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 Fe²⁺ 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 x 10⁴ 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 cefuroxime. 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, spectrophotometric, 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 spectrophotometric (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 nucleophylic attack 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 cefazolin 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...
with hexacyanoferate (III) to form PB complex. The absorbance of this complex is measured at the $\lambda_{\text{max}}$ 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 aliquate 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 $\lambda = 700$ nm against a reagent blank after 30 min. The calibration curves were drawn and regression equation calculated.

**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 hexacyanoferate (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 $\lambda_{\text{max}}$ of 700 nm and molar absorptivity of $3.0 \times 10^4$ l.mol$^{-1}$
Penicilloaldehyde has been reported as the ultimate degradation product of most β-lactam antibiotics (Desphande 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 effect 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
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 FeCl₃ 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 attained 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 $\mu$g/ml for cef-
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 determinations 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 readily available and the instrumentation versatile and adaptable. The method is recommended for the routine determination of the selected antibiotics in pure and in pharm-

Table 1. Assay parameters and regression analysis.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression Equation</th>
<th>LR µg/ml</th>
<th>n</th>
<th>R²</th>
<th>DL µg/ml</th>
<th>QL µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>A = 0.038C + 0.015</td>
<td>2 - 20</td>
<td>7</td>
<td>0.994</td>
<td>0.369</td>
<td>1.116</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>A = 0.026C + 0.075</td>
<td>2 - 20</td>
<td>7</td>
<td>0.995</td>
<td>0.350</td>
<td>1.06</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>A = 0.028C + 0.053</td>
<td>2 - 18</td>
<td>6</td>
<td>0.991</td>
<td>0.322</td>
<td>0.975</td>
</tr>
</tbody>
</table>

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

Table 2. Analysis of some pharmaceutical preparations.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Labeled Amount (mg)</th>
<th>Amount found (mg)</th>
<th>% Recovery (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>250</td>
<td>253.1</td>
<td>100.9 ± 0.37</td>
</tr>
<tr>
<td>B</td>
<td>250</td>
<td>255.7</td>
<td>103.5 ± 1.50</td>
</tr>
<tr>
<td>C</td>
<td>750</td>
<td>744.4</td>
<td>99.2 ± 1.10</td>
</tr>
<tr>
<td>D</td>
<td>500</td>
<td>506</td>
<td>101.3 ± 1.80</td>
</tr>
<tr>
<td>E</td>
<td>500</td>
<td>493.4</td>
<td>98.7 ± 1.30</td>
</tr>
</tbody>
</table>

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)
aceutical preparations as an alternative to the already existing methods.

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REFERENCES


