
A New Spectrophotometric Method for the Determination of Ceterizine Hydrochloride in Pharmaceutical Preparations and Biological Samples

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A sensitive and rapid extractive spectrophotometric method was developed for the assay of ceterizine hydrochloride in bulk drug, pharmaceutical preparations, urine and blood samples. The method was based on the formation of a chloroform soluble ion-pair complex between ceterizine hydrochloride with thymol blue in an acidic buffer. The complex showed absorption maximum at 413 nm with molar absorptivity of 4.2×10^3 l/cm.mol. The system obeyed Beer's law in the concentration range of 3-60 μ g/ml ($r=0.9992$). The results obtained by the proposed method were compared statistically by means of Student t-test and by the F- test with those of the reported method. The two were shown to be in good agreement. No interference was observed from common pharmaceutical excipients.

Ceterizine dihydrochloride (CTZH) is a second-generation antihistamine with high specificity and potent H₁ receptor antagonist activity. It is used in the treatment of perennial and seasonal allergic rhinitis and also for chronic urticaria¹. In literature, chromatographic² and HPLC^{3,4} procedures have been reported for the assay of CTZH. A UV spectrophotometric method is also reported⁵, which was applied to the analysis of CTZH in syrups. However, parabens interfered with its direct determination and sensitivity of the method was not reported. No spectrophotometric method is reported so far for the determination of CTZH in biological samples. Hence, we aimed at developing a new sensitive spectrophotometric method for the determination of CTZH in pharmaceutical preparations and in biological samples. Ion-pair extraction spectrophotometry has received a considerable attention for quantitative assay of many pharmaceutical drugs⁶⁻⁸. In the present investigation, we report the development of accurate, reproducible and adequately sensitive extractive spectrophotometric method based on the formation of chloroform soluble ion-association complex between CTZH with thymol blue (TB) in an

acidic buffer. The proposed method was validated by application to marketed formulations and by comparison with a reported method.

EXPERIMENTAL

All spectral measurements were made on a Hitachi UV/vis spectrophotometer model U-2001 using 1 cm matched quartz cells. The pH measurements were made with Schott Gerate pH meter CG 804. Elemental analysis of the complex was performed on Thermoquest CHN analyzer (EA 1110). CTZH was supplied gratis by Dr. Reddy's Laboratories Ltd., Hyderabad. Dosage forms of CTZH, manufactured by different firms, were obtained commercially. All other chemicals were of analytical reagent or pharmaceutical grade. Quartz-processed high purity water was used throughout.

Stock solution of CTZH was prepared by dissolving 50 mg of the drug in 50 ml distilled water in a calibrated flask. A 0.05 % solution of TB was prepared in distilled water. Series of buffer solutions of KCl-HCl (pH 1.0-2.2), NaOAc-HCl (pH 1.99-4.92), NaOAc-AcOH (pH 3.72-5.57) and potassium hydrogen phthalate-HCl (pH 2.2-3.6) were prepared using standard methods⁹⁻¹¹.

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Assay methodology for pure drug:

Aliquots of standard solution of CTZH (3-60 µg/ml) were transferred into a series of 125 ml separating funnels followed by 2 ml of buffer (potassium hydrogen phthalate-HCl, pH 3.6) and 5 ml of 0.05 % TB. Ten millilitres of chloroform was added to each of the separating funnels and the contents were shaken well. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbance of the yellow coloured species was measured at 413 nm against a reagent blank. A calibration graph was plotted.

Assay procedure for pharmaceutical preparations:

Twenty tablets were weighed and powdered. An amount of the powder equivalent to 20 mg of the drug was weighed

into a 100 ml volumetric flask. Seventy millilitres of distilled water was added and shaken thoroughly for about 15-20 min. The contents were diluted to the mark with distilled water, mixed well and filtered through a Whatman filter paper No. 40 to remove the insoluble matter. Twenty-five millilitres of the filtrate was diluted to 100 ml and an aliquot was analyzed using the procedure described earlier. The results are shown in Table 1 and are compared with those of the reported spectrophotometric method¹².

In respect of syrup and suspension, 20 ml equivalent to 20 mg of drug were transferred into a 250 ml separating funnel. The sample was rendered alkaline to litmus with 6 M ammonia solution, which was added in excess by 1 ml. The mixture was then extracted with 3X15 ml portions of chloroform. The chloroform extracts were evaporated to dry-

TABLE 1: ANALYSIS OF PHARMACEUTICAL PREPARATIONS OF CTZH.

Drugs*	Label claim (mg/tablet or ml)	Recovery** ± SD, %	
		Reported method ¹²	Proposed method
Alerid syrup ^a	1	98.8±1.10	98.1±0.95, F=1.34, t=0.97
Alerid tablet ^a	10	98.9±0.75	98.0±0.71, F=1.11; t=0.91
Ceterzine tablet ^b	10	97.5±1.12	97.6±1.32, F=1.38; t=0.96
Cetzine syrup ^b	10	99.4±0.78	99.1±0.85, F=1.18; t=1.19
Cetiriz syrup ^c	1	98.1±0.93	97.6±0.95, F=1.04; t=1.11
Cetiriz tablet ^c	10	98.8±1.11	99.1±0.97, F=1.19; t=1.11
Cetriset-D tablet ^d	10	98.7±1.04	98.4±0.96, F=1.17; t=1.04
Zirtin tablet ^e	10	98.9±0.98	98.7±1.16, F=1.18; t=1.06
Zirtin tablet ^e	10	98.9±0.98	98.7±1.06, F=1.17; t=1.06
Zyrtec tablet ^f	10	99.8±0.61	101.5±0.68, F=1.24; t=1.12
Zyncet tablet ^g	20	101.9±1.08	102.2±0.92, F=1.37; t=1.21
Zyncet suspension ^g	10	98.0±1.04	96.8±0.94, F=1.22; t=1.05
Sizon forte tablet ^h	10	99.1±0.55	99.0±0.69, F=1.57; t=1.06
Alzine tablet ⁱ	10	99.4±0.67	99.1±0.88, F=1.72; t=0.85
Cetrine tablet ^j	10	99.8±0.75	101.1±0.92, F=1.50; t=0.86
Coszin tablet ^k	10	99.0±0.52	99.1±0.38, F=1.87; t=1.08

**Recovery value by the proposed method is the mean of five determinations. *Marketed by: a, Cipla; b, Glaxo Lab; c, Alkem; d, Sun Pharmaceuticals; e, Torrent Pharmaceuticals; f, UNI-UCB; g, Unichem, h; Systopic Laboratories Limited; i, Core Healthcare Ltd.; j, Dr. Reddy's labs. Ltd.; k, CFL Pharmaceuticals Ltd. The calculated F- and t- values refer to 95 % confidence limits.

ness and the residue was dissolved in 0.1 M HCl and made up to 100 ml with distilled water. The solution was diluted to get 100 µg/ml of drug and an aliquot was analyzed as above. The results are shown in Table 1 and are compared with those of the reported method¹².

Recovery experiments were performed using the method of addition. A fixed amount of pure sample solution was added to one of the three different concentrations of the standard drug solution. The total amount of the drug was then determined using the proposed methods and the amount of the added drug was calculated by difference.

Assay procedure for drug in urine:

A known amount of CTZH was added to 5 ml of urine sample. To this was added 0.5 g of lead nitrate to precipitate out the chlorides present. The solution was filtered and the excess of lead present in the filtrate was removed by adding 8 M sulphuric acid. The solution was again filtered. The pH of the filtrate was adjusted to 3.6. A suitable amount of an aliquot was analyzed for the quantification of CTZH as described for pure drug.

Assay procedure for drug in blood:

One millilitre of blood was spiked with a known amount of CTZH before the addition of sodium citrate. The citrated blood was deproteinated with trichloroacetic acid and filtered. The filtrate was diluted with distilled water to 100 ml in a calibrated flask. An appropriate amount of an aliquot was taken, neutralized with dilute sodium hydroxide solution and analyzed as described for pure drug.

Assay procedure for drug in a synthetic mixture:

Synthetic mixture containing talc, starch (160 mg each), sucrose, lactose (80 mg each), gelatin (120 mg) and magnesium stearate (200 mg) and 25 mg of CTZH were prepared and a portion of the mixture containing known amount of CTZH was weighed accurately. The drug was extracted with distilled water, filtered and the residue was washed 5 times with distilled water. The filtrate and the washings were then combined in a 100 ml calibrated flask, diluted up to the mark with distilled water and the amount of CTZH was determined as described earlier.

RESULTS AND DISCUSSION

CTZH reacts with TB in acidic buffer to give a chloroform-soluble yellow coloured ion-association complex which exhibits an absorption maximum at 413 nm where the reagent blank absorbs negligibly. The optimum reaction conditions for quantitative determination of the ion-pair com-

plexes were established via a number of preliminary experiments. The optimum volume of the reagent was studied. It was observed that 5 ml of TB was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in the presence of various buffers such as KCl-HCl (pH 1.0-2.2), NaOAc-HCl (pH 1.99-4.92), NaOAc-AcOH (pH 3.72-5.57) and potassium hydrogen phthalate-HCl (pH 2.2-3.6). It was noticed that the maximum colour intensity and constant absorbances were observed in potassium hydrogen phthalate-HCl (2 ml, pH 3.6) buffer. Several organic solvents were tried for the effective extraction of the coloured species from aqueous phase. Chloroform was found to be the most suitable solvent as it was observed that only one extraction was adequate to achieve a quantitative recovery of the complex. Shaking times of 0.5 to 2 min produced a constant absorbance and hence a shaking time of 1 min was used throughout. There was no appreciable change in the absorbance or colour of the product if the order of addition of the reactants was varied. The absorbances of the complexes were found to be stable for more than 12 h. The drug to reagent ratio was found to be 1:1 as evaluated from Job's method. The complex was isolated in solid form by evaporating the chloroform layer on a water bath. The analytical data of the isolated solid complex also confirmed the composition to be 1:1 for drug to reagent.

A linear correlation was found between absorbances and concentration of CTZH in the range of 3-60 µg/ml. The equation for one representative calibration curve is $A_{413nm} = 0.00622C + 0.1159$, where A and C refers to absorbance and concentration of the drug in µg/ml. The slope, intercept and correlation coefficient obtained by linear least squares treatment of the results, molar absorptivity and Sandell's sensitivity values are presented in Table 2. The precision and accuracy of the proposed method was checked by using a solution containing 40 µg/ml of CTZH. The RSD values were found to be less than 1.1 %.

The extent of interference by commonly associated excipients such as magnesium stearate, starch, talc, gelatin, dextrose, lactose and sucrose was determined by measuring the absorbance of a solution containing 10 µg/ml of CTZH. An error of ± 2 % in the absorbance readings was considered tolerable. The proposed method was found to be free from interferences by the excipients in the levels found in dosage forms. This was quite clear from the data obtained on the analysis of synthetic mixtures. The RSD value was found to be 1.04 for six replicates of 40 µg/ml of CTZH.

TABLE 2: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA.

Parameter	Value
λ_{max} (nm)	413
Beer's law limits ($\mu\text{g/ml}$)	3-60
Molar absorptivity (l/mol.cm)	4.2×10^3
Sandell's sensitivity (ng/cm ² /0.001 absorbance units)	111.11
Correlation coefficient (r)	0.9992
Regression equation (Y) *	
Slope	0.0062
Standard deviation on slope, (S _b)	0.005
Intercept	0.1159
Standard deviation on intercept, (S _a)	0.052
Standard error of estimation, (S _e)	0.0387
Relative standard deviation (n = 6), %	0.87
Range of error ^c (95 % confidence limits), %	± 0.54

*Y=a+bX where X is the concentration in $\mu\text{g/ml}$. ^cFor six replicate analysis within Beer's law limits.

The applicability of the proposed method to the assay of dosage forms was examined by analysing tablets, syrups and suspension marketed under different trade names. The results obtained were compared statistically by Student t-test and by the variance ratio F-test with those obtained by the reported method¹². The Student t-values at 95 % confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and the reported method. It was also observed that the variance ratio F-values calculated for p=0.05 did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and the reported method¹². The results are tabulated in Table 1.

The results of the analysis of urine and blood samples are shown in Table 3. As no spectrophotometric method for the analysis of CTZH in biological samples is currently available the data obtained by the proposed method could not be compared for its validation. However, the data of the analy-

TABLE 3: ANALYSIS OF CTZH IN URINE AND BLOOD SAMPLES.

Sample	CTZH present, $\mu\text{g/ml}$	CTZH found* $\mu\text{g/ml}$	Recovery \pm SD, %
Blood 1	5.0	4.97	99.4 \pm 0.81
Blood 2	10.0	9.96	99.6 \pm 0.79
Blood 3	15.0	15.1	100.7 \pm 1.04
Urine 1	5.0	4.96	99.2 \pm 0.77
Urine 2	10.0	9.98	99.8 \pm 0.69
Urine 3	15.0	14.91	99.4 \pm 0.92

* Average of five determinations.

sis was supported by RSD values.

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