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Spectrophotometric determination of Cetirizine: Dyes as analytical reagents

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Abstract
Extractive spectrophotometric methods for the determination of Cetirizine using dyes viz., bromocresol green, bromothymol blue and Tropaeolin OO as analytical reagents have been developed and described. The developed methods involved the formation of coloured ion-pair complexes of Cetirizine with dyes in acidic medium. The ion-pair complexes of Cetirizine, with bromocresol green, bromothymol blue and Tropaeolin OO, quantitatively extracted into chloroform, absorbed maximally at 410, 414, and 410nm. The stoichiometry of the complex in three cases is found to be 1:1. The Beer's law is obeyed in the concentration ranges 2.5-30µg/ml. The effect of concentration of dye, pH and interference of excipients have been studied and optimized. The limits of detection and quantification have been determined for all the three methods. These methods have been validated as per the guidelines of International Conference on Harmonization and have been applied to the determination of Cetirizine in commercial tablets and results of analysis were validated statistically through recovery studies. Thus the newly developed methods are accurate, efficient and stable.

Keywords: spectrophotometry, cetirizine, cetcip tablet, bromocresol green, bromothymol blue, tropaeolin OO, Ion-pair complex, ICH validation

1. Introduction
Chemically, Cetirizine (Fig 1) is (±)- [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy] acetic acid, dihydrochloride. It is a histamine H1-receptor antagonist of non-sedating type. Cetirizine is used in the treatment of allergic rhinitis, conjunctivitis and urticaria [202].

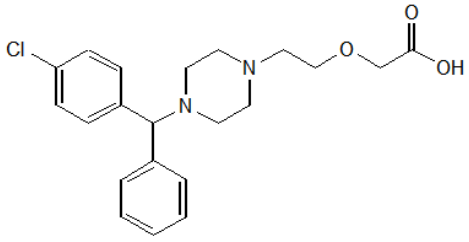


Fig 1: Structure of Cetirizine

The literature survey revealed that various analytical methods were employed and reported to accomplish the quantification of Cetirizine in pharmaceutical dosage forms. Such techniques include: Spectrophotometry [1, 2], HPTLC [3], HPLC [4, 5], HPLC-TMS [6] and LC/MS [7]. The Cetirizine was quantified in biological fluids by HPLC [8-11], TLC-Densitometry [12] and LC-Tandem Mass Spectrometry [13]. Recently, extractive spectrophotometric determination of cetirizine in pure and pharmaceutical form was developed by Sevgi Tatar Ulu [14]. UV Spectrophotometry [15-17], Extractive spectrophotometry [18-20], Capillary electrophoretic [21] and Raman Scattering [22] are important methods reported for the estimation of Cetirizine. Development of methodology for the determination of cetirizine hydrochloride in medicines [23] was also reported. Thorough survey of literature on Cetirizine revealed that the quantification using bromocresol green (BCG), bromothymol blue (BTB) and Tropaeolin OO (TpOO) as analytical reagents has not been reported yet. This prompted to develop new simple and cost-effective quantitative methods for the determination of Cetirizine using these dye stuffs.

2. Materials and Methods
2.1 Instruments
For recording UV-Vis spectra of the study, SHIMADZU 140 double beam spectrophotometer and ELICO SL 210 UV-Visible double beam spectrophotometer with quartz cells of 10 mm path length have been used. For *p*H measurements, an Elico model Li-120 *p*H meter was employed.

2.2 Materials
The dyes *viz*., Bromocresol green, Bromothymol blue and Tropaeolin OO of analytical grade supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The solvent Chloroform (HPLC grade) and AR grade HCl and Sodium acetate supplied by SD Fine Chemicals, Mumbai were used in the study. The drug, Cetirizine was procured as gift sample from Hetero Drugs Pvt. Ltd, Hydeabad, Telangana.

2.3 Methods
Method A
Method A involves the interaction of Cetirizine with bromocresol green to form ion-pair complex, extractable into

chloroform. This ion-pair complex absorbs around 410 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BCG was used. The required pH of reaction mixture was maintained at 3.5 using CH_3COONa-HCl buffer.

Method B

Method B involves the interaction of Cetirizine with bromothymol blue to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 414 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BTB was used. The required pH of reaction mixture was maintained at 2.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

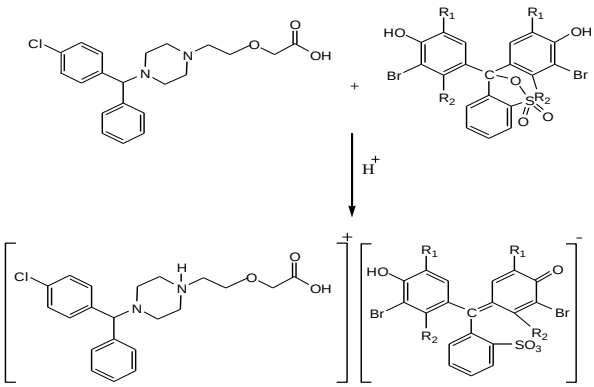
Method C

Method E involves the interaction of the Cetirizine with Tropaeolin OO (Tp OO) to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 410 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of Tropaeolin OO (Tp OO) was used. The required pH of reaction mixture was maintained at 2.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

3. Results and Discussion

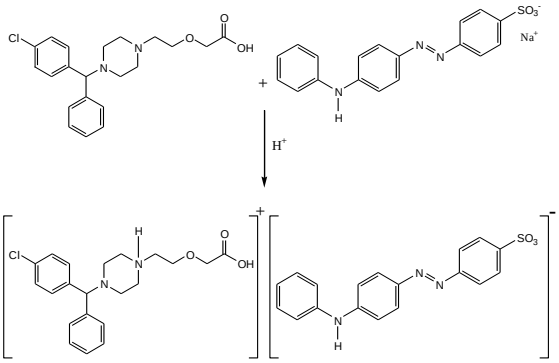
3.1 Formation of Ion-pair complexes

Cetirizine forms ion-pair complex with bromocresol green (BCG) and bromothymol blue (BTB) in acidic medium. Cetirizine contains two tertiary nitrogen atoms of which the nitrogen attached to carboxylic acid chain acts as a donor. Hence, the protonation takes place at this nitrogen atom and finally forms ion-pair complex with sulphonic acid group of BCG or BTB. The sulphonic group of dye undergoes dissociation to form quinoid group by opening of lactoid group in acide medium which can be attributed to the colour of ion-pair complexes. The plausible reaction mechanism is proposed and given in Scheme 1.



Scheme 1: Cetirizine-Dye ion pair complex

Bromocresol green : R_1 = Br, R_2 = $-CH_3$
Bromothymol blue : R_1 = isopropyl, R_2 = $-CH_3$
Scheme 2 shows the ion pair complex formation between the Cetirizine and TpOO.



Scheme 2: Cetirizine-TropaeolinOO ion-pair complex

The developed methods are based on the interaction of Cetirizine to form ion-pair complexes with dyes viz., BCG, BTB and TpOO. The ion-pair complexes of Cetirizine, quantitatively extracted into chloroform, absorbed maximally at 410, 414, and 410nm with use of the cited dyes respectively (Fig 2A, 2B and 2C) where the reagent blank under similar experimental conditions showed no absorption. The developed methods can be applied for the quantification of Cetirizine in pharmaceutical industries. 0.025% aqueous solutions of dyestuffs and CH_3COONa-HCl acid buffers of *p*H 3.5, 2.5 and 2.5 were used to get stable ion-pair complexes of Cetirizine with the mentioned dyes. Appropriate pH values are maintained in all the experiments with the help of a *p*H meter.

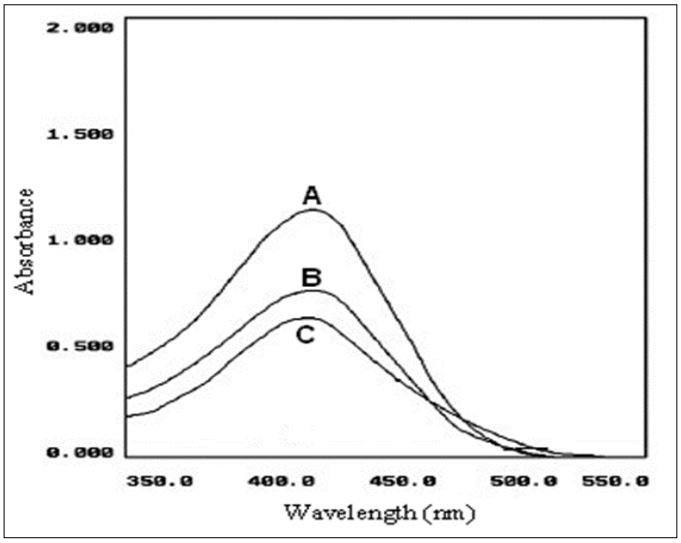


Fig 2: Absorption spectra of Cetirizine-dye complex extracted into 10 ml chloroform

A. Drug = 20 μg ml^{-1} + 5 ml of 0.025% BCG + 5 ml of pH 3.5 buffer
B. Drug = 22.5 μg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.5 buffer
C. Drug = 22.5 μg ml^{-1} + 5 ml of 0.025% TpOO + 5 ml of pH 2.5 buffer

3.2 Calibration curves for the methods

Different aliquots of solution of Cetirizine were taken into separating funnels. 5 ml of CH_3COONa-HCl buffer (of *p*H 3.5, 2.5 and 2.5) and 5 ml of 0.025% aqueous solution of dye were added. The total volume of the contents in the flask was made up to 20 ml with distilled water. To this, 10 ml of chloroform was added and the contents were thoroughly shaken for 5 min in order to form a stable ion-pair complex. The flask was kept aside for 5 min to allow the organic and aqueous layers to separate. The absorbance of stable colored solution was recorded around 417 nm against blank similarly prepared. The determination of pure Cetirizine and its pharmaceutical forms were carried out using the same procedure developed. The calibration curves (Fig 3) are constructed which are linear over the concentration ranges which are in permissible range.

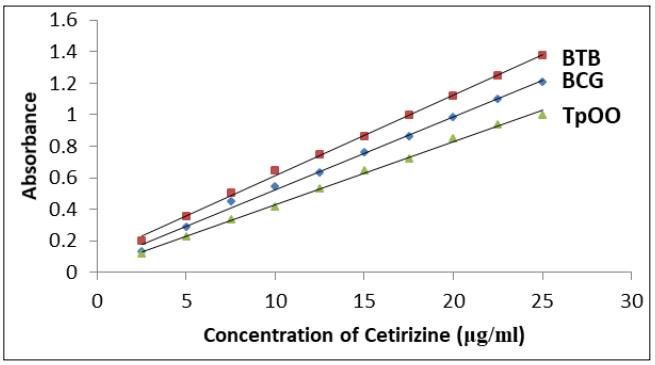


Fig 3: Calibration graphs for Cetirizine-BCG, BTB and TpOO ion-pair complexes

The statistical data for the regression equations for the developed methods and optical characteristics of ion-pair complexes of Cetirizine with dyes are presented in Table 1.

Table 1: Optical characteristics and statistical analysis for the regression equation of the proposed methods for the estimation of Cetirizine

Parameters	Extraction methods with[b]		
	BCG	BTB	TpOO
λ_{max} (nm)	410	414	410
Beer's law limit (µg ml^{-1})	3.0-30	2.5-25	2.5-25
Molar absorptivity (L mol^{-1} cm^{-1})	20605	21252	18018
Formation constant, K, M^{-1}	$1.54x10^6$	$1.72x10^6$	$1.43x10^6$
Sandell sensitivity (µg cm^{-2})	0.022	0.0217	0.0256
Slope (specific absorptivity), b	0.0446	0.046	0.039
Intercept (a)	0.087	0.0975	0.0187
Correlation coefficient (r)	0.999	0.999	0.999
Standard deviation of intercepts (% n=6)	0.0122	0.0091	0.0081
Limit of detection, µgml^{-1}	0.33	0.27	0.274
Limit of quantification, µgml^{-1}	1.00	0.83	0.832
Regression equation[a]	Y=0.0446C ± 0.087	Y=0.046C ± 0.0975	Y=0.039C ±0.0187

[a]With respect to $Y=bc+a$, where C is the concentration (µg ml^{-1}) and *Y* is absorbance
[b]Six replicate samples

3.3 Procedure for the assay of pure drug

Five different solutions of pure Cetirizine drug in the range of calibration curve were chosen for conducting recovery experiments, the results of which are presented in Table 2 along with relative standard deviations for the methods developed.

Table 2: Application of proposed methods for the estimation of Cetirizine in pure form

Taken (µg/ml)	Proposed methods						Reference method Recovery (%)
	Found (µg/ml)			Recovery (%)			
	BDG	BTB	TpOO	BDG	BTB	TpOO	
6	5.596	5.97	6.007	99.26	99.57	100.11	101.23
10	9.962	9.95	9.93	99.62	99.53	99.33	99.98
14	13.99	13.95	13.9	99.94	99.654	99.34	101.25
18	17.98	17.91	18.007	99.91	99.5	100.04	101.56
							101.45
							99.45
							99.26
							101.52
RSD (%)				0.3152	0.065	0.428	0.9721
Mean±SD				99.686 ±0.314	99.57±0.065	99.71 ±0.427	100.71 ±0.9791
t-test				2.16	0.0438	1.857	
F-test				1.027	2.436	1.932	

3.4 Procedure for the assay of dosage forms

Ten tablets of Cetcip 30mg were taken and grounded to powder and dissolved in doubly distilled water. The solution was stirred thoroughly, filtered through a Whatman No. 42 filter paper, and taken into a 100 ml standard flask and diluted with required doubly distilled water. The recovery

experiments were carried out by selecting different aliquots of this solution which come in the range of calibration curve for the determination of drug in its dosage form. Table 3 represents the results of the recovery experiments for the assay of dosage forms.

Table 3: Application of proposed methods for the estimation of Cetirizine in pharmaceutical form

Taken (µg/ml) Cetcip 30mg	Proposed methods						Reference method Recovery (%)
	Found (µg/ml)			Recovery (%)			
	BDG	BTB	TpOO	BDG	BTB	TpOO	
6	5.989	5.97	5.98	99.83	99.58	99.72	99.96
10	9.980	9.93	9.91	99.81	99.35	99.09	101.25
14	13.87	13.93	13.93	99.1	99.51	99.52	101.56
18	17.94	17.91	47.98	99.68	99.5	99.9	101.84
							99.84
							99.98
							99.56
							101.36
RSD (%)				0.345	0.0968	0.349	0.9100
Mean±SD				99.61 ±0.344	99.49 ±0.096	99.56 ±0.347	100.66 ±0.9161
t-test				1.670	1.75	1.445	
F-test				1.230	0.0966	1.256	

3.5 Stoichiometry

The Job's continuous variation method for the determination of molar ratio between Cetirizine and dyestuffs [24] was followed. The solutions of Cetirizine and dyestuffs (BCG, BTB and TpOO) with same concentrations of 8 x 10^{-5}*M* each were mixed in varying the volume ratios such that the total volume of each mixture was maintained constant. The absorbance of each mixture solution was measured and plotted against the mole fraction of the drug (Fig 4). It is confirmed that 1:1 drug & dye molar ratio exists in all the complexes formed between Cetirizine and each BCG, BTB and TpOO. The formation constants [25, 26] were also determined and found to 1.54 x 10^6, 1.72 x 10^6 and 1.43 x 10^6 K M^{-1} for complexes with BCG, BTB and TpOO respectively.

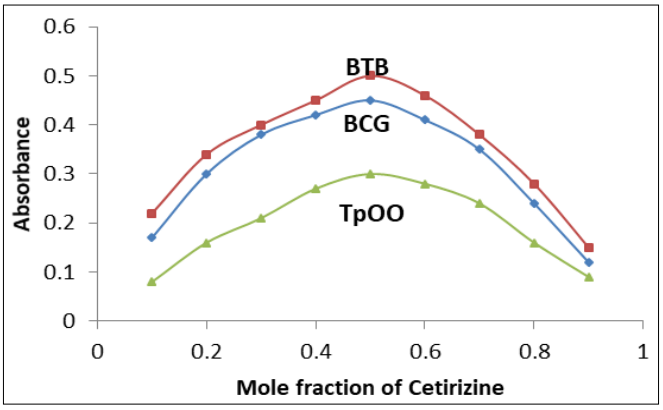


Fig 4: Continuous-variations study of drug-dye systems [Cetirizine] = [Dye] = 8x10^{-5}M

3.6 Optimization of the factors effecting the absorbance

The effect of pH on the absorbance of ion-pair complexes of Cetirizine with BCG, BTB and TpOO was studied using CH_3COONa-HCl buffer. It is evident from the Fig 5 that the absorbance of complexes with BCG, BTB and TpOO was found to be constant within the *p*H ranges 2.2-3.8, 2.0-3.0, and 2.0-3.0 respectively. Thus, all the absorbance measurements were made at *p*H 3.5, 2.5 and 2.5 with BCG, BTB and TpOO respectively.

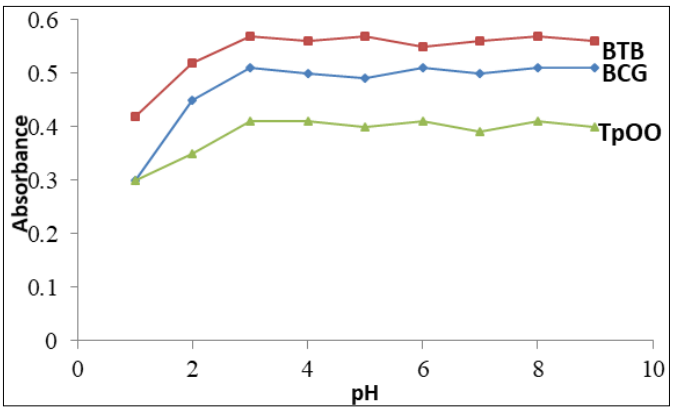


Fig 5: Effect of pH [Cetirizine] = 8µg ml^{-1}, [Dye] = 5ml of 0.025%

Different volumes of BCG, BTB and TpOO were added separately to a constant volume (8 µg ml^{-1}) of Cetirizine for studying the effect of concentration of dye on the absorbance of ion-pair complex. It is evident from Fig 6 that the absorbance gradually increases with the volume of dye upto 3.0 ml, beyond which no change in the absorbance was observed. Hence, in all the experiments carried out with Cetirizine for its determination, 5 ml of dye was used.

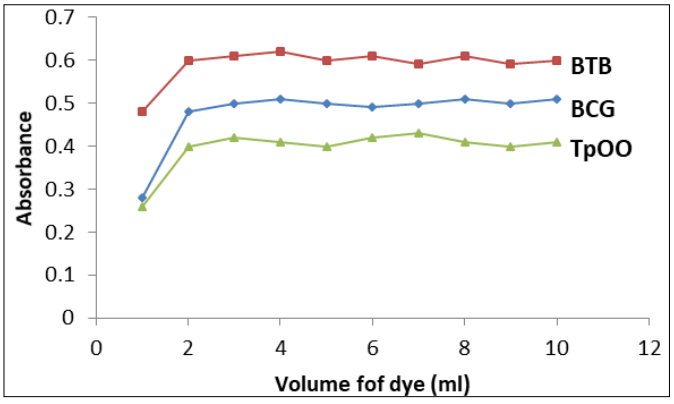


Fig 6: Influence of the volume of 0.025% dye [Cetirizine] = [8µg ml^{-1}]

3.7 Effect of foreign substances

The effect of the presence of foreign substances (excipients) along with Cetirizine has been studied choosing the concentration level at 8 µg ml^{-1}. Experiments on systems with 10 ml of sample and known amount of foreign substance were carried out adopting the procedures of proposed methods. The results of these experiments and tolerance limits were tabulated in Table 4. It is appropriate to mention that any interference by the common excipients found in tablets is completely ignored as the drug content from the powdered tablets was extracted into chloroform.

Table 4: Interference study in the estimation of Cetirizine

Sl. No	Excipients	Tolerance limit (µg ml^{-1})
1	Microcrystalline cellulose	86
2	Starch	148
3	Lactose	127
4	Povidone	55
5	Silicon dioxide	65
6	Titanium dioxide	48

3.8 Validation of the proposed method

The methods developed for the quantification of Cetirizine using dye stuffs viz., BCG, BTB and TpOO have been validated in terms of guidelines prescribed by ICH [27] for method validation. The terms mentioned in ICH *viz*. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation for the proposed methods were studied.

For comparison with a reference method, the student t-test and variance F-test were performed. The results of Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries are presented in Table 1. Six replicate determinations were carried out to test the precision of the proposed methods. It is found that the coefficient of variation was less than 1.2% for all the procedures.

The performance order of the developed methods is found to be BTB > BCG > TpOO. The results of the developed methods presented in Table 3 and Table 4 were compared to those achieved by reference method in terms of *t*-test at 95% confidence level. It is observed, in all the cases, that the results achieved by developed methods and those by reference methods were identical in terms of statistical data.

The results obtained by the proposed methods proved that these methods can be considered as standard methods. These methods are simple and sensitive with high precision and accuracy. Comparative t- and F-tests develop the confidence on the applicability of the methods in pharmaceutical formulations. The results obtained are satisfactorily accurate and precise as indicated by the excellent percent recovery. The optical parameters and statistical comparison validated these methods for application in routine analysis of Cetirizine in pure and dosage forms.

4. Conclusions

Bromocresol green, bromothymol blue and Tropaeolin OO can be used as analytical reagent for the estimation of Cetirizine. Cetirizine forms ion-pair complexes with these reagents in 1:1 proportion. The ion-pair complexes are extractable into chloroform and offer a basis for assay of the drug. The developed methods are simple, sensitive, and reproducible and can be used for routine analysis of Cetirizine in pure and formulation forms.

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