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A New Validated Stability indicating RP-HPLC Method for the Quantitative Analysis of Phentermine Hydrochloride in Tablet Dosage Form

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A B S T R A C T

A novel stability indicating RP-HPLC method was developed for the estimation of Phentermine hydrochloride in tablet dosage form. The separation was achieved on C_{18} (250 mm x 4.6 mm x 5 µm) column using a mobile phase composition of 0.2% Triethyl amine in water (pH- 3.5 with OPA) and methanol (70:30 % *V/V*). Eluents were detected at 210 nm at 1 ml/min. Stress studies were performed with milder conditions followed by stronger conditions so as to get sufficient degradation around 20%. A total of three degradation products were detected and separated from analyte. The linearity of the proposed method was investigated in the range of 25 - 175 µg/ml for Phentermine. The limit of detection and limit of quantification was found to be 1.89µg/ml and 5.67µg/ml respectively. Precision % RSD was found to be less than 2% and the mean recovery was between 99-101%. The method was validated according to ICH guidelines

Keywords: Phentermine hydrochloride, RP-HPLC method, Stress degradation, ICH guidelines

A R T I C L E I N F O

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1. Introduction

High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. HPLC is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. phenyl-tertiary-butyl amine contracts and forms as Phentermine which is used as psychostimulant drug. Phentermine is used as a substitute for amphetamine. Phentermine is used medically as an appetite suppressant. Medically Phentermine is used as a diet pill for obese persons [3,4] .

Phentermine is a USFDA approved drug for weight loss in combination with exercise, a healthy diet and in combination with other drugs like topiramate . Various brands manufacture phentermine in various dosage forms like Vites, Adipex and Qsymia (previously known as Qnexa) Phentermine is a TAAR1 agonists where the mechanism of action is activation of TAAR1 in monoamine neuron. Some of the trade name of Phentermine are Acxion (MX), Adipex P (immediate release), Adiphene (India), Anoxine-AM, Ionamin (slow-release resin, Australia, discontinued in the US), Duromine (slow-release resin, New Zealand, Australia and South Africa), Metermine (slow-release resin, Australia) e.t.c. Kavitha k.y.et.al, developed and validated a RP-HPLC method for the determination of phentermine and topiramte in pure and its pharmaceutical dosage form my present work aims to develop a A new validated stability indicating rp-hplc method for the quantitative analysis of phentermine hydrochloride in tablet dosage form. Various UV Spectrophotometric and RP-HPLC methods are reported.

2. Materials and Methods

Materials:

An analytical pure Phentermine hydrochloride was obtained as gift sample from Symed Labs, Hyderabad (India). HPLC Solvents Methanol used is manufactured by Merck, Mumbai (India). Phentaramine tablet was procured from local market.

Preparation of standard drug solutions

Stock solution of Phentermine hydrochloride was prepared by dissolving 10 mg of Phentermine hydrochloride in separate 10 ml of volumetric flask with small quantity of Methanol. The mixture was sonicated for about 10 min and then made up to volume with Methanol. From the stock solution 100µg/ml of Phentermine hydrochloride was prepared.

3. Results and Discussions

Method Development:

In the present work the drug Phentermine hydrochloride was scanned in 200-400 nm. The λmax was found to be 210 nm. A representative chromatogram of Phentermine hydrochloride was shown in Fig.1. A representative spectrum of λmax and Infrared spectrum of Phentermine hydrochloride was shown in Fig.2 and Fig.3. The developed method was validated as per ICH guidelines.

Chromatographic conditions

The mobile phase consisted of methanol, 0.2% tri ethyl amine in the ratio of 30:70 v/v contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 10min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min and the injection volume was 20µl. The eluents were monitored at 210 nm.

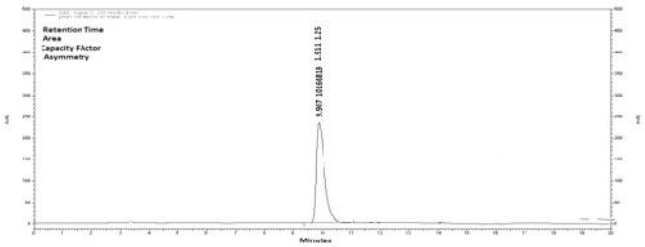

Figure 1: Chromatogram of Phentermine hydrochloride

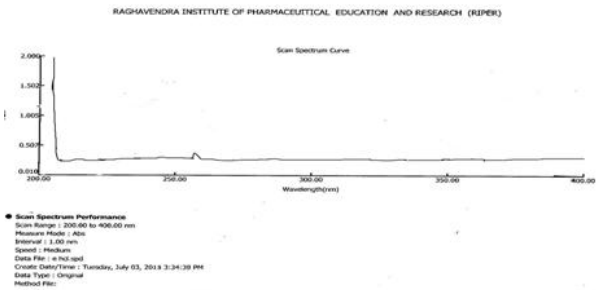

Figure 2: UV Spectrum of Phentermine hydrochloride

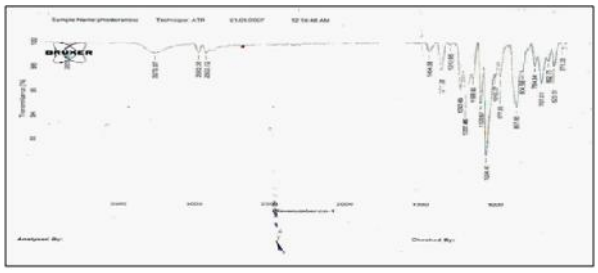

Figure 3: IR Spectrum of Phentermine hydrochloride

Method Validation

The method was validated according to the ICH guidelines [10 and 11]. The following validation Characteristics was addressed: specificity and selectivity, linearity, assay, accuracy, precision, limits of detection and quantitation, robustness, ruggedness.

Accuracy:

Accuracy of the method was determined by performing the recovery experiments. Known amount of the standard at 50%, 100% and 150% levels was fortified to the degradation sample. Peak area of the standards was calculated by the difference of peak area between fortified and unfortified samples. Six replicate samples of each concentration level were prepared and the percentage

recovery at each level (n=6) was determined for Phentermine hydrochloride, the results obtained are in good agreement with the added amounts. The results were shown in table 1.

Precision:

Intraday and interday precision was evaluated by injecting six different replications of 100 µg/mL of Phentermine hydrochloride. For intraday variation, sets of six replicates of the optimized concentrations was analyzed on the same day; for inter- day variation, six replicates was analyzed on six different days. The intra-day and inter-day precision (% RSD) was found to be less than 2%. The results was shown in table 2 & table 3, indicating that the method was precise.

Limits of detection and Quantification:

The LOD was defined as the lowest concentration of Phentermine hydrochloride resulting in a signal-to-noise ratio of 3:1 and LOQ was expressed as a signal-to-noise ratio of 10:1. The LOD and LOQ obtained were 1.89 µg/ml and 5.67 µg/ml, respectively.

Robustness:

Robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in mobile phase pH (±0.2), organic phase composition (90% to 110%), column temperature (±5˚C) and flow rate (± 0.2 mL min-1).System suitability parameters like USP plate count, USP tailing and Resolution were checked and they found to be within the limits.

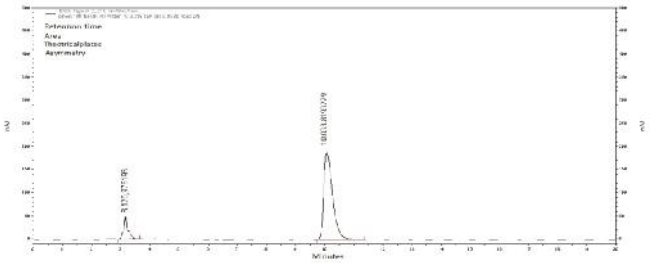

Figure 5: Acidic degradation of Phentermine hydrochloride

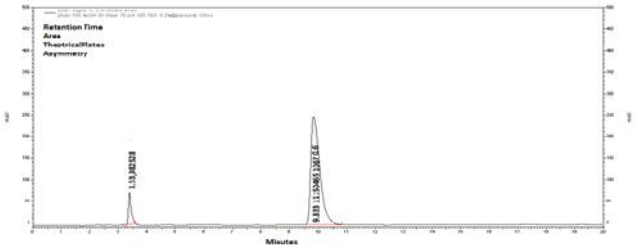

Figure 6: Oxidative Stress studies

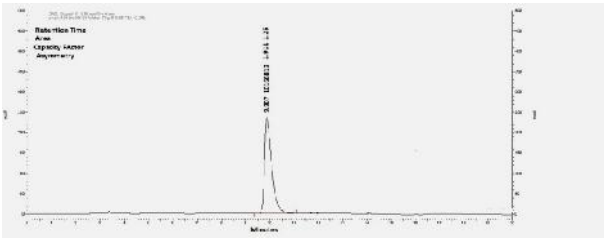

Figure 7: Thermal Stress studies

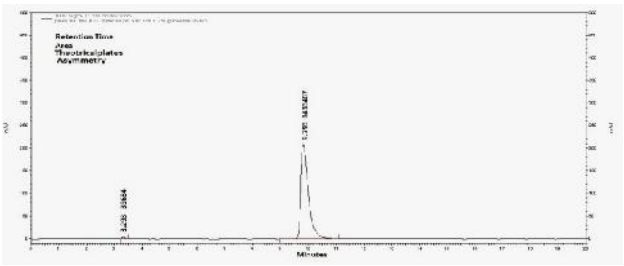

Figure 8: Photolytic Stress studies

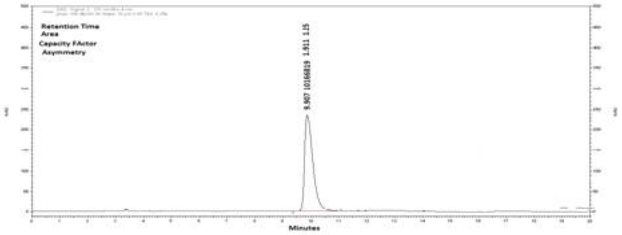

Figure 9: Neutral Stress studies

Table 1: Results of Accuracy

QC conc. (µg/mL) (A)	Recovery level	Addeddrug (µg/mL) (B)	Peak Area mean ± SD (n=3)	Amount Found ± SD (µg/mL)	% Recovery	%RSD
100	50%	50	14828612±105039.	35.76±0.30	99.33	1.3
100	100%	100	19046492±71344.4	39.81±0.34	99.94	0.6
100	150%	150	24341858±96105.9	44.22±0.36	100.5	0.7

Table 2: System Precision Data

Drug Inj No.	Conc. (µg/ml)	Rt	Peak area
1	100	9.90	10166819
2	100	9.92	10136819
3	100	9.91	10236819
4	100	9.95	10136819
5	100	9.98	10336819
6	100	9.97	10156819
7	100	10.05	10146819
8	100	9.94	10234819
mean ± S.D		9.95±0.029	1095924 ± 4217363
%RSD		0.291519	413.707598

Table 3: Intra and Inter-day Precision of Phentermine hydrochloride

S.No	Concentration (mcg/mL)	Peak area ±S.D (n=3)	
		Intraday	Inter day
1	25	2647683±14142.135	2637683±7523.56
2	125	1274858±7071.067	1257848±9327.75
3	175	1626035±60827.625	1625025±6472.83

Table 4: Robustness study

Parameter	Conditions	Rt	Area (n=3)	% Assay	Remarks
Optimized	1ml/min, M:W :30:70W :210nm	9.907	10166812±13048	100	-------
Flow rate	0.9ml/min	10.787	1114119±27614	111.47	Not Robust
	1.1ml/min	8.713	90597976±79133	90.42	Not Robust
Mobile phase	M:W:28:72	9.925	1019983±11327	100.01	Robust
	M:W:32:68	9.542	10336242±55754	100.26	Robust
Wavelength	217nm	8.180	38206187±55482	106.40	Not Robust
	223nm	8.180	27259297±91503	94.45	Not Robust

Table 5: Acidic degradation of Phentermine hydrochloride at room temperature

S.No.	Time (hr)	Peak area (n=3) Mean ± SD	% Degradation
1	0	30942543 ±903932	4.562
2	3	30924857 ± 686482	6.432
3	6	30348462 ± 2520940	8.548
4	2	29902948 ± 219	9.062

Table 6: Degradants formed during acidic degradation at room temperature

S .NO.	Degradants	Retention time	Peak area(n=3) Mean ± SD
1	D_1	3.1	9761832±577352

Table 7: Base Degradation Studies

S.NO.	Degradants	Retention time	Peak area Mean ± SD
1	D_1	3.023	225293±8294.78
2	D_2	3.143	379037±5712.24

Table 8: Assay of Phentermine hydrochloride formulation

S.No	Formulation	Label claimed (mg/tab)	Amount found (mg) (n=3)	Assay	%RSD
1	Adipex-p	37.5mg	25.31mg	101.26%	0.875

4. Conclusion

In present work a new method was developed and forced degradation studies were carried out for the estimation of Phentermine hydrochloride in bulk and pharmaceutical dosage form. Forced degradation HPLC method was developed with the mobile phase system of MeOH: Water (0.2% TEA in the ratio of 30:70 v/v p^H adjusted to 3.5 with orthophosphoric acid). The flow rate of 1ml/min was used on C_{18} column (250×4.6 mm, 5µm particle size). The retention time of Phentermine hydrochloride was observed at 9.907 min The developed and validated stability indication HPLC method is found be linear, accurate, precise, specific and robust. Hence the method can be used routinely for estimation of Phentermine hydrochloride in formulations.

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6. References

[1] Skoog W. Fundamental of Analytical chemistry, saunders college, publishing, 7th edition,1992
[2] http://www.pharmtech.com/pharmtech/article/articleDetail.jspid=89002.
[3] Kavitha K.Y, Geetha. G and Hariprasad R. RP-HPLC method development and validation for determination of phentaramine and toperamate in

bulk and dosage forms. Int. Res. j. Pharm, 2014, 5 (7): 613-618.

[4] Patel NS, Tandel FB, Patel YD, Thakkar KB. Development and validation of stability-indicating HPLC method for simultaneous estimation of cefixime and linezolid. Indian J Pharm Sci, 2014, 76: 535-40.

[5] Satish A. Patel and Jinalben and V Patel. Rp-HPLC method for simultaneous estimation of cefixime trihydrate and linezolid in tablet dosage form. IJPCBS, 2013, 3(2): 372-379.

[6] Dolly T. Gadhiya, Hina. Bagada. Simultaneous equation method for the estimation of cefixime trihydarte and linezolid in their combined tablet dosage form by uv-visible spectrophotometry. International Bulletin of Drug Research,2013, 3(5): 29-38.

[7] Patel DP, Goswami K, Patel M. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Cefixime and Linezolid in Combined Dosage Form. Int J Pharm Res Scholars, 2012, 1 (4): 1-12.

[8] P. Radhakrishnanand, D.Subba Rao, & V. Himabindu.A Validated Method for Determination of the Enantiomeric Purity of Darifenacin in Bulk Drug, Extended Release Tablet. Chromatographia. vol 68, issue 11, 2008, 1059-1062.ssue 2; 2010: 254-255.

[9] Barry kaye, William J.Herron, Paul V.macrae, Sylvia Robinson, Rapid, Solid Phase Extraction Technique for the High-Throughput Assay of Darifenacin in Human Plasma analytical Indian J Pharm Sci, 2014,76 : 535-40.

[10] ICH, Q2 (R1) Validation of Analytical Procedure, Test and Methodology, International Conference on Harmonization, Geneva, 2005.

[11] B. Mohammed Ishaq; H. Abdul Ahad; Shaik Muneer; S. Parveen; B. Famida. Analytical Method Development and Validation for the Estimation for Temozolomide in Phsophate Buffer pH 2.0 as a solvent by UV Spectroscopy, Int. Res. J. Pharm, 5(1), 2014.