Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 805 (2004) 211–214
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simple determination of terbutaline in dog plasma by column-switching liquid chromatography

Y. Zhang, Z.R. Zhang*

No. 17, Section 3, Renmin Nan Road, West China School of Pharmacy, Sichuan University, 610041, PR China

Received 10 July 2003; received in revised form 25 February 2004; accepted 25 February 2004

Available online 1 April 2004

Abstract

Terbutaline is a β-adrenergic receptor antagonist that acts as a bronchodilator in the treatment of asthma and chronic bronchitis. In the present work, a column-switching high-performance liquid chromatographic method was developed to monitor terbutaline sulphate in dog plasma. The system consists of a C_2 pre-column (PC) and a C_{18} analytical column connected in series via a switching valve. Atenolol was used as the internal standard. Good linearity was achieved in the range of 5–800 ng/ml plasma. The mean intra- and inter-assay variation coefficients for this analysis were 2.3 and 4.7%, respectively. The average recovery for terbutaline was 87.4% from plasma. The mean concentration after three freeze-thaw cycles was 99.4% of the normal value. The analytical sensitivity and accuracy of this assay is adequate for characterisation of the pharmacokinetics of oral administration of terbutaline to dogs and has been successfully used to provide pharmacokinetic data using pulsatile and immediate-release tablets.

Keyword: Terbutaline

1. Instruction

Terbutaline, 1-(3,5-dihydroxyphenyl)-2-(tert-butylamino) ethanol, is a selective β_2-receptor agonist widely used in the treatment of bronchial asthma. Asthma symptoms tend to become severe at 2:00 and 8:00 a.m.; it is inconvenient for patients to take medications at mid-night. Terbutaline pulsatile tablets with different lag times were developed in our laboratory [1] to satisfy the dosing requirements for treatment of asthma. Terbutaline in samples from pharmacokinetic studies has been analysed by GC-MS [2] or capillary electrophoresis [3], however, most investigators have used HPLC. Several HPLC methods have been developed, using MS [4–6], electrochemical [7] or fluorescence detection [8] to ensure adequate analytical sensitivity. The determination of drugs in biological fluids using HPLC usually requires several sample preparation processes or off-line solid phase extraction [7], which are time-consuming and may lead to variability in the results. There are several reported HPLC methods based on column-switching. Electrochemical detection was applied in some of these methods [9–11], which may not be available in many laboratories, or MS [6] and some particular materials [12] were utilised in other methods.

Automated column-switching liquid chromatographic systems have the advantage that the enrichment and clean-up of sample is performed by on-line liquid–solid extraction via a short pre-column (PC) for removal of other matrix components. With this automated column-switching technique, the drug level in plasma is easily determined by a UV detector, without loss of accuracy and sensitivity.

The objective of the present work has been to develop a more generally accessible method with a column-switching system and UV detection for the analysis of terbutaline, in which plasma samples could be applied to HPLC only with simple deproteinisation without multiple preparation steps. Since a UV detector is widely available in most laboratories, this method is easily adopted compared with present methods for the determination of terbutaline. This method is sensitive with a limit of de-

* Corresponding author. Tel.: +86-28-85501566; fax: +86-28-85456898.
E-mail address: zrzzl@vip.sina.com (Z.R. Zhang).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.02.037

tection at 1.5 ng/ml, which can meet the requirements of pharmacokinetics studies. In the present study, pharmacokinetics of terbutaline in beagle dogs after oral administration of terbutaline pulsatile tablets and immediate-release tablets as reference formulation was investigated using this method.

2. Experimental

2.1. Chemicals and reagents

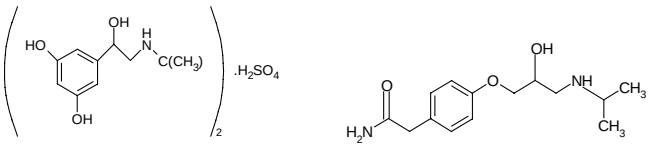
Terbutaline sulphate (Fig. 1, I) was kindly supplied by Kanghong Medical Co. (Chengdu, China). Atenolol (Fig. 1, II) used as internal standard was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of I and II were 99.4 and 99.6%, respectively. Methanol and acetonitrile (HPLC grade) were purchased from Sanli Chemical Factory (Zhejiang, China). Water was prepared in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Dog plasma was from Department of Pharmacology, Sichuan University (Sichuan, China). All other chemical and reagents used were of analytical grade.

The reference formulation was an immediate-release tablet (AstraZeneca, Jiangsu, China) containing terbutaline sulphate 30 mg. Terbutaline pulsatile tablets with lag times of 2 and 10 h were placed in size one gelatin capsules to form pulsatile capsules. Each kind of pulsatile tablet contains terbutaline sulphate 30 mg.

2.2. Apparatus

HPLC determinations were performed with a Shimadzu® model 10A*vp* liquid chromatographic system (Chiyoda-Ku, Tokyo, Japan) consisting of two gradient pumps (LC-10AT), an UV-Vis detector (SPD-10A) operated at 220 nm, a six-port valve and a 500 μl sample loop. The system was controlled by a system controller (SCL-10A) and a personal computer.

The pre-column was dry-packed in-house with Bondesil C_2 material (40 μm particle size, 50 × 4.6 mm). The analytical column was a Shimadzu Shim-Pack C_{18} reversed-phase column (5 μm particle size, 150 × 6 mm) (Chiyoda-Ku, Tokyo, Japan) protected by a Shimadzu Shim-Pack guard column (5 μm particle size, 10 × 2.5 mm).



I II

Fig. 1. Chemical structures of terbutaline (I) and atenolol (II).

2.3. Preparation of calibration standards

A concentrated stock solution of terbutaline was prepared at a concentration of 250 μg/ml in deionized water and was further diluted into 0.025–4 μg/ml for the preparation of plasma calibration standards. Atenolol, internal standard, was diluted to 3.66 μg/ml. All solutions were stored at −20 °C in dark glass.

A plasma calibration curve was constructed with ten different standards covering the expected concentration range (5–800 ng/ml). Different standard solutions (100 μl each) were transferred into test tubes containing 500 μl of drug-free dog plasma and 100 μl of atenolol solution. The calibration curve was produced by linear regression of peak area ratios (terbutaline to atenolol) against their respective concentrations.

2.4. Sample preparation and separation

The mixture was deproteinized with 1 ml acetonitrile, vortexed for 3 min and centrifuged for 5 min at 1500 × *g*. A 0.5 ml aliquot of supernatant fluid was injected onto the pre-column, which was then washed with pure water at a flow-rate of 1 ml/min to enrich terbutaline from the plasma sample. In contrast, the analyte were eluted from the analytical column with a mobile phase of 0.03 M phosphate buffer solution (PBS, pH 3.5):methanol:acetonitrile (94:3:3, v/v) at flow-rate of 1 ml/min at 40 °C. Four minutes after injection, the switching valve was rotated to back-flush mode, coupling the pre-column in line with the analytical column. This mode allowed the separation mobile phase to flow in a back-flush mode through the pre-column and transfer the enriched analytes from the pre-column to the analytical column with an elution mobile phase at a flow-rate of 1 ml/min for separation. The pre-column was re-equilibrated with water prior to next injection.

2.5. Animal experiment

Three male and three female beagle dogs (weighing 10–12 kg) were divided into a test group and a control group at random. The dogs were fasted for 15 h with a free supply water before drug administration. The dogs were administered, in a crossover design, either the immediate-release tablets containing terbutaline sulphate 30 mg (control group) or the pulsatile tablets with different lag times (test group). The formulations were swallowed with 200 ml water. The dogs were provided a standardised meal 6 h after drug intake. Blood samples of control group were collected into heparinised polystyrene tubes at time 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 9, 11 and 15 h. Blood samples of test group were collected at time 0, 2, 2.5, 3, 4, 5, 7, 8, 9, 11, 12, 14, 15, 18, 19, 23, 26 and 28 h. Plasma samples were obtained from the supernatant of centrifuged blood and they were immediately frozen and kept at −20 °C until assay. There was a 1-week washing-out time between each treatment period.

3. Results and discussion

3.1. Extraction procedure

Although the column-switching sample-preparation technique offers the possibility of directly analysing and pre-concentrating an analyte, an additional isolation procedure may be necessary for example to remove proteins that could otherwise harm the liquid chromatographic system [13]. Therefore, a simple depreteinization was applied to prolong the lifetime of the pre-column. No striking change was observed even after more than 250 plasma samples (500 μl each) had been analysed by this pre-column.

The first step in the set-up of a switching system involved selection of a suitable pre-column, which would retain the drug and remove the endogenous components. A short C_2 pre-column was chosen for this purpose. C_2 materials showed the most favourable retention characteristics for terbutaline and weak retention for other matrix components when compared with C_{18} packing materials. The next stage was to find two compatible and miscible eluents of different elutropic strength. The washing mobile phase ideally would have poor elution capability on the pre-column in order to ensure maximum concentration of the sample with minimum band broadening. In this case, deionized water was found to provide adequate concentration and was compatible with an aqueous-based mobile phase (analytical mobile phase). Full elution of the drug from the pre-column and minimisation of band broadening was achieved by eluting in a back-flush direction.

Complete separation of terbutaine and atenolol (I.S.) can be achieved by an analytical mobile phase of PBS–methanol or PBS–acetonitrile. But there always was an inverse or concave system peak at the retention time of terbutaline to distort the peak shape. However, the system peak disappeared when mixture of methanol and acetonitrile on the halves were applied. It was estimated that the inverse or concave system peak resulted from methnol or acetonitrile was counteracted by the mixture of the two solvents in equal proportion.

To determine the optimum wash time for the samples on the pre-column, plasma aliquots containing 50 ng/ml drug were injected onto the concentration column. The wash time was varied between 1 and 6 min, and it was found that an optimum was obtained at 4 min, in that it provided good clean-up of the plasma components without causing the drug to elute.

3.2. Selectivity, stability and recovery

Typical chromatograms obtained from blank plasma, plasma sample and drug-spiked plasma are shown in Fig. 2. The retention times of terbutaline and the I.S. were approximately 19 and 24 min, respectively, with complete baseline resolution between peaks of interest. The selectivity of the assay was investigated by injecting the blank plasma.

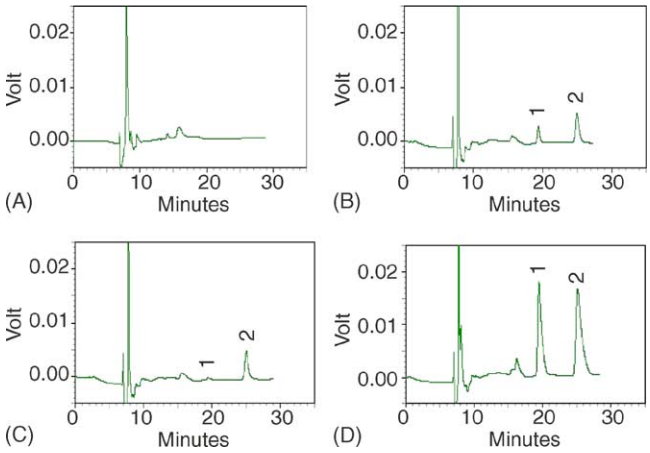


Fig. 2. Representative chromatograms of (A) blank plasma, (B) dog plasma sample (TB 86.4 ng/ml, AT 183 ng/ml), (C) sample close to the LOQ (3.4 ng/ml), (D) plasma spiked with TB and AT (TB 800 ng/ml, AT 700 ng/ml). (1. terbutaline; 2. atenolol.)

No chromatographic interference derived from endogenous substance or system peaks was observed.

The stability of terbutaline was investigated after storing spiked plasma samples (100 ng/ml) at −20 °C for 2 months. During the storing period, three freeze-thaw cycles were carried out. The mean concentration following this storage period was 99.4% (R.S.D. = 1.2%, $n = 3$) of the normal value, indicating that plasma samples containing terbutaline were stable at −20 °C for at least 2 months.

Recoveries of terbutaline were assessed at three concentration levels by comparing the results obtained from the spiked plasmas to standard solutions. Table 1 showed the mean recoveries of terbutailne at the concentrations tested. The mean recovery was 87.4% for terbutaline from dog plasma.

3.3. Linearity, limit of detection and precision

The calibration curve was linear with a correlation coefficient of 0.999 over the concentration range of 5–800 ng/ml. This linear correlation had a slope of (0.0025±0.0004) and an intercept of (−0.016±0.019). The limit of detection for this method defined as a signal-to-noise ratio of 3:1, was 1.5 ng/ml. The limit of quantification (LOQ) was defined as the lowest drug concentration, which can be determined with a within-day relative standard deviation R.S.D.≤20% [14,15]. The LOQ was estimated as 3 ng/ml.

The precision of the present method was investigated by repeated analysis of TB-spiked plasma samples at 15.5,

Table 1
Assay recoveries of terbutaline from plasma

Spiked concentration (ng/ml)	Recovery (%)	R.S.D. (%)
15.5	87.0	3.5
206.7	86.4	1.7
826.9	88.8	1.3

Table 2
Intra- and inter-assay precision measurement

Spiked concentration (ng/ml)	Observed concentration (ng/ml)	R.S.D. (%)
Intra-assay		
15.5	15.0	2.3
206.7	199.2	2.6
826.9	825.1	2
Inter-assay		
15.5	15.7	6.1
206.7	206	4.8
826.9	835.3	3.2

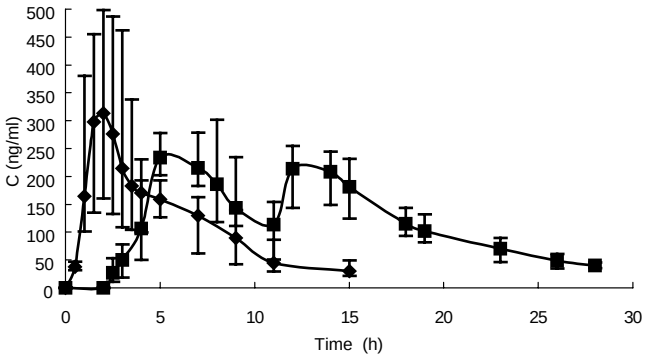


Fig. 3. Plasma concentration–time profiles of terbutaline after oral administration of pulsatile tablets and immediate-release tablets: (■--■,), test group; (◆-◆), control group.

206.7, 826.9 ng/ml. The results of intra-assay and inter-assay was reproducible with an average intra-day C.V. less than 2.6%, and an average inter-day C.V. less than 6.1% (Table 2).

3.4. Assay application

The mean plasma profiles of the terbutaline pulsatile tablets and reference tablets are shown in Fig. 3. Two plasma concentration peaks were observed in the test group, representing the first and second pulsed-release with different lag times respectively. The pharmacokinetic parameters were estimated by the 3p87 program (The Chinese Society of Mathematical Pharmacology). A one-compartment model without lag-time was applied to estimate the parameters of immediate-release tablets, and a one-compartment model with lag-time was applied for terbutaline pulsatile tablets. Statistical moment was applied to calculate area under curve (AUC). The main pharmacokinetic parameters are listed in Table 3.

In the test group, T_{max} was 5 h for the first pulsed-release and 12 h for the second pulsed-release. T_{lag} was defined as the extrapolated intersection with the time axis. In this case, T_{lag} were 2.35 and 9.87 h for the two pulsed-releases, respectively. While in the control group, no obvious lag-time was observed and T_{max} was about 2 h. A *t*-test was carried out to evaluate the difference of the T_{max} between the two formulations. The *P*-value was smaller than 0.01 indicating

Table 3
Mean pharmacokinetic parameters and statistical analysis ($n = 6$)

Parameters	Pulsatile tablets	Immediate-release tablets	*t*-Test
T_{max} (h)	5 ± 0.5 12 ± 1.5	2.1 ± 0.6	$P < 0.01$
C_{max} (ng/ml)	234.1 ± 32.2 213.9 ± 40.4	328.3 ± 125	$P < 0.01$
T_{lag} (h)	2.4 ± 0.4 9.9 ± 1.1	0.2 ± 0.04	$P < 0.01$
AUC_{0-tn}(h μg/ml)	3275 ± 431	1691 ± 377	$P < 0.01$
$AUC_{0\to\infty}$(h μg/ml)	3539 ± 434	1879 ± 426	$P < 0.01$
MRT (h)	14.6 ± 0.6	5.2 ± 0.6	$P < 0.01$

that there was statistical difference of the T_{max} between the two formulations. These parameters showed that delayed release could be fulfilled using terbutaline pulsatile tablets to satisfy the necessary time concentration profile for treatment of asthma.

4. Conclusion

The analytical method described above is useful for quantification of terbutaline in dog plasma for pharmacokinetic studies. This system employs column-switching with off-line deproteinisation. The method shows good overall recovery, accuracy, precision, and a low detection limit of terbutaline with UV detection. There was no evidence of instability of terbutaline in plasma following freeze-thaw cycles or after 2 months of storage at −20 °C. The present method may be useful for the routine determination of terbutaline in pharmacokinetic study.

References

[1] Y. Zhang, Z.R. Zhang, F. Wu, J. Control Relat. 89 (2003) 47.
[2] S.E. Jacobsson, S. Tohsson, C. Lindberg, L.A. Svensson, Biomed. Mass Spectrom. 7 (1980) 265.
[3] M. Petersson, K.G. Wahlund, S. Nilsson, J. Chromatogr. A 841 (1999) 249.
[4] L. Gao, G.J. Wang, Y. Wang, J.G. Sun, Acta Pharm. Sinica 34 (1999) 535, in Chinese.
[5] S.B. Black, R.C. Hansson, J. Anal. Toxicol. 23 (1999) 113.
[6] Y.Q. Xia, D.Q. Liu, R. Bakhtiar, Chirality 14 (2002) 742.
[7] V.L. Herring, J.A. Johnson, J. Chromatogr. B 741 (2000) 307.
[8] P.T. McCarthy, S. Atwal, A.P. Sykes, J.G. Ayres, Biomed. Chromatogr. 7 (1993) 25.
[9] S. Bergquist, L.E. Edholm, J. Liq. Chromatogr. 6 (1983) 559.
[10] K.A. Sagar, M.T. Kelly, M.R. Smyth, J. Chromatogr. 577 (1992) 109.
[11] L.E. Edholm, B.M. Kennedy, Chromatographia 16 (1982) 341.
[12] P. Chiap, O. Rbeida, B. Christiaens, Ph. Hubert, D. Lubda, K.S. Boos, J. Crommen, J. Chromatogr. A 975 (2002) 145.
[13] C.F. Pilar, H.H. Rosa, S.C. Adela, J. Chromatogr. 619 (1993) 177.
[14] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[15] S. Liu, M. Kamijo, T. Takayasu, S. Takayama, J. Chromatogr. B 767 (2002) 53.