ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 927–937

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of terbutaline sulfate and its degradation products in pharmaceutical formulations using LC

N. Daraghmeh [a], M.M. Al-Omari [a], Z. Sara [a], A.A. Badwan [a], A.M.Y. Jaber [b,*]

[a] *The Jordanian Pharmaceutical Manufacturing and Medical Equipment Co. Ltd, PO Box 94, Naor 11710, Jordan*
[b] *Chemistry Department, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia*

Received 14 November 2001; received in revised form 15 March 2002; accepted 1 April 2002

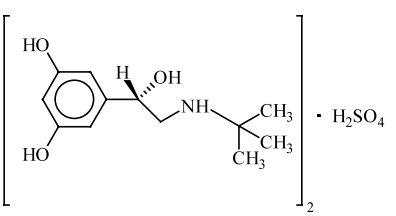
Abstract

There is a lack of information concerning analysis of terbutaline sulfate and quantification of its related substances particularly in the liquid dosage forms. This work aimed at developing and validating an HPLC method for determination of terbutaline sulfate and its possible degradation products, namely, 3,5-dihydroxybenzoic acid, 3,5 dihydroxybenzaldehyde and 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl) amino]-ethanone that might appear as impurities in the starting material as well as in the solid and liquid formulations. The chromatographic system used consisted a Hypersil 100 $C_{18,}$ 150 × 4.6 mm (5 μm) column, a mobile phase of ammonium acetate (0.15 M) and glacial acetic acid (pH of 4.0, 96:4 v/v) with a flow rate of 2 ml min^{-1} and a UV detector set at 270 nm. The degree of linearity and the characteristic statistical parameters of the calibration curves including the limit of detection (LOD) and limit of quantitation (LOQ) were estimated for terbutaline sulfate and its degradation products. The method was found to be specific, stability indicating, accurate, precise and robust.

Keywords: Terbutaline sulfate; Chromatographic purity; Stability indicating assay; HPLC

1. Introduction

Terbutaline sulfate is a synthetic β_2-adrenoceptor that is used as a bronchodilator in the treatment of bronchial asthma. It is known chemically as ±-α-[(*tert*-butylamino) methyl]-3,5-dihydroxybenzyl alcohol sulfate. It exists as a racemic mixture and has the chemical structure shown below.



Terbutaline sulfate

HPLC methods were extensively used to quantify terbutaline sulfate in its dosage forms and as anticipated, these methods differ in columns used,

* Corresponding author. Tel.: +966-3-860-2611; fax: +966-3-860-4277.
E-mail address: amjaber@kfupm.edu.sa (A.M.Y. Jaber).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00216-9

detection methods and mobile phase components. Generally, HPLC methods using UV detectors were the most acceptable for terbutaline sulfate among workers in this field [1–8]. In addition, HPLC in conjunction with electrochemical and fluorescence detectors was also used to analyze terbutaline sulfate in different dosage forms and in biological fluids [9–11].

Generally, in international pharmacopoeias USP, BP and EU and USP forum, different HPLC assay methods are available for the official analysis of terbutaline sulfate in its dosage forms. HPLC method adopted by the USP was based on the C_8 column kept at 40 °C with a mobile phase containing a mixture of water, methanol, tetrahydrofuran and sodium 1-octanesulfonate as ion pair [12]. In the BP, the HPLC system consisted C_{18} column and a mobile phase made of a mixture of acetonitrile, 0.005 M sodium 1-octanesulfaonate and glacial acetic acid (pH 3.4; 21:79, v/v) [13]. UV detection at 280 nm was used in the two methods, however methods of separation and quantification were different.

The chromatographic profile of degraded terbutaline sulfate suggested that the degradation of terbutaline sulfate in aqueous solutions into 3,5-dihydroxybenzoic acid and 3,5-dihydroxybenzaldehyde is enhanced by the presence of oxygen, low pH and low levels of metals [14,15]. Recently, determination of one of the related substances in the terbutaline sulfate drug, namely, 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl) amino]-ethanone, was introduced in BP, EU and USP forum [13,16,17]. The separation of these degradation products was achieved on a C_{18} silica gel column using a mobile phase of a mixture of methanol, 0.05 M ammonium formate solution (pH 3.0; 230:770, v/v) and sodium 1-hexanesulfonate as an ion pair. Also, the degradation products, 3,5-dihydroxybenzoic acid and 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl) amino]-ethanone were only identified as related substances of terbutaline sulfate in the BP and EUP [13,14].

A stability indicating method by which the drug and its degradation products are determined simultaneously is always demanding. Although the substances related to terbutaline sulfate that appear as oxidative degradation products have been identified, no method was reported for simultaneous determination of all these components in the presence of the formulation excipients of the tablet or liquid dosage forms. Consequently, validation of a newly developed HPLC method for this mixture of components was the main objective of this study. Furthermore, the method has been tested for both solid and liquid formulations.

2. Experimental

2.1. Instrumentation

An HPLC unit equipped with diode array detector module 168 and programmable pump module 125 (System Gold, Beckman, USA) in conjunction with a Hypersil 100 ODS (5 μm) column, 15 cm × 4.6 mm ID (Shandon, England) was used. A low intensity UV light provided by ultraviolet rays sterilizer (Ah Poong AP602) connected to Germicidal lamp G15T8-AN 15W (Sankyo Denki, Japan) was used for the photochemical stability studies.

2.2. Materials

All reagents used were of analytical and HPLC grade (Merck, Germany). Deionized water was used for all reagent preparations. Terbutaline sulfate was of BP grade (Lake Chemicals and Neuland companies, India). 3,5-Dihydroxybenzoic acid (compound 1) and 3,5-dihydroxybenzaldehyde (compound 2) were obtained from Aldrich, Germany and 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl) amino]-ethanone (compound 3) was from Neuland, India. Inactive drug excipients for tablet dosage form include: lactose monohydrate of EP grade (DMV, Netherlands), maize starch of BP grade (National Starch and Chemical, Germany), and magnesium stearate of NF grade (Mallinkcrodt, USA). Inactive ingredients for liquid dosage form include: carboxymethyl cellulose 400–600 cps of USP grade (Aqualon, Netherlands), sodium saccharin of EP grade (Dr Hesse and CIE, Germany), sodium benzoate of EP grade (Merck, Germany), anhydrous citric acid of EP grade (Jungbunzlauer, Germany), sodium citrate of EP grade (Jungbunzlauer, Germany) and apricot

flavor 41.077 of food grade (Flachsmann, Switzerland).

2.3. Optimized chromatographic conditions

Isocratic elution technique was utilized with the column maintained at room temperature. The mobile phase used consisted of a mixture of 0.15 M ammonium acetate and glacial acetic acid (pH 4.0; 96:4, v/v). The mobile phase was filtered and degassed by sonication before use. The flow rate was kept at 2.0 ml min^{-1}, the injection loop size was 200 μl and the UV detector was set at 270 nm.

2.4. Solutions

2.4.1. Standard solutions

An accurately weighed quantity of terbutaline sulfate or related substances was dissolved in water and diluted quantitatively to obtain solutions of known concentrations (about 150 μg terbutaline sulfate ml^{-1} or 0.75 μg of related substance ml^{-1}).

2.4.2. Synthetic mixture solutions

7.5 mg of each of the related substances were dissolved and the volume was adjusted to 100 ml with water. 2.0 ml of the resulted solution were transferred to 200 ml volumetric flask, 30 mg terbutaline sulfate, and 800 mg of a mixture of the tablet formulation excipients were added and the volume was adjusted to 200 ml with water. The suspension was shaken for 15 min, and then centrifuged. The syrup synthetic mixture solutions were prepared in the same way but a volume of the syrup containing about 30 mg terbutaline sulfate, and 100 ml of the liquid formulation excipients were added instead of terbutaline sulfate, and the tablet formulation excipients added above.

2.4.3. Tablet and liquid dosage form solutions

Accurately weighed quantities of powdered tablets or accurately measured volumes of the liquid dosage form equivalent to 15 mg terbutaline sulfate were shaken with 100 ml water for 15 min and centrifuged. Each of the final solutions had a concentration of about 150 μg terbutaline sulfate ml^{-1}.

2.5. Chromatographic procedure

200 μl samples were injected into the chromatograph and the HPLC chromatograms were recorded at a detector setting of 270 nm. The relative standard deviation for six replicate injections of the standard preparation were not greater than 2.0%, the resolution between terbutaline sulfate and its related substances was not less than 2.0 and the tailing factor was not more than 3.

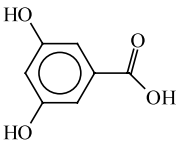
3. Results and discussion

3.1. Developing the HPLC method

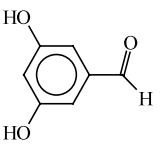
Preliminary HPLC trials have been carried out to investigate the possibility of determining terbutaline sulfate in the presence of its related substances (potential oxidative products) and formulation excipients. Table 1 shows various trials that have been carried out to investigate the optimum chromatographic conditions needed for appropriate peak separation of terbutaline sulfate and its related substances. During this phase, a column of Hypersil trade name was used. Several mobile phase blends were tried starting with a mixture of water and methanol (8:2, v/v), followed by introduction of ammonium acetate salt in combination with different organic modifiers. Various HPLC parameters (Table 1) were manipulated in order to achieve reasonable peak areas for all components and good resolution that comply with HPLC system suitability. The mobile phase components and its pH, the flow rate and column dimensions were optimized as shown in Table 1. A wavelength of 270 nm was found to be suitable to detect terbutaline sulfate and its possible degradation products simultaneously. The mobile phase used here is characterized by its simplicity where organic modifiers such as methanol or acetonitrile have not been used.

The relative retention times for terbutaline sulfate, compound 1, compound 2 and compound 3 were 1.0, 0.23 and 0.62 and 0.81, respectively; the

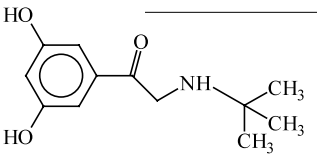
response factors (concentration/peak area) determined for these compounds using 5 μg solute ml^{-1} were 2.09, 0.51, 0.15 and 0.042, respectively. The structural formulas for the related substances are given below.



Compound 1



Compound 2



Compound 3

3.2. Specificity

HPLC chromatograms for synthetic solutions tablet (Fig. 1A) and syrup (Fig. 1B) formulations of terbutaline sulfate mixed with its related substances show that terbutaline sulfate, the related substances and the formulation excipients are well separated.

To assess the suitability of the method as a stability indicator, the specificity was demonstrated by stimulating the degradation of terbutaline sulfate in solid and in syrup formulations using 1 M HCl, 1 M NaOH and various oxidizing agents as well as the light effect under different stress conditions as shown in Table 2. The results indicated a decrease in the recovery of terbutaline sulfate and detection of degradation products under all stress conditions studied except that for hydrochloric acid case where a full recovery of terbutaline sulfate was observed and degradation products were absent. The related substances, compound 1 and compound 2 have been detected in almost all cases with amounts of less than 1% (Table 2). Table 2 also shows the presence of unknown degradation products especially for the case of nitrous acid where a degradation product of high UV absorptivity has been observed at a relative retention time of 0.78.

Table 1
Preliminary trials made to determine the optimum chromatographic system

Mobile phase components	pH	Column	Flow rate ($ml\ min^{-1}$)	Remarks
Water:methanol (80:20, v/v)	–	Hypersil 100 C_{18} 5 μm (150 × 4.6 mm)	1.0	Fast elution with bad resolution and no peak separation.
0.15 Ammonium acetate:methanol (90:10, v/v)	6.8	Hypersil 100 C_{18} 5 μm (150 × 4.6 mm)	1.0	High tailing factor for terbutaline sulfate peak and bad resolution between the peaks of terbutaline sulfate compound 2.
0.15 Ammonium acetate:methanol (90:10, v/v)	4.0	Hypersil 100 C_{18} 5 μm (150 × 4.6 mm)	1.0	Fast elution with bad resolution between the peaks of terbutaline sulfate and compound 2.
0.15 Ammonium acetate:acetonitrile (90:10, v/v)	6.8	Hypersil 100 C_{18} 5 μm (150 × 4.6 mm)	1.0	Fast elution that led to peaks overlapping.
0.05 M ammonium acetate:glacial acetic acid (96:4, v/v)	4.0	Hypersil 100 C_{18} 5 μm (150 × 4.6 mm)	2.0	Short peak heights for the solutes. High tailing factor.
0.15 M ammonium acetate:glacial acetic acid (96:4, v/v)	4.0	Hypersil 5 μm (250 × 4.6 mm)	2.0	Long retention time and high tailing factor for the solute peaks.
		Hypersil 5 μm (150 × 4.6 mm)	1.0	Long retention time and high tailing factor for the solute peaks.
		Hypersil 100 C_{18} 5 μm (150 × 4.6 mm	2.0	Resolution and peak characteristics were good. Acceptable retention time for all solute peaks.

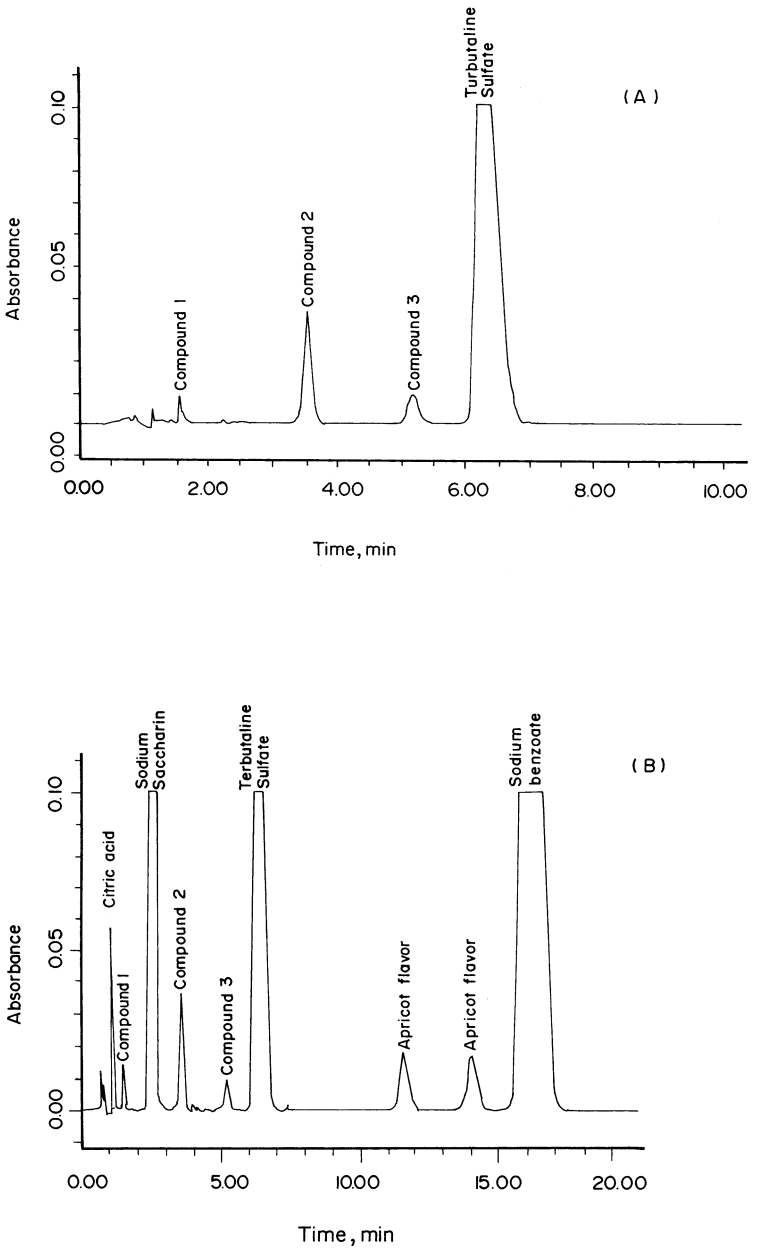


Fig. 1. HPLC chromatograms for (A) Synthetic solution of terbutaline sulfate in the drug-matrix of the tablet formulation dosage form; (B) Synthetic solution of terbutaline sulfate in the drug-matrix of the liquid dosage form.

Not only the degradation products have been observed by this HPLC method, quite few of the drug excipients have also been separated with good resolution when HPLC chromatograms were recorded for samples of terbutaline sulfate in a synthetic liquid formulation dosage form (Fig. 1B). It was also possible to detect a degradation product when HPLC chromatograms were recorded for a commercial sample of terbutaline sulfate (Fig. 2). It is obvious that the method has

successfully differentiated between the active moiety, terbutaline sulfate, and the oxidative degradation product, compound 3.

Photodegradation of turbutaline sulfate was demonstrated by exposing sample of terbutaline sulfate in its solid form or in solution to daylight or low intensity UV light. Table 2 shows the appearance of noticeable percentages of the degradation products, compound 1, compound 2 and compound 3 as a result of photodegradation of terbutaline sulfate.

3.3. Calibration curves, sensitivity and accuracy

The calibration curves for terbutaline sulfate and its related substances were constructed covering two concentration levels; high level (about 78–248 μg ml^{-1}) for the purpose of terbutaline sulfate analysis in tablets and syrups and low level (about 0.4–1.5 μg ml^{-1}) for the low concentrations of terbutaline sulfate and its related substances (Table 3). The correlation coefficients observed for all calibration curves constructed for terbutaline sulfate and its related substances were about 0.9999 in most cases but not less than 0.9952 (Table 3). The rest of linearity parameters of the calibration curves for terbutaline sulfate and its related substances are given in Table 3.

The limits of detection (LOD) and limits of quantification (LOQ) have been estimated from the calibration curves of terbutaline sulfate and its related substances as three and ten times of the

Table 2
Degradation of terbutaline sulfate under various stress conditions

Degradation conditions	Terbutaline sulfate	Degradation products[a]	
	% Recovery	Relative retention	%
1 M HCl/65 °C/18 h	100.7	–	–
0.1 M NaOH/65 °C/6 h	90.7	0.11	3.3
		0.17	4.8
		0.23 (Compound 1)	0.5
		0.62 (Compound 2)	0.3
		2.31	4.7
2.5 M H_2O_2/65 °C/10 h	95.4	0.23 (Compound 1)	0.1
		0.62 (Compound 2)	0.1
		0.9	0.2
0.005 M $KMnO_4$/25 °C/1 h	61.9	0.23 (Compound 1)	0.4
		0.62 (Compound 2)	0.2
		1.29	0.6
		1.63	3.9
0.025 M Nitrous acid/25 °C/5 min	85.1	0.23 (Compound 1)	0.5
		0.30	0.6
		0.78	110.4
Water/sunlight/3 weeks	88.5	0.23	0.13
		0.33	0.42
		0.62 (Compound 2)	0.01
		0.83 (Compound 3)	0.38
		0.88	0.46
Powder/sunlight/3 weeks	99.9	0.23 (Compound 1)	0.05
		0.62 (Compound 2)	0.02
Powder/UV light/7 days	93.9	0.11	0.11
		0.23 (Compound 1)	0.05
		0.62 (Compound 2)	0.02
		0.83 (Compound 3)	0.15
		0.88	0.06

[a] The recovery of unknown degradation product was calculated using terbutaline sulfate as a reference standard.

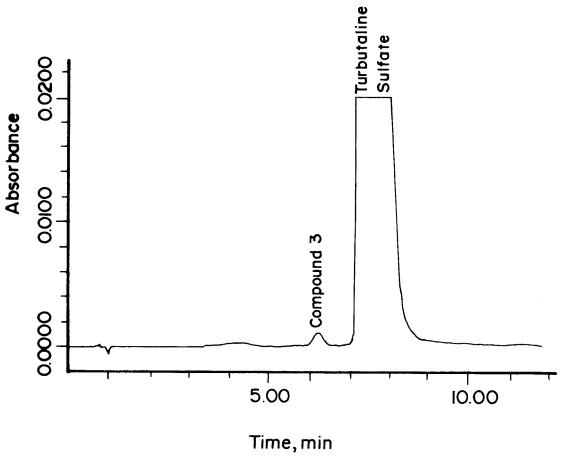


Fig. 2. HPLC chromatogram for a sample of commercial terbutaline sulfate. Compound 3 is 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethyl)amino]-ethanone.

noise level for LOD and LOQ, respectively. The values for LOD and LQD are given in Table 3.

The accuracy of the method was tested by analyzing various samples of terbutaline sulfate and all other related substances at various concentration levels in either pure solutions or in solutions comprising the drug-matrix used in solid and liquid formulations. The results were expressed as percent recoveries of the particular components in the samples. Table 4 shows that the overall percent recoveries of terbutaline sulfate from the tablet and liquid dosage forms were >99 and the RSD values were $\leq 0.5\%$. However, the related substances in tablet and liquid dosage forms matrices showed overall percent recoveries in the range of 93.9–102.1% with relative standard deviations ranging from 1.5 to 3.3%.

3.4. Repeatability and reproducibi1ility

Various samples containing about 150 μg ml^{-1} terbutaline sulfate in tablet and liquid synthetic matrices were analyzed by two independent analysts (six samples each) over 1 day and various days. Considering each one of the analysts, the short term precision showed overall percent recoveries in the range of 98.8–102.9 with RSD ranging from 0.2 to 0.7%. The long term precision for analysis of terbutaline sulfate in all samples of the tablet and liquid dosage forms (24 samples) showed % recovery and %RSD of 100.1 and 1.7, respectively.

Another set of samples, each containing about 150 μg ml^{-1} terbutaline sulfate and 0.75 μg ml^{-1} of each of its related substances (compound 1, compound 2 and compound 3) in tablet and liquid synthetic matrices were analyzed as above. The short term precision gave overall percent recoveries, for each analyst, in the range of 95.4–99.9 with RSD ranging from 1.2 to 4.2%. The long term precision for the analysis of the related substances in samples of tablet and liquid dosage forms (24 samples of each compound) showed, respectively, percent recoveries and %RSDs of 97.3 and 3.13 for

Table 3
Linearity of calibration curves for terbutalene sulfate and its related substances

Parameters	Compound				
	Terbutaline sulfate	Terbutaline sulfate	Compound 1	Compound 2	Compound 3
Calibration range (μg ml^{-1})	0.38–1.51	77.7–248.6	0.38–1.51	0.38–1.51	0.38–1.52
Correlation coefficient	0.9952	0.9999	0.9976	0.9989	0.9989
Slope	0.523	0.433	0.579	4.84	2.13
95% Confidence limits for the slope	0.456–0.591	0.423–0.443	0.527–0.632	4.54–5.14	1.95–2.32
Intercept	0.0036	0.158	0.0068	0.16	0.022
95% Confidence limits for the intercept	−0.061–0.069	−1.47–1.79	−0.044–0.058	−0.12–0.45	−0.16–0.20
LOD (μg ml^{-1})	0.11	–	0.08	0.05	0.07
LOQ (μg ml^{-1})	0.37	–	0.26	0.18	0.25

Number of points on each regression line is five.

Table 4
Accuracy for terbutaline sulfate and its related substances in tablet and liquid dosage forms (average of three replicates)

Compound	Tablet dosage form			Liquid dosage form		
	Quantity added ($\mu g\ ml^{-1}$)	Quantity found ($\mu g\ ml^{-1}$)	% Recovery	Quantity added ($\mu g\ ml^{-1}$)	Quantity found ($\mu g\ ml^{-1}$)	% Recovery
Terbutaline sulfate	77.20	77.23	100.3	7.50	7.54	100.5
	153	152.2	99.5	15.10	15.00	99.3
	244.8	244.5	99.9	24.10	24.05	99.8
Overall% recovery			99.9			99.9
%RSD			0.4			0.5
Compound 1	0.47	0.45	95.7	0.38	0.37	97.4
	0.72	0.67	93.1	0.76	0.76	100.0
	1.21	1.13	93.4	1.15	1.11	96.5
Overall% recovery			94.1			98.0
%RSD			1.5			1.9
Compound 2	0.45	0.46	102.2	0.38	0.38	100.0
	0.67	0.68	101.5	0.75	0.77	102.7
	1.17	1.16	99.1	1.10	1.14	103.6
Overall% recovery			100.9			102.1
%RSD			1.6			1.8
Compound 3	0.38	0.35	92.1	0.38	0.38	99.2
	0.76	0.74	97.4	0.75	0.72	96.0
	1.14	1.05	92.1	1.13	1.11	98.2
Overall% recovery			93.9			97.8
% RSD			3.3			1.7

compound 1, 99.3 and 1.53 for compound 2, and 97.1 and 3.75 for compound 3.

3.5. Robustness

Samples of the analytical solutions (standard or synthetic solution preparations) were tested for HPLC stability over various periods of time (24, 6 or 3 h) by analyzing them before and after the storage period. The percent differences of terbutaline sulfate recoveries from standard solutions of tablet and liquid dosage forms were in the range of 2.0 to -0.9 (Table 5), indicating the possibility of using analytical solutions of terbutaline sulfate over a period of 24 h without degradation. Although not as good as the case for terbutaline sulfate, solutions of synthetic mixtures of terbutaline sulfate and the three related substances showed an acceptable stability over storage periods of 3 or 6 h for compound 1 and compound 3 (Table 5). However, compound 2 in such mixtures showed significant instability where its recovery after a storage time of 3 h was about 90% or even less (Table 5). Furthermore, its recovery has decreased to about 70% of its initial value after 6 h of storage time. Meanwhile, the recovery of compound 1 has increased to reach up to about 110% or more after 3 h of storage time and as high as 130% after 6 h storage time. The increase in the recovery of compound 1 may be ascribed to the oxidation of 3,5-dihydroxybenzaldehyde (compound 2) into3,5-dihydroxybenzoic acid (compound 1). Thus, it is recommended to use fresh solutions of compound 2 to avoid its oxidation to compound 1.

The system suitability was checked by studying the effect of various parameters on the percent

recovery of terbutaline sulfate and its related substances. The parameters include the wavelength of detection (265, 270 and 275 nm), flow rate (1.7, 2.0 and 2.3 ml min^{-1}), filtration system (nylon, centrifuge or cellulose acetate), shaking time (10, 15 and 20 min) and mobile phase ratio (buffer:acetic acid, v/v 95:5 (pH 3.7), 96:4 (pH 4.0) or 97:3 (pH 4.3)). Percent recoveries of terbutaline sulfate for synthetic samples of tablet and liquid dosage forms analyzed under various conditions were in the range of 98.5–100.5 with overall %RSDs and tailing factors of 0.1–0.7 and 1.1–1.8, respectively (Table 6). Percent recoveries for the related substances, compound 1, compound 2 and compound 3 in tablet and liquid dosage forms under all conditions studied were in most cases better than 95% with %RSDs in the range of 0.2–9.1. All these results indicate that the method is robust

4. Conclusion

An HPLC method was developed for the assay of terbutaline sulfate and its related compounds in tablet and liquid dosage forms using an aqueous mobile phase mixture without organic modifier. The method showed well separated HPLC peaks for terbutaline sulfate, the drug excipients and the related compounds: 3,5-dihydroxybenzoic acid,

Table 5
Stability of standard and assay solutions of terbutaline sulfate and related substances under ambient conditions

Compound	Solution	% Recovery					
		Tablet dosage form			Liquid dosage form		
		Fresh	Stored	%Difference	Fresh	Stored	%Difference
Terbutaline sulfate[a]	Standard solutions	100.0	100.4	−0.4	100.0	99.4	0.6
		99.6	98.6	1.0	99.2	98.2	1.0
		99.2	98.8	0.4	100.8	98.8	2.0
	Synthetic solutions	99.6	100.5	−0.9	99.4	99.7	−0.3
		99.6	100.2	−0.6	99.1	99.2	−0.1
		99.4	99.5	−0.1	99.3	99.3	0.0
Compound 1[b]	Standard solutions	96.0	105.3	−9.7	–	–	–
		97.5	105.8	−8.5	–	–	–
		99.6	109.0	−9.4	–	–	–
	Synthetic mixture solutions	91.0	107.8	−18.5	100.4	99.0	1.4
		95.3	109.9	−15.3	100.0	100.4	−0.4
		95.5	116.2	−21.7	100.2	99.1	1.1
Compound 2[b]	Standard solutions	100.3	90.3	9.9	–	–	–
		101.9	90.6	11.0	–	–	–
		98.4	86.4	12.1	–	–	–
	Synthetic mixture solutions	97.3	89.1	8.4	101.4	105.0	−3.6
		97.0	88.6	8.6	100.0	99.4	0.6
		96.6	89.5	7.3	99.0	102.1	−3.1
Compound 3[c]	Standard solutions	99.6	98.4	1.2	–	–	–
		100.5	102.8	–2.3	–	–	–
		99.1	102.4	−3.3	–	–	–
	Synthetic mixture solutions	102.5	94.1	8.2	101.0	100.4	0.6
		100.1	96.9	3.2	100.4	102.2	−1.8
		98.4	97.2	1.2	100.7	101.3	−0.6

Difference (%) = (quantity found in fresh solution − quantity found in stored solution)/(quantity found in fresh solution) × 100.
[a] Storage time 24 h.
[b] Storage time 3 h.
[c] Storage time 6 h.

Table 6
Effect of different parameters on the percent recovery of terbutaline sulfate and its related substances

Parameter		% Recovery in tablet dosage form				%Recovery in liquid dosage form			
		Terbutaline sulfate	Compound 1	Compound 2	Compound 3	Terbutaline sulfate	Compound 1	Compound 2	Compound 3
Mobile phase ratio	95:5 (pH 3.7)	99.2	98.3	100.3	97.6	99.4	87.2	107.7	101.7
	96:4 (pH 4.0)	99.0	97.9	98.8	98.6	100.0	101.2	107.8	97.8
	97:3 (pH 4.3)	99.8	100.0	99.2	97.8	99.3	101.8	108.0	98.6
Flow rate (ml min^{-1})	1.7	99.0	96.7	95.1	98.6	99.1	100.2	116.0	99.5
	2.0	99.0	97.9	98.8	98.6	100.0	101.2	107.5	97.8
	2.3	98.7	96.1	97.7	96.7	98.7	90.0	118.6	109.9
Wavelength (nm)	265	100.5	96.8	95.6	98.7	99.2	98.7	100.9	104.4
	270	99.0	97.9	98.8	98.6	99.0	101.2	107.8	97.8
	275	100.5	97.3	97.7	98.0	100.0	95.5	97.0	95.2
Shaking time (min)	10	98.8	97.9	99.1	96.0	–	–	–	–
	15	99.0	97.9	98.8	98.6				
	20	99.0	96.4	98.9	95.0				
Filtration	Nylon	98.5	81.2	96.5	95.7	–	–	–	–
	Centrifuge	99.0	97.9	98.8	98.6				
	Cellulose acetate	99.0	96.0	88.0	97.3				

Compound 1: 3,5-dihydroxybenzoic acid; compound 2: 3,5-dihydroxybenzaldehyde; compound 3: 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethyl)amino]-ethanone.

3,5-dihydroxybenzaldehyde and 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl) amino]-ethanone. The method is specific, stability indicating and robust, thus it can be used for development and quality control purposes that include, assay of the drug substance in its solid and liquid dosage forms. All statistical values (percent recovery, RSD, %difference, confidence limits of the slope and intercept, LOD and LOQ) calculated from the calibration curves were within the acceptable limits.

Acknowledgements

JPM and KFUPM are thanked for the support of this research project.

References

[1] V.D. Gupta, Journal of Liquid Chromatography 9 (1986) 1065–1074.
[2] J.E. Kountourellis, C. Markopoulou, Journal of Liquid Chromatography 12 (1989) 3279–3286.
[3] M.T. Acekrmans, J.L. Beckers, F.M. Everaerts, I.G.J.A. Seelen, Journal of Chromatography 590 (1992) 341–353.
[4] H.L. Rau, A.R. Aoor, P. Gundu-Rao, Indian Drugs 28 (1991) 385–387.
[5] G.A. Jacobson, G.M. Peterson, Journal of Pharmaceutical and Biomedical Analysis 12 (1994) 825–832.
[6] S.N. Tenjarla, R. Allen, B. Mitchell, Journal of Liquid Chromatography 18 (1995) 1603–1615.
[7] M. Vasudevan, S. Ravisankar, G. Manesh, J. Ravi, Indian Drugs 37 (2000) 489–492.
[8] Y. Zou, Y. Qin, M. Liang, Y. Huang, Q. Yu, Zhongguo Yaoxue Zazhi 35 (2000) 473–474.
[9] W.L. Childress, Journal of the Association of Analytical Chemistry 70 (1987) 974–976.
[10] K.A. Sagar, M.T. Kelly, M.R. Smyth, Journal of Chromatography—Biomedical Applications 115 (1992) 109–116.
[11] K.H. Kim, D.S. Kim, S.P. Hong, O.S. Keon, Archive Pharmaceutical Research 23 (2000) 26–30.
[12] United States Pharmacopeia (USP 24) and National formulary (NF 19), United State Pharmacopeial Convention, Rockville, MD, 2000, pp. 1604–1607.
[13] British Pharmacopoeia (BP 2000), British Pharmacopoeia Commission, Market Towers, London, 2000, pp. 1477–1478 and 2149–2152.
[14] L.A. Svensson, Acta Pharmaceutica Suecica. 9 (1972) 141–146.
[15] S. Ahuja, P. Liu, J. Smith, in: 45th International Congress of Pharmaceutical Sciences, Montreal, Canada, 1985, September 2–6.
[16] European Pharmacopoeia, Supplement 2000, Council of Europe, Strasbourg Cedex, France, 2000, pp. 1239–1240.
[17] Pharmacopeial Forum, United State Pharmacopeial Convention, Rockville, MD 26, 2000, pp. 752–753.