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DETERMINATION OF PROPIVERINE HCL IN PURE AND TABLET DOSAGE FORMS BY MEANS OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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ABSTRACT
A simple, sensitive, and robust reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of propiverine HCl in pure and tablet dosage forms. In contemporary Ace C-18 (250×4.6 mm, 5µm) column was used with a mobile phase containing a mixture of potassium dihydrogen phosphate buffer (pH-2.5) and Acetonitrile in the ratio of 70:30%v/v) at ambient temperature. The flow rate was maintained at 1.0 ml/min. Results were determined at 220 nm with a fixed wave length PDA detector. The linearity for propiverine HCl was found between 10 - 60 µg/ml with the retention time of 5.262 respectively. Validation parameters like and system suitability parameters LOQ, LOQ, assay, accuracy, precision and robustness were determined for propiverine HCl with the present developed method.

KEYWORDS: Propiverine HCl, RP-HPLC, Method development, Validation.

INTRODUCTION
Propiverine HCl[1,2] [**Fig 1**], (1-methylpiperidin-4-yl) 2,2-diphenyl-2-propoxyacetate;hydrochloride is an anticholinergic drug used for the treatment of urinary urgency, frequency and urge incontinence, all symptoms of overactive bladder syndrome. It has an empirical formula of $C_{23}H_{30}ClNO_3$ with molecular weight of 403.947 g/mol.

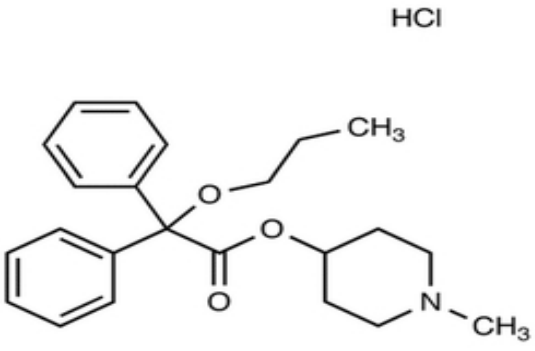

Figure 1: Chemical structure of propiverine HCl.

It is formulated and sold in the pharamcy in the brand name **Mictonorm** oral tablets (Label claim 15mg of propiverine HCl) used for the symptomatic treatment of urinary incontinence and/or increased urinary frequency and urgency in patients with overactive bladder syndrome or neurogenic detrusor over activity (detrusor hyper reflexia) from spinal cord injuries, e.g. transverse legion paraplegia.

As per the above literature studies only a few reports are available on the determination of propiverine HCl in biological fliuds.[3-8] In the present paper the author designed in the development of simple, sensitive and robust analysis for propiverine HCl in formulations by using RP-HPLC.

MATERIALS AND METHODS
i) Instrumentation
The present chromatographic assay was performed on Waters 2695 HPLC system with Photodiode Array detector 2996 with data handling system and with Ace C-18 (250 × 4.6 mm, 5 µm) column. Empower 2 solutions was utilized for the present study. Chemicals were weighed using electronic balance Denver.

ii) Chemicals and Reagents
Pure standard of Propiverine HCl (99%) was procured as gifted sample from R.L Fine chemicals, Yelahanka New Town, Banganapalle, Andhra Pradesh and its formulation [Mictonorm; Label claim 15mg of propiverine HCl) was purchased and imported from Dr. Omar-Shalaby Pharmacy, Egypt. All the chemicals such as Potassium dihydrogen phosphate (AR-grade), Trimethyl amine (AR-grade) and Acetonitrile (HPLC Grade) used in the present study were obtained from

Merck chemicals Pvt. Ltd. HPLC Grade water was used in the preparation of mobile, buffer, standard and sample solutions.

a) Mobile Phase preparation

Mobile phase was prepared by mixing of potassium dihydrogen phosphate buffer (pH-2.5) and Acetonitrile in the ratio of 70:30 %v/v). Prior to use this mobile phase was sonicated for 15min and filter through 0.45µ membrane filter.

Buffer Solution preparation: Weigh accurately 1.36g of potassium dihydrogen phosphate and dissolve it in 1000ml of HPLC Grade water. And adjust the pH to 2.5 with 5% Triethyl amine filter through 0.45µm nylon membrane filter and degassed.

b) Dliuent preparation: It was prepared by diluting HPLC Water and methanol in the ratio of (50:50%v/v).

c) Preparation of Standard Stock and working Solutions

Standard stock solution of propiverine HCl was prepared by accurately weighing 25mg of propiverine HCl in to a 25mL volumetric flask and make up to volume with diluent. This stock solution was stored at 2-8°C and was protected from light. From the above standard stock solution working standard solutions of propiverine HCl in the concentration range of 10µg/ml to 60µg/ml were prepared by diluting aliquots of stock solution of propiverine HCl in 10ml volumetric flasks with the mobile phase. The linearity of response of propiverine HCl was determined by injecting 20µL of the above prepared working standard solutions into the prescribed column. Prior to injection the working standard solutions were filtered through a 0.45µm membrane filter respectively.

d) Preparation of Sample Solution

For this ten oral tablets of Mictonorm (Label claim 15mg of propiverine HCl) purchased and imported from Dr. Omar-Shalaby Pharmacy, Egypt were weighed accurately and grinded to fine powder. The powder equivalent to 100mg of propiverine HCl sample was transferred into a 100ml volumetric flask containing 50ml of diluent, sonicated to dissolve, degassed and made up to the volume by the diluent and filtered. Further, dilutions were made to obtain solutions of final concentration within the linearity range, and the procedure previously described for working standard solution was then followed.

RESULTS AND DISCUSSION

i. Method development and Optimization

Optimization of the present method was done by altering mobile phase composition, pH, column packing, flow rate, temperature, detection wavelength and the effects on retention and peak shape were monitored for the selected drug. The final chromatographic conditions have been set for stationary phase giving satisfactory resolution and run time with Ace C-18 (250 × 4.6 mm, 5 µm) column. A series of mobile phases varying the pH and volume fractions of methanol and water have been also tested with changing the pH and the best results obtained by use of mobile phase consisting of methanol: water (70:30%v/v). The flow rate of 1.0 ml/min and detection wavelength of 220nm was chosen in the present study. From the described experimental conditions a well resolved, sharp peak with clear baseline separation of propiverine HCl with a retention time of 5.262 was obtained which is represented in **Fig 2.**

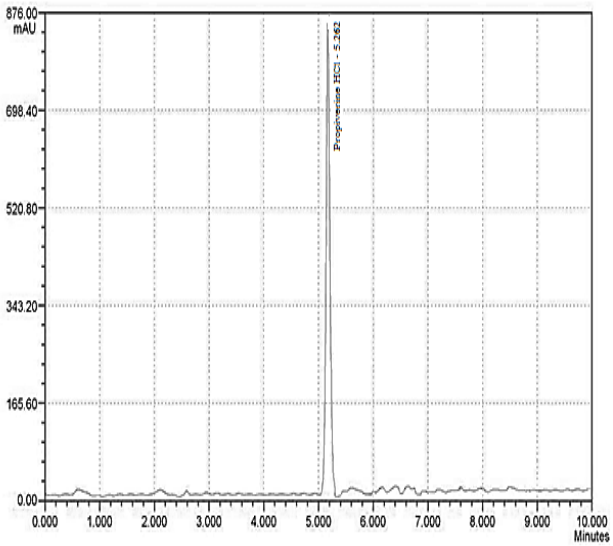

Figure 2: Chromatogram of Propiverine HCl Standard.

ii. Validation of the method

The proposed RP-HPLC method was validated according to the ICH guidelines[9] for several parameters that include the following.

a. Specificity

The specificity of present method was determined by analyzing the interference of blank and placebo solutions with propiverine HCl sample solution using the above described experimental conditions. The HPLC chromatograms of the blank and placebo displayed no other peak within the retention time of 5.262min making the developed analytical method highly specific for the analysis of propiverine HCl in tablet dosage form respectively.

b. System suitability

System suitability was ascertained by running six replicates of one fixed standard solution of propiverine HCl containing concentration 40µg/mL respectively. The number of theoretical plates and the tailing factor were within the acceptance criteria of > 2000 and ≤ 2, respectively, representing good column efficiency and optimum mobile phase composition (**Table 1**) demonstrated the suitability of the system for the analysis.

Table 1: System suitability parameters for propiverine HCl with the proposed RP-HPLC method.

Name of the compound	Retention Time	Theoretical Plates	Tailing Factor	Peak Area
Propiverine HCl	5.262	21234	1.15	424576

c. Linearity

The linearity of the present RP-HPLC method was evaluated by recording the chromatograms of six solutions of concentrations ranged from 10 to 60ug/ml of the target concentration of propiverine HCl. Peak area were recorded for each concentration were recorded and the linear regression curve was plotted using the concentration vs. peak area for propiverine HCl (**Fig 3**). Statistical analysis of the calibration curve was done and a linear response was observed in the concentration range of 10µg/mL to 60µg/mL with regression equation of the **y = 10808.203x +8053.400 (r2= 0.9997)** for propiverine HCl respectively. The results of linearity, limit of detection and limit of quantification were presented in **Table 2**.

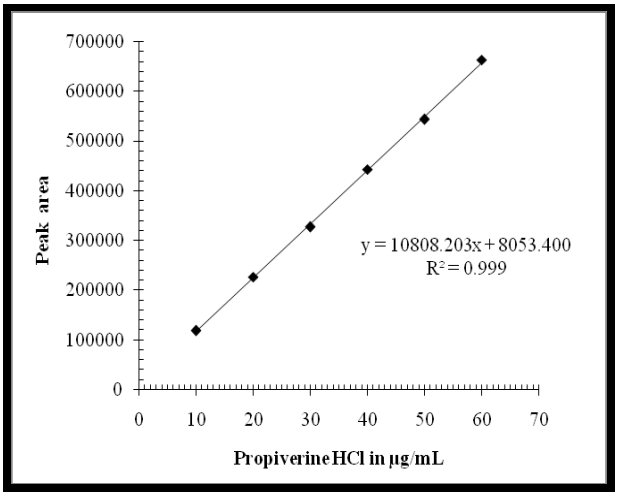

Figure 3: Linearity curve of propiverine HCl.

Table 2: Linearity studies of propiverine HCl with the proposed method.

Concentration (µg/mL)	Peak area
10	118235
20	226723
30	326424
40	441919
50	542495
60	662247
Slope	10808.203
Intercept	8053.400
r2	0.9997
LOD (µg/mL)	1.389
LOQ (µg/mL)	4.632

The Limit of detection and limit of quantification was determined and it was found to be 1.389µg/mL and 4.632µg/mL for propiverine HCl which indicated that the concentration in micrograms level can be quantified with acceptable accuracy and precision.

d. Precision

The precision of the present method (system repeatability) was assessed by calculating %RSD values for six determinations at 100% (40µg/ml) of the test concentration of standard and sample solutions of propiverine HCl. The results are reported in **Table 3**. The %RSD values were ranged from 0.334 & 0.732%(less than 2), revealing the present RP-HPLC method is precise.

Table 3: Results of method precision of propiverine HCl with the proposed method.

S.No	Retention time	Peak Area
Solution-1	5.259	447923
Solution-2	5.262	441919
Solution-3	5.238	445627
Solution-4	5.224	442556
Solution-5	5.234	446798
Solution-6	5.22	439458
Avg*	5.2395	444046.8
Std Dev*	0.017547	3252.635
% RSD*	0.334	0.732

*Average of three determinations; SD=Standard Deviation; %RSD= Percentage Relative standard deviation.

e. Accuracy

The accuracy of the current method was made by analyzing different extracted samples of various tablets. The accuracy was determined by interpolation of replicates (n = 3) peak areas of three accuracy standards (50%, 100% and 150%) and their respective chromatograms are recorded. The peak area responses are depicted in **Table 4**. The results illustrated the best recovery values for propiverine HCl that ranged from 99.80 - 99.90% respectively, revealing the good accuracy of the developed method.

Table 4: Recovery studies (Accuracy) of propiverine HCl with the proposed method.

S.No	25% AREA	100% AREA	150% AREA
Injection-1	115392	446964	662247
Injection-2	114356	445784	664549
Injection-3	117454	447433	667865
Avg *	115734	446727	664887
Amt Recovered*	9.98	19.96	59.94
%Recovery*	99.80	99.80	99.90

*Average of three determinations.

f. Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in chromatographic conditions. The factors chosen for this study were varying the flow rate by ± 0.2ml/min and the detection wavelength by ±5nm and the appropriate chromatograms are recorded. The results shown in **Table 5** revealed, that there were no remarkable variations in the system suitability parameters for for propiverine HCl were observed by varying above experimental conditions, which indicated the reported method as robust.

Table 5: Results of robustness study of propiverine HCl with the proposed method.

Robust Conditions		Propiverine HCl		
		Theoretical Plates	Rt	Peak Area
Flow Rate	**0.8 ml/Min**	24754	5.465	453654
	1.2 ml/Min	19592	4.970	445325
Wavelength	**215nm**	19596	5.136	466407
	225nm	24228	5.118	449924

g. Ruggedness

Ruggedness of the developed method is accessed by the analysis of the same samples (Under a variety of conditions, such as in different labs, different analysts, with different instruments, in different days etc. In the present study using the defined experimental conditions the analysis of propiverine HCl carried out on different days and the results were recorded that are shown in **Table 6**. The % RSD for retention time and peak area are less than 2, revealed the ruggedness of the proposed method.

Table 6: Ruggedness Studies of propiverine HCl with the proposed method.

S No	Analyst-1	Analyst -2
	Area	Area
Injection-1	447923	447263
Injection-2	441919	445631
Injection-3	445627	435050
Injection-4	442556	435439
Injection-5	446798	447586
Injection-6	439458	443285
Avg*	444046.8	442375.7
Std Dev*	3252.635	5731.325
% RSD*	0.732	1.295

*Average of three determinations; SD=Standard Deviation; %RSD= Percentage Relative standard deviation

h. Determination of assay of sample

The proposed RP-HPLC method was applied for the estimation of propiverine HCl in tablet dosage form Mictonorm (Label claim 15mg of propiverine HCl). The results are shown in **Table 7.** The high recovery (99.99%) values confirmed the appropriateness of the proposed RP-HPLC method for the routine analysis of propiverine HCl in tablet dosage forms.

Table 7: Analysis of marketed tablets of propiverine HCl with the proposed method

Drug	Label claim	Quantity Found*	%RSD*	%ASSAY*
Mictonorm	15mg	14.99	0.523	99.99%

*Average of three determinations; SD=Standard Deviation; %RSD= Percentage Relative standard deviation.

CONCLUSIONS

In conclusion a new RP-HPLC method was developed and validated for propiverine HCl in pure and formulations. Chromatographic separation for propiverine HCl was achieved at ambient temperature on Ace C-18 (250 × 4.6 mm, 5 µm) column using of mixture potassium dihydrogen phosphate buffer (pH-2.5) and acetonitrile in the ratio 70:30%v/v as mobile phase at a flow rate of 1.0ml/min and detection was carried out by absorption at 220 nm using photodiode array detector respectively. Excellent linearity, mean recovery studies for precision, ruggedness, robustness and sensitivity was attained for propiverine HCl when the present RP-HPLC method was validated as per ICH guidelines.On the credentials described above it is made known that the developed RP-HPLC method can be successfully used for the quality control of propiverine HCl in bulk product and pharmaceutical dosage form.

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