Journal of Chromatography, 577 (1992) 109–116
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6315

Analysis of terbutaline in human plasma by high-performance liquid chromatography with electrochemical detection using a micro-electrochemical flow cell

Kamal A. Sagar, Mary T. Kelly and Malcolm R. Smyth*

School of Chemical Sciences, Dublin City University, Dublin 9 (Ireland)

(First received December 5th, 1991; revised manuscript received February 7th, 1992)

ABSTRACT

A high-performance liquid chromatographic method is described for the determination of terbutaline in human plasma in the range 1–35 ng/ml. Detection was achieved using a carbon fibre micro-electrochemical detector and a column-switching system. The micro-electrode cell has advantages over conventional glassy carbon electrode-based detection systems in that it is easy to prepare, flexible in its operation and suffers less trouble from problems such as air bubbles and leaks. Furthermore, it has a better detection limit for terbutaline (0.8 ng/ml) to that obtained using a conventional glassy carbon electrode flow detector (2 ng/ml). Sample clean-up was by on-line solid-phase extraction with column switching, providing a method which was sensitive and reproducible, where the mean overall coefficient of variation was 5.60% and drug recovery in excess of 86% at the concentration levels studied.

INTRODUCTION

Terbutaline, 1-(3,5-dihydroxyphenyl)-2-(*tert.*-butylamino)ethanol, is a sympathomimetic (or adrenergic) drug which affects those neurotransmitters which mediate sympathetic nerve impulses [1]. It has clinical application primarily as a bronchodilator, where it is widely used in the prophylaxis of bronchospasms. Plasma concentrations of unchanged drug associated with effective therapy are in the range 10–30 pmol/ml [2]. The analysis of terbutaline is important both in pharmaceutical research and clinical chemistry, and because the drug is widely prescribed, it can sometimes be taken in over-dose. Because of the low concentrations encountered in biological fluids, the complexity of the sample and the reactivity of the drug to trace impurities, sensitive and selective detection is necessary for its determination in plasma.

Multi-dimensional column chromatography (now widely known as column switching) is a powerful technique for the separation of multi-component samples. Fractions from one chromatographic column are selectively transferred onto one or more secondary columns for further separation. This technique has been applied in thin-layer chromatography [3] and gas chromatography (GC) [4]. The principle of on-line pre-concentration of drugs from biological samples in high-performance liquid chromatographic (HPLC) systems has been shown to be advantageous for drug analysis in human plasma [5]. Pre-concentration is usually carried out on relatively short (10–30 mm in length) pre-columns, packed with a sorbent employed in HPLC, such as chemically bonded silicas [6].

Electrochemical detection (ED) combined with HPLC (LC–ED) offers the capability of selective and sensitive determination of electroactive drug species, with little interference from biological matrix interferences [5] and with low limits of de-

0378-4347/92/$05.00

tection [7,8]. In recent years, carbon fibre microelectrodes have found increasing use in bioanalytical applications, and their application has been previously reported [9,10]. Microelectrodes have a number of distinct advantages [9,11]. For instance, the ohmic (*iR*) drop is usually very small, capacitive charging currents are reduced, and the rate of mass transport to and from the electrode surface increases as the electrode area decreases, thus establishing a steady-state response quite rapidly.

Hitherto, only methods based on gas chromatography–mass spectrometry (GC–MS) [12,13] and ED employing a glassy carbon electrode have been useful for low level analysis of terbutaline in human plasma [2]. This report describes an LC–ED method using a micro-flow cell based on a carbon fibre working electrode [14]. The method was found to compare favourably with a glassy carbon electrode when applied to the same conditions of analysis and separation and provided a limit of detection of at least 0.8 ng/ml. In addition, the on-line technique of sample preparation, which was used for extraction, is less laborious and time-consuming than liquid–liquid partition, thus providing an assay which is sensitive, cost-effective and expedient in its execution.

EXPERIMENTAL

Materials and reagents

Terbutaline was obtained from Sigma (Poole, UK) and analytical-grade sodium dihydrogenphosphate from Merck (Germany). Orthophosphoric acid and diethylamine (Analar grade) were supplied by BDH (Poole, UK) and HPLC-grade methanol was supplied by Labscan Analytical Sciences (Dublin, Ireland). Human plasma, obtained from Beaumont Hospital (Dublin, Ireland), was frozen until required and thawed at room temperature. Water was distilled and then further purified using a Milli-Q purification system (Millipore, Milford, MA, USA). The preconcentration column was packed with Corasil C_{18} reversed-phase material (37 μm) (Waters, Milford, MA, USA). Carbon fibres, 14 μm in diameter, were obtained from Avco (Lowell, MA, USA). The surface of these fibres has no external coating. Silver epoxy was purchased from RS components (Corby, UK) and the glassy carbon used in the comparison studies was obtained from EG & G Princeton Applied Research (Princeton, NJ, USA).

Construction of carbon fibre flow cell

The carbon fibre working electrode was inserted through the centre of a 25-mm-length polyethylene tubing (2 mm × 0.5 mm I.D.) as reported previously [14]. The silver phosphate reference electrode was prepared by first connecting a silver wire (0.1 mm in diameter) to the anode, and a platinum electrode to the cathode of a 1.5 V battery, after which the assembly was immersed for 2 min in a solution of 1 *M* phosphoric acid. The wire was then inserted into a polyethylene tube (15 mm × 1 mm I.D.) one end of which was plugged with a ceramic porous rod 2 mm × 1 mm I.D. (Reagecon, Clare, Ireland). The tube was then filled with an internal reference solution containing 1 *M* phosphoric acid, and this end was closed by heating. A 2-cm piece of stainless-steel tubing (1 mm O.D., 0.2 mm I.D.) served as the counter electrode. The working, reference and counter electrodes were mounted in a T-tube arrangement such that the mobile phase eluent passed first through the working electrode and then via the counter electrode, to waste. A diagram of the micro-electrode flow cell is shown in Fig. 1.

Instrumentation and operating conditions

Chromatographic separation was based on the work of Jarvie *et al.* [15] following a slight modification to the ratio of methanol to aqueous component to obtain a suitable retention time for terbutaline. The drug was separated on a Spherisorb ODS (10 μm) column, 25 cm × 4.6 mm I.D. (HPLC Technology, Macclesfield, UK), which was protected by a guard column with C_{18} packing material. The mobile phase consisted of 0.067 *M* phosphate buffer (pH 5)–methanol–40 g/l sodium dodecyl sulphate (SDS)–diethylamine (DEA) in a ratio of 45:55:0.5:0.02 (v/v). Prior to the addition of SDS and DEA, the mobile phase

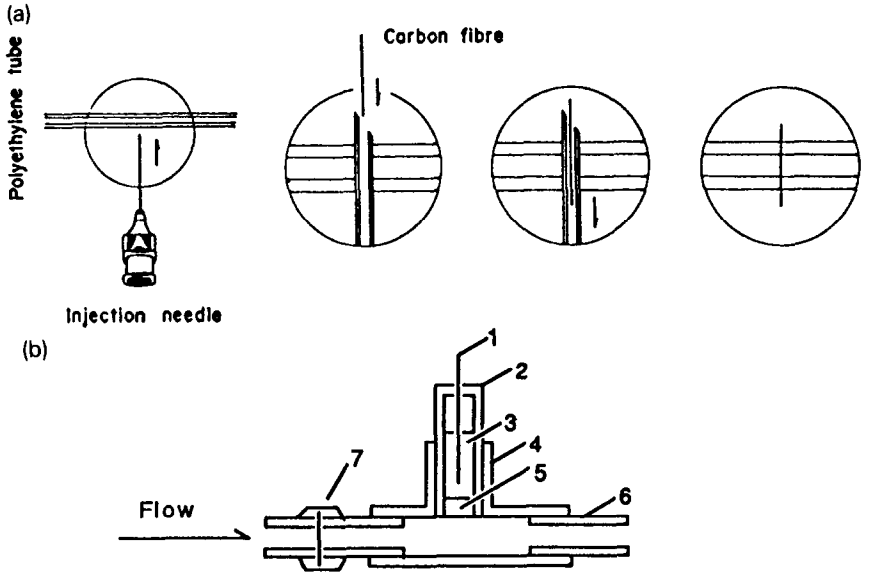

Fig. 1. (a) Method of fixing a carbon fibre into polyethylene tube. (b) Diagram of the carbon fibre micro-flow cell consisting of (1) silver wire coated with Ag_3PO_4, (2) reference electrode body, (3) internal reference solution, (4) T-tube, (5) ceramic rod, (6) stainless-steel counter electrode and (7) fibre flow electrode.

was filtered and degassed by sonication. The prepared eluent was delivered by a Waters Model 501 HPLC pump at a flow-rate of 1 ml/min. Before use, the column was conditioned by passing 100 ml mobile phase adjusted to contain 10 times the standard concentration of SDS and 2.5 times the standard concentration of DEA. Sample introduction was via a Rheodyne (Cotati, CA, USA) Model 7010 injection valve, fitted with a 20-μl loop for direct injection. For the purposes of extraction by column switching, the injector was fitted with a 1-ml loop and a second pump (pump A), and the concentration columns were connected to the analytical assembly via a Rheodyne Model 7000 six-port switching valve.

The concentration column (10 cm × 1.5 mm I.D.) was dry-packed in-house with Corasil material. The loading/washing eluent delivered by pump A was filtered degassed deionised water. The operation of this instrument arrangement has been previously described [16]. Oxidative amperometric measurements were performed using an EG & G Princeton Applied Research Model 400 EC potentiostat connected to the flow cell by crocodile pins. The drug was detected amperometrically by employing a potential of +1.3 V at the working electrode. The resultant signals were recorded on a Philips Model PM8261 X-t recorder (Eindhoven, Netherlands) at a chart speed of 300 mm/h. The peak currents (measured as peak heights on the recorder) as a function of concentration were then measured for quantitative analysis.

Standard solutions and calibration curves

Stock solutions equivalent to 0.2 mg/ml drug in water were freshly prepared. These were diluted and added to drug-free plasma aliquots to generate spiked plasma standards in the concentration range 1–35 ng/ml. Each calibration point was run in triplicate over three consecutive days.

Extraction procedure

Aliquots of the drug in plasma (1 ml) were introduced via the injector port and swept onto the concentration column by water from pump A, whereupon the drug was retained on the sorbent while the gross plasma interferents were eluted to waste (Fig. 2). After a pre-determined wash time, the valve was rotated to position 2, which caused

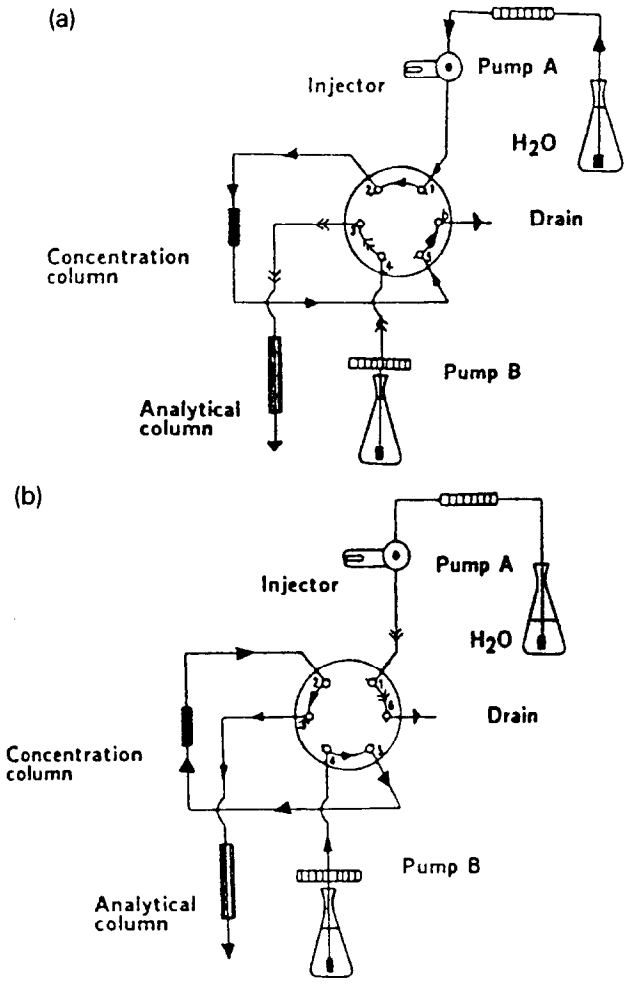

Fig. 2. Column-switching assembly, used for retaining terbutaline in the pre-concentration column (a) and eluting the drug from the pre-concentration column (b).

the analytical mobile phase to flow in a back-flush mode through the concentration column whence terbutaline was desorbed and swept onto the analytical column for separation.

RESULTS AND DISCUSSION

Hydrodynamic studies

The hydrodynamic voltammogram for terbutaline was obtained by injecting 35 ng/ml drug into the chromatograph with the detector set at different working electrode potentials *vs.* Ag/Ag_3PO_4. The hydrodynamic voltammogram shown in Fig. 3 indicates that a working potential

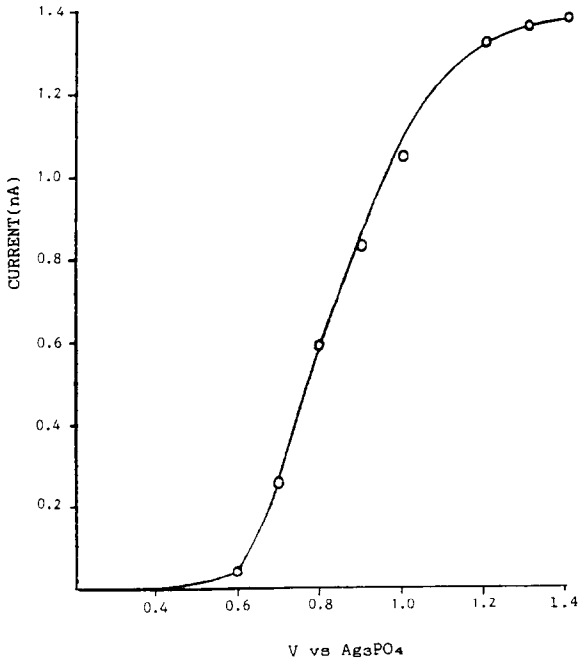

Fig. 3. Hydrodynamic voltammogram of terbutaline obtained by injecting 35 ng into the chromatogram with detection using the carbon fibre electrode at different potentials (*vs.* Ag/Ag_3PO_4).

of +1.4 V was required for maximum response. When the potential was greater than +1.3 V, both background current and the noise level increased rapidly. Accordingly, a potential of +1.3 V was used in subsequent experiments.

Optimisation of extraction procedure

Pre-column selection. Terbutaline can be extracted into organic solvents, either as a zwitterion [13] or as an ion pair [17,18]. Such off-line methods are time-consuming and may cause loss of the analyte through the formation of emulsions or artefacts, or by adsorption of the drugs onto glassware. Solid-phase extraction was chosen to circumvent some of these problems, and the column-switching technique described in this paper provided the requisite extraction selectivity in a reasonable time frame.

The first step in the set-up of the switching system involved selection of a suitable pre- (or concentration) column which would retain the drug. A short stainless-steel pre-column (10 mm × 1.5 mm I.D.) was chosen for this purpose. Pellicular

C_{18} material showed the most favourable retention characteristics for terbutaline when compared to C_8 and cyano packing materials and was therefore chosen as the pre-column packing. The next stage was to find two compatible eluents of different elutropic strengths [19]; one weakly eluting solvent to concentrate terbutaline on the pre-column and a second strongly eluting solvent to elute terbutaline off the pre-column and onto the analytical column. The choice of solvents is also important in terms of their mutual miscibility, as even slight incompatibility could result in a slug of solvent travelling down the analytical column partially carrying sample components which may cause band broadening. The washing/loading eluent (designated mobile phase A) ideally would have poor elution capability on the pre-column in order to ensure maximum concentration of the sample with minimum band broadening. Degassed, deionised water was found to provide adequate concentration and was compatible with an aqueous-based mobile phase (mobile phase B). This effected full elution of the drug from the concentration column, and any possible band broadening was minimised by eluting in a backflush direction. Typical chromatograms of drug-free plasma and a plasma extract spiked with terbutaline at the 30 ng/ml level are shown in Fig. 4a and b, respectively.

Boundary conditions. To determine the optimum wash time for the samples on the concentration column, plasma aliquots containing 10 ng/ml drug were injected onto the concentration column. The wash time (defined as the length of time between injection and switching of the valve) was varied between 1 and 3 min, and it was found that a maximum response was obtained at 1.7 min, in that it provided good clean-up of the plasma components without causing the drug to elute. Seven replicate injections of plasma samples containing 10 ng/ml terbutaline yielded a relative standard deviation (R.S.D.) of 5% for terbutaline.

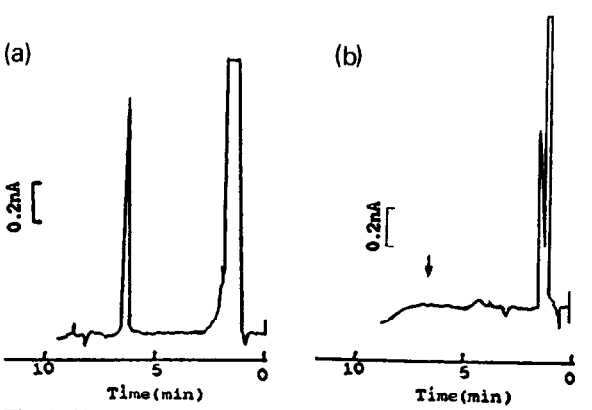

Fig. 4. Chromatogram obtained by analysis of drug-free plasma extract (a) and an extract of plasma spiked with 30 ng/ml terbutaline (b).

Electrode pre-treatment

A variety of methods have been devised for pre-treating carbon fibre electrodes, including laser treatment [20] and electrochemical activation [21]. The reason electrode pretreatment is required is that its surface changes with time due to either the adsorption of species from solution or chemical changes on the electrode surface itself. These changes often result in variations in sensitivity, reproducibility and selectivity.

One protocol which improved the peak current and reduced the noise to its orginal level involved the anodisation of the microelectrode at +1.4 V for 10 s, then cathodisation at −1.4 V for 10 s *vs.* Ag/Ag_3PO_4, followed by equilibration for 5 min. It has been proposed [22] that the improvement in performance is a result of the removal of surface contaminants or inhibitory layers which hinder electron transport. Thus, extended lifetime can be obtained after electrochemical pre-treatment, and the effect of the procedure described above is shown in Fig. 5b. The effect of pre-treat-

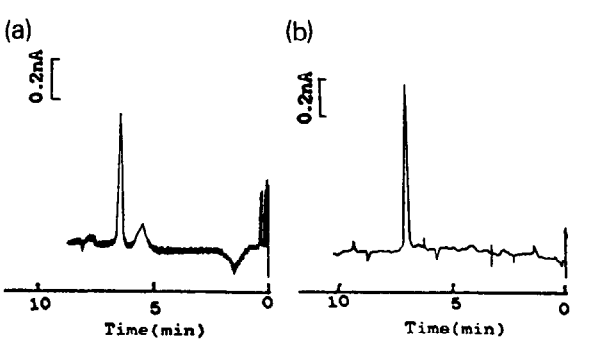

Fig. 5. Chromatograms of 20 ng/ml terbutaline obtained by analysing the drug (a) before and (b) after electrochemical pre-treatment of the working electrode surface.

ing the electrode is to increase its current response, which is probably due to activation of the surface, producing quinoidal functionalities [23]. It also seemed to stabilise the noise contribution due to the charging current. Another source of noise in the system might be the potentiostat, which was designed for a conventional glassy carbon working electrode and tailored to analyses at higher currents. A lower noise level would be expected with a potentiostat more suitable for the lower current encountered with the carbon fibre electrodes.

Assay performance

The linearity of the method was determined by constructing a calibration curve in the concentration range 0–35 ng/ml terbutaline in plasma, and, as shown from the data in Table I, the method is linear over this range with correlation coefficients of greater than 0.999 on each of three successive days.

The precision or reproducibility of the assay was evaluated in terms of the variability both between and within batches of replicate analyses at the concentrations 3, 5, 10, 20 ng/ml terbutaline in plasma. Within-batch or intra-assay variability was determined by analysing seven replicates at each of the concentrations. A calibration curve based on the mean (of seven) detector response values was constructed and individual peak current values were then interpolated on the regression line to yield seven new values of x (*i.e.* amount found) at each concentration. The mean, standard deviation (S.D.) and coefficient of variation (C.V.) for each amount found were then calculated, with the intra-assay being expressed as the mean C.V. over the entire concentration range.

Between-batch (inter-assay) variability was calculated by constructing a calibration curve on each of three consecutive days in the same concentration range as above. Peak current values were interpolated on the individual regression lines to yield three new values of amount found at each concentration level. Between-batch variability was then calculated by obtaining the mean C.V. over the calibration range. Results from this experiment are presented in Table II, and they show that the overall intra-assay C.V. was 4.4%, while the corresponding value for the inter-assay variability was 5.6%.

Recovery of terbutaline from plasma was assessed by comparing the peak current of extracted plasma samples at the 3, 8, 15 and 30 ng/ml levels with the peak current of authentic (unextracted, aqueous) standards, which were direclty injected (*i.e.* without column switching) onto the analytical column at all concentration levels. The results presented in Table III show that drug recovery was greater than 86% and usually greater than 91%. The minimum detectable concentration was found to be 0.8 ng/ml terbutaline in plasma, at which the signal-to-noise ratio was 3:1. This limit of detection compares favourably with previous methods for determination of terbutaline in biological fluids, and the method is capable of monitoring the therapeutic range of the drug.

Comparison with conventional glassy carbon-based detector

The performance of the micro-electrochemical flow cell was compared to a conventional macro-glassy carbon electrode flow cell, the electrode of which was polished successively with small-particle-size silicon carbide, alumina, diamond paste

TABLE I

CALIBRATION DATA FOR TERBUTALINE

Day	Regression equation	Correlation coefficient (r)
1	$y = 3.9 \cdot 10^{-2}(\pm 0.0004) + 0.0067(\pm 0.0001)x$	0.9997
2	$y = 3.8 \cdot 10^{-2}(\pm 0.0003) + 0.0041(\pm 0.0003)x$	0.9998
3	$y = 3.7 \cdot 10^{-2}(\pm 0.0004) + 0.0027(\pm 0.0002)x$	0.9999

TABLE II

INTRA-AND INTER-ASSAY VARIABILITY

Amount added (ng/ml)	Amount found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
Intra-assay (n = 7)		
3	2.9 ± 0.2	7.2
5	4.7 ± 0.2	5.3
10	9.6 ± 0.3	3.0
20	19.9 ± 0.4	2.3
Mean		4.4
Inter-assay (n = 3)		
3	3.0 ± 0.2	8.1
5	5.2 ± 0.3	7.0
10	10.1 ± 0.4	4.3
20	20.2 ± 0.50	3.0
Mean		5.6

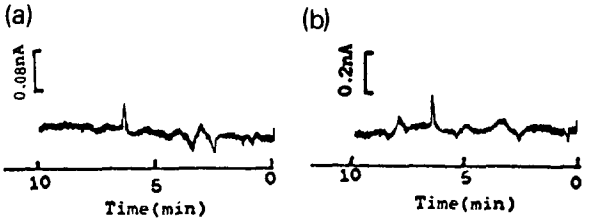

Fig. 6. Chromatograms obtained by analysis of (a) 1 ng/ml terbutaline using the carbon fibre micro-electrode at +1.3 V *vs.* Ag/Ag_3PO_4 and (b) 2 ng/ml terbutaline using a macro-glassy carbon electrode at +1.3 V *vs.* Ag/Ag_3PO_4.

and then subjected to ultrasonic cleaning [24]. As shown in Fig. 6, the detection limit of the carbon fibre electrode was shown to be approximately half that obtained using the macro-glassy carbon electrode. Similar findings have been reported by Luscombe and Bond [25] for the determination of copper in urine using platinum disc micro-electrodes instead of a conventional carbon disc macroelectrode, and in our laboratory, using a carbon fibre microelectrode for determination of salbutamol in human plasma [26] in place of a glassy carbon electrode. In addition, the cell design can overcome many problems associated with a conventional glassy carbon-based detection system. For example, the glassy carbon electrode requires frequent polishing, which can be quite tedious, fluid can leak from the cell, air bubbles can form in the cell, and a high electrolyte concentration is needed in order to reduce the *iR* drop in the cell caused by the large surface area.

CONCLUSION

An HPLC method for the determination of terbutaline in human plasma, based on pre-column switching and electrochemical detection using a carbon fibre flow cell, has been described. The column-switching technique permitted selective retention of terbutaline of the pre-column and provided satisfactory clean-up to avoid any interferences due to endogenous plasma components. The micro-flow cell described here is simple to prepare and easy to manage. The cell has the advantage of a small *iR* drop, virtually insignificant capacitive charging currents and a rapid response due to rapid mass transport to and from the working electrode. The limit of detection of the method was found to be 0.8 ng/ml,

TABLE III

RECOVERY STUDY

Terbutaline concentration (ng/ml)	Detector response (nA)		Recovery (%)	Coefficient of variation (%)
	Extracted standards	Authentic standards		
3	0.12	0.14	86	9.2
5	0.59	0.63	93	6.0
8	0.31	0.34	91	7.4
30	1.21	1.26	96	3.5

the mean overall C.V. was 5.60%, and drug recovery was in excess of 86% over the concentration levels investigated.

REFERENCES

1 R. McLean, in A. Burger (Editor), *Medical Chemistry*, Interscience Publishers, New York, 1960, p. 595.
2 L. E. Edhom, B. M. Kennedy and S. Bergquist, *Chromatographia*, 16 (1982) 341.
3 K. Randerath, *Thin Layer Chromatography*, Academic Press, New York, 1965, p. 51.
4 N. J. Sagliano, T. V. Reglione and R. A. Hartwick, *Chem. Anal.*, 98 (1989) 643.
5 M. Telting-Diaz, M. T. Kelly, C. Hua and M. R. Smyth, *J. Pharm. Biomed. Anal.*, in press.
6 C. E. Werkhoven-Goewie, C. de Ruiter, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.*, 255 (1983) 79.
7 C. E. Lunte, J. F. Wheeler and W. R. Heinehman, *Anal. Chim. Acta*, 200 (1987) 101.
8 P. T. Kissinger, C. S. Bruntlett and R. E. Shoup, *Life Sci.*, 28 (1981) 455.
9 L. A. Knecht, E. J. Gutherie and J. W. Jorgenson, *Anal. Chem.*, 56 (1984) 479.
10 S. P. Kounaves and J. B. Young, *Anal. Chem.*, 61 (1989) 1469.
11 E. B. Tay, S. B. Khoo and S. W. Loh, *Analyst*, 114 (1989) 1039.
12 R. A. Clare, D. S. Davies and T. A. Baillie, *Biomed. Mass Spectrom.*, 6 (1979) 31.
13 A. Jacobsson, S. Jonsson, C. Lindberg and L. A. Svensson, *Biomed. Mass. Spectrom.*, 7 (1980) 265.
14 C. Hua, K. Sagar, K. McLaughlin, M. Jorge and M. R. Smith, *Analyst*, 116 (1991) 1117.
15 D. R. Jarvie, A. M. Thompson and E. H. Dyson, *Clin. Chim. Acta*, 108 (1987) 313.
16 M. T. Kelly and M. R. Smyth, *J. Pharm. Biomed. Anal.*, 7 (1989) 1757.
17 J. G. Leferink, I. Wagemaker-Engels, R. A. A. Lamont, R. Pauwels and M. J. van der Straeten, *J. Chromatogr.*, 143 (1977) 299.
18 R. Modin and M. Johansson, *Acta Pharm. Suec.*, 8 (1971) 561.
19 C. E. Goewie and E. A. Hogendoorn, *J. Chromatogr.*, 410 (1987) 211.
20 T. G. Sterlin and A. G. Ewing, *Anal. Chem.*, 63 (1991) 194.
21 J. Wang, P. Tuzhi and V. J. Villa, *J. Electroanal. Chem.*, 234 (1987) 119.
22 W. J. Blaedal and R. A. Jenkins, *Anal. Chem.*, 46 (1974) 1952.
23 T. J. O'Shea, A. C. Garcia, P. T. Blanco and M. R. Smyth, *J. Electroanal. Chem.*, 307 (1991) 63.
24 G. N. Kamau, W. S. Willis and J. F. Rusling, *Anal. Chem.*, 57 (1985) 545.
25 D. L. Luscombe and A. M. Bond, *Anal. Chem.*, 62 (1990) 27.
26 K. S. Sagar, C. Hua, M. T. Kelly and M. R. Smyth, *Electroanalysis*, in press.