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RP-HPLC METHOD FOR ESTIMATION OF RESIDUAL P-CHLOROPHENOL CONTENT IN CHLORPHENESIN

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ABSTRACT

A Novel, sensitive and selective HPLC method was newly innovated and proves the validity for determination of residual p- chlorophenol content in Chlorphenesin. The reverse phase chromatography illusion was achieved on Spolar-C18; 15.0cm 4.6mm 5µm of a particle size analytical column by with a blend of Buffer solution and Acetonitrile in a ratio of 1:1 v/vwith1ml per min flow rate at an ambient temperature and detection was carried out at 230nm. A clear chromatography peak was identified with a retention time of 3.602 min for p- chlorophenol. The developed reverse phase technique was validated with reference of ICH guidelines validation parameters specificity, precision, linearity, LOD, LOQ, accuracy, solution stability and robustness and the method show exceptional linearity and correlation coefficient of p-chlorophenol was 0.999. The % mean recovery of the p-Chlorophenol was in between 100 to 110 of the specification limits (<30ppm) and %RSD for repeatability was less than 1%. Therefore, the proposed validated method shall be in use for the residual content of p-chlorophenol content in Chlorphenesin during regular and quality-control analysis.

INTRODUCTION:

The estimation of impurities in bulk drug substances and pharmaceutical formulations is one of the mainly important fields of activity in a modern industrial analysis. Impurity is anything that is not the drug matter or an excipient in the drug product. According to ICH [1], impurity profile of a drug material is "A description of the identified and unidentified Impurities exists in a new drug substance."Impurity profiling is measured to be the ordinary name of analytical behavior, the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical preparations. The significance of drug impurity profiling is that it affords data which can straightly donate to the safety and efficacy of drug therapy by minimizing the impurity-related adverse effects of drug materials and the preparations made thereof. In current

years, the significance of assay methods for characterizing the quality of bulk drug [2].The manufacturing of active pharmaceutical ingredients (API) under GMP (, Canadian Drug and Health Agency has emphasized on the pure necessities of drug substances and products. Regulatory authorities have to make sure the quality of pharmaceutical formulations accessible in the market substance has decreased noticeably in the same time the good manufacturing practice) circumstances require sufficient to organize of the quality of the diverse ingredients concerned in the synthesis. Various regulatory establishments like ICH, the USFDA importance of impurity profiling is incessantly increasing.Chlorphenesin is a broad spectrum antimicrobial material active aligned with bacteria, fungi and yeast. In the concentration of 0.10 to 0.30% it has bactericidal activity against Gram (+) and Gram (-) bacteria, fungicidal action against Aspergillusniger and Penicilliumpinophilum (fungi), and is also active against Candida albicans and Saccharomyces cerevisiae (yeasts) [3] and thus classified as an anti fungal agent for dermatological use by the WHO. Chlorphenesin is being used as a polyvalent preservative in1386 cosmetic products and personal care products including creams, lotions, gels, aqueous and alcoholic solutes, sprays, makeup products, shampoos, conditioners and foams. Overdose of Chlorphenesin includes nausea and drowsiness. The major adverse effect of this antimicrobial agent is allergic contact sensitivity [4].The Chlorphenesin the form of carbamate salt is used as a central muscle relaxant to treat muscle pain and spasms. Other effects include sedation, anxiolysis and dizziness [5,6]Chlorphenesin is a derivative of chlorophenol.Chemically,, it is 3-(4-Chlorophenoxy)-1,2-propanediol or 3-(4-chlorophenoxy)propane-1,2-diol having a molecular formula of $C_9H_{11}ClO_3$, Chemical structure of Chlorphenesin is shown figure 1(a). It is a white or pale cream colored crystal, slightly soluble in water and freely soluble in alcohol (95%), soluble in ether, slightly soluble in fixed oils. It is prone to degradation in alkaline hydrolysis and transformed into p-chlorophenol. The chemical structure of 4-chlorophenol is shown in figure 1 (b). 4-chlorophenol is a synthetic reactant of Chlorphenesin and so it is considered as an impurity in Chlorphenesin bulk drug. PCP is a White to pale yellow crystals, crystalline powder, soluble in ethanol. Pharmacologically, PCP is labeled in the PubChem Catalogue as Local antibacterial agent in root canal therapy [7], Topical antiseptic in ointments [8]. It also considered as toxic as it irritates the eyes, skin and the respiratory tract. From the above mentioned information, it is clear that PCP is important in the field of pharmaceutical analysis mainly as a manufacturing impurity in Chlorphenesin bulk powder. The maximum allowed limit of PCP in Chlorphenesin is not more than 30ppm.Chlorophenols are recognized by EPA as persistent environmental pollutants [9].

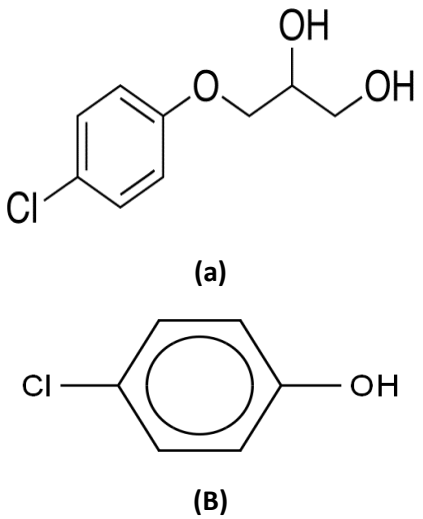


Fig.1 Chemical structures of (A) Chlorphenesin (B) P-Chlorophenol

Chlorophenols can be detected by several techniques, although the gas and liquid chromatographic methods have been realized as the most suitable. Various analytical methods were published for the determination of chlorophenol in drinking

and environmental water samples by gas chromatography mass spectrometry [10-12] , by converting into derivatives [13] by liquid chromatography [14-19] and Gas Chromatography Coupled with an Electron-Capture Detector [20] and in soil by liquid chromatography [21], by microbial and chemical methods [22], and also in the commercial formulation by LC [23]. Detection of P-chlorophenol as 4-amino anti pyrine derivative by liquid chromatography. Study for the presence of synthetic impurity in Chlorphenesin bulk drug is not reported. So, need was felt to develop a validated Chromatographic method for estimation of 4-Chlorophenol content. In present research work, the quality of the Chlorphenesin bulk drug has been assessed by determining 4-chlorophenol (Mfg. Impurity of Chlorphenesin) content using reversed phase high performance liquid chromatography method.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Chlorphenesin drug was obtained as a contribution sample from salicylates and Chemicals Pvt. Ltd, Hyderabad, India. Acetonitrile and purified water of HPLC grade and P-Chlorophenoland other chemicals of A.R grade were purchased from E. Merk (India) Ltd. Worli, Mumbai, India. The 0.45µ nylon filters were purchased from Millipore.

2.2. Instruments

The HPLC system (Shimadzu) Auto sampler separation module LC2010CHT consisted of a high pressure pump and 10 µL capacity injector loops. The system was well equipped with empower 2 software for monitoring and processing of data. The analytical column used was Spolar C18; (150mm x 4.6mm; 5µm). Other types of equipment like Afcofet analytical balance module ER200A/0410014was used for weighing of the materials. Degassing of the mobile phase was done by ultrasonic bath sonicator.

2.3. Chromatographic condition

The mobile phase consisted of acetonitrile and buffer solution in the ratio of 1:1v/v. Samples were analyzed using the following parameters. Flow rate; 1 ml/min, injection volume; 10 ml, run time; 10 min, column oven temperature; ambient and detection wave length; 230 nm.

2.4 . Method development

Any analytical method was not reported for the estimation of P-chlorophenol content in Chlorphenesin. Hence it was significant to start the method development using reverse phase liquid chromatography as it is commonly used and C-18 columns are also available. RP-HPLC method was developed for the estimation of P-chlorophenol content in Chlorphenesin. The mobile phase was selected based on the sensitivity of the process, the time necessary for the analysis, easily available solvents and simplicity of preparation. The mobile phase was premixed and filtered through a 0.45µm filter and sonicated for 10min to remove gases. Optimization of the mobile phase was taken based on various parameters such as retention time, the number of theoretical plates and resolution. Mobile phase was remixed and filtered through a 0.45µm filter. Optimization of the mobile phase was taken based on various parameters such as retention time, the number of theoretical plates and resolution.

2.5 .Preparation of Standard solution

P-Chlorophenol standard of about 30 mg was weighed accurately and added to 100 ml of volumetric flask and dissolved, then diluted to 100 ml with the mobile phase. 10 ml of this solution was taken into 100 ml of volumetric flask and was diluted to 100 ml with the mobile phase.

2.6. Preparation of sample solution

50.0 mg of sample substance was weighed and transferred to 50 ml of volumetric flask and dissolved and diluted to 50ml with the mobile phase. 10 ml of this solution was taken in 100 ml volumetric flask and made up to the volume with the mobile phase.

2.7. Preparation of buffer solution: 2.95 g of potassium Di-hydrogen phosphate and 0.53g of Di-potassium hydrogen orthophosphate were weighed and dissolved in 1000 ml of purified water.

2.8. Preparation of mobile phase: Acetonitrile and phosphate buffer were mixed in a ratio of 1:1 v/v. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µm filter under vacuum.

2.9. Procedure for method development: A mobile phase of Acetonitrile and phosphate buffers in the ratio of 1:1% v/v was found to be the most suitable mobile phase for ideal separation of P-chlorophenol. The mobile phase was pumped through the Spolar C18columnhaving a length of 150mm x 4.6mm; and 5µm particle size with a flow rate of 1 ml/min. The column was at an ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of drug solution.10µl of standard and sample solutions were injected into the chromatography system and the area for the P- chlorophenol peak was measured. The detection of drug was monitored at 230nm. The run time was set at10 min. Under these optimized chromatographic conditions, the retention time for the p-Chlorophenol was 3.602min.and Chlorphenesin was 2.273 min. The typical chromatograms of both standard and sample solutions are given in figures3 and 4. From the peak, the content of P-chlorophenol was calculated using the following formula.

$$\text{Content of } {}_{P}\text{-Chlorophenol in ppm} = \frac{A_T \times C_S \times P \times 1000}{A_S \times C_T \times 100}$$

Where, A_T = Peak Response (Area) of 4-Chlorophenol in sample
$A_{S=}$ Mean Peak Response (Area) of 4-Chlorophenol in Standard
C_S= Concentration of Standard solution (0.03mg/mL)
$C_{T\,=}$Concentration of Sample Solution (0.1mg/mL)
P = Purity of 4-Chlorophenol Standard

2.1.0: Method validation: The validation of analytical method verifies that the characteristics of the method if they satisfy the requirements of the method. The Proposed method was validated according to ICH guidelines for specificity, linearity, accuracy, precision, limit of detection and quantification, robustness and solution stability [24].

3. RESULTS AND DISCUSSION

3.1. System suitability: After equilibration of column with mobile phase, the blank preparation (twice) and five replicate injections of 10µl of 4-Chlorophenol standard solution were injected through an auto injector into the optimized chromatography method and the chromatograms were recorded. The system suitability parameters were measured and the results are shown in Table 1 and the chromatogram is shown in Fig.2. The results have been demonstrated with respect to %RSD, USP plate count and USP peak tailing. The %RSD for the five replicate injections was less than 2%.

Specificity: The specificity of the analytical method was determined by injecting a blank (mobile phase), 4-chlorophenol and sample preparations into the chromatography system under the same experimental conditions. The chromatograms were compared.

Table 1. System suitability Results

Injection No	RT (min)	Peak area	Peak tailing	Plate count
1	3.602	1189358	1.235	45213
2	3.601	1190481	1.234	45759
3	3.600	1190994	1.235	45515
4	3.599	1191055	1.233	45650
5	3.600	1190765	1.235	45185
Mean	3.600	1190531		
%RSD	0.03	0.06		

Table 2. Percentage of interference Results of the blank**.**

Name	%Interference	
	RT	Peak Area
4-Chlorophenol	3.602	1189358
Blank	3.592	639
%Interference	0.05%	

Table 3. Calibration Plot Results for 4-Chlorophenol.

S. No	Name	Correlation coefficient	Slope
1	4-Chlorophenol	0.99954	40408.2

Table 4. Results of 4-Chlorophenol at LOQ level.

Injection No	LOD conc. (µg/mL)	Peak area	LOQ conc. (µg/mL)	Peak area
1	0.048	1748	0.150	6487
2	0.048	1743	0.150	6432
3	0.048	1770	0.150	6438
4	0.048	1798	0.150	6420
5	0.048	1744	0.150	6423
6	0.048	1744	0.150	6388
Mean		1751		6431
%RSD		0.62		0.50

Table 5. Recovery Results of 4-chlorophenol

% Recovery level	Spike Amount (ppm)	Amount found (ppm)	%Recovery
Quantitation Level	0.1472	0.1613	109.6
	0.1472	0.1599	108.6
	0.1472	0.1600	108.7
50	14.7196	15.0951	102.6
	14.7196	15.0420	102.2
	14.7196	15.0682	102.4
80	23.5514	24.0252	102.0
	23.5514	24.2117	102.8
	23.5514	24.1906	102.7
100	29.4392	29.6307	100.7
	29.4392	29.6039	100.6

	29.4392	29.6154	100.6
	35.3270	35.6723	101.0
120	35.3270	35.6397	100.9
	35.3270	35.5971	100.8
	44.1588	44.4444	100.6
150	44.1588	44.4573	100.7
	44.1588	44.4417	100.6

Table 6. Repeatability Results of 4-chlorophenol

Preparation No	RT (min)	Content (ppm)
1	3.544	5.7
2	3.547	5.4
3	3.55	5.5
4	3.55	5.6
5	3.548	5.6
6	3.543	5.4
Mean	3.547	5.52
%RSD	0.08	1.71

Table7. Solution Stability Results of 4-chlorophenol content

Interval (hours)	Content (ppm)	% difference
0	5.7	-
6	5.6	1.7%
14	5.7	0.0%
30	5.7	0.0%
72	5.6	1.7%

Table 8. System suitability results of 4-chlorophenol for Robustness study

Parameter	variation	System suitability parameters			% Difference		
		RT (min)	Response	Tailing	RT (min)	Response	Tailing
Original Method	None	3.602	1189358	1.235	-	-	-
Buffer strength(KH_2PO_4) g/L	2.70 &0.48	3.573	1197067	1.233	0.81	-0.65	0.16
	3.25 &0.58	3.528	1204761	1.097	2.05	-1.30	11.17
Wavelength (+ 2nm)	232 nm	3.592	1050985	1.238	0.28	11.63	-0.24
	228 nm	3.592	1239696	1.236	0.28	-4.23	-0.08
Flow rate	0.9 ml/min	4.000	1320665	1.243	-11.08	-11.04	-0.65
	1.1 ml/min	3.277	1081791	1.237	9.02	9.04	-0.16
Temperature	23°C	3.637	1187512	1.244	-0.97	0.16	-0.73
	27°C	3.534	1199511	1.247	1.89	-0.85	-0.97
% Organic modifier (Acetonitrile; %v/v)	45	3.565	1188158	1.230	1.03	0.10	0.40
(Acetonitrile; %v/v)	55	3.561	1192137	1.235	1.14	-0.23	0.00

Table 9. Results for content of4-Chlorphenol in Chlorphenesin from Robustness study

Robust Conditions		Content (ppm)	% Difference
Original Method	None	5.7	-
Buffer strength	2.70 &0.48	5.7	0.0
(KH_2PO_4) g/L	3.25 &0.58	5.7	0.0
Wavelength	232 nm	5.6	1.7
(+ 2nm)	228 nm	5.7	0.0
Flow rate	0.9 ml/min	5.7	0.0
	1.1 ml/min	5.7	0.0
Temperature	23°C	5.6	1.7
	27°C	5.6	1.7
% Organic modifier	45	5.7	0.0
(Acetonitrile; %v/v)	55	5.7	0.0

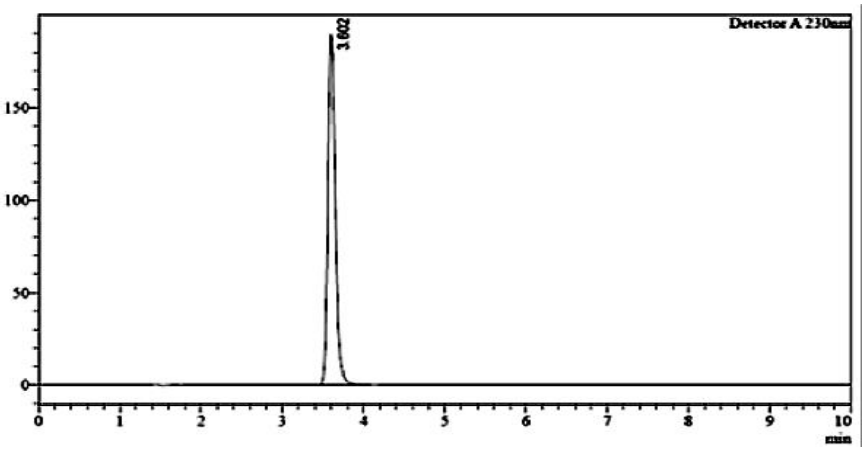


Fig.2 Typical Chromatogram of System suitability

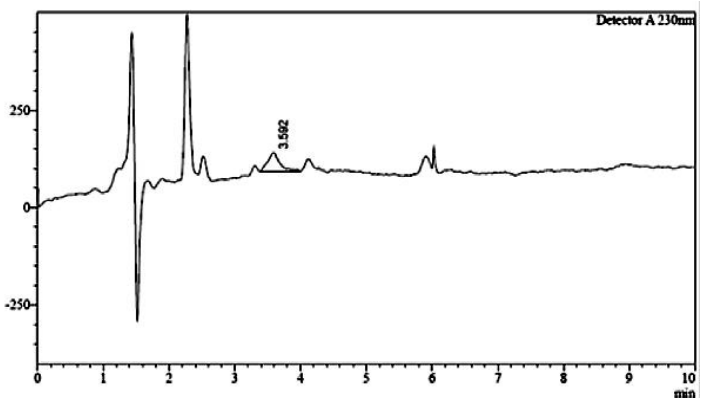


Fig.3 Typical chromatogram of Blank (Mobile phase)

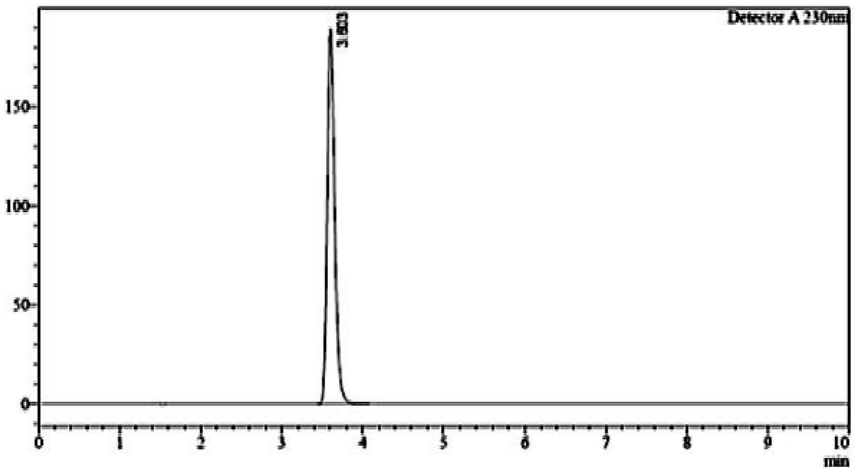
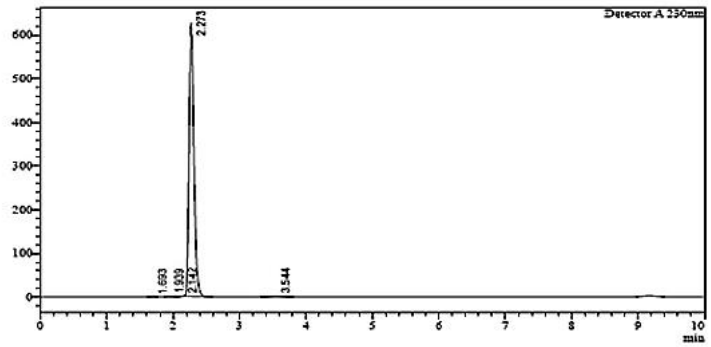


Fig.4 Typical chromatogram of 4-Chlorophenol Standard 30ppm



Peak Table

Detector A 230nm

Peak#	Ret. Time	Name	Area	Height	Area%	Resolution(USP)
1	1.693	RT:1.692	1916	429	0.058	–
2	1.939	RT:1.939	6697	1367	0.203	1.761
3	2.142	RT:2.142	2488	708	0.075	1.814
4	2.273	RT:2.273	3268524	626228	98.987	1.148
5	3.544	4-CHLOROPHENOL	22362	2159	0.677	6.095
Total			3301987	630891	100.000	

Fig. 5 Typical chromatogram of Chlorphenesin (Un-spike)

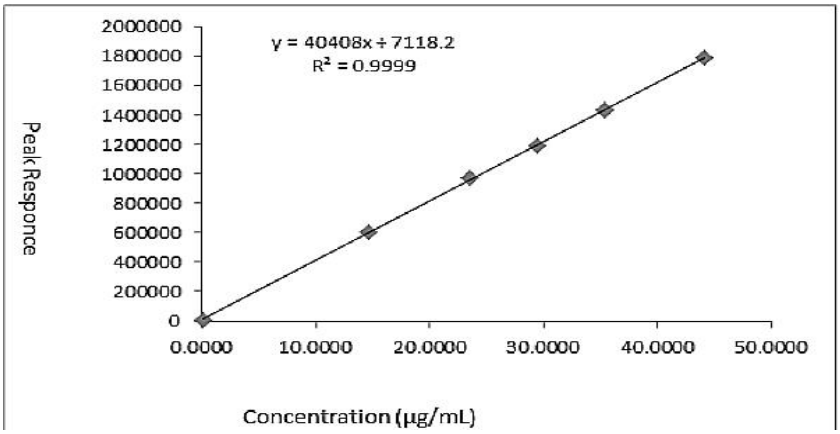
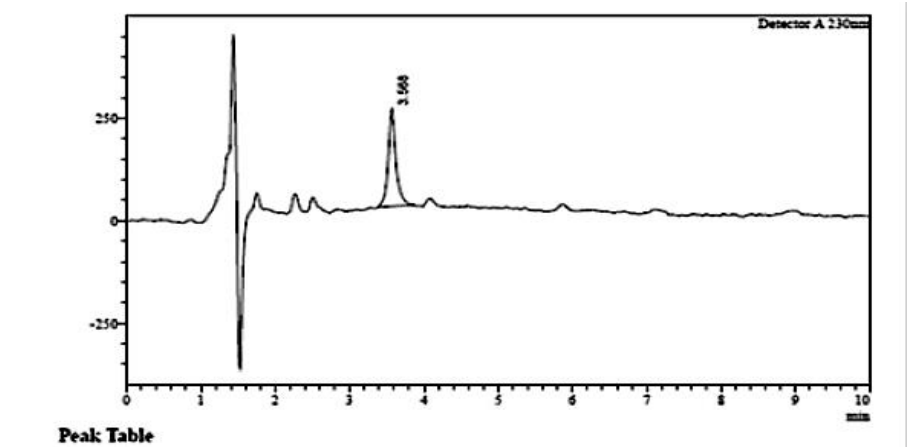


Fig.6 Typical Calibration curve for 4-Chlorophenol

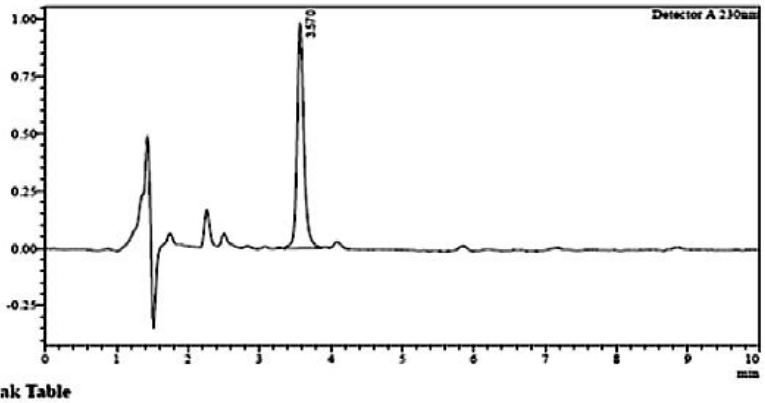


Peak Table

Detector A 230nm

Peak#	Ret. Time	Name	Area	Height	Area%	Resolution(USP)
1	3.568	4-CHLOROPHENOL	1748	238	100.000	--
Total			1748	238	100.000	

Fig.7 Typical Chromatogram from LOD solution



Peak Table

Detector A 230nm

Peak#	Ret. Time	Name	Area	Height	Area%	Resolution(USP)
1	3.570		6487	976	100.000	--
Total			6487	976	100.000	

Fig.8 Typical Chromatogram from LOQ solution

The retention time for standard and sample were identical i.e. 3.602 min for 4-chlorophenol and 2.273 min for Chlorphenesin. Since there was no interference of impurities and it was observed from the chromatogram of blank that there was interference at the retention time of the 4-Chlorophenol peak. The %interference of Blank peak was less than 1% and results are shown in Table 2 and Chromatograms are shown from Fig.3-5.

3.3. Linearity & Range: The linearity was evaluated by measuring the peak area response of all solutions over the range of the Quantitation limit (0.5% to 150% to the Specification of 4-Chlorophenol). Six concentrations were injected in replicate across the range. A linear correlation was obtained between the peak area and concentration of 4-chlorophenol for LC method as shown in Fig. 6. The linearity was validated by the value of correlation coefficients of the regression (R = 0.99954).The results are shown in Table 3.

3.4. Limit of Detection and Quantitation

The detection and quantitation limits of 4-chlorophenol were determined by the signal-to-noise ratio (S/N) method. This ratio for LOD is 3:1 and LOQ is 10:1.Solution of 4-chlorophenol was prepared around its quantitation limit (QL) concentration and injected in six replicates. The %RSD for the area of replicate injections was less than 10%.The results

are listed in Table 4 and the chromatograms are in Fig.7 and 8.

3.5. Accuracy (Recovery Studies) The Accuracy of the method was evaluated through recovery experiments and was performed by spiking known amount of 4-Chlorophenol at Quantitation limit, 50%, 80%, 100%, 120% and 150% of 30ppm in Sample Preparation. Each level was analyzed in triplicate and percent recovery was calculated. The recovery was found to be in between 100% and 110% and the results are summarized in Table 5.

3.6. Precision: Repeatability (Intraday precision) study was evaluated by spiking six injections of test solution to the specification level with respect to 100 mg of test weight. The peak responses of 4-Chlorophenol were measured and % relative standard deviation of 4-chlorophenol content was found to be less than 2. The results are summarized in the Table 6

3.7. Solution Stability: The solution stability was determined by leaving the un-spiked sample solution of 4-Chlorophenol at room temperature for 6, 14 30 and 72 hrs. The peak areas of 4-Chlorophenol were recorded, the content of 4-Chlorophenol was calculated and the results were compared with that of a freshly prepared solution. The %RSD values for solution stability experiments were calculated and found to be less than 2.0%. All samples were found to be stable for up to 72 hours. The results are shown in Table 7

3.8. Robustness: Robustness of the method were established by estimating the content of 4-Chlorophenol in Chlorphenesin under small but intentionally modified chromatographic conditions specified under the method like flow rate, buffer strength in % v/v, mobile phase composition, wavelength and column temperature on lower and higher side of the authentic values. The %RSD of retention time and response for 4-Chlorophenol from standard solution and the %RSD of 4- chlorophenol content under these customized chromatographic conditions was less than 2.0% and the difference in results between modified chromatographic conditions and the Precision was less than 2.0% of absolute value. Hence, the method was robust for determination of 4-chlorophenol content in Chlorphenesin and results are summarized in Tables 8 and 9.

4 CONCLUSION: The proposed RP-HPLC method can be successfully applied for the determination of residual *P*-Chlorophenol in Chlorphenesin bulk drug. The validated method is simple, sensitive, precise, specific, accurate, robust and linear. The good validation criteria of the propose method allow its use in quality control laboratories.

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