Full Length Research Paper

Spectrophotometric determination of some cephalosporin antibiotics using Prussian blue reaction

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A simple, sensitive and accurate spectrophotometric method of analysis of ceftriaxone, cefotaxime and cefuroxime in pharmaceutical dosage forms has been developed and validated. The method is based on the formation of Prussian Blue (PB) complex. The reaction between the acidic hydrolysis product of the antibiotics (T = 70°C) with the mixture of Fe^{3+} and hexacyanoferate (III) ions was evaluated for the spectrophotometric determination of the antibiotics. The maximum absorbance of the coloured complex occurred at λ = 700 nm and the molar absorptivity is 3.0×10^4 l.mol^{-1} cm^{-1}. Reaction conditions have been optimized to obtain PB complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the PB complex were found to increase linearly with increase in concentrations of ceftriaxone, cefotaxime and cefuroxime, which corroborated with the correlation coefficient values. The linear range of the calibration graph was 2 - 20 µg/ml for ceftriaxone and cefotaxime and 2 - 18 µg/ml for cefuroxine. The proposed method was successfully applied to the determination of the selected antibiotics in bulk drugs and pharmaceutical formulations and the results obtained agree well with the labeled contents.

Key words: Cephalosporins, β-lactam antibiotic, spectophotometric, Prussian Blue.

INTRODUCTION

Ceftriaxone, cefotaxime and cefuroxime are among the cephalosporin antibiotics widely used in contemporary clinical practice. These drugs have been found very useful in pre and post operative chemotherapy against infections in abdominal, pelvic, orthopaedic, cardiac, pulmonary, oesophageal and vascular surgery (Gerald and Merl, 1990). Owing to this strategic importance, various spectophotometric (Reddy et al., 2002; Issopoulos, 1989, 1996) and chromatographic (Hartman and Rodiger, 1976; Coman et al., 2003; Eric-Jovanovic et al., 1998; Nabi et al., 2004) methods for their assay have been reported. Some of these methods, however, are not easily adaptable in developing countries with poorly equipped laboratories.

The β-lactam ring present in these drug molecules has been shown to be enormously liable to nucleophilic atta-

ck in presence of acid and alkali or even neutral molecules. Several methods for quantitative estimation of β-lactam antibiotics have been based on the measurement of colour reaction of their degradation products and are used as well accepted methods (Deshpande et al. 2004). This property has been exploited in the present investigation.

We report here the formation and application of Prussian Blue (PB) complex in the development of a sensitive spectrophotometric method for the determination of the mentioned antibiotics. The formation of PB Complex is a classical qualitative test used to detect Fe (II) using hexacyanoferate (III) (Brown and Lemay, 1988). Farhadi et al. (2002) employed this test for the quantitative estimation of ampicillin, amoxicillin and cefazoline in pharmaceutical preparations. Acid hydrolysis of β-lactam antibiotics has been shown Figure 1 to produce penicilloaldehyde as the ultimate degradation product (Deshpande et al., 2004). This compound has aldehyde functional group and thus capable of converting Fe (III) to Fe (II); the latter reacts

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(a) Ceftriaxone sodium

I

(b) Cefotaxime sodium

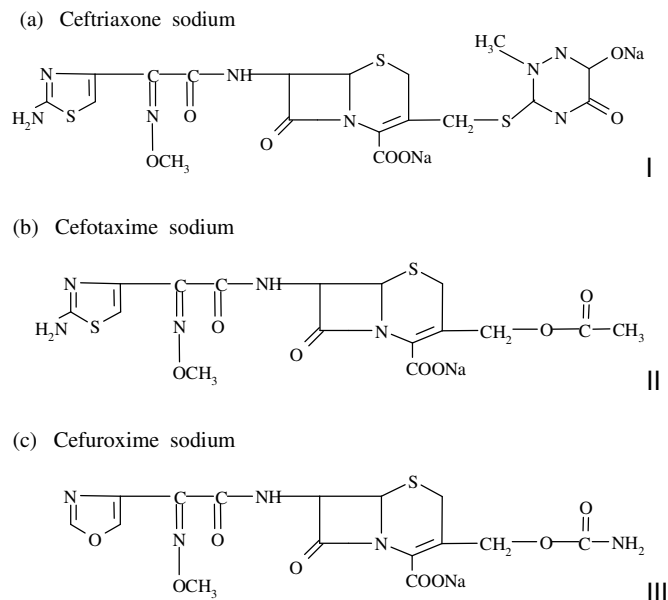

II

(c) Cefuroxime sodium

III

Figure 1. Chemical structures of the studied antibiotics.

with hexacyanoferate (III) to form PB complex. The absorbance of this complex is measured at the λ_{max} 700 nm.

MATERIAL AND METHODS

Materials and reagents

Ceftriaxone sodium, cefotaxime sodium (De Santos), cefuroxime sodium (GSK). All reagents used were analytical grade. Freshly distilled water was used throughout.

Spectral and absorbance measurements were made on UV 2102 PC spectrophotometer (UNICO) by using 1 cm quartz cells.

Absorption spectra

The chromogenic reagent was prepared by mixing 1 ml of 0.03 M $FeCl_3$ and 0.25 ml of 0.008 M Hexacyanoferrate (III) and making up to 10 ml, and the absorption Spectra determined. An aliquote of the drug solution was mixed with 2 ml of 0.1 M HCl in a test tube and the solution heated at 70°C for 30 min. The mixture was allowed to cool and 1 ml of 0.03 M $FeCl_3$ and 0.25 ml of 0.008 M of hexacyanoferrate (III) added and mixture made up to 10 ml with distilled water. A deep blue colour was developed after 20 min and the wavelength of maximum absorption was determined.

Calibration curve

Aliquots of the standard solution (0.1 mg/ml) of the drugs were transferred to a series of 10 ml volumetric flask containing 2 ml of 0.1 M HCl. The mixtures were placed in a thermostat adjusted to 70°C for 30 min and the chromogenic reagents added as described above. Absorbance values were measured at λ = 700 nm against a reagent blank after 30 min. The calibration curves were drawn and regression equation calculated.
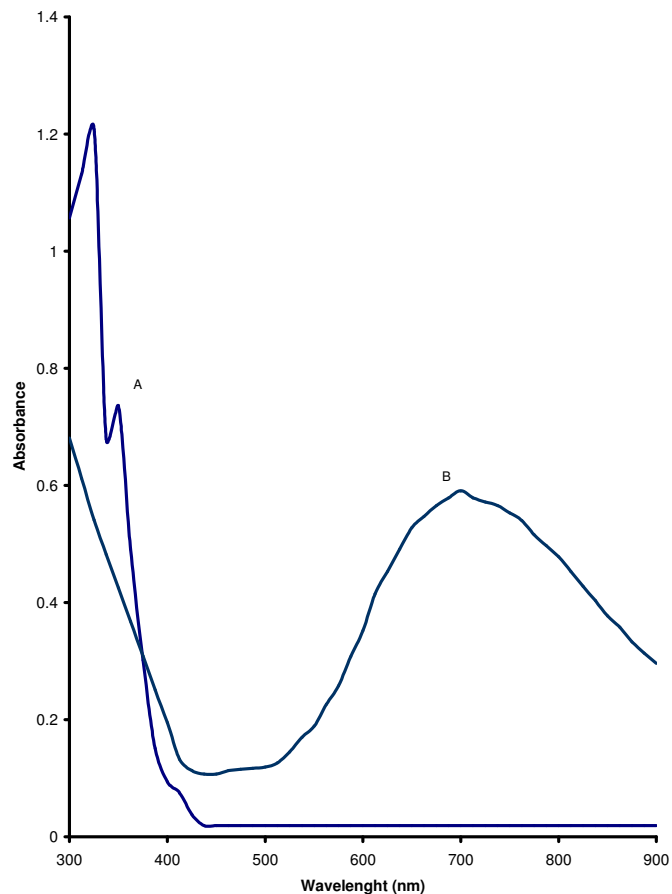

Figure 2. Visible spectrum of (A) chromogenic reagent [ferric chloride + hexacyanoferrate (III)] and (B) A + hydrolysis product of ceftriaxone.

Determination in pharmaceutical preparations

The preparations were obtained from local sources in various forms (vials and tablets). Accurately weighed quantity of the powdered tablet or vial equivalent to 250 mg/L of the drugs I - III were transferred into 100 ml volumetric flasks, and the proposed spectrophotometric method was performed on aliquot portions of the resulting sample solutions. The amount of drug present in the sample solution was computed from the calibration curve.

RESULTS AND DISCUSSION

Formation of PB complex has been employed in qualitative detection of Fe (II). A deep blue complex is formed by the reaction between Fe (II) and hexacyanoferrate (III) (Brown and Leman, 1988). As shown in Figure 2A, the chromogenic reagent i.e. Fe (III) mixed with hexacyanoferrate (III) in acidic media did not show any strong absorption in the visible region of the spectrum. However, after adding the acidic hydrolysis product of the studied antibiotics, the spectrum changed as depicted in Figure 2B due to the formation of PB complex. The complex has a λ_{max} of 700 nm and molar absorptivity of 3.0×10^4 l.mol^{-1}
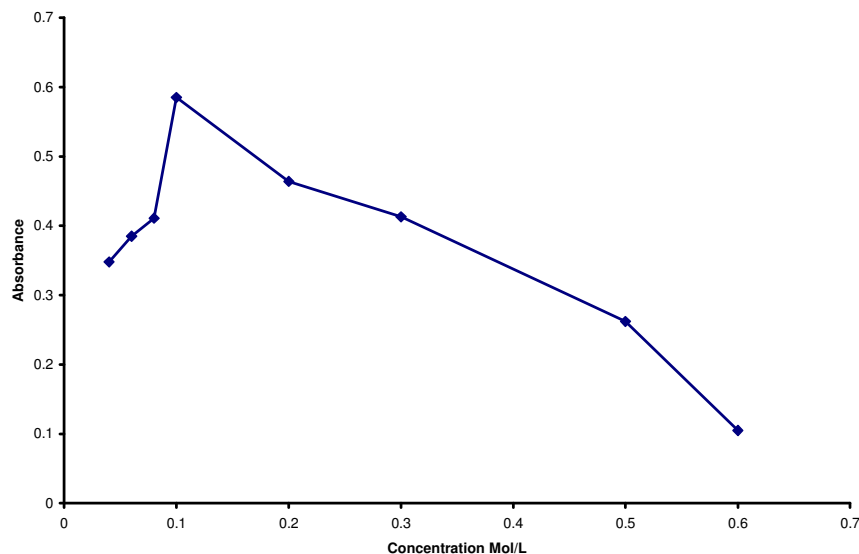
Figure 3. Effect of concentration of HCl used for hydrolysis of ceftriaxone within a period of 30 min at T = 70°C on the absorbance of PB complex.
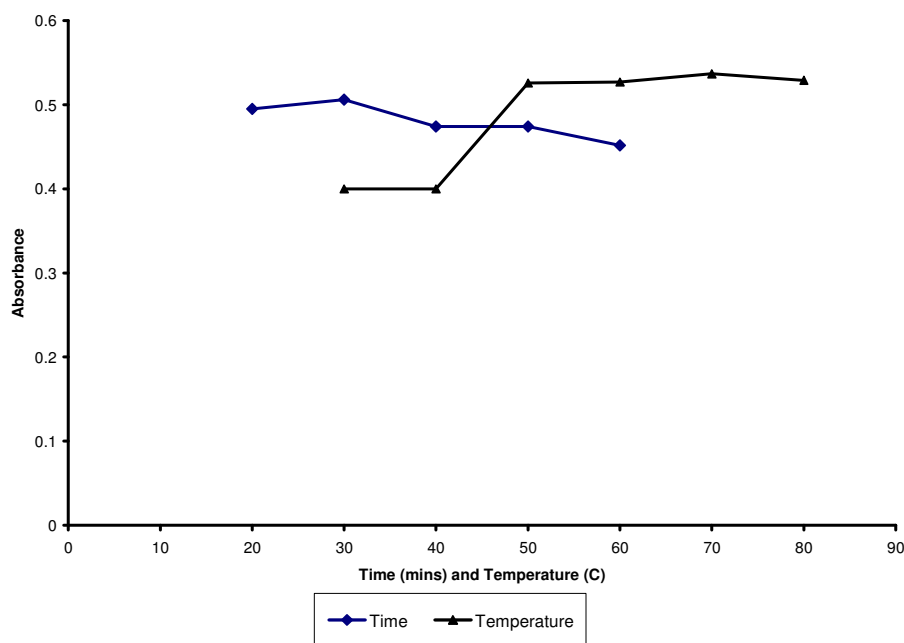

Figure 4. Effect of acidic hydrolysis temperature within a period of 30 min and time of acidic hydrolysis at 70°C on the absorbance of PB complex related to the reaction of hydrolysis of ceftriaxone.

cm^{-1}. Penicilloaldehyde has been reported as the ultimate degradation product of most β-lactam antibiotics (Deshpande et al., 2004; Bentley and Southgate, 1988). This product reduces Fe (III) to Fe (II), the latter reacting with potassium hexacyanoferrate (III) to form the PB complex (Farhadi et al., 2002).

The optimum conditions for the reaction were carefully studied. Among the mineral acids, HCl was shown to provide high intensity colour and faster reaction. The eff-

ect of the various concentrations of HCl used in the acidic hydrolysis step of the drugs is shown in Figure 3 for ceftriaxone. Optimal concentration of 0.1 M was obtained and selected for further study. Absorbance values dropped drastically at higher HCl concentration indicating possible degradation products other than the aldehydes. Effects of temperature of hydrolysis and time of heating shown in Figure 4 for ceftriaxone indicates that hydrolysis is complete at 70°C after 30 min. Similar results were
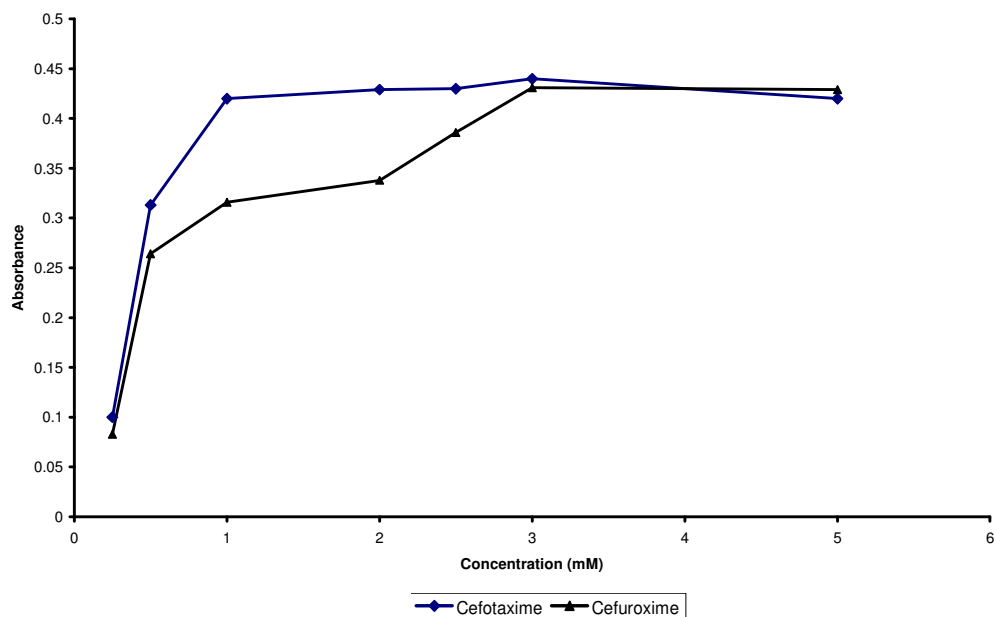
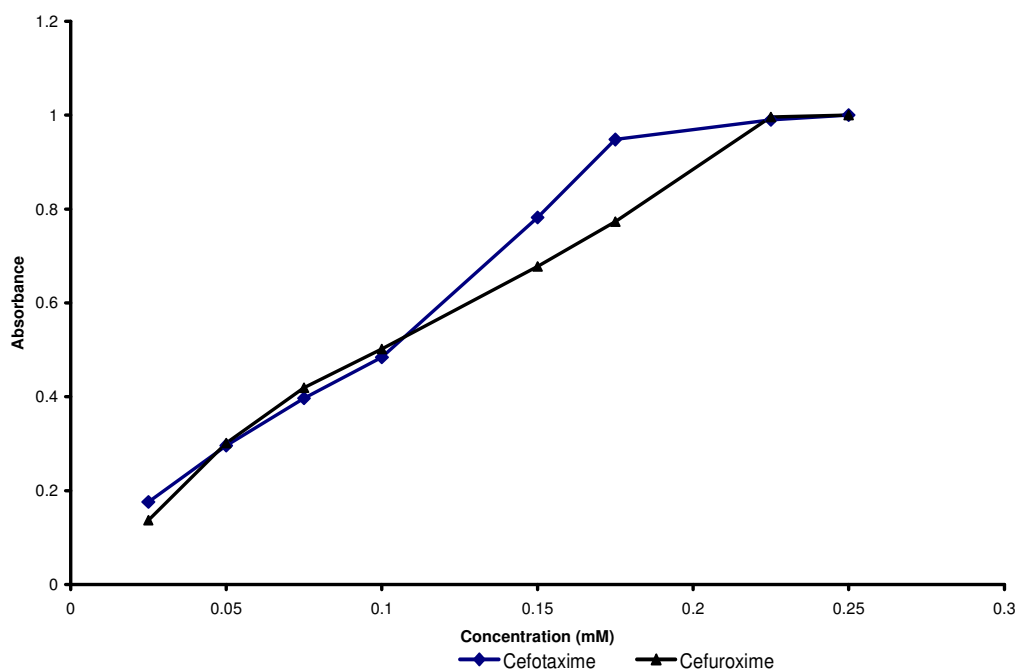**Figure 5.** Effect of concentration of Fe (III).

Figure 6. Effect of concerntration of Hexacyanoferrate (III).

obtained for cefotaxime and cefuroxime.

The effect of reagent concentrations on the colour intensity of the complex was also studied. As shown in Figures 5 and 6, it was found that 1ml of 0.03 M $FeCl_3$ and 0.25 ml of 0.008 M hexacyanoferrate (III) were sufficient for maximum colour development. It was also found that maximum absorbance of the PB complex was attai-

ned at 20 and 30 min for ceftriaxone, and cefuroxime respectively (Figure 7). The colour was stable for up to 50 min.

Under the optimum conditions chosen for the reaction, a linear relationship was found between the absorbance $\lambda = 700$ nm of PB complex and the concentration of the drugs in the concentration range of 2-20 µg/ml for cef-

Figure 7. Effect of Time.
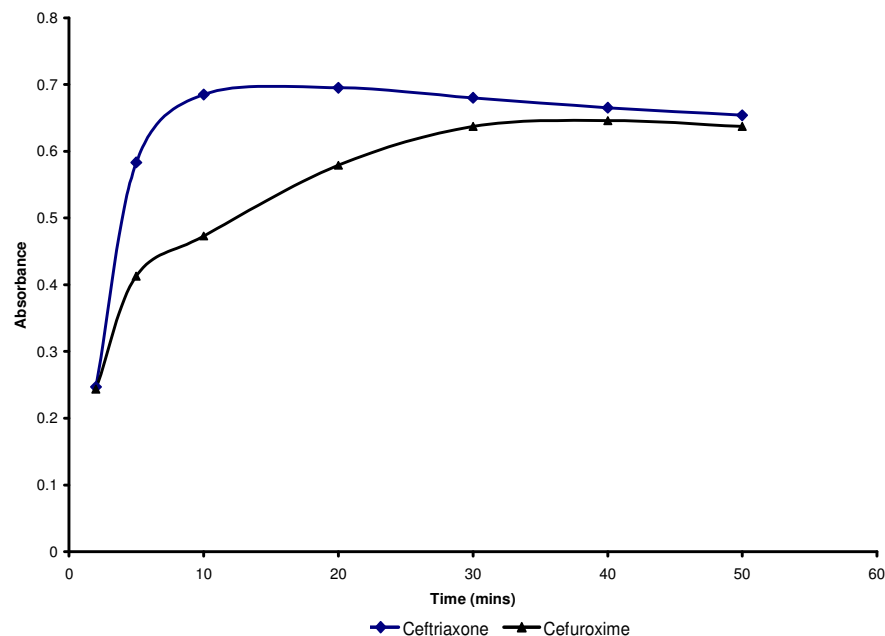
Table 1. Assay parameters and regression analysis.

Drug	Regression Equation	LR µg/ml	n	R2	DL µg/ml	QL µg/ml
Ceftriaxone	A = 0.038C + 0.015	2 - 20	7	0.994	0.369	1.116
Cefotaxine	A = 0.026C + 0.075	2 - 20	7	0.995	0.350	1.06
Cefuroxime	A = 0.028C + 0.053	2 - 18	6	0.991	0.322	0.975

A = Absorbance, C = Concentration in µg/ml, LR = Linear Range, DL = Detection Limit,
QL = Quantitation Limit.

Table 2. Analysis of some pharmaceutical preparations.

Formulations	Labeled Amount (mg)	Amount found (mg)	% Recovery (Mean ± SEM)
A	250	253.1	100.9 ± 0.37
B	250	255.7	103.5 ± 1.50
C	750	744.4	99.2 ± 1.10
D	500	506	101.3 ± 1.80
E	500	493.4	98.7 ± 1.30

A = Cefuroxime (as Cefuroxime Axetyl)
B = Cefuroxime (as Cefuroxime Axetyl)
C = Cefuroxime (as Cefuroxime sodium)
D = Ceftriaxone (as Ceftriaxone sodium)
E = Cefotaxime (as Cefotaxime sodium)

triaxone and cefotaxime and 2 - 18 µg/ml for cefuroxime. Regression analyses of the Beer's law plots reveal a good correlation and also the calculated detection and quantitation limits (ICH Topic Q2B, 1996) indicate the high sensitivity of the proposed method (Table 1).

The proprietary drugs containing the antibiotics were analyzed by the proposed method. Five replicate deter-minations were carried out and the results obtained as shown in Table 2 appear to be highly satisfactory.

The proposed method is simple, sensitive, accurate and inexpensive. The reagents employed are cheap and rea-dily available and the instrumentation versatile and adap-table. The method is recommended for the routine deter-mination of the selected antibiotics in pure and in pharm-

aceutical preparations as an alternative to the already existing methods.

ACKNOWLEDGMENTS

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