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Short communication

Simple method for determination of terbutaline plasma concentration by high-performance liquid chromatography

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Abstract

A method is described in which low nanomolar concentrations of terbutaline in plasma can be quantitated by use of a standard isocratic high-performance liquid chromatography system with electrochemical detection. Samples were prepared for injection by solid-phase extraction and preserved from degradation by addition of glutathione. Terbutaline and internal standard metaproterenol were resolved from plasma constituents on a single C_{18} column by ion-pairing chromatography. The method is precise and accurate for measurement of freebase concentrations as low as 4.4 nmol/l (1 ng/ml). © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Due to its low concentration in plasma, terbutaline in samples from pharmacokinetic studies was initially analyzed only by GC–MS [1–3]. However, many investigators do not have these analytical capabilities. Thus, several HPLC methods have been developed. Most of the available assays rely on column switching, both with [4,5] and without sample pretreatment [6] before injection. Such methods require two or more pumps, detectors and columns, as well as microprocessor-controlled switching equipment. A few HPLC methods utilizing standard isocratic chromatography systems have also been published. However, they were developed for analysis of samples from 'brittle asthma' patients taking large doses [7], or patients who had overdosed [8]. Thus, these assays do not have the sensitivity required for accurate analysis of samples obtained during pharmacokinetic studies that employ normal therapeutic doses of the drug. In this report, we describe a simple method for measurement of terbutaline concentrations in plasma following normal therapeutic doses.

2. Experimental

2.1. Materials

2.1.1. Drug standards and dosage forms

Terbutaline hemisulfate and metaproterenol hemisulfate were obtained from Sigma (St. Louis, MO, USA). Terbutaline sulfate for injection was

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manufactured by Novartis Pharmaceuticals (Summit, NJ, USA).

2.1.2. Extraction materials

Solid-phase extraction columns were 3 ml polypropylene columns packed with 200 mg of C_{18} bonded phase from J.T. Baker (Phillipsburg, NJ, USA). Reagent-grade monobasic and dibasic sodium phosphates were from Mallinckrodt Specialty (Chesterfield, MO, USA). Absolute ethanol was USP grade from McCormick Distilling (Weston, MO, USA). Molecular biology-grade ammonium chloride and SigmaUltra-grade reduced glutathione were from Sigma (St. Louis, MO, USA). Syringe filters were 4-mm diameter, 0.2-μm porosity nylon membrane units from Alltech Associates (Deerfield, IL, USA).

2.1.3. Mobile phase materials

Monobasic potassium phosphate and anhydrous dibasic sodium phosphate were reagent grade from Mallinckrodt. The remaining mobile phase reagents were of HPLC grade and obtained from commercial sources. The 47-mm diameter, 0.2-μm porosity polyvinylidene difluoride (PVDF) filtration membranes were from Gelman Sciences (Ann Arbor, MI, USA).

2.2. Apparatus

The mobile phase was pumped through the system by a reciprocating piston pump (Model LC 10-AD, Shimadzu Scientific Instruments, Columbia, MD, USA). Samples were injected using a variable injection volume autoinjector (Model AS-300, Spectra Physics Analytical, Fremont, CA, USA). The prepacked 5-μm C_{18} guard column was from Alltech Associates. The analytical column was a 150×4.6-mm Dynamax column from Rainin Instrument (Woburn, MA, USA) packed with 5-μm, 100-Å pore size, Microsorb C_{18} silica stationary phase. Analytes were detected using a Coulochem II amperometric detector with a Model 5011 high sensitivity flow cell and a Model 5020 guard cell, all from ESA (Bedford, MA, USA) The detector signal was processed on a Shimadzu model CR501 computing integrator.

2.3. Chromatographic conditions

The mobile phase was 25 m*M* phosphate buffer, pH 7.4:methanol (77:23, v/v), with 2 m*M* 1-octanesulfonic acid. It was filtered, degassed by sonication and pumped through the system at a flow-rate of 0.7 ml/min, at room temperature. The electrochemical detector guard cell was set at +700 mV potential. The analytical cell screen electrode was set at +450 m*M* and the analytical electrode was set at +700 m*M* potential. The signal filter was set to 0.2 s. These potentials were based on recommendations from an application note from the detector manufacturer [9] and experiments in our laboratory showing terbutaline begins to oxidize at a potential of about +450 mV. This low potential was used for the analytical cell screen electrode to improve sensitivity, realizing this would also cause a loss of selectivity. Detector response peaked at about +1000 m*M*, but running at this potential caused rapid loss of response, due to fouling of the electrode by oxidizable materials from the sample. The potential of +700 mV was selected for the analytical electrode to minimize fouling of the electrode and also to minimize the size (and thus interference) of the glutathione peak. Running at +700 mV required periodic storing of the column and rinsing the system with a mobile phase containing 0.9 *M* acetic acid to remove materials adsorbed to the electrodes.

2.4. Extraction procedure

The extraction procedure was based on that described by Kennedy et al. [5] with minor modifications. Two milliliters of plasma were added to a culture tube. Twenty μl of a 1 ng/μl solution of metaproterenol (I.S.) in methanol and 1 ml of 10 m*M* sodium phosphate buffer (pH 7.5) were then added, and the samples mixed. Solid-phase extraction columns were preconditioned with 2×3 ml of ethanol, followed by 2×3 ml water. Plasma samples were then passed through the columns. They were next rinsed with 2×3 ml of water. Receiver tubes containing 50 μl of 50 m*M* glutathione were then placed inside the vacuum manifold. The drugs were eluted from the columns with 1 ml of ethanol:50 m*M* ammonium chloride buffer, pH 8.5 (95:5, v/v). The

samples were then dried under nitrogen in a water bath at 30°C, reconstituted with 200 μl of mobile phase, vortex-mixed, and transferred to 1.5-ml microcentrifuge tubes. The tubes were centrifuged at 13 400 *g* for 2 min. Each sample was then passed through a syringe filter into a conical polypropylene autosampler vial. The autosampler was programmed to inject 170 μl of each sample.

2.5. Calibration and reproducibility

Standard curves were produced by injecting extracted samples prepared from blank plasma stocks that had been spiked to concentrations of 15, 7, 5, 3 and 1 ng freebase per ml of plasma (66.6, 31.1, 22.2, 13.3 and 4.4 nmol/l, respectively). The linear equation describing the relationship between terbutaline concentration and the peak height ratio was determined using weighted linear regression analysis, with the method of weighting chosen as $1/\text{concentration}^2$ for each standard. The coefficient of determination was used as an estimate of goodness-of-fit, calculated as the regression sum of squared residuals/total sum of squared residuals. However, the final decision on the goodness-of-fit was made based on the control sample results.

Within-day variability and precision were measured using control samples made by spiking blank plasma to concentrations of 10, 6 and 2 ng freebase per ml plasma (44.4, 26.6 and 8.9 nmol/l, respectively). Five extracted samples were injected at each control concentration on one day. Between-day variability and precision were evaluated by injecting one extracted sample at each control concentration each day for 5 days. Control sample concentrations were determined from the standard curve run on each day of analysis.

2.6. Extraction efficiency

Extraction efficiency was determined at 10, 6 and 3 ng/ml (44.4, 26.6 and 13.3 nmol/l). For each concentration, extraction efficiency was calculated by comparing the mean peak height obtained from four injections of standards in mobile phase with the peak heights in four extracted plasma samples.

2.7. Pharmacokinetic evaluation

Subjects were caffeine and alcohol free for at least 12 h before and throughout the study day, and avoided all over the counter medications for 24 h prior to the study. Chronic prescription medications, except for antihypertensive drugs, were continued and none of the concurrent medications appeared to cause interference with the assay, except gemfibrozil (and/or its metabolites). Indwelling venous catheters were inserted into the forearm and terbutaline sulfate was administered by constant rate infusion at a dose of 0.11 μg/kg per min for 60 min. Blood samples were collected before initiation of the infusion, every 10 min during the infusion, and at 30, 60, 90, 120, 180, 240, 300 and 360 min after the infusion.

3. Results and discussion

Representative chromatograms resulting from use of the assay described herein are shown in Fig. 1. These include chromatograms of an extracted, blank plasma sample, and extracted subject plasma samples. As can be seen in Fig. 1, the retention times of the I.S. (metaproterenol) and terbutaline were approximately 12 and 21 min, respectively, with complete baseline resolution between peaks of interest. The metaproterenol peak elutes on the tail of a large glutathione peak, but the integrator was able to measure metaproterenol's peak height reliably. The terbutaline peak eluted after the signal had returned to baseline.

Extraction of terbutaline using the described procedure was both consistent and efficient. The mean extraction efficiencies at terbutaline concentrations of 13.3 and 44.4 n*M* were 93±5% (C.V.=5.7%) and 91±8% (C.V.=6.1%), respectively. Extraction efficiency of the I.S. was 70±4% (C.V.=6.1%). Extraction efficiency was consistent over the concentration range.

Standard curves were linear over the concentration range, with coefficients of determination greater than 0.9980. The mean±SD of the slope and intercept of the regression lines were 0.1132±0.0104 and −0.0030±0.0070, respectively. Table 1 summarizes the results of the within- and between-day measurements of precision and accuracy. We found the limit

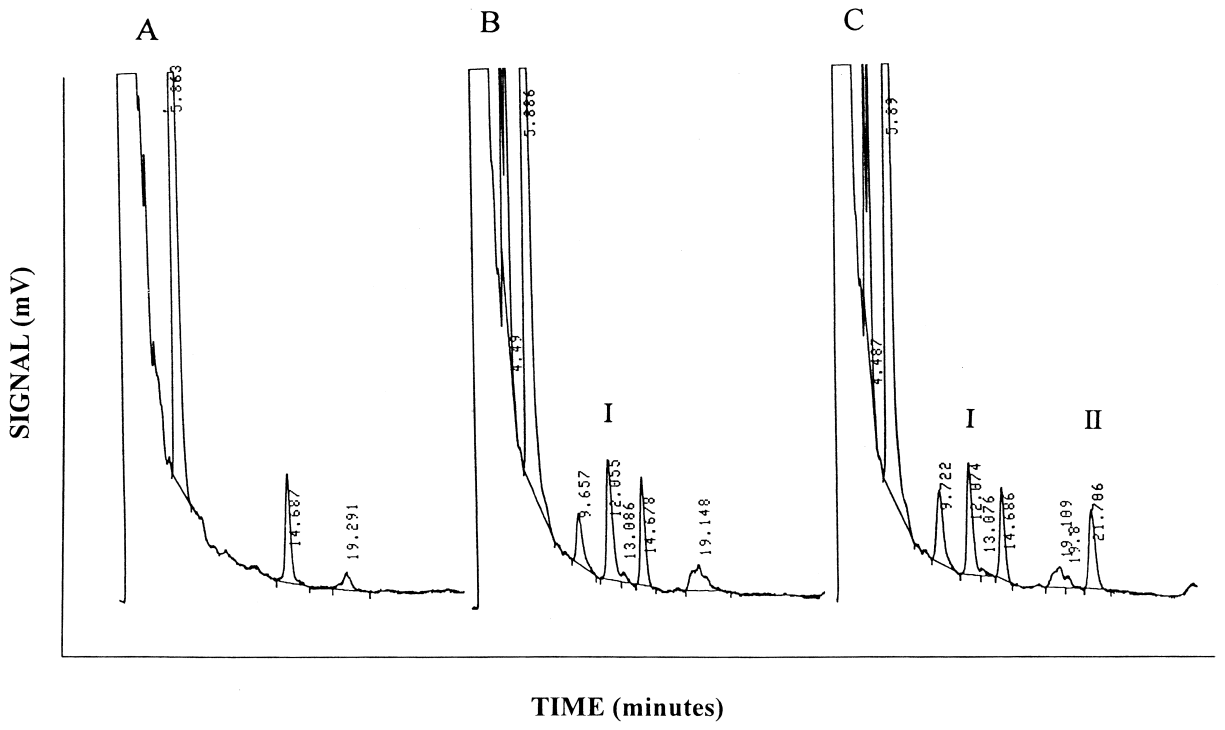

Fig. 1. Representative chromatograms of (A) extracted blank plasma, (B) extracted subject sample at time zero, (C) extracted subject sample; terbutaline concentration, 8.1 ng/ml (35.8 nmol/l). Chromatographic peaks: (I) metaproterenol (internal standard), (II) terbutaline.

of detection (defined as a signal-to-noise ratio of 4:1) to be about 0.4 ng terbutaline on column or approximately 0.2 ng/ml using the procedures we described.

Table 1
Precision and accuracy in the determination of terbutaline in plasma

Spiked concentration[a] (nmol/l)	Observed concentration[b] (nmol/l)	Accuracy (obs/actual) (%)	Precision (C.V.) (%)
Within-day variation ($n=5$)			
44.4	47.45	107	7.10
26.6	25.57	96	2.65
8.9	8.61	97	3.48
4.4	4.49	101	6.46
Between-day variation ($n=5$)			
44.4	46.07	104	8.91
26.6	25.70	97	5.99
8.9	8.74	98	7.20
4.4	4.36	98	6.36

[a] All concentrations reported as freebase.
[b] Reported observed concentrations represent the mean values from all experiments.

When the limit of quantitation is defined as that concentration which can be determined with a relative error of the mean of less than ±10%, and relative standard deviation of 10% or less, the limit of quantitation was found to be 1 ng/ml.

Finally, the assay was successfully employed for a clinical pharmacokinetic study. The assay allowed us to easily quantitate terbutaline plasma concentrations in all 23 study patients. A representative plasma concentration–time profile is shown in Fig. 2. The range of maximal observed concentrations (C_{max}) found in our study was 5.46–17.87 ng/ml, and the median was 9.06 ng/ml. The 17.87 ng/ml C_{max} was the only one of 344 samples that was >15 ng/ml, thus our standard curve range of 1–15 ng/ml was appropriate for determination of terbutaline plasma concentration following the dose we administered. The average coefficient of determination for the log-linear portion of the concentration–time profiles was 0.97±0.02, which is excellent for the terminal phase of a pharmacokinetic curve, and suggests the assay provides accurate and precise measurements of

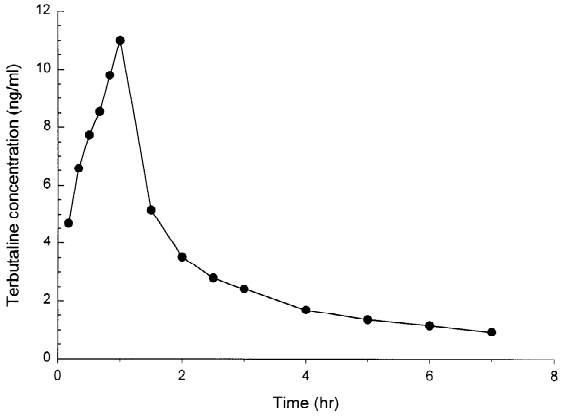

Fig. 2. Representative terbutaline plasma concentration–time curve in a hypertensive patient, as determined by the assay described herein.

plasma concentration with little variability across samples.

In three of 23 subjects, a small but quantitatable peak (equal to 1.1, 1.2 and 2.6 ng/ml terbutaline) eluted at the terbutaline retention time in the time zero (predose) sample. Potential explanations for this observation are that: (a) the subjects actually began receiving their terbutaline infusion just prior to collection of the time zero sample in which case the observed terbutaline concentrations would be real, and explained by nursing error; (b) concomitant drugs produced a peak that coeluted with the terbutaline peak; or (c) the peak represents coelution of some endogenous substance. It is difficult to attribute any one of these potential explanations to all three patients. The patient with the 2.6 ng/ml zero time sample was on no other medications at the time of the study, nor does this appear to be an endogenous peak since the last five samples (2–6 h after the dose) were all lower than 2.6 ng/ml. For this patient we believe the most likely explanation is sample contamination. This is because the nurses recorded having great difficulty with catheter insertion and blood sample collection in this patient. Additionally, the patient's 10-min sample concentration was lower than that at zero time, further suggesting problems with collection/labelling of the zero time sample. The other two patients had much lower zero time concentrations and these may represent an endogenous peak in these patients since all their samples had concentrations greater than 1 ng/ml (whereas concentrations dropped below 1 ng/ml by 7 h in most patients). Additionally, one of these patients was on low dose buspirone at the time of study. Based on this information, we conclude there may be an endogenous substance that coelutes with terbutaline in less than 10% of patients. Given the small size of this peak, the fact that it occurs in a small percentage of patients and does not impact pharmacokinetic calculations, we do not believe it poses any problem related to the clinical utility of the assay.

4. Conclusions

The assay described is a clinically useful, relatively simple HPLC assay for quantitating terbutaline concentrations in plasma resulting from a normal therapeutic dose. It employs an efficient solid-phase extraction procedure, and uses a standard isocratic method to separate the sample components on a single column. This assay provides a simple alternative to GC–MS or bimodal HPLC for those who wish to undertake pharmacokinetic studies of terbutaline.

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