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Validated UV-Spectrophotometric Determination of Velathamate Bromide in Pure and Pharmaceutical Formulation

Meghana. G.S, Gurupadayya B.M*. Sowmya. S and Vasavi.T.N
Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Mysore – 570 015, Karnataka, India

ABSTRACT

Aim: The present work is aim to develop simple, extraction free and UV spectrophotometric method for the quantitative estimation of valethamate bromide in bulk drug and pharmaceutical formulations. **Method:** A simple double beam UV spectrophotometric method has been developed and validated with different parameters according to ICH guidelines. **Results and discussion:** The drug showed strong absorption at 227 nm and linearity in the concentration range of 50-300 µg/ml. Recovery studies for valethamate bromide was found to be 99.9%. The % RSD values were found to be within the range and found not interference from excipients and the proposed method was statistically validated. **Conclusion**: The results showed that the proposed method is suitable for the precise, accurate and rapid determination of valethamate bromide in bulk drug and its formulations.

INTRODUCTION

Valethamate bromide is chemically phenyl-2methyl-valerianic acid-diethyl- 1- amino-ethyl ester-bromo methylate[1] (Fig. 1). The drug is official in BP and INF. It is effective, fast, acting anticholinergic and musculotrophic agent. It is a synthetic analog of atropine, which is found to reduce spasm by its parasympatholytic action and results in cervical dilatation[2].
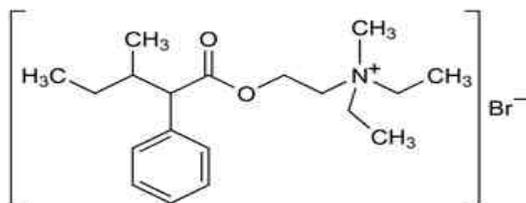

Fig. 1. Chemical structure of Velathamate bromide

Literature survey reveals that only one method was developed for the quantification of Valethamate bromide in pharmaceutical formulation by HPTLC method[3]. So here we developed sensitive UV spectrophotometric method for the assay of Valethamate bromide using sodium hydroxide as solvent and validated the method according standard ICH guidelines.

*Corresponding author.
Dr. B.M.Gurupadayya
Professor,
Pharmaceutical Analysis Group,
Dept. of Pharmaceutical Chemistry,
J S S College of Pharmacy, J S S University,
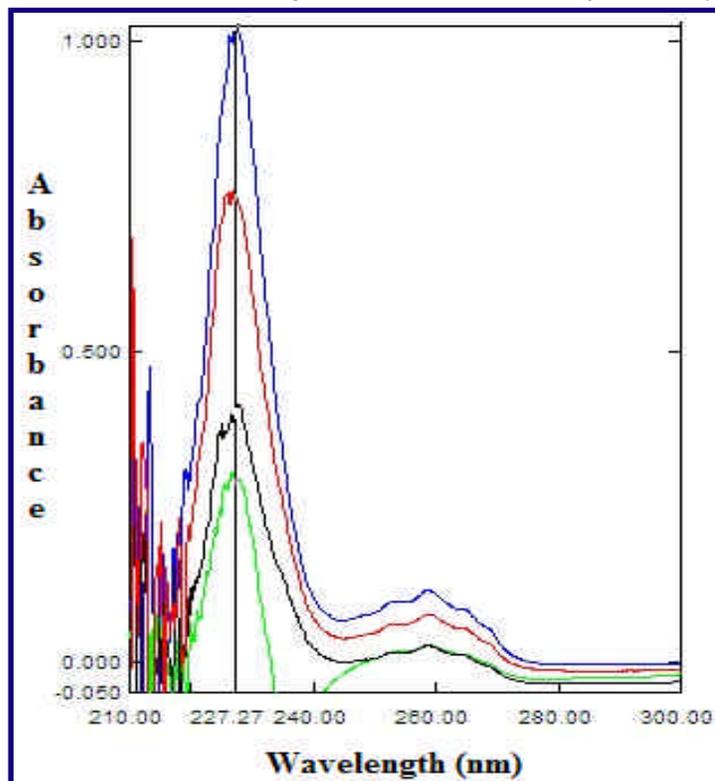*Shivarathreeshwara Nagar,*
Mysuru-570 015, Karnataka, India.

EXPERIMENTAL

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Shimadzu electronic balance was used for weighing the samples.

Reagents and solutions

All employed chemicals were of analytical grade and high-purified water was used for the preparation of the all the reagent. Valethamate bromide pure sample was obtained as a gift sample from RL fine chemicals ltd, Bangalore, India.

5N Sodium hydroxide solution.

It is prepared by dissolving 20 grams of sodium hydroxide in 100 ml of distilled water.

Preparation of standard drug solution

Valethamate bromide Stock solution

A stock solution of velathamate was prepared by dissolving 100 mg in 100 ml of 5N sodium hydroxide (1000 µg/ml). Aliquots of stock solution of Valethamate of 0.5.0-3.0 ml (1000µg/ml) were transferred into a series of 10 ml volumetric flasks. The solution was diluted to sodium hydroxide up to the mark. The resultant solution of each was measured at 227 nm against the reagent blank. The overlay absorption spectra and calibration curve were represented in the Fig. 2 and 3 respectively.

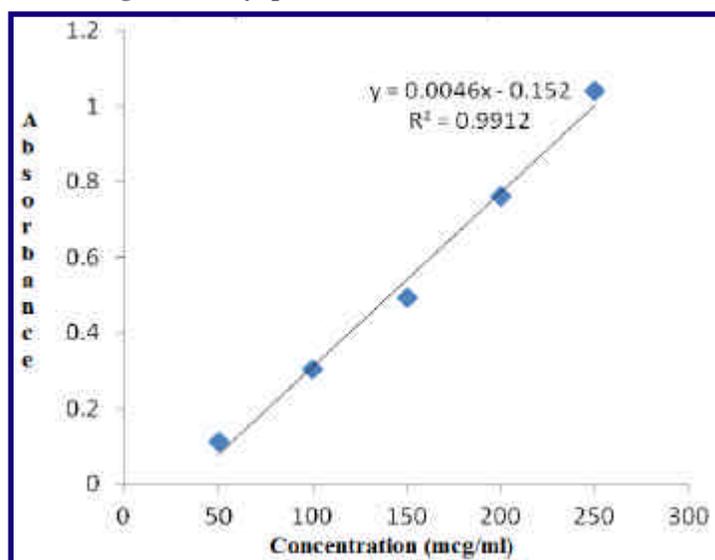**Fig. 2: Overlay spectra of Valethamate bromide**

Fig. 3: Linearity graph of Valethamate Bromide

RESULT AND DISCUSSION

Validation of the method[4]
Specificity

The spectra obtained from tablet and pure drug solution was identical with an equivalent concentration of valethamate bromide. It was concluded that the excipients did not interfere with quantification of valethamate bromide in this method and the proposed method could be considered specific.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determined with precision and accuracy using the method. The linearity ranges are found to be in the range of 50-300 µg/ml.

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations [– lower concentration (LC, 80%), intermediate concentration (IC, 100%) and higher concentration (HC, 120%)] were prepared from independent stock solutions and analysed. Accuracy was assessed as the percentage relative error and mean % recovery. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug to a known pre analyzed formulation sample and the total concentration was determined using the proposed methods.

Precision

Repeatability was determined by using different levels of drug concentrations (same as in accuracy study), prepared from independent stock solutions and analyzed. Inter-day, intra-day and inter instrument variations were studied to determine intermediate precision of the proposed analytical methods. Different levels of drug concentrations (6 times) were prepared three different times in a day and studied for intra-day variation. The same procedure was followed for three different days to study inter-day variation. One set of different levels of the concentrations was reanalyzed using the Shimadzu 1800 UV–VIS spectrophotometer connected to computer with UV-PC software was for studying the inter-instrument variation. The per cent relative standard deviation (% R.S.D.) of the predicted concentrations from the regression equation was taken as precision. Precision studies were also carried out using the real samples of valethamate bromide in a similar way to standard solution to prove the usefulness of the method. The values recovery and precision of the methods were shown in Tables 2 and 3.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for Method A and Method B by the proposed method were determined using calibration standards. LOD and LOQ are calculated as 3.3 σ/s and 10 σ/s, respectively, where s is the slope

Table 1: Optical parameters of the method

Parameters	Method
λ_{max}, nm	227
Beer's law limits, μgmL^{-1}	50-250
Sandell's sensitivity,$\mu g/cm^2/0.001$ A.U.	0.4504
Regression equation, $y = mx + c$	Y= 0.0046x- 0.152
Slope, m	0.0046
Intercept, c	0.152
Limit of detection, $\mu g/ml$	10.16
Limit of quantification, $\mu g/ml$	30.80
Molar extinction coefficient,L mol^{-1} cm^{-1}	5.8728×10^4
Correlation coefficient (r)	0.9912

Table 2: Recovery study of the method

Level of recovery (%)	Amount of formulation (µg/ml)	Amount of Pure drug (µg/ml)	Total amount of drug (µg/ml)	% Recovery
50	100	50	150	101.53
	100	50	150	
	100	50	150	
100	100	100	200	99.34
	100	100	200	
	100	100	200	
150	100	150	250	102.10
	100	150	250	
	100	150	250	

Table 3: Precision study of the method

Conc. (µg/ml)	Intraday	Interday
150	0.493	0.489
150	0.489	0.478
150	0.478	0.48
150	0.485	0.488
150	0.487	0.493
150	0.488	0.487
Average	0.48666	0.4858
Std. Dev.	0.00457	0.0052
%RSD	0.9391	1.0722

of the calibration curve and s is the standard deviation of y-intercept of regression equation. LOD and LOQ are found to be 10.16 µg/ml and 30.80 µg/ml respectively.

The validity of the methods for the assay of valethamate bromide was examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure valethamate bromide were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

Robustness

The robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important procedure variables such wavelength (226 nm and 228 nm). The results obtained from the different conditions were not similar compared to the optimum conditions and none of these variables significantly affected the assay of valethamate bromide and the proposed method could be considered robust.

Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 150 µg/ml of valethamate bromide using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts. Thus, the proposed methods could be considered rugged.

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of in pure valethamate bromide form and in pharmaceutical preparations. Method validation has been demonstrated by a variety of tests for specificity, sensitivity, linearity, precision, accuracy, recovery, and stability. The result of analysis suggests the applicability, reproducibility, and utility of the method for direct estimation of valethamate bromide in quality control laboratories.

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