Development and Validation of Pholcodine In Pharmaceutical Formulation – A New Rp-HPLC Method

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Abstract **A validated HPLC method is developed to determine Pholcodine in pharmaceutical formulation by using rate of flow at 1.0ml/min is employed on Symetry or Luna C8 5µm (4.6 x 150 mm) or equivalent. As a mobile phase, mixture of Methanol, Water and Acetonitrile ratio in the range of 70:20:10/v/v is prepared. The wavelength is detected as 220nm (UV) and 100 µl sample is injected. By using Run time as 10minutes, the flow rate is found to be 1.5 ml per minute. The% R.S.D Pholcodine was 0.4. The LOD was found to be 15µg/ml and the LOQ was found to be 25µg/ml. The mean Percentage recovery for Pholcodine is found that 80%. By using the guidelines from ICH this method is validated. This proposed HPLC method can be applied successfully for the common quality control analysis. Therefore, proposed developed method is simple and is better than other methods reported in the literature.**

Key Words - Pholcodine, HPLC, UV detection, Mobile phase, LOQ, Recovery.

I. INTRODUCTION

Pholcodine(Fig:1) is found in certain cough lozenges.[1] The preparation is almost exclusively an oral solution; typically 5 ml. Adult dosages are generally 5 to10 ml Maximum 4 times per day.[2] Pholcodine now largely replaces the previously more common codeine linctus, as it has a much lower potential for dependence. Pholcodine is not prescribed in the United States where it is classed as a Schedule I drug, the most highly controlled drug category, which includes the likes of heroin, LSD and ecstasy.[3] It is a class B substance in the United Kingdom but can be purchased over-the-counter in most UK pharmacies.[4]-[5] M.V.BasaveswaraRao et.al.,[6] proposed, developed and validated Buspirone. They are used the flow rate of Isocratic elution at 1.0mL per minute is identified on a symmetry C18 (250 × 4.6mm, and the particle size as 5µm). Water, acetonitrile and methanol were taken in the range of 45V/V, 35V/V and 20V/V. The wavelength is 210nm. The injected sample is 20µL. 7.057min. is the Retention time for buspirone. Below the value two, the percentage RSD for both precision and accuracy of the method is identified. The proposed method is used for the regular method of analysis for the estimate of Buspirone which is used as tablet form. Najmul Hasan et.al.,[7] described accurate, precise, simple Ion-pairing stability indicating, specific and Reverse phase High performance liquid chromatography for the determination of paracetamol and cetirizine Hydrochloride. In this development they are used mobile phase as Acetonitrile, Buffer and Sulfuric Acid in the propotions of 45, 55 and 0.3v respectively. The rate of flow is1.0 mL min^{-1} is identified. The wave length was 230nm by using Hibar® Lichrosorb® C_{18} column. The correlation coefficients of 0.9977, 0.9998, 0.9984, and 0.9997 the relative recoveries were found that 99.3% for cetrizine hydrochloride, 99.5% for paracetamol, 99.8% for methyl paraben and 98.7% for propylparaben. The LOD and LOQ were in the range of 0.3-2.7 ng ml^{-1} and 0.1-0.8 ng ml^{-1}. Jagdish Kakadiya et.al.,[8]developed PMZ and PCM in pharmaceutical dosage form. By using Hyperchrom ODS-BP (4.6 mm X 250 mm, 5 µm) column separation was carried out. Methanol and Water with 1% TEA are used in the proportions as 30V/V and 70V/V are as eluent. Wavelength was found to be 250nm and rate of flow is 1.0 ml/minute. Rt of Promethazine hydrochloride and Paracetamol are 3.10min. and 1.72 min. respectively.

II. EXPERIMENTAL

2.1 CHEMICALS AND REAGENTS: Pholcodine reference sample is purchased from Cipla where as from merck acetonitrile and methanol are procured.

2.2. EQUIPMENT & ANALYTIC FUNCTION: Drug analysis is performed with the help of a Luna C8 5µm (4.6 x 150 mm), a LC-P7000 isocratic pump, a 20µL sample and a detector-LC-UV7000 which is running on PEAK Chromatographic Software version of 1.06. Isocratic elution with Methanol, Water and Acetonitrile in the range of 70(V/V),20(V/V) and 10(V/V) with the help of PH-5.5 is used at a rate of flow 1.0mL/minute. Fresh mobile phase is

prepared and after sonication for five minutes if any gas is there which is removed.

2.3. STOCK AND WORKING STANDARD SOLUTIONS: Pholcodine is taken as 60mg. Working standard solution and diluents are passed in 100mL volumetric flask after that it is subjected to complete dissolving then by using the solvent again the volume is make to the mark. After that 5mL of the stock solution is pipette out and which is transferred into a 100mL volumetric flask after that distilled water is added to make up the solution to 100ml. If any filtrates are found which are subjected to filteration through 0.45µm nylon paper. Again 30µg/mL mL solution is prepared with the help of 100ml solution. The calibration curve is drawn with in the concentrations range of 5.0 to 30.0µg/mL working standard solutions. To perform the experiment calibration solutions are prepared daily and subjected to immediate analyzation after preparation.

2.4. PHOLCODINE TABLETS THEIR ASSAY: Pholcodine tablet was weighed to 20mg. An average weight is computated. Exactly weighed 20mg of Pholcodine is passed to a 100mLflask. To this, Diluent to be added to Pholcodine which is well mixed to and make up to the mark in the volumetric flask. The ingredients are mixed well. If any filterate is found that filterate is filtered by 0.45µm filter paper. 1mL of stock solution is pipetted out and transafered in 100mL flask and dilution is made up to mark with suitable diluent. 30µg/mL solution was prepared from the filtered sample. Into the HPLC System this solution is injected. For the determination Pholcodine peak area was measured.

2.5. VALIDATION PROCEDURE: As per ICH guidelines the main objective of this proposed validation method is that this method is accurate. In this method different parameters were studies like intermediate precision, repeatability, system suitability, specificity, accuracy and stability and. Constructed different plots by using different concentrations within the range of 05.0µg/mL-30.0µg/mL prepared set of three for the identification of linearity. Pholcodine peak area is drawn against to concentration to get the calibration graph. By using linear regression linearity is measured. By using five replicate injections of Pholcodine the repeatability is determined by using fresh Pholcodine solution. This method is repeated on two successive days for the intermedidate precision. Precision values are reported as percentage RSD and Pholcodine area is measured. By using ambient temperature in the range of 30±15◦C stability values are identified in three days.

III. RESULTS AND DISCUSSION

3.1. CHROMATOGRAPHIC CONDITIONS: The Stationary phase selection purely relies on nature of the sample, molecular weight and miscibility. Preferably by using colum - RP the Pholcodine drug in polar compounds is analyzed. C8 column is selected for this reaction. For RP colums nonpolar substance is highly attractive. As a mobile phase Methanol: water: acetonitrile mixture was used, and mobile composition effect of Rt on pholcodine is measured. The concentrations of the water, acetonitrile and methanol are optimized to give symmetric peak with short run time.

3.2. METHOD – VALIDATION

3.2.1 SPECIFICITY: Components should be presented for both the solvent and placebo solutions, which with the Pholcodine. Peak purity results shows that the Pholcodine peak is pure – i.e. the PA < TH. PA represents the purity angle and TH represents threshold angle. The solutions were subjected to injection with the help of conditions represented in analysis method. Components are not seen in the peak of Pholcodine, and the peak Purity results indicated that peak of Pholcodine should be considered as spectrally pure.

3.2.2 DETECTION LIMIT: The Limit of detection can be defined as the minimum conc. of the substance is measured and identified by the useage of analyte, by using the conditions in the experiment. At a notified stage it removes the analyte strength either above to the value nor below to the value. Maximum allowable carryover for Pholcodine is 0.0892 mg. Standard solutions are passed two times, after the total results average is noted, the results are showed in the graph. Eight solutions with different concentrations like 0.90, 0.45, 0.225, 0.1125, 0.056, 0.0140, 0.0030 mg/swab of Pholcodine working concentrations are prepared, which are passed by using analysis method. A curve of linear regression is drawn. The results were tabulated in Table: 1 and graph is shown in the figure: 8

3.2.3. LINEARITY: To construct five-point graph three independent determinations were performed by using various concentration ranges starting from 5.0–25.0µg/milli Liter. To the pholcodine along with equivalent drug the linear relationship between peak areas concentrations were measured. The calibration curve is constructed by using statistical data which is shown in Table 2.

3.2.4. PRECISION: Under subjected conditions percentage for a test procedure express degree of scatter in between a series of evaluations which are observed from multiple sampling of exact homogeneous sample. An amount of material which is to be predetermined limit was placed on a stainless steel and subjected to swabbing. The precision is repeated by using six samples which are prepared in the following manner. Six replicate injections of working standard solutions are passed with the help of analysis. The percentages recovery for the peak response is identified. Solution must be made up immediately before use and

protected from light. The precision should be continued for all the six samples. The % recovery should not exceed to 65%. The result is tabulated in the Table: 3. Which indicates good method precision.

3.2.5. STABILITY: Sample and standard solutions containing stability can be determined by using temperature 30 ±15◦C. After three days the triplicate solutions are checked after storage, obtained data was compared with fresh samples. For every 48 hrs it was observed that the solutions are stable. In this time the values does not decreases below 98%.

3.2.6. SYSTEM SUITABILITY: As per the analysis of method Total 06 replicate injections of working standard solution are passed by using injector. For the peak the values of % RSD were identied. To the total peak responses what ever the %RSD values are there those values are not exceeded 5.0%. Therefore, The total analytical system satisfy with the essentials stated by the system suitability. The values so obatined were shown and given in the form of table: 4

V. CONCLUSION

A method RP-HPLC was developed and validated to determine Pholcodine. This method so proposed is very simple and rapid. This proposed and developed method provided accurate results, gives precise and acts as specific. The chromatographic flow rate is 1.5mins provides the analysis of large number of samples in a short period time. Due to this reason the method presented here is suitable to the analysis of Pholcodine in pharmaceutical dosage forms (Figure 3, Table 4).

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REFERENCES

[1] inovapharma.com

[2] Jump up BNF (2007). British National Formulary 54. BMJ Publishing Group Ltd., RPS Publishing, London. Page 175.

[3] Jump up US Food and Drug Administration. (2009) "Legislation - Controlled Substances" http://www.fda.gov/RegulatoryInformation/Legislation/ucm148726.htm

[4] Jump up "Boots Night Cough Relief"

[5] Jump up "Care Pholcodine Linctus".

[6] M.V.BasaveswaraRao,1 A.V.D.Nagendrakumar,2 SushantaMaiti,1 andGuttikondaRaja1, "Validated RP-HPLC Method for the Determination of Buspirone in Pharmaceutical Formulations"; Chromatography Research International; 2011, Article ID 232505, 3 pages doi:10.4061/2011/232505.

[7] Najmul Hasan,1,* Mathurot Chaiharn,2 Umair Ali Toor,1 Zulfiqar Ali Mirani,3 Ghulam Sajjad,4 Nawab Sher,5 Mubashir Aziz,6 and Farhan Ahmed Siddiqui7; "Development, Validation and Application of RP-HPLC Method: Simultaneous Determination of Antihistamine and Preservatives with Paracetamol in Liquid Formulations and Human Serum"; Open Med Chem J.; 2016; 10: 33–43.

[8] Jagdish Kakadiya*1, Naiya Parmar1 , Nehal Shah1; " Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Promethazine Hydrochloride And Paracetamol In Combined Liquid Formulation"; Asian Journal of Research in Biological and Pharmaceutical Sciences; 2014; 2(1): 11 – 26

FIGURES AND TABLES

Fig: 1 Structure of Pholcodine

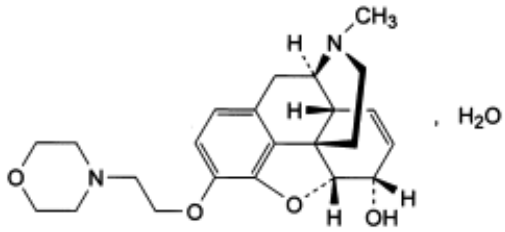

Fig: 2 Chromatogram 1 Solvent – No significant peak detected

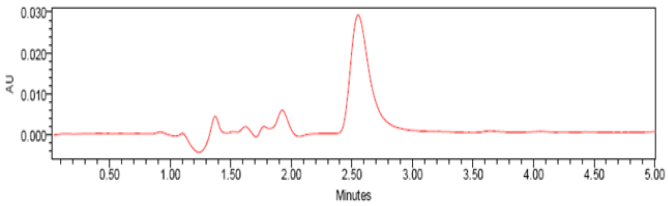

Fig: 3 Chromatogram 2 Drug active – Peak due to Pholcodine.

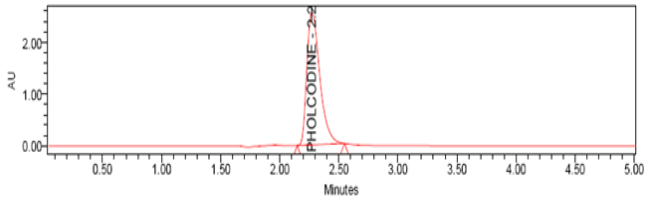

Fig: 4 Chromatogram 3 Product – Peak due to Pholcodine.

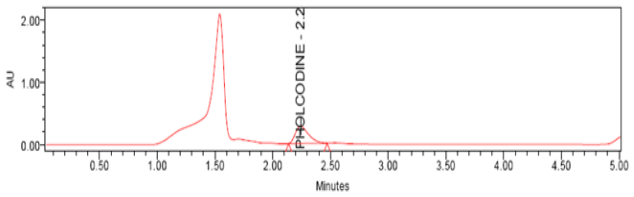

Fig: 5 Chromatogram 4 Active – UV stress: Peak due to Pholcodine.

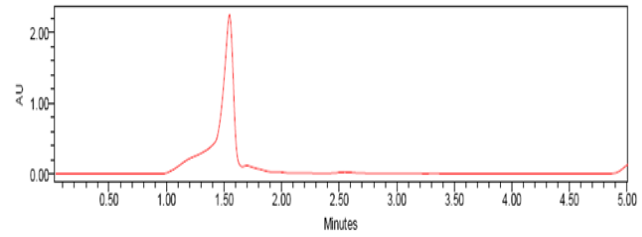

Fig: 6 Chromatogram 5 Detergent- No significant peak detected

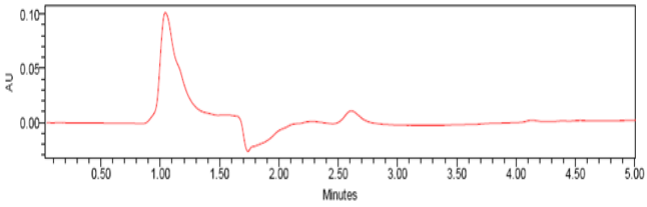

Fig: 7 Peak purity: 1 Purity angle < Threshold: 8.621 < 48.847

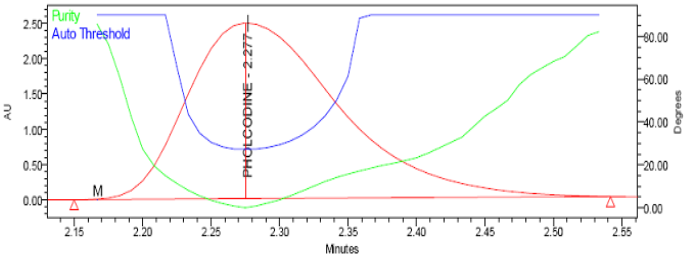

Fig: 8 Peakpurity: 2 Purity angle < Threshold: 1.014 < 90.000

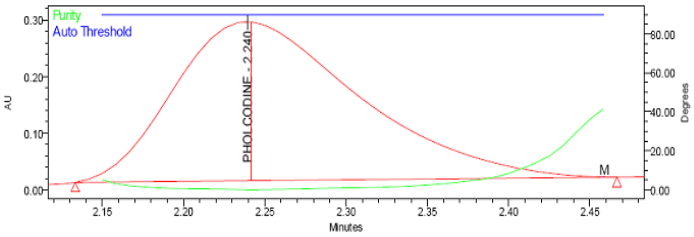

Fig: 9 Calibration curve

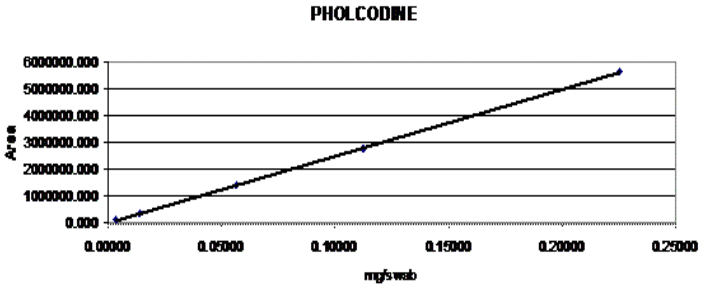

Table : 1 Average Response

Concentration mg/swab	Response 1	Response 2	Average Response
0.22538	5616838	5617161	5616999.500
0.11269	2781811	2801657	2791734.000
0.05634	1395550	1396117	1395833.500
0.01409	380489	381319	380904.000
0.0030	121946	123297	122621.500

Table : 2 Regression analysis of calibration curve

Parameters	Values
Calibration range (µg/mL)	5 - 30
Slope	21727542
Intercept	122621.500
Correlation coefficient (r2)	0.898

Table :3 Precision Values

Sample	% Recovery
1	68
2	83
3	70
4	89
5	90
6	79
Mean	80

Table : 4 System Suitability Results

Sample	Pholcodine Area
1	21708835
2	21839877
3	21801640
4	21746045
5	21643499
6	21625358
Mean	21727542
% RSD	0.4