

High Performance Liquid Chromatographic Determination of Butamyrate Citrate in Pharmaceutical Preparation

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An HPLC method was developed and validated for the determination of butamyrate citrate. The HPLC separation was achieved on a diol column (300 × 4.6 mm) packed with 5.0 μm particle size using a mobile phase of ammonium acetate buffer (pH = 6.5) and methanol (750:250, v/v) at a flow rate of 1.4 ml min⁻¹. The UV detector was operated at 225 nm. The method was validated for specificity, linearity, precision, accuracy and robustness. The retention time was 5.9 min. The proposed method provided linear responses within the concentration range 75-225 μg ml⁻¹ with LOD and LOQ values of 0.69 and 2.29 μg ml⁻¹, respectively. Correlation coefficient (*r*) of the regression equation was 0.9999. The method was found to be precise, accurate, and reproducible.

Keywords: Butamyrate citrate, Pharmaceutical preparation, HPLC, Validation

INTRODUCTION

Butamyrate citrate 2-(2-diethylaminoethoxy) ethyl 2-phenylbutanoate (Fig. 1) is widely used as a central cough suppressant [1-2]. The drug is mainly prescribed in the form of syrup. A literature survey reveals that only two methods are described referring to both the relative bioavailability of different butamyrate citrate preparations after single dose oral administration [3] and to the determination of the compound using optical compensation method [4].

To date, only one paper has reported on the determination of butamyrate citrate in formulations using UV spectrophotometry and HPLC [5]. Moreover, there is no pharmacopeia method for the analysis of this drug. Therefore, it is very important, and will be of a real advantage, to develop

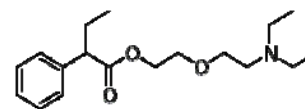


Fig. 1. Chemical structure of butamyrate.

a suitable analytical method, with less expensive reagents and short analysis time than other reported methods, for the measurement of butamyrate citrate in pharmaceutical preparations.

The purpose of this study was to develop a simple, sensitive, and reliable method for the determination of the drug which could be applied in quality control laboratories. To achieve this aim, a liquid chromatographic assay was developed in this research.

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EXPERIMENTAL

Chemicals and Solutions

Methanol and Acetonitrile were of HPLC grade from Arcos lab (Germany). Triethylamine and *ortho*-phosphoric acid were of analytical grade from Arcos lab (Germany). Ammonium acetate was of analytical grade from Scharlau (Spain).

Stock solution of 0.5 mg ml⁻¹ of butamyrate citrate was prepared by dissolving it in mobile phase and keeping in a refrigerator until being used. Standard calibration solutions were prepared by dilution of the stock solution using the mobile phase. These solutions were considered at six different levels which were 50%, 80%, 100%, 120% and 150% of the test concentration. Six replicate injections of standard solution containing a constant concentration of 150 µg ml⁻¹ were prepared in mobile phase.

Sample solution was prepared by dissolving 20 ml of butamyrate citrate syrup (containing 30 mg of butamyrate citrate) in 100 ml mobile phase. Then, 10.0 ml was diluted to 20 ml with mobile phase.

Apparatus and Chromatographic Conditions

The LC system consisted of a Shimadzu LC-2010C pump and degasser, connected to a UV detector. Injection was performed using auto sampler model (L2010A, Tokyo, Japan). The instrument was connected to LC Solution program as software.

The mobile phase was prepared by mixing ammonium acetate buffer and methanol (750:250 v/v). Choosing this mobile phase led to a high resolution with a good symmetry. Before mixing with organic solvent, the final pH of ammonium acetate buffer was adjusted to the desired value (pH = 6.5). It was then filtered through a 0.45 µm membrane and degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 1.4 ml min⁻¹. Detection of the analytes was carried out at 225 nm. Injection volume of the analyte was set to a constant volume of 20 µl using a sample loop.

The calibration curve for butamyrate citrate was constructed by plotting the peak area of the drug against the drug concentration.

Recovery Studies

To demonstrate the accuracy of the proposed method and to see whether there was any interference from excipients used in the dosage forms, recovery studies were employed by adding known amounts of butamyrate citrate to placebo. HPLC samples were then prepared and the resulting mixtures were analysed as described for pharmaceutical dosage forms.

Accelerated Degradation Studies

All degradation experiments in solution were performed at a drug concentration of 500 µg ml⁻¹. For acid and basic degradation, 10 ml of stock solution was heated with 10 ml of 0.1 M HCl or 0.1 M NaOH at 80 °C for 12 h, and then neutralized by adjusting the pH to 7.0. For photo and thermal decomposition experiments, separate solutions of pure drug samples in the mobile phase (500 µg ml⁻¹) were prepared. These solutions were exposed to ultraviolet light (254 nm) for 24 h and kept at 80 °C for 8 h. Another solution which was exposed to 10% solution of hydrogen peroxide was also kept at ambient temperature for 24 h in order to observe short term stability of the stock solutions.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

Initially, the mobile phase used was acetonitrile:water (450:550 (v/v)), then changed to acetonitrile:buffer solution (450:550) at pH = 6.0. The ratios of the solvents were changed, and found to have no significant change in chromatographic results.

Finally, a mobile phase consisting of 750 ml ammonium acetate buffer solution of pH 6.5 and 250 ml methanol, at a flow rate of 1.4 ml min⁻¹, was used. The elution time for butamyrate citrate was 5.9 min using a diol column (300 × 4.6 mm) packed with 5.0 µm particle size (Fig. 2).

Validation of the Method

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines [6]. The method under study was extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability. The precision (%relative standard deviation, RSD) was expressed with respect to the intra- and

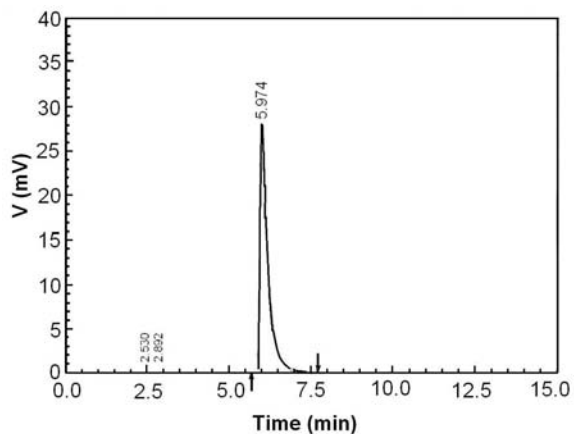


Fig. 2. Chromatogram of butamirate citrate working standard.

inter-day variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery of the known amount of the active pharmaceutical ingredient to the placebo. After validation, the developed method was applied to pharmaceutical dosage forms containing butamirate citrate.

System suitability. System suitability tests are an integral part of a liquid chromatographic method, and they were used to verify that the proposed method was able to produce good resolution between the peaks of interest with high reproducibility. The system suitability was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (N), resolution (R) and tailing factors (T).

System suitability requirements for butamirate citrate were a R.S.D. of peak areas and retention times less than 2%. Peak resolution (R) was greater than 2.0 between two adjacent peaks. Theoretical plate numbers (N) were at least 2000 for each peak and USP tailing factors (T) less than 1.5.

The results of system suitability test in comparison with

the required limits are shown in Table 1. According to the results presented, the proposed method fulfils these requirements within the accepted limits.

Stability/specificity. Specificity can be described as the capability of the method to accurately measure the response of the analyzed compound with no interferences originating from sample matrix. High percentage recovery observed with assay samples of pharmaceutical dosage forms, including standard addition to the placebo experiments, indicates that the proposed method was not affected by interferences from excipients used in formulations.

An alternative approach, *i.e.* accelerated degradation studies, was adopted to demonstrate the validity of the method as well as provide an evidence for the specificity of the proposed method. The approach was tried by designing intentional degradation experiments using acid, base, heat, UV-light (254 nm) and oxidation. Results obtained from stress tests have been summarized in Figs. 3a-3e. It is clear from the figures that there is no interference from either butamirate citrate or degrading components. Interestingly, the method is able to separate the drug from its degrading components.

Linearity. The calibration curve for butamirate citrate was obtained in the range of standard solutions (75-225 $\mu\text{g ml}^{-1}$). The linearity of this method was evaluated by linear regression analysis, which was calculated by the least square method.

A linear simple regression by the least squares method was applied. The representative linear equation was $y = 3401.64X - 10263.81$ ($n = 6$, $r^2 = 0.9999$). RSD of the slope at the linearity range was found to be 0.21%, indicating the repeatability of the calibration curve. Table 2 represents calibration characteristics for butamirate citrate.

Limit of detection (LOD) and limit of quantification (LOQ). ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard

Table 1. System Suitability Results of the Proposed Method

	N	R	T	RSD (%)	
				t_R	Peak area
Butamirate citrate	2397.1	10.0	1.17	0.92	0.22
Required limits	$N > 2000$	$R > 2.0$	$T > 1.5$	< 2.0	< 2.0

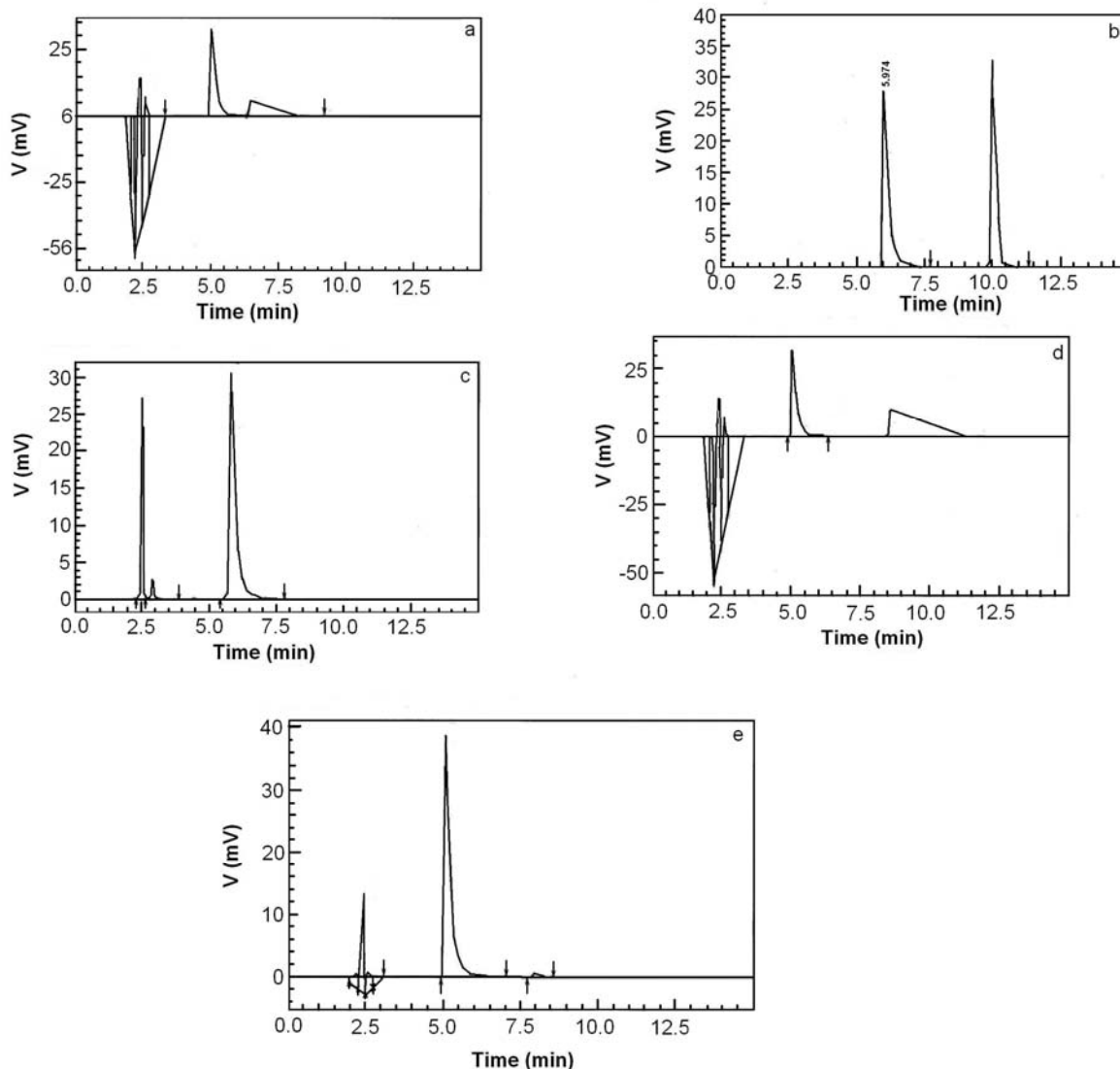


Fig. 3. Chromatogram of degradation experiments using: (a) 0.1 M HCl, (b) 0.1 M NaOH, (c) 0% H₂O₂, (d) UV-light at 254 nm, (e) heat.

deviation of the response and the slope of the calibration curve.

In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criteria, respectively; where σ is the standard deviation of the peak area ratios and s is the slope of the corresponding calibration curve. The LOD and LOQ values of the developed method are presented in Table 2.

Precision. The precision of the proposed method was assessed as repeatability and intermediate precision performing five replicate injections of three different sample

solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily (Table 3). These experiments were repeated over a 2-day period to evaluate day-to-day variability (intermediate precision). As can be seen in Table 3, the %RSD values of the measurements ranged between 0.22 and 0.89%. The %RSD of assay results obtained in the intermediate precision study were not greater than 0.57%, confirming the good precision of the proposed method between days.

Accuracy. Accuracy of the proposed method was established by recovery experiments. In this study, we added

Table 2. Characteristics of Butamyrate Citrate Calibration Plot^a

Linear range ($\mu\text{g ml}^{-1}$)	75.0-225.0
Slope	3401.64
%RSD of slope	0.21
Intercept	-10263.81
%RSD of intercept	0.15
Correlation coefficient (r)	0.9999
Limit of detection ($\mu\text{g ml}^{-1}$)	0.69
Limit of quantification ($\mu\text{g ml}^{-1}$)	2.3

^aMean of six injections.

citrate (n = 6). The mean percentage recovery obtained after six repeated experiments was found to be 44.38 (Table 4), indicating that the results are accurate and precise and there is no interference from the common excipients used in the pharmaceutical dosage forms.

CONCLUSIONS

The validated HPLC method has been proved to be precise and reliable. Using this procedure, it is possible to perform quantitative analysis of butamyrate citrate in pharmaceutical dosage forms within a short analysis time which is essential in

Table 3. Summary of Intra-Day (Repeatability) and Inter-Day (Intermediate Precision) Variability Data for Determination of Butamyrate Citrate

Real concentration ($\mu\text{g ml}^{-1}$)	Intra-day measured concentration ($\mu\text{g ml}^{-1}$) ^a		Inter-day measured concentration ($\mu\text{g ml}^{-1}$) ^b	
	Mean	%RSD	Mean	%RSD
80	80.58	0.71	79.89	0.42
150	150.92	0.22	149.74	0.57
200	199.18	0.89	199.48	0.38

^aMean values represent five different sample standards for each concentration. ^bInter-day precision was determined from five different runs over a 2-days period.

known amounts of butamyrate citrate onto a placebo mixture. Results obtained from the recovery studies are given in Table 4. The recovery experiments show mean recoveries of 100.3 with RSD value of 0.25.

High recovery results obtained from the proposed method for the analysis of butamyrate citrate syrup indicate that this assay procedure can be used for quantification and routine quality control analysis of this commercial sample.

Application to Pharmaceutical Products

On the basis of the above results, the proposed method was applied to the determination of butamyrate citrate in syrup dosage forms. Figure 4 shows representative chromatogram obtained from the analysis of butamyrate citrate in syrup. The differences between the amount claimed and those assayed were very low and the RSD values were within the acceptable range mentioned by pharmacopoeias. The mean values of 15.04 mg with %RSD of 0.25 was obtained for butamyrate

Table 4. Statistical Analysis of Assay Results and Recovery Experiments in Commercial Samples

Analysis of pharmaceutical dosage form	Value
Label claim (mg in 10 ml)	15 mg
Mean of amount found (mg in 10 ml) ^a	15.0
Confidence limit ^b	± 0.0080
%Recovery	100.3
%RSD	0.25
Recovery analysis	
Added (mg)	45.0
Mean of amount found (mg) ^a	44.4
Confidence limit ^b	± 0.05
%Recovery	98.6
%RSD	0.45

^aMean values represent six determinations. ^bCalculated value for 95% confidence level.

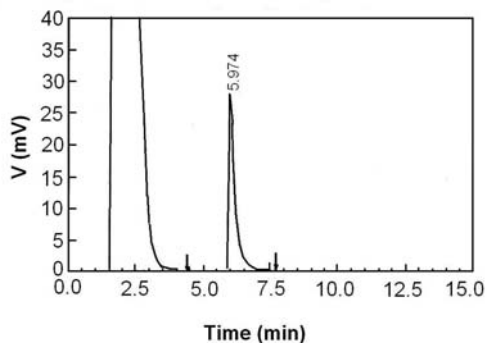


Fig. 4. Chromatogram of butamyrate citrate in pharmaceutical preparation.

routine analysis of pharmaceutical dosage forms. The developed method was validated as described in ICH guideline. System suitability, specificity, linearity, LOD, LOQ values, within- and between-day precision and accuracy of the proposed technique were obtained during the validation studies. Based on the above-mentioned detailed validation studies, it is likely to conclude that the proposed method has the advantages of simplicity, repeatability, sensitivity, less

expensive reagents and short analysis time than the other reported methods, which make it suitable for the studies of formulated samples in routine quality control applications.

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