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# Utility of 2,3-dichloro-5,6-dicyano-p-benzo-quinone for kinetic charge transfer determination of certain cephalosporins

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## ABSTRACT

A simple and rapid kinetic spectrophotometric method has been developed for determination of ceftriaxone, cefixime and cefpodoxime in pure forms and in their pharmaceutical formulations. The method is based on the reaction of these drugs as n-electron donors with 2,3-dichloro-5,6-dicyano-*p*-benzo-quinone (DDQ) as π-acceptor. The orange-red colour formed due to the formation of charge transfer complex was monitored spectrophotometrically by measuring the absorbance at 460 nm. The factors affecting the reaction were studied and optimized. The stoichiometry of the reaction was determined and the reaction pathway postulated. The initial rate and fixed time methods were utilized for constructing the calibration graphs. The graphs were linear in the concentration ranges of 0.024-1.00 mg $ml^{-1}$ and 0.024-2.00 mg $ml^{-1}$ using the initial rate and fixed time methods respectively. Ultraviolet–visible, infrared and $^{1}H$-nuclear magnetic resonance techniques were used to study the formed complex. The analytical performance was fully validated, and the results were satisfactory. The proposed method has been successfully applied for the determination of studied drugs in pharmaceutical formulations and the results obtained were in good agreement with those obtained by the reference methods.



## KEYWORDS

Kinetic spectrophotometry;
Charge-transfer complex;
Cephalosporins;
Pharmaceutical analysis.

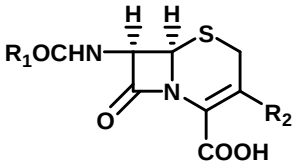
## INTRODUCTION

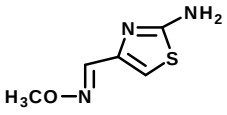
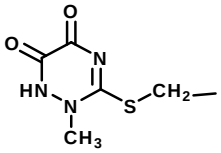
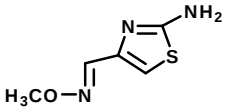
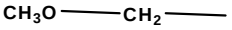
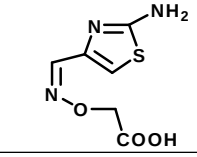
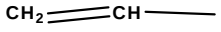
Ceftriaxone, cefixime and cefpodoxime are members of an important class of valuable clinical antibiotics, cephalosporins. They consist of a fused ß-lactam-Δ3- dihydrothiazine two-ring system, known as 7-aminocephalosporanic acid, and vary in their side chain substituents at C3 (R2), and C7 (acylamido, R1). The chemical structure of studied cephalosporins is shown in TABLE 1. They are used for the treatment of the infection caused by both gram-negative and gram-positive bacteria[1]. Several methods have been reported for the determination of these cephalosporins in pure form, in pharmaceutical preparations and in biological fluids. These methods include spectrophotometry[2-6], fluorometry[7,8], liquid chromatography[9-19], electrophoresis[20-22] and electrochemical[23-26] methods. The literature is still poor in analytical procedure based on kinetics, especially for determination of drug in commercial dosage forms. Kinetic spectrophotometric methods are becoming of great interest in chemical and pharmaceutical analysis[27]. No attempts have yet been made to determine cephalosporins by any kinetic charge transfer method .Our proposed method provides some

important advantages over other reported methods that can be summarized as follows:

1- The kinetic study of reaction has the advantage of avoiding the interference of coloured and/or turbidity background of samples and reduces the time required for the reaction.
2- High selectivity due to the measurement of the increase of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.
3- Simplicity owing to elimination of some experimental steps such as filtration and extraction prior to absorbance measurements.

**TABLE 1 : Structural formula of the studied cephalosporins.**




| Name | $R_1$ | $R_2$ |
|---|---|---|
| Ceftriaxone |  |  |
| Cefpodoxime |  |  |
| Cefixime |  |  |

## EXPERIMENTAL

### Apparatus

• Spectronic™ Genesys™ 2PC. Ultraviolet/Visible spectrophotometer (Milton Roy Co, USA) with matched 1 cm quartz cell was used for all measurements connected to IBM computer loaded with winspec™ application software.

• Jennway® 6505, Ultraviolet/Visible spectrophotometer (London, U.K.)

### Materials and reagents

All the materials were of analytical reagent grade. Samples of cephalosporin were generally supplied by their respective manufacturers and were used without further purification:

* Ceftriaxone sodium (T3A Pharma Group, Assiut, Egypt).
* Cefixime (SIGMA Pharmaceutical Industries, S.A.E. Egypt)
* Cefpodoxime (Sanofi-Aventis Egypt S.A.E. under licence of Sanofi-Aventis, France)
* 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Sigma Chemical Co.,USA) was prepared as 2 mg $mL^{-1}$ in methanol in case of ceftriaxone and 4 mg $mL^{-1}$ in methanol in case of cefixime and cefpopdoxime.
* Methanol (El-Nasr Chemical Co., Egypt).

### Pharmaceutical formulations

Cefotrix® vials (T3A Pharma Group, Assiut, Egypt), labeled to contain 500 mg ceftriaxone sodium per vial.
Ximacef® capsules (SIGMA Pharmaceutical Industries, S.A.E. Egypt) labeled to contain 400 mg cefixime per capsule.
Orelox® tablets (Sanofi-Aventis Egypt S.A.E. under licence of Sanofi-Aventis, France) labeled to contain 100mg cefpodoxime per tablet.

### Preparation of standard solution

Into 50 mL volumetric flask, 80-500 mg drug was weighed accurately and dissolved in 5 mL methanol, completed to volume with the same solvent and diluted quantitatively to obtain the suitable concentrations.

### General analytical procedure

### Initial rate method

Aliquots of 0.024-10 mg $mL^{-1}$ of studied drugs working standard solutions were pipetted into a series of 10 mL volumetric flask, 1.2 mL of DDQ (10.57 x $10^{-3}$ M for ceftriaxone and 21.11 x $10^{-3}$ M for cefixime and cefpodoxime) was added to each flask and then diluted to volume with methanol. The content of mixture of each flask was mixed well and the increase in absorbance at 460 nm was recorded as a function of time for 20 min against reagent blank treated similarly. The initial rate of the reaction ($\nu$) at different concentrations was obtained from the slope of the tangent to absorbance time curves. The calibration graphs were constructed by plotting the logarithm of the initial rate of the reaction ($\log\nu$) versus logarithm of molar concentration of the studied drugs (log C).

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### Fixed time method

In this method, the absorbance of each sample solution at preselected fixed time (5 minutes) was accurately measured and plotted against the final concentration of the drug.

### Stoichiometric study

Job's method of continuous variation[28] was employed. Master equimolar solutions of ceftriaxone with DDQ ($1.0\times10^{-3}$ M) and cefixime and cefpodoxime with DDQ ($3.0\times10^{-3}$ M), were prepared in 5.0 ml methanol, and completed to volume with the same solvent. A series of 10-ml portions of master solutions of each drug with the acceptor (DDQ) was made up comprising different complementary proportions (0:10, 1:9, …, 9:1, 10:0) in 10-ml calibrated flasks. The absorbance of the resulting solutions was measured after 20 minutes at 460 nm against reagent blanks treated similarly.

### Determination of the studied drugs in pharmaceutical formulations

### Analysis of capsules and tablets

The content of 20 capsules or tablets were evacuated or powdered and mixed well, A quantity of the powder equivalent to 2 g was transferred into a 50-ml calibrated flask, dissolved in 10 ml methanol, swirled and sonicated for 2 min, completed to volume with the same solvent, shaken well for 15 min and filtered, rejecting the first portion of the filtrate and then proceeding as in the general procedure.

### Analysis of vials

A quantity of the powder equivalent to 100 mg was transferred into a 50-ml calibrated flask and dissolved in 5 ml methanol, then proceeding as in the analysis of capsules and tablets.

### Preparation of the complexes for infrared measurements

To 2 ml of 0.05 M ceftriaxone, as a representative example, in methanol, 2 ml of 0.05 M of DDQ in methanol was added in a round-bottom flask containing ~30 ml methanol and stirred for 30 min. The solvent was evaporated under reduced pressure and the resulting oily residue was dried over calcium chloride.

### Solutions for $^1$H-nuclear magnetic resonance measurements

A 30 mg of ceftriaxone were dissolved in 1 ml $d_6$-DMSO. One milliliter containing an equimolar amount of DDQ in the same solvent was added and used directly for $^1$H-nuclear magnetic resonance ($^1$H-NMR) measurements.

## RESULTS AND DISCUSSION

The reaction of DDQ with basic nitrogenated drugs results in the formation of intense orange-red products that exhibit absorption maxima at 460 nm. Figure 1 show the absorption spectra of ceftriaxone, as a representative example, DDQ and ceftriaxone-DDQ reaction product.

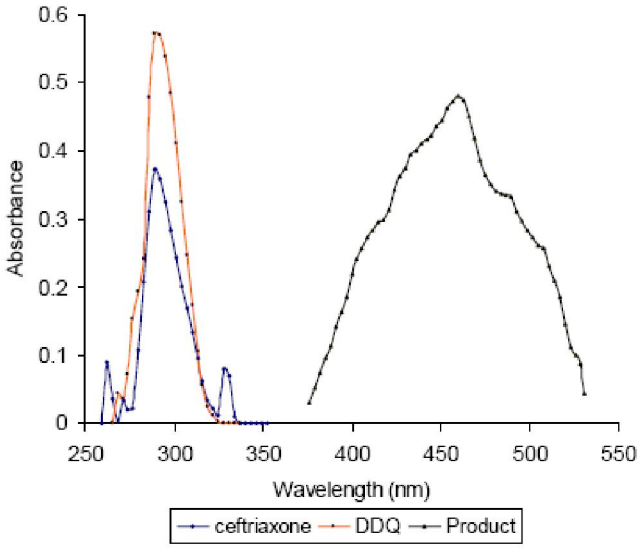



**Figure 1 : Absorption spectra of ceftriaxone sodium 1.3 mg mL$^{-1}$, DDQ 4.0 mg mL$^{-1}$ and ceftriaxone (88µg mL$^{-1}$)-DDQ complex in methanol.**

The band may be attributed to formation of DDQ radical anion[29]. In polar solvents such as methanol, complete electron transfer from the donor to the acceptor moiety takes place with the formation of radical ions according to the following equation

$$D + A \longrightarrow \underset{\text{Complex}}{D\text{-}A} \xrightarrow{\text{polar solvents}} \underset{\text{Radical ions}}{D^{\cdot+} + A^{\cdot-}}$$

### Optimization of variables

### Effect of diluting solvent

The effect of different solvents on the formation and stability of each drug-DDQ complex was studied. It was found that methanol is the most suitable solvent for producing the highest stable colour.

### Effect of reaction time

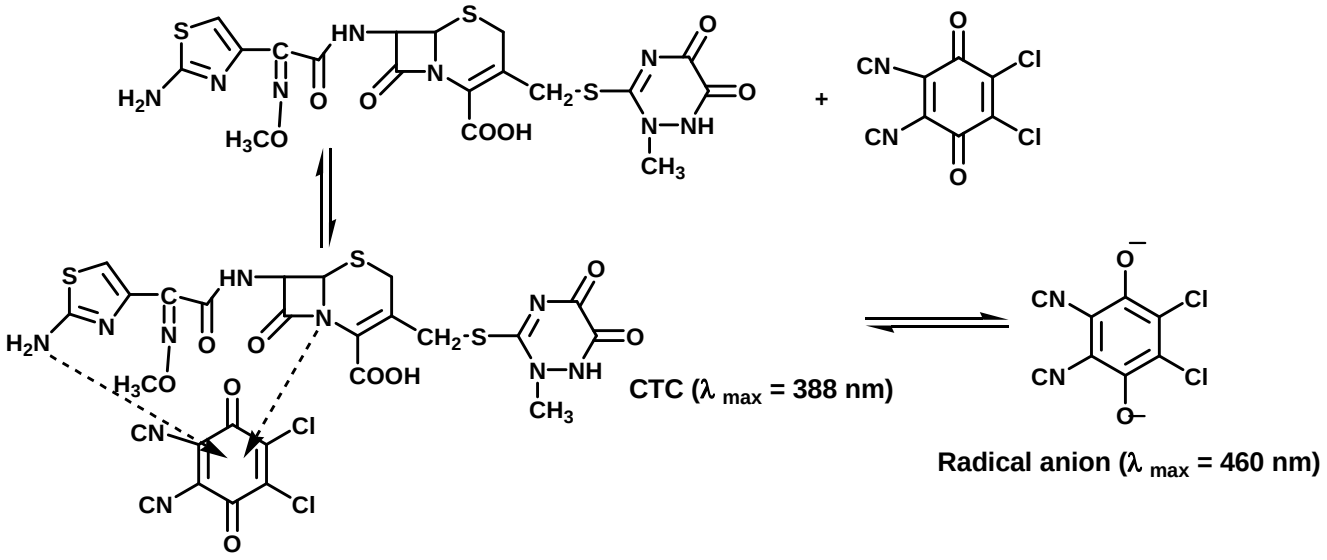
The optimum reaction time was determined by following the colour development at ambient temperature (25±5°C). Complete colour development was attained after 20 min with all investigated drugs, and the colour remain stable for at least a further 20 min.

### Effect of DDQ concentration

The absorption intensity increases by increasing concentration of DDQ and reaches its maximum value at 1.0 mL of 8.81 M X $10^{-3}$ M for ceftriaxone and 17.62 M X $10^{-3}$ M for cefixime and cefpodoxime, after which no further increase. Thus, the adoption of 1.2 mL of DDQ in the final solution proved to be adequate for the maximum concentration of cephalosporin used in determination process.

### Stoichiometry *and reaction mechanism*

Using Job's method of continuous variation[28], the molar ratio of DDQ to each of the studied drugs was 1:1. The reaction mechanism is based on the interaction between the tertiary amine moiety of the chosen drugs as n-electron donors and DDQ to form a stable charge transfer complexes or radical anions. The dissociation of donor-acceptor (DA) complex was promoted by the high ionizing power of the solvent (methanol). In this solvent, complete electron transfer from the donor to the acceptor moiety takes place followed by formation of DDQ-radical anions as predominant chromogens[29,30]. Scheme 1 shows the suggested reaction pathway between ceftriaxone, as representative example for the studied drugs, and DDQ.




**Scheme 1 : Suggested reaction mechanism for CTC of ceftriaxone with DDQ.**

### Kinetics of the reaction

Under the optimum conditions, the absorbance time curves of the investigated cephalosporins with DDQ were constructed (Figures 2, 3 for ceftriaxone as representative example). The initial rate of the reaction was determined from the slope of tangents of the absorption time curves. The order of the reaction with respect to DDQ was determined by studying the reaction at different concentrations of DDQ with fixed concentration of investigated cephalosporins. The plot of initial rate ($\Delta A/\Delta t$) against initial absorbance was linear passing through origin indicating that the initial order of the reaction with respect to DDQ was 1. The order with respect to investigated cephalosporins was evaluated by measuring the rate of the reaction at several concentrations of cephalosporins at a fixed concentration of DDQ reagent. This was done by plotting the logarithm of initial rate of the reaction versus logarithm of molar concentration of investigated cephalosporins and was found to be 1. However under the optimized experimental conditions, the concentrations of cephalosporins were determined using relative excess amount DDQ solution. Therefore pseudo-zero order conditions were obtained with respect to their concentrations.

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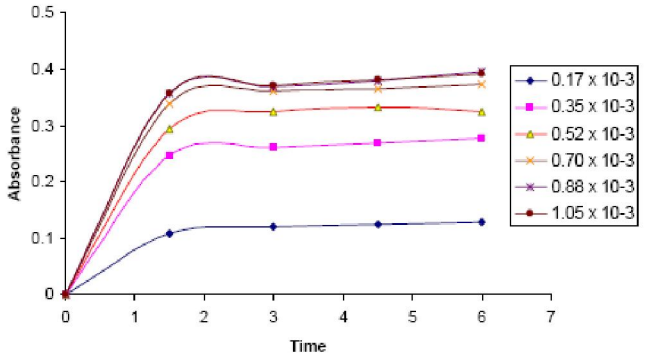



**Figure 2 : Absorption versus time for the reaction between ceftriaxone 1.7 x $10^{-4}$ M and DDQ (different molar concentrations)**

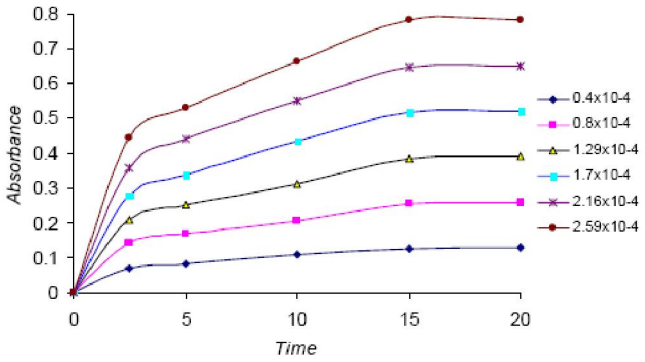



**Figure 3 : Absorption time curve using different molar concentration of ceftriaxone with 1.05 x $10^{-3}$ M ml of DDQ**

## Quantitation methods

### Initial rate method

The initial rate of the reaction would follow pseudo-first order and were found to obey the following equation: $\nu = \Delta A/\Delta t = K' C^n$ where $\nu$ is the reaction rate, A is the absorbance, t is the measuring time, K′ is the pseudo-first order rate constant, C is the molar concentration of cephalosporins and n is the order of the reaction. The logarithmic form of the above equation is written as follows: $\text{Log } \nu = \log \Delta A/\Delta t = \log K' + n \log C$.

Regression analysis using the method of least square was performed to evaluate the slopes, intercepts and correlation coefficients. The analytical parameters and results of regression analysis are given in TABLE 2. The value of n (≈ 1) in the regression equation confirmed that the reaction of cephalosporins with DDQ was pseudo first order with respect to cephalosporins concentration. The limits of detection (LOD) were calculated and results obtained confirmed adequate sensitivity of the proposed method and consequently their capabilities to determine low amount of cephalosporins.

**TABLE 2: Analytical parameters for the initial rate method for determination of investigated cephalosporins with DDQ.**

| Investigated cephalosporin | Linear range, M x$10^{-3}$ ( )mg $ml^{-1}$ | Least square equation log V = log K′ + n log c | | Correlation coefficient ( r ) | LOD µg $ml^{-1}$ |
|---|---|---|---|---|---|
| | | Intercept (log K′) | Slope (n) | | |
| Cefotriaxone | 0.4-2.59 (0.024-0.144) | -2.589 | 1.017 | 0.9990 | 0.0886 |
| Cefixime | 0.44-2.2 (0.2-1) | -1.704 | 1.031 | 0.9996 | 0.0585 |
| Cephpodoxime | 0.4-2.3 (0.2-1) | -1.678 | 0.9856 | 0.9999 | 0.0266 |

### Fixed time method

In this method, the absorbance of the reaction solution containing varying amount of cephalosporins was measured at preselected fixed time. Calibration plots of absorbance versus the concentration of cephalosporins at fixed time were established for each investigated cephalosporins. The regression equation, correlation coefficients and detection limits are given in TABLE 3. The lowest detection limit was obtained at fixed time of 15 min. However the fixed time of 5 minutes showed a wider concentration range for quantification. According to international conference of harmonization (ICH) guideline for validation of analytical procedures[31], the detection limit is not required to be part of validation procedure for assay. Therefore on the basis of wider concentration range and less time of analysis, the fixed time of 5 min. was recommended for determination.

### Validation of the proposed method

**Concentration range**[31] is established by confirming that the analytical procedure provides a suitable degree of precision, accuracy and linearity when applied to the sample containing amount of analyte within or at the extreme of the specified range of the analytical procedure[31,32]. In this work, concentration ranging from 0.4 x $10^{-3}$M to 2.59 x $10^{-3}$M were studied for the investigated drugs in the initial rate method and concentration ranging from 0.024 to 2.0 mg $mL^{-1}$ were studied for the investigated drugs in the fixed time method (at preselected fixed time of 5 min.). The whole set of experiments were carried out through this range to ensure the validation of the proposed procedure. Linear calibration graphs were obtained for all the studied drugs by plotting the logarithm of initial rate of the reaction versus logarithm of molar concentration of analyte in the sample (in initial rate method) within the specified range (Figure 4.):

**TABLE 3: Analytical parameters for fixed time method of the kinetic charge transfer parameter for determination of investigated cephalosporins.**

| Reaction time | Linear range (mg mL$^{-1}$) | Intercept (a) | Standard deviation of intercept (Sa) | Slope (b) | Standard deviation of slope ($S_b$) | correlation coefficient (r) | LOD (µg mL$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Ceftriaxone | | | | | | | |
| 2.5 | 0.024-0.28 | 0.024 | 0.0084 | 0.4216 | 0.0079 | 0.9984 | 0.0059 |
| 0.05 | 0.024-0.232 | 0.114 | 0.0072 | 0.5421 | 0.0082 | 0.9992 | 0.0039 |
| 10 | 0.024-0.168 | -0.003 | 0.0070 | 0.7181 | 0.0104 | 0.9995 | 0.0029 |
| 15 | 0.024-0.144 | 0.007 | 0.0035 | 0.8733 | 0.0060 | 0.9999 | 0.0012 |
| 20 | 0.024-0.144 | 0.001 | 0.0044 | 0.8730 | 0.0075 | 0.9999 | 0.0011 |
| Cefixime | | | | | | | |
| 2.5 | 0.2-2 | 0.029 | 0.0119 | 0.1856 | 0.0038 | 0.9983 | 0.1930 |
| 5 | 0.2-1.6 | 0.018 | 0.0098 | 0.2245 | 0.0039 | 0.9991 | 0.1309 |
| 10 | 0.2-1.2 | 0.005 | 0.0103 | 0.2921 | 0.0529 | 0.9993 | 0.1057 |
| 15 | 0.2-1.0 | 0.006 | 0.0047 | 0.3662 | 0.0028 | 0.9999 | 0.0387 |
| 20 | 0.2-1.0 | 0.005 | 0.0049 | 0.3600 | 0.0029 | 0.9999 | 0.0406 |
| Cephpodoxime | | | | | | | |
| 2.5 | 0.2-2 | 0.004 | 0.0103 | 0.1759 | 0.0033 | 0.9986 | 0.1758 |
| 5 | 0.2-1.6 | 0.011 | 0.0093 | 0.2043 | 0.0037 | 0.9990 | 0.1365 |
| 10 | 0.2-1.2 | -0.001 | 0.0086 | 0.2679 | 0.0044 | 0.9995 | 0.0959 |
| 15 | 0.2-1.0 | 0.005 | 0.0045 | 0.3232 | 0.0027 | 0.9999 | 0.0417 |
| 20 | 0.2-1.0 | 0.001 | 0.0032 | 0.3228 | 0.0019 | 0.9999 | 0.0299 |

**Log ν = log ΔA/Δt = log K′ + n log C (where n ≈ 1)**

or by plotting the absorbance of the studied drugs versus the drug concentration (in fixed time method) within the specified range. Linearity was studied for both initial rate and fixed time methods indicated by the good values of correlation coefficient (r) (TABLE 2, 3).

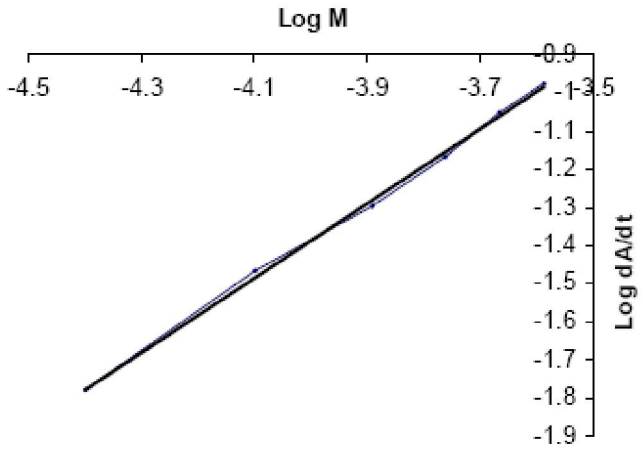



**Figure 4 : Calibration plot of logarithm rate of the reaction against logarithm molar concentration of ceftriaxone for initial rate method.**

**Accuracy**[32] was checked at three concentration levels within the specified range, six replicate measurements were recorded at each concentration level. The results were recorded as percentage recovery ± standard deviation (TABLE 4, 5).

**TABLE 4 : Evaluation of accuracy of the analytical procedure for ceftriaxone using initial rate and fixed methods.**

| Method | Recovery %* 72 µg mL$^{-1}$ | 96 µg mL$^{-1}$ | 120 µg mL$^{-1}$ |
|---|---|---|---|
| Initial rate | 99.40 ± 1.121 | 99.54 ± 0.412 | 99.65 ± 0.288 |
| Fixed time | 99.66 ± 0.518 | 99.63 ± 0.399 | 99.60 ± 0.600 |

*** Mean of 6 replicate ± SD**

**TABLE 5 : Evaluation of accuracy of the analytical procedure for cefixime and cefpodoxime using initial rate and fixed methods.**

| Method/ Drug | Recovery %* 400 µg mL$^{-1}$ | 600 µg mL$^{-1}$ | 800 µg mL$^{-1}$ |
|---|---|---|---|
| Initial rate | | | |
| Cefixime | 99.93 ± 0.197 | 100.32 ± 0.444 | 99.87 ± 0.459 |
| Cefpodoxime | 99.65 ± 0.409 | 99.62 ± 0.496 | 99.9 ± 0.576 |
| Fixed time | | | |
| Cefixime | 100.30 ± 0.521 | 100.38 ± 0.445 | 99.78 ± 0.435 |
| Cefpodoxime | 99.88 ± 0.387 | 99.73 ± 0.476 | 99.65 ± 0.485 |

*** Mean of 6 replicate ± SD**

**Precision**[32] was checked at three concentration levels, eight replicate measurements were recorded at each

concentration level; the results are summarized in (TABLE 6). The calculated relative standard deviations were below 2.2 % indicating excellent precision of the proposed procedure at both levels of repeatability and intermediate precision.

**TABLE 6 : Evaluation of precision of the initial rate and fixed time methods of the proposed kinetic charge transfer method for determination of investigated cephalosporins.**

| Drug | Amount taken (μg mL$^{-1}$) | Recovery (%±S.D) | |
|---|---|---|---|
| | | Initial rate method | Fixed time method |
| Cefotriaxone | 72 | 100.16 ± 0.336 | 99.28 ± 0.497 |
| | 96 | 99.34 ± 0.451 | 99.94 ± 0.439 |
| | 120 | 100.06 ± 0.167 | 99.54 ± 0.642 |
| Cefixime | 400 | 100.26 ± 0.488 | 100.02 ± 0.462 |
| | 600 | 99.98 ± 0.444 | 100.32 ± 0.920 |
| | 800 | 100.12 ± 0.517 | 100.40 ± 0.394 |
| Cefpodoxime | 400 | 99.84 ± 0.483 | 99.82 ± 0.545 |
| | 600 | 99.88 ± 0.466 | 101.04 ± 0.611 |
| | 800 | 100.62 ± 0.449 | 99.56 ± 0.365 |

**Specificity and interference** the specificity of the method was investigated by observing any interference encountered from common excipients of the pharmaceutical formulations such as starch, magnesium stearate, and Talc. It was found that these compounds did not interfere with the results of the proposed method.

**Limit of detection (LOD)**[31] was calculated based on standard deviation of intercept and the slope of calibration curve. The limit of detection was expressed as[32]:

$$LOD = 3\sigma / S$$

where σ is the standard deviation of intercept. S is the slope of calibration curve.

The results are summarized in (TABLE 2, 3) indicating good sensitivity of the proposed method. According to USP *XXV* validation guidelines[32], the calculated LOD values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOD equations were actually detected in these experiments.

**Limit of quantitation (LOQ)** was calculated based on standard deviation of intercept and slope of calibration curve. In this method, the limit o quantitation is expressed as[32]:

$$LOQ = 10\sigma / S$$

The results indicating adequate sensitivity of the proposed method. According to USP XXV validation guidelines[33], the calculated LOQ values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOQ equations were actually quantitated in these experiments.

## Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged such as reaction time and DDQ concentration. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D. % of the same procedure applied by two different instruments on different days. The results showed no statistical differences between two different instruments on different days, suggesting that the developed method was robust and rugged.

## Application to pharmaceutical dosage forms

The initial rate and fixed time methods of the proposed kinetic charge transfer method for determination of investigated cephalosporins have been tested on commercial pharmaceutical dosage forms. The concentration of the investigated cephalosporins was computed from its responding regression equations. The results of proposed method (initial rate and fixed time) were statistically compared with those of reported methods[5,8], in respect to accuracy and precision. The obtained mean recovery values were 99.06 ± 0.792 -100.16 ± 0.650 (TABLE 7) which ensures that there is no interference of other active additives present in the studied formulations.

**TABLE 7 : Determination of studied drugs in their pharmaceutical dosage forms using initial rate and fixed time methods.**

| Drug | Pharmaceutical dosage form | Proposed method ± SD (n = 5) | | Reported methods ± SD (n = 5) |
|---|---|---|---|---|
| | | Initial rate | Fixed time | |
| Ceftriaxone | Cefotrix® | 100.16 ± 0.650<br>t = 0.305*<br>F = 3.743* | 99.76 ± 0.439<br>t = 1.213<br>F = 1.708 | 100.06 ± 0.336 |
| Cefixime | Ximacef® | 99.06 ± 0.792<br>t = 0.998<br>F = 2.444 | 99.50 ± 0.469<br>t = 0.4193<br>F = 1.168 | 99.48 ± 0.507 |
| Cefpodoxime | Orelox® | 100.02 ± 0.589<br>t = 1.554<br>F = 1.350 | 99.78 ± 0.526<br>t =0.918<br>F =1.078 | 99.48 ± 0.507 |

***Tabulated value at 95 % confidence limit; *t* = 2.306 and F = 6.388**

In the *t*- and F- tests, no significant difference were found between the calculated and theoretical value of both the proposed and reported methods at 95% confidence level. This indicates good precision and accu-

racy in the analysis of investigated cephalosporins in pharmaceutical dosage forms.

### Investigations on the structure of the charge-transfer complexes (CTC)

The presence of bands at 460 nm indicates the possible CTC formation of the type n–π complexes. The formation of such complexes was also confirmed by both IR and $^1$H-NMR measurements.

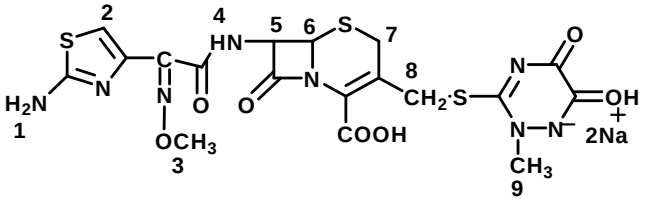
The majority of infrared measurements on such complexes have been concerned with the shifts in the vibrational frequencies of donors or acceptors (or both). Decreases in the vibration frequency of a particular band have been used as evidence for a particular site of a charge-transfer interaction[29]. The infrared spectra of the complexe show some differences compared with the sum of the spectra of the two components. This was used to distinguish between weak charge-transfer complexes and the products of electron-transfer or proton-transfer reactions[30].

The IR spectra of DDQ shows strong bands at 2340 and 1664 cm$^{-1}$ corresponding to C≡N and C=O of quinone stretching, respectively. The band at 2340 cm$^{-1}$ was shifted to 2300 cm$^{-1}$. The IR spectra of ceftriaxone shows strong bands at 1730, 1637, 3315-3435, 1595 and 1637 cm$^{-1}$ corresponding to β-lactam C=O stretching mode, amide C=O stretching mode, N-H stretching mode of the hydrogen bonded amide group, oxime C=N stretching mode and asymmetric carboxylate stretching mode. The band at 3315-3435 cm$^{-1}$ was shifted 3300-3405 cm$^{-1}$. This was attributed to change in electron density due to charge transfer complex and hydrogen bonding exists in the complex formed.

In $^1$H-NMR, generally, the protons of the donor are shifted to a lower field (paramagnetic shift). The $^1$H-NMR spectra of the complex of ceftriaxone with DDQ was recorded in $d_6$-DMSO together with the spectra of the free drug (TABLE 8).

In the $^1$H-NMR spectra of the complex, 6-H and 7-H are downfield shifted and 2-$CH_2$ are not affected, and as reported in the literature[34] with similar compounds of related β-lactam antibiotics, it can be preliminary suggested that the sulphur atom is not taking part in the CTC formation and that the lactam nitrogen atom is probably the donor centre.

**TABLE 8 : $^1$H-NMR spectra ($d_6$-DMSO) of ceftriaxone sodium and its DDQ complex**




| Ceftriaxone-DDQ complex | Ceftriaxone | Proton |
|---|---|---|
| 3.51 | 3.5-3.7 | 9 |
| 3.80 | 3.80 | 8 |
| 4.30 | 4.32 | 7 |
| 4.7 | 3.8 | 3 |
| 5.02 | 5.05 | 6 |
| 5.64 | 5.60 | 5 |
| 6.64 | 6.64 | 2 |
| 9.52 | 9.52 | 4 |

## CONCLUSION

The initial rate and fixed time methods are accurate, time saving, do not require prior extraction procedure and have the advantages of simplicity, sensitivity and reproducibility.

The proposed method (initial rate or fixed time) is sensitive enough to determine small amounts of these drugs, therefore can be used for quality control and routine determination of drugs in pharmaceutical dosage forms where precision, time and cost effectiveness of analytical methods are important. In addition to ultraviolet–visible spectrophotometry, infrared and $^1$H-NMR spectroscopy could also be used to study the possible site of interaction between the donors and acceptor.

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