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Research Article

Estimation of Valethamate bromide in pharmaceutical dosage forms by HPTLC

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ABSTRACT

A simple, rapid, sensitive and highly precise High Performance Thin Layer Chromatographic Method has been developed for the estimation of Valethamate bromide in bulk and pharmaceutical dosage forms. HPTLC was performed on CAMAGLINOMAT IV, TLC scanner version 3.20, using n-butanol and glacial acetic acid (9:1) (v/v) gas mobile phase. The Chromatogram was developed in CAMAG twin trough glass chamber containing mobile phase. The TLC plates were scanned at 299nm in schimadzu dual wavelength scanner, and R_f value of valethamate bromide was found to be 0.37. The LOD and LOQ values were found to be 6µg/ml and 18µg/ml respectively. The linearity of Valethamate bromide was found to be 20-100µg/ml and gives correlation coefficient of 0.9985.

Keywords: Valethamate bromide; Methanol; Glacial acetic acid; n-butanol; HPTLC; Validation.

INTRODUCTION

Valethamate bromide is diethyl (2-hydroxyethyl) methyl ammonium 3-methyl-2-phenyl valerate bromide (British Pharmacopoeia- 2003; Merck index, 2001). It is official in INF 13th edition. Valethamate bromide is used as an antispasmodic drug (Tripathi KD, 2008). Literature review shows no method reported for the analysis of valethamate bromide by HPTLC. The present work deals with development and validation (I.C.H. Harmonized Tripartite guidelines,1996) of valethamate bromide in bulk drug and various pharmaceutical dosage forms by HPTLC (Indian Pharmacopoeia-1996,Gurdeep R Chatwal 1984, Sethi PD, 1996;).

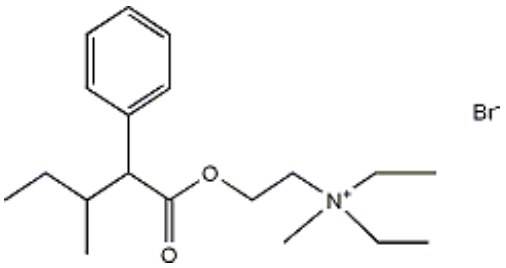

Figure 1: Structure of valethamate bromide

MATERIALS AND METHODS

INSTRUMENT USED

CAMAG LINOMAT IV (Schimadzu Dual Wavelength Scanner), Silica HPTLC Plate, CAMAG Sample Applicator, CAMAG twin trough glass chamber, Hamilton Syringe - 25 µl, CAMAG TLC Scanner Version 3.20.

CHEMICALS AND REAGENTS

Glacial acetic acid- HPLC grade from E-Merck and n-Butanol- Analytical grade from qualigens.

MOBILE PHASE

The mobile phase is prepared by mixing 45ml of n- butanol with 5ml of glacial acetic acid to get the required volume.

CHROMATOGRAPHIC CONDITIONS

The stationary phase used was silica gel G 60F254 precoated plates. Mobile phase used in the study was a mixture of n- Butanol: glacial acetic acid in the ratio 9:1(v/v). Development chamber used was a Camag twin trough glass chamber (20 cm x 10 cm) saturated with filter paper for 10 mins. Deuterium lamp was used and detection was carried out at wavelength 229 nm. A migration distance of 70mm and band width of 3 mm was applied. The distance between the tracks is 10 mm.

ESTABLISHMENT OF R_f VALUES

For determining the R_f values, working standard solutions were prepared by diluting sufficient quantity of stock solution to get the concentration of 60 µg/ml. The solution is then spotted on the TLC plates and the R_f values for Valethamate Bromide is established.

The R_f value of valethamate bromide is 0.37

CONSTRUCTION OF CALIBRATION CURVE

Varying quantities of the stock solution was suitably diluted with methanol to obtain the concentration of 20-100 µg/ml of valethamate bromide. The solution is then spotted on the TLC plates by using automatic application device; Chromatographic plate was then developed in a saturated twin trough chamber containing

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the mobile phase. After development, the plates were scanned at 229nm and the peak areas were measured and given in the table no: 01. Calibration curve was constructed by plotting concentration against peak area as shown in figure no: 02. The densitogram is given in figure no: 03.

Table 1: Linearity and range of valethamate bromide (HPTLC)

S.No	Concentration µg/ml	Peak area
1	20	161.2
2	40	331.6
3	60	476.4
4	80	632.8
5	100	795.6

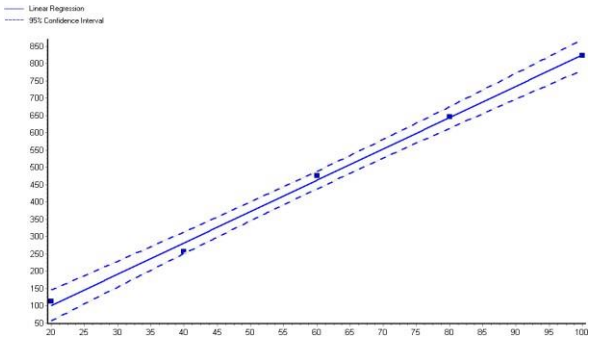

Figure 2: Linearity graph of Valethamate Bromide HPTLC

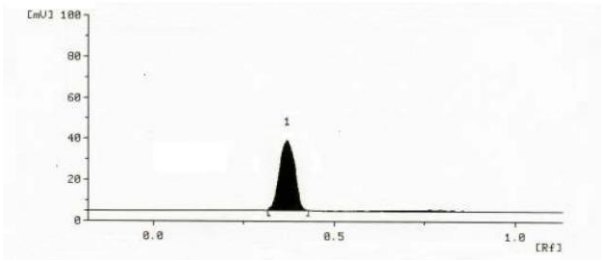

Figure 3: Standard chromatogram of Valethamate Bromide HPTLC

SAMPLE ANALYSIS

ORALSOLID DOSAGE FORMS

Twenty tablets were weighed and crushed to finely powdered material. Aliquot quantity of powder was accurately weighed and transferred to a 100ml volumetric flask and dissolved in methanol, and made up to 100ml with methanol. From this solution, further dilutions were made in methanol to get the required concentration. The solution is then spotted on the TLC plates by using automatic application device. The chromatographic plate was then development the plates were scanned at 229nm and the peak areas were measured. The amount of Valethamate bromide was calculated from the regression equation.

Amount of drug in each tablet is given by

$$\frac{Area\ of\ sample}{Area\ of\ standard} \times Wt\ of\ standard \times Dilution\ factor \times \frac{Average\ weight}{Weight\ taken}$$

The results were furnished in the table 02. The densitogram is given in Figure 04.

PARENTERALS

About 10 injections were taken and the contents were mixed; from this solution suitable dilutions were made in methanol to get the required concentration and preceded as above. The amount of the drug in each injection is calculated by using the formula given below.

Amount of drug in each injection

$$\frac{Area\ of\ sample}{Area\ of\ standard} \times Wt.of\ standard \times Dilution\ factor \times \frac{Volume\ of\ injection}{Volume\ taken}$$

The results were furnished in the table 02.

RECOVERY STUDIES

If was performed to assess the accuracy of the analytical method. The recovery experiments were carried out in triplicate by adding a known amount of drug to the pre-analyzed sample and the percentage recovery was calculated.

The statistical parameters, LOD & LOQ, and system suitability parameters are given in table 04 and 05 respectively.

RESULTS AND DISSCUSSION

The method was validated as per ICH guidelines (I.C.H Harmonized Tripartite Guidelines, 1996). The retention factor (R_f) of valethamate bromide was found to be 0.37 and the linearity range to be 20-100 µg/ml. Correlation coefficient (0.9965) indicates good linearity between concentration and peak area. The variance of

Table 2: Estimation of valethamate bromide HPTLC

S.No	Valethamate bromide	Label claim (mg/tablet)	Amount found (mg/tablet)	Standard deviation (S.D)	Relative S.D
1	Tablet	10	9.9961	0.0166	0.1671
2		10	9.9732		
3		10	9.9637		
1	Injection	8	7.9906	0.0135	0.1697
2		8	8.0094		
3		8	7.9906		

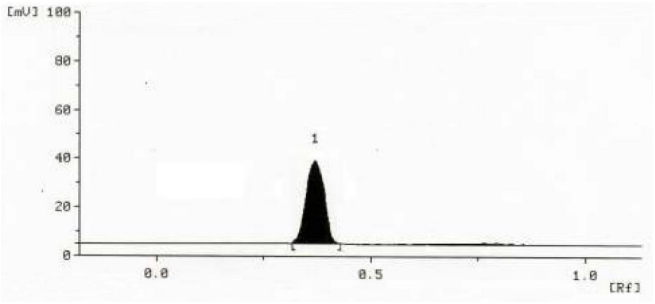

Figure 4: Sample chromatogram of valethamate bromide HPTLC

Table 3: Recovery of valethamate bromide HPTLC

S.No	Valethamate bromide	Amount of Drug in sample (mg)	Amount of drug added (mg)	Amount Recovered (mg)	Percent Recovery
1	Tablet	10	1	1.0042 0.9812 1.0003	100.42 98.12 100.03
2	Injection	8	0.8	0.7993 0.8060 0.7960	99.92 100.76 99.50

Table 4: LOD AND LOQ of valethamate bromide HPTLC

S.No	Parameter	Valethamate bromide
1	LOD	6 µg/ml
2	LOQ	18 µg/ml

Table 5: System suitability parameters (HPTLC)

S.No	Parameter	Valethamate bromide
1	Rf	0.37
2	Asymmetry factor	1.06
3	Theoretical plates/meter	1027
4	linearity	20-100 µg/ml

ruggedness (0.1697) for HPTLC proves the suitability of the proposed method. The regression of valethamate bromide concentration over peak area was found to be Y= (9.048+8.891) here, Y= concentration of the drug, x= peak area ratio. The percentage recovery indicating that the proposed method is highly accurate. The densitogram and the value pertaining to evaluation are given in the above tables 1-5 and figures 2-4

CONCLUSION

The proposed method was found to be simple, precise, rapid and sensitive for routine quantitative determination. The amount of drug recovered by the above methods was in good agreement with the label claim and the percentage recovery of 100.76% in HPTLC indicates the reproducibility of the proposed method.

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