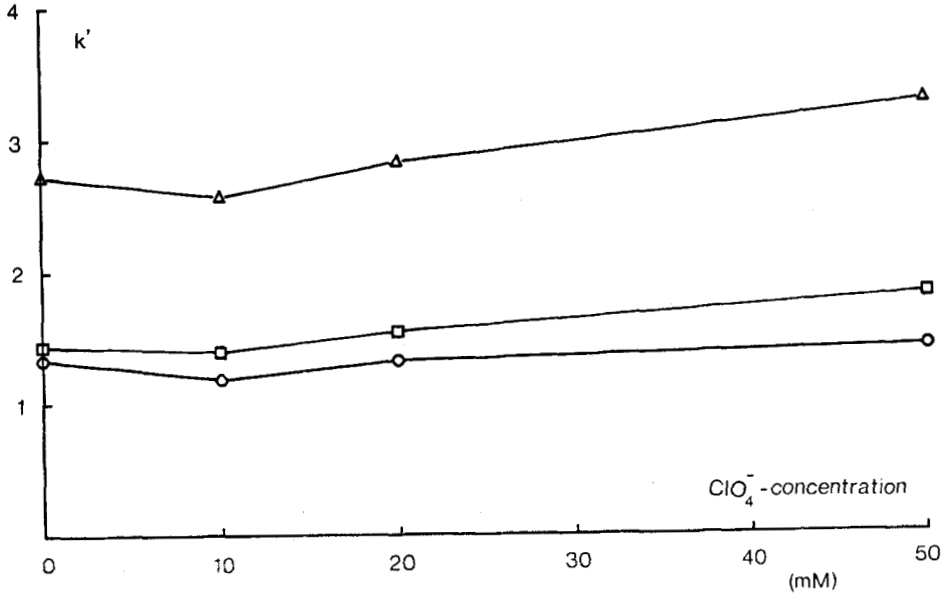

FIGURE 5

Relationship between capacity factors, k', and the perchlorate concentration of the eluent. Symbols as in Fig. 4.

terfering peak is influenced by the amount of sodium perchlorate. For some batches of Nucleosil C_{18}, separation could be achieved without the addition of sodium perchlorate. However, in these cases the retention of terbutaline and internal standard were increased. Although sodium perchlorate determines the position of the interfering peak, it does not appreciably affect the chromatography of terbutaline and the internal standard (Fig. 5).

Electrochemical detection

In electrochemical detection, precision and accuracy can often be enhanced by the use of an appropriate internal standard, which also serves to correct for fluctuations in the detector response. The internal standard used in this work, is chemically similar to terbutaline and their electrochemical properties are thus very much alike (Fig. 6).

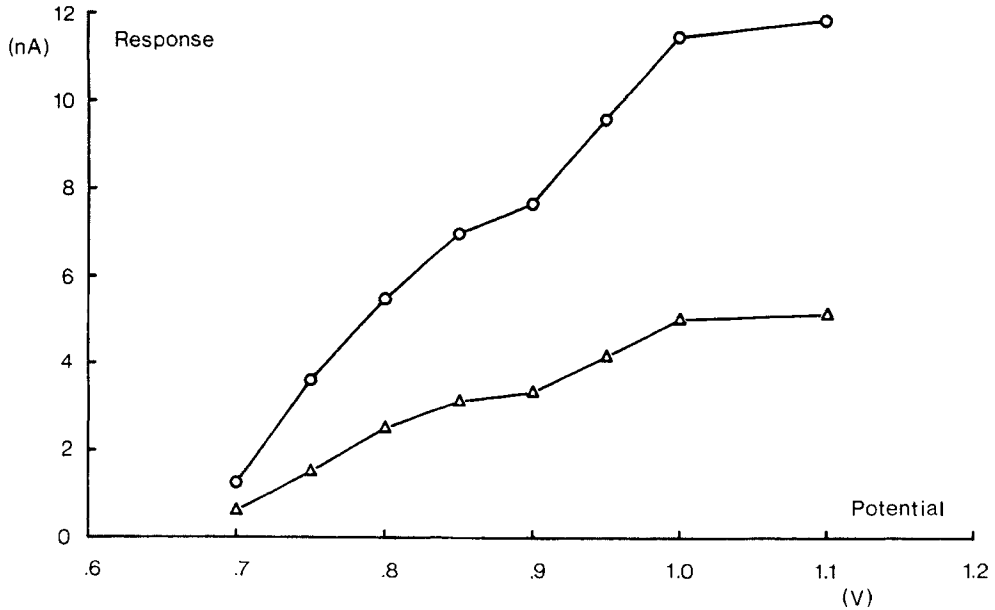

FIGURE 6

Hydrodynamic voltammograms of terbutaline (○) and internal standard (△). Eluent as in Fig. 3. The reference electrode was Ag/ /AgCl (3 M NaCl).

A pH of 6, in the mobile phase, was found optimal regarding peak symmetry and column life time. Increasing pH gave little extra detector response. The hydrodynamic voltammograms indicate a plateau at + 0.9 V and this potential was found to be optimal. Sensitivity was sufficient and no problems of contamination of the glassy carbon electrode were found. However, at potentials above + 1.0 V, irreproducible results were obtained with contamination of the electrode.

Method validation

Since the principal aim of the present work was to evaluate whether LC-EC is a suitable alternative for the analysis of terbutaline in human plasma, a thorough method validation is not presented at this stage. At present selectivity, accuracy, precision and recovery have been tested on a limited material. Future work will give more information on the usefulness of the proposed method, especially in clinical work.

The selectivity of the method was tested by co-chromatography of the principal terbutaline metabolite (2). Also we have not found any interfering peaks in plasma from a number of investigated healthy volunteers.

The accuracy of the method was tested by comparison with a GC-MS method (5) in the range 5 - 50 pmole ml^{-1} , using plasma from healthy volunteers after oral intake of 5 or 10 mg of Bricanyl® (terbutaline sulphate). See Fig. 7. The data are presented in Table II. Linear regression gave a line with equation

$$(LC\text{-}EC) = 1.014 \quad (GC\text{-}MS) + 1.12$$

$$r = 0.9980$$

A convenient way to show differences in the comparison of two methods is to plot the relative values found by the two methods against the mean values found by the two methods as proposed by Eksborg (14). See Fig. 8. The plot clearly shows that slightly higher values are obtained with the LC-EC method. At low concentrations (5 pmole ml^{-1}) the LC-EC method becomes less accurate and also the precision becomes poorer. See Table III. This is partly due to difficulties in establishing a correct baseline. A sloping baseline is often encountered when using electrochemical detectors at high sensitivity. This problem can be partly overcome by using a column switching technique (15).

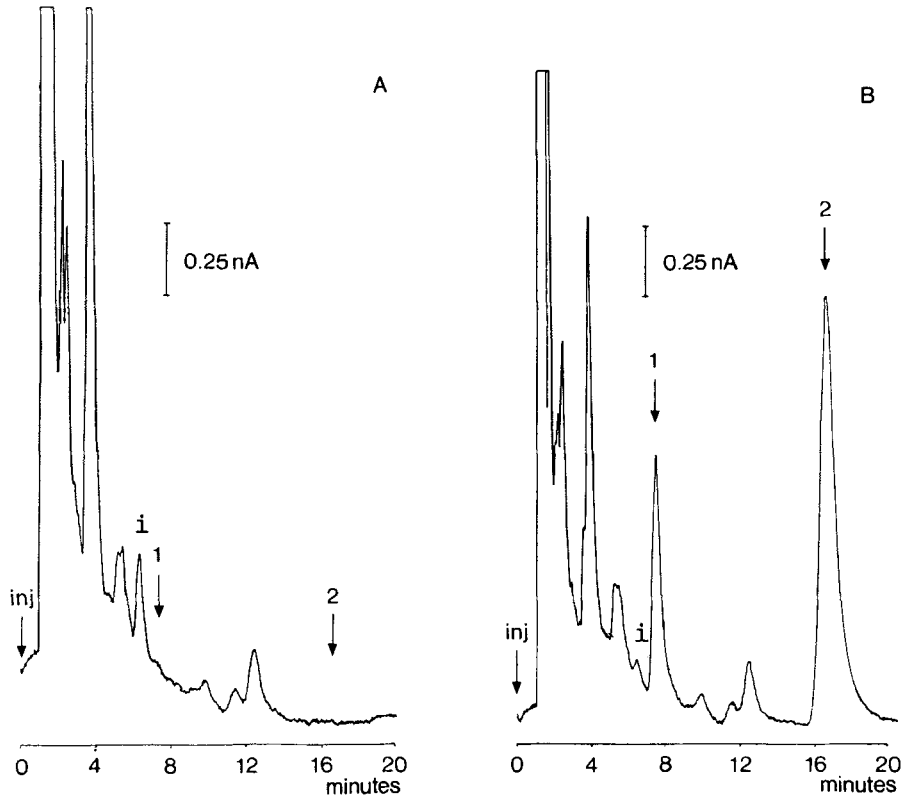

FIGURE 7

Chromatograms of blank plasma (A) and plasma from a healthy volunteer (12 pmole ml^{-1}) after a single oral dose of Bricanyl (B). Conditions as in Fig. 3. 1 = terbutaline, 2 = internal standard, i = interfering peak, the magnitude of this peak varied inconsistently.

TABLE II

Comparison of LC-EC with GC-MS. Plasma samples were drawn from healthy volunteers after oral intake of 5 or 10 mg Bricanyl® (terbutaline sulphate)

Subject	Hours after intake	GC-MS	LC-EC	Mean value	Ratio LC-EC/GC-MS
		pmole ml^{-1}	pmole ml^{-1}	pmole ml^{-1}	
1	2	22.6	24.6	23.6	1.09
1	6	8.3	8.9	8.6	1.07
2	2	24.8	25.6	25.2	1.03
2	6	5.5	6.8	6.2	1.24
3	2	31.1	33.7	32.4	1.08
3	6	13.9	15.1	14.5	1.09
3	10	5.9	7.0	6.4	1.19
4	2	6.2	7.6	6.9	1.23
4	6	15.8	18.0	16.9	1.14
4	10	4.9	6.5	5.7	1.33
5	2	43.5	44.6	44.1	1.02
5	6	15.5	15.8	15.6	1.02
5	10	7.6	7.9	7.8	1.04
6	2	13.6	16.0	14.8	1.18

TABLE III

Intra-day variation

Conc. level (pmole ml^{-1})	5	50
% Rel. stand. dev.	8	0.3
n	4	4

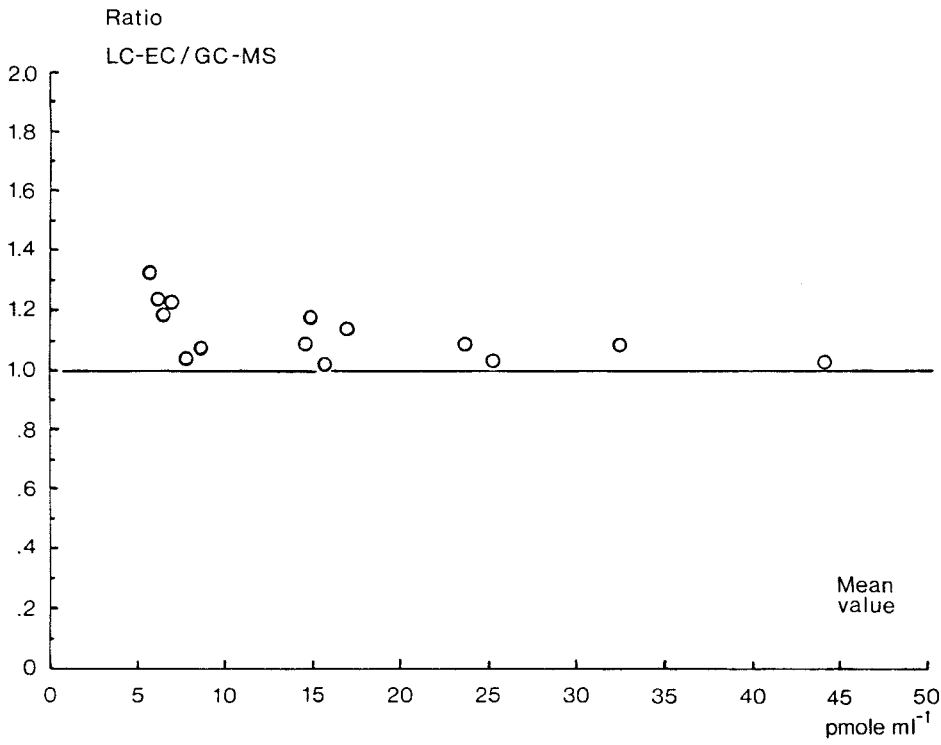

FIGURE 8

Plot of the ratio between the values found with LC-EC and GC-MS against the mean values found by the two methods.

TABLE IV

Absolute recovery (%)

Enrichment column	100
Total procedure	91 ± 2

Linear regression of data from the calibration curve gave the following equation and correlation coeffecient

$Y = 0.282\ (\pm\ 0.001) * X - 0.028\ (\pm\ 0.005)$

$r = 0.9986\ (\pm\ 0.0006)$

$n = 3$ (three calibration curves obtained on three different occasions)

The absolute recovery of terbutaline at 50 pmole ml^{-1} was measured as described in Experimental. The data are presented in Table IV.

CONCLUSIONS

A new, simple liquid chromatographic method has been developed for the assay of terbutaline in human plasma. It is considered that the method will be useful for both clinical and pharmacokinetic studies. Hopefully it will be an important complement to GC-MS methods, which still will be required for pharmacokinetic work at concentrations below 5 pmole ml^{-1} in plasma. As a further development of the technique presented here, we are presently investigating a column switching configuration based on the on-line coupled enrichment column. Preliminary results indicate that this system can be easily automated.

ACKNOWLEDGEMENT

We wish to thank Drs C. Lindberg and S. Jönsson for performing the GC-MS analyses of terbutaline.

The authors are also indepted to Professor B. Smith and Drs O.Borgå and L. Nyberg for helpful discussions. Mrs A. Toräng is acknowledged for her skilful typing of the manuscript.

One of the authors is grateful to AB Draco for financial support during the investigation.

REFERENCES

1. Andersson, K.-E., Owman, C. and Westling, H. (Editors), Acta Pharm. Toxicol. 44 (Supplement II), (1979)

2. Nilsson, H.T., Persson, K. and Tegner, K., Xenobiotica 2, 363 (1972)

3. Leferink, J.G., Wagemaker-Engels, I., Maes, R.A.A., Lamont, H., Pauwels, R. and Van der Straeten, M., J. Chromatogr. 143, 299 (1977)

4. Clare, R.A., Davies, D.S. and Baillie, T.A., Biomed. Mass Spectrom. 6, 31 (1979)

5. Jacobsson, S.-E., Jönsson, S., Lindberg, C. and Svensson, L.-Å., Biomed. Mass Spectrom. 7, 265 (1980)

6. Modin, R. and Johansson, M., Acta Pharm. Suecica 8, 561 (1971)

7. Kissinger, P.T., Bruntlett, C.S. and Shoup, R.E., Life Sci. 28, 455 (1981)

8. Hansson, C., Edholm, L.-E., Agrup, G., Rorsman, H., Rosengren, A.-M. and Rosengren, E., Clin. Chim. Acta 88, 419 (1978)

9. Edholm, L.-E., Hansson, C., Agrup, G., Rorsman, H., Rosengren, A.-M. and Rosengren, E., J. Chromatogr. 162, 7 (1979)

10. Armentrout, D.N., McLean, J.D. and Long, M.W., Anal. Chem. 51, 1039 (1979)

11. Svensson, L.-Å., Björklund, A and Lindvall, O., Acta Chem. Scand. B29 341 (1975)

12. Lankelma, J. and Poppe, H., J. Chromatogr. 149, 587 (1979)

13. Van Vliet, H.P.M., Bootsman, Th.C., Frei, R.W. and Brinkman, U.A. Th., J. Chromatogr. 185, 483 (1979)

14. Eksborg, S., Clin. Chem. 27 (7), 1311 (1981)

15. Edholm, L.-E., Kennedy, B.-M. and Bergquist, S., in preparation.