Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 7, pp. 591–596, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Determination of albuterol concentrations in human plasma using solid-phase extraction and high-performance liquid chromatography with fluorescence detection

R.E. BLAND,† R.J.N. TANNER,† W.H. CHERN,‡ J.R. LANG*‡ and J.R. POWELL‡

† *Department of Biochemical Pharmacology, Glaxo Group Research Ltd, Ware, Herts SG12 0DJ, UK*
‡ *Department of Clinical Pharmacokinetics, Pharmaceutical Development, Glaxo Inc., Five Moore Dr., Research Triangle Park, NC 27709, USA*

Abstract: A fast, simple, and accurate method for determining albuterol concentrations in human plasma has been developed and validated for use in routine clinical analyses. This method involves a solid-phase extraction procedure using silica cartridges and normal-phase high-performance liquid chromatography with fluorescence detection. Regression analysis showed the method was linear over the standard curve range 1–16 ng ml^{-1}. The percent recovery for albuterol and the internal standard, bamethane, at 5 ng ml^{-1} was found to be >90%. The newly developed method has been applied in the analysis of plasma samples from patients and healthy volunteers.

Keywords: *Albuterol; high-performance liquid chromatography; solid-phase extraction; fluorescence detection; human plasma; routine sample analysis; pharmacokinetic studies.*

Introduction

Albuterol, 2-(tert-butyramino)-1-(4-hydroxy-3-hydroxy-methylphenyl)ethanol, also referred to as salbutamol in Europe, is the active ingredient in Ventolin® products. Albuterol is a relatively selective β_2-adrenergic bronchodilator, which has been used to relieve bronchospasm in patients with reversible obstructive airway diseases for almost 20 years [1, 2]. The estimated therapeutic plasma concentration of oral albuterol preparation is between 5.5–7.5 ng ml^{-1} with a half-life of approximately 4–6 h [3, 4].

For many years, the only published procedures for quantitating albuterol plasma concentrations involved gas chromatography with mass spectrometry [5, 6]. These methods required time-consuming derivatization procedures and used elaborate instrumentation not available in most clinical laboratories. Recently, numerous HPLC procedures have been published utilizing fluorescence and electrochemical detection, which are techniques commonly used in routine analysis [7–12]. However, the methods involved tedious sample extraction procedures [7–10], long run times [7, 8, 10], ion-pair reagents and solvent modifiers [7, 9, 10, 12], or complicated chromatography conditions involving column switching designs [12].

This paper describes an HPLC method using a simple solid-phase extraction procedure. The method employs normal phase chromatography with a run time of approximately 8 min. This method has been shown to be sensitive, accurate, reproducible and is suitable for analysing a large number of samples to support bioavailability and pharmacokinetic studies.

Experimental

Reagent and materials

Albuterol sulphate standards (batch No. WC01456) was acquired from the Biochemical Pharmacology Department, Glaxo Group Research (Ware, UK). Bamethane (α-[(butylamino)methyl]-4-hydrobenzenemethanol) sulphate (lot 66C-0151) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol (HPLC grade) were obtained from Burdick and Jackson Co. (Muskegeon, MI, USA). Ammonium acetate anhydrous was purchased from Aldrich Chem-

* Author to whom correspondence should be addressed.

ical Co. (Milwaukee, WI, USA). Bond-Elut® SPE silica cartridges, 100 mg (Part No. 601101, SI Lot No. 012689) were supplied by Analytichem International (Baltimore, MD, USA).

Instrumentation and conditions

The vacuum manifold box which holds 50 solid-phase extraction cartridges was obtained from Glaxo Group Research, UK [13]. The Speed Vac Concentrator with a −60°C refrigerated trap was purchased from Savant Instruments Co. (Farmingdale, NY, USA). The HPLC system consisted of a Spectra-Physics SP8780 autosampler, a Kratos Spectraflow 400 pump and a Kratos Spectraflow 980 fluorescence detector. Detector parameters were 0.005AUF, 2 s response time, an excitation wavelength set at 225 nm. With such a low excitation wavelength, we were able to eliminate the cut-off filter to achieve maximal sensitivity. The chromatographic data were acquired by the Maxima Chromatography Workstation (version 2.1, Dynamic Solutions, Ventura, CA, USA) on an IBM/AT computer interfaced with a local area network Netware (Novell Inc., Provo, UT, USA).

An upright precolumn filter with 2 μm replacable frit was used to protect the 3-μm Spherisorb® silica analytical column (4.6 mm × 10 cm). The mobile phase consisted of 0.25% ammonium acetate (pH 7.5) aqueous solution (2 M) in methanol, which was filtered and degassed by continuous helium sparging. The analytical column and its prefilter were kept in a column heater (Jones Chromatography) at 40°C. Back pressure of the system was maintained under 1500 psi with a 1 ml min^{-1} flow rate.

Standard solution

An aqueous solution containing 1 μg ml^{-1} of albuterol base was prepared and stored at 4°C and used to prepare calibration standards for 7 days. An aqueous stock solution of 0.1 μg ml^{-1} bamethane base was prepared and separated into 5 ml aliquots and stored in individual glass vials at −20°C. During the study, an aliquot was thawed daily and used in the sample preparation. Fifty microlitres (5 ng bamethane base) of this solution was added to each calibration standard, quality control sample, and subject sample analysed.

Preparation of calibration standards

Aliquots of 50, 100, 200, 400 and 800 μl of albuterol standard solution were added to drug-free control plasma to give a final volume of 50 ml corresponding to 1, 2, 4, 8 and 16 ng ml^{-1} standards. Each standard was separated into 2.5 ml aliquots and stored at −20°C until required during the method validation and study analysis. For clinical sample analysis, three concentrations of quality control plasma samples were prepared and stored by a similar procedure at 1.9, 7.5 and 15 ng ml^{-1}, and were analysed along with unknown samples. Albuterol has been found here to be stable when stored between −10 to −20°C for up to 54 weeks.

Preparation of plasma samples

One-millilitre aliquot of a subject plasma, standard, or quality control sample and 50 μl of internal standard solution were transferred to a prelabelled culture tube and well mixed. The solid-phase (silica) extraction columns were preconditioned by washing with 1 ml of acetonitrile followed by 1 ml of deionized water. The wash solvents were allowed to pass through with minimum vacuum (<50 mmHg) which was released immediately after the solvents eluted from all the cartridges. One millilitre of the previously mixed plasma sample was transferred into the preconditioned column and minimum vacuum was applied. When all plasma in the cartridge reservoir had been removed, the vacuum was increased to 500 mmHg for 2 min and then released. Each column was then washed with 1 ml of deionized water followed by 1 ml of acetonitrile under minimum vacuum until all the wash solvents eluted from the cartridges. The columns were dried by full vacuum for an additional 2 min. Albuterol and bamethane were then eluted from the silica sorbent by rinsing twice with 1 ml of methanol under minimum vacuum until no effluent was observed. The sample tubes containing the column effluents were carefully removed from the collection rack and placed into the Speed Vac concentrator. Methanol was evaporated to dryness under reduced pressure at 50°C for approximately 1 h. The extracted samples were reconstituted in 200 μl mobile phase, with 100 μl injected into the HPLC system.

Results and Discussion

Using the described chromatography procedure, albuterol and bamethane can be easily

separated (Rs = 1.6), with retention times of approximately 4.7 and 3.9 min, respectively. Chromatograms of extracted blank plasma, albuterol and internal standard spiked plasma are shown in Fig. 1, and Fig. 2 shows typical chromatograms from extracted patient samples. Although an endogenous peak eluted at approximately 5 min, its presence did not interfere with the albuterol peak and was not considered a problem. Injections of solutions containing theophylline, metaproterenol, isoproterenol, epinephrine and terbutaline revealed no peak interferences.

The strategy in developing this procedure was to design a rugged method with a short run time, utilizing a simple solid-phase extraction coupled with the sensitivity and selectivity characteristics of fluorescence. In comparison,

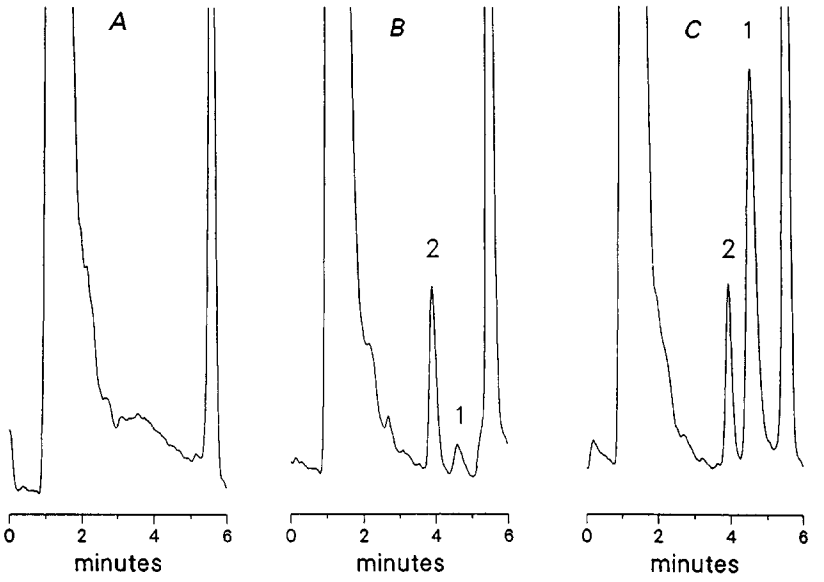

Figure 1
Chromatograms of 1-ml plasma extracts. (A) Blank plasma; (B) blank plasma spiked with 1 ng ml^{-1} albuterol and 5 ng ml^{-1} internal standard; (C) blank plasma spiked with 16 ng ml^{-1} albuterol and 5 ng ml^{-1} internal standard. Peaks: 1 = Albuterol; 2 = internal standard (bamethane).

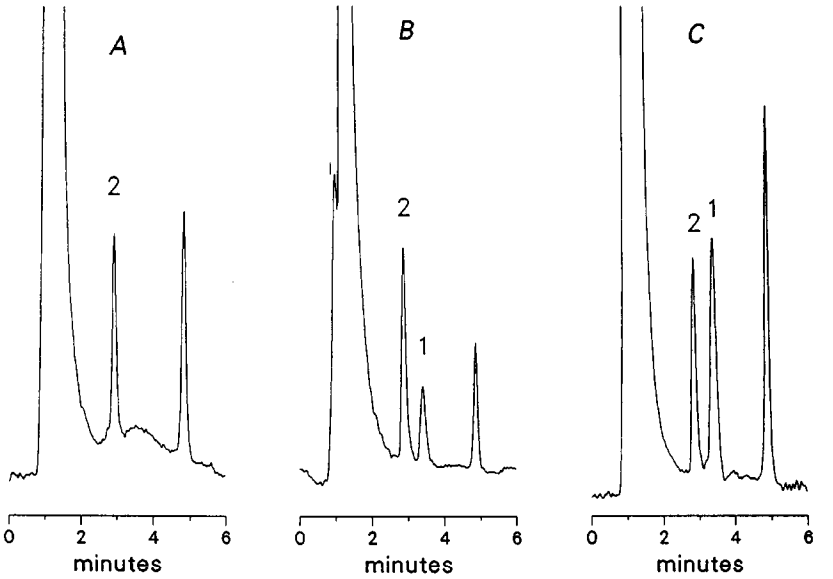

Figure 2
Chromatograms obtained by analysis of 1-ml plasma from a patient 4 h after the treatment of (A) placebo; (B) Volmax® tablet, 4 mg; (C) Volmax® tablet, 8 mg. Peaks: 1 = Albuterol; 2 = internal standard.

the referenced fluorescence and electrochemical procedures involved tedious ion-pair extractions [7], complicated solid-phase extractions [8, 9], and electrode stability and selectivity problems [8, 9, 11, 12] and thus, are not desirable for use on a routine basis.

Linearity, precision and accuracy

The reported method was found to be linear ($r \geq 0.9900$) over the standard curve range from 1 to 16 ng ml^{-1}, based on the peak area ratio of albuterol to bamethane. The slopes ranged from 0.171 to 0.203 and the estimated intercepts did not differ significantly from the origin.

The reproducibility of the method was assessed by statistical analysis of the daily standard curves and quality control samples. Within-run precision was determined by analysing six replicates at each drug concentration. The relative standard deviations (RSD) were found to range from 2.4 to 10.3% throughout the standard curve. Between-run precision was determined by analysing duplicates at each standard concentration over four different days. Results from between-day runs gave RSDs between 5.8–9.6%. The statistical analysis from the within-run and between-runs is shown in Table 1. Extrapolated results from the quality control samples gave similar RSDs to those obtained from the standard curves, and the percent bias was found to range from 3.4 to 13.6%, as shown in Table 2. These results clearly demonstrate the reproducibility and accuracy of this method. The limit of detection based on a signal-to-noise ratio (2:1) was found to be 0.5 ng ml^{-1}.

Recovery

Various types of sorbents including C_2, C_8, C_{18}, CN and silica were evaluated to determine their extraction efficiencies. The results from our investigations showed that C_2, C_8 and C_{18} columns did not provide the selectivity necessary to separate albuterol from endogenous compounds, whereas the cyano and silica sorbents cleaned the plasma samples adequately. However, the recovery of albuterol from the cyano columns was not reproducible and was considered unacceptable. Both albuterol and bamethane were extracted efficiently, and their recoveries using silica extraction columns were found to be reproducible.

The absolute recovery of albuterol and bamethane were determined by comparing the peak areas of extracted plasma standards to non-extracted standards at equivalent concen-

Table 1
Within-day and between-day variability of albuterol assay

Nominal standard concentration (ng ml^{-1})	Within-day precision: Calc. mean* concentration (ng ml^{-1})	RSD (%)	Bias (%)	Between-day precision: Calc. mean† concentration (ng ml^{-1})	RSD (%)	Bias (%)
1	0.95	7.6	5.0	1.11	9.6	11.0
2	1.82	10.3	9.0	2.01	6.7	0.5
4	3.89	5.5	2.8	4.00	6.9	0.0
8	7.70	3.2	3.8	7.78	7.2	2.8
16	15.80	2.4	1.3	16.07	5.8	0.4

*$n = 5$.
†$n = 8$.

Table 2
Analysis results from quality control samples

QC nominal concentration (ng ml^{-1})	Extrapolated concentrations (ng ml^{-1}): Run 1	Run 2	Run 3	Run 4	Run 5	Mean	RSD (%)	Bias (%)
1.0	—	1.66	1.51	1.98	1.62	1.62	10.9	13.6
		1.49	1.39	1.70	1.60			
7.5	6.66	6.68	7.82	7.56	6.58	7.17	6.2	4.4
	7.13	7.41	6.76	7.46	7.65			
15.0	14.23	14.02	13.28	14.62	15.06	14.49	4.8	3.4
	14.38	15.10	14.21	14.23	15.78			

trations. The mean recovery of bamethane at 5 ng ml^{-1} was 97.2% with a RSD of 7.3% (n = 24). The overall recovery of albuterol was found to be 97.7%, as shown in Table 3.

Table 3
Recovery of albuterol from human plasma

Plasma albuterol concentration (ng ml^{-1})	Percentage recovery (mean* ± SD)	RSD (%)
1	106.3 ± 8.6	8.1
2	89.3 ± 10.6	11.8
4	99.8 ± 12.3	12.4
8	97.3 ± 6.5	6.7
16	97.1 ± 12.2	12.6

*n = 4.

Clinical studies

This method has been used to obtain albuterol plasma concentrations in several clinical trials including bioavailability studies in healthy volunteers and efficacy studies in

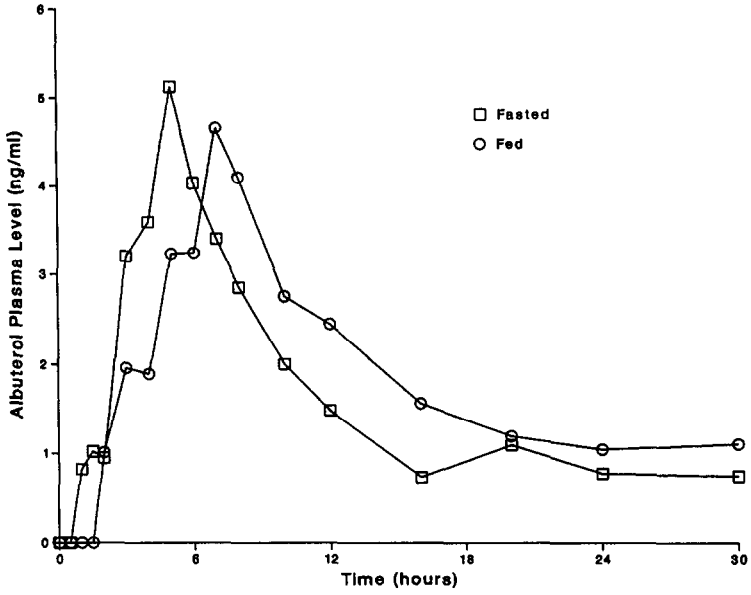

Figure 3
Plasma albuterol concentration–time curves in a normal volunteer following oral administration of 4 mg Volmax®. (A) Fasting, no food allowed 8 h prior to and 4 h after the dose; (B) fed, Volmax® tablet was given immediately following a standard breakfast.

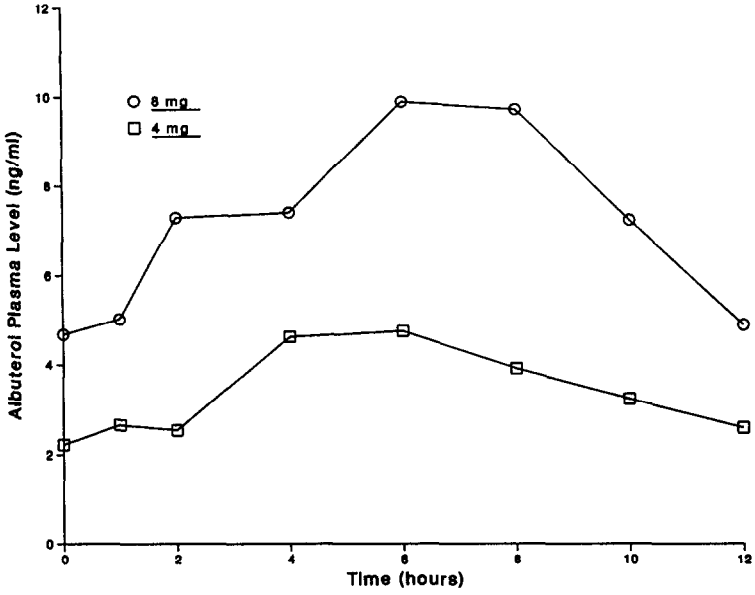

Figure 4
Plasma albuterol concentration–time curves at steady state in a patient on the seventh day following Volmax® administration every 12 h. (A) Volmax®, 4 mg; (B) Volmax®, 8 mg.

patients. Approximately 80 samples per day can be analysed with a typical run time of 8–10 h. Figure 3 shows the plasma concentration curve in a normal young male subject after receiving a single 4 mg dose of Volmax® while fasting and postprandial. We were able to monitor the albuterol plasma concentrations up to 30 h after the dose. The typical plasma concentration–time curves in a patient on the seventh day following administration of a Volmax® tablet every 12 h is shown in Fig. 4. These examples clearly demonstrate that the quality of data provided by this assay allows accurate and precise pharmacokinetical analyses.

Conclusion

We have successfully developed a simple and reliable method for quantitating albuterol concentrations with improved sensitivity over previously published fluorometric methods [7, 10]. The procedure utilizes a solid-phase extraction using silica columns with an overall recovery >97%. The method has been used in numerous clinical studies involving thousands of samples.

Acknowledgement — The administrative assistance provided by Elsbeth van Tongeren is gratefully acknowledged.

References

[1] V.A. Cullem, J.B. Farmer, D. Jack and G.P. Levy, *Br. J. Pharmacol.* **35**, 141 (1969).
[2] J.B. Farmer and G.P. Levy, *Br. J. Pharmacol.* **35**, 358 (1969).
[3] M.L. Powell, M. Weisberger, R. Gural, M. Chungh, J.E. Patrick, E. Radwanski and S.S. Symchowics, *J. Pharm. Sci.* **74**, 217 (1985).
[4] J.W. Jenne and R.C. Ahrens, *Drug Ther. Asthma* **31**, 213 (1987).
[5] R.J.N. Tanner, L.E. Martin and J. Oxford, *Anal. Proc.* **20**, 38 (1983).
[6] M. Weisberger, J.E. Patrick and M.L. Powell, *Biomed. Mass Spectrom.* **10**, 556 (1983).
[7] M.J. Hutchings, J.D. Paull and D.J. Morgan, *J. Chromatogr.* **227**, 423 (1983).
[8] D.R. Jarvis, A.M. Thompson and E.H. Dyson, *Clinica Chim. Acta* **168**, 313 (1987).
[9] Y.K. Tan and S.J. Soldin, *J. Chromatogr.* **311**, 311 (1984).
[10] B. Oosterhuis and C.J. Van Boxtel, *J. Chromatogr.* **232**, 327 (1982).
[11] T. Emm, L.J. Lesko, J. Leslie and M.B. Perkal, *J. Chromatogr.* **427**, 188 (1988).
[12] N. Kurosawa, S. Morishima, E. Owada and K. Ito, *J. Chromatogr.* **305**, 485 (1984).
[13] R.E. Bland, C. Harrison, L.E. Martin, J. Oxford and R.J.N. Tanner, *Int. J. Environ. Anal. Chem.* **18**, 25 (1984).