

# Sensitive and Robust RP-HPLC Method for Determination of Valethamate Bromide in Pharmaceutical Formulation

Umang Shah<sup>1,\*</sup>, Tarang Talaviya<sup>2</sup>, Anuradha Gajjar<sup>3</sup>

<sup>1,2,3</sup>Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, Charusat Campus, Gujarat

**\*Corresponding Author:**

Email: umangshah.ph@gmail.com

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## ABSTRACT

The present study depicts the developed and validated simple, reliable, sensitive and robust RP-HPLC method for the determination of Valethamate bromide in pharmaceutical formulation. The chromatographic system consisted of LC 2010cHT, Luna HPLC analytical C<sub>18</sub> 100 A<sup>0</sup>, 250 X 4.6 mm, 5 µm columns and the mobile phase containing acetonitrile: water in the ratio of (20:80) % v/v. Detection was carried out by using PDA detector at 200 nm. Retention time (R<sub>t</sub>) of Valethamate bromide was 4.62 min. Method shows to linear response in the range of 5-30 µg/ml (r<sup>2</sup>=0.9975). LOD and LOQ were 0.22 and 0.68 µg/ml, respectively. Method was validated according to ICH Q2 (R1) guidelines. Parameters taken into consideration for validation were linearity, precision, specificity, accuracy, robustness. %RSD values for all the parameters were <2%. Accuracy of the method after standard addition of the drug was within range 99.67-100.66%. Robustness study was performed using 2<sup>3-1</sup> factorial design. The developed method can be successfully applied to the pharmaceutical formulation for determination of Valethamate bromide.

**Keywords:** Valethamate bromide (VLB); RP-HPLC; ICH guideline; Validation; Factorial Design

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## INTRODUCTION

Valethamate Bromide (VLB) chemically, it is N, N-Diethyl-N-methyl-2-(3-methyl-I-oxo-2-phenylpentyl) oxyl ethanaminium bromide (Fig.1).<sup>1-3</sup> VLB is an antispasmodic drug used to induce the labour.<sup>4</sup> Literature survey reveals that only HPTLC<sup>5</sup> method is reported for the estimation of Valethamate bromide in pharmaceutical dosage form. The present work develop an alternative analytical procedure based on RP-HPLC for estimation of Valethamate Bromide in bulk and pharmaceutical dosage form and validate the developed method as per ICH Q2(R1) guideline<sup>6</sup> and perform the robustness study by applying full factorial design.

## MATERIALS AND METHODS

### Reagents and chemicals:

The Valethamate Bromide (VLB) reference standard was kindly gifted by TTK healthcare Ltd (Hyderabad, India). Acetonitrile (ACN) was procured from Loba Chemi Pvt. Ltd. and HPLC grade water was prepared by Mili-Q - DQ5 smart pack system, Millipore.

### Apparatus:

Chromatographic separation was performed on a Shimadzu HPLC system consisting of pump (LC 20AT Shimadzu), detector PDA (SPD - M20A, Shimadzu), injection system (Rheodyne System 20 µl loop), oven (CTO -10AS, Shimadzu), Column (Luna HPLC analytical C18 100 A<sup>0</sup> column 250 \* 4.6 mm, 5 µm.). The elution was carried out isocratically at flow rate of 0.4 ml/min using acetonitrile: water (20: 80, % v/v) as a mobile phase. The Detection wavelength selected was 200 nm. Shimadzu AUX 220 analytical balance was used for weighing. The result of system suitability parameter was shown in Table 1.

### Preparation of standard stock:

Accurately weighed 10 mg of VLB was transferred to 100 ml volumetric flask. The volume was made up to the mark with mobile phase to obtain stock solution of VLB having concentration 100 µg/ml.

**Preparation of solutions for construction of calibration curve:**

Aliquot (0.5, 1, 1.5, 2, 2.5 and 3 ml) of VLB from their stock solution were withdrawn and transferred into individual 10 ml of volumetric flask and volume was made up to the mark with mobile phase. The concentrations of resulting solutions were (5, 10, 15, 20, 25, 30 µg/ml), respectively.

**Assay of marketed formulation:**

Ten ampoules were broken and solution was filtered into the 250 ml volumetric flask. From this solution 1 ml aliquot was withdrawn and transferred into 100 ml of volumetric flask and 70 ml of distilled water was added. The mixture was sonicated for 20 min and diluted up to the mark with distilled water and filtered through whatman filter paper no.41. The final concentration of the solution was 80 µg/mL. From this solution 18.75 ml aliquot was withdrawn into 100 ml of volumetric flask and diluted up to the mark with mobile phase. Solution contains VLB 15 (µg/ml). The analysis procedure was repeated six times for injectable formulation and result was shown in **Table 2**.

**Validation of the developed method:**

The developed method was validated according to ICH Q2 (R1)<sup>6</sup>. As per the guideline the method was subjected to validation by performing the parameters like Linearity and range, precision, accuracy, repeatability, specificity, robustness and sensitivity. Robustness was performed using 2<sup>3-1</sup> factorial design.

**Linearity:**

Under proposed experimental conditions, the relationship between the area and the concentration of VLB was studied. The calibration curve was plotted between concentrations versus area by the prepared concentration of 5 - 30 µg/ml of stock solution, and r<sup>2</sup> value was found to be 0.9997 (**Table 3**).

**Repeatability:**

The precision of the methods was checked by repeated measurement (n = 6) of the peak area of standard solution of VLB 15 µg/ml without changing the parameters for the method over a short interval of time. The relative standard deviation (% RSD) was found to be less than 2%, which indicates that the proposed method is repeatable.

**Precision:**

Intraday and interday precision were carried out through replicating analysis (n=3) for 3 concentrations (5, 15 and 30 µg/mL). For interday precision, the analysis was carried out for three consecutive days at the same concentration level as used in intraday precision. And the intraday precision was carried out by using three concentrations at different time interval in a day. The area was recorded as % RSD (**Table 3**).

**Specificity:**

The prepared 15 µg/mL standard and sample solutions of VLB were injected and check any other excipients interference occurs or not.

**Accuracy:**

The accuracy of the method was determined by calculating recoveries of VLB by method of standard additions. Known amount of VLB (15 µg/ml) was added at 80, 100 and 120% level to a pre-quantified sample solution, and the amount of VLB was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve. The values of % recovery for analysis of formulation are found within 98-102% which shows that the method was accurate for analysis of marketed formulation.

**LOQ & LOD:**

A calibration curve was prepared using concentrations in the range of 5 - 30 µg/ml for VLB (expected detection limit range). The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantitation limit.

LOD and LOQ were determined using the following equation;

$$\text{LOD} = 3.3 \times \sigma / S \text{ and } \text{LOQ} = 10 \times \sigma / S$$

Where,  $\sigma$  is the standard deviation of the response

S is the slope of the calibration curve.

### Robustness:

Robustness testing was performed by experimental design approach.  $2^{(n-1)}$  factorial designs for testing of three factors are the most commonly used designs for robustness testing of chromatographic methods. The proposed HPLC method was tested for robustness using factorial design with four experiments. The parameters that were varied are detection wavelength, mobile phase ratio, and flow rate of the mobile phase.

The p values for the parameters selected for the robustness study were greater than 0.05 hence the selected model for robustness study passes the test and none of the above parameters affect significantly to the results given by the method. Hence, the method was found to be robust. (Fig. 3)

### RESULTS AND DISCUSSION

Optimization of mobile phase was performed based retention time, number of theoretical plates, tailing factor and peak shape obtained for VLB. The mobile phase acetonitrile: water (20:80) was found to be satisfactory and gave well-resolved peak for VLB with acceptance criteria for system suitability test. The retention time for VLB was 4.76 min. The Chromatogram of VLB under optimized mobile phase condition was shown in Fig. 2.

The calibration curve for VLB was obtained by plotting the peak area of VLB versus the concentration of VLB over the range of 5 -30  $\mu\text{g/ml}$ , and it was found to be linear with  $r^2 = 0.9975$ . The data of regression analysis of the calibration curves are shown in Table 3. The detection limit for VLB was 0.22  $\mu\text{g/ml}$  and quantitation limit was 0.68  $\mu\text{g/ml}$ . The validation parameters are summarized in Table 3.

The recovery of VLB was found to be in the range of 99.67 – 100.66%. The system suitability test parameters are shown in Table 1. The liquid chromatographic method was applied to the determination of VLB in their combined dosage forms (injectable dosage form). The result for VLB was comparable with the corresponding labeled amounts. Robustness was performed using factorial design ( $2^{3-1}$ ) (Table 4). The factors selected for robustness were flow rate, mobile phase composition, detection wavelength. For all the factors the p values obtained were higher than 0.05 (Table 5) which indicates the factors have no significant effect on the response. Hence the method is robust.

Proposed study describes a new RP-HPLC method for the estimation of VLB in injectable dosage form. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of VLB injectable dosage form.

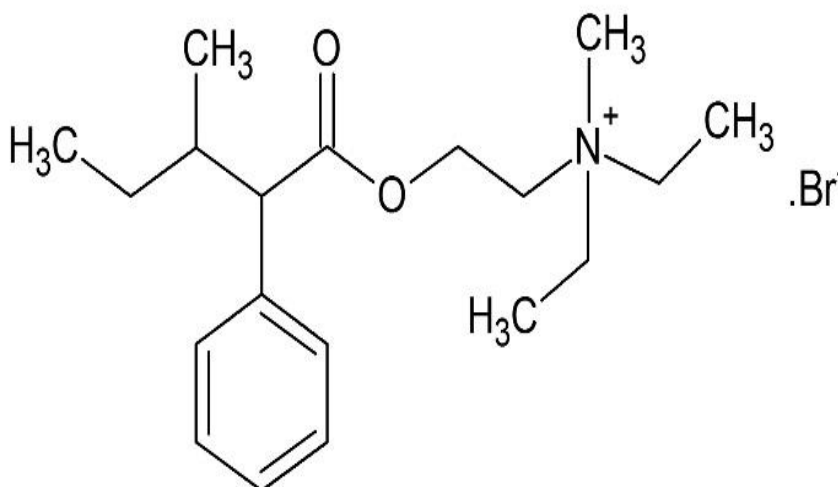
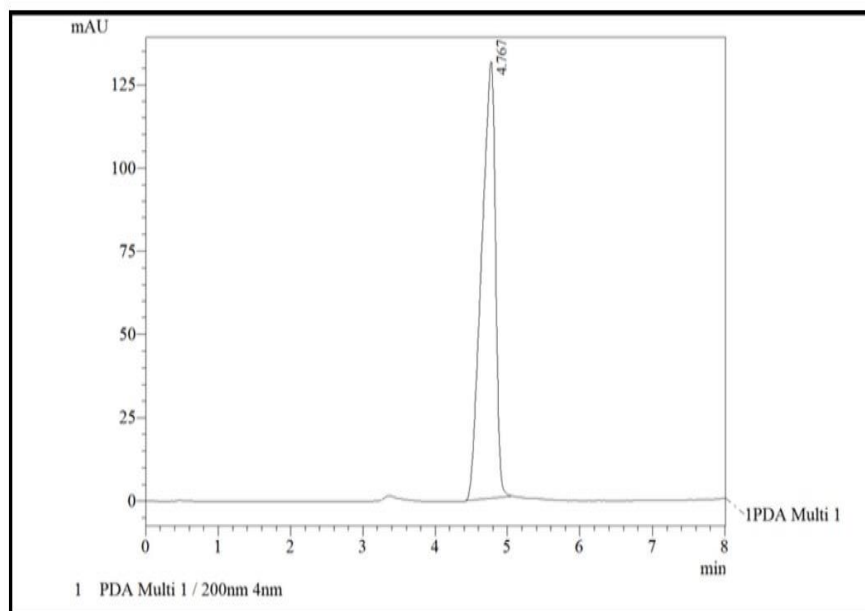
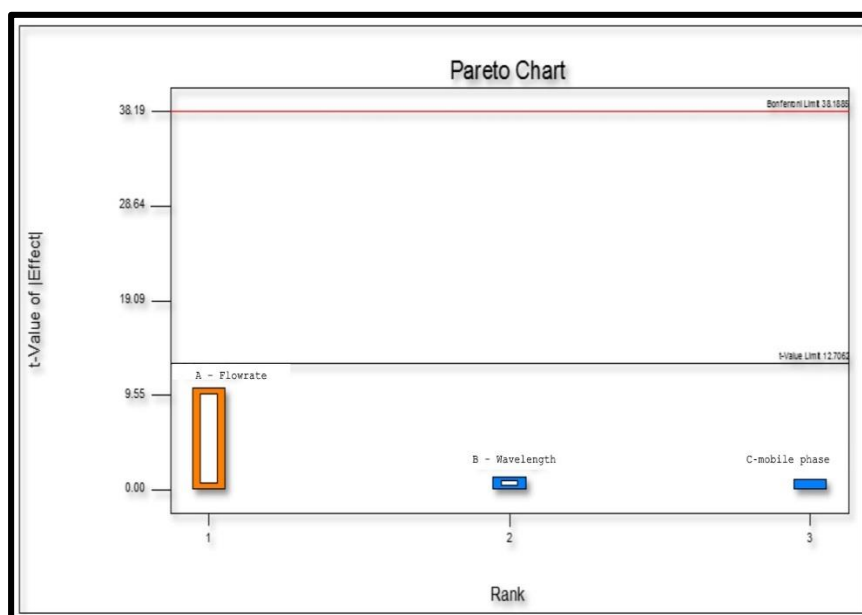


Fig. 1: Structure of VLB



**Fig. 2: Chromatogram of VLB under optimized mobile phase condition [Acetonitrile: Water (20: 80, % v/v)]**



**Fig. 3: Pareto chart for robustness study**

**Table 1: System suitability test parameters for VLB**

System Suitability Parameters	VLB
Retention Time	4.76
No. of theoretical plates	2062
Tailing Factor	1.11

**Table 2: Assay of pharmaceutical formulation of VLB by developed RP-HPLC method**

Actual Concentration ( $\mu\text{g/mL}$ )	Peak Area (AU) $\pm$ SD	Concentration found ( $\mu\text{g/mL}$ )	%Recovery	%RSD
15	963812 $\pm$ 14619.1	14.75	98.33	1.52

**Table 3: Summary of Validation Parameters**

Validation Parameters	VLB
Linearity ( $\mu\text{g/ml}$ ) (n=6)	5-30 $\mu\text{g/ml}$
Regression equation (n=6)	$Y=63468x+53708$
Correlation co-efficient ( $r^2$ )	0.9975
%Recovery (n=3)	99.66 -100.66%
Repeatability (%RSD <sup>a</sup> )( n=6)	0.50
Intraday precision (% RSD <sup>a</sup> )( n=3)	0.20-0.96
Interday precision (% RSD <sup>a</sup> )( n=3)	0.37-1.06
LOD ( $\mu\text{g/ml}$ )	0.22
LOQ ( $\mu\text{g/ml}$ )	0.68

RSD<sup>a</sup> indicates relative standard deviation; VLB is Valethamate Bromide

**Table 4: Factorial design ( $2^{3-1}$ ) for robustness study**

Run	Mobile Phase Strength	Detection Wavelength	Flow Rate
1	-1	+1	-1
2	+1	-1	-1
3	-1	-1	+1
4	+1	+1	+1

**Table 5: Levels of robustness study and p value**

Parameters	Optimized Condition	High level(+1)	Low level (-1)	p-value
Flow rate	0.4	0.5	0.3	0.0619
Detection wavelength	200nm	198 nm	202 nm	0.9381
Mobile phase ratio	ACN: Water(20:80)	ACN: Water(88:12)	ACN: Water(72:28)	0.4232

## CONCLUSION

The developed method was validated as per ICH Q2(R1) guideline and was found to be within the prescribed limits. It can be concluded that developed method is simple, rapid, accurate, and specific. The method is suitable for routine quality control analysis of Valethamate bromide in pharmaceutical formulation.

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