

Highly Accurate and Reliable RP-HPLC Approach for the Measurement of Valethamate Bromide in Pharmaceutical Compounds

V.Haribaskar, M.Gobinath, B.Prathap & D.Ramesh

ABSTRACT

The developed and confirmed RP-HPLC technique for the measurement of Valethamate bromide in pharmaceutical formulation is presented in this paper. The method is simple, reliable, sensitive, and robust. The mobile phase was composed of acetonitrile and water in a ratio of 20:80 % v/v. The chromatographic system included LC 2010cHT, Luna HPLC analytical C18 100 A^o, 250 X 4.6 mm, 5 μm columns. At 200 nm, a PDA detector was used for detection. The half-life of valethamate bromide was 4.62 minutes. In the 5-30 μg/ml range, the method demonstrates a linear response ($r^2=0.9975$). LOQ was 0.68 μg/ml and LOD was 0.22 μg/ml. Following the requirements laid forth by ICH Q2 (R1), the method was verified. Linearity, precision, specificity, accuracy, and robustness were the parameters that were validated. There was less than a 2% RSD for all of the metrics. The method's accuracy ranged from 99.67 to 100.66% after the typical addition of the medication. A research was conducted to assess robustness using a 23-1 factorial design. The described approach may be used to determine the concentration of Valethamate bromide in pharmaceutical formulations.

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

INTRODUCTION

N, N-Diethyl-N-methyl-2-(3-methyl-I-oxo-2-phenylpentyl) oxyl ethanaminium bromide is the chemical name for valethamate bromide (VLB) (Fig.1). An antispasmodic medication called 1-3 VLB is used to induce labor.⁴ Valethamate bromide in medicinal dose form has only been documented to be estimated using the

HPTLC5 technique in the literature. This research used a complete factorial design to conduct a robustness analysis and validate the established technique according to the ICH Q2(R1) guideline⁶, and it used RP-HPLC as an alternate analytical approach for estimating valethamate bromide in both bulk and pharmaceutical dose form.

Department of Pharmacy
Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524001
A.P. India

MATERIALS AND METHODS

Reagents and chemicals:

A reference standard of Valethamate Bromide (VLB) was generously provided by TTK healthcare Ltd of Hyderabad, India. We used the Mili-Q - DQ5 smart pack equipment from Millipore to create HPLC grade water, and Loba Chemi Pvt. Ltd. supplied the acetonitrile (ACN).

Apparatus:

Chromosome separation was carried out using a Shimadzu HPLC system that included the following components: pump (LC 20AT Shimadzu), detector (SPD - M20A, Shimadzu), injection system (Rheodyne System 20 μ l loop), oven (CTO - 10AS, Shimadzu), and column (Luna HPLC analytical C18 100 A^o column 250 * 4.6 mm, 5 μ m). An isocratic elution was performed using a mobile phase consisting of acetonitrile and water (20:80, % v/v) at a flow rate of 0.4 ml/min. A wavelength of 200 nm was chosen for detection. The analytical balance that was used for weighing was a Shimadzu AUX 220. One can see the outcome of the system appropriateness parameter in Table 1.

Preparation of standard stock:

The 10 milligrams of VLB was carefully added to the 100 milliliter volumetric flask. To get a stock solution of VLB with a concentration of 100 μ g/ml, the volume was filled up to the mark using mobile phase.

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 μ g/ml), respectively.

Assay of marketed formulation:

The 250 ml volumetric flask was filled with filtered solution after ten ampoules were shattered. One milliliter was taken from the solution, placed in a 100 milliliter volumetric flask, and then 70 milliliters of pure water was added. Following 20 minutes of sonication, the liquid was diluted with distilled water to the mark and filtered using whatman filter paper no. 41.A final concentration of 80 μ g/mL was achieved in the solution. An 18.75 ml portion of the solution was transferred to a 100 ml volumetric flask and then diluted with mobile phase until it reached the desired concentration. The solution contains 15 micrograms of VLB. For the injectable formulation, the analysis was carried out six times, and the results are shown in Table 2.

Validation of the developed method:

The developed method was validated according to ICH Q2 (R1)⁶. 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 2³⁻¹ 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 μ g/ml of stock solution, and r² 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 μ g/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, σ 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 µg/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 µg/ml and quantitation limit was 0.68 µg/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.

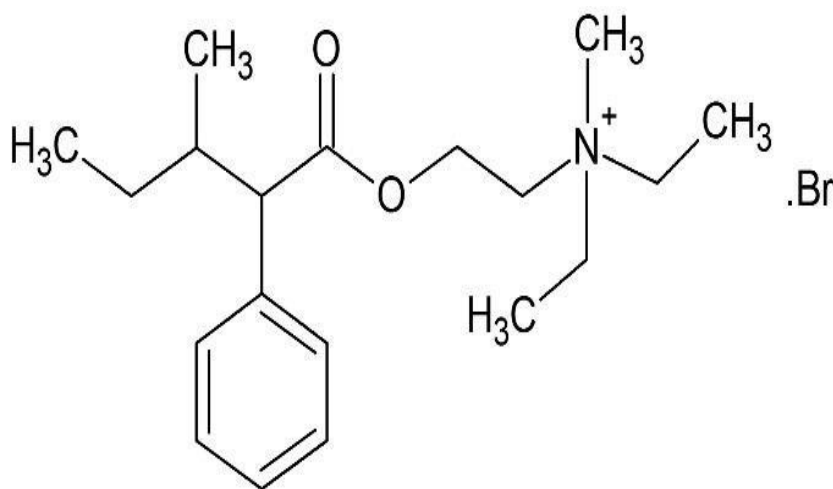


Fig. 1: Structure of VLB

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

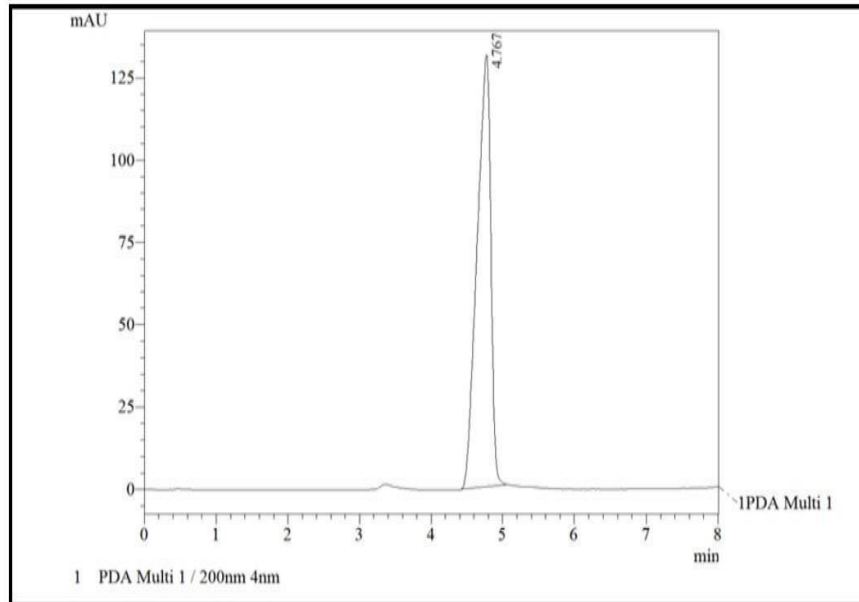


Fig. 2: Chromatogram of VLB under optimized mobile phase condition [Acetonitrile: Water (20: 80, % v/v)]

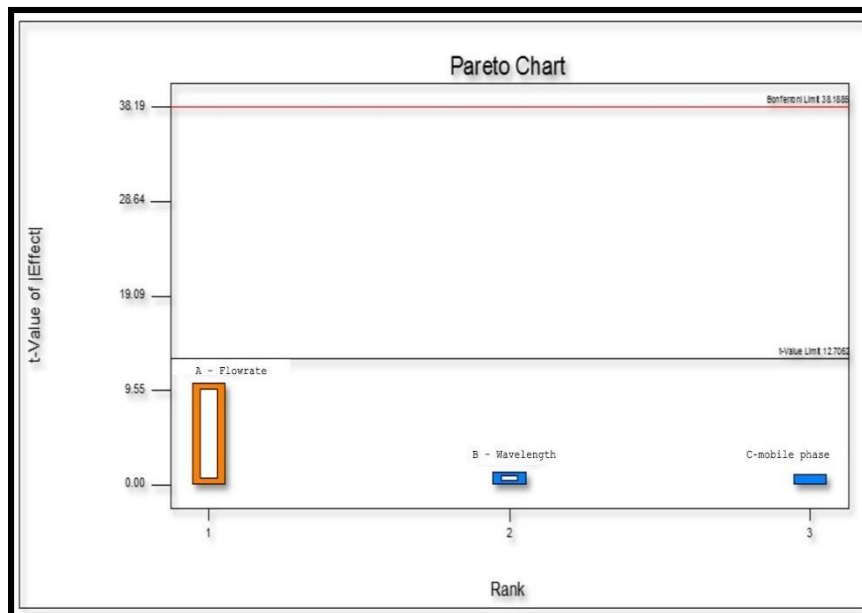


Fig. 3: Pareto chart for robustness

study Table 1: System suitability test

parameters for VLB

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

Actual Concentration (µg/mL)	Peak Area (AU)±SD	Concentration found (µg/mL)	%Recovery	%RSD
15	963812±14619.1	14.75	98.33	1.52

Table 3: Summary of Validation Parameters

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

RSD^a indicates relative standard deviation; VLB is Valethamate Bromide

Table 4: Factorial design (2³⁻¹) 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 validated technique was determined to be within the specified limitations according to the ICH Q2(R1) guideline. Simple, quick, accurate, and specific describe the created procedure. This technique works well for the regular examination of pharmaceutical formulations containing Valethamate bromide for quality control purposes.

ACKNOWLEDGEMENTS

The team thanks TTK healthcare Ltd (Hyderabad, India) for providing gift samples of VLB. The team also acknowledges Charotar University of Science & Technology, Changa

(CHARUSAT) for providing the necessary infrastructure to carry out the research.

REFERENCES

1. Merck Inc., New Jersey, USA, 2001, 1764, The Merck Index, 13th edition.
2. 3. Martindale, Ph.P. Pharmaceutical Press, 2005, The Complete Drug Reference, 34th edition, 491.
3. Section 3, pages 2257 and 2544, British Pharmacopoeia, 2003, published by the British Pharmacopoeial Commission and housed at the Department of Health,

Social Services, and Public Safety.

4. 4. In 2008, Jaypee Brothers Medical Publishers Ltd. published Tripathi's Essential of Medical Pharmacology, sixth edition, in New Delhi.
5. The authors of the article "Estimation of Valemthamate Bromide in Pharmaceutical Dosage Form by HPTLC" (Venugopal V., Harshita M.,

Nikitha B., Vikram B., Sunil L., Prasad M., Subhash G., Sowjanya G.) published in the International Journal of Research in Pharmaceutical Science in 2012 range from 373 to 375.

6. International Conference on Harmonization, 2005: Text and Methodology Q2 (R1) on Validation of Analytical Procedures (2006).