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Method Development and Validation of Pinaveriumbromide in Bulk Form by UV - Visible Spectroscopy

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Abstract: ***The aim of this work was to develop and validate a simple estimation of Pinaverium bromide in bulk by using UV spectroscopic method. The method was developed using n - butyl alcohol as the solvent and absorbance was observed at 285nm. Beers law was obeyed the concentration ranges from 10 - 50µg/ml. Calibration curve shows a linear relationship between the absorbance and concentration. The line equation Y=0.0888x+0.033 with correlation coefficient (r) of 0.9999 was obtained. The method was validated as per ICH guidelines. The method was validated statistically and by recovery studies. The percentage recovery was found to be in the range of 98%. The %RSD value was found to be less than 2.***

Keywords: Pinaverium Bromide, UVvisible spectroscopy, method validation, pharmaceutical analysis, nbutyl alcohol

1. Introduction

UV spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200nm to 400nm. Compounds which are coloured, absorb radiation from 400nm - 800nm. But compounds which are colourless absorb radiation in UV region. In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, thereby the molecules undergo transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and pathlength as given by Beer - Lambert's law.

The mathematical equation for Beer - lambert's Law
A = εct
WHERE, A=absorbance or optical density or extinction coefficient
ε = Molecular extinction coefficient
C = Concentration of drug
T= Path length.

Drug Profile

IUPAC Name:
4 - [(2 - Bromo - 4, 5 - dimethoxyphenyl) methyl] - 4 - [2 - [2 - (6, 6 - dimethyl - 4 - bicyclo [3.1.1] heptanyl) ethyl] morphotin - 4 - ium bromide.

Structure:

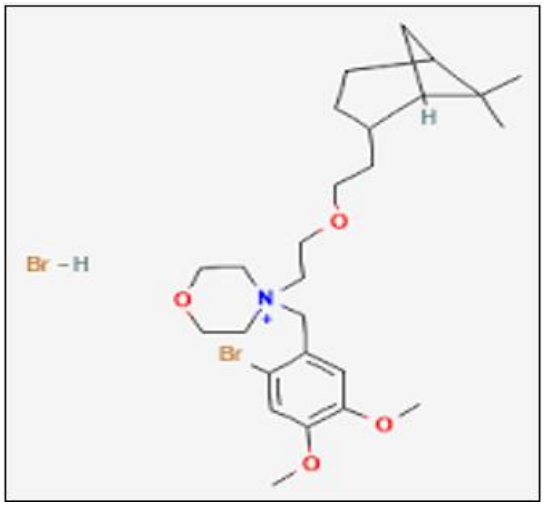

Figure 10: Shows Structure of pinaverium bromide

Common name: Pinaverium bromide
Molecular formula: C_{26} H_{41}, Br_2NO_4
Category: Antispasmodic
Molecular mass: 591.4g/mol
Appearance: White powder
Generic Name: DICETEL and ELDICET
Brand Name: DICETEL and in ARGENTINA as Nulite.
Solubility: Insoluble in ethyl acetate and soluble in organic solvents.

Mechanism of Action:
Pinaverium bromide is a locally acting spasmolytic agent of the digestive tract. It's mechanism of action relies upon inhibition of calcium ion entrance into smooth muscle cells (calcium antagonist effect) which leads to relaxing the muscles in the walls of the gut.

USES:
It is used in the treatment of irritable bowel syndrome.

2. Materials and Methods:

Chemicals and Reagents

Table 1: Shows Chemicals Used for Experimental work

Chemicals or Solvents	Grade	Manufacturer
Pinaverium Bromide	-	Gift sample
N - Butyl alcohol	LR	Virat labs, Mumbai - 02

Instrument:

Table 2: Shows Information about Instrument

S. No	Instrument	Model
1	Double beam UV Visible Spectrophotometer	LABMAN
2	UV Sources	Deuterium lamp Elico SL -
3	Software	UV ANALYST
4	Analytical balance	Acutek

3. Experimental Work

Method Development:

Solubility Studies:

The solubility studies performed using various solvents are listed below:

Table 3: Shows Solubility Studies of Pinaverium Bromide.

S. No	Solvent	Result
1.	Ethanol	Soluble
2.	Chloroform	Soluble
3.	N - Butyl alcohol	Soluble
4.	Cyclohexane	Soluble
5.	Acetonitrile	Soluble
6.	Acetone	Soluble
7.	Carban tetra chloride	Soluble
8.	Distill Water	Sparingly Soluble
9.	Ethyl Acetate	Insoluble

Among all the solvents in which drug is soluble. We have selected N - Butyl alcohol as a solvent to use in the entire experiment.

Determination of Absorption Maxima by UV/Visible Spectroscopy.

- Weigh accurately 25mg of drug and dissolve in 25ml of volumetric flask gives 1000µg/ml which is (1mg/ml).
- Take 1ml of solution from above from above solution and make up the volume with solvent upto 10ml which gives 100µg/ml this solution is termed as Stock Solution.
- Take 1ml from the above stock solution and make up the volume upto 10ml with solvent in 10ml volumetric flask. It is known as Sample Solution.
- The above solution was scanned in the Uv region 200 - 400nm.
- The maximum absorption takes place at 285nm.

Figure: Shows Wavelength of Pinaverium Bromide

Method validation:

Linearity

Form solution having conc.100µg/ml, various conc ranges 10 µg/ml - 50 µg/ml solution was prepared and linear relationship was observed between absorbance and concentration.

Precision:

Precision of the methods was studied as intra - day, inter - day.

- **Intra - day Precision** - Intra - day study was performed by examining a 10µg/ml, concentration of drug for three times in the same day.
- **Inter - day Precision**– Inter - day precision was performed by examining a 10µg/ml, concentration of drug for three times in next day.

Accuracy

The Accuracy of the method was evaluated by recovery studies at three different levels i. e.75%, 100%, and 125%. The recovery studies were carried out by the addition of a known amount of standard solution of pinaverium bromide to pre - analysed solutions. The resulting solutions then re - analysed by the proposed method.

Limit of detection (LOD)

LOD for pinaverium bromide by the proposed method was determined on the response and slope of the regression coefficient.

$LOD = 3.3 \times \sigma/S$

Where, σ = standard deviation, S = linearity curve slope

Limit of quantization (LOQ)

Limit of quantization for pinaverium bromide by the proposed method was determined on the response and slope of the regression coefficient.
LOQ = 10 × σ/S
Where, σ = standard deviation, S = linearity curve slope.

Ruggedness
The ruggedness is a degree of reproducibility of test result under verification of condition such as a different analyst, different instruments, and different days. To determine ruggedness of the proposed method, the sample solutions of 10µg/ml of pinaverium bromide was prepared by different analysts and analyzed.

4. Results and Discussion

Accuracy:

Table 4: Shows accuracy results of Pinaverium Bromide

S. No	Level	Concentration (Actual value)	Concentration (Calculated value)	Percentage Recovery
1	75%	7.5µg/ml	7.352	98%
2	100%	10µg/ml	9.85	98.5%
3	125%	12.5µg/ml	11.10	88.8%

The accuracy of the Pinaverium bromide was performed. The percentage RSD values are found to be with in the limit.

2). Precision
a) Intra Day Precision:

Table 5: Shows intraday precision results of Pinaverium Bromide

S. No	Concentration	Absorbance
1	Sample 1	0.135
2	Sample 2	0.138
3	Sample 3	0.136
4	Sample 4	0.132
5	Sample 5	0.133
6	Sample 6	0.131
Standard Deviation		**0.00263**
Mean		**0.1341**
% RSD		**1.97**

Table 6: Shows intraday precision results of Pinaverium Bromide

S. No	Concentration	Absorbance
1	Sample 1	0.165
2	Sample 2	0.162
3	Sample 3	0.168
4	Sample 4	0.165
5	Sample 5	0.163
6	Sample 6	0.167
Standard Deviation		**0.0028**
Mean		**0.165**
% RSD		**1.38**

The intraday precision on Pinaverium bromide was performed and the %RSD of the second was found to be with in the limit.

b) Inter Day Precision:

For 1st Day:

Table 7: Shows interday precision results of Pinaverium Bromide

S. No	Sample	Concentration	Absorbance
1	Sample1	10µg	0.181
2	Sample 2	10µg	0.185
3	Sample 3	10µg	0.183
4	Sample 4	10µg	0.182
5	Sample 5	10µg	0.186
6	Sample 6	10µg	0.182
Standard Deviation			**0.0019**
Mean			**0.183**
% RSD			**1.0**

For 2nd Day:

Table 8: Shows interday precision results of Pinaverium Bromide.

S. NO	Sample	Concentration	Absorbance
1	Sample1	10µg	0.14
2	Sample 2	10µg	0.145
3	Sample 3	10µg	0.148
4	Sample 4	10µg	0.143
5	Sample 5	10µg	0.142
6	Sample 6	10µg	0.143
Standard Deviation			**0.0027**
Mean			**0.143**
% RSD			**1.8**

For 3rd Day:

Table 9: Shows interday precision results of Pinaverium Bromide.

S. No	Sample	Concentration	Absorbance
1	Sample1	10µg	0.165
2	Sample 2	10µg	0.168
3	Sample 3	10µg	0.164
4	Sample 4	10µg	0.163
5	Sample 5	10µg	0.167
6	Sample 6	10µg	0.166
Standard Deviation			**0.0018**
Mean			**0.1655**
% RSD			**1.08**

The interday precision on Pinaverium bromide was performed. The % RSD value was found to be within the limit.

3) Linearity

Table 10: Shows Linearity results of Pinaverium Bromide.

S. NO	Concentration	Absorbance
1	25%	0.090
2	50%	0.180
3	75%	0.270
4	100%	0.350
5	125%	0.445
Slope		**0.088**
R^2 Value		**0.9997**
Y - Intercept		**0.0003**

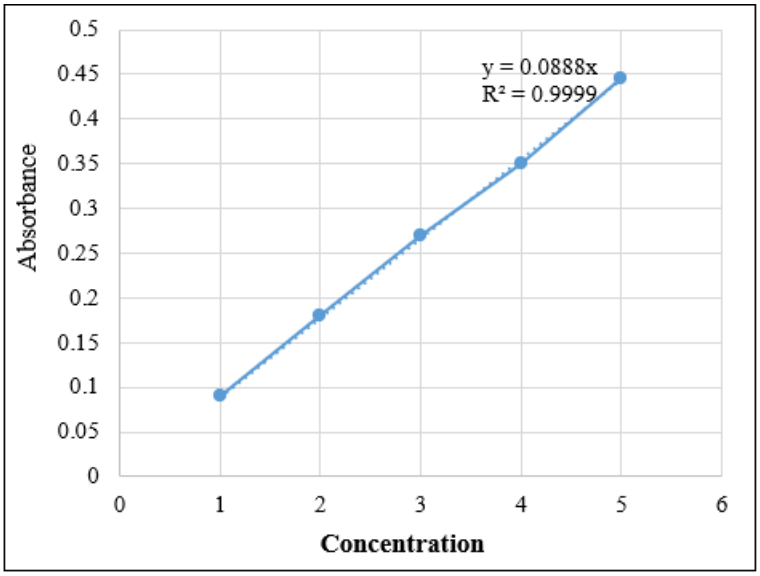

Figure 13: Shows Calibration Curve of Pinaverium Bromide

4) RUGGEDNESS:

Analyst – 1

Table 11: Shows Ruggedness results of Pinaverium Bromide.

S. NO	Concentration	Absorbance
1	Sample 1	0.152
2	Sample 2	0.156
3	Sample 3	0.158
4	Sample 4	0.154
5	Sample 5	0.157
6	Sample 6	0.155
Standard Deviation		**0.0021**
Mean		**0.155**
% RSD		**1.39**

Analyst – 2

Table 12: Shows Ruggedness results of Pinaverium Bromide

S. No	Concentration	Absorbance
1	Sample 1	0.190
2	Sample 2	0.185
3	Sample 3	0.196
4	Sample 4	0.184
5	Sample 5	0.176
6	Sample 6	0.198
Standard Deviation		**0.0082**
Mean		**0.964**
% RSD		**0.0085**

Analyst – 3

Table 13: Shows Ruggedness results of Pinaverium Bromide

S. NO	Concentration	Absorbance
1	Sample 1	0.158
2	Sample 2	0.155
3	Sample 3	0.158
4	Sample 4	0.163
5	Sample 5	0.155
6	Sample 6	0.154
Standard DeviatioN		**0.0033**
Mean		**0.157**
% RSD		**2.10**

The Ruggedness of the Pinaverium bromide was performed. The %RSD values are found to be within the limit

5) LOD AND LOQ:

Table 14: Shows LOD and LOQ results of Pinaverium Bromide

S. NO	Parameter	Sample Name
1	LOD	0.0675
2	LOQ	0.2045

5. Conclusion

As per the result and discussion, it was concluded that most method development and validation are successfully done on Pinaverium bromide. Which starts with the solvent selection according to particular drugs solubility within it. Then go for the proposed analytical method that is simple, easy, and cost - effective. The proposed method is exact and accurate. The UV spectrophotometric method offers adequate data quickly and plays a significant role in the method development and validation. The Linearity is obeyed in the concentration range of 10 - 50µg/ml with correlation coefficient 0.9999 by UV spectroscopy. The methods were precise as %RSD values for repeatability, interday precision and intraday precision are found to be less than 2. Accuracy of proposed methods determined by recovery studies and good % recovery (80 - 120) of drug obtained indicates that the methods are accurate. The methods are also found to be rugged and robust as % RSD values are less than 2. The limit of detection and limit of quantification of proposed methods are found to be 0.0675 and 0.2045µg/ml. Hence, conclude that UV spectroscopy method can be used to analysis of Pinaverium bromide.

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