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ORIGINAL ARTICLE **OPEN ACCESS**

Analytical Method Development and Validation of Stability Indicating RP-HPLC Method for Assay of Phenylephrine Hydrochloride Injection

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ABSTRACT

The current proposed methodology of the research is to determine the assay present in phenylephrine hydrochloride by using high-performance liquid chromatographic method. the developed method was validated for their accuracy and reproducibility. Reversed-phase chromatography was performed on Waters 2489 UV 2695 pump, Waters 2998 PDA2695 pump Software Empower3 photodiode array detector using Partsil 10 ODS, Hichrom C18, 250 mm x 4.6 mm x 10µm column with gradient elution program contain Buffer solution pH 3.0 as Mobile phase A and 100% methanol as Mobile phase B. UV detection at 280nm.Recovery and Linearity was observed well within the limits(R2 = more than 1.0 for concentration range of LOQ to 150% level for linearity and the %recovery was within the ICH acceptance limits of 97-103%). The method was validated as per ICH guidelines. The RSD for intermediate precision (<3.0% RSD) precision were found to be less than 2 %. The percentage recovery was in good agreement with the labeled amount in the pharmaceutical formulations. From the method validation data, it can be concluded that the method is simple, specific, precise and accurate for the determination of Phenylephrine hydrochloride in pharmaceutical formulations.

Keywords: *Phenylephrine hydrochloride, Estimation of related substances, HPLC*

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INTRODUCTION

Phenylephrine hydrochloride (Figure 1.1) chemically known as (1R)-1-(3-hydroxyphenyl)-2-(methylamino) ethanol hydrochloride. Molecular formula $C_9H_{13}NO_2.HCl$, molecular weight 203.67.Phenylephrine is an agonist of α_1-adrenoceptors. Phenylephrine Hydrochloride Injection is indicated for the treatment of clinically important hypotension resulting primarily from vasodilation in the setting of anesthesia. [1] Phenylephrine hydrochloride is a sympathomimetic agent with mainly direct effects on adrenergic receptors. It has predominantly alpha-adrenergic activity and is without significant stimulating effects on the central nervous system at usual doses. Phenylephrine Injection is indicated in adults and children for the treatment of hypotensive states e.g., circulatory failure, during spinal anesthesia or drug induced hypotension. [2-3] It is extensively hydrolyzed in the blood. Phenylephrine hydrochloride diluted to a concentration of 100 mug/mL in 0.9% sodium chloride injection was stable for at least 30 days when stored in polypropylene syringes at -20°C, 3-5°C, and 23-25°C. Intravenous infusion of phenylephrine hydrochloride, the observed effective half-life was approximately 5 minutes. There are several methods reported in the literature to estimate the content of Phenylephrine hydrochloride in the bulk drug and formulations using different methods such as titrimetric, UV spectroscopy and HPLC. However, no method for estimating for specific phenylephrine hydrochloride injection there are other drugs are combined with phenylephrine injection has been published. also, no method for determination of development and validation of stability indicating RP-HPLC method for estimation assay of phenylephrine hydrochloride injection. [4] In this work, a simple analytical method was developed and validated to estimate assay of phenylephrine hydrochloride through the use of reverse phase liquid chromatography in accordance with the ICH guidelines. In this work we develop a simple, fast and accurate reverse phase liquid chromatic method for the determination of phenylephrine hydrochloride.

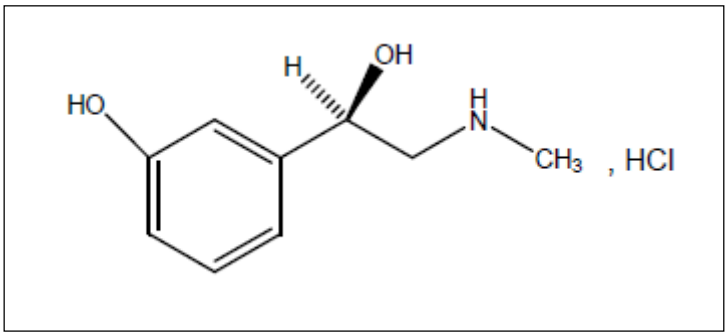

Figure 1.1: Chemical Structure of phenylephrine hydrochloride.

MATERIAL AND METHODS

Reagents & Chemicals

Sodium 1-octanesulfonate, Methanol, Ortho-phosphoric acid 88%, were obtained from Merck (India). All chemicals were of an analytical grade and used as received.

Chromatographic conditions [5-7]

Chromatographic separation was achieved by using a Waters 2489 UV 2695 pump, Waters2998 PDA 2695 pump Software Empower3 photodiode array detector using Partsil 10 ODS, Hichrom C18, (250 mm x 4.6 mm x 10µmparticlesize) column with gradient elution program contain Buffer solution pH 3.0 as Mobile phase A and 100% methanol as Mobile phase B at a flow rate of 1.0 mL/min.

Table 1 Chromatographic Conditions

Time	Mobile Phase A	Mobile Phase B
0	70	30
9	70	30
10	30	70
13	30	70
15	70	30
18	70	30

With UV detection at 280 nm. Column maintained at temperature 25 ºC, sample temperature10ºC. The overall run time was 20 min and the flow rate were 1.0 mL/min. 20 µL of sample was injected into the HPLC system. Retention time of phenylephrine hydrochloride is 8.3 minutes.

Preparation of Diluent: [8]

Prepare a mixture of Methanol and water in the ratio of 50:50 v/v respectively; adjust pH 3.0 with 3M Ortho-phosphoric acid and mix.

Preparation of Blank:

Diluent was used as Blank.

Preparation of Standard Solution: [9-11]

Weigh and transfer accurately about 50 mg of USP Phenylephrine Hydrochloride WS in 25 mL volumetric flask. Add 10 mL of water, sonicate to dissolve and dilute up to the mark with diluent, mix.
Further dilute 5 mL of this solution to 25 mL with diluent, mix.

(Concentration of standard solution: 400 ppm)

Preparation of Sample Solution:

Transfer 1mL of Phenylephrine Hydrochloride sample into 25 mL volumetric flask. Dissolve and dilute up to the mark with diluent, mix.

(Concentration of sample solution: 400 ppm)

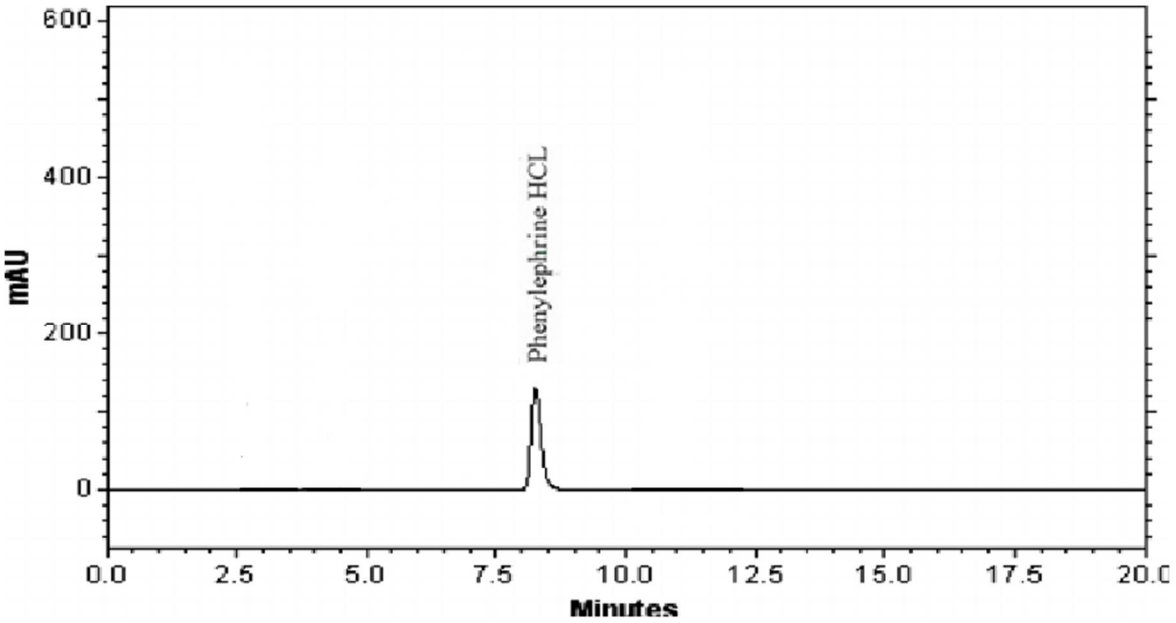

Figure:1.2 Standard chromatogram of phenylephrine hydrochloride by proposed method.

METHOD VALIDATION [13-14]

System Suitability

Performed the system suitability by injecting the standard solution for six times as per recommendations from US pharmacopeia. Calculate the theoretical plates and tailing form a peak.

Table:1.1Summary of system suitability.

Retention time of phenylephrine HCL	Tailing factor for phenylephrine HCL peak	Theoretical plates for phenylephrine HCL peak
8.3	1.1	16780

Specificity

Inject Blank (Diluent), Standard solution, Placebo solution, Epinephrine Bitartrate, Phenylephrine related compound C, Phenylephrine related compound D, Phenylephrine related compound E, Norphenylephrine hydrochloride (1% sample concentration) Sample solution and sample solution spiked with impurities (1%) onto the HPLC.

Table: 1.2 Summary of results for Specificity (Identification, Interference and Peak purity)

Solution		Phenylephrine HCl peak		
		Retention time (min.)	Purity angle	Purity threshold
Blank	-	-	-	-
Placebo	**10 mg/mL**	-	-	-
Standard solution	--	8.345	0.280	0.476
Sample solution	**10 mg/mL**	8.214	0.388	0.477
Phenylephrine related compound-C	**--**	9.770	2.989	3.697
Phenylephrine related compound-D	**--**	14.221	6.143	7.448
Phenylephrine related compound-E	**--**	14.684	2.229	2.257
Norphenylephrine Hydrochloride	**--**	7.484	11.700	14.745

Linearity and Range:

Linearity range Evaluated linearity in the range of 50% to 150% of the working concentration level. The working concentration of Phenylephrine Hydrochloride is 400 ppm for Phenylephrine Hydrochloride Injection; IV (Infusion) USP 10mg/mL, 50mg/5mL, 100mg/10mL. The range is 50% to 150%.

Table: 1.3 Summary of results for Linearity

Level (%)	Concentration in ppm (Phenylephrine HCl)	Response		
		1	2	Mean
50	200	1757417	1760763	1759090
80	320	2835973	2810659	2823316
100	400	3509133	3486708	3498071
120	480	4203245	4180499	4191872
150	600	5227680	5216731	5222206
Co-Relation Coefficient (R)		**1.000**		
Regression (r^2)		**1.000**		
Slope		**8367.549**		
Intercept		**41550.541**		
Working Level Area		**3498071**		

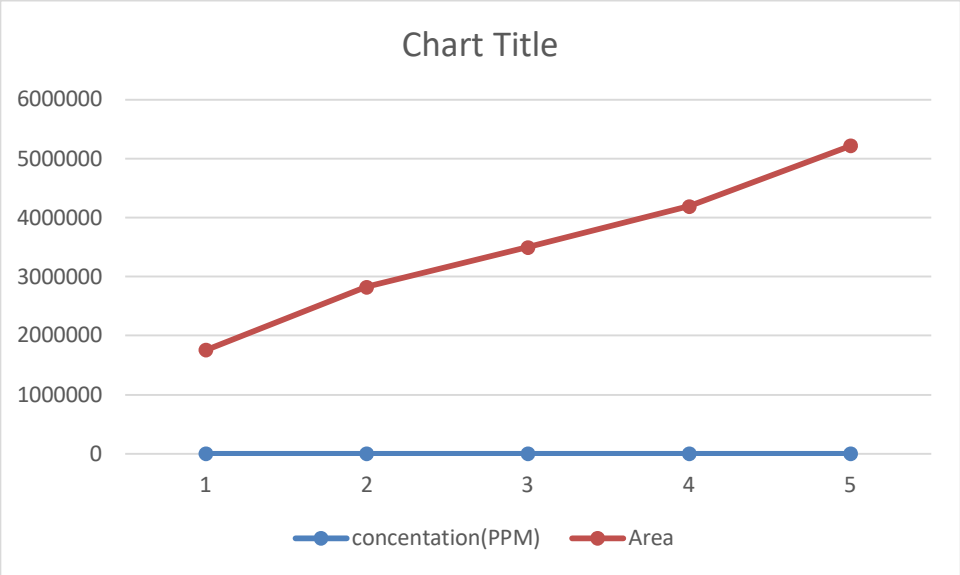

Figure: 1.3 Linearity and range for Phenylephrine Hydrochoride

Accuracy (Recovery):

Accuracy has been Evaluated accuracy at three levels, 50% to 150% of working concentration level of Phenylephrine Hydrochloride Injection; IV (Infusion) USP 10mg/mL, 50mg/5mL, 100mg/10mL. The working concentration of Phenylephrine Hydrochloride is 400 ppm.

Table:1.4: Summary of results for Accuracy and Mean % Recovery for Phenylephrine HCl

Level	Conc. added (µg/mL)	Peak Area	Conc. recovered (µg/mL)	% Recovery	Mean % Recovery
50%	200.670	1756976	198.136	98.7	99.0
	200.670	1770853	199.701	99.5	
	200.670	1757971	198.249	98.8	
100 %	401.340	3520625	397.025	98.9	98.7
	401.340	3508020	395.604	98.6	
	401.340	3507921	395.593	98.6	
150 %	602.010	5268076	594.087	98.7	98.7
	602.010	5267991	594.078	98.7	
	602.010	5270776	594.392	98.7	

Precision:

System Precision:

Single injection of Blank (Diluent) and six replicate injections of standard solution were injected on the system.

Table:1.5 Summary of results for System precision

S. No.	Area
1	3594746
2	3599373
3	3597738
4	3601863
5	3594210
6	3540965
Mean	**3588149**
%RSD	**0.65**

Method Precision:

Six independent sample preparations were prepared for all strength and injected in the HPLC.

Table:1.6 Summary of results for Method precision

Sample No.	10 mg/mL
	% Assay
1	101.4
2	101.0
3	100.9
4	101.0
5	101.3
6	101.2
Mean	**101.1**
% RSD	**0.19**

Intermediate Precision (Ruggedness):

Evaluated Intermediate precision by performing the method precision sample of same batch on different HPLC system and with different column. Evaluated the reproducibility- by comparing the results obtained from Ruggedness with those obtained from Method precision.

Table 1.7 Summary of results for Intermediate precision

Sample No.	10 mg/mL
	% Assay
1	100.7
2	99.8
3	100.5
4	101.5
5	100.4
6	100.6
Mean	**100.6**
% RSD	**0.55**

Robustness:

Changed following parameters in experimental conditions, measured the area of standard and sample preparations and calculated the % release. Change in chromatographic conditions.

Table:1.8 Summary of results for Robustness

Changes in parameters	Values	% RSDv of standard area	Resolution of phenylephrine HCL	% Correlation	%Mean assay
High flow rate (+0.1mL/min)	1.1 mL/min	0.41	2.42	99.2	100.1
Low flow rate (-0.1mL/min)	0.9 mL/min	0.36	2.60	99.5	100.7
High wavelength (+2 nm)	282nm	0.76	2.84	99.9	101.4
Low wavelength (-2 nm)	278 nm	0.75	2.84	99.9	101.4
High column temperature (+ 5°C)	30°C	0.10	2.84	100.4	101.5
Low column temperature (- 5°C)	20°C	0.32	2.43	98.8	101.4

Solution Stability:
The Standard and sample preparation were kept for more than 24 hours at sample temperature condition and injected time to time continuously in order to check the solution stability.

DISCUSSION AND CONCLUSION:
A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Partsil 10 ODS, Hichrom C18, (250 mm x 4.6 mm x 10µm particle size). Injection volume of 20µl is injected and eluted with the Buffer solution pH 3.0 as Mobile phase A and 100% methanol as Mobile phase B over gradient program, which is pumped at a flow rate of 1.0 ml/min. Detection, was carried out at 280 nm. There is no interference from blank. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness, and stability of solution.[11-12] For Selectivity, the chromatograms were recorded for standard and sample solutions of Phenylephrine hydrochloride Selectivity studies reveal that the peak is well separated and there is no interference of standard solution peak at the retention time of sample solution peak. Therefore, the method is selective for the determination of assay in Phenylephrine hydrochloride. The linearity results for Phenylephrine hydrochloride are specified concentration range are found satisfactory, with a correlation coefficient1.000. Calibration curve was plotted and correlation co-efficient for Phenylephrine hydrochloride found to be 1.000. The accuracy studies were shown as % recovery for Phenylephrine hydrochloride at 50%,100% and 150%. The limit of % recovered shown is in the range of 90 and 110% and the results obtained were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed %recovery of the Phenylephrine hydrochloride99.0-98.7 respectively. this indicates that the developed method is more accurate and reproducible over the range specified. For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of Phenylephrine hydrochloride. [13] The RSD for % assay of six independent samples should not be more than 2.0%.and the results were found to be within the acceptance limits. The absolute difference of % assay should not be more than ± 2.0, when compared to the freshly prepared sample solution. The data shows that cumulative % RSD is less than 2.0 standard solution is stable up to 38 hrs at 10ºC.[14] The absolute difference of % Assay is within limit. sample solution is stable up to 35 hrs at 10ºC, the data shows that cumulative % RSD is less than 2.0 standard solution is stable up to 52 hrs at 25ºC, the absolute difference of % Assay is within limit. sample solution is stable up to 22 hrs at 25ºC.Hence, the chromatographic method developed for Phenylephrine hydrochloride is rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of the active pharmaceutical ingredients for assurance of its quality in active pharmaceutical ingredients and different formulation. Further as part of future course of extended research, the method can be further applied directly for estimating impurities in pharmaceutical substances and formulation in commercial labs as well as can be extended for identifying the impurities in the drug substances.

Table: 1.9 Summary of results for Stability in analytical solution at 10°C

Standard Solution Stability			Sample Solution Stability	
Time (HRS)	Standard area	Cumulative % RSD	Time	% Assay
Initial	3594746	NA	Initial	101.4
12	3609090	0.65	9	101.5
23	3578850	0.63	20	100.8
27	3620662	0.68	25	101.8
38	3651957	0.89	35	102.5

Table: 1.10 Summary of results for Stability in analytical solution at 25°C

Standard Solution Stability			Sample Solution Stability	
Time (HRS)	Standard area	Cumulative % RSD	Time	% Assay
Initial	3516085	0.24	Initial	100.9
17	3462780	0.60	15	99.9
24	3503827	0.25	22	101.6
52	3618706	1.13	38	104.3

REFERENCES

1. Dagariya, R. K., Barad, M. B., &Kalele, U. A. (2021). Stability Indicating Method Development and Validation For Simultaneous Estimation Of Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate In Their Combined Syrup Dosage Form By Reverse Phase High Performance Liquid Chromatography. *Tropical Journal of Pharmaceutical and Life Sciences*, *8*(3), 01-16.
2. Kumar, A., Sharma, R., Nair, A., & Saini, G. (2012). Development and validation of RP-HPLC method for simultaneous estimation of nimesulide, phenylephrine hydrochloride, chlorpheniramine maleate and caffeine anhydrous in pharmaceutical dosage form. *Acta Pol Pharma*, *69*(6), 1017-22.
3. Sanchaniya, P. M., Mehta, F. A., & Uchadadiya, N. B. (2013). Development and validation of an RP-HPLC method for estimation of chlorpheniramine maleate, ibuprofen, and phenylephrine hydrochloride in combined pharmaceutical dosage form. *Chromatography Research International*, *2013*.
4. Heydari, R. (2008). A new HPLC method for the simultaneous determination of acetaminophen, phenylephrine, dextromethorphan and chlorpheniramine in pharmaceutical formulations. *Analytical letters*, *41*(6), 965-976.
5. El Yazbi, F. A., Hassan, E. M., Khamis, E. F., Ragab, M. A., &Hamdy, M. M. (2016). Development and validation of a high-performance thin-layer chromatographic method for the simultaneous determination of two binary mixtures containing ketorolac tromethamine with phenylephrine hydrochloride and with febuxostat. *Journal of chromatographic science*, *54*(5), 819-828.
6. Mahesh, P., Swapnalee, K., Aruna, M., Anilchandra, B., &Prashanti, S. (2013). Analytical method development and validation of acetaminophen, caffeine, phenylephrine hydrochloride and dextromethorphan hydrobromide in tablet dosage form by RP-HPLC. *Inventi Journal of Pharmaceutical Science Invention*, *2*, 9-15.
7. Al-Uzri, W. A. (2019). Determination of phenylephrine hydrochloride in pharmaceutical preparations using spectrophotometric method. *Asian J. Pharm. and Clinical Rese*, *12*(5), 1-5.
8. Patel, B. S., Nayak, P. P., Parmar, R. R., & Shah, D. A. (2014). Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Ebastine and Phenylephrine Hydrochloride in Pharmaceutical Dosage. *J. Pharm. Anal. Qual. Assur.*, *3*, 1-7.
9. Douòa, M., &Gibala, P. (2010). Fast HPLC method using ion-pair and hydrophilic interaction liquid chromatography for determination of phenylephrine in pharmaceutical formulations. *Journal of AOAC International*, *93*(5), 1436-1442.
10. Patel, S. M., Patel, C. N., & Patel, V. B. (2013). Stability-indicating HPLC method for simultaneous determination of aspirin and prasugrel. *Indian Journal of Pharmaceutical Sciences*, *75*(4), 413.
11. Kang, W., Liu, K. H., Ryu, J. Y., & Shin, J. G. (2004). Simultaneous determination of ebastine and its three metabolites in plasma using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, *813*(1-2), 75-80.

12. Soni, L. K., Narsinghani, T., & Saxena, C. (2011). Development and validation of UV-Spectrophotometric assay protocol for simultaneous estimation of ebastine and phenylephrine hydrochloride in tablet dosage form using simultaneous equation method. *International Journal of ChemTech Research*, *3*(4), 1918-1925.
13. ICH, I. (2005, November). Q2 (R1): Validation of analytical procedures: text and methodology. In *International conference on harmonization, Geneva*.
14. ICH, I. (2003). Harmonized tripartite guideline, stability testing of new drug substances and products Q1A (R2). In *International Conference on Harmonisation*.

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