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# ASSAY OF CEFPROZIL IN BULK AND ITS PHARMACEUTICAL FORMULATIONS BY VISIBLE SPECTROPHOTOMETRY

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## ABSTRACT
Four simple and sensitive visible spectrophotometric methods (A, B, and C) have been described for the estimation of Cefprozil (CEF). The methods that are based on the formation of radical anion with the involvement of basic nitrogen in CEF (donor) and quinones [2,3-dichloro-5,6-dicyano-p-benzoquinone(DDQ), chloranilic acid (DHQ), 2,3,5,6-tetrachloro-p-benzoquinone (TQ)] (acceptor). The variable parameters in all these methods have been optimized and the chemical reactions involved are presented. The results obtained in the three methods are statistically validated and recoveries range from 99.7 to 101.3%. Common excipients used in additives in pharmaceutical preparations do not interfere in the proposed methods.
**Keywords:** Cefrprozil, DDQ, DHQ, TQ, Spectrophotometric, Phrmaceutical formulations.

## INTRODUCTION
Cephalosporins are penicillin- resistant antibiotics with significant activity both gram positive and gram negative bacteria. The key intermediate for semi synthetic production of a large number of Cephalosporins is 7-cephalosporanic acid, which is formed by hydrolysis of cephalosporins C produced by fermentation. Cefprozil (CEF) is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral, intravenous administration and chemically known as (6$R$,7$R$)-7-[($R$)-2-($p$-hydroxyphenyl) acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid monohydrate. A number of methods such as spectrophotometric[1-10] and HPLC[11-21] were reported for the estimation of CEF. Literature survey revealed that only two visible spectrophotometric[6,7] method was reported for it quantitative determination in bulk drug and pharmaceutical formulations. Hence there is a need to develop to sensitive and flexible spectrophotometric methods for the assay of CEF.

A direct chemical analysis based on the reactivity of the intact molecule with out cleavage is not frequently encountered. The methods that are based on the charge transfer complexation are usually rapid and simple to perform.π-acceptors such as 2, 3-dichloro-5,6-dicyano-p-benzoquinone(DDQ), 2,3,5,6-tetrachloro-p-benzoquinone(TQ), chloranilic acid (DHQ) are known to yield charge transfer complexes with a variety of electron donors. The present work describes an improved direct simple analytical procedure that can be applied at quality control laboratories for the analysis of Cefprozil.

## EXPERIMENTAL
### Instrument
A Systronics model 117 UV – Visible spectrophotometric with 1cm matched quartz cells was used for spectral and absorbance measurements in the UV and visible regions respectively.

**Materials**

All reagents used were of Analytical Grade and freshly prepared solutions were always used. DDQ (Fluka, $4.4 \times 10^{-3}$M) solution in acetonitrile for Method A, DHQ (Sd-Fine, 0.1%, $4.785 \times 10^{-3}$M) solution in methanol for Method B, TQ (BDH, 0.1%, $4.067 \times 10^{-3}$M) solution in 1.4-dioxane for Method C were prepared.

**Standard Drug Solution**

Stock solution (1mg/1ml) of CEF for method A and B was prepared by dissolving 100 mg of it in 100ml of methanol and for method C was prepared by dissolving 100 mg of it in 100ml of 1,4-dioxane. The working standard solution of CEF of the required strength was prepared by further dilution of stock solution of CEF with Acetonitrile (method A ), Methanol (method B) and 1,4-Dioxane (method C).

**Recommended Procedures**

   (a) **For Bulk Samples**

**Method A:** Aliquots of standard drug CEF solution (0.5-2.5 mL, 400 μg/mL) in acetonitrile were delivered into 10ml were delivered into 10 mL graduated tubes. Then 2mL of ($4.4 \times 10^{-3}$M) DDQ in acetonitrile was added and kept aside for 20 min (CEF). The volume was made upto 10 mL with acetonitrile and read at 460 nm against reagent blank during the stability period (15-60min). The amount of drug present was computed from the appropriate calibration curve

**Method B:** Aliquots of standard drug CEF solution (0.5 – 2.5 mL, 500 μg/mL), was transferred into 10mL-graduated tubes. 2.0 mL of ($4.785 \times 10^{-3}$M) DHQ in methanol was added and kept aside for 5 min. Then the volumes of the contents were made upto 10 mL with methanol and read at 540 nm for CEF against a reagent blank within 30 min. The amount of drug was computed from the appropriate calibration curve.

**Method C:** Aliquots of standard drug CEF solution (0.5 – 2.5 mL, 500 μg/mL) in dioxan were delivered into 10 ml graduated tubes. Two mL of ($4.067 \times 10^{-3}$M) TQ in 1, 4-dioxan, followed by dioxan was added for bringing the volume to 7 mL. The final volume was brought to 10 mL with dimethyl formamide and the absorbance was measured against a reagent blank at 580 nm for CEF within the stability period (15-60min). The amount of the drug present was computed from the appropriate calibration graph.

   (b) **For Pharmaceutical Formulations:**

An accurately weighed amount of tablet powder equivalent to 100 mg of CEF was extracted with isopropanol (4 x 15ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in Acetonitrile (method A ), Methanol (method B) and 1,4-Dioxane (method C) to get 1mg/mL solution. The working standard solution of CEF of required strength prepared by further dilution of the stock solution of CEF with required solvent in the respective method and analyzed under procedure described for bulk samples.

## RESULTS AND DISCUSSION

The optimum conditions for the colour development of method were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table -2. The precision of each method was found by measuring absorbances of six replicate samples containing known amounts of drug and the results obtained are incorporated in Table-2. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and is presented in Table 2. The accuracy of each method was ascertained by comparing the results by proposed and reference methods (UV) statistically (Table-3). This comparison shows that there is no significant difference between the results of proposed methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods, the additives and excipients that are usually present in tablets do not interfere in the assay

of proposed methods.  As an additional check of accuracy of the proposed methods, recovery experiments were performed by adding a fixed amount of the drug to the pre-analyzed formulations.  The amount of drug found, the  % recovery was calculated in the usual way.

**Chemistry**

The interaction of any of the investigated compounds with poly halo and polycyanoquinone $\pi$-acceptors in nonpolar solvents was found to produce colored charge-transfer complexes with low molecular absorptivity values. In polar solvents such acetonitrile or methanol, complete electron transfer from donor to the acceptor moiety takes place with formations of intensely colored radical ions with higher molar absorptivity values according to the following scheme.

$$D^{..} + A \rightarrow \underset{Complex}{(D-A)} \xrightarrow{Polar\ solvent} A^{-.} + D^{+.}$$
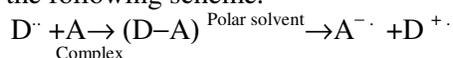
The dissociation of the D-A Complex is promoted by the high ionizing power of the acetonitrile and the resulting bands of the named drugs with acceptors are similar to the maxima of radical anions of the acceptors obtained by the iodide reduction method.

Acetonitrile was considered an ideal solvent as it afforded maximum sensitivity yield of radical anions in addition to its high solvating power of the reagents. Methanol gave maximum sensitivity in case of DHQ and 1,4-dioxane gave maximum sensitivity in case of TQ.

The interaction of CEF with TQ, DHQ, DDQ, gave a colored chromogens with a strong absorption maxima in different solvents given in Table-1

## CONCLUSION

The proposed methods are applicable for the assay of drug (CEF) and have the advantage of wider range under Beer's law limits.  The decreasing order of sensitivity and $\lambda_{max}$ among the proposed methods are C>B>A respectively.  The proposed methods are simple, selective and can be used in the routine determination of CEF in bulk samples and formulations with reasonable precision and accuracy.

Table-1: Reaction time and intensity in polar solvent

| Acceptor | Reaction time | Solvent | Absorption Maxima |
|---|---|---|---|
| TQ | 10 | 1,4-dioxane | 580 |
| DHQ | 5 | Methanol | 540 |
| DDQ | 20 | Acetonitrile | 460 |

Table-2: Optical Characteristics, Precision and Accuracy of the Proposed Methods for CEF

| Parameters | DDQ | DHQ | TQ |
|---|---|---|---|
| $\lambda_{max}$ (nm) | 460 | 540 | 580 |
| Beer's Law limits  ($\mu$g/ml) | 20-100 | 25-125 | 25-125 |
| Molar absorptivity (l mol$^{-1}$cm$^{-1}$) | $3.321\times10^3$ | $2.306\times10^3$ | $2.025\times10^3$ |
| Correlation coefficient (r) | 0.9999 | 0.9999 | 0.9999 |
| Sandell's sensitivity ($\mu$g/cm$^2$/ 0.001 absorbance unit) | 0.123 | 0.177 | 0.201 |
| Regression Equation ( y = a + bc) (i)Slope (b) | 0.0081 | 0.0055 | 0.0050 |
| (iii) Intercept (a) | -0.0016 | 0.0118 | -0.0006 |
| Relative Standard Deviation  * | 0.3474 | 0.2505 | 0.227 |
| % Range of error (confidence limits) (i) 0.05 level | 0.290 | 0.209 | 0.191 |
| (ii) 0.01 level | 0.430 | 0.310 | 0.281 |

 *Average of six determinations considered.

Table-3: Assay of CEF in pharmaceutical formulations

| Pharmaceutical formulations[#] | Labelled amount (mg) | Amount found by Proposed Methods* | | | Reference method[##] | %Recovery by Proposed methods** | | |
|---|---|---|---|---|---|---|---|---|
| | | Method A | Method B | Method C | | Method A | Method B | Method C |
| Tablet I | 250 | 250.63±1.28 F=3.36 t=0.49 | 250.56±1.37 F=3.85 t=0.41 | 250.69±1.08 F=2.39 t=1.25 | 250.09 ±0.69 | 100.25 ±0.51 | 100.22 ±0.55 | 100.27 ±0.43 |
| Tablet II | 250 | 248.82±1.92 F=1.17 t=0.65 | 249.10±2.27 F=1.64 t=0.23 | 250.85±2.09 F=1.39 t=0.56 | 249.63 ±1.77 | 99.52±0.77 | 99.64±0.91 | 100.34 ±0.83 |
| Tablet III | 250 | 250.90±1.24 F=1.01 t=0.43 | 248.51±1.87 F=2.26 t=1.36 | 249.13±1.67 F=1.81 t=1.24 | 250.45 ±1.24 | 100.36 ±0.50 | 99.40±0.75 | 99.65±0.66 |
| Tablet IV | 250 | 250.82±2.44 F=2.34 t=0.69 | 251.67±2.01 F=1.60 t=1.25 | 249.66±2.39 F=2.25 t=0.32 | 249.26 ±1.59 | 100.32 ±0.97 | 100.42±0.81 | 99.86±0.96 |

[#] Four different batches of tablets from a pharmaceutical company.
[##] Developed in the laboratory using methanol solvent ($\lambda_{max}$ 253 nm).
*Average ± standard deviation of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, t = 2.57, F = 5.05.
**After adding 3 different amounts of the pure labelled to the pharmaceutical formulation, each value is an average of 3 determinations.

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