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Method Development and Validation of Assay of Chlorpromazine Hydrochloride Tablet Formulation Using Ultra Violet Visible Spectrophotometry

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Abstract

The International Conference on Harmonisation [Q2 (R1)] guidelines was applied for this one-step spectrophotometric estimation of chlorpromazine hydrochloride in neurazine tablet formulation. The method was found to be simple, safe, sensitive, and validated for the assay of chlorpromazine hydrochloride using bromophenol blue, citrate buffer pH 3, and water as diluents. It was also found to be an accurate, reproducible, and cost-effective quality-control tool for the routine analysis of chlorpromazine hydrochloride in standard and pharmaceutical forms.

Keywords: Spectrophotometry; Validation; Chlorpromazine hydrochloride; International Conference on Harmonisation

Introduction

The IUPAC name of chlorpromazine hydrochloride (Cpn-HCl) is 3-(2-chlorophenothiazine-10-yl) propyl dimethyl-amine. It is an important compound in the group of phenothiazine and is used for psychoses control, including schizophrenia, mania, and severely disturbed or agitated behavior. It is also used for the relief of nausea and vomiting, pre-operative anxiety, and intractable hiccups. Chlorpromazine is very sensitive to light and easily darkens when exposed to light [1,2].

Chlorpromazine is absorbed from the gastrointestinal tract. It is metabolized extensively, and 12 different metabolites are known. Less than 1% is excreted unchanged. Most metabolites are excreted in the urine. The half-life of chlorpromazine is variable at approximately 30 hours. Liquid concentrates may have greater bioavailability than tablets [3,4].

Due to its importance, analysts have been eager to investigate new methods for chlorpromazine detection. The British Pharmacopoeia (BP) and US Pharmacopoeia (USP) listed the official method of Cpn detection [5,6]. It depends on non-aqueous potentiometric titrimetry or spectrophotometry in the UV region [7,8]. Different analytical methods and techniques have been developed for Cpn determination, including chromatographic [9-11], potentiometric [12-15], voltammetric [16,17], fluorimetric [18-20], and UV-visible spectrophotometric [21-24] techniques. Quantitative analysis, using UV-visible absorption spectroscopy, is one of the most widely used methods [25,26]. Analysts use this method due to its important spectrophotometry characteristics:

1. Wide applicability: The method is widely applicable for absorbent and nonabsorbent species. Inorganic, organic, and biochemical species that can absorb UV-visible radiation are subjected directly to quantitative analysis. On the other hand, nonabsorbent species can be converted to absorbent species by reacting with a chromophoric reagent to yield a product that absorbs UV-visible radiation.

2. High sensitivity: The method shows a high sensitivity of a very high detection limit range from 1×10^{-4} M to 1×10^{-5} M, which can be extended to higher limits with specific modifications.

3. Moderate to high selectivity: The maximum wavelength can be found (that which only the analyte absorbs) by rearranging the UV-visible range from 200-700 nm. This makes preliminary extractions unnecessary.

4. Good accuracy: Accuracy can be measured by the relative error. If it ranges from 1-5%, it is considered low. It can be decreased with specific modifications and precautions.

5. Ease and convenience: Spectrophotometric methods are easy and rapidly performed with high accuracy and precision.

This paper describes the development and the application of UV-visible spectrophotometric analysis of Cpn in standard form and pharmaceutical preparations. Compared to the spectrophotometric methods used, the proposed method has many advantages, including the use of aqueous medium, fewer steps in the preparation procedure, high sensitivity and accuracy, and finally, simple instrumentation. The method is subjected to validation and verification to match international standards. The objective of validating this method is to demonstrate the suitability of the determination of Cpn in standard and pharmaceutical formulations. The method also has to be successfully verified to work in different laboratories. The elements of validation and verification were performed by the application of specificity, linearity, precision, accuracy, solution stability study, robustness, and system suitability [27-29].

Experiment

Materials

All chemicals used were of analytical grade. Cpn-HCl was purchased from Sigma (Fw: 355.3), neurazine tablets (100 mg/tablet)

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were purchased from Misr Co. for Pharmaceuticals, Egypt, and bromophenol blue indicator (BrPB) was purchased from Sigma (Fw: 691.94).

Diluent preparation

Distilled water was used as a diluent.

Standard preparation

A bromophenol blue (1×10^{-3} M) and Cpn-HCl (1×10^{-3} M) stock solution was prepared to be used for the preparation of standard solutions. In 100 mL volumetric flasks, 15 mL BrPB, 3 mL citrate buffer (pH 3), and different aliquots of Cpn-HCl (1×10^{-3} M) were added to prepare five standard solutions of Cpn-HCl ranging in concentration from 2.553-16 ppm. The five standard solutions were scanned for the maximum wavelength (nm) previously assigned, and a calibration curve was set to perform the linearity test.

Pharmaceutical test preparation

Ten tablets of neurazine formulation were ground and weighed (4.4870 g). Each tablet contained 100 mg active ingredient of Cpn-HCl. To prepare the stock solution (1×10^{-3}) of the formulation, 0.1594 g of the ground tablets were weighed and dissolved with distilled water in a 100 mL volumetric flask. The stock solution was diluted to prepare (1×10^{-4} M). A test solution was made by adding 15 mL bromophenol blue, 3 mL citrate buffer (pH 3), and 10 mL neurazine (1×10^{-4} M) (ppm). The solution was tested by the extrapolation method to determine the concentration of the neurazine test sample.

Instrumentation

A UV-visible double beam spectrophotometer with matched quartz cell (1 cm) (model no. Evolution 201) by Thermo Scientific, 81 Wyman Street Waltham, Massachusetts, US was used.

Results and Discussion

Development and optimization of the spectrophotometric method

The selection of the proper wavelength in this method depends on the sample, diluent, and test solution. Spectrophotometric quantitative determination of chlorpromazine needs the development of rugged and suitable conditions after testing different parameters, such as diluents, buffer, buffer concentration, and other chromatographic conditions. Different trials with varied compositions of diluents and buffer should be conducted to choose the optimum conditions.

Selection of wavelength

The standard solution and the pharmaceutical test forms were scanned by UV-visible spectrophotometer between 200 and 700 nm on spectrum mode before and after using BrPB (Figures 1 and 2). Both spectra showed that Cpn-HCl has a maximum wavelength λ_{max} at 314. Bromophenol blue was scanned to avoid the interferences of wavelengths (Figure 3). The spectrum pf BrPB showed a λ_{max} at 590 nm. However, the addition of BrPB as chromophore agent generated a new λ_{max} 413 nm and the reduction of λ_{max} at 314 nm as seen in Figure 4. In this case, the selected λ_{max} for both the standard and formulation forms was 413 nm.

Method validation

Specificity: Both the standard solution and the test solution were scanned separately. Their maximum wavelengths were recorded, and each peak was checked for its resolution from the nearest peak (Figure 4).

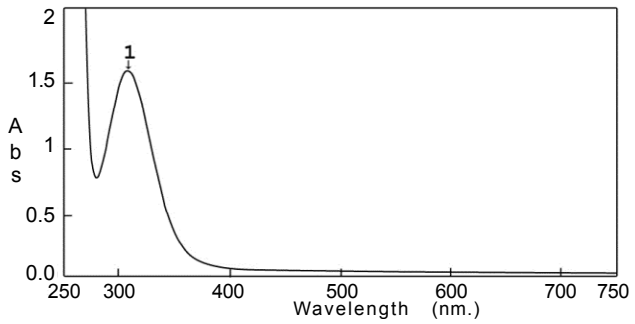

Figure 1: UV-visible spectrum of chlorpromazine in standard solution.

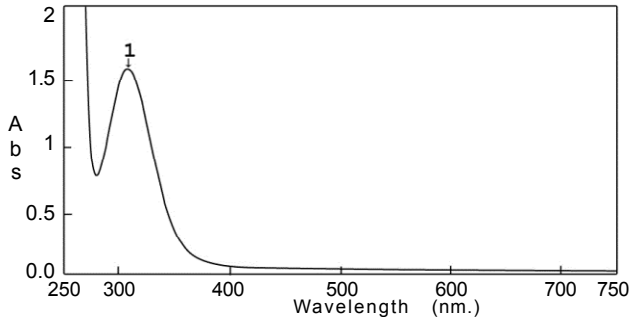

Figure 2: UV-visible spectrum of chlorpromazine in pharmaceutical solution.

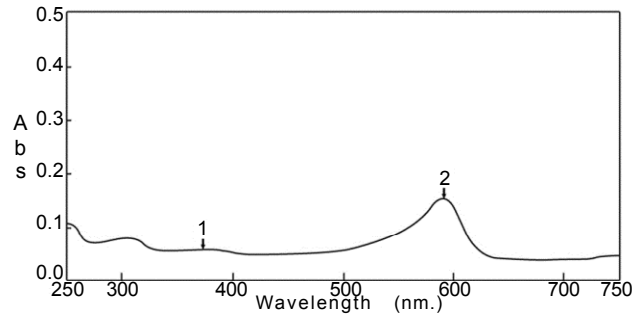

Figure 3: UV-visible spectrum of bromophenol blue and diluent solution.

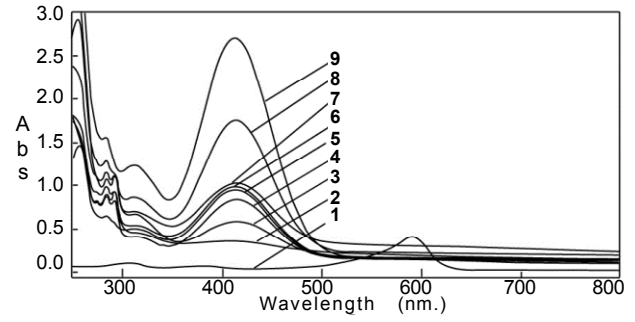

Figure 4: UV-visible overlay spectra of chlorpromazine standard solution compared with bromophenol blue spectrum(1: bromophenol, 2: 2.5 ppm, 3: 3.55 ppm, 4: 5.33 ppm, 5: 7.10 ppm, 6: 8.825 ppm, 7: 10.659 ppm, 8: 24.87 ppm, 9: 39.08 ppm).

Linearity: Known concentrations of the standard solutions ranging from 0 to 10.659 ppm were scanned to set a calibration curve of six points. The response of the drug was linear, and the linear regression equation was y=0.0666x+0.0123 with correlation co-efficient R^2=0.988 (Table 1, Figure 5).

Precision: The analysis of six samples of the standard solution was carried out as per procedure and as per normal weight to ascertain the precision. The method was found to be precise, as the % RSD values for the repeatability and intermediate precision studies were <0.360 and <0.488, respectively (Table 2).

Accuracy: Standard quantity equivalent to 5%, 100% and 125% is to be added in sample by calibration method were scanned for their absorbance. The results showed that the best recoveries (93 to 96.20%) of the standard drug, indicating that the method was accurate (Table 3). Comparisons of standard and pharmaceutical solutions for the determination of accuracy are shown in Tables 3 and 4.

Conc (ppm)	Absorbance
0	0.000
2.5	0.180
3.55	0.223
5.33	0.417
7.106	0.489
8.825	0.615
10.659	0.691

Table 1: Conc. vs. Abs. table for linearity study.

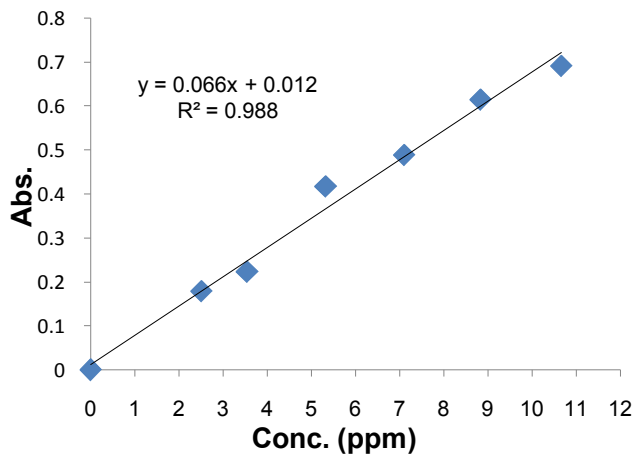

Figure 5: Linearity curve for chlorpromazine standard test.

Sample No	% Assay	
Set	Intraday	Interday
1	97.8	95.3
2	97.2	95.2
3	97.3	95.0
4	97.5	95.2
5	97.6	94.9
6	96.8	96.2
Mean	97.4	95.3
SD	0.350	0.465
%RSD	0.360	0.488

Table 2: Evaluation of data of precision study.

% Recovery Level	% Recovery	Mean % Recovery	SD	% RSD
50%	94.1	94.1	0	0
	95.1		0.005	0.005
	93.0		0.005	0.005
100%	95.3	95.3	0.008	0.008
	95.2		0.009	0.009
	95.3		0.015	0.015
125%	96.2	96.2	0.008	0.008
	96.2		0.008	0.008
	96.2		0	0

Table 3: Evaluation data of accuracy study.

Time (Hrs.)	Abs. Standard	Abs. Pharmaceutical
0	0.367	0.352
2	0.365	0.349
4	0.365	0.348
6	0.363	0.347
8	0.361	0.345
Limit at (2hr)*	0.545	0.800
Limit at (4hr)*	0.545	1.130
Limit at (6hr)*	1.080	1.140
Limit at (8hr)*	1.660	1.920

*Limit can be calculated by the formula:

$$\text{Limit} = \frac{Abs.of\ standard\ intial - Abs.of\ standard\ at\ diffrent\ time}{Abs.of\ standard\ intial} \times 100$$

Table 4: Evaluation data of solution stability study.

Sl no.	Abs. at 412 nm	Abs. at 413 nm	Abs. at 414 nm
1	0.365	0.366	0.364
2	0.364	0.365	0.364
3	0.365	0.366	0.365
4	0.364	0.365	0.364
5	0.365	0.366	0.364
6	0.366	0.367	0.365
Mean	0.365	0.366	0.364
SD	0.000753	0.000753	0.000516
% RSD	0.200	0.200	0.140

Table 5: Evaluation data of robustness study.

Solution stability study: The standard and test solutions were stable up to 8 hr. at room temperature, and the results did not decrease below the minimum percentage (Table 5).

Robustness: The robustness should be considered during the development phase, and it depends on the type of procedure studied. It should exhibit reliability of analysis with respect to conscious procedure variations in method parameters. Variations in analytical conditions should be stated in the procedure. Table 6 shows the robustness study during the dissent conditions. The assay value of the test solution was not affected, and it matched the actual value. System suitability was satisfactory, and, as a result, it was concluded that the analytical method was robust.

System suitability: Before each validation run, a system suitability test of the method was performed. Six replicate readings of the standard preparations were measured and % RSD was calculated. Acceptance

Sl no.	Absorbance
1	0.367
2	0.365
3	0.365
4	0.366
5	0.366
6	0.366
Average	0.366
SD	0.000753
% RSD	0.200

Table 6: Evaluation data of system suitability study.

criteria for system suitability, % RSD of standard reading not more than 2.0%, were fulfilled during all validation parameters (Table 6).

Conclusion

The presented method meets the specific acceptance criteria of analytical method validation as per International Conference on Harmonisation (ICH) Q2 (R1) guidelines. Elements of validation—specificity, precision, linearity, accuracy, robustness, and system stability—were approved. Thus, it was concluded that the method is valid for determining chlorpromazine in pure and pharmaceutical preparations.

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