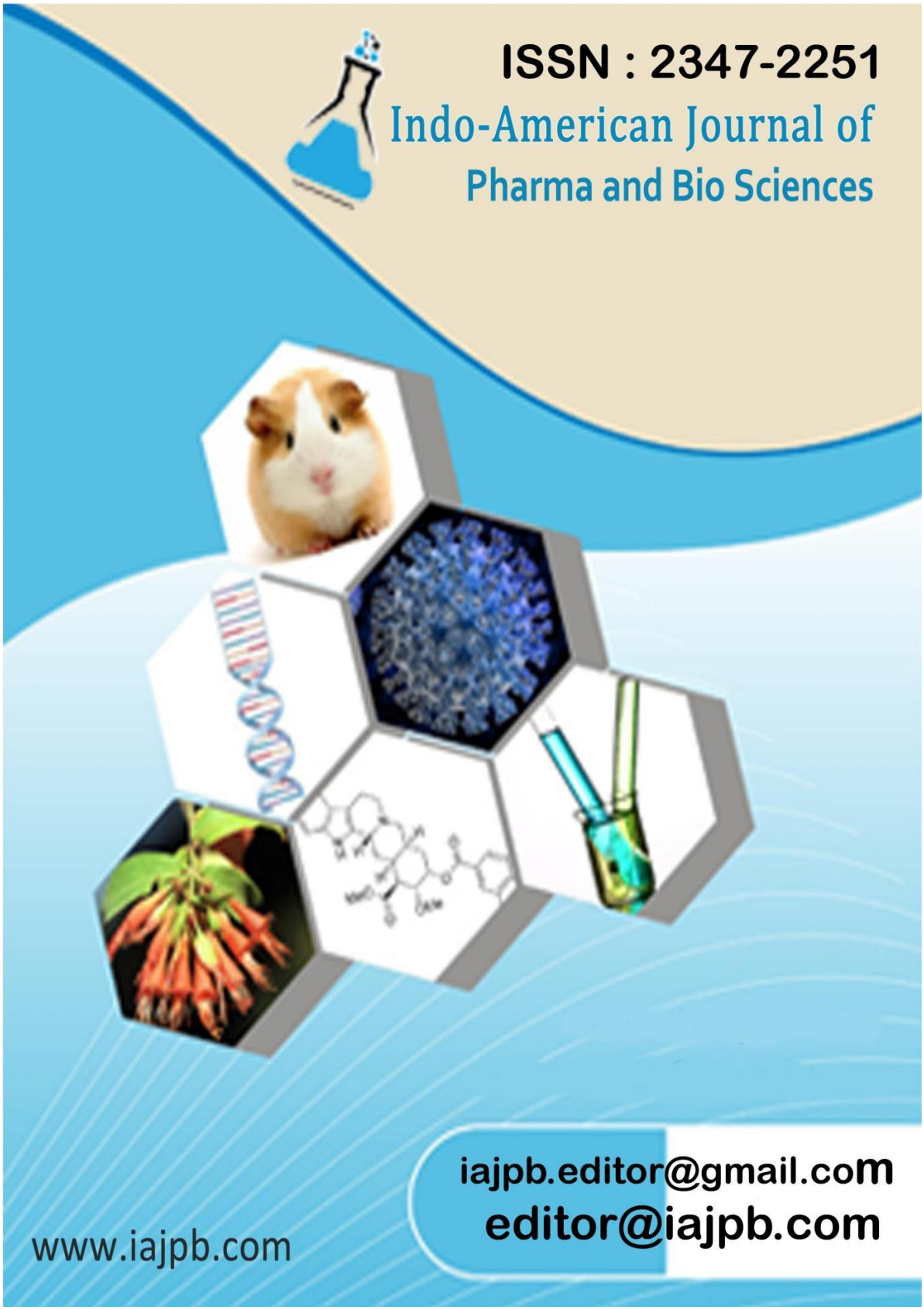
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Creation and Use of the Spectrophotometric Method for Cefacolor Determination in Pharmaceutical Formulations

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Abstract

A simple, fast and sensitive spectrophotometric method for the determination of cefaclor in pharmaceutical raw and dosage forms based on reaction with ninhydrin is developed, optimized and validated. The purple color (Ruhemenn's purple) that resulted from the reaction was stabilized and measured at 560 nm. Beer's law is obeyed in the concentration range of 4-80 µg mL^{-1} with molar absorptivity of 1.42×10^5 L $mole^{-1}$ cm^{-1}. All variables including the reagent concentration, heating time, reaction temperature, color stability period, and cefaclor/ninhydrin ratio were studied in order to optimize the reaction conditions. No interference was observed from common pharmaceutical adjuvant. The developed method is easy to use, accurate and highly cost-effective for routine studies relative to HPLC and other techniques.

Keywords: spectrophotometric; Cefaclor; pharmaceutical formulations.

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INTRODUCTION

Cefaclor belongs to the family of antibiotics known as the cephalosporins (cefalosporins). The cephalosporins are broad-spectrum antibiotics which are used for the treatment of septicaemia, pneumonia, meningitis, biliary-tract infections, peritonitis, and urinary-tract infections. The pharmacology of the cephalosporins is similar to that of the penicillins, excretion being principally renal. Cephalosporins penetrate the cerebrospinal fluid poorly unless the meninges are inflamed. Cefaclor is active against many bacteria, including both Gram-negative and Gram- positive organisms.[1,2]

Numerous analytical procedures have been reported for the determination of cefaclor in its pure form, in pharmaceutical prepa- rations and in biological fluids. The USP XXII recommends the hydroxylamine colorimetric assay.[3] HPLC methods were described for the determination of cefaclor in human plasma and urine.[4,5] Also iodometric[6] and polarographic[7] determination of cefaclor has been reported. A spectrofluorimetric method is described for the determi- nation of cefaclor in formulations and biological fluids.[8] Only two spectrophotometric methods[9,10] are available for the determination of cefaclor in pharmaceutical raw and dosages forms. The first method is less sensitive, while the second involves the degradation of the product.

Many of these methods are either not sensitive enough or require complicated and expensive instruments, or are subjected to interferences from other ions, suffer from narrow calibration range, toxicity of the reagents used, or more or less are time consuming.

The proposed method in the present paper offers the advantages of simplicity, rapidity and specificity without the need of extraction or degradation having higher selectivity range than most of the existing spectrophotometric methods. The aim of this work is to develop fast, sensitive, accurate and extraction free spectrophotometric method for the routine analysis of cefaclor in pharmaceutical quality control laboratories.

EXPERIMENTAL

Apparatus

All spectrophotometric measurements were carried out using a spectrophotometer (U 1100 Hitachi, Japan) with silica glass cell of 1 cm thickness. Officially calibrated Pyrex glassware was used throughout this study.

Materials

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Lahore Chemical and Pharmaceutical Works, Lahore, Pakistan supplied pure cefaclor. Standard stock solution of cefaclor was prepared by dissolving 50 mg pure drug in 30 mL of phosphate buffer solution of pH 8 and diluted to the mark in 100 mL calibrated flask with same solvent. In the same manner, a stock solution of 2×10^{-3} mol L^{-1} of drug and ninhydrin were also prepared. These solutions are stable for a period of 3 d when refrigerated (4 °C). A 1 mol L^{-1} phosphate buffer solution of pH 8.0 was prepared by dissolving 13.61 g of potassium dihydrogen phosphate (Merck) in a 100 mL measuring flask and made up to volume with distilled water. The pH was adjusted by 0.1 mol L^{-1} solution of sodium hydroxide.

Recommended analytical procedure

Different aliquots of standard stock solution equivalent to 4-80 µg mL^{-1} were transferred into a series of 10 mL volumetric flasks, 2 mL of ninhydrin was added and the mixture was heated in a water bath at 80 ± 5 °C for 15 min. The flasks were cooled and the volume was made up to the mark with phosphate buffer. The absorbance was measured at 560 nm against a reagent blank.

Analysis of dosage forms

Capsules

10 capsules were accurately weighed; the contents of the capsules were removed as completely as possible and mixed. The capsule shells were cleaned and accurately weighed, and the net weight of the capsule contents was calculated. An accurately weighed portion of the mixed capsule contents equivalent to 50 mg cefaclor was trans- ferred into 100 mL volumetric flask. The mixture was sonicated for few minutes, diluted to volume with the phosphate buffer, mixed and filtered through Whatman filter paper 41. A portion of the filtrate was transferred into 10 mL volumetric flask and used for the determination of the drug content following the procedure described above.

Suspensions

A quantity of powder equivalent to 50 mg of cefaclor was trans- ferred into a 100 mL calibrated flask, dissolved in phosphate buffer, and manipulated as described in the analysis of capsules.

Determination of molar ratio of the reactions

The Job's method of continuous variation[11,12] was employed to establish the stoichiometry of the colored product. A 2×10^{-2} mol L^{-1} standard solution of cefaclor and a 2×10^{-2} mol L^{-1} solution of ninhydrin were used. A series of solutions was prepared in which the total volume of drug and reagent was constant (2.0 mL). The reagents were mixed in various proportions (0:10, 1:9, . . . 9:1, 10:0) and dilu- ted in a 10 mL calibrated flask with Phosphate buffer. The reactions were allowed to proceed at (80 ± 5 °C). The reaction mixtures were completed to volume with phosphate buffer, and the absorbance was measured at 560 nm against reagent blank

RESULTS AND DISCUSSION

Ninhydrin is a well-established reagent for the determination of cer- tain amines, amino acids and thiophenes.[13] It has been extensively used in the determination of the compounds of pharmaceutical importance.[14-16]

Reaction involved and optimization of reaction conditions

It was suggested that the reactions of ninhydrin with amine, amino acids and imino acids all proceed by the same mechanism to give diketohydrindylidene-diketohydrindamine or the Ruhemann's purple.[17] This compound would further react with amino group of cefaclor to give the product, which absorb maximally at 560 nm as shown in Figure1. The optimum conditions for determination of cefaclor were established.

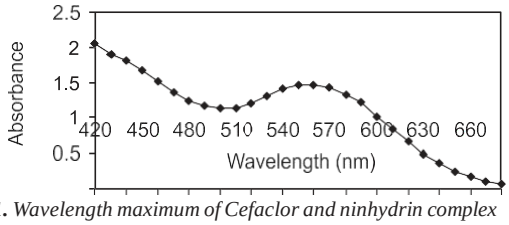


Figure 1. *Wavelength maximum of Cefaclor and ninhydrin complex*

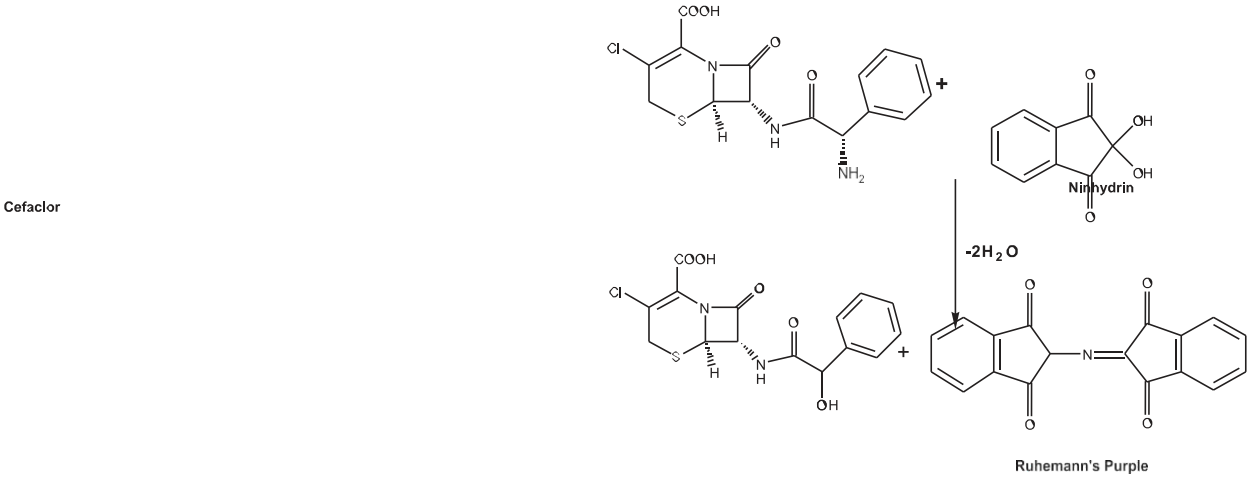
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Cefaclor reacts with ninhydrin reagent in phosphate buffer me- dium via oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored reaction product Ruhemenn's purple with λ_{max} at 560 nm (Scheme 1). To optimize the reaction conditions, we have investigated a number of parameters such as heating time, reagent concentration, temperature, pH, stability of color and solvent. Varying one variable and observing its effect on the absorbance of the colored product established the optimum reaction conditions.

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Stoichiometry of the colored product

The molar ratio of the colored product formed between cefaclor and ninhydrin reagent was investigated by applying the continuous variation Job's method using an equimolar solution of the drug and the reagent. The result indicated that the colored product was formed at the 1:2 ratio shown in the reaction Scheme 1 and Figure 2.



Scheme 1. *Proposed reaction between cefaclor and ninhydrin*

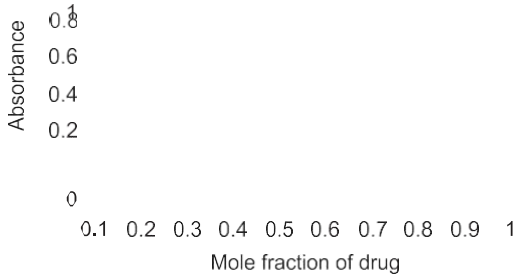


Figure 2. *Continuous variation plot for the reaction of Cefaclor and ni- nhydrin*

Effect of heating time

A 4.0 mL aliquot of a 100 µg mL^{-1} cefaclor solution was mixed with 2 mL of ninhydrin solution. The reaction mixture was heated on a water bath at 80 ± 5 °C. A colored product was obtained and the color intensity reached its maximum value after 15 min of heating. After reaching the ambient temperature, the reaction mixture was transferred to a 10 mL volumetric flask and diluted to the mark with pH 8.0 phosphate buffer. Hence, the absorbance was measured after 15 min of heating. The results are shown in Figure 3.

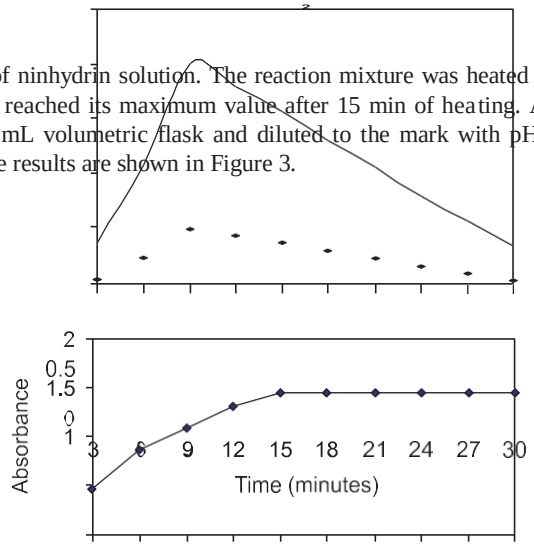


Figure 3. *Effect of heating time on color development*

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Effect of ninhydrin concentration

To 4 mL of 100 µg mL^{-1} cefaclor solution, different volumes (0.5-4 mL) of 0.2 % ninhydrin were added. The reaction mixtures were heated for 15 min on a water bath at 80 ± 5 °C. The colored product was di- luted to 10 mL with phosphate buffer of pH 8 and the absorbance was measured against a reagent blank at 560 nm. The results showed that the highest absorbance was obtained with 2 mL of ninhydrin solution that remained unaffected with higher amounts (Figure 4).

Figure 6. *Beer's law verification range*



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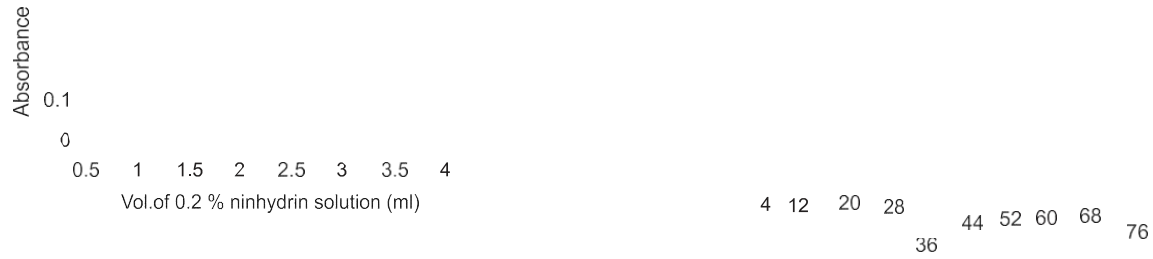


Figure 4. *Effect of reagent concentration on color development*

Effect of temperature

To 4 mL of 100 µg mL^{-1} cefaclor solution, 2 mL of ninhydrin were added. The reaction mixtures were heated for 15 min on a water bath at 20-100 °C. The colored product was diluted to 10 mL with phosphate buffer of pH 8 and the absorbance was measured against a reagent blank at 560 nm. The results showed that the highest ab- sorbance was obtained at 80 ± 5 °C (Figure 5). The developed color was stable for 2 h.

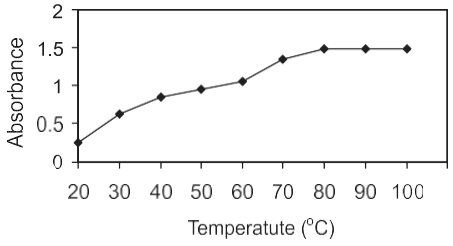


Figure 5*. Effect of temperature on color development*

Effect of pH on color development

The ninhydrin reacts with cefaclor in basic medium. It is reported that pH effect under certain conditions on the ninhydrin reaction may have a fluctuating effect.[18] In our study, sample absorbance increased with an increase of the pH of the sample solution from 7.0 to 8.0, thus, phosphate buffer with pH 8.0 was selected for further studies.

Effect of solvents

Different dilution solvents such as water, ethanol, methanol, isopropanol, acetone, dioxan and acetonitrile have been tried, but water gave the best results.

Analytical parameters of proposed method

Beer's law limit, molar absorptivity, detection limit and correlation coefficient were obtained by least square treatment of results. The linear relationship was found between absorbance at λ_{max} and concentration of drug in the range 4-80 µg mL^{-1} (Figure 6). High values of Correla- tion coefficient (r=0.9998) and small value of intercept validated the linearity of calibration curve and obedience to Beer's law.

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The apparent molar absorptivity and Sandell sensitivity values together with the limits of detection (LOD) and quantification (LOQ) compiled in Table 1 are indicative of the high sensitivity of the propo- sed method. The LOD and LOQ were calculated using LOD = 3.3 s/S and LOQ = 10 s/S, where s is the standard deviation of seven blank determinations and S is the slope of the calibration curve.

Table 1. Spectral data for the reaction of cefaclor with ninhydrin

Parameters	Values
λ_{max} (nm)	560
Beer's law limits (µg mL^{-1})	4-80
Sandell sensitivity (µg cm^{-2})	2.7×10^{-2}
Detection limit (µg mL^{-1})	1.61
Quantification limit (µg mL^{-1})	5.31
Molar absorptivity (L $mole^{-1}$ cm^{-1})	1.42×10^{5}
Regression equation, Y[a]	
Slope (b)	3.69×10^{-2}
Intercept (a)	-1.12×10^{-2}
Correlation coefficient (r)	0.9998

$Y^a = a + bX$ Where Y is the absorbance in a cell of 1cm path length and X is concentration in µg mL^{-1}

Application of proposed method

Commercially available capsules and suspensions were success- fully analyzed for the cefaclor content by the proposed method. The assay results are presented in Table 2. As can be seen from the Table 2, the results obtained agreed with the label claim and also those of the reference method.[19] The performance of the proposed method was judged further by the Student's t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the method is as accurate and precise as the reference method. The accuracy and validity of the proposed method was further ascertained by performing recovery studies. The pre-analyzed capsule powder/suspension solution was spiked with pure cefaclor at three levels and the total was found by the proposed method. Each determination was repeated three times. The results summarized in Table 3 reveal good recoveries of pure drug added and non-interference from capsule excipients such as magnesium stearate, lactose and talc and suspension excipients such as sucrose, cellulose gum, aerosil, citric acid, and sodium benzoate.

The proposed method is favorably compared with other existing spectrophotometric methods as shown in Table 4.

Interference liabilities

Table 2. Results of Analysis of commercial formulations containing cefaclor and statistical comparison with reference method[19]

Brand name and dosage form	Label claim	% found ± SD		t-test	F-test
		Proposed method	Reference method		
Capsules					
Ceclor[a]	250 mg	101.5 ± 2.5	100.9 ± 1.8	1.61	2.17
Cefanol[b]	250 mg	99.6 ± 1.8	99.6 ± 2.1	1.11	4.10
Cefalor[c]	250 mg	100.8 ± 2.3	100.2 ± 3.2	1.72	1.13
Suspensions					
Ceclor	125mg/mL	100.4 ± 2.3	101.1 ± 1.9	2.40	3.25
Cefanol	125mg/mL	101.2 ± 1.7	100.8 ± 2.1	0.50	1.48
Cefalor	125mg/mL	99.8 ± 2.8	100.2 ± 1.3	0.46	2.13

Percent found is the mean value of five determinations. Marketed by: aAli Gohar Pharmaceuticals. bAbbott Laboratories. cBosch Pharmaceuticals.

Table 3. Results of recovery study by standard addition method

Formulation and dosage form	Cefaclor in sample (mg)	Pure cefaclor added (mg)	Total cefaclor found (mg)	Pure cefaclor recovered (%)
Ceclor	250	5	255.4	100.1
Capsules	250	10	260.6	100.2
(250 mg)	250	15	264.8	99.92
Cefanol	125	10	134.9	99.93
Suspension	125	20	145.5	100.3
(125 mg/mL)	125	30	155.2	100.1

Percent recovered is the mean value of three determinations

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Table 4. Comparison of the proposed method with existing spectrophotometeric methods

S. No.	λ_{max} (nm)	Beer's law range µg mL^{-1}	Molar absorptivity L mol^{-1} cm^{-1}	Reagent	Ref.
1	520	40-400	0.84	Chloranilic acid	9
2	340	1.8-55	-	Degradation with phosphate buffer of pH 10	10
3	560	4-80	1.42×10^5	Ninhydrin	Present work

Table 5. Percent recovery of the cefaclor in the presence of various excipients

Excipients	Amount taken (µg mL^{-1})	% Recovery ± RSD (n =5)
Magnesium stearate	60	100.2 ± 0.45
Lactose	300	99.21 ± 0.36
Talc	60	99.45 ± 0.25
Sucrose	100	100.4 ± 0.85
Cellulose gum	90	101.2 ± 0.71
Aerocil	100	99.31 ± 0.15
Citric acid	80	99.9 2 ± 0.40
Sodium benzoate	70	99.56 ± 0.36

maceutical dosage forms, interference liabilities were carried out to explore the effect of common excipients that might be added during formulations. Samples were prepared by mixing known amount (20 µg mL^{-1}) of the drug with various amounts of the common excipients: Magnesium stearate, Lactose, Talc, Sucrose, Cellulose gum, Aerocil, Citric acid and Sodium benzoate. The analysis of these laboratory prepared samples was carried out using the recommended procedure, and the recovery values were determined. No interference was found from these excipients and the recovery values were 99.21-101.2 % (Table 5). This indicated the absence of interference liabilities from these excipients.

CONCLUSION

The data given above reveal that the proposed method is simple, accurate and sensitive with good precision and accuracy. With this method one can do rapid analyses at low cost without losing accu- racy. The proposed method can be used as alternative method to the reported ones for the routine determination of cefaclor in pharma-ceutical raw and dosage forms. This encourages its successful use in routine analysis of cefaclor in quality control laboratories.

REFERENCES

1. Hebert, A.; Sigman, E.; Levy, M.; *J. Am. Acad. Dermatol.* **1991**, *25* , 805.
2. Parra, F.; Igea, J.; Martín, J.; Alonso, M.; Lezaun, A.; Sainz, T.; *Allergy* **1992**, 47, 439.
3. Mays, D. L.; Bangert, F. K.; Cantrell, W. C.; Evans, W. G.; *Anal. Chem.* **1975**, 47, 2229.
4. Kovach, P. M.; Lantz, R. J.; Brier, G.; *J. Chromatogr*. **1991**, *567*, 129.
5. Nakata, M. C.; *J. Chromatogr*. **1982**, 228, 429.
6. Yamana, T.; Tsuji, A.; *J. Pharm.* Sci. **1976**, 65, 1563.
7. Rodrigues, L. N. C.; Zanoni, M. V. B.; Fogg, A.G.; *J. Pharm.Biomed. Anal*. **1999**, *21*, 497.
8. Aly, F. A.; Hefnawy, M. M.; Belal, F.; *Anal. Lett*. **1996**, *29*, 117.
9. Saleh, G. A.; Askal, H. F.; Darwish, I. A.; El-Shorbagi, A. N.; *Anal. Sci.* **2003**, *19*, 281.
10. Ivama,V. M.; Rodrigues, L. N. C.; Guaratini, C. C. I.; Zanoni, M. V. B.; *Quim. Nova* **1999**, *22*, 201.
11. Job, P.; *Anal. Chem.* **1936**, *16*, 97.
12. Job, P.; *Advanced Physicochemical Experiments*, 2nd ed., Oliner and Boyd: Edinburgh, 1964, p. 54.
13. Fiegel, F.; *Spot Tests in Organic Analysis*, Elsevier Publishing Co.: London, 1960.
14. Raza, A.; Ansari, T. M.; Rehman, A.; *J. Chin. Chem. Soc*. **2005**, *52*, 1055.
15. Raza, A.; Ansari, T. M.; Rehman, A.; *Anal. Sci.* **2005**, *21*, 1133.
16. Rahman, N.; Singh, M.; Hoda, M. N.; *J. Braz. Chem. Soc*. **2005**, *16*, 1001.
17. McCaldin, D. J.; *Chem. Rev*. **1960**, *60*, 39.
18. Sheng, S.; Kraft, J. J.; Schuster, S. M.; *Anal. Biochem*. **1993**, *211*, 242.
19. *British Pharmacopoeia*, HM Stationary Office: London, 2003, p. 267.