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Quantitative determination of diphenhydramine in syrup formulation by titrimetry and extractive ion-pair spectrophotometry

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ABSTRACT

A simple and rapid spectrophotometric method is proposed for the determination of diphenhydramine hydrochloride in a pharmaceutical formulation. The method is based on ion-pair complexation with methyl orange (MO) at pH 3 .0 (phthalate buffer). The yellow ion-pair complex was extracted with chloroform and estimated at 420 nm. Validation parameters for the method were established following standard guidelines. The calibration curve was found to be linear over the range of 20 to 100 µg/ml with a correlation coefficient of 0.9822. The method gave good recovery with relative standard deviation (% RSD) of between 0.38 and 1.11 while precision was between 0.457 and 0.774 for inter-day and 0.556 and 0.908 for intra-day. The limit of detection (LOD) and limit of quantitation (LOQ) were 3.1 and 10.3 µg/ml respectively. The proposed method was found to produce results comparable to the official titrimetric method and so it was employed in the determination of diphenhydramine hydrochloride in a formulation with excellent results.

Keywords: Diphenhydramine, Methyl orange, Ion-pair complexation, Titrimetry

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INTRODUCTION

Diphenhydramine is an ethanolamine derivative and a histamine H1-receptor antagonist. It is primarily used as an antitussive agent in cough preparations. It also possesses sedative, antiemetic and anticholinergic properties. [1] Chemically, it is known as 2-diphenylmethoxy-N, N-dimethylethanamine.

The British Pharmacopoeia [2] and the European Pharmacopoeia [3] both describe titrimetric methods for diphenhydramine while the USP [4] describes a liquid chromatographic method for the drug. Several unofficial analytical methods have also been reported for determining diphenhydramine hydrochloride in pharmaceutical preparations, [5] fluorometry, [6] electrochemical analysis [7, 8, 9] and spectrophotometry. [10, 11, 12, 13, 14] Other techniques previously used for determination of the drug include capillary electrophoresis, [15, 16, 17] atomic absorption. [18, 19] Chromatographic methods such as HPTLC, [20, 21] TLC-densitometry, [22] Gas Chromatography, [23, 24, 25] HPLC [26, 27, 28, 29, 30] and FT-Raman spectroscopy [31] have also been used. Some of the reported methods suffer from limitations such as time-consuming procedure, interference from substances and/or relatively high cost of instrumentation.

Ion - pair spectrophotometry, due to its simplicity, relative selectivity and sensitivity and ease of application has become very popular for quantitative determination of many drugs possessing suitable functional groups that can be exploited in the analysis. Methyl orange which is a readily available indicator in most laboratories has been selected as the ion- pairing reagent in the present effort which is aimed at developing a simple analytical procedure for diphenhydramine. This technique can be used during field work and in resource-poor laboratories. This proposed method was validated according to ICH guidelines in order to ascertain its suitability for assaying diphenhydramine in syrup formulation.

MATERIALS AND METHODS

Equipment

A Shimadzu UV-Visible spectrophotometer (Model 1250, Japan) with matched 1 cm quartz cells was used for all spectral measurements. The pH measurements were carried out using a calibrated digital pH meter (GallenKamp, England).

Chemicals and reagents

All chemicals used were of analytical grade and procured as follows: Chloroform, Ethyl acetate, Diethyl ether, Dichloromethane, Hydrochloric acid, Potassium hydrogen phthalate (Sigma Aldrich, Germany). Double distilled water was used to prepare all solutions. Freshly prepared solutions were used for method development and validation. Methyl orange (BDH Chemicals Limited, Poole, England). Standard diphenhydramine hydrochloride was obtained from Prof. Ikoni Ogaji of Department of Pharmaceutics and Pharmaceutical Technology, University of Jos, Nigeria. Several brands of the expectorant containing diphenhydramine hydrochloride were purchased from a retail pharmacy store in Jos, Nigeria.

Preparation of Solutions

Standard Drug Solution

Standard stock solution (100 µg/ml) of diphenhydramine hydrochloride was prepared by dissolving appropriate weight (0.1 g) of pure drug in 10 ml of double distilled water and the volume made up to the mark in a 100 ml volumetric flask. Working standard solutions were prepared by suitable dilution of this stock with water.

Phthalate buffer solution

Potassium hydrogen phthalate buffer solution (pH 3.0) was prepared by standard method. [32]

Methyl orange dye (0.15% w/v)

This was prepared by dissolving the appropriate weight of methyl orange (MO) in 10 ml methanol and diluted to 100 ml with distilled water.

Titrimetry

The official assay method (aqueous acid-base titration) as described in the British Pharmacopoeia [2] was employed to determine the drug content in the five commercial brands.

Extractive ion-pair spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) and Construction of calibration curve

Aliquots of diphenhydramine hydrochloride standard solution (100 µg/ml) were transferred into a series of 100 ml separating funnels. To each separating funnel 2.0 ml of methyl orange solution (0.05% w/v) and 1.0 ml potassium hydrogen phthalate–HCl buffer of pH 3.0, were added and the volume of the aqueous layer adjusted to 10 ml with distilled water. The mixture was extracted twice with 5.0 ml chloroform by shaking for 2.0 minutes and allowed to stand for separation of the two phases. The chloroform layers extracted were combined. Absorption spectrum of the yellow diphenhydramine-methyl orange ion- pair complex was obtained by scanning the chromogen from 350 to 800 nm. Subsequent absorbance measurements were carried out at 420 nm against the reagent blank. All measurements were made at room temperature (25 ± 2°C). The procedures were repeated for other analyte aliquots and calibration plot was generated using absorbance versus concentration.

Determination of Diphenhydramine in Syrup dosage form

A quantity of the syrup equivalent to 7 mg of the API was measured and transferred into 100 ml volumetric flask and the volume made up to mark with distilled water and further dilution carried out to obtain test solution of 100 µg/ml diphenhydramine. The general procedure described above was subsequently used for the determination of diphenhydramine concentration. Five different brands of diphenhydramine syrup were analyzed using the proposed method.

METHOD VALIDATION

Accuracy

The accuracy of the proposed method was evaluated through recovery studies whereby a known quantity of the pure drug was used to spike pre-analyzed samples before being subjected to the analytical procedure previously described.

Precision

Precision of the proposed method was determined by evaluating the inter-day and intra-day variation which were obtained by replicate analysis (n = 5) of calibration standards at three different concentration levels, five times per day on five consecutive days.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) of the method was established using the formula: LOD = 3 *s*/*k* and LOQ = 10 *s*/*k*, where *s* is the standard deviation of replicate determination values under the same conditions as for the analysis in the absence of the analyte, and *k* is the slope of the calibration graph.

OPTIMIZATION OF REACTION CONDITIONS

The optimum reaction conditions for complex formation and stability were also investigated. Experiments were therefore conducted to study the effects of pH, reaction time, reagent concentration, extraction solvent and reagent volume. The stability of the ion- pair complex was also evaluated over 24 hours.

RESULTS AND DISCUSSION

Absorption spectra

Diphenhydramine is a basic drug containing an amino group in its structure and is present in positively charged form at acidic pH. When it is treated with methyl orange which is an acidic dye (present mainly in anionic form) at pH 3.0 3.0 a yellow ion-pair complex was formed which was extracted with chloroform. The absorption spectrum of the ion- pair complex so formed was measured at 420 nm against a blank solution. All other spectral measurements were therefore carried out at this wavelength.

Optimum reaction conditions for complex formation

Selection of the extracting solvent

The effect of several extracting organic solvents on the ion-pair complex was examined. Chloroform, ethyl acetate, diethyl ether and dichloromethane were tried to compare the extractability of the coloured ion pair complex from the aqueous phase. Chloroform was found to be the most suitable solvent because of its higher selectivity for the ion-pair complex and the shortest time to reach the equilibrium between both chloroform and aqueous phases.

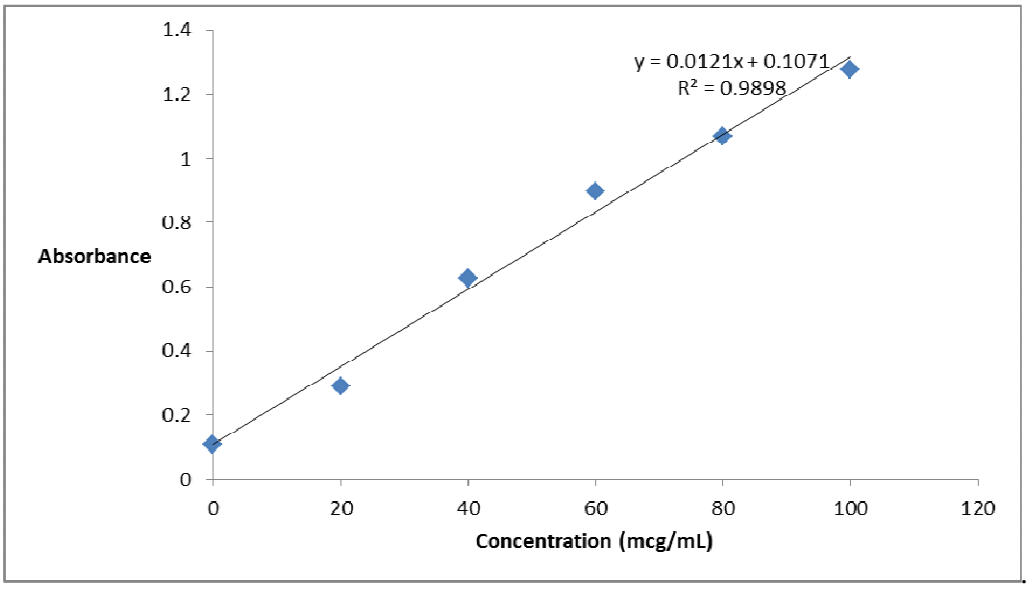

Figure 1: Calibration curve for diphenhydramine-methyl orange ion pair complex

Effects of pH on the ion-pair formation

The effect of pH was studied by extracting the coloured complex in the presence of potassium hydrogen phthalate–HCl buffer at various pH values ranging from pH 1.0 to 5.0. It was noticed that the maximum colour intensity and highest absorbance value were observed in potassium hydrogen phthalate–HCl buffer of pH 3.0 (Figure 2). Furthermore, 1.0 ml of phthalate buffer gave maximum absorbance and reproducible results.

Effects of reagents concentration

The effect of the reagent concentration was studied by measuring the absorbance of solutions containing a fixed concentration of diphenhydramine and varied amounts of the ion-pairing reagent. Maximum colour intensity of the complex was achieved with 1.0 ml of MO (0.15 % w/v) as seen in figure 3.

Effect of Reaction time

The optimum reaction time was investigated from 0.5 to 4.0 min by following the colour development at ambient temperature (25 ± 2°C). Complete colour intensity was attained after 2.0 min of mixing (Figure 4).

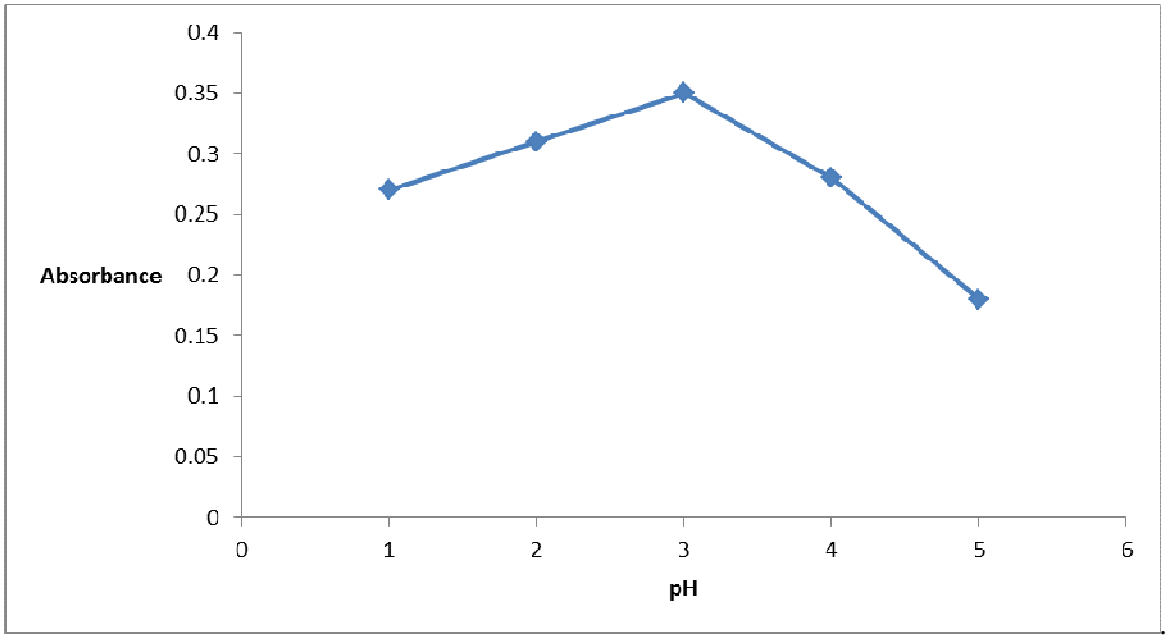

Figure 2: Effect of pH on diphenhydramine-methyl orange ion pair complex

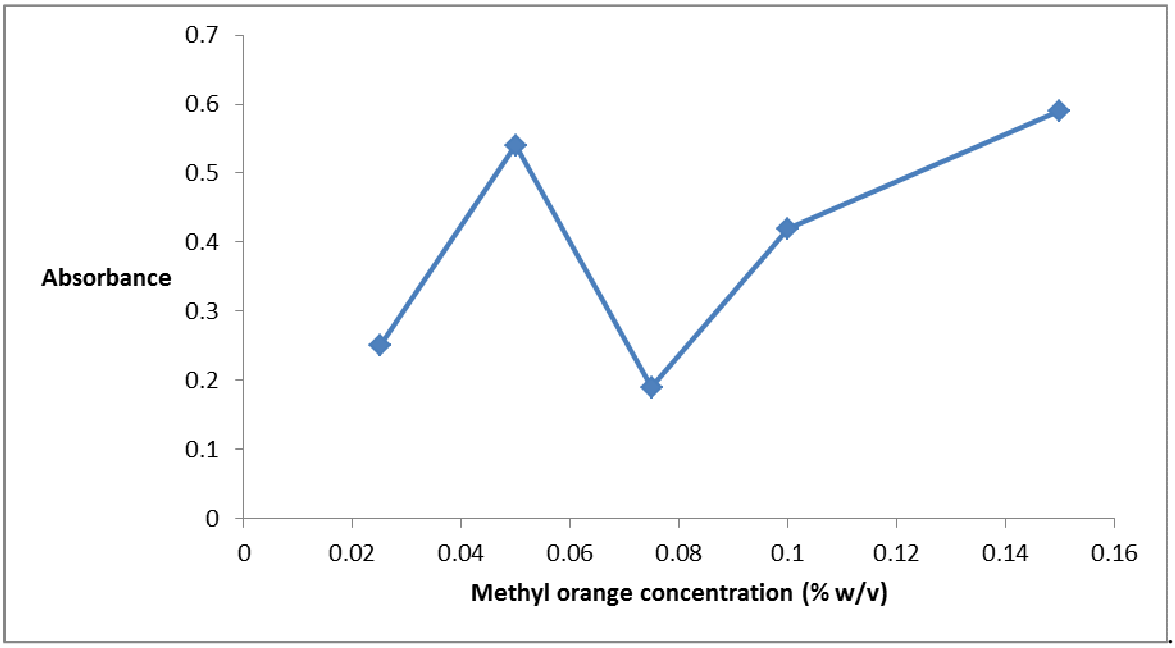

Figure 3: Effect of methyl orange concentration on diphenhydramine-methyl orange ion pair complex

Method validation

The plot of absorbance versus drug concentration was linear between 20 and 100 µg / ml. The regression equation shown below satisfied all the conditions investigated.

A_{abs} = 0.0121x + 0.1071 (correlation coefficient = 0.9898)

where A_{abs} = absorbance of analyte, x = concentration of analyte in the final mixture in moles.

Molar absorptivity and Sandells' sensitivity were calculated to be 78,180.26 L mol^{-1} cm^{-1} and 1.56 µg cm^{-2} respectively. The precision of the method was assessed and the inter-day variation ranged between 0.457 and 0.774 % while the intra-day variation ranged from 0.556 to 0.908 %. The yellow coloured complex was found to be stable beyond 24 hours. The limit of detection (LOD) and limit of quantitation (LOQ) were determined to be 3.1 µg / mL and 7.3 µg / mL respectively.

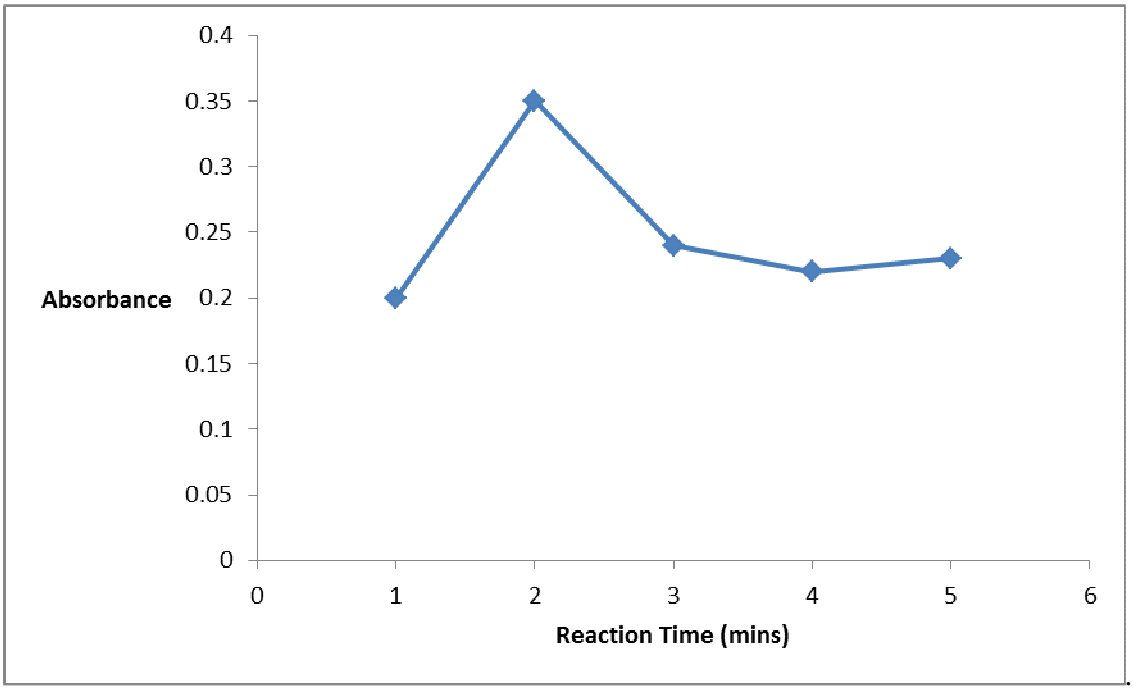

Figure 4: Effect of reaction time on diphenhydramine-methyl orange ion pair complex

Analysis of pharmaceutical preparations

The proposed method was applied successfully to the determination of diphenhydramine in five commercial syrup brands. The results of the analyses are shown in in Table 1. Recovery values were found to range between 99.47 and 103.81 %. The results obtained by the developed method were compared to the official titrimetric method [2] by means of student's t-test and it was found that there were no significant differences between the proposed method and the official method. The assay results were also in good agreement with the label claims.

Table 1: Determination of diphenhydramine in pharmaceutical formulation (syrup)

Brand	Official Titrimetric method - BP, 1988 (n = 5)	Proposed Method (n = 5)
A	105.05 ± 0.23	104.82 ± 0.50
B	101.97 ± 0.15	100.11 ± 0.21
C	99.21 ± 0.63	99.47 ± 0.10
D	104.64 ± 0.38	102.84 ± 0.22
E	103.80 ± 0.35	103.81 ± 0.42

CONCLUSION

The proposed method has been shown to be simple and cheap utilizing readily available reagents. Another advantage of the ion-pair spectrophotometric method is that it can be used for the determination of individual drugs in a complex matrix of the syrup and this makes it very useful for field analysis in particular. The results obtained demonstrate that the method can be applied for the routine analysis of various brands and formulation of diphenhydramine hydrochloride.

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