

Evaluation of Antihistamine in Pure and in Dosage forms by Least Square Regression Method

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(Received on 3rd July 2012, accepted in revised form 8th May 2013)

Summary: A charge transfer complex is formed when antihistaminic drug react with *m*-dinitrobenzene in basic medium. This complex is used for the determination of antihistamine (cyclizine hydrochloride) in pure and pharmaceutical formulations. The purple color complexes formed are quantified spectrophotometrically at 540nm. Different statistic parameters like standard deviation, relative standard deviation, slope; intercept and coefficient of determination are calculated. The proposed method can be used successfully for routine analysis of cyclizine hydrochloride in pure and in dosage forms.

Keywords: Cyclizine hydrochloride, *m*-dinitrobenzene, spectrophotometry.

Introduction

Cyclizine hydrochloride is a piperazine derivative (Fig. 1), is described as sedating antihistamine with antimuscarinic activity. It is used as an antiemetic in the management of nausea and vomiting including motion sickness, postoperative nausea and vomiting, after radiotherapy and in drug-induced nausea and vomiting. Cyclizine is also used for the symptomatic treatment of vertigo caused by Menieres disease and other vestibular disturbance [1].

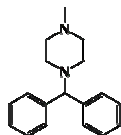


Fig. 1: Structure of cyclizine.

Considering the importance of this drug various efforts had been put in to define methods for its evaluation which range from simplest to complex e.g. , high performance liquid chromatography [2-4], gas chromatography [5-7], gas chromatography-mass spectrometry [8], nitrogen-phosphorous gas chromatography [9], thin layer chromatography [10-12], over pressed layer chromatography [13], capillary electrophoresis [14, 15], voltammetry [16], spectrophotometry [17] and colorimetry [18-21].

Chromatographic techniques (GC-MS) require stringent control of conditions and skillful operator. In addition to that these techniques are known to have high RSD value (upto 3.5% and peak area has poor precision) [5] and chromatogram takes

long time (20 minutes) to develop [3]. Relatively better RSD values are observed when spectrophotometric methods are used. The spectrophotometric methods either carried out in UV region or require extraction in organic solvents before absorbance measurements. [20, 21]. Furthermore color is stable for very short time e.g. one hour while in other method interference of vitamin B1 with dye is observed [19]. The literature revealed that most of the methods have poor sensitivity, short color stability, interference, extraction requirement, and need expensive or objectionable chemicals.

The idea behind the study was to develop a new spectrophotometric method for the determination of cyclizine hydrochloride in pure and its dosage forms. It was found that drug react with *m*-dinitrobenzene in basic media to give purple coloured complex having absorbance at 540nm. The reaction obeys Beer's law in the concentration range 50 µg to 200 µg /ml. The present method is simple, accurate, precise and sensitive and can be used for determination of pure cyclizine hydrochloride in pure and in pharmaceutical formulations.

Results and Discussion

Absorption Spectrum of Coloured Complex

Cyclizine hydrochloride reacts with *m*-dinitrobenzene at 100°C in basic media to give purple complex, the absorption spectra of which under optimum conditions lies at 540 nm (Fig. 2).

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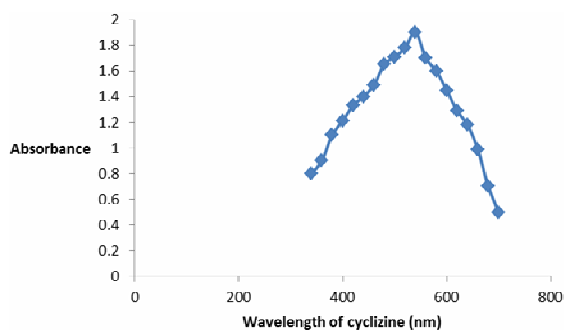


Fig. 2: Absorption spectra of cyclizine hydrochloride.

Effect of Temperature and Heating Time

Studies for the effect of temperature show that at 100 °C the colour intensity was maximum (Fig. 3). The colour remained stable more than 24 h. A water bath was used to carry out the temperature studies. The effect of heating time on colour intensity (Fig. 4) shows that heating for 80 seconds at 100 °C gave the maximum colour, above and below this time and temperature the colour intensity decreased. The contents of the test tube were cooled to room temperature prior to dilution and measurement of absorbance.

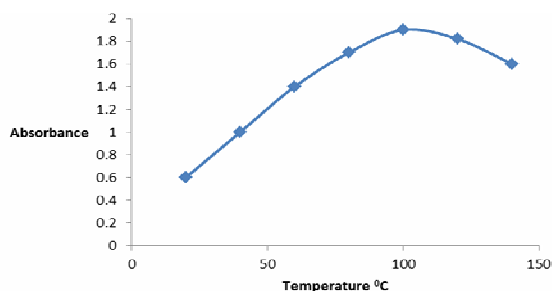


Fig. 3: Effect of temperature.

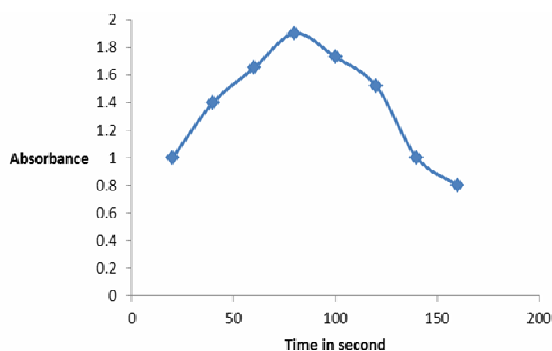


Fig. 4: Effect of heating time.

Effect of Colour Producing Reagent and pH

m-dinitrobenzene was used as a colour producing reagent. It was found that 40 mg/mL of *m*-

dinitrobenzene gave maximum absorbance (Fig. 5) at pH 13.6 (Fig. 6). The probable mechanism of the colour reaction is that on addition of sodium hydroxide, piperazine moiety is generated thus furnishing a pair of electrons for interaction with electron deficient *m*-dinitrobenzene. A charge transfer is formed having absorption maxima at 540 nm.

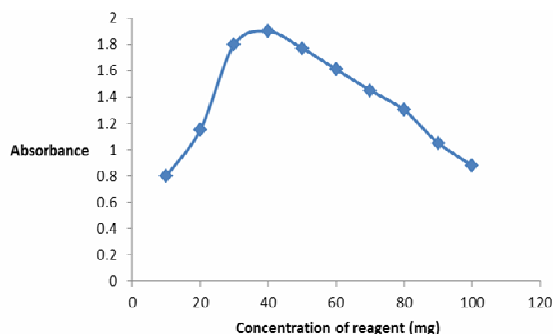


Fig. 5: Effect of reagent.

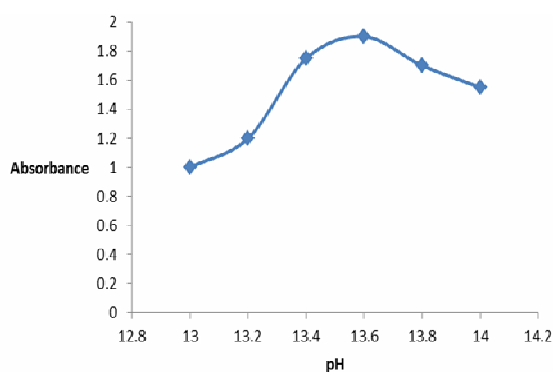


Fig. 6: Effect of pH.

Charge transfer complexes are formed by interaction between the basic -N- of antihistamine as electron donor and electron acceptor in this case dinitrobenzene [22]

Effect of Organic Solvents

Different organic solvents (such as chloroform, *n*-hexane, xylene, acetone, benzene, dichloromethane) were tested for colour extraction and for stability but none of the solvents used were effective. Hence the present method is simple and gives good results without the extraction for measurement.

Analytical Figures of Merit

The results for the determination of cyclizine hydrochloride are shown in Table-1 and 2,

which show the sensitivity, validity and repeatability of the method. It is also reasonably precise and accurate, as the amount taken from identical samples is known and the amount found by the above procedure does not exceed the relative standard deviation 0.35 % which is the replicate of five determinations. The optimization has been done at lower analyte concentration. The calibration graph is linear in the range 50 ug to 200 $\mu\text{g mL}^{-1}$. The apparent molar absorptivity calculated was 0.2878×10^5 and the regression ($Y=mx+b$) was calculated by the method of least squares each of which was the average of five determinations [23]. The regression coefficient of determination (r^2) comes out to be 0.995.

Table-1: Determination of cyclizine hydrochloride from pure solution.

cyclizine hydrochloride taken ($\mu\text{g/ml}$)	cyclizine hydrochloride found* ($\mu\text{g/ml}$)	Relative standard Deviation (%)
50	45.5	0.35
60	59.9	0.26
70	69.6	0.22
80	80.1	0.19
90	90.3	0.17
100	99.8	0.15
140	139.50	0.11
200	205.50	0.076

*Every reading is an average of five independent measurements.

Table-2: Optical characteristics, precision and accuracy of the proposed methods.

Parameters	Values
λ_{max} (nm)	540
Molar absorptivity ($\text{mol}^{-1} \text{cm}^{-1}$)	0.2878×10^5
Regression equation (Y^*)	
Slope (b)	0.0095
Intercept (a)	-0.003
Regression coefficient of determination (r^2)	0.995
Relative standard deviation (RSD%)**	0.35
% range of error (confidence limit) at 95% confidence	9.92 ± 0.0248 %

* $Y = a + bc$ where c is the concentration of analyte ($\mu\text{g/mL}^{-1}$) and Y is the absorbance unit.

Interferences

The quantitative assessment of tolerable amounts of different organic compounds under the experimental conditions is given in Table-3. Various amounts of diverse interfering compounds were added to a fixed amount of cyclizine hydrochloride (1 mg/mL) and the recommended procedure for the spectrophotometric determination was followed.

Applications

The proposed method is successfully applied for the quality control of pure cyclizine hydrochloride and in the pharmaceutical dosage form as shown in Table-4.

Table-4: Determination of cyclizine hydrochloride in pharmaceutical preparations.

Drug	Pharmaceutical Preparation	Amount present (Manufacturer's specifications) (mg)	Amount found* (mg)	Percentage Recovery (%)
Sample 1	Tablet	10	9.99	99.5
Sample 2	Syrup	2.5	2.48	99.2

*Every reading is an average of five determinations.

Table-3: Quantitative assessment of tolerable amounts of other drugs.

Drugs	Maximum amount not Interfering* (%)
Aspirin	250
Paracetamol	150
Flubiprofen	100
Phentoin sodium	200
Indomethacin	100
Ibuprofen	270
Chlorpheniramine maleat	100
Metamizole	400
Diclofenac sodium	150
Fluoxetine-HCl	100
Atenolol	300
Caffeine hydrate	350
Ergotamine tartrate	400

*The value is the percentage of the drug with respect to 100 $\mu\text{g/mL}$ of cyclizine hydrochloride that causes ± 0.01 change in absorbance.

Experimental

Apparatus and Reagent

Cecil CE-2041 spectrophotometer with 1 cm quartz cell was used to measure the absorbance and graduated pipettes were employed. Analytical grade chemicals and doubly distilled water were used. Standard solution (w/v) (1.0 mg/mL) of cyclizine hydrochloride (GlaxoSmithKline, 35-Dockyard Road, WestWharf, Karachi, Pakistan) was prepared by dissolving cyclizine hydrochloride (100 mg) in distilled ethanol and the volume was made up to 100 mL with ethanol to give a stock solution, which was diluted further as required. A 2 % (w/v) *m*-dinitrobenzene (BDH) was prepared in ethyl alcohol (BDH) and 1 N sodium hydroxide (E. Merck) was prepared in distilled water.

General Procedure

To an aliquot of cyclizine hydrochloride containing 50 μg to 200 $\mu\text{g/mL}$ was added 1.5 mL of 2 M sodium hydroxide, 2.0 mL of 2 % (w/v) *m*-dinitrobenzene and the contents were heated for 80 seconds in a water bath at 100°C, cool and the volume was made upto 10 mL with ethyl alcohol. The resulting absorbance of the purple colour was measured at 540 nm, employing all reagents except cyclizine hydrochloride as a blank. The experiment was repeated with different volumes of standard cyclizine hydrochloride solution and a calibration curve was prepared (Fig. 7). The color reaction obeys Beer's law from 50 ug to 200 $\mu\text{g mL}^{-1}$ of cyclizine hydrochloride.

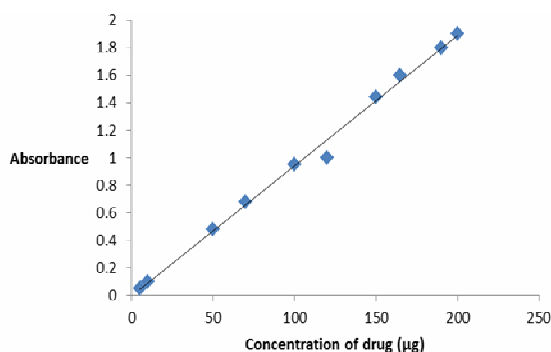


Fig. 7: Standard calibration curve of cyclizine hydrochloride.

Procedure for Studying the Interfering Compounds

To an aliquot containing 1.0 mg/mL of cyclizine hydrochloride different amounts of various compounds (1 mg/mL) were added individually until the solution showed the same (± 0.01) absorbance as that of pure cyclizine hydrochloride solution without the addition of the organic compound, under experimental conditions, as described in the general procedure. The value was calculated as the percentage of organic compound with respect to the amount of cyclizine hydrochloride.

Procedure for Determination of Cyclizine Hydrochloride in Pharmaceutical Preparations

Tablets

Tablets containing 10mg cyclizine hydrochloride were powdered, weighed, dissolved in water and filtered. The filtrate was diluted with distilled water to get 1 mg/mL solution of cyclizine hydrochloride. An aliquot containing 50 µg to 200 µg mL⁻¹ was taken and the procedure was followed as described above and the absorbance was measured at 540 nm. The quantity per tablet was calculated from the standard calibration curve.

Syrup

The content of syrup containing 2.5ml per mg of cyclizine hydrochloride was dissolved in distilled water and filtered. After filtration 1mg/mL solution of cyclizine hydrochloride was prepared. An aliquot containing 50 to 200 µg/10mL was taken and drug concentration was measured as described under general procedure. The quantity of cyclizine hydrochloride per mL of syrup was calculated from standard calibration curve.

Conclusions

The new spectrophotometric method for the determination of cyclizine hydrochloride is simple, reliable and sensitive. The colour reaction does not require stringent conditions nor many reagents or solvents. The method can be successfully applied to the microdetermination of cyclizine hydrochloride either in pure or pharmaceutical preparations. The advantage of proposed method is that no extraction is required prior to the determination of cyclizine hydrochloride and color is stable for more than 24 hour as compared to reported method [19]. Hence for routine pharmaceutical analysis, the spectrophotometric methods are more rapid and economical when compared to chromatographic procedure. A significant advantage of a spectrophotometric determination is its application to the determination of individual compounds. This aspect of spectrophotometric analysis is of major interest in the analytical pharmacy, since it offers a distinct possibility of quality control in the assay of pharmaceutical dosage formulation.

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