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DEVELOPMENT AND VALIDATION OF A NEW AND STABILITY INDICATING LC METHOD FOR ANALYSIS OF PINAVERIUM BROMIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, and stability indicating reverse phase high performance liquid chromatographic assay method was developed for pinaverium bromide in the presence of its degradation products generated from decomposition studies.LC separation was achieved isocratic mode on a Zorbax SB C8 (4.6x250) mm, 5 µm column using mobile phase containing solution A (0.1% ortho phosphoric acid) with solution B (acetonitrile) (30:70) (v/v) at flow rate 1.0 ml/min. The UV detector was operated at 245 nm and temperature was 25˚C. The retention time was 4.84 min and linearity was observed in the concentration range of 20-150µg/ml with correlation coefficient of 0.9999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. The method was successfully validated as per International Conference on Harmonization (ICH) guidelines. pinaverium bromide undergoes degradation under acidic, basic, oxidation, dry heat and photolytic conditions, degradation impurities did not interfere with the retention time of pinaverium bromide, and assay method is thus stability indicating.

KEYWORDS: Pinaverium. validation. HPLC. Stability indicating

INTRODUCTION

Pinaverium is a drug used for functional gastrointestinal disorders. It acts as a calcium channel blocker and helps to restore the normal contraction process of the bowel. It is most effective when taken for a full course of treatment and is not designed for immediate symptom relief or sporadic, intermittent use. Pinaverium is available as tablets at the dose of 50 mg in the market under the brand name of ELDICET. Pinaverium is chemically 4-[(2-Bromo-4, 5-dimethoxyphenyl) methyl]-4-[2-[2-(7,7-dimethyl-2-bicyclo[3.1.1]heptanyl) ethoxy] ethyl] morpholin-4-ium bromide with empirical formula is $C_{26}H_{41}BrNO_4.Br$ and molecular weight 591.42.

Various methods in the literatures involve determination of Pinaverium bromide in human plasma by LCMS/MS (1), GC/MS (2) pharmacokinetics, pharmacodynamics (3-14). However no method is available for assay of pinaverium bromide in bulk drug and pharmaceutical dosage form. In the present work we have developed a new, simple precise and stability indicating method for determination of pinaverium bromide in bulk drug and pharmaceutical dosage form.

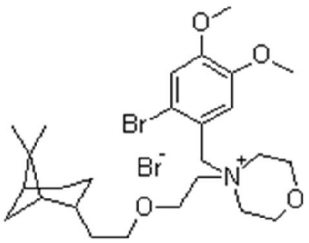


Figure 1: Structure of Pinaverium bromide

EXPERIMENTAL

Chemicals & Reagents

Pinaverium is available as tablets with brand name ELDICET was purchased from local market, containing Pinaverium 50mg. HPLC grade acetonitrile and AR grade ortho Phosphoric acid were purchased from Merck, Mumbai. High pure water was prepared by using Millipore Milli-Q plus purification system.

Chromatographic Conditions

A Alliance e2695 separation module (Waters corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. Buffer consisted of 1.0 ml of ortho phosphoric acid in 1000 ml of water. A, Zorbax SB C8 (4.6x250) mm 5 µm column and isocratic mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The isocratic program was fixed as (30:70). Water:Acetonitrile(50:50)v/v used as diluent. The column oven maintained at 25˚C with 1.0ml flow rate. An injection volume 10µl was used. The elution compounds were monitored at 245 nm.

Preparation of Stock and standard solutions

Accurately 100mg of Pinaverium bromide standard dissolved in 100ml diluent to get a concentration of 1000µg/ml. Further 10ml of stock solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml.

Preparation of Tablets for assay

The formulation tablets of ELDICET were crushed to give finely powdered material. Powder equivalent to 100mg of drug was weighed and transferred to the 100ml flask added 10ml diluent and placed in an ultrasonicator for 10minites made up to the volume with diluent, and filtered through a 0.45µm nylon syringe filter. 10ml of this solution was taken into 100 ml flask and diluted volume with diluent to get concentration 100µg/ml.

Forced Degradation studies

Acid Degradation studies

Acid decomposition was carried out in 0.2N HCL at concentration of 1000µg/ml Pinaverium bromide and after refluxation for 24hours at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (f). The results are tabulated in table 4.

Alkali Degradation studies

Base decomposition was carried out in 0.2N NaOH at concentration of 1000µg/ml Pinaverium bromide after refluxation for 24hours at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3(h). The results are tabulated in table 4.

Oxidation

Oxidation was conducted by using 6%H2O2 solution at room temperature for 24hours, 10ml of solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml filtered and injected. The resulting chromatogram is shown in fig.3 (j). The results are tabulated in table 4.

Temperature Stress studies

1g of Pinaverium bromide sample was taken into a petridish and kept in oven at 80˚c for 7days. 100mg of sample was taken into 100 ml flask diluted volume with diluent, further 10ml to 100ml made up with diluent. The results are tabulated in table 4.

Photo stability

1g of Pinaverium bromide was taken in to a petridish and kept in photo stability chamber 200 W.hr/m^2 in UV Fluorescent light and 1.2M LUX Fluorescent light. 100mg of sample was taken in 100ml flask, dissolved in diluent, further 10ml in 100ml flask diluted volume with diluent. The results are tabulated in table 4.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

To develop a rugged and suitable HPLC assay method for the determination of Pinaverium bromide , the analytical condition were selected after the consideration of different parameters such as diluents, buffer, organic solvent for mobile phase, column and other chromatographic conditions (12). Initial trails were performed with different composition of buffer (acetate and formate) and organic phase (methanol, teterhydrofuran) with different column like c8, phenyl, cyno, amino and basic but Pinaverium bromide peak shape was not good. Finally 0.1% ortho phosphoric acid in water and acetonitrile with isocratic and Zorbax SB C8 (4.6x250) mm 5 µm column was optimized. Different diluents were tried to dilute sample like water, buffer, methanol, tetrahydrofuran and mixture of water: methanol and water: teterhydrofuran, buffer: methanol and buffer: acetonitrile. Pinaverium bromide was not dis-

solved, finally (water: acetonitrile) (50:50) % v/v was optimized. The detection wavelength was chosen as 245nm for Pinaverium bromide because they have better absorption and sensitivity at this wavelength (fig-2). Hence selected method was best among the all trails by many aspects.

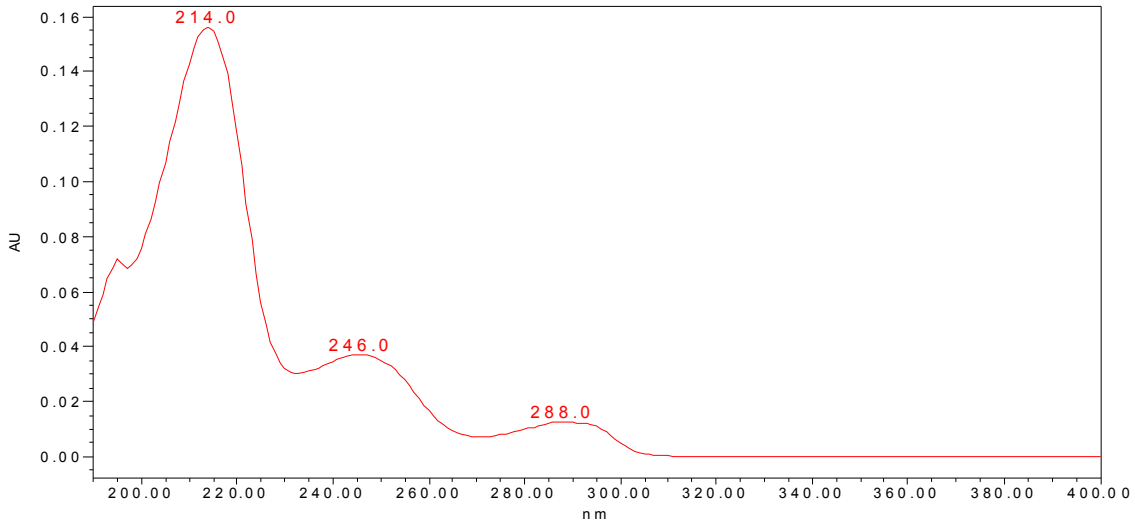


Fig-2 wavelength spectrum of Pinaverium bromide

Method Validation

Precision

The precision for assay method was established by evaluating method precision and intermediate precision study. Method precision was determined by analyzing six independent assays were performed and calculated the % RSD for replicate assay determinations. Intermediate precision of the analytical method was determined by conducting method precision on another day and another analyst under same experiment condition. The result obtained for method precision and intermediate precision are shown in table 3. The percentage of RSD was calculated. The %RSD range was obtained as 0.18 and 0.22 for method precision and intermediate precision respectively (Table 3) which is less than 2% indicating that the method is more precise.

Accuracy

The accuracy of the method was estimated by determination of recovery for three concentrations (corresponding to 50,100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. The drug concentrations of Pinaverium bromide were calculated, the results obtained are shown in table 2. The percentage recovery was found to be 99.84-99.99% with %RSD 0.09 - 0.49(<2.0%) indicating that the method is more accurate (table 2)

LOD and LOQ

The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of test solutions of known concentrations within the linearity range. Precision study was also carried out at the LOQ level by injecting six pharmaceutical preparations. The LOD and LOQ were to be 0.023µg/ml and 0.078µg/ml respectively. The %RSD value was noticed to be less than 2.0% at LOQ concentration level.

Linearity

The linearity plot was prepared with six concentration levels (24, 48, 96,120,144 and 180 µg/ml of Pinaverium bromide). These concentration levels were respectively corresponding to 20, 40, 80,100,120 and 150 % of test solution concentration. The results obtained are shown in table 1. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure 4).

Robustness

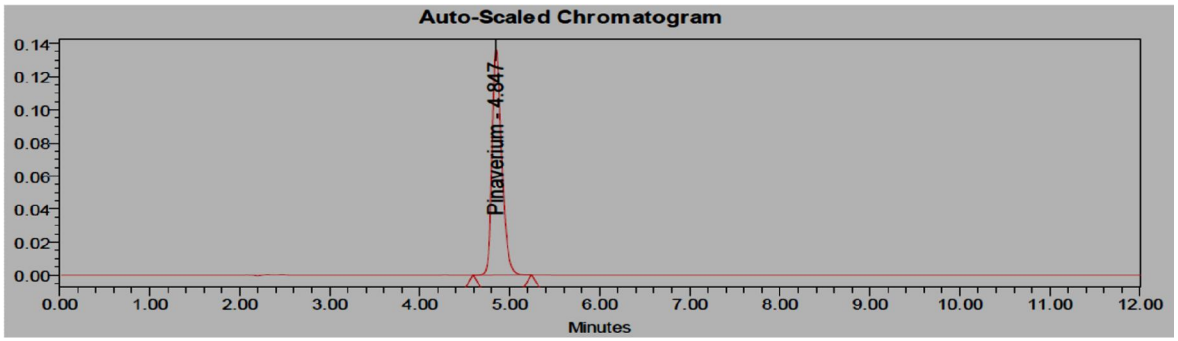
Robustness of method was checked by making slight deliberate changes in chromatographic conditions like flow rate (±0.1 ml/min), PH (±0.1units) and column temperature (±5˚c). In the all above varied conditions, the components of the mobile phase were held constant. The results are tabulated in table 5.Under all the deliberately varied chromatographic conditions, the reproducibility of results was observed to be reasonably good. Hence the proposed method has good robustness for the assay of Pinaverium in bulk and dosage forms

Solution stability and Mobile phase stability

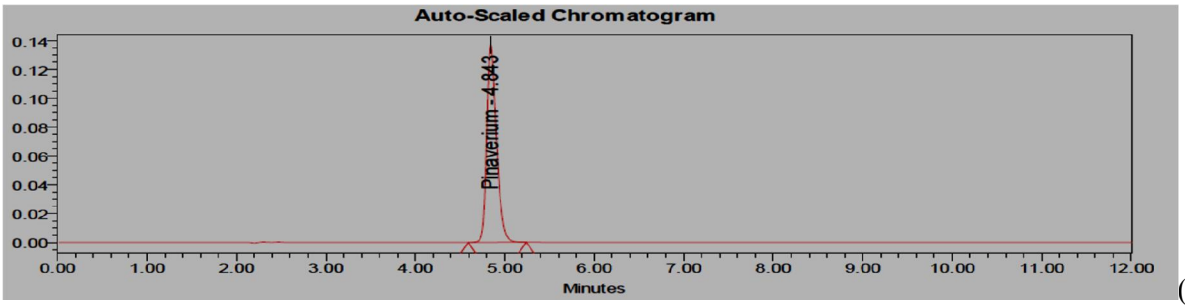
Solution stability checked for stability of standard and sample solutions. Solution stability checked at each interval initial 2,4,6,8,12,16,20 and 24 hours. For standard solution stability and sample solution stability %assay value calculated at each interval. %RSD (NMT 2.0%) between initial assay value and assay value obtained at predetermined time interval calculated.

Forced Degradation Studies

Stress studies on Pinaverium were carried out under oxidation, thermal stress, photolysis, acid and alkali hydrolysis conditions. Significant degradation was observed in acid (fig 3f) and base (fig 3j) of Pinaverium . There was no significant degradation of Pinaverium upon exposure to dry heat at 80˚c for 7days, photolysis and peroxide oxidation total impurity increased to 0.10%, 0.70% and 0.48% which indicated that the drug was stable against these stress conditions. The developed method revealed that there was no interference from the impurities, degradation products and excipients to determine the assay of drug substance in pure and pharmaceutical formulation.


Auto-Scaled Chromatogram
Pinaverium - 4.847
Minutes

(a)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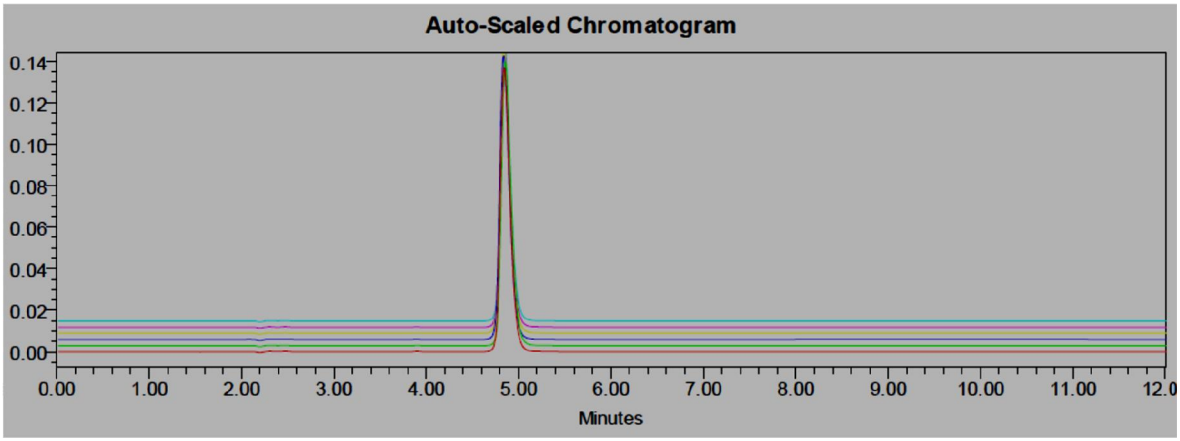
Pinaverium - 4.843
Minutes

(b)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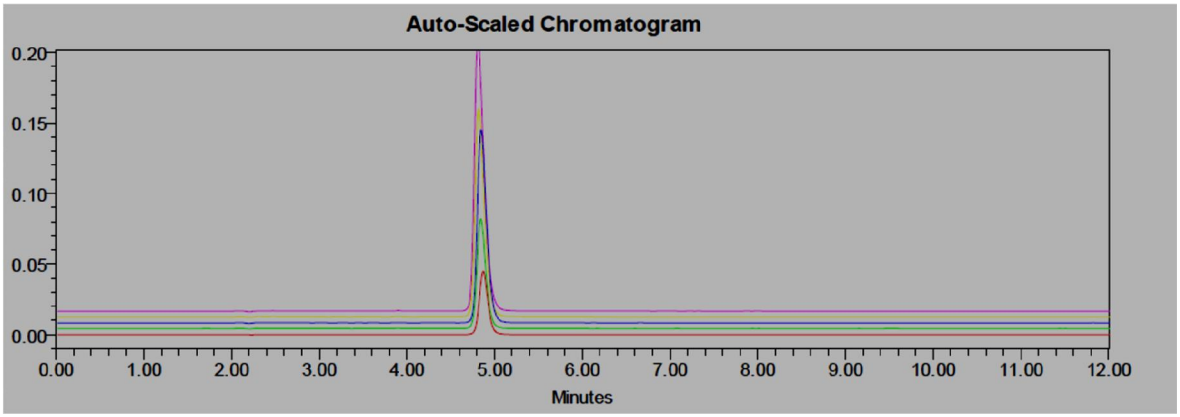
(c)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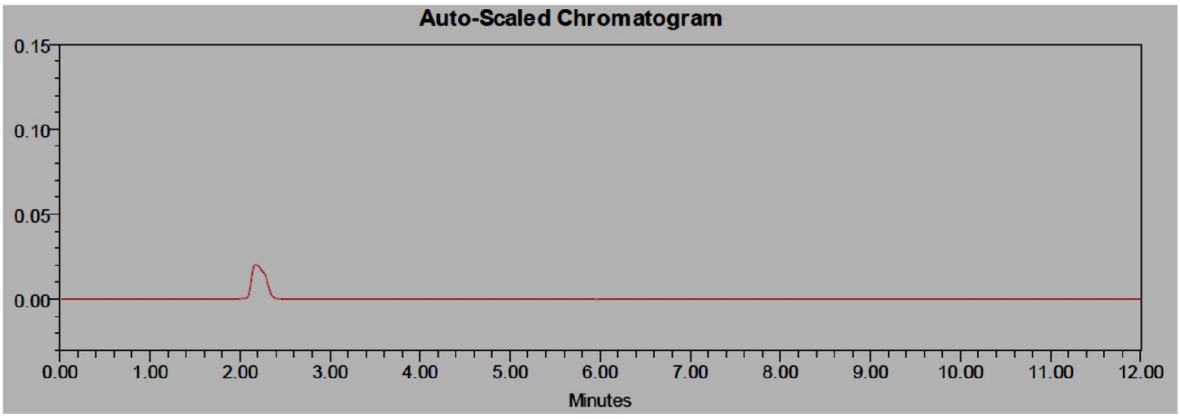
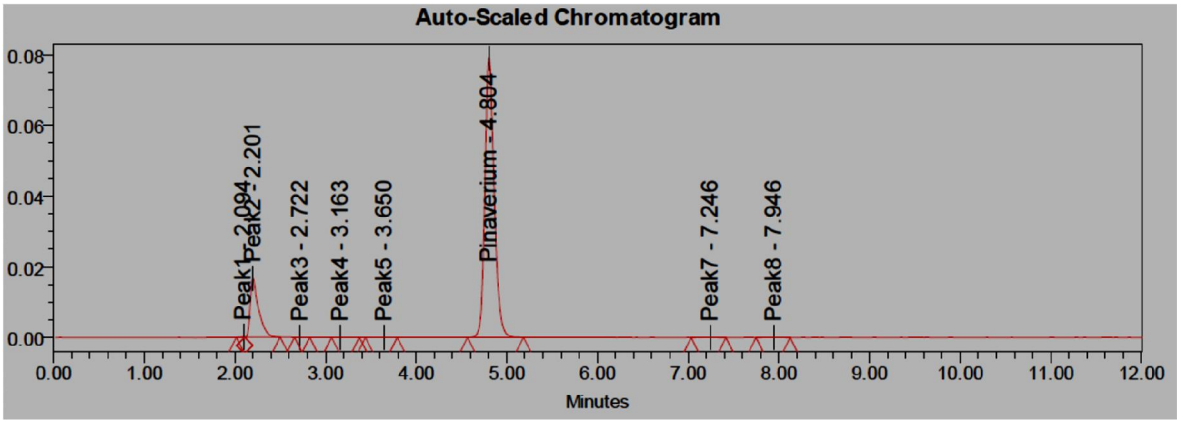
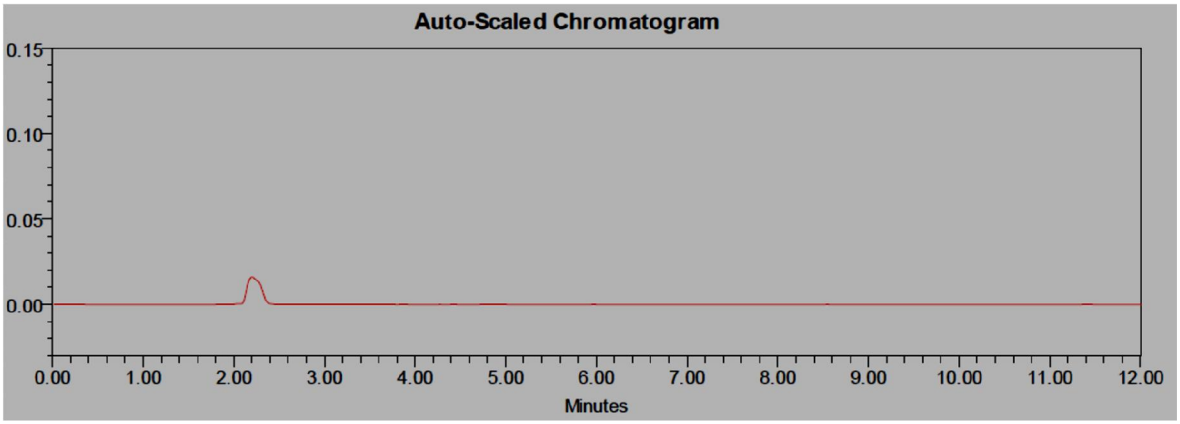
(d)


Auto-Scaled Chromatogram
0.15
0.10
0.05
0.00
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes

(e)


Auto-Scaled Chromatogram
0.08
0.06
0.04
0.02
0.00
Peak1 - 2.094
Peak2 - 2.201
Peak3 - 2.722
Peak4 - 3.163
Peak5 - 3.650
Pinaverium - 4.804
Peak7 - 7.246
Peak8 - 7.946
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes

(f)


Auto-Scaled Chromatogram
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0.10
0.05
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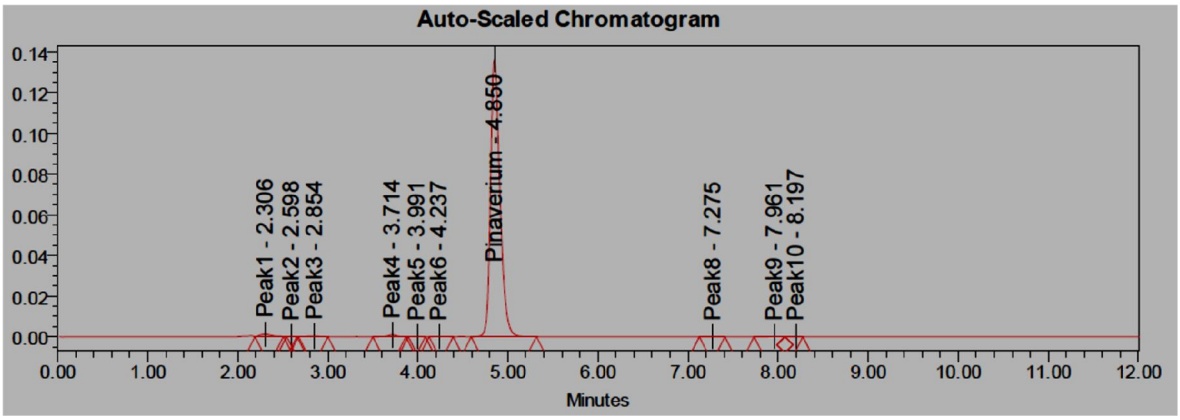
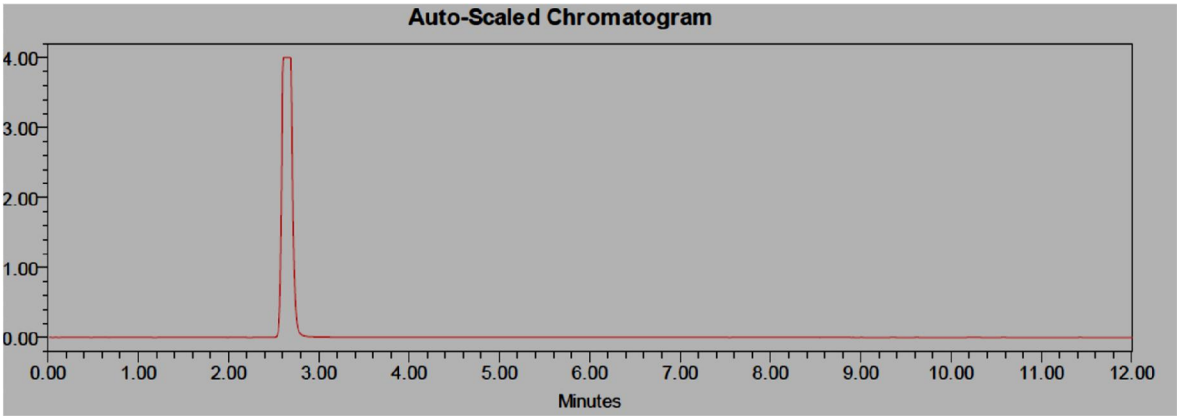
Minutes

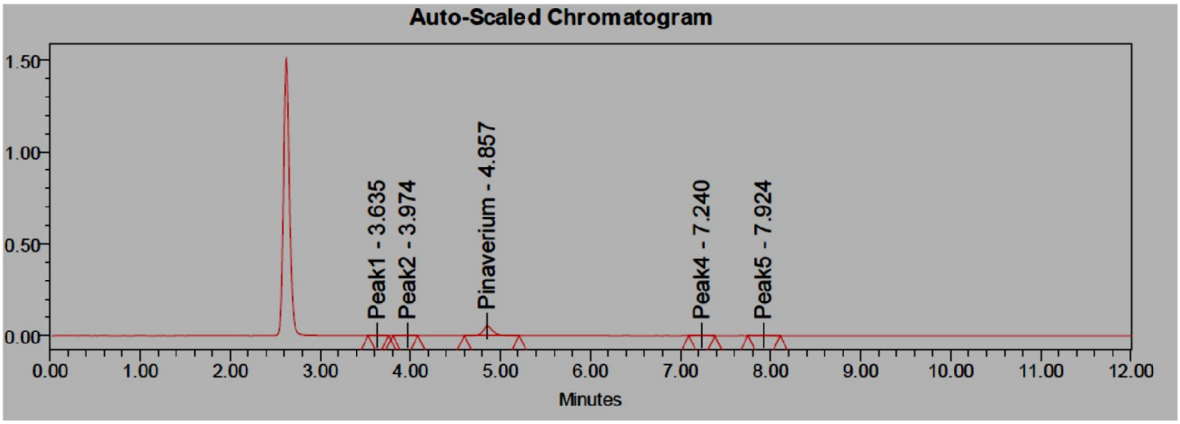
(g)



(h)



(i)



(j)

Fig-3 Typical chromatograms of (a) Standard (b) Sample (c) precision injections (d) Linearity injections (e) Acid blank(f) Acid sample (g) Base blank (h) Base sample (i) Peroxide blank (j) Peroxide sample

Fig-4 Linearity Pinaverium

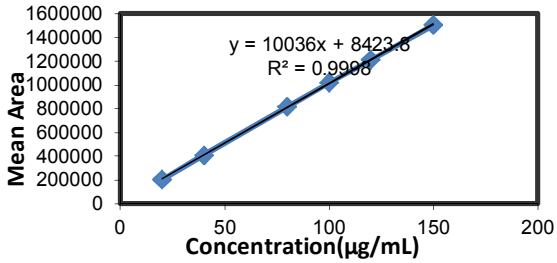


Table-1 Results for linearity of Pinaverium

Linearity level	%Level	Area
1	20	204172
2	40	408375
3	80	816625
4	100	1020863
5	120	1212426
6	150	1506317
Correlation co-efficient		0.999921
	intercept	8423.77
	slope	10035.76

Table-2 Recoveries study for Pinaverium bromide

Accuracy (Recovery) study							
Accuracy Level	**Set No**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
50%	1	49.94	50.12	100.36	99.84	0.48	0.49
	2	50.00	49.88	99.76			
	3	50.06	49.76	99.40			
100%	1	100.18	99.96	99.78	99.97	0.24	0.24
	2	100.08	99.98	99.90			
	3	100.02	100.26	100.24			
150%	1	149.96	150.04	100.05	99.99	0.09	0.09
	2	150.10	149.94	99.89			
	3	150.08	150.14	100.04			

Table-3 Precision results for Pinaverium bromide

Study	Set no	Assay (%)	Mean assay(%)	Stdev	RSD%
Method precision	1	100.25	100.05	0.18	0.18
	2	99.78			
	3	99.96			
	4	100.14			
	5	100.22			
	6	99.96			
Intermediate precision	1	99.76	99.94	0.22	0.22
	2	99.64			
	3	100.18			
	4	100.12			
	5	100.08			
	6	99.88			

Table-4 forced degradation results for Pinaverium bromide

Stress condition (%)	Drug recovered (%)	Drug decomposed
Standard drug	100	
Acid degradation	84.07	15.93
Alkali degradation	97.99	2.01
Oxidation degradation	99.52	0.48
Thermal degradation	99.90	0.10
Photolytic degradation	99.30	0.70

Table -5 Robustness results for Pinaverium bromide

Robust conditions	variation	Retention time(min)	USP Tailing	USP Plate count
Flow	0.9ml	5.28	1.14	9721
	1.0ml	4.84	1.08	9806
	1.1ml	4.42	1.03	9998
Temperature	20˚c	4.96	1.1	9765
	25˚c	4.84	1.08	9806
	30˚c	4.59	1.04	9986
%Acetonitrile	65	5.08	1.12	9728
	70	4.84	1.08	9806
	75	4.53	1.03	9998

CONCLUSIONS

A validated RP-HPLC method has been developed for determination of Pinaverium bromide in presence of degradation impurities. The proposed method was found to be a new, simple, precise, linear, accurate and specific. Degradation impurities did not interfere with the retention time of Pinaverium bromide, and assay method is thus stability indicating.

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