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UPLC Method Development and Validation for the Determination of Chlophedianol Hydrochloride in Syrup Dosage Form

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Abstract:

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of chlophedianol hydrochloride in bulk drug and syrup dosage form. The method employed with Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) in a gradient mode, with mobile phase of methanol and acetonitrile in the ratio of 65:35 %v/v. The flow rate was 0.1 ml/min and effluent was monitored at 254 nm. Retention time was found to be 1.130±0.005 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ)in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20-100 µg/ml respectively. The LOD and LOQ values were found to be 2.094(µg/ml)and 6.3466(µg/ml)respectively. No chromatographic interference from syrup excipients and degradants were found. The proposed method was successfully used for estimation of chlophedianol hydrochloride in syrup dosage form.

1. Introduction

Chlophedianol hydrochloride also referred to as clofedanol chemically is 1-(2-chlorophenyl)-3-(dimethylamino)-1-phenylpropan-1-ol; hydrochloride (Pubchem, Chlophedianol, 2016)(Figure 1). It is practically described as a centrally acting cough suppressant used in the treatment of dry cough(Centrix Pharmaceutical Announces Clofera., 2009). Chlophedianol hydrochloride has local anaesthetic and antihistamine properties and may have anticholinergic effects at high doses(NHS Choices, 2016; Schachter, S.C et al 2016, eMedicinehealth 2016).

Analytical methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological samples in pharmaceutical industry (Nguyen D.T, et al., 2006, Katharina Sterz et al., 2013and Ashok kumar et al., 2012). The validation of a specific method must be demonstrated through laboratory experiments by routinely analysing the samples. (Lyon, R. C et al., 2006)

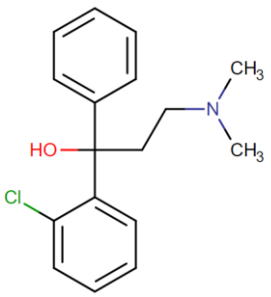

Figure.1: Molecular Structure of Chlophedianol hydrochloride, 1-(2-chlorophenyl)-3-(dimethylamino)-1-phenylpropan-1-ol;hydrochloride

2. Experimental:

2.1 Materials:

Chlophedianol hydrochloride (98.90 % purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad).

HPLC grade methanol, acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water was used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were of analytical grade.

The syrup dosage (Ulone 25mg/5mL) labelled to contain 25mg per 5mLin 100 mL of container for chlophedianol hydrochloride. All chemicals used were of pharmaceutical or special analytical grade.

2.2 Instrumentation:

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis.
The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Band line sonerex sonicator was used for pH adjustment.

2.3 Chromatographic Conditions:

Table 1: Chromatographic Conditions of the validating method

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm)
Mobile Phase	Methanol and acetonitrile in the ratio of 65:35 %v/v
Flow rate	0.1mL/min
Run time	10 Min.
Column Temperature	Maintained at 25°C
Injection volume	20 µL
Detection wavelength	254 nm
Diluent	Mobile Phase

2.4 Preparation of standard stock solution:

2.4.1 Preparation of diluent:

In order to achieve the separation under the optimized conditions after experimental trials it can be summarized,that stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution, good sensitivity and with good resolution. The flow rate was maintained 0.1 mL min-1 shows good resolution. The PDA detector response of chlophedianol hydrochloride was studied and the best wavelength was found to be 254 nm showing highest sensitivity.

The mixture of two solutions methanol and acetonitrile in the ratio of 65:35 %v/v was used, the buffer 0.5 M phosphate buffer (pH adjusted to 4.5 with triethylamine) with gradient programming was used as mobile phase at 0.1 mL/min was found to be an appropriate mobile phase for separation of chlophedianol hydrochloride. The column was maintained at 25°Ctemperature.

2.4.2 Preparation of internal standard solution:

Weigh accurately about 10 mg of acetaminophen into a clean and dry 100 mL volumetric flask, dissolve with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20µ membrane filter. Acetaminophenis taken as internal reference standard as it is most detectable compound for the proposed wavelength and mobile phase.

2.4.3 Preparation of chlophedianol hydrochloride standard solution:

Transfer accurately about 10mg of chlophedianol hydrochloride into 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20µ membrane filter. Linearity was determined in the range of 20-100 µg mL^{-1}.

3. Results and discussions

3.1 Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

3.2 Linearity and Range:

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample.
The calibration curve showed good linearity in the range of 20-100µg/mL for chlophedianol hydrochloride (API) with correlation coefficient of 0.9965. A typical calibration curve has the regression equation of y = 86973.4x + 4341977.2 for chlophedianol hydrochloride . Results are given in Table 2.

3.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ of chlophedianol hydrochloride were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of chlophedianol hydrochloride was found to be 2.094 (µg/ml) and the LOQ of was found to be 6.3466 (µg/ml). Results are given in table 2.

3.4 Precision:

The Precision of the method was studied in terms of intraday and interday precision of sample injections (25.68 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.15%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.14%. Low % RSD values indicate that the method is precise. The results are given in table 3.

3.5 Robustness:

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed UPLC method for the analysis of chlophedianol hydrochloride. The results are given in table 5.

3.6 Accuracy:

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

3.7 Analysis of formulation:

Assay studies for the analysis of spray- dosage formulation ofChlophedianol hydrochloride. Fixed chromatographic conditions were made use for the analysis of formulation and was found to be 92.59%.

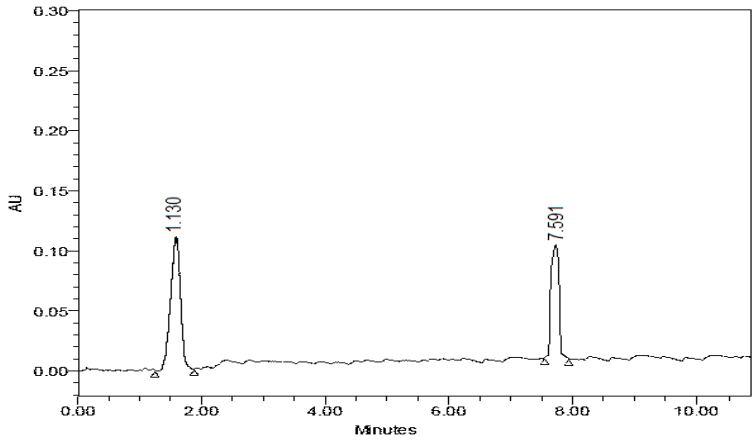

Figure 2: Optimized chromatogram of Chlophedianol hydrochloride and internal standard using mobile phase of Methanol and acetonitrile in the ratio of 65:35 %v/v

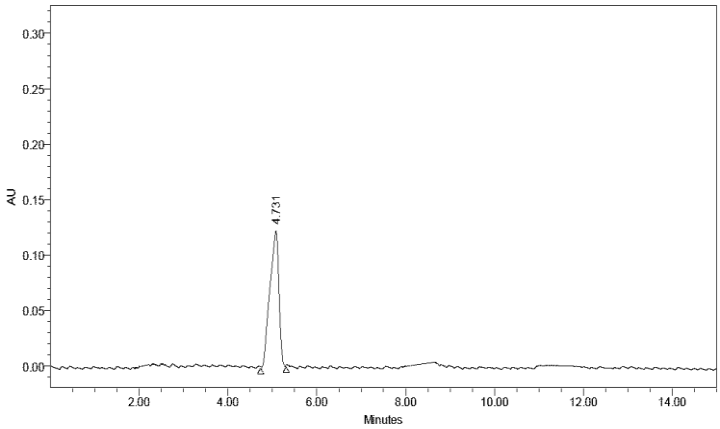

Figure. 3: Standard Chromatogram ofChlophedianol hydrochlorideusing mobile phase of Methanol and acetonitrile in the ratio of 65:35 %v/v

Table 2: Summary of validation parameters for the proposed method

PARAMETER	Chlophedianol hydrochloride
Linearity	20- 100µg/ml
Intercept (c)	4341977.2
Slope (m)	86973.4
Correlation coefficient	0.9965
LOD	2.094 (µg/ml)
LOQ	6.3466 (µg/ml)

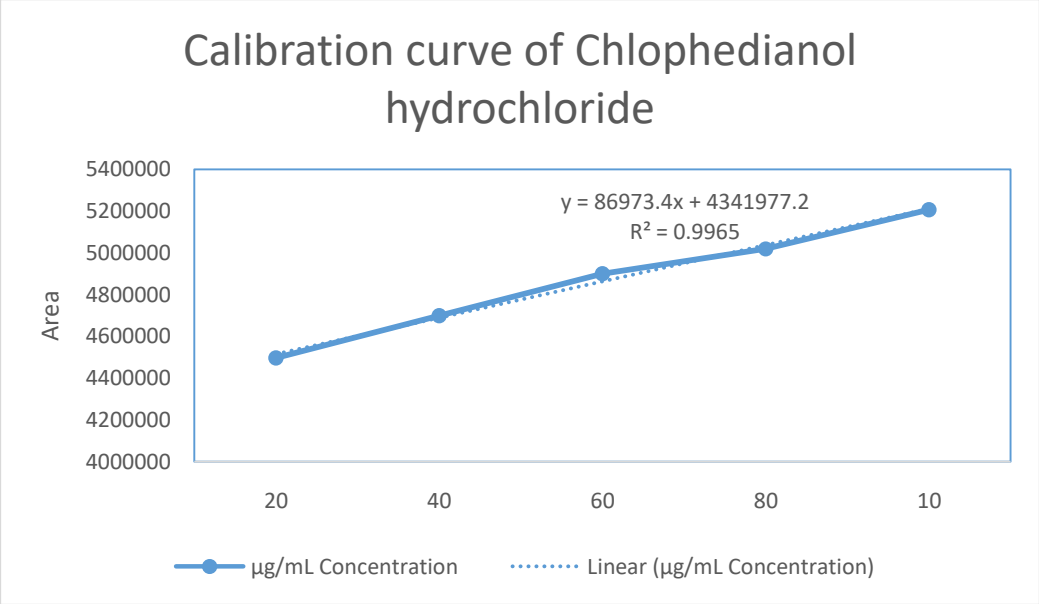

Figure 4: Calibration curve of Chlophedianol Hydrochloride

Table 3: Results of Precision Studies

Replicate	Chlophedianol hydrochloride		
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	25.68	4546389	98.90%
2		4552071	98.77%
3		4554099	98.71%
4		4556291	98.68%
5		4558244	98.64%
6		4555351	98.70%
Average			98.73%
Std.Dev			0.0920
% RSD			0.09%
Standard weight			25.68mcg
Standard potency			98.90 %

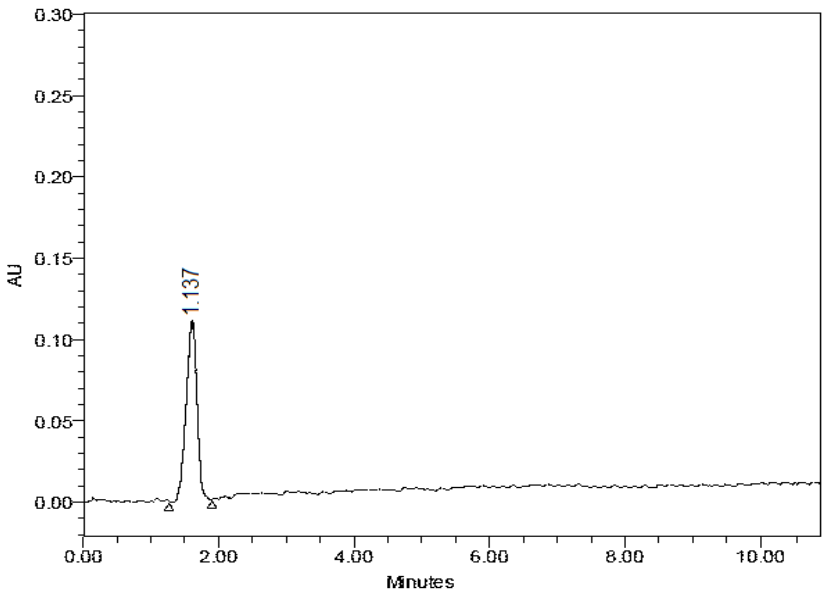

Figure. 5: Chromatogram Showing accuracy results

Table 4: Results of accuracy study

Chlophedianol hydrochloride						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	09.95	09.75	97.98	98.61	0.6977	0.71%
100	19.90	19.60	98.49			
150	29.85	29.66	99.36			

Table 5: Results of Robustness Studies

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	4557141	0.03%
	Actual	4559365	
	Plus	4560043	
Temperature	**Low**	4558912	0.01%
	Actual	4559724	
	Plus	4560254	
Wavelength	**Low**	4558864	0.01%
	Actual	4559671	
	Plus	4560113	

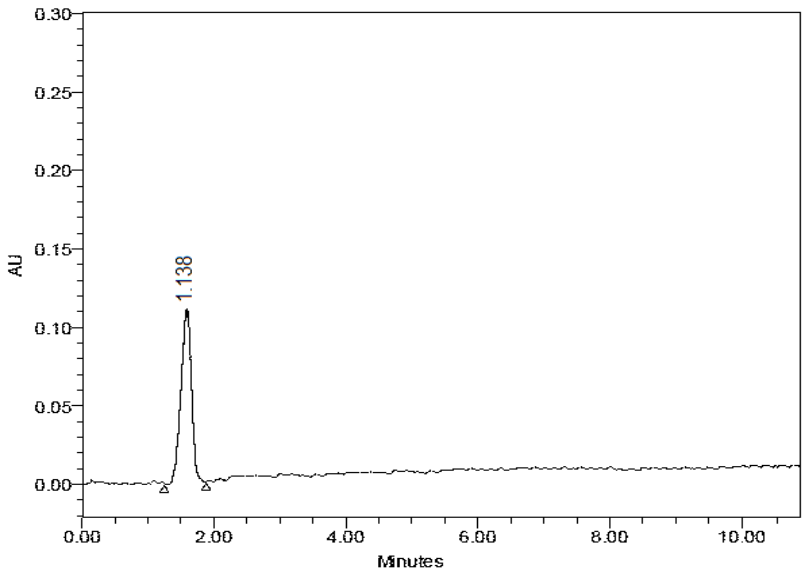

Figure 6: Chromatogram of Assay Studies

4. Conclusion

The method provides selective quantification of Chlophedianol hydrochloride withoutinterference from blank affirming precise method. The proposed method ishighly sensitive, reproducible, specific and rapid. The method was completely validatedshowing satisfactory data for all the method validation parameters.

The developed method was robust in the separation and quantification of Chlophedianol hydrochloridein syrup dose. This method can be used for the routine analysis ofproduction samples. The information presented herein could be very useful for qualitymonitoring of bulk samples and as well employed to check the quality during stabilitystudies. The current method is validated for theassay study of the formulation and was found to be beneficial.

Conflict of interest

None declared

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