Research Article

Rapid Ultra High Performance Liquid Chromatographic Assay of Pinaverium Bromide and its Stability studies

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ABSTRACT

A rapid and validated stability indicating UPLC method for the determination of pinaverium bromide in bulk and formulation is developed. The selectivity of the method was confirmed by carrying out stressed study on pinaverium bromide at different conditions such as acidic, basic, oxidative, photolytic and thermal. The peak corresponding to pinaverium bromide was found to be free from degradants confirming the selectivity of the method. Further, the method was validated according to the ICH guidelines. The selectivity of the method was further confirmed by standard addition technique on pinaverium bromide in tablet. The precision and accuracy of the method are within the ICH limit.

Keywords: Assay, C-18 column, Pharmaceutical, PNB, Ultra high performance liquid chromatography.

INTRODUCTION

Pinvarerium bromide is chemically known as 4-(2 Bromo-4,5-dimethoxy-benzyl)-4-{2-[2-(6,6-dimethyl -bicyclo[3.1.1]hept-2-yl)-ethoxy]-ethyl}-morpholin-4-ium (Fig. 1). Pinaverium bromide is a locally acting spasmolytic agent of the digestive tract. It acts upon inhibition of calcium ion entrance into smooth muscle cells (calcium-antagonist effect). In humans, pinaverium facilitates gastric emptying and decreases intestinal transit time in patients with constipation.[1]

There were only two analytical methods reported for the determination of PNB.[2,3] The method involves extraction, use of internal standard and reduction of analyte/internal standard using Raney-Nickel.[2] Thus for this complicated and time consuming method, an alternative method with faster analysis time and less complicated steps were proposed.[3] Although both the methods are equally sensitive and applicable to blood sample, there lack a macro-quantitative method for the determination PNB in routine quality control laboratories. More recently, we[4] have reported a microgram determination of pinavarium bromide using titrimetric technique.

In this regard, we proposed a simple and rapid UPLC method for the determination of pinavarium bromide. Selectiveness of the method was confirmed by performing stressed degradation study and standard addition technique. The method has been validated in-accordance with the ICH guidelines.

Experimental

Chemicals and materials

Potassium dihydrogen orthophosphate (Merck co), potassium hydroxide and Methanol (Rankem) all chemicals were HPLC grade .Pure penavirum bromide obtained from jubilant life science india . milli pore water were used in throughout the experiment. Analysis was carried out using waters Aquity UPLC with UV detector. The chromatographic column used was Acquity UPLC BEH C18 100mm* 2.1 mm 1.7 µm particle size.

Mobile phase preparation

Buffer solution was prepared by dissolving 2.82 g of potassium dihydrogen orthophosphate in 2000ml Millipore water pH adjusted to 6.5 with KOH solution. Mixture of 550 ml buffer and 450 ml methanol was mixed and filtered through 0.22 nylon membrane filter. This solution was used as diluent for sample and standard preparation.

Instrumental parameters

Isocratic flow rate of mobile phase was maintained at 0.4ml/min the column temperature kept at 40 ^{o}C the injection volume was 1 µl. The elution was monitored at 220 nm the retention time of sample is 1.5 min.

Preparation of stock solution

A stock standard solution of PNB was prepared by dissolving 50mg of drug sample dissolved in 100 ml diluent (500 ppm). Further, 5ml of the solution was diluted to 50ml with diluents (50ppm).

Procedure

Working standard solutions containing 10ppmto 400 ppm of PNB was prepared by serial dilution of stock solution. Aliquots of 1µl were injected (six injection) and eluted with the mobile phase under reported chromatographic conditions. Average peak area vs the concentration of PNB in ppm was plotted.

Preparation of tablets extracts and assay procedure

Ten tablets of PNB (50mg PNB each Eldecet) were weighed and ground into a fine powder using mortar and pestle. A quantity equivalent to 50mg of PNB was weighed into 100ml volumetric flask, dissolved in 60ml diluent, sonicated for 20 min an diluted up to the mark with diluent. The solution was filtered through 0.22mm nylon membrane filter. Obtained solution was further 5.0 ml diluted to 50 ml with diluents.

Table 1: Linearity

Conc. (ppm)	Retention Time	Area	Height	USP Tailing	USP Plate Count
0.7	1.521	6124	2100	1.45	6011
10	1.522	75916	24844	1.45	5854
25	1.522	252405	82332	1.45	5815
40	1.525	393700	127032	1.46	5708
50	**1.526**	**492940**	**157870**	**1.45**	**5610**
100	1.49	985517	297850	1.45	4722
150	1.526	1476322	411857	1.44	4207
200	1.525	1969531	503779	1.42	3368
400	1.489	3912055	991256	1.40	2803

Correlation	0.99996
Regression	0.99992
Intercept	1211.6
Slope	9771.4

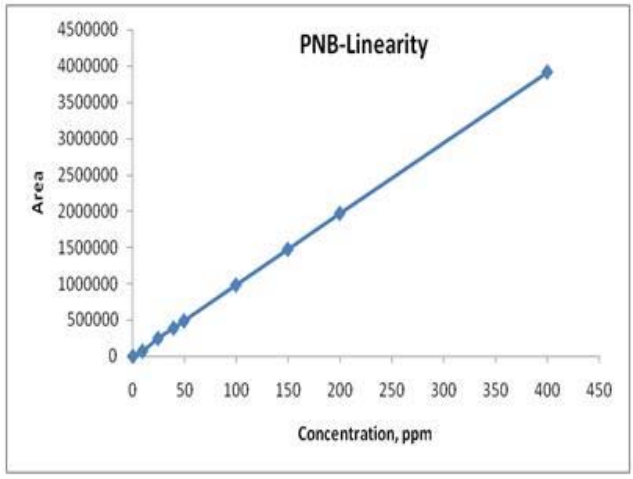


Figure 1: Linearity graph

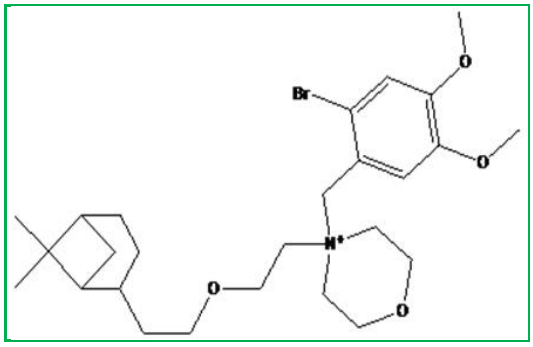


Figure 2: Structure of Pinaverium bromide

Table 2: Precision

Preparation	Retention Time	Area	Height	USP Tailing	USP Plate Count
1	1.541	494096	153560	1.45	5366
2	1.517	485862	152804	1.45	5312
3	1.512	487798	154074	1.45	5323
4	1.509	483222	153135	1.45	5334
5	1.506	485435	154004	1.45	5326
6	1.504	483101	153243	1.45	5313
Avg	1.515	486585.6667	153470	1.45	5329
Std. dev.	0.01361	4078.71895	503.06699	0.00000	19.91984
% RSD	0.899	0.838	0.328	0.000	0.374

Procedures for Method Validation

Preparation of calibration curve

Stock standard was prepared by dissolving 50mg sample in 100ml diluent. Working standard solution containing 10 to 400ppm of drug was prepared by serial dilution of stock standard solution. Aliquots of 1µl (6 injections) were injected. Average peak area v/s concentration of PNB was plotted. Ref table 1

Accuracy and Precision

To determine the accuracy and intraday precision, pure PNB solutions of three different concentrations were analyzed in six replicates each during the same day. Mobile phase was injected as the blank solution before the sample injection and the RSD (%) values of peak area and retention time were calculated ref table-2

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were determined by the signal to noise (S/N) ratio method. These were obtained by a series of dilutions of the PNB stock solution. Precision study was performed at the LOQ level also. LOQ solution was injected six times (n=6) and calculated the % RSD values for the obtained peak area and retention time.

Robustness studies

The robustness of the method was studied by varying the two parameter .i.e. wavelength and column temperature. The results were tabulated in table 3.

Solution stability and mobile phase stability

The stability of mobile phase and PNB solution was carried out by injecting sample solution into the chromatographic system in different time intervals. The peak area was recorded in the time intervals of 3, 6, 12, 18 and 24 hrs. The RSD of retention time and peak area was calculated and tabulated in table 5.

Table 3: Robustness

Condition	Variation	Area Pression (n=3)	% RSD
Wavelength	218	494256 494590 494389	0.03
	220	494029 494123 494361	0.03
	222	493970 493862 494084	0.02
Temperature	38	494376 493892 493645	0.08
	40	494933 494462 494384	0.06
	42	493456 493789 494205	0.08

Recovery studies by standardisation method

Recovery of the method was performed by standard addition technique. Previously analyzed tablet solution was co-spiked with the known concentration of pinavarium bromide and the percent recovery was checked. The method was found to be accurate and specific. The RSD of retention time and peak area was calculated and tabulated in table 4.

Forced degradation studies

Forced degradation studies of drug were carried out on solid and solution state in accordance to ICH regulatory guidelines. The objective of the forced degradation was to demonstrate the method was capable to separate the all possible impurities in drug.

Acid hydrolysis

Pipette 5ml 500 ppm sample solution into 50ml volumetric flask, 2ml of 5 N HCl added and kept under 80°C for 3hours naturalised with NaOH . Diluted to 50ml with diluent. 1µl above solution injected to chromatographic system.

Base hydrolysis

Pipette 5ml 500 ppm sample solution into 50ml volumetric flask, 2ml of 5 N NaOH added and kept under 80°C for 3hours naturalised with HCl. Diluted to 50ml with diluent. 1µl above solution injected to chromatographic system.

Thermal degradation studies

Sample was dried at 105°C for 24 hours 50 mg sample was dissolved in 10ml diluent and diluted to 100ml with diluents. Further 5ml diluted to 50ml with diluent. 1µl above solution injected to chromatographic system.

Photolytic degradation

The photolytic degradation of a drug was studies by exposing the drug to UV light for 24hours and 50 mg sample was dissolved in 10ml diluent and diluted to 100ml with diluents. Further 5ml diluted to 50ml with diluent. 1µl above solution injected to chromatographic system.

Oxidation studies

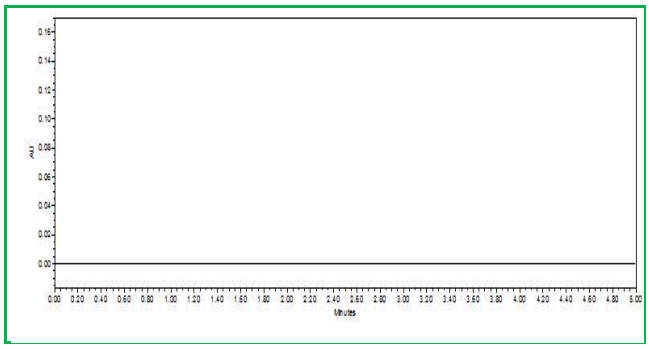
Pipette 5ml of 50 ppm sample solution into 50ml volumetric flask, 2ml of 5% H_2O_2 added and kept under 80°C for 3hours.Diluted to 50ml with diluent. 1µl above solution injected to chromatographic system

Table 4: Recovery study by standard addition method

Tablet brand name*	Nominal amount, mg	ppm PNB in tablet	PPM pure PNB added	Total Area	RSD (%)
Eldicet	**50**	50	25	744869 750678 748537	0.39
		50	50	985563 983476 987432	0.20
		50	75	1273904 1269890 1266589	0.29

Table 5: Solution stability (API concentration was 50 µg/ mL)

Time, h	Peak area	Retention time (min)	RSD, Peak area	RSD, Retention time
3	493729 494023 493962	1.522 1.525 1.529	0.03	0.23
6	493556 494089 493812	1.536 1.528 1.526	0.05	0.35
12	493389 493741 494084	1.534 1.527 1.538	0.07	0.36
18	493456 493789 494205	1.540 1.533 1.536	0.08	0.23
24	493970 493862 494084	1.519 1.523 1.526	0.02	0.23



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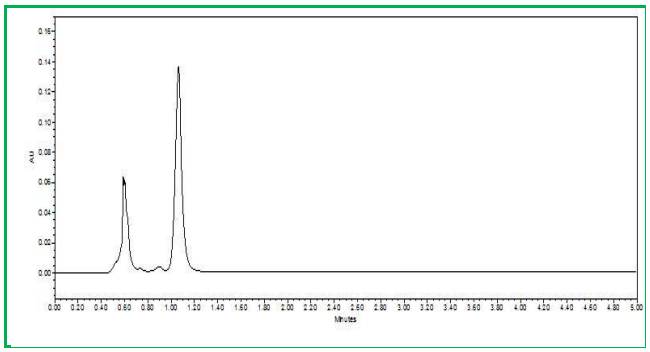

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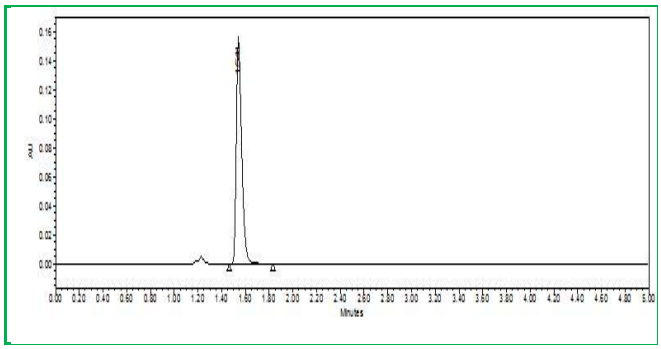

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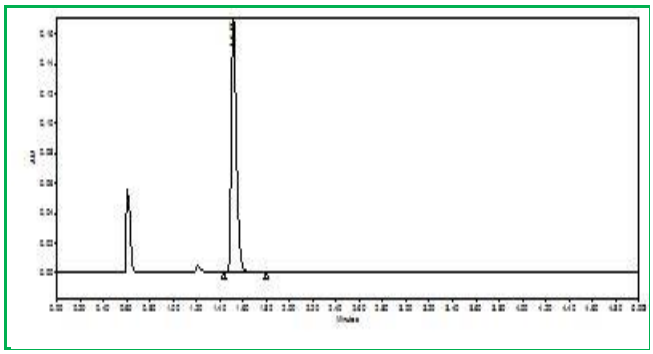

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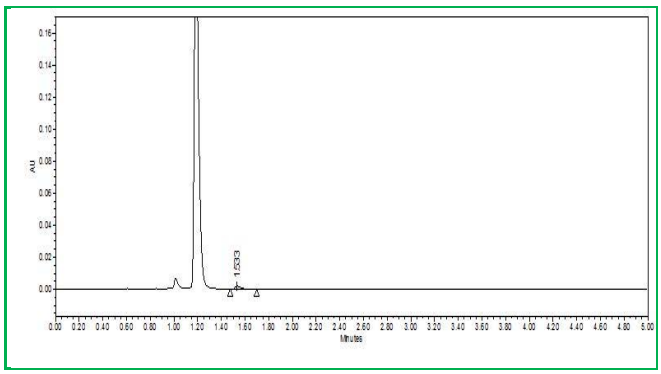

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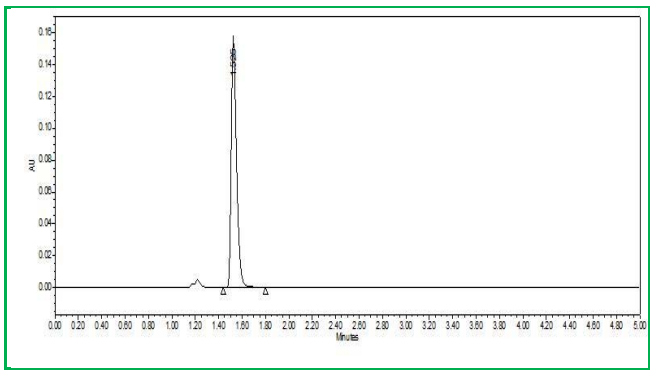

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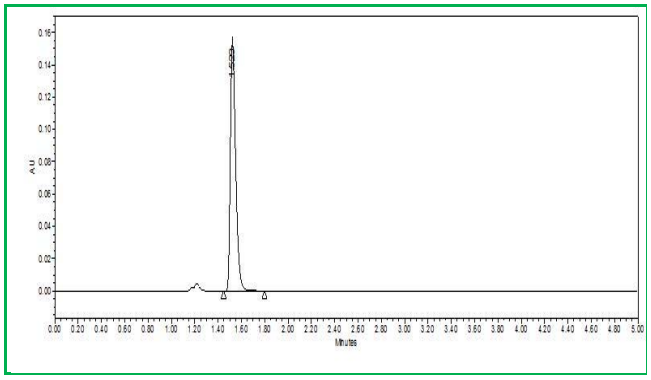

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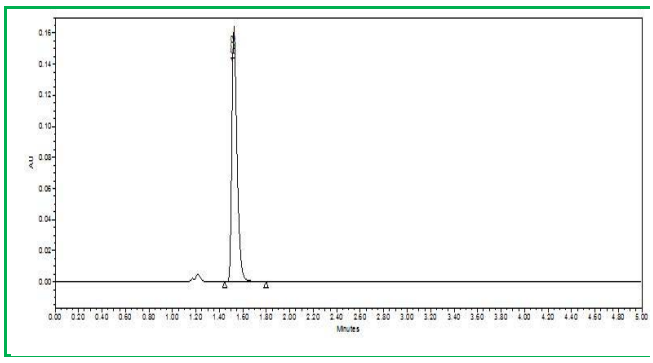

(h)

Figure 3: a. Blank Chromatogram; **b.** PNB-50ppm sample; **c.** PNB-50ppm (Photolysis); **d.** PNB-50ppm (2mL 5M NaOH / 80°C / 3 HRs); **e.** PNB-50ppm (2mL 5% H2O2 / 40°C / 3 HRs); **f.** PNB-50ppm (2ml 5M HCl / 80°C / 3 HRs); **g.** PNB-50ppm (105°C / 24 HRs); **h.** Tablet chromatogram.

RESULTS AND DISCUSSION

Stability indicating assay method was developed using different combinations of mobile phase, pH, columns and organic modifier in order to resolve all interfering peaks. Methanol as organic modifier was found to resolve the peaks and isocratic elution mode was adopted to shorten the run time which also have the advantage of continuous analysis without column re-equilibration time. During the validation of the method the correlation coefficients R^2 is less then 0.99996 in the calibration plots which gives the good linearity (Table 1). The analyte peak in the tablet solution was completely separated from the excipients showing the specificity of the method. The robustness of the method was carried out by a small variation in different parameter like wavelength and temperature. In slight variation of these parameters, method does not show any significant changes in the resolution as tabulated (Table 3).

Forced degradation studies

The development of stability indicating assay method for active pharmaceutical ingredients is paramount importance. The drug substances tend to degrade into often carcinogenic or mutagenic at elevated conditions. When these conditions are mimic in a laboratory at short period, a similar step of degradation occurs and thereby a developed method is checked for its selectivity. In this study, a various probable stress conditions such as acidic, basic, oxidative, thermal and UV irradiations are performed and degradants are resolved by modifying various experimental conditions. Finally, a developed method was validated in accordance to the ICH guidelines.

CONCLUSION

A simple, rapid and stability indicating assay method for pinavarium bromide is presented. Method has been checked for its selectivity by forced degradation study at different conditions such as acidic, basic, thermal, photolytic and oxidative. The method was tuned to resolve all interfering peaks. A validation based on ICH guideline was performed and can be use in regular quality control laboratories.

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