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# Development of high performance liquid chromatography method for buspirone in rabbit serum: Application to pharmacokinetic study

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ABSTRACT

A simple and sensitive high performance liquid chromatographic (HPLC) method for quantification of buspirone (BUSP) in rabbit serum was developed and validated. BUSP and internal standard (IS), diltiazem hydrochloride were extracted into dichloromethane and separated using an isocratic mobile phase, on a Kromasil $C_8$ column. The eluent was monitored by UV detector at 235 nm and at a flow rate of 1.0 mL $min^{-1}$. The linearity range of proposed method was 1–3000 ng $mL^{-1}$. The intra-day and inter-day coefficient of variation and percent error values of the assay method were less than 15% and mean recovery was more than 97 and 96% for BUSP and IS, respectively. The method was found to be precise, accurate, and specific during the study. The method was successfully applied for pharmacokinetic study of buspirone after application of reservoir based transdermal therapeutic system of BUSP to rabbits.



## 1. Introduction

Buspirone (Fig. 1a), 8-[4-(4-pyrimidin-2-yl-piperazin-1-yl) butyl]-8-azaspiro [4,5] decane-7,9-dione, an azaspirone, is an anxiolytic drug that has dopaminergic, noradrenergic and serotonin-modulating properties used in the treatment of generalized anxiety disorders [1]. It is available under the trade names of Ansial®, Anxiolan®, Busansil®, Buspar®, Buspirol®, Narol®, Neurosine®, Pasrin-10®, Paxon®, Relax®, Sorbon®, Tensispes®, Tran-Q® and Xiety®. Buspirone (BUSP) is rapidly absorbed from the gastrointestinal tract and the peak plasma concentrations occur after about 40–90 min. The protein binding is very high (95%). BUSP undergoes extensive first-pass metabolism that results in low oral bioavailability. Most metabolites are inactive, although oxidative dealkylation produces an active metabolite, 1-(2-pyrimidinyl)-piperazine which is about 20–25% as potent as parent drug. The major metabolite is 5-hydroxybuspirone. The metabolites are excreted mainly in urine (65%) and faeces (35%). The serum therapeutic concentration range is 0.9–5 μg $L^{-1}$ [2]. For pharmacokinetic studies, a sensitive method that allows an accurate measurement of low concentration of BUSP in biological fluid is required.

Few analytical methods previously have been reported for estimation of BUSP in biological fluids. However, no literature is available for the estimation of BUSP in rabbit serum and to estimate the BUSP a simple and sensitive method is required. The serum of two different species differs in their vascular components; therefore separate studies in rabbit serum will provide relevant information. Hence, United States Food and Drug Administration initiated partial validation to overcome the matrix effects [3]. Chromatographic methods reported for BUSP in blood [4], human plasma [5–9], serum [10] and urine [11] include based on liquid chromatography [4–10]; gas chromatographic-mass spectrometry [12] and radioimmunoassay [13]. However, these methods have various limitations, cost, time consuming sample clean-up, laborious extraction steps and long run times which are not suitable in all conditions. Some methods utilize electrochemical detector [9] with column switching procedure [10]. Moreover, the liquid chromatography and mass spectrometry techniques [4,5] are not available for most laboratories because of its special requirement and financial reasons.

BUSP is a potent anxiolytic agent it could cause dizziness in human beings [14], hence rabbits were selected as an animal model for conducting pharmacokinetic study. In this paper the estimation of BUSP concentrations after application of a reservoir based transdermal therapeutic system was described. The present work was aimed at developing a sensitive HPLC method for determination of BUSP in rabbit serum. The advantages of present method include small sample volume, simple and single step extraction procedure using inexpensive chemicals, less organic solvent consumption, short run time and does not require special type of detector such as mass spectrometric detector. Protein precipitation was selected

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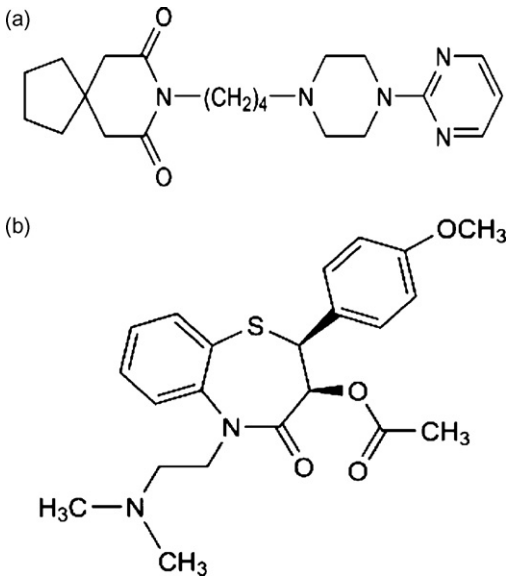


**Fig. 1.** Structure of (a) buspirone and (b) diltiazem.

because it had obvious advantages such as shorter processing time, fewer steps, and good sample clean up. We also demonstrate the applicability of this method for pharmacokinetic study in rabbits.

## 2. Experimental

### 2.1. Materials

Buspirone hydrochloride and diltiazem hydrochloride pure samples were gifted by Dr Reddys Laboratories (Hyderabad, India) and Divi's Laboratories Ltd (Hyderabad, India) respectively. Acetonitrile, methanol (HPLC grade), potassium dihydrogen orthophosphate (GR grade) and ortho phosporic acid (GR grade) were purchased from Merck (Mumbai, India). Double distilled water was used during the entire HPLC procedure.

### 2.2. Chromatographic conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AT solvent module, SPD10A UV–visible detector with LC10 software. The analytical column used was C8 column (Kromasil, 250 mm × 4.6 mm i.d, particle size 5 μ) at a temperature of 30 °C. The mobile phase consists of acetonitrile, potassium phosphate buffer (10 mM, pH 4.6) 35:65 v/v, the pH was adjusted to 4.6 with ortho phosporic acid. The elute was monitored at 235 nm, at a flow rate of 1 mL $min^{-1}$. The injection volume was 50 μL and detector sensitivity was set to 0.005 AUFS.

### 2.3. Preparation of the calibration standards and quality control (QC) samples

The stock solutions of BUSP and diltiazem hydrochloride (DILT) were prepared in methanol at a concentration of 1.0 mg $mL^{-1}$ each. Diltiazem hydrochloride was used as an internal standard (Fig. 1b). The working solutions of 10 μg $mL^{-1}$ and 1.5 μg $mL^{-1}$ were prepared by appropriately diluting the stock solutions of BUSP and DILT, respectively. BUSP working solution was used to prepare the spiking stock solutions for construction of nine point calibration curve (1, 5, 10, 50, 100, 500, 1000, 2000 and 3000 ng $mL^{-1}$) and QC samples at three different levels (2.5, 1250 and 2750 ng $mL^{-1}$). All the stock solutions were refrigerated (4 °C) when not in use. Calibration standards and QC samples were prepared in bulk by spiking 100 μL of respective spiking stock solutions to 0.5 mL of control rabbit serum and then aliquoted. These were stored at −20 °C until analysis.

### 2.4. Sample preparation for analysis

Aliquot (0.5 mL) of the rabbit serum containing BUSP was pipetted into screw capped tubes and 100 μL of an internal standard (1500 ng $mL^{-1}$ of DILT) was added and vortexed for 2 min. Phosphate buffer (500 mM of potassium dihydrogen phosphate) saturated with sodium chloride solution of 250 μL was added, vortexed for 3 min followed by the addition of 5 mL of dichloromethane. This was vortexed for 5 min and centrifuged at 5000 rpm for 15 min. The dichloromethane layer (4.5 mL) was separated and allowed to evaporate in vacuum oven (Sheldon Manufacturing Inc., Cornelius, USA). The evaporated residue was reconstituted with 150 μL of mobile phase and 50 μL of the reconstituted sample was injected in to the HPLC system.

### 2.5. Assay validation

The assay was validated according to the guidelines [3,15] with respect to linearity, range, LOD, LOQ and precision etc. The intra and inter-day precision and accuracy of the assay were determined by percent coefficient of variation (C.V) and percent relative error (R.E) values, respectively. Samples containing 2.5, 1250 and 2750 ng $mL^{-1}$ concentrations were spiked for the determination of precision and accuracy. Five replicates at each concentration were processed as described in the sample preparation on day 1, 3, 5 and 10 to determine intra-day and inter-day precision and accuracy. The limit of detection (LOD) was determined using the signal-to-noise ratio (s/n) of 3:1 by comparing test results from samples with known concentrations of analytes with blank samples.

### 2.6. Recovery

The extraction recovery was determined by standard addition for QC samples at concentration of 2.5, 1250 and 2750 ng $mL^{-1}$ of BUSP and one concentration (150 ng $mL^{-1}$) for IS. Five replicates of each QC sample were extracted by the above mentioned sample preparation and injected into the HPLC system.

### 2.7. Stability studies

To ensure the reliability of the results in relation to handling and storing of serum samples and stock standard solutions, stability studies were carried out at three different concentration levels 2.5, 1250 and 2750 ng $mL^{-1}$. Freeze and thaw stability for three cycles was determined over three freeze-thaw cycles by thawing at room temperature for 2–8 h and then refreezing at −20 °C for 12–24 h. The stability of spiked rabbit serum stored at room temperature (bench top stability) was evaluated for 12 h. The long-term stability was assessed by carrying out the experiment after 30 days of storage at −20 °C. The stock solution stability of BUSP and DILT each 1000 ng $mL^{-1}$ were determined at room temperature for 12 h and upon refrigeration (4 °C) for 20 days. The concentration of BUSP after each storage period was related to the initial concentration as determined for the samples that were freshly prepared.

### 2.8. Robustness

To determine the robustness of the developed method experimental conditions were purposely altered and the resolution of BUSP and DILT was evaluated. The effect of percent organic strength (acetonitrile 35 ± 2%), flow rate (1.0 ± 0.2 mL), buffer concentration (10 ± 5 mM), temperature (30 ± 5 °C) and pH of buffer (4.6 ± 0.2)