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The Use of Chloranilic Acid for the Spectrophotometric Determination of Three Antihistamines*

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Cyproheptadine hydrochloride (CPH), methdilazine hydrochloride (MDH) and promethazine theoclate (PMT) were determined in their pure state and in pharmaceutical formulations by a simple spectrophotometric method. The determination was based on the formation of a charge-transfer complex between chloranilic acid as a π-acceptor and the studied drugs as n-donors in an acetonitrile-chloroform mixture. The spectra, various experimental parameters, the stoichiometry and the stability of the complexes formed were investigated. The complexes formed were found to absorb at 520 nm. Beer's law is obeyed in the concentration ranges 25-125, 20-100 and 25-150 μg ml^{-1}, for CPH, MDH and PMT respectively. The corresponding values of molar absorptivity and Sandell sensitivity are 1.48 x 10^3, 1.56 x 10^3 and 1.75 x 10^3 1 mol^{-1} cm^{-1} and 217.39, 212.44 and 284.63 ng cm^{-2}, respectively. The applicability of the method was demonstrated by the determination of the studied drugs in commercial tablets and syrup, and the results were statistically evaluated.

Introduction

The three antihistaminic drugs investigated are of diverse chemical types, and many chemical methods have been reported for their assay.

Cyproheptadine hydrochloride (CPH) is a serotonin and a histamine antagonist with anticholinergic and sedative effects. It also blocks voltage sensitive calcium channels in pancreatic islet cells and smooth muscle. CPH is also used as an appetite stimulant to assist weight gain. The majority of methods found in the literature for the determination of cyproheptadine were devoted to biological materials. Methods for the assay of the drug in pharmaceutical preparations are few and include uv-spectrophotometry[1], visible spectrophotometry [2–4], ion-selective electrode based potentiometry[5,6], gas chromatography[7,8] and high performance liquid chromatography[9–11], though the last two techniques are not always widely available.

Methdilazine hydrochloride (MDH) belongs to the phenothiazine class of drugs. Medicinally, it has been used as an antihistamine and it is also found to possess antipruritic action. Several spectrophotometric

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methods based on such diverse reactions as oxidative coupling[12,13], complex formation[14], condensation[15], ion-association complex formation[16] and radical cation formation using sodium cobaltinitrite[17] and iodic acid[18] have been reported for the determination of MDH in tablets and syrup.

Promethazine theoclate (PMT) is an antihistamine/H_1 receptor antagonist. It is also a phenothiazine derivative which has very little dopaminergic action. It is primarily an antihistamine with additional sedative and antiemetic actions. PMT is used in the treatment of nausea and vomiting associated with migraine labyrinthine disorders and radiation therapy. Literature on the methods reported for the assay of PMT is scarce. Davidson[19] determined the drug in pharmaceutical formulations by a differential spectrophotometric method. A condensation reaction involving PMT and formaldehyde in strong sulphuric acid at 4-5 °C and subsequent measurement of the coloured product at 551 nm was reported[20]. Recently, a flow-injection method with cerium (IV) oxidation of the drug and measurement of the coloured radical cation was described by Martinez and Garcia[21].

Charge-transfer complexes result from a donor-acceptor mechanism of a Lewis acid base reaction between two or more different chemical constituents. The formation of electron donor-acceptor complexes can be rapidly assessed for their validity as a simple quantitative analytical method for many drug substances which can act as electron donors. Chloranilic acid (CAA) as a π-acceptor has been successfully utilized for the spectrophotometric determination of a variety of electron donating basic compounds including some antimalarials[22], tranquilizers[23], diethylcarbamazine citrate[24] and ketomine hydrochloride[25]. In the work described in this paper, the reagent was utilized to develop a simple, precise and accurate method for the spectrophotometric determination of three antihistamines, CPH, MDH and PMT, either in their pure form or in tablet and syrup preparations.

Experimental

Apparatus

A Systronics model 106 digital spectrophotometer (AHMEDABAD, INDIA) with 1 cm glass cells were used for absorbance measurements.

Reagents

Pharmaceutical grade CPH, (Cipla Inida, Ltd.), MDH (Glaxo Allenburgs Ltd.) and PMT (Rhone Poulenc, India) were used as working standards. CAA (s.d. Fine. Chem. India, Ltd.) solution, 0.1%, was freshly prepared in acetonitrile. All other reagents and solvents used were of analytical grade.

Standard solutions

An accurately weighed amount of drug salt CPH, MDH or PMT equivalent to 100 mg of the base was dissolved in about 20 ml distilled water. The solution was quantitatively transferred into a separating funnel, made alkaline with ammonia solution and shaken with five 20 ml portions of chloroform. The extracts were pooled by filtration through a filter paper containing anhydrous sodium sulphate into a 100 ml standard flask and made up to volume with chloroform to provide a stock standard 1 mg ml^{-1} solution of the base. This was diluted to get a working concentration of 500 μg ml^{-1}.

Procedures

Calibration graphs

Serial volumes of standard solutions ranging from 0.25 to 1.25 ml (CPH), 0.20 to 1.00 ml (MDH) or 0.25 to 1.50 ml (PMT) were transferred to 5 ml standard flasks and the volume was brought to 2 ml by adding requisite volumes of chloroform. Then, 0.50 ml of 0.1% CAA reagent was added and the volume was brought to 5 ml with acetonitrile and the absorbance was measured at 520 nm against a reagent blank prepared simultaneously. The calibration graph in each instance was prepared by plotting the measured absorbance versus drug concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Analysis of tablets

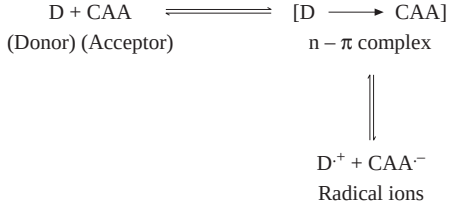
Twenty tablets were finely powdered and mixed. An accurately weighed quantity equivalent to the drug base concentration mentioned in the standard solution preparation was transferred to a 100 ml standard flask and extracted with 60 ml of distilled water by vigorous shaking for 20 min. For avomine tablets (PMT), 2 ml of 0.1 M HCl was used to aid the dissolution of the salt. The solution was then made up to volume with water, mixed and filtered. In a separating funnel, a 50 ml portion of the filtrate was made alkaline to litmus paper with ammonia solution and extracted with four successive 10 ml portions of chloroform, which were pooled, dried over anhydrous sodium sulphate, and diluted accurately to 50 ml with chloroform as the standard solution. This was further diluted to obtain the working solutions with chloroform. A suitable aliquot was treated as described under the preparation of calibration graphs.

Analysis of syrup

An accurately measured volume of the mixed dilosyn syrup (MDH) equivalent to 50 mg of drug base was quantitatively transferred into a separating funnel and treated as described for tablets except that final volume was 50 ml. After diluting this 1 mg ml^{-1} solution suitably, an appropriate volume was subjected to analysis.

Results and Discussion

Chloranilic acid (CAA) solution in acetonitrile gives an absorption spectrum with an absorption maximum at 430 nm. On addition of chloroformic solution of CPH, MDH or PMT to CAA solution, a bathochromic shift to a longer wavelength is obtained at room temperature (Figure 2). This new absorption band formed is the result of the formation of charge-transfer complex through the interaction of CAA as a π-acceptor and the studied drugs as n-donors followed by the formation of radical anion according to the following scheme[26]:



The formation of a radical anion in such molecular interactions has been established by electron-spin resonance measurements[27–29].

Optimization of experimental variables

The influence of various factors on the colour development was studied to determine the optimal conditions, such as reagent concentration and choice of solvent. CAA solution in various solvents failed to give quantitative results. However, CAA in acetonitrile readily reacted stoichiometrically with all the three antihistamines investigated forming a single purple chloranilic acid radical anion. Acetonitrile proved to be most suitable diluting solvent as it gives good solvating capacity for CAA, and gives the highest yield of the radical anion. Solvents such as chloroform, 2-propanol, dichloroethane, 1,4 – dioxane were not suitable, because the complex formed in these solvents either had low absorbance or was precipitated on dilution. The reaction between the drugs and CAA in acetonitrile was instantaneous and the product remained stable for at least 24 h.

Maximum absorbance was obtained when 0.5 ml of 0.1% CAA solution was used in a total volume of 5 ml. Figure 3 shows the relationship between the absorbance and concentration of chloranilic acid at a fixed drug concentration.

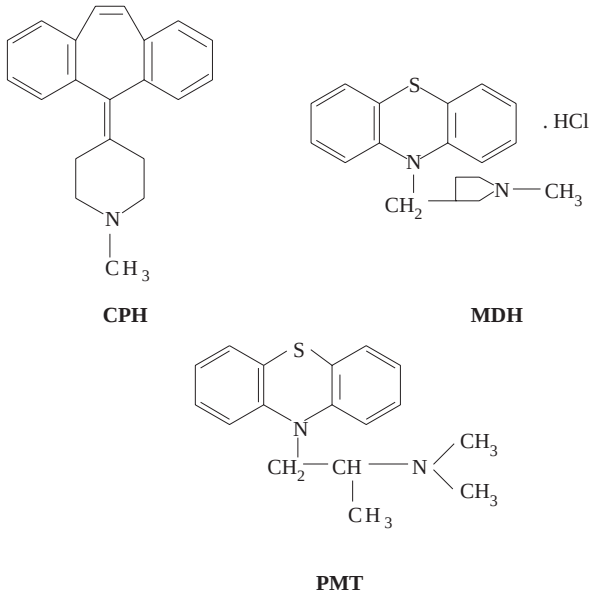


Figure 1. Structures of the antihistamines studied

Molecular ratio and formation constant

The stoichiometry of the reaction between CAA and CPH, MDH or PMT was studied by Job's method of continuous variations[30]. The plot (Figure 4) indicates that the interaction occurs through the formation of 1:1 (donor: acceptor) complex in all three cases. This is in agreement with the presence of a single tertiary nitrogen atom in CPH, MDH or PMT molecule (Figure 1). The formation constant (K) and molar absorptivity ($\in$) of the drug – CAA complex were determined by using the Benesi-Hildebrand equation[31]:

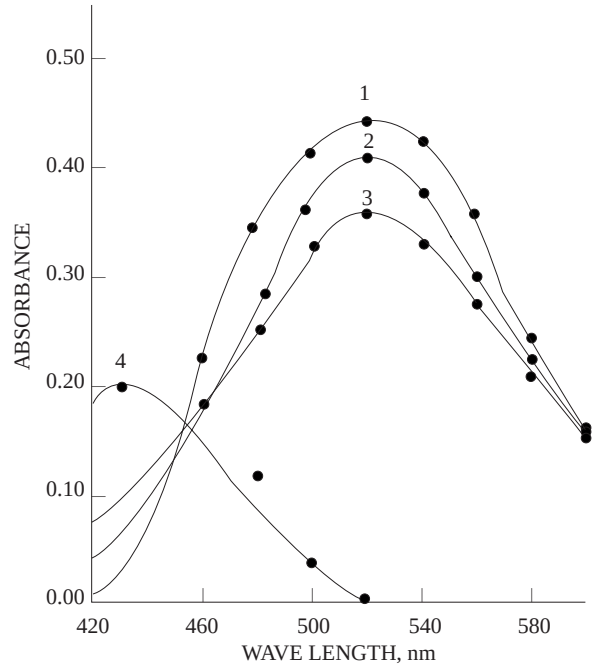


Figure 2. Absorption spectra of the CT complex formed between the drugs and CAA. 1. CPH (100μg ml^{-1}) 2. PMT (125 μg ml^{-1}) 3. MDH (80 μg ml^{-1}) 4. Blank

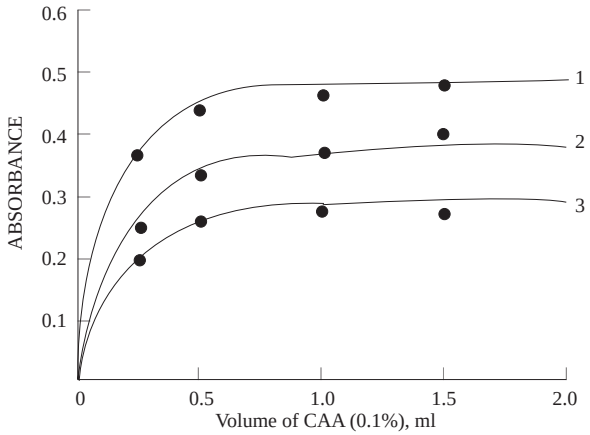


Figure 3. Effect of CAA concentration on complex formation 1. CPH (100 μg ml^{-1}) 2. PMT (100 μg ml^{-1}) 3. MDH (60 μg ml^{-1})

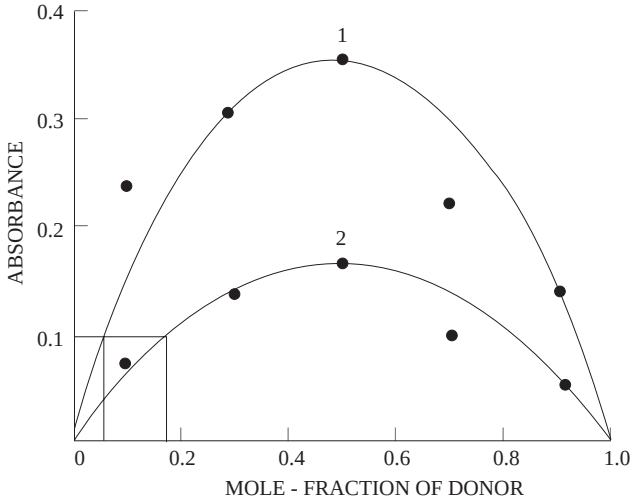


Figure 4. Job's Continuous Variation plots for MDH 1. 1.68 x 10^{-3} M2. 0.84 x 10^{-3} M

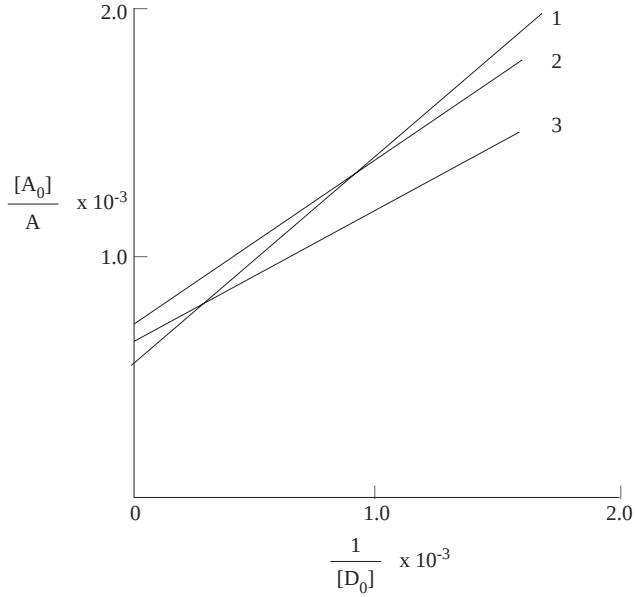


Figure 5. Benesi-Hildebrand plots of CT complexes of CPH, MDH and PMT with CAA, 1 PMT, 2 CPH, 3. MDH

$$\frac{[A_0]}{A} = \frac{1}{K}[D_0] \in + \frac{1}{\in}$$

where K is the formation constant, A is the absorbance and $\in$ is the molar extinction coefficient of the complex, and $[A_0]$ and $[D_0]$ are the initial concentrations of the acceptor and donor, respectively. Formation constants were obtained from the ratio of intercept to slope and $\in$ from the inverse intercept of Benesi-

Hildebrand plots (Figure 5), which were prepared by keeping the concentration of CAA constant, and lower than the varied concentrations of the drugs as described in the procedures. The results of the study are presented in Table 1.

Table 1. Analytical parameters for the charge-transfer complexes of CPH, MDH and PMT with CAA.

Parameter	CPH	MDH	PMT
Beer's law limits, μg ml^{-1}	25-125	20-100	25-150
Limit of detection, μg ml^{-1}	1.07	0.96	1.43
Limit of quantification, μg ml^{-1}	3.57	3.21	4.76
Molar absorptivity, l mol^{-1}cm^{-1}	1.48 x 10^3 a 1.42 x 10^{3}b	1.56 x 10^3 a 1.53 x 10^3 b	1.75 x 10^3 a 1.81 x 10^{3}b
Sandell sensitivity ng cm^{-2} per 0.001 absorbance unit	217.39	212.44	284.63
Regression equation*			
Intercept, a	0.0284	0.001	0.0296
Slope, b	0.0041	0.0046	0.0031
Confidence interval of intercept,α	0.0284 ± 0.0776	0.001 ± 0.9506	0.0296 ± 0.0672
Confidence interval of slope, β	0.0041 ± 0.0009	0.0046 ± 0.0143	0.0031 ± 0.0006
Correlation coefficient, r	0.9976	0.9990	0.9997
Formation constant, K.	1.02 x 10^3 b 1.21 x 10^3 c	1.16 x 10^3 b 1.23 x 10^3 c	0.68 x 10^{3}b 0.80 x 10^3 c

* Y = a + bX where Y is the absorbance for concentration, X in μg ml^{-1}.
a : from Beer's law data.
b : from Benesi-Hildebrand plot.
c : from Turner and Anderson plot.

Analytical data

Beer's law limits, detection limits, molar absorptivity and Sandell sensitivity values are given in Table 1. Regression analysis indicated that the values of the intercept are small; the values were 0.0284, 0.0010 and 0.0296 for CPH, MDH and PMT respectively. Correlation coefficient values range from 0.9976 to 0.9997, suggesting a perfect linearity between the absorbance and concentration of drugs in the Beer's law limits studied.

Accuracy and precision

The accuracy of the method was established by performing seven replicate analyses on solutions containing four different amounts (within the Beer's law limits) of each drug and calculating the percentage error. The precision was ascertained by calculating the relative standard deviation (RSD) for seven determinations at each level. The range, percent error, standard deviation (SD), and RSD (%) are given in Table 2. The comparision of the actual difference between the mean and the true value ($\overline{x}$ - μ) with the largest difference that could be expected as a result of indeterminate error (± ts / $\sqrt{n}$) is made in the last two columns of Table 2. It is clear from the results that at all four levels (concentrations) studied, the values of ($\overline{x}$ - μ) are less than ± ts / $\sqrt{n}$ indicating that no significant difference exists between the mean and true values.

Table 2. Evaluation of accuracy and precision of the method

Antihistamine studied	Concentration taken, μg ml^{-1}	Concentration found*, μg ml^{-1}	Range, μg ml^{-1}	Error, %	S, μg ml^{-1}	RSD, % (n = 7)	$\overline{x} - \mu$	$\pm \frac{ts}{\sqrt{n}}$
CPH	50	49.32	2.35	1.36	0.96	1.96	0.68	0.86
	75	74.46	1.81	0.72	0.68	0.92	0.54	0.61
	100	99.89	0.50	0.11	0.45	0.46	0.11	0.41
MDH	20	20.14	0.69	0.70	0.34	1.73	0.14	0.22
	60	60.32	0.89	0.53	0.44	0.74	0.32	0.39
	100	100.35	1.66	0.35	0.57	0.57	0.35	0.51
PMT	50	50.08	1.71	0.16	0.77	1.53	0.08	0.68
	75	75.40	1.04	0.53	0.55	0.73	0.40	0.49
	100	100.79	2.81	0.79	0.95	0.94	0.79	0.85

* Mean value of seven determinations at each level
$\overline{x}$ = mean value; μ = true value; t = 2.36 for n = 7 at 95% confidence level.
S = standard deviation; RSD = relative standard deviation.

Application of the method to formulations

The method was applied successfully to the determination of CPH, MDH and PMT in their dosage forms. The results presented in Table 3 reveal that the recoveries were in the range of 98.76-100.97%, reflecting the high accuracy and precision of the method as indicated by low RSD values.

Table 3. Results of assay of CPH, MDH and PMT in their dosage forms

Drug	Dosage form* and brand name	Label claim, mg/tablet	Found, $ mg	Mean recovery, % (n = 7)	RSD, % (n = 7)
CPH	Ciplactin tablets[a]	4.00	3.98	99.54	1.04
	Ciplactin syrup[a]	2 mg/5ml	2.01	100.97	0.37
	Practin tablets[b]	4.00	3.96	99.16	2.21
	Practin syrup[b]	2 mg/5 ml	1.98	99.20	0.92
	Peritol tablets[c]	4.00	3.95	98.76	0.97
	Peritol syrup[c]	2mg / 5ml	2.01	100.68	0.40
MDH	Dilosyn tablets[d]	8.00	8.10	101.25	1.13
	Dilosyn syrup[d]	4 mg/ 5 ml	4.02	100.55	0.54
PMT	Avomine tablets[e]	25.00	25.01	100.04	1.82

* Marketed by: a. Cipla; b. Merind; c. Themis Chemicals; d. Glaxo Allenburgs; e. Rhone-Poulenc.
$ Average of seven determinations.

Interferences

Excipients and fillers added to formulations were tested for their interference in the procedure. Fortunately, such auxillary substances as starch, talc, lactose, gelatin, magnesium stearate and sodium alginate exhibited no interference, since in the proposed method the free base is extracted prior to the instant complexation with CAA. This is clearly indicated from the results obtained for dosage forms (Table 3).

Conclusions

The developed method is rapid, simple and fairly sensitive. It has the advantages of a wide range of determination and high accuracy. The complex formed is stable for at least 24 h, thus permitting quantitative analysis to be carried out with good reproducibility.

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