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Quantitative Determination of Few Commercial Drugs by Using NBS and Rhodamine-B Couple :A Spectrophotometric Study

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Abstract:
Simple, sensitive and accurate methods are developed for the spectrophoto metric determination of five drugs, *viz*., Cefixime, Cefoxitine sodium, Cefdinir, Guaifenesin and Levocetrizine based on their reactivity towards N-Bromosuccinamide(NBS) . The method involves addition of a known excess of N-bromosuccinimde to drugs in acidic medium (1*M* HCl) and the residual amount of oxidant (NBS) is estimated with Rhodamine-B dye. The absorbance was measured at 557 nm. These methods have been applied for the determination of above drugs in their pure form as well as in tablet formulations. The method has been validated in terms of guidelines of International Conference on Harmonization (ICH).

Key Words: Drugs, NBS, Rhodamine-B, Spectrophotometry, Quantification and Validation.

1. INTRODUCTION

Cefixime (CFX) (6R, 7R)-7[[2-(2-amino-1, 3-thiazole-4yl)-2-(carboxymethoxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-carboxylic acid, is third-generation cephalosporin antibiotic [1]. It is one of the important medicine for the treatment of antibacterial infections including gonorrhea, urinary-tract infections, otitis media, pharyngitis and lower respiratory-tract infections especially bronchitis. The mechanism of cefixime is stops bacteria by preventing cell wall synthesis. A literature survey has revealed several analytical methods were reported for the estimation of CFX like UV spectrophotometry [2-4], HPLC [5-6], RP-HPLC [7], HPTLC [8], Capillary electrophoretic method [9] and Voltammetry [10].

Cefoxitine sodium (CEFO) (6S, 7R)-3-(carbamoyloxy methyl)-7-methoxy-8-oxo-7-[(2-thiophen-2-ylacetyl) amino]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, is second generation cephalosporin antibiotic [11] and widely used as an antimicrobial agent. It has broad spectrum activity against Gram positive and Gram negative bacteria. Many methods were reported for its estimation of CEFO such as HPLC [12-13], colorimetry [14-15], Fluorimetry [16], spectrophotometry [17-18] and LC-MS [19].

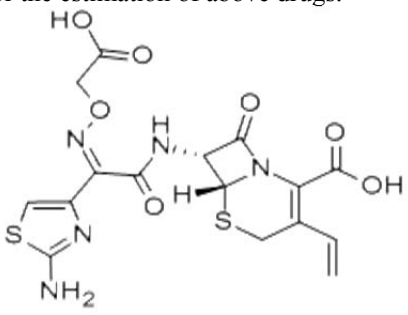
Cefdinir (CFD) 8-[2-(2-amino-1, 3-thiazol-4-yl)-1-hydroxy-2-nitroso-ethenyl] amino-4-ethenyl7-oxo-2-thia-6-azabicyclo [4.2.0] oct-4-ene-5-carboxylic acid, is third generation cephalosporin antibiotic [20] used for ear infections, soft tissue infections, respiratory tract infections, including sinusitis, strep throat and community-acquired pneumonia. Many methods have been developed for the estimation of CFD including HPTLC [21], UV Spectrophotometry [22-23], RP-HPLC [24-25] and LC-MS [26].

Guaifenesin (GUA) (RS)-3-(2-methoxyphenoxy) propane-1, 2-diol, is an expectorant and used to reduce chest congestion caused by the common cold, infections, or allergies [27] .It may help control symptoms but does not treat the cause of symptoms or speed recovery. Several methods have been reported in literature for the estimation of GUA such as RP-HPLC [28-31], UV Spectrophotometry [32-35], LC-MS [36] and Voltammetry [37].

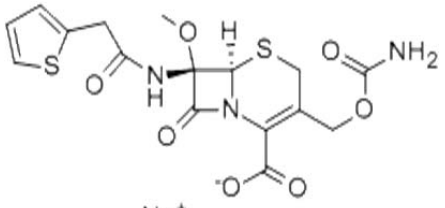
Levocetrize (LEV) 2-[2-[4-[(R)-(4-Chlorophenyl)-phenyl methyl] piperazin-1-yl] ethoxy] acetic acid dihydrochloride, is a third generation non-sedative antihistamine [38]. It is an R-enantiomer of racemic cetirizine and works by blocking histamine receptors. Levocetrizine is selective, potent, H1-antihistamine compound indicated for the treatment of allergic rhinitis and chronic idiopathic urticarial. A literature survey has revealed UV Spectrophotometry [39-41], HPLC [42-44] and LC-MS [45] methods were developed for estimation of LEV.

Structures of five drugs are shown in Fig. 1.

Through survey of literature on the above mentioned drugs revealed that quantification based on use of NBS as oxidizing reagent and Rhodamine-B as analytical reagent have not been yet reported. The present work is an attempt to develop accurate, simple, sensitive and cost effective methods for the estimation of above drugs.



Cefixime



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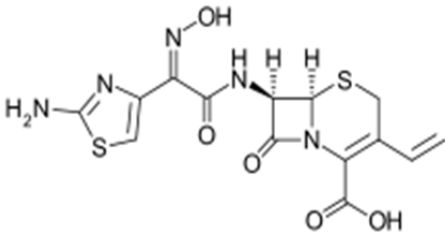
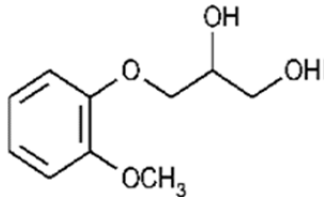
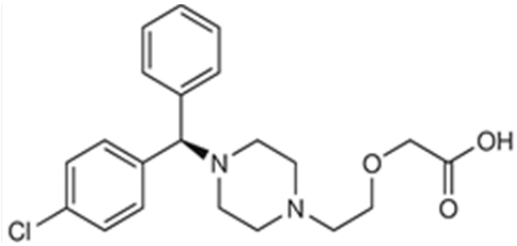


Cefdinir



Guaifenesin



Levocetrizine

Fig 1 Structures of five drugs

2. EXPERIMENTAL

2.1 Instrumentation

All absorption spectra were recorded on Elico SL 210 UV-Visible Double beam spectrophotometer as well as on Thermo Nicolet 1000 single beam spectrophotometer using matched pair of Quartz cells of 10mm path length. A high precision analytical balance was used for weighing the reagents.

2.2 Materials and Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade and all solutions are prepared afresh every day. Double distilled water was used throughout the investigation.

An approximately 0.01*M* NBS stock solution was prepared by dissolving N-bromo succinimide (Himedia Laboratories pvt. Ltd, Mumbai) in 100 ml standard flask with double distilled water. The solution was kept in an amber colored bottle and was further diluted with distilled water appropriately to get 70 µg mL^{-1}.

A 500 µg mL^{-1} of Rhodamine-B was prepared by dissolving the dye (S.D. Fine Chem. Ltd., Mumbai) in 100 ml standard flask with double distilled water. The dye solution was further diluted to get 50 µg mL^{-1}.

Concentrated Hydrochloric acid (S.D. Fine Chem., Mumbai, India) was diluted appropriately with double distilled water to get 1 *M* HCl.

Pharmaceutical grade drugs were kindly supplied by Hetero Drugs Pvt. Ltd. Hyderabad. A stock standard solution of drugs were prepared by dissolving accurately weighed 20 mg of drug transferred in 100ml volumetric flask and made up to mark with distilled water. The solution was diluted stepwise to get required concentrations.

2.3 Method development

Aliquots of pure drug solution (1 to 7 mL) were transferred into a series of 10 mL calibrated flasks. To each flask, 1 mL of HCl was added, followed by 1 mL of NBS solution (70 µg mL^{-1}). The contents were mixed and they were set aside for 10 min under occasional shaking. Finally, 1 mL of Rhodamine- B solution (50 µg mL^{-1}) was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 557 nm against a reagent blank after 10 min.

2.4 Construction of Calibration Curves

The calibration curve was plotted by taking concentration (µg mL^{-1}) of the drugs in X-axis and absorbance in Y-axis. The calibration curves were constructed by taking absorbance data in six replicate experiments. The absorbance to concentration called relative response is calculated. Those points falling between 95% to 105% of the average relative response are only considered for construction of calibration .The linearity graphs are shown in Fig. 2.

2.5 Accuracy and Precession studies

Accuracy of the methods developed are determined from the recovery studies on pure drug sample. At least four known concentration of solutions of drugs in Beer's law limit were taken and recovery studies were performed. Excellent recovery showed the validity of the calibration curves for each drug.

Precession of the method is demonstrated by repeating experiment (n=6) and %RSD is worked out. %RSD being less than 2 in each case speaks the high precession of the methods. Accuracy and precession values of pure drug samples are given in table 2.

2.6. Analysis of Pharmaceutical preparations

Three tablets of (Suprax-200mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of cefixime was transferred in 100ml volumetric flask and made up to mark with water. And the solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration.

One vial of (CEFO-1gr) was reconstituted with 10mL of water for injection or as per labeling to get 95mg/mL of cefoxitin. Then entire contents was withdrawn from the vial using a suitable calibrated Hamilton syringe & transferred

in to 100mL volumetric flask and diluted to volume with double distilled water and mixed well. The solution was filtered through whatman No.42 filter paper. It was further diluted to get required concentration for the analysis of the drug.

Two tablets of (Omnicef-300mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of cefdinir was transferred in 100ml volumetric flask. The solution was shaken well for 15 minutes and filled with water up to mark. The solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration.

Three tablets of (Guaifenesin-2000mg) were weighed and ground in to fine powder. The powder equivalent to10mg of guaifenesin was transferred in 100ml volumetric flask and made up to mark with water. The solution was filtered through Whatman No.42 filter paper. It was further diluted to get required concentration for the analysis of the drug.
Twenty tablets of (Xyzal-5mg) were weighed and ground in to fine powder. The powder equivalent to10mg of levocetrizine was transferred in 100ml volumetric flask and made up to mark with water. The solution was filtered through Whatman No.42 filter paper. It was further diluted to get required concentration for the analysis of the drug.
The drug solutions obtained from tablet formulations were subjected to oxidation by excess NBS and subsequent determination of NBS and Rhodamine-B was carried out. The concentration of the tablet solutions falling in Beer's law limit were selected for the assay of drug in the tablet. An excellent tally between the concentration of drugs taken and found indicated the applicability of the methods for formulations.

3. Results And Discussions

The proposed spectrophotometric methods are indirect and are based on the determination of the excess of NBS after allowing the oxidation reaction to complete in acidic medium. The excess of NBS was determined by reacting it with a fixed amount of Rhodamine-B dye. The NBS is capable to oxidize drugs and readily bleach the colour of dye. The absorbance λ max (557nm) increased linearly with increasing concentration of a given drug.
Acidic medium (Hydrochloric acid) was found to be a convenient medium for this method. For a quantitative reaction between drug and NBS, a contact time of 10 min was found sufficient.

4. Analytical Data

Under optimum conditions a linear correlation was found between absorbance at λ max and concentration of all drugs in the ranges given in table 1. Sensitivity parameters such as molar absorptivity, Sandell sensitivity are also presented in Table 1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and is also given in table 1.
The LOD and LOQ were determined based on the standard deviation of the y-intercept and the slope of the calibration curves and presented in table1.

4.1 Linearity and Range

The linearity of the analytical procedure is its ability to obtain the best results which is directly proportional to the concentration of analyte in the sample. The calibration curves of Cefixime, Cefoxitine sodium, Cefdinir, Guaifenesin and Levocetrizine by the proposed method were found to be linear of the ranges of 1.6-11.2 µg mL^{-1}, 2-14 µg mL^{-1}, 1.2-8.4µg mL^{-1}, 2.4-16.8 µg mL^{-1} and 2-14 µg mL^{-1}.

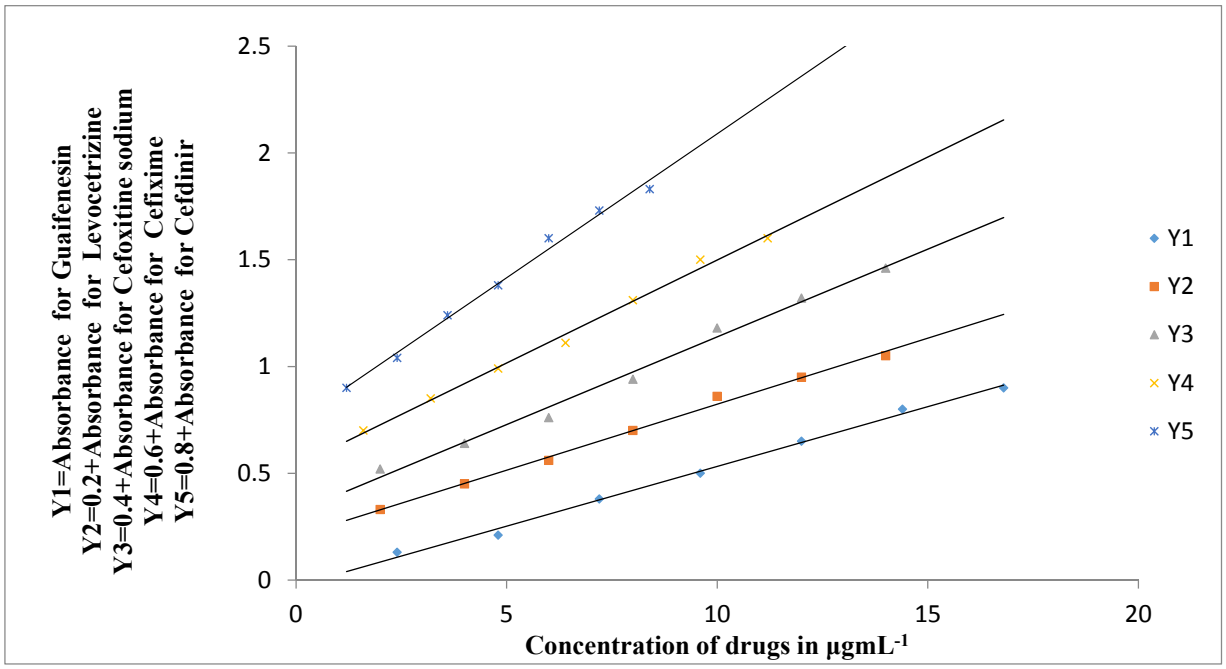


Fig 2 Construction of calibration curves for five drugs

Table 1: Analytical and regression parameters of spectrophotometric method

Parameter	CFX	CEFO	CFD	GUA	LEV
λmax, nm	557	557	557	557	557
Beer's law limits μg mL^{-1}	1.6-11.2	2-14	1.2-8.4	2.4-16.8	2-14
Molar absorptivity, L mol^{-1} cm^{-1}	2.85×10^4	27.5×10^4	3.33×10^4	1.23×10^4	26×10^3
Sandell sensitivity μg cm^{-2}	0.104	0.123	0.769	0.017	0.163
Limit of detection μg mL^{-1}	0.410	0.553	1.177	0.701	0.96
Limit of quantification μg mL^{-1}	0.125	1.67	3.56	1.964	2.92
Intercept, (a)	-0.0657	-0.0857	-0.0586	-0.0271	0.0057
Slope, (b)	0.0964	0.0811	0.1348	0.056	0.0618
Correlation coefficient, (r)	0.994	0.9951	0.992	0.995	0.9952
Standard deviation of intercept (Sa)	0.0012	0.0136	0.0481	0.0119	0.0181
Regression equation, Y	0.096X-0.065	0.081X-0.085	0.138X-0.058	0.056X-0.027	0.061X+0.005

X=Concentration of drug

Table 2: Determination of accuracy and precision of the methods on pure drug samples

Drug	Taken (μg mL^{-1})	Found (μg mL^{-1})	Er (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
CFX	2.0 4.0 6.0	2.0 4.03 5.99	0.00 0.75 0.16	100.00 100.75 99.83	0.488	100.19 ±0.489
CEFO	3.0 5.0 7.0	3.02 5.01 7.0	0.66 0.20 0.00	100.66 100.2 100.00	0.337	100.28 ±0.338
CFD	1.5 3.0 4.5	1.53 3.02 4.5	2.00 0.66 0.00	102.00 100.66 100.00	1.01	100.88 ±1.01
GUA	3.0 6.0 8.0	3.01 5.97 8.01	0.33 0.50 0.12	100.33 99.5 100.1	0.425	99.97 ±0.428
LEV	3.0 5.0 7.0	2.96 5.0 7.01	1.33 0.00 0.14	98.66 100.00 100.14	0.820	99.6 ±0.817

Table 3: Results of assay of tablets by proposed method and statistical evaluation

Tablet	Drug in tablet (μg mL^{-1})	Drug Found (μg mL^{-1})	Er (%)	Recovery (%)	RSD (%)	Reference method Mean± SD	Proposed method Mean ± SD	t-test	F-test
Suprax-200mg (CFX)	2 4 6	1.98 4.02 6.03	1.00 0.50 0.50	99.00 100.5 100.5	0.86	99.94±0.39	100±0.86	0.129	4.861
CEFO-1gr (CEFO)	3.5 5.5 7.5	3.47 5.5 7.49	0.85 0.00 0.13	99.14 100.00 99.86	0.46	100.57±0.80	99.66±0.46	-1.77	0.328
Omnicef-300mg (CFD)	4 6 8	4 5.96 8.03	0.00 0.66 0.37	100.00 99.33 100.37	0.52	99.15±0.51	99.9±0.52	1.918	1.03
Guaifenesin-2000mg (GUA)	5 7 9	5.01 6.98 9.04	0.20 0.28 0.44	100.2 99.71 100.44	0.36	99.63±0.30	100.11±0.37	2.171	1.51
Xyzal-5mg (LEV)	4.5 6.5 8.5	4.49 6.45 8.51	0.22 0.76 0.11	99.77 99.23 100.11	0.44	99.38 ±0.56	99.7±0.44	0.966	0.616

***Average of four determinations**

5. CONCLUSION

The proposed method was found to be very simple, rapid and cost effective than some of the reported methods. The method is suitable for the determination of above drugs in tablet formulation without interference from commonly used excipients. The solvent used for this method are inexpensive and simple to prepare, and could be used in a quality control laboratory for routine drug analysis. Hence this method can be valid for application in laboratories lacking liquid chromatographic instruments.

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