Future Journal of Pharmaceutical Sciences

RESEARCH

Open Access

Spectrophotometric studies of low-cost method for determination of ceftriaxone using a novel charge transfer acceptor, N-(2,4-dinitr o-1-naphthyl)-p-toluenesulphonamide

O. E. Umoh[1,2*], O. A. Adegoke[2] and D. E. Effiong[1]

Abstract

Background: This study reports a new, cost-effective and validated method for the determination of ceftriaxone (CFR). The method involved charge transfer (CT) complexation reaction of ceftriaxone (as n-electron donor) and N-(2,4-dinitro-1-naphthyl)-p-toluenesulphonamide {N-(2,4-DN1NL) PTS} as π-electron acceptor to form a complex. Ultraviolet, infrared and ^{1}H NMR spectra of CFR, N-(2,4-DN1NL) PTS and adduct were then studied to predict the site of interaction between the donor and acceptor.

Result: The complex formed had deep golden-yellow colour, having a new absorption band at 440 nm. Molar absorptivity of 1.667×10^5 L M^{-1} cm^{-1} was obtained. The complexation reaction was completed at 30 °C optimal temperature within 10 min. Acetonitrile was found to be the best diluting solvent for optimal detector response and the complex was stable (absorbance unchanged) at room temperature for hours. At concentration of 1.708–11.956 µg mL^{-1}, with low limits of detection of 0.143 µg mL^{-1}, Beer's law was observed. Between-day recovery statistics of CFR from quality control samples were 102.15 ± 0.062 (% RSD = 0.61, n = 12) over three days. The site of interaction of donor and acceptor molecules, as revealed through infrared (IR) and proton nuclear magnetic resonance studies (^{1}H NMR) and the formation of charge transfer complex is through intermolecular hydrogen bonding between the amino group of the donor and the acidic proton of the acceptor. Common tablet excipients, as observed, did not interfere with the analytical method and no significant difference existed between the results of this new method and the high performance liquid chromatographic procedures ($p > 0.05$) documented in the USP. The new CT procedure described in this paper is not only simple but also fast, accurate and precise. Also, the reactions were carried out at room temperature compared to previously described procedures.

Conclusions: This novel method could therefore be adopted as a fast but cost-effective alternative for the qualitative and quantitative assessment of CFR in its pure and dosage form. It could find usefulness in on-the-spot detection of counterfeit drugs and in field inspections with reliable accurate results that compares with established methods.

Keywords: Spectrophotometry, Charge transfer reaction, Ceftriaxone, N-(2,4-dinitro-1-naphthyl)-*p*-toluenesulphonamide, Complexation reaction

Background

Ceftriaxone disodium salt (CFR) is Disodium (6R,7R)-7-[[(2*Z*)-(2-aminothiazol-4-yl) (methoxyimino) acetyl] amino]-3-[[(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl) sulphanyl] methyl]-8-oxo-5-thia-1-azabi-

*Correspondence: offiongumoh@uniuyo.edu.ng

[1] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria
Full list of author information is available at the end of the article

© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

cyclo [4.2.0]oct-2-ene-2-carboxylate 3.5 hydrate [1]. Its structural formula is $C_{18}H_{16}N_8Na_2O_7S_3$ and the molecular weight, 662.0 g/mol.

This off-white or yellowish, crystalline powder is slightly hygroscopic, very soluble in water, very slightly soluble in ethanol, but sparingly soluble in methanol [1]. Its possession of aminothiazole, 7-α-iminomethoxy groups at 7-α-position, and 1, 2, 4-triazine ring gives greater antibacterial activity as a third generation cephalosporin [2]. Little wonder it is a widely used broad spectrum injectable antibiotics in hospital settings.

Cephalosporins are classified based on spectrum of antimicrobial activity into generations [3, 4]. The newer generations have significant Gram-negative antimicrobial properties than preceding generations with members in the fourth-generation specifically having longer half-lives with reduced dosing frequency [5].

Several analytical methods have been reported for the quantitative determination of cephalosporins in dosage forms. These include spectrophotometric methods which are based on charge transfer complex reactions, flow injection analysis, spectrophotometric methods using, Fe^{3+} hexacyanoferate (111) ion, metol-chromium (vi), molybdophosphoric acid, variamine blue, condensation of cefotaxime with 1,2-naphthaquinone-4-sulphonic acid in alkaline medium [6–15]; others are first-derivative spectrophotometry and HPLC analysis, direct titration and indirect spectrophotometry, spectroscopic and RP-HPLC methods and spectrofluorometry, using fluorescamine at specific pH ranging of neutral to alkaline [16–19]. The use of adsorptive stripping voltammetry, permanganate-based chemiluminescence and the pharmacopoeial methods have been documented [20–23].

While most of these methods are cumbersome, the charge transfer complex formation techniques are usually rapid and simple to perform. Thus, the ability of the acidic hydrogen (-NH) of N-(2,4-dinitro-1-naphthyl)-*p*-toluenesulphonamide to protonate the ring amine group of ceftriaxone when in association in acetonitrile to form charge transfer complex is investigated in this work as a means of spectrophotometric determination of ceftriaxone. Further studies on the ultraviolet, infrared and ^{1}H NMR spectra of CFR, N-(2,4-DN1NL) PTS and the adduct were carried out to predict the site of the interaction between the donor and acceptor. N-(2,4-dinitro-1-naphthyl)-*p*-toluenesulphonamide {N-(2,4-DN1NL) PTS} and ceftriaxone (CFR) were selected based on our previous work on screening of seven nitroaromatics [24] which showed evidence of charge transfer reaction. This was then used to develop an alternative simple, accurate and precise method for the determination of CFR in bulk sample and dosage form.

This present work will be useful in both qualitative and quantitative on-the-spot cost-effective analysis of ceftriaxone in its pure and bulk forms with high accuracy and precision. Hence, it can be applied to detect counterfeit and adulterated ceftriaxone products with high degree of precision and reliable accuracy especially in resource-limited economies where advanced technologies, such as HPLC, are not readily available.

Method

Chemicals and reagents

The reagents and solvents of analytical grade were used and include acetone, ethanol, methanol, chloroform, ethyl acetate, 1, 4-dioxan, sulphuric acid, hydrochloric acid, perchloric acid, acetic anhydride, glacial acetic acid, acetonitrile, petroleum ether, diethyl ether (all BDH, England); N-(2,4-dinitro-1-naphthyl)-*p*-toluenesulphonamide (Sigma-Aldrich, USA).

Drug sample

Ceftriaxone (CFR) powder for injection was extracted, crystallised and recrystallised in our laboratory.

Equipment

Digital analytical balance (KERN AL 220-4, Kern and Sohn own DKD Calibration Laboratory, Germany); Mettler analytical balance (H 80, Mettler, UK); UNICO UV-2100 series (Shanghai Instruments Company Ltd; China), Ultrasonic bath (Langford, Ultrasonic sonomatic®; UK); UV lamp (Jenway UV 7804, U.K); Vortex mixer (XH-C, Wincom Co. Ltd, China); Struart melting point apparatus (SMP 11 with thermometer, Struart Co. Ltd, England); Oven (Techmel AISET YLD-2000, Techmel, U.S.A.), HPLC equipment (Agilent Technologies 1260 Infinity, U.S.A.); UV/VIS spectrophotometer (Spectrumlab 752s., B. Bran Scientific and instrument company, England); infrared spectrophotometer (Spectrum Two FT-IR Perkin Elmer and Perkin Elmer FT-IR BX II, U.S.A); ^{1}H NMR spectrometer (Bruker DPX 400, Rheinstetten, Germany).

Software utilised in the study

i. Chem ^{1}H Estimate, C S ChemDRAW Pro®. Version 6.0. Cambridge Software Corporation, Cambridge, MA. 02,140 USA www.camsoft.com/support/
ii. Spartan'10 Graphical User Interface for Windows, Macintosh and Linuh version 1.0.1, Wavefunction, Inc. CA 9612; California. www.wavefun.com
iii. ACD/NMR Processor, Academic Edition, version 12.0. ACD Labs Inc. Toronto, Ontario, Canada. www.acdlabs.com

iv. ACD/ChemSketch (Freeware), Version 12.01. ACD Labs Inc. Toronto, Ontario, Canada. www.acdlabs.com
v. Spinus ^{1}H NMR Processor (on-line). www.nmrdb.org
vi. Mestrenova (Mnona 11). Mestrelab Research S. L. Feliciano Barrera, 9B. Bajo 15,706, Santiago de Compostela, Spain. www.mestrelab.com

Preparation of standard solutions of the donor and acceptor

Stock solution of donor, CFR (1708 µg mL^{-1}, corresponding to 2.58×10^{-3} mol L^{-1}) was prepared in methanol. A 0.010 g quantity of N-(2,4-DN1NL) PTS powder was weighed into a clean 10 mL volumetric flask and acetonitrile (5 mL) was added. This was then shaken until it had dissolved appreciably. The solution was then made up to 10 mL mark with acetonitrile to make 0.1% w/v (2.58×10^{-3} mol L^{-1}) N-(2,4-DN1NL) PTS solution.

Instrumentation and physical measurements

Electronic absorption spectra

The electronic absorption spectra were recorded in the region 600–200 nm using UV spectrophotometer (UNICO UV/VIS-2100 series, China).

Infrared spectra

The infrared spectra of the prepared solid charge transfer complex were measured using KBr discs on infrared spectrophotometer (Spectrum Two FT-IR Perkin Elmer, USA).

1H NMR spectra

^{1}H NMR spectra were obtained on ^{1}H NMR spectrometer (Bruker Advance II 400 MHz, Rheinstetten, Germany) using TMS as an internal reference and DMSO-D_6 as the solvent.

Evidence of reaction between N-(2,4-dinitro-1-naphthyl)-p-toluenesulphonamide and CFR

Sample solution of N-(2,4-DN1NL) PTS (0.1% w/v) was prepared in acetonitrile, while equimolar concentration of CFR (0.00258 M) was made in methanol. A 0.1 mL solution of CFR was added into test tube containing 0.1 mL N-(2,4-DN1NL) PTS. The colours produced immediately and after 5 min were noted.

Thin layer chromatography

The thin layer chromatographic examination was conducted using three different solvent systems, chloroform/ethyl acetate/methanol (4:4:2) for the ceftriaxone using N-(2,4-dinitro-1-naphthyl)-*p*-toluenesulphonamide as charge transfer reagent.

Another 0.1% w/v solution of N-(2,4-DN1NL) PTS was prepared in acetonitrile while equimolar concentrations of ceftriaxone (0.00258 M) was made in methanol as stock solutions. Pre-coated TLC plates were thereafter spotted and developed with the stock solution of the drug, N-(2,4-DN1NL) PTS, and the adduct to show reaction occurred. The corresponding retardation factor, R_f, of the spots was thereafter calculated.

Selection of analytical wavelength

To select the appropriate wavelength for the coloured product, a spectrophotometric scan of the reaction mixture was done after diluting to 10 mL with acetonitrile. The absorption maxima of the reaction product relative to N-(2,4-DN1NL) PTS was noted.

Selection of the maximum wavelength of the investigated drug

A spectroscopic scan of the investigated drug was carried out after appropriate dilutions so as to choose the maximum wavelength for reference purposes. This was further used as evidence of the reaction of the investigated drug with N-(2,4-DN1NL) PTS.

Optimisation studies

Optimal temperature

The temperature requirement for optimal colour formation was determined at six temperature levels of 30, 40, 50, 60, 70 and 80 °C using two time levels of 5 and 20 min.

Aliquot of stock solution of drug (0.1 mL) was added to 0.5 mL of N-(2,4-DN1NL) PTS solution in test tubes and the resulting mixture was mixed using a vortex mixer followed by incubation at 30, 40, 50, 60, 70 and 80 °C for 5 and 20 min. Each determination was done in duplicate. Using the same procedure, the blank mixture was also prepared to eliminate the contributions due to reagent (N-(2,4-DN1NL) PTS) and methanol.

The reaction mixtures were diluted to 10 mL with acetonitrile and the absorbance was recorded at 440 nm for CFR using UV/VIS-spectrophotometer. The optimum temperature was taken as the temperature that corresponds to the maximal absorbance of the sample. Blanks were prepared for every other method of optimization and validation mentioned below.

Optimal time

The time requirement for complete reaction was optimised at temperature of 30 °C using time levels of 0, 2, 5, 10, 15, 20, 25 and 30 min. Aliquot of CFR stock solution (0.1 mL) was added to 0.5 mL of N-(2,4-DN1NL) PTS solution in test tubes and the reaction mixtures

were mixed using a vortex mixer followed by incubation at 30 °C for 0, 2, 5, 10, 20 and 30 min. The reaction was terminated by making up to 10 mL by acetonitrile at the specified times. Each determination was done in duplicate. The absorbance reading of the complex was recorded at 440 nm after terminating the reaction. The optimal reaction time was taken as the time corresponding to the maximal absorbance of the sample.

Effect of diluting solvent after charge transfer reaction

The diluting solvent requirement for the method development was determined using the optimised procedures described.

Methanol, acetonitrile, 1, 4-dioxan and ethanol were used as the diluting solvent. Each of these solvents was used to make up the reaction mixture (0.5 mL of 1000 µg / mL of N-(2,4-DN1NL) PTS stock solution and 0.1 mL of drug candidate's stock solution after incubation at 30 °C for 10 min) to 10 mL. In each case, the absorbance reading of the resulting mixture was recorded at 440 nm using UV–visible spectrophotometer.

Stoichiometric ratio determination

The optimal mole ratio required in the reaction was determined using Job's method of continuous variation [25]. The optimal mole ratio was taken as one that gave the highest absorbance for the reaction products of N-(2,4-DN1NL) PTS with CFR. Equimolar solutions (0.00258 M) of N-(2,4-DN1NL) PTS, and CFR stock solution were prepared in their respective solvents.

The molar concentration prepared for the studied drug (CFR) was 1.708 mg/mL, equivalent to 1708 µg/mL.

Into nine (9) different test tubes, 0.00, 0.20. 0.25, 0.40, 0.50, 0.60, 0.70, 0.75 and 1.00 mL of N-(2,4-DN1NL) PTS stock solution was added. The solutions were made up to 1 mL with CFR stock solution. These mixtures represented mole ratios of N-(2,4-DN1NL) PTS and the drug candidate (CFR) used (0:1, 1:4, 1:3, 1:1.5, 1:1, 1.5:1, 2.33:1, 3:1 and 1:0). The mixtures were incubated at 30 °C for 10 min for CFR, made up to 10 mL with acetonitrile and the absorbance readings recorded at 440 nm.

Validation studies

The validation studies were carried out in line with the international conference on harmonisation (ICH) guidelines [26] and described below;

Calibration curves

Calibration curves were prepared on each of three days using the optimised procedures described. The slope, intercept and coefficient of determination (r^2) were determined. Also are, limit of detection (LOD) and limit of quantitation (LOQ).

A 0.10 mL of N-(2,4-DN1NL) PTS stock solution was added to each of the eight test tubes containing aliquot of CFR stock solution (0.000, 0.005, 0.010, 0.015 0.020, 0.025 0.030, and 0.035 mL). The mixtures were incubated at 30 °C for 10 min, made up to 5 mL with acetonitrile and the absorbance readings, recorded at 440 nm. This process was repeated three times and on each occasion, freshly prepared drug candidate's stock solution was used. The average absorbance reading was obtained from the determinations, and used to generate the calibration curve.

Assessment of accuracy and precision

Recoveries of standard concentrations of the drug (CFR) from the reaction matrix were carried out on each of the three successive days in order to determine the accuracy and precision of the proposed method. These were determined by using quadruplet of samples of different concentrations of drug candidate's stock solutions, (0.010, 0.020 and 0.030 mL), which corresponds to low, medium and high concentrations on three different days. To each of the samples, 0.1 mL of N-(2,4-DN1NL) PTS stock solution was added.

The reaction mixtures were incubated at 30 °C for 10 min, made up to 5 mL with acetonitrile and the absorbance readings, recorded at 440 nm. The accuracy and precision of the new method were carried out in three successive days.

Assessment of analytical method interference liabilities

The effect of commonly used tablet excipients were studied by carrying out sample determination from matrices containing each of lactose, starch, magnesium stearate, talc, gelatin and a mixture of the excipients.

Six test tubes were used for this study. Five of the test tubes contained little quantities of each of the aforementioned excipients while the sixth contained a mixture of all the above excipients. To each of the test tubes, 0.02 mL of CFR and 0.1 mL of N-(2,4-DN1NL) PTS stock solutions were added.

The reaction mixtures were incubated at 30 °C for 10 min, made up to 5 mL with acetonitrile and the absorbance readings, recorded at 440 nm which was used for calculating percentage recoveries. The results were in quadruplet.

Dosage forms analysis

The new procedure was thereafter applied to the determination of different brands of CFR in pharmaceutical preparations.

A 0.02 mL of CFR stock solution (equivalent to 6.832 µg of ceftriaxone) was reacted with 0.1 mL N-(2,4-DN1NL) PTS stock solution and incubated at 30 °C for 10 min.

Using the same procedure, the blank mixture was also prepared to eliminate the contribution due to reagent (N-(2,4-DN1NL) PTS) and methanol.

The solutions were made up to 5 mL each with acetonitrile and their absorbance measurements recorded at 440 nm.

The United States Pharmacopoeia's [27] method of high performance liquid chromatography (HPLC) for CFR was used as a standard procedure.

Data analysis was carried out using F ratio and Student's t test at 95% confidence level ($p > 0.05$).

Preparation of solid charge transfer complex

The solid charge transfer complex between the donor and acceptor was prepared by mixing equimolar amount of donor and acceptor in acetonitrile, stirred for about 45 min. The resulting colour complex solution was allowed to evaporate slowly at room temperature where the solid precipitated after reduction of volume of the solvent. The separated complex was filtered off, washed several times with acetonitrile, and then collected and dried. The melting point and colour were noted.

Results

Electronic absorption spectra

The absorption spectrum of CFR in methanol and N-(2,4-DN1NL) PTS in acetonitrile are shown in Fig. 1. Strong change in colour was observed upon mixing methanolic solution of CFR with N-(2,4-DN1NL) PTS in acetonitrile.

The result of TLC analysis is presented in Table 1.

The optimisation conditions for the reaction are given in Fig. 2a–c. The temperature and time conditions studied and optimised for the N-(2,4-DN1NL) PTS developed method were found to be 30 °C (room temperature) and 10 min as shown below in Fig. 2a, b, respectively.

Optimised diluting solvent for the formation of the charge transfer complex is shown in Fig. 2c.

Table 1 Result of TLC analyses: mobile phase system used: CHCl3:EtOAc:MeOH (4:4:2 v/v)

Samples	(R_f) Retardation factor values
CFR (drug)	0.014
CFR-N-(2,4-DNINL) PTS (complex)	0.014, 0.83
N-(2,4-DN1NL) PTS	0.83

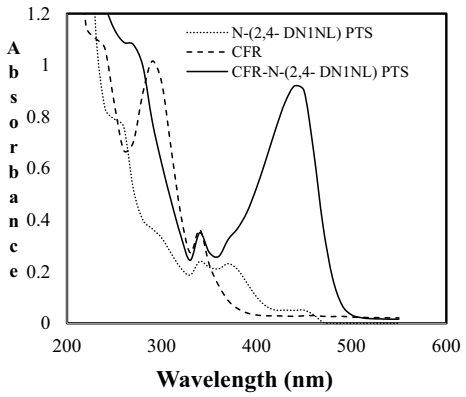


Fig. 1 The overlaid absorption spectra of reagent (N-(2,4-DN1NL) PTS), drug (CFR) and adduct formed. The spectra were produced following UV scanning of the reagent, drug and adduct at wavelength range of 600–200 nm. The non-continuous lines represent the reagent, dashed lines, and the drug, while the continuous lines refer to the drug adduct

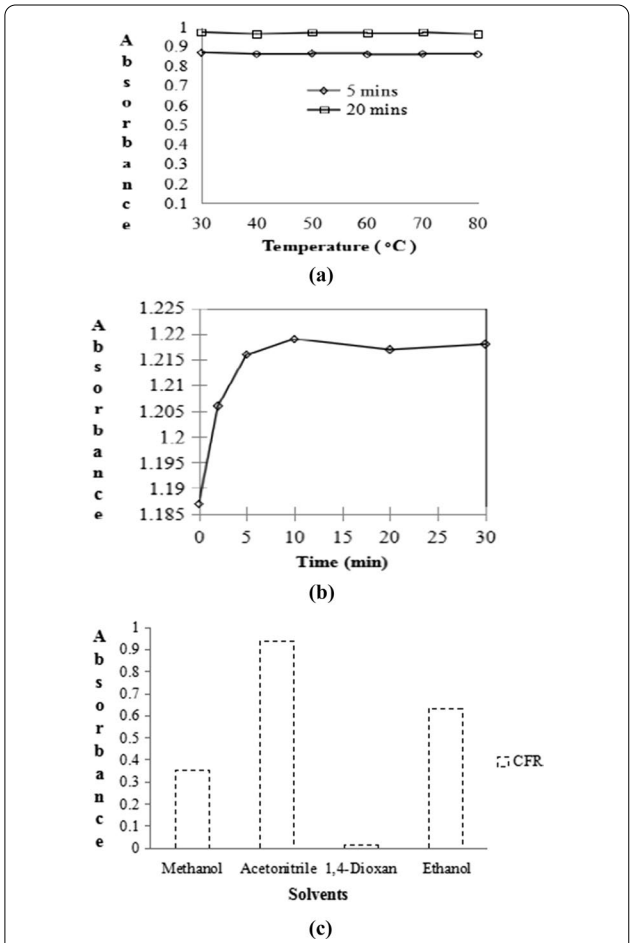


Fig. 2 **a** Optimisation of reaction temperature for charge transfer formation between CFR and N-(2,4-DN1NL) PTS. **b** Optimisation of reaction time for charge transfer formation between CFR and N-(2,4-DN1NL) PTS at 30 °C. **c** Optimisation of the diluting solvent for charge transfer formation between CFR and N-(2,4-DN1NL) PTS complex

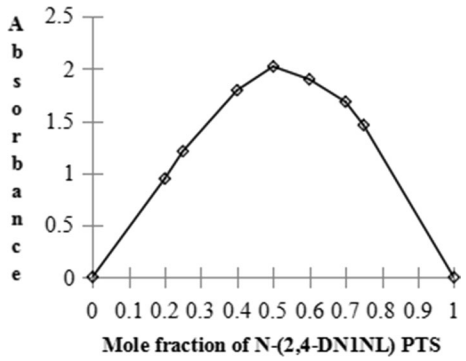


Fig. 3 Job's plot of charge transfer complex of N-(2,4-DN1NL) PTS with CFR

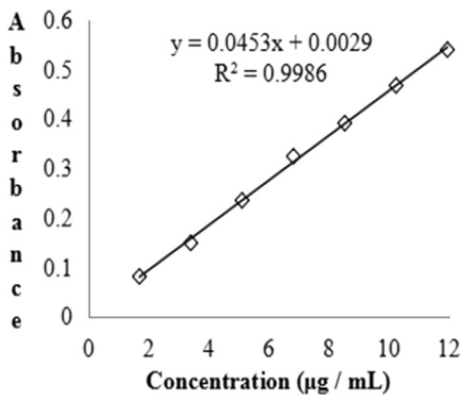


Fig. 4 Calibration line for the charge transfer formation between CFR and N-(2,4-DN1NL) PTS

Figure 3 represents the continuous variation method curve according to Job's method.

The results of validation studies on the calibration curve which were thereafter conducted on three successive days using the optimised conditions earlier outlined are shown in the calibration line (Fig. 4) prepared over the three days' period, while the analytical and validation parameters for the reactions of CFR are presented in Table 2.

The Benesi–Hildebrand plot for CFR and N-(2,4-DN1NL) PTS is presented in Fig. 5.

The physicochemical parameters for the charge transfer formation between CFR and N-(2,4-DN1NL) PTS are presented in Table 3.

The accuracy and reproducibility of the new charge transfer reaction procedure for the determination of CFR from quality control samples are presented in Table 4.

The results of the assessment for interference liability of the complex CFR-N-(2,4-DN1NL) PTS are presented in Table 5.

Table 2 Analytical and validation parameters for the reaction of CFR with N-(2,4-DN1NL) PTS

Parameter	CFR
Beer's law limits (µg mL^{-1})	1.708–11.956
Coefficient of determination (R^2)	0.9986
LOD (µg mL^{-1})	0.143
LOQ (µg mL^{-1})	0.434
Molar abs. (LM^{-1} cm^{-1})	1.667×10^5
Formation constant (K)	3.0×10^3
Sandell's sen. (µg cm^{-1} per 0.001 Abs unit)	3.97
Regression equation[a]	y = 0.0453x + 0.0029
Slope, b	0.045291
Intercept, a	0.002943
Correlation coeff., r	0.9993
Std. dev. of slope	0.000765
Std. dev. of intercept	0.005846
CI of slope, α	0.00070611
Clof intercept, β	0.00147219

CI stands for confidence interval (at 95%)

[a] signifies the mean value of the calibration curve for three days

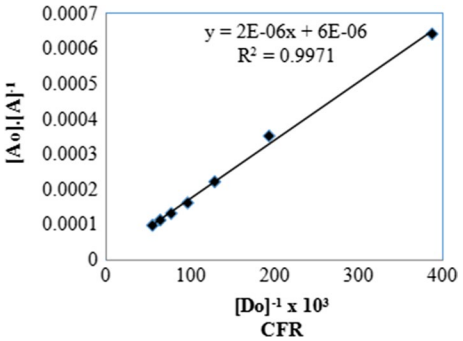


Fig. 5 Benesi–Hildebrand plot for charge transfer formation between CFR and N-(2,4-DN1NL) PTS

The application of this new method in the determination of CFR in different commercial tablet samples is shown in Table 6.

The infrared spectra of the acceptor {N-(2,4-DN1NL) PTS}, drug (CFR) and complex {(CFR-N-(2,4-DN1NL) PTS} showing infrared bands are presented in Fig. 6a–c, respectively.

The Spinus predicted chemical shifts of ^{1}H NMR spectra of N-(2,4-DN1NL) PTS and CFR showing atom identity (ID) and chemical shifts (δ), arranged according to protons and their chemical environments are presented in Figs. 7a, b and 8a, b, respectively.

Also, the ^{1}H NMR spectrum of the formed complex {CFR-N-(2,4-DN1NL) PTS} in deuterated dimethyl

Table 3 Physicochemical parameters for the formation of charge transfer (CT) complex between CFR and N-(2,4-DN1NL) PTS

Drugs	λ_{max} (nm)	Transition energy (E) (eV)	Oscillator frequency (*f*)	Transition dipole moment (μ_{EN}) (Debye)	Resonance energy (R_N) (eV)	Ionisation potential (I_D) (eV)	Dissociation energy (W) (eV)
CFR	440	2.821	414.47	195.59	3.664	9.24	5.317

Table 4 Intra-day and inter-day assessment of accuracy and precision for CFR-N-(2,4-DN1NL) PTS adduct

Assessment	Amount taken (μg mL^{-1})	Amount found (μg mL^{-1})	Recovery (%)	RSD (%)	Relative error (%)
Intra-day[a]	3.416	3.479	101.84 ± 0.82	0.82	1.84
	6.832	6.956	101.81 ± 0.59	0.58	1.81
	10.248	10.444	101.91 ± 0.77	0.75	1.91
Inter-day[b]	3.416	3.490	102.17 ± 0.82	0.81	2.17
	6.832	6.974	102.08 ± 0.66	0.64	2.08
	10.248	10.473	102.20 ± 0.72	0.70	2.20

RSD relative standard deviation

[a] Average of six determinations

[b] average of 12 determinations

Table 5 Interference liability for CFR-N-(2,4-DN1NL) PTS adduct

Adduct	Excipients	% recovery ± S.D[n]
CFR-N-(2,4-DN1NL) PTS	Lactose	103.11 ± 0.95
	Starch	103.27 ± 0.42
	Talc	102.38 ± 0.55
	Gelatin	102.46 ± 0.87
	Magnesium stearate	102.78 ± 1.15
	Mixture	102.94 ± 0.42

Mean values, n = 4

sulphoxide (DMSO-D_6) and sections of the spectrum are presented in Fig. 9a–c

Discussion

A deep golden-yellow colour which does not belong to any of the reactants was observed for CFR-N-(2,4-DN1NL) PTS complex indicating the existence of charge transfer interaction between the drug and acceptor. The colour remained unchanged for more than five hours in the laboratory environment. As shown in Fig. 1, CFR produced new compound with the reagent giving a pronounced bathochromic shift and hyperchromic effect. Optimal difference in absorptivity that was found at 440 nm for the charge transfer complex also confirms charge transfer interaction between the drug and the acceptor. Neither the drug nor the acceptor has measurable absorptions in the region of CT absorption of the resultant complex.

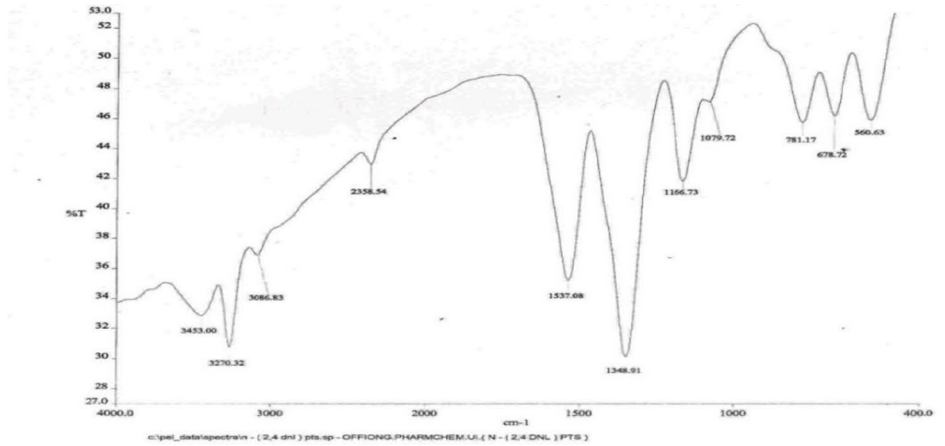
The mechanism of the appearance of this new band can be explained thus: CFR is a nitrogenous compound and hasan electron rich centre capable of donating lone pairs of electrons to electron-deficient species. On the other hand, besides the two nitro groups (-NO_2) of N-(2,4-DN1NL) PTS that draw electrons from the rings, the –SO_2 group and the additional methyl sulphonamide ring could cause the hydrogen of the secondary amide (-NH) to be partially acidic thereby partaking in intra-molecular

Table 6 Assay results of different commercial brands of CFR dosage forms

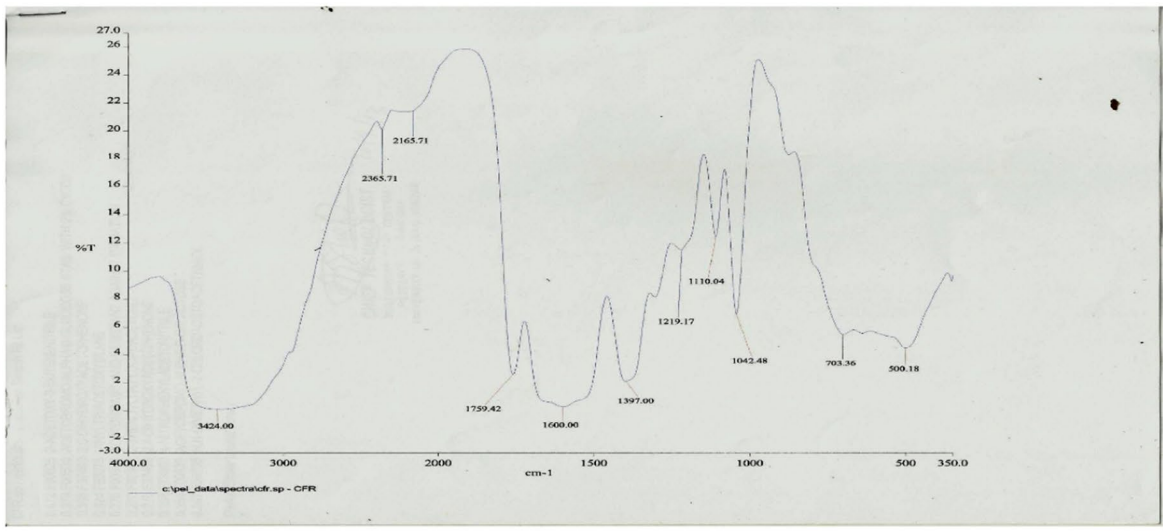
Brands coded	Proposed method[a] (%) ± SD	95% CI (of 6.832 μg/ml)	Official method[a] (%) ± SD	P-value[b] F test T test
Du®	103.32 ± 0.39	7.059 ± 0.029	104.39 ± 1.00	0.192 0.106
Dfx®	102.14 ± 0.29	6.978 ± 0.022	101.90 ± 0.59	0.318 0.390
Sy®	103.16 ± 0.32	7.000 ± 0.050	103.17 ± 0.56	0.365 0.953
Ebt®	102.46 ± 0.71	7.022 ± 0.022	102.69 ± 2.54	0.074 0.872

[a] Mean values, n = 6. Content of ceftriaxone stated by USP, 2007 = 90–115%

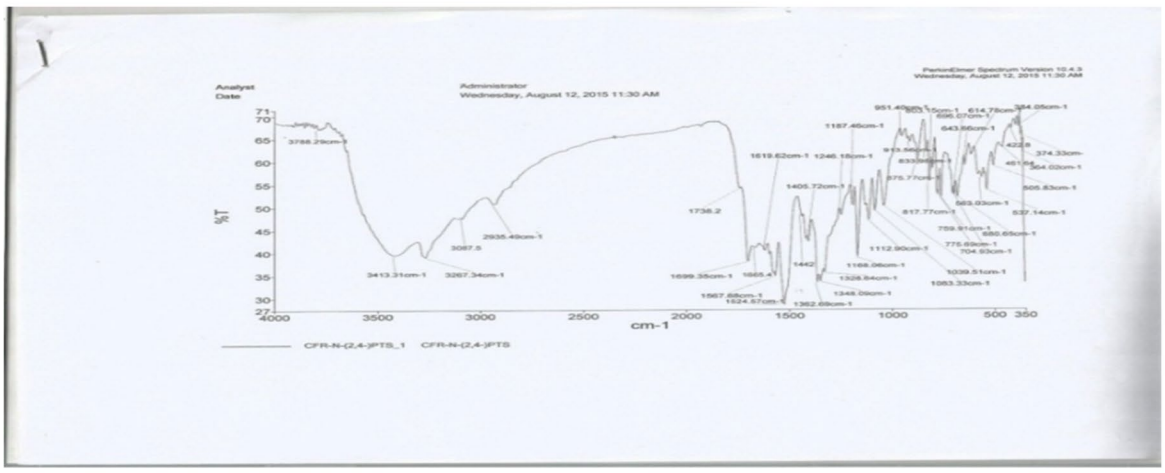
[b] Statistical analyses done between the results obtained from the proposed method and the BP official HPLC method



(a)

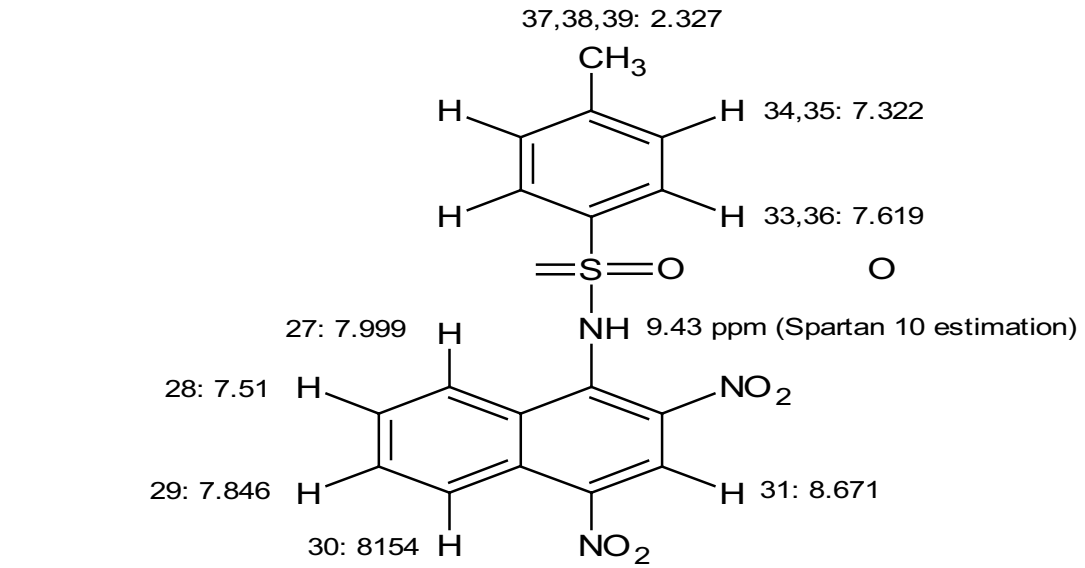


(b)

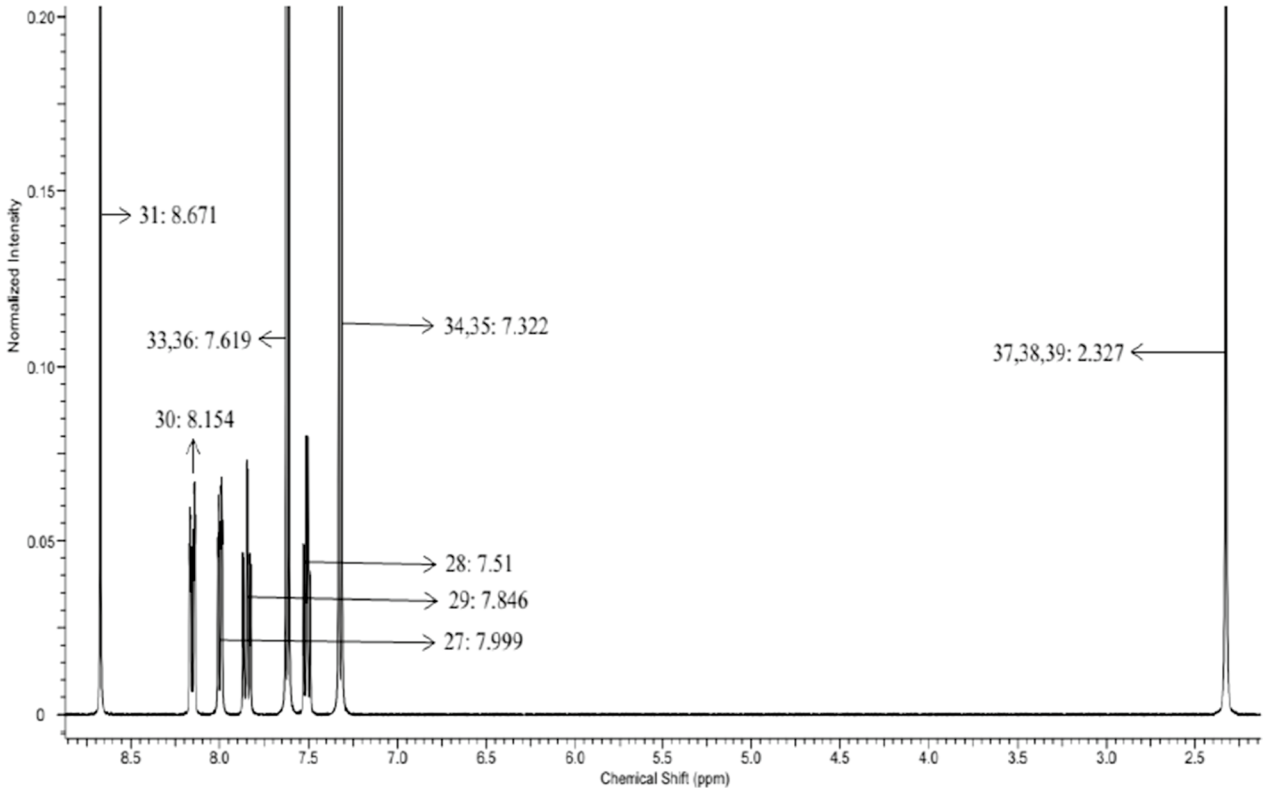


(c)

Fig. 6 **a** The infrared spectrum of N-(2,4-DN1NL) PTS, **b** the infrared spectrum of CFR, **c** the infrared spectrum of CFR-N-(2,4-DN1NL) PTS complex



(a)



(b)

Fig. 7 **a** Spinus predicted chemical shifts of N-(2,4-DN1NL) PTS arranged according to protons. **b** Spinus predicted ^{1}H NMR spectrum of N-(2,4-DN1NL) PTS showing atom ID and chemical shift

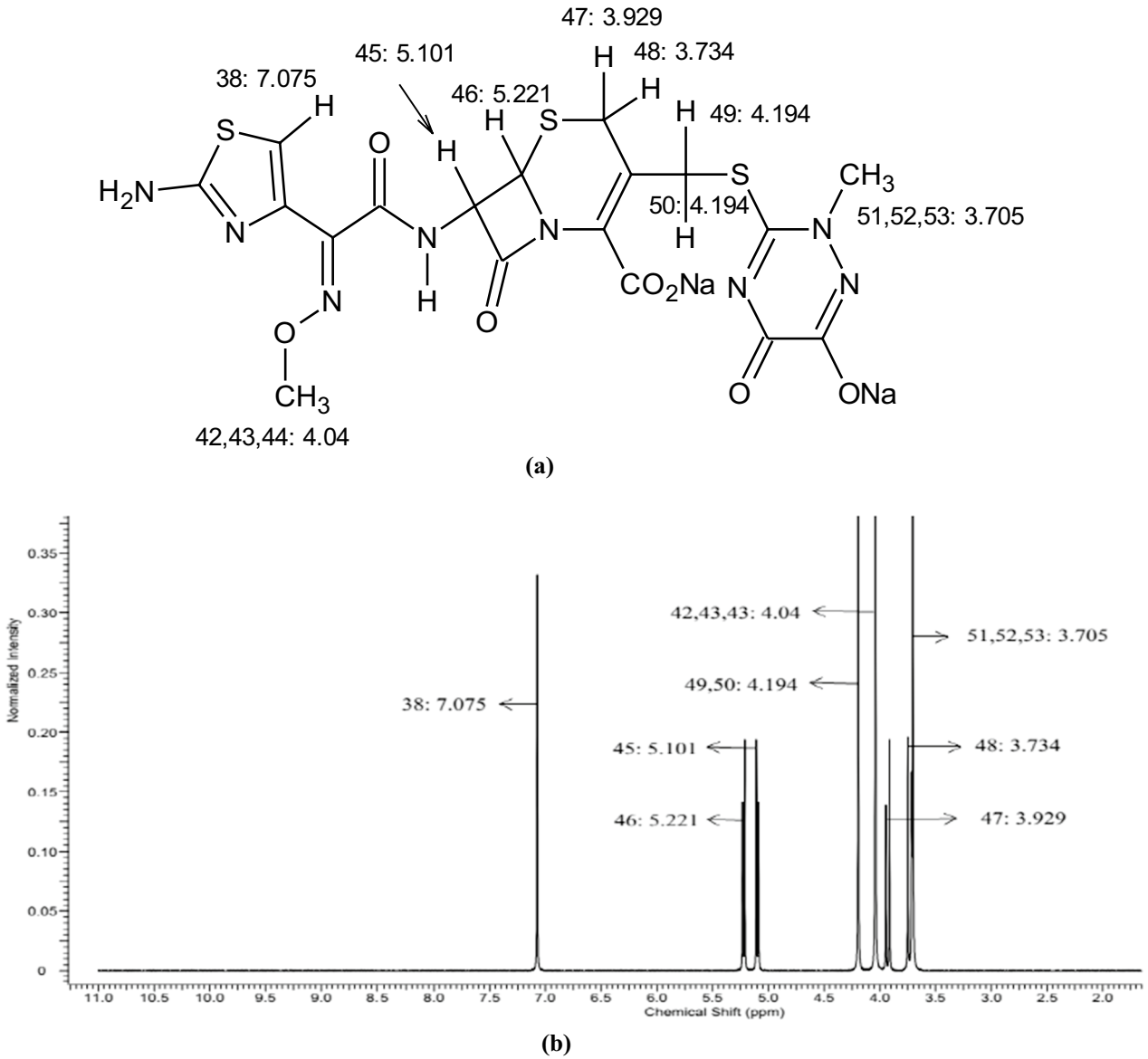


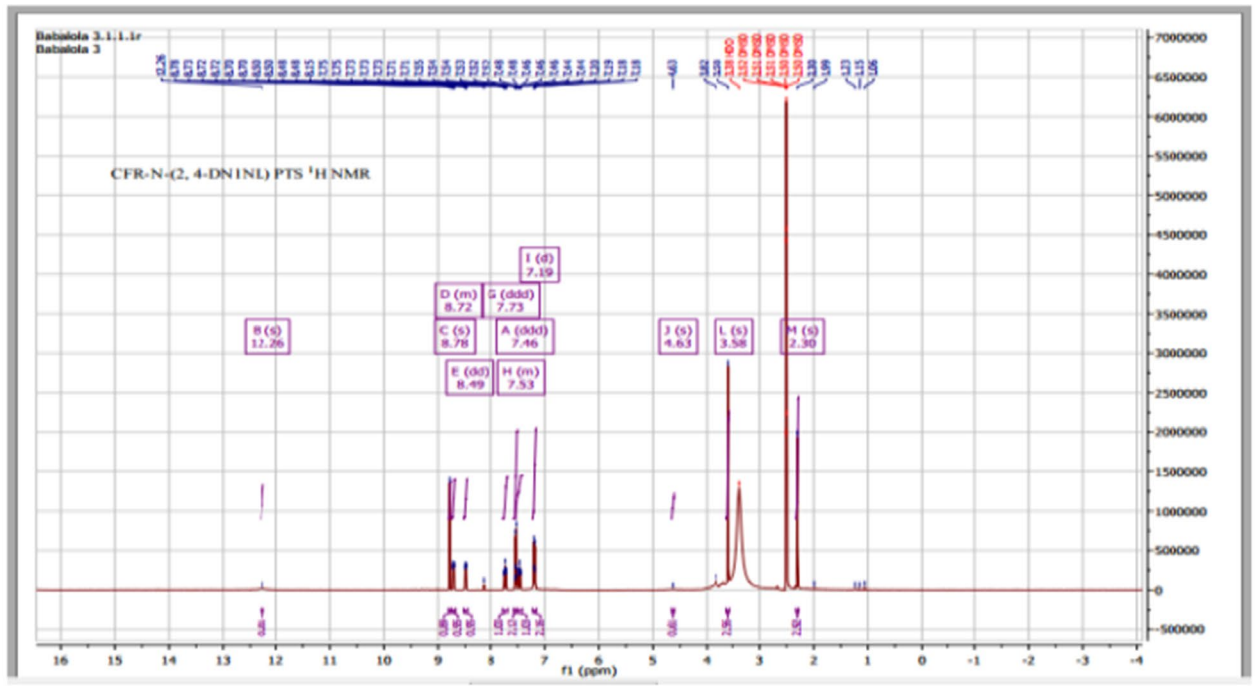
Fig. 8 **a** Spinus predicted chemical shifts of CFR arranged according to protons. **b** Spinus predicted ^{1}H NMR spectrum of CFR showing atom ID and chemical shift

and intermolecular hydrogen bond formation, trying to assume resonance stabilisation. The resultant effect will be the protonation of the ring amine group of the donor (CFR) forming intermolecular hydrogen bonding which resulted in n-π charge transfer complex formation as shown in Scheme 1. Presumably, the arrangement could lead to a tautomer.

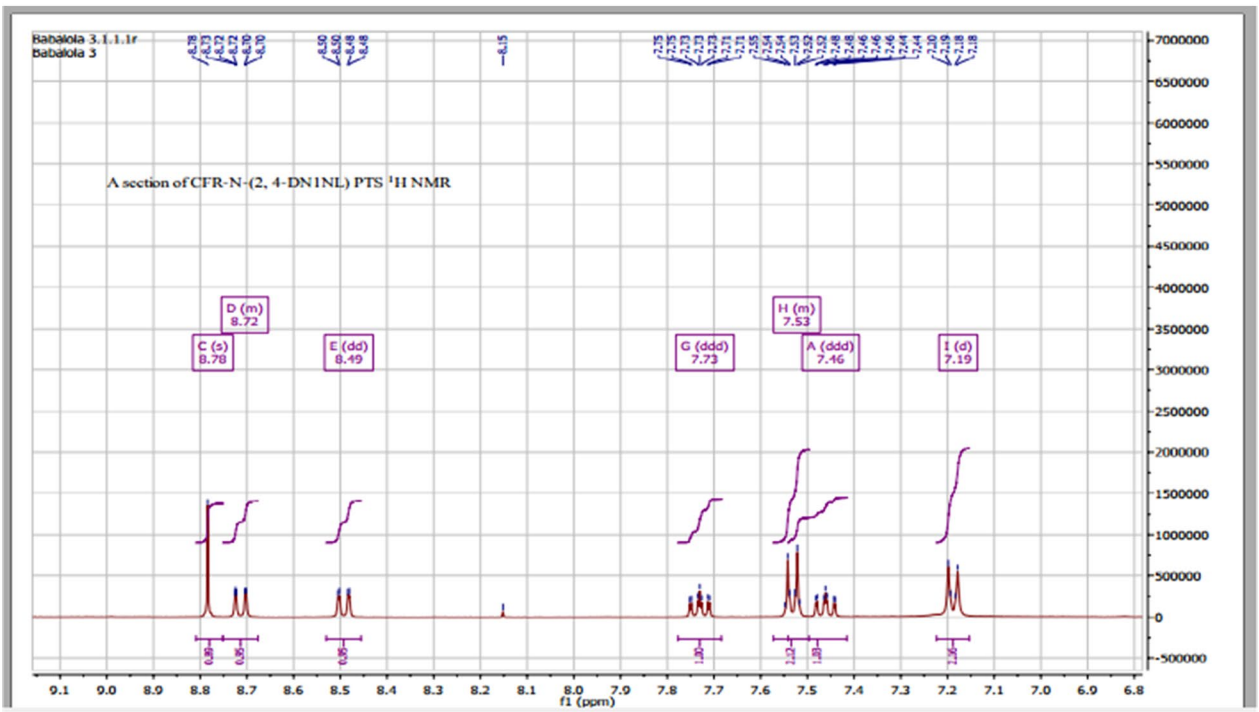
This illustration and explanation also qualifies N-(2,4-DN1NL) PTS as charge transfer reagent for being able to form bathochromic shift and hyperchromic effect when involved in charge transfer interaction with CFR. The new long wavelength absorption band formed is attributed to the formation of N-(2,4-DN1NL) PTS radical anion resulting from the complete transfer of charge from the donor (D) to the acceptor (A) according to Scheme 2.

Thin layer chromatography

TLC analysis was carried out to further ascertain the formation of charge transfer complex between the studied drug (CFR) and reagent, N-(2,4-DN1NL) PTS). The results of TLC confirmed the purity of the CFR, as only one clear spot in the column of drug sample. The

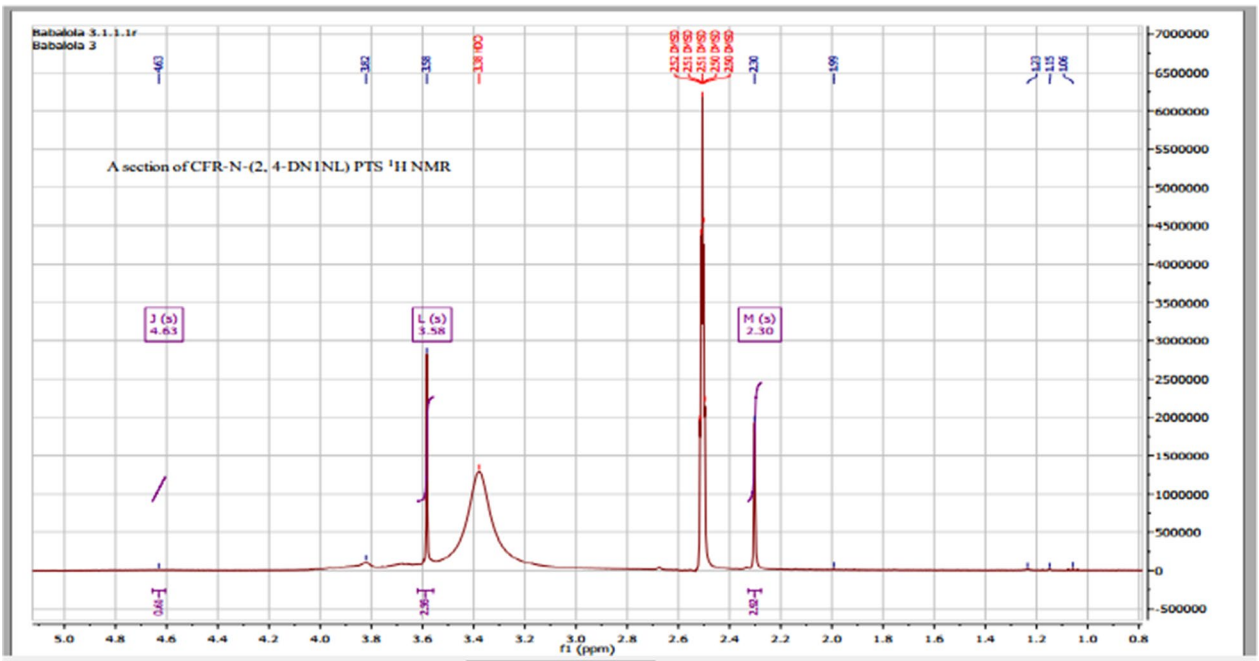


(a)



(b)

Fig. 9 **a** The ^{1}H NMR of the formed complex, {CFR-N-(2,4-DN1NL) PTS} in deuterated dimethylsulphoxide. **b** A section of the ^{1}H NMR of the formed complex, {CFR-N-(2,4-DN1NL) PTS} in deuterated dimethylsulphoxide. **c** A section of the ^{1}H NMR of the formed complex, {CFR-N-(2,4-DN1NL) PTS} in deuterated dimethylsulphoxide



(c)

Fig. 9 continued

presence of two spots in the column of the complex indicates two entities in the complex. This inability of having one spot is presumably due to incorrect solvent system that could convey both entities as a single spot along the solvent front. Also, since the complex is held by weak electrostatic forces, such as Van der Waals, the inability of having one spot could also be attributed to the size of the complex, symmetry and electronic structure change upon the formation of the complex that these weak forces could no longer hold together as it travels along the solvent front. It could also imply that there is an unreacted drug since the droplets were not combined stoichiometrically.

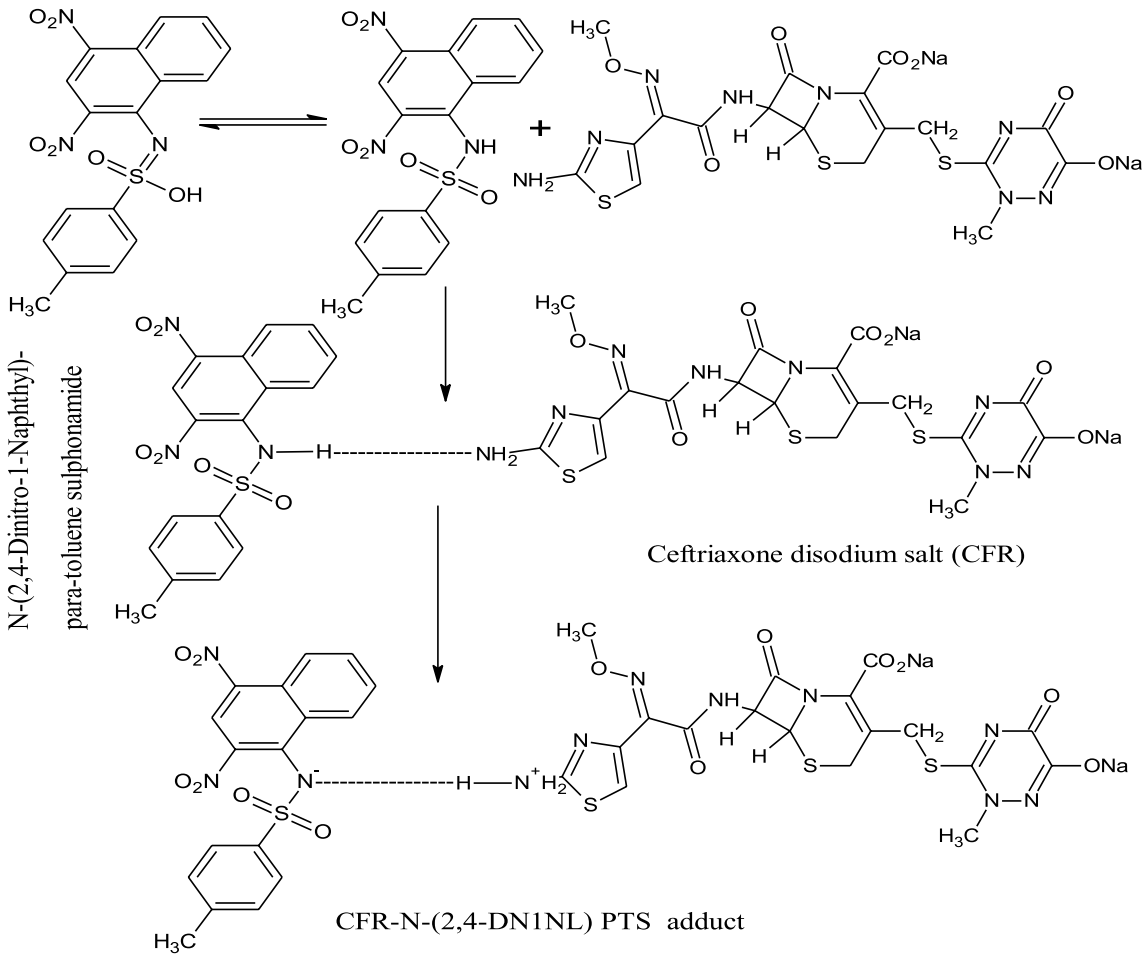
Optimization studies

The optimum temperature studied and determined for charge transfer complex formation of CFR and N-(2,4-DN1NL) PTS as presented in Fig. 2a shows that at five and twenty minutes' interval chosen, N-(2,4-DN1NL) PTS was found to optimally combine with CFR at 30 °C (room temperature). It was also observed that the absorptivity of the complex was decreasing with a rise in temperature from 30 to 80 °C. This gradual decline in absorptivity was confirmed by the reduction in intensity of deep golden-yellow colour produced with N-(2,4-DN1NL) PTS as temperature and incubation time were increased. Such change (in stability) observed in molecular charge transfer is very common in charge transfer complexes as elevated temperatures lead to the destruction of intermolecular binding forces of attraction between ion pairs. Thus, the optimum temperature of 30 °C was chosen for the reaction between N-(2,4-DN1NL) PTS and CFR.

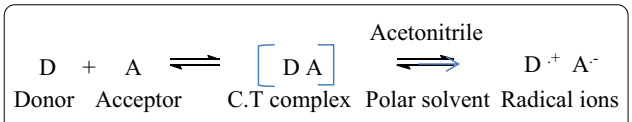
The influence of time on the reaction of CFR with N-(2,4-DN1NL) PTS was also monitored between 0 to 30 min' time limits. The results as presented in Fig. 2b showed a steady increase in absorbance values from 0 min to the highest at 10 min for the complex. A slight or no reduction in absorbance of the charge transfer complex was obtained beyond 10 min. This further confirmed that the system for the analysis of the studied drug using N-(2,4-DN1NL) PTS as reagent was suitable and relatively stable. Thus, the optimal temperature and time of 30 °C and 10 min, respectively, were taken for the reaction of CFR with N-(2,4-DN1NL) PTS.

Effect of diluting solvent after charge transfer reaction

Different polar solvents were used for the reactions of N-(2,4-DN1NL) PTS with CFR so as to choose the best for the formation of charge transfer complex. Acetonitrile was considered to be an ideal solvent for the colour reaction as it offered excellent solvent capacity for N-(2,4-DN1NL) PTS giving the highest yield of radical anion as indicated by high absorptivity value (Fig. 2c).



Scheme 1 Proposed mechanism and site of charge transfer formation for CFR-N-(2,4-DN1NL) PTS adduct



Scheme 2. General presentation of C.T reaction between donor and acceptor molecules

A possible explanation is because acetonitrile possesses the highest dielectric constant of all the solvents examined. This property, not only helped in the formation of N-(2,4-DN1NL) PTS radical anions, but also led to a complete transfer of charge from the donor to the acceptor. The maximum colour intensity afforded by acetonitrile may also be attributed to its high solvating power for π acceptors [28]. On the other hand, solvents (methanol and ethanol) increased the absorbance of the blank solutions thereby reducing the absorbance of the complexes formed. The absorbance of 1, 4-dioxan was rather very low.

Stoichiometric ratio determination

The composition of the formed charge transfer complexes was determined by applying Job's method of continuous variation [25] as presented in Fig. 3.

The maximum light absorption of the complex of CFR-N-(2,4-DN1NL) PTS varied with the ratios of the drug (CFR) and the reagents {N-(2,4-DN1NL) PTS} at the optimised reaction conditions (Fig. 3). At the optimal condition of temperature and time for the assay of CFR using N-(2,4-DN1NL) PTS as reagent, the stoichiometric ratio for CFR was determined using Job's method of continuous variation [25].

When two species at their total concentration interacts, the complex formed would be at its greatest concentration in their ratio at the point of combination in the complex [29]. Therefore, the plots of absorbance versus the mole fraction of N-(2,4-DN1NL) PTS (Fig. 3) showed a

change of slope at a mole fraction of 0.5 corresponding to a maximum absorbance before the absorption decreases as the mole fraction changed from 0.5. This observation also points to the fact that when two equal volumes of equimolar concentrations (0.00258 M) of N-(2,4-DN1NL) PTS and CFR stock solution is combined, it will produce the highest possible absorbance reading at a mole fraction of 0.5. This signifies that the drug, CFR and N-(2,4-DN1NL) PTS reacted in a 1:1 ratio.

Therefore, the mole ratio of 1:1 represented by 0.5 mL of equimolar stock solutions (0.00258 M) of CFR and 0.5 mL of the reagent {N-(2,4-DN1NL) PTS} with the highest value of absorbance was chosen as the stoichiometric ratio.

Validation studies

This followed the ICH guidelines [26].

Determination of linearity of response and calibration curve

The calibration curve for the CFR-N-(2,4-DN1NL) PTS adduct was prepared from the concentration range of (0.00–34.16) µg/mL using 20 µg/mL of N-(2,4-DN1NL) PTS. The linearity of response for the charge transfer reaction of CFR-N-(2,4-DN1NL) PTS adduct was taken from the concentrations from the region of the curve which obeys Beer–Lambert's law.

The calibration curve was used to generate the regression line equation for the assay of CFR by the new charge transfer method.

Excellent calibration data were obtained for the average determination of the calibration curves constructed on each of three successive days for the studied drug complex with N-(2,4-DN1NL) PTS as reagent. The linear regression line equation with coefficient of determination is presented on the calibration plot in Fig. 4. The slope and intercept of the calibration line gave a small 95% confidence limit. This makes this method suitable to detect small changes in the concentration of the drug under investigation. The correlation coefficient, Beer's law range, molar absorptivity and Sandell's sensitivity were also estimated (Table 2). The small value of Sandell's sensitivity and high value of absorptivity (ε) indicate the high sensitivity of the proposed method for analysis of the studied drug (CFR).

The LOD and LOQ for the drug (CFR) were $0.143 \mu g mL^{-1}$ and $0.434 \mu g mL^{-1}$, respectively (Table 2). The limit of detection (LOD) is the minimum level at which the analyte can be reliably detected for the drug candidate (CFR). It was estimated according to the current ICH guidelines using the expression 3.3σ/S. The limit of quantitation (LOQ), on the other hand, is the lowest concentration that can be measured with acceptable accuracy and precision was also estimated according to the current ICH guidelines using the expression 10σ/S. In both cases, σ is the standard deviation of replicate determination values under the same conditions for the sample analysis but in the absence of the analyte (the blank, n = 6) and S is the sensitivity, namely the slope of the calibration graph.

The Benesi–Hildebrand plot for charge transfer formation between CFR and N-(2,4-DN1NL) PTS

The absorbance values obtained in the calibration curve plot for CFR were plotted as a function of ratio of the molar concentration of the donor/acceptor ($[D_0]$: $[A_0]$) in line with the Benesi–Hildebrand equation [30] shown in Eq. (1).

$$\left(\frac{[A_0]}{A} = \frac{1}{K_{CT}\varepsilon_{CT}}.\frac{1}{[D_0]} + \frac{1}{\varepsilon_{CT}}\right) \tag{1}$$

where $[A_0]$ is the initial concentration of the acceptor (N-(2,4-DN1NL) PTS, A is the absorbance of the charge transfer band, $[D_0]$ is the initial concentration of the donor (CFR), K_{CT} is the formation constant of the new charge transfer band, and ε_{CT} is the molar absorptivity. A plot of $[A_0]/A$ against $1/[D_0]$ will yield intercept as $1/\varepsilon_{CT}$ and the slope as $1/K_{CT}.\varepsilon_{CT}$ from where the formation constant and the molar absorptivity were obtained (Fig. 5). The concentration of the acceptor was kept greater than the donor and fixed so that a wide concentration range could be adopted. The data obtained from the plot (Fig. 5) revealed relatively higher molar absorptivity and formation constant for the studied drug complex with N-(2,4-DN1NL) PTS at room temperature as presented in Table 2.

The parameters characterising the charge transfer complex are presented in Table 3. The characteristics of the complex CFR-N-(2,4-DN1NL) PTS is explained by calculating these physicochemical parameters which give reasons for the tendency or otherwise of the intermolecular charge transfer complex formed. The transition energy of the complex was calculated using the equation $E = h\nu_{CT}$ where h is Planck's constant and ν_{CT} is the wavenumber of the absorption peak of the charge transfer complex. The value obtained in electron volt (eV) was 2.821 using the applicable λ_{max} of 440 nm.

The oscillator strength (f) and transition dipole moment (μ_{EN}) were also calculated, as presented in Table 3. The oscillator strength, a non-dimensional quantity used to predict the transition probability of the charge transfer band and the transition dipole moment of the charge transfer complex were obtained from Eqs. (2) and (3), respectively [31]

$$f = 4.32 \times 10^{-9}[\varepsilon \Delta \nu_{\frac{1}{2}}] \tag{2}$$

$$\mu_{EN} = 0.095\left[\frac{\varepsilon_{CT}\Delta\nu_{\frac{1}{2}}}{\Delta\nu}\right]^{\frac{1}{2}} \quad (3)$$

where $\Delta\nu_{1/2}$ is the half-width, i.e. the width of the band at half the maximum absorption, and $\Delta\nu \approx$ wavenumber at the absorption maximum. The ionisation potential, I_D, of the donor in the charge transfer complex was calculated using the empirical equation by Aloisi and Pigantro [32] as presented in Eq. 4.

$$I_D(\text{eV}) = 5.76 + 1.53 \times 10^{-4}\nu_{CT} \quad (4)$$

where ν_{CT} is the wavenumber of the charge transfer band in cm^{-1}. The resonance energy of the complex (R_N) in the ground state is obtained from Eq. (5) derived by Brieglieb [33].

$$\varepsilon_{CT} = 7.7 \times 10^4/[h\nu_{CT}]/R_N - 3.5 \quad (5)$$

where ε_{CT} is the molar absorptivity of the complex at the maximum of the charge transfer absorption, $h\nu_{CT}$ is the transition energy of the complex. The resonance energy calculated for the new molecular complex is also presented in Table 3. The dissociation energy (W) of the formed charge transfer complex between the drug candidates and N-(2,4-DN1NL) PTS was calculated from the transition energy ($h\nu_{CT}$), ionisation potential of the donor (I_D) and the electron affinity of N-(2,4-DN1NL) PTS ($E_A = 1.1$) using Eq. (6) [34].

$$h\nu_{CT} = I_{CT} - E_{CT} - W \quad (6)$$

The value for dissociation energy was 5.317 eV. From Table 3, these physicochemical parameters followed a certain pattern. The results showed that the formed complex between N-(2,4-DN1NL) PTS and CFR was stable. The ionisation potential of the donor (I_D) was 9.24 eV which indicates that CFR is a good *n*-electron donor because of the high value. Thus, the electrons were readily donated to N-(2,4-DN1NL) PTS. The value of the I_D also showed that the primary amino group found in the drug is a good electron donor. The transition energy (E = 2.821 eV) is lesser than a third of the ionisation energy; hence, the complex is produced easily because the energy barrier required for electronic transition is readily removed. This is another evidence that the complex was produced readily. On the other hand, the dissociation energy ($W = 5.317$ eV) was far less than I_D suggesting a minimal decomposition of the charge transfer complex at room temperature where the reaction took place. This supports the fact that complex decomposes gradually at higher temperatures. The formed complex also has high resonance energy giving stability to the complex. This is very important considering the bulkiness of the drug and their stability after donation of a lone pair electron to N-(2,4-DN1NL) PTS.

Generally, the physicochemical parameters obtained by using N-(2,4-DN1NL) PTS as an acceptor also proves that the intermolecular complex formed between the drug and the reagent was stable at room temperature (30 °C) where the reaction occurred.

Recovery studies

Assessment of accuracy and precision

The recovery of CFR pure sample from the method gave good results on the 3-day assessment.

Three different concentration levels of drugs were used in the charge transfer reactions with N-(2,4-DN1N) PTS to assess accuracy and precision of the proposed method over a 3-day period. The procedures were carried out using four replicates.

The results of intra-day (same day) and inter-day (three consecutive days) assay are summarized in Table 4. (RSD). The low percentage relative standard deviation (RSD) for CFR obtained, (RSD ≤ 0.82% for intra-day) and (RSD ≤ 0.81% for inter-day) confirmed the proposed method to be very precise. Alsothe low percentage relative error (RE %) confirmed the accuracy of the proposed method. Thus, the method showed high precision and good accuracy {(RE ≤ 1.91% for intra-day) and (RE ≤ 2.20% for inter-day)}.

Interference liabilities for analysis of CFR-N-(2,4-DN1NL) PTS adduct using tablet excipients

The effect of additives commonly employed in dosage forms on the assay method was studied for CFR-N-(2,4-DN1NL) PTS adduct. The procedure was carried out using four replicates.

The interference study was performed in order to show the effect of possible interfering species on the reaction of the drug (CFR) under investigation with the N-(2,4-DN1NL) PTS reagent. The interference liability of the proposed method was investigated by observing any interference encountered from some common excipients (such as lactose, starch, talc, magnesium stearate and gelatin) in the pharmaceutical formulations.

There was no interference from the commonly utilised excipients. Gelatin and magnesium stearate (generally used as binders and lubricants, respectively), however, gave high absorbance from the recovery studies. This effect was removed by preparing blank mixtures to eliminate the contribution due to the reagent, N-(2,4-DN1NL) PTS and methanol. Thus, the accuracy and selectivity of this developed method was not affected by the presence of these excipients.

Dosage forms analysis

To evaluate the applicability of this proposed analytical method to the quantification of the CFR in commercial dosage forms (mainly injections), the results obtained by the proposed method were compared to those of the official method by applying Student's t test for accuracy and F ratio test for precision.

The proposed and the official HPLC methods [27] were applied for the assay of CFR injections, respectively. The results obtained for different brands of CFR using both methods were statistically compared. The values obtained from the proposed method for CFR were between 102.14–103.32 ± 0.29–0.71%, while those for the official method range from 101.90–104.39 ± 0.56–2.54 (Table 6). The F ratio test and Student's t test carried out revealed no significant differences in the contents of the four brands of CFR as assessed by both methods at 95% confidence level. This also suggests a similarity in precision and accuracy in the assay of CFR injections. Likewise, the low values of percentage standard deviation (% SD ≤ 0.71) for CFR are also indication of good accuracy. It can therefore be concluded that the proposed method is suitable for the assay of CFR injections with good comparable analytical performance and could be used as an alternative analytical method to the official one.

The benefits of this developed method for the assay of CFR are simplicity, high accuracy and high precision besides the low-cost and speed. Furthermore, the contributions (excess absorbance) due to slight absorption of the reagent and the drug-dissolving solvent (methanol) at the analytical wavelength (λ_{max}) were removed (by using blank solution containing the reagent and methanol) from the absorbance of the complex thereby obtain the net (effective) absorbance of the complex. Consequently, the hyperchromic effect contributed by the reagent is removed conferring to the proposed method correct slope and intercept that gave good recoveries for good precision and accuracy.

Other advantages of the proposed assay method include that of performing all measurements in the visible region (λ_{max} of 440 nm), use of 30 °C (room temperature) as reaction temperature and also the use of reagent and instrumentation that is easily obtainable. Thus, this method could find application in resource-limited economies where technologies such as HPLC are not readily available.

Evidences for the formation of charge transfer complex {CFR-N-(2,4-DN1NL) PTS} and determination of the site of interaction between donor (CFR) and acceptor {N-(2,4-DN1NL) PTS} molecules

Infrared (IR) analysis

The formation of the charge transfer complexes when donors and acceptors are involved in chemical reactions is well evident when comparing the main IR bands of the reactants (donors and acceptors) to the spectra of the products. It is realised that the bands of the reactants in the complexes showed small shifts in intensities when they are compared with those of the reactants. This can be as a result of changes in the symmetry and electronic structure during charge transfer complex formation.

The vibrational frequency shifts of the donors or acceptors (or both) have been the major concern during the measurements of IR bands of charge transfer complexes. The evidence for a particular site of a charge transfer interaction is always confirmed by observing the decrease in vibrational frequency of a particular band [28, 35, 36]. It is also noticed that the complexes showed some differences when their spectra were compared with those of the reactants. Thus, this was employed in differentiating between weak CT complexes and electron-transfer products or proton-transfer reactions [28, 35, 36].

Infrared (IR) spectral studies

The infrared spectra of the acceptor {N-(2,4-DN1NL) PTS}, donor (CFR) and the complex {CFR-N-(2,4-DN1NL) PTS} are shown in Fig. 6a–c.

The comparison of the infrared bands revealed that the β-lactam carbonyl of CFR was shifted from 1759.42 cm^{-1} to1738.20 cm^{-1} when compared with the complex. This vibrational shift proves the formation of charge transfer complex.

Apart from using UV and IR measurement to confirm the formation of CFR-N-(2,4-DN1NL) PTS, IR and ^{1}H NMR were also used to establish the site of interaction of the donor (CFR) and acceptor {N-(2,4-DN1NL) PTS}. Thus, the site of interaction was predicted by comparing the IR and ^{1}H NMR spectra of the complex with the spectra of the donor and acceptor.

The IR spectrum of CFR-N-(2,4-DN1NL) PTS complex is characterised by the absence of bands at 3453.00 and 3270.32 cm^{-1} {found in N-(2,4-DN1NL) PTS} and 3424.00 cm^{-1} (seen in CFR).

These asymmetric and symmetric stretching vibrations of the amino groups, υ_{as} (NH_2) and υ_{s} (NH_2) appearing at 3413.31 and 3267.34, in the IR spectrum of the complex {CFR-N-(2,4-DN1NL) PTS} were shifted from 3424.00 cm^{-1} in the spectrum of the donor (CFR), confirming the formation of C.T. complex [38, 39]. These stretching vibrations of amino groups are also seen as disturbances for the amino groups which reflect the sensitivity of the groups towards hydrogen bonding interactions [28]. Therefore, the recorded band at 2935.40 cm^{-1} is presumably attributed to υ ($^{+}NH_3$), confirming the migration of acidic proton (-NH) of N-(2,4-DN1NL) PTS to the nitrogen of the primary amine of the donor (CFR) through intermolecular hydrogen bonding as shown

in reaction mechanism in Scheme 1. This is confirmed by the reduction in the intensity or broadening in the stretching of N–H group of N-(2,4-DN1NL) PTS because of intermolecular hydrogen bond formation in the complex [40].

^{1}H NMR analysis

Generally, to establish the site of interaction for charge transfer complex (CTC) formation, ^{1}H NMR spectra of the complexes of the investigated drugs were measured in deuterated dimethyl sulphoxide (DMSO-D6) together with the spectra of the drugs to monitor the shift in protons.

In this study, the ChemNMR ^{1}H Estimation, Spinus prediction [37] and Spartan 10 (computational chemistry) software were used to predict the chemical shifts of ^{1}H NMR of the donor and acceptor before comparing these shifts with the ^{1}H NMR of the complex that was dissolved in DMSO-D6 and measured using BRUKER AVANCE II 400 MHz spectrometer.

^{1}H NMR spectral studies

The proton transfer from N-(2,4-DN1NL) PTS to the donor (CFR) was further confirmed by studying and comparing the predicted ^{1}H NMR spectra of the reactants (Figs. 7b, 8b)) with the formed complex in deuterated dimethyl sulphoxide DMSO-D_6 that were measured using BRUKER AVANCE II 400 spectrometer (shown in Fig. 9a–c).

The ^{1}H NMR spectrum of N-(2,4-DN1NL) PTS (Fig. 7a, b) showed the following peaks: The singlet of H-31 showing signals downfield because of the additional deshielding effect by the adjacent nitro groups. The position of absorption for the (H-31) proton was predicted at about $\delta = 8.671$ ppm while two protons (H-27, H-30) displayed a double of doublets at $\delta = 7.999$, and 8.154 ppm and protons of (H-28, H-29) showed double triplets at $\delta = 7.51$ ppm and $\delta = 7.846$ ppm due to large *ortho* coupling with two protons (H-27, H-30) in N-(2,4-DN1NL) PTS. The rest of the methyl benzene protons showed the following chemical shifts depending on their nearness to the deshielding effect of the SO_2 group in the molecule. The doublet (H-33, H-36) showed signals downfield at $\delta = 7.619$ ppm compared with the doublet (H-34, H-35) at $\delta = 7.322$ ppm, while the signal for methyl protons (H-37, H-38, H-39) is seen at $\delta = 2.327$ ppm.

The arrangement of peaks in N-(2,4-DN1NL) PTS (Fig. 7b) are also seen in the complex {CFR-N-(2,4-DN1NL) PTS by inspection at positions $\delta = 8.78$, 8.72, 8.49, 7.73, 7.53, 7.46, 7.19 and 2.30 when comparing the types and positions of the peaks found in the donor (CFR) and acceptor {N-(2,4-DN1NL) PTS} with the peaks found in the complex.

The protons of CFR are also assigned chemical shifts at positions, $\delta = 8.15$, 4.63, 3.82 and 3.58 in the complex as shown in Fig. 9a–c. The differences noticed in the chemical shifts (δ) of the reactants when compared to the complex are presumably due to the fact that those of the reactants were predicted besides the symmetry and chemical environment of the protons when reactants come together in charge transfer reaction. Another presumption is that the association of the reactants in charge transfer reaction also brings about different kinds of signals and multiplets because of the molecular size of reactants, number of protons, kinds of protons and their chemical environments.

It was found that, the acidic proton (-NH) of N-(2,4-DN1NL) PTS (Fig. 7a) is assigned at 9.43 ppm (Spartan 10 estimation).

This ^{1}H NMR signal of the acidic proton of N-(2,4-DN1NL) PTS (9.43 ppm) disappeared in the complex {CFR-N-(2,4-DN1NL) PTS}. Instead new peaks were observed at $\delta = 12.26$, 1.99 and 1.15 that can be attributed to N^-----H—N^+ [38], $^+NH_2$ and/or other exchangeable protons in the complex. This interaction supports the transfer of the acidic proton (-NH) of N-(2,4-DN1NL) PTS to the ring nitrogen of the donor in accordance with infrared results [39]. These suggestions also point to the site of interaction of donor and acceptor molecules as well as the formation of charge transfer complex through intermolecular hydrogen bonding between the amino group of the donor (CFR) and acidic proton of the acceptor {N-(2,4-DN1NL) PTS}. The proposed site of interaction of donor (CFR) and acceptor {N-(2,4-DN1NL) PTS} molecules is shown in Scheme 1.

It is also observed in the ^{1}H NMR spectra of Fig. 9a–c that the peaks at positions H-45 ($\delta = 5.101$) and H-46 ($\delta = 5.221$) were absent although seen in ^{1}H NMR spectrum of CFR, IR spectrum of CFR-N-(2,4-DN1NL) PTS and by the presence of β-lactam C=O band in IR of drug (Figs. 8b, 6c, b, respectively). This could presumably be attributed to the effect of protonation by another intermolecular hydrogen bond formation at the nitrogen ring of β-lactam thereby removing the partial stabilisation of the C=O group which was allowed through delocalization of the lone pair of electrons on the adjoining N-atom [41]. The absence of such stabilisation may result in hydrolysis of the β-lactam bonds by the ^{1}H NMR solvent (DMSO-D_6) which was used to dissolve the complex. In addition, a careful look at the ^{1}H NMR spectrum of the complex also revealed the presence of water, d_1 (HDO) that may also cause the hydrolysis of β-lactam bond.

Conclusion

The proposed method is the first attempt of using N-(2,4-DN1NL) PTS as reagent in charge transfer reactions for the purpose of developing an analytical method for the assay of a dosage form. It holds promise for ready utilisation, especially in the third world countries were sophisticated facilities like HPLC with electrochemical detection are not readily available.

Also, we conclude that the molecular complex formed between N-(2,4-DN1NL) PTS and CFR is through electron and proton transfer.

Abbreviations

µm: Micrometre; °C: Degree Celsius; A: Acceptor molecule; D: Donor molecule; Abs: Absorbance; BP: British Pharmacopoeia; CFR: Ceftriaxone disodium salt; CFR-N-(2,4-DN1NL) PTS: Ceftriaxone-N-(2,4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; G: Gram; Hr: Hour; HPLC: High-performance liquid chromatography; IP: International Pharmacopoeia; Kg: Kilogramme; M.O.: Molecular orbital; Mg: Milligramme; Min: Minutes; mL: Millilitre; N-(2,4-DN1NL) PTS: N-(2,4-dinitro-1-naphthyl)-*p*-toluene sulphonamide; Nm: Nanometre; P: Level of significance; RSD: Relative standard deviation; SD: Standard deviation; TLC: Thin layer chromatography; USP: United States Pharmacopoeia; UV: Ultraviolet–visible; **E**: Molar absorptivity; NAC: Nitro aromatic compound; CNAC: Chloronitroaromatic compound; H_2SO_4: Sulphuric acid; Conc.: Concentrated; ppt.: Precipitate; Eqn.: Equation; CTC: Charge transfer complex; E: Transition energy; *F*: Oscillator frequency; μ_{EN}: Transition dipole; R_N: Resonance energy; I_D: Ionisation potential; eV: Electron volt; W: Dissociation energy; K: Formation constant.

Acknowledgements

The authors wish to express profound gratitude to Professor C. P. Babalola, Professor S. O. Idowu, Dr. B. B. Samuel, Dr. Aderigbe and all the laboratory staff members of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan.

Author contributions

OAA conceived the concept of the work and supervised it. OEU designed the work and carried out the laboratory experiments, obtained and analysed data and prepared a draft of the manuscript. DEE gave inputs and prepared the final copy of the manuscript. All authors read and approved the final manuscript.

Funding

This work was partly funded by the University of Uyo, Nigeria as a staff development training assistance to encourage the proficiency of staff members in postgraduate studies and research excellence. The University, however, does not influence or determine the direction of the research. The course of the research was solely determined by the authors.

Availability of data and materials

All data and materials used to make inferences and conclusion of this work have been provided in the blinded copy. Any further data that may be requested shall be provided.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors approved the manuscript for publication.

Competing interests

All authors declare no competing interests.

Author details

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

Received: 8 August 2022 Accepted: 19 December 2022
Published online: 03 January 2023

References

1. British Pharmacopoeia (2013) Incorporating the requirements of the 7th edition of the European Pharmacopoeia as amended by supplements 7.1 to 7.5. The British Pharmacopoeia Commission Secretariat, 5th. Floor, 151 Buckingham Palace Road, London. SWWIW 9S:291–295
2. Podolsky ML (1998) Cures out of chaos: how unexpected discoveries led to breakthroughs in medicine and health. Harwood Academic Publishers
3. Singh JA (1999) New cephalosporins. Semin Pediatr Infect Dis 10(1):14–22. https://doi.org/10.1016/S1045-1870(99)80005-3
4. Perez-Inestrosa E, Suau R, Montañez MI, Rodriguez R, Mayorga C, Torres MJ, Blanca M (2005) Cephalosporin chemical reactivity and its immunological implications. Curr Opin Allergy Clin Immunol 5(4):323–330
5. eMedExpert.com-Comprehensive information on prescription drugs
6. Gamal AS, Hassan FA, Ibrahim AD, Abdel-Nasser AE (2003) Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. Anal Sci 19:281–287
7. Gamal AS, Hassan FA, Mohammed FR, Mohammed AO (2001) Use of charge-transfer complexation in the spectrophotometric analysis of certain cephalosporins. Talanta 54:1205–1215
8. Eman YF, Gehad GM, Farag AB, Yussof EB (2011) Utility of pi and sigma acceptor reagent for the spectrophotometric determination of cephotaxim sodium antibacterial drug via charge transfer complex formation. Insight Pharm 1(4):47–54
9. Al-Monani IF (2001) Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. J Pharm Biomed Anal 25(5–6):715–717
10. Metwally FH, Alwarthan AA, Al Tamimi SA (2001) Flow-injection spectrophotometric determination of certain cephalosporins based on the formation of dyes. Farmaco 56(8):601–607
11. Nkeoma NO, Godwin ICN, Nkechinyere NU, Festus BCO (2007) Spectrophotometric determination of some cephalosporin antibiotics using Prussian blue. Sci Res 2(8):342–347
12. Alaa SA, Gamal HR (2004) Spectrophotometric determination of certain cephalosporins in pure form and in pharmaceutical formulations. Spectrochim Acta Mol Biomol Spectrosc 60(12):2831–2835
13. Issopoulos PB (1989) Spectrophotometric determination of certain cephalosporins using molybdophosphoric acid. Part 11. Determination of cefadroxil, cefpirin, ceforanide and cefuroxime. Analyst 114(2):237–239
14. Pasha C, Narayana B (2008) A simple method for the spectrophotometric determination of cephalosporins in pharmaceuticals using variamine blue. Department of Post Graduate Studies and Rsearch in Chemistry, Mangalore University, Mangalagangothri-574199, Karnataka, India
15. Aswani KCH, Anil KT, Gurupadayya BM, Navya SS, Rahul RMB (2010) Novel spectrophotometric determination of valacyclovir and cefotaxime using 1,2-naphaquinone-4-sulphonic acid sodium in bulk and pharmaceutical dosage form. Arch Appl Sci Res 2(4):278–287
16. El-Gindy A, El-Walily AF, Bedair MF (2000) First derivative spectrophotometric and LC determination of cefuroxime and cefadroxil in urine. J Pharm Biomed Anal 23(2–3):341–352
17. Murad IHH, Eyad SMA, Rasheed MAQJ (1998) Direct titration and indirect spectrophotometric determination of selected cephalosporins. Acta Ploniac Pharm Drug Res 55(2):93–97
18. Sonil S, Nitin D, Dinesh KJ, Lalit KT, Mahendra S (2010) Spectrophotometric and RA-HPLC methods for simultaneous determination of cefpodoxim proxetil and clavulanate potassium in combined tablet dosage form. Am Eurasian J Sci Res 5(2):88–93
19. Hefnawy M, El-Shabrawy Y, Belal F (1999) Spectrofluorometric determination of alpha-aminocephalosporins in biological fluids and pharmaceutical preparations Journal of Pharmceutical Biomedical. Analysis 21(4):703–707

20. Farghaly OA, Hazzazi OA, Rabie EM, Khodari M (2008) Determination of some cephalosporins by adsorptive stripping voltammetry. Int J Electrochem Sci 3:1055–1064
21. Fatma AA, Nawal AA, Abdlrahman AA (1998) Permanganate-based chemiluminescence analysis of cefadroxil monohydrate in pharmaceutical samples and biological fluids using flow injection. Talanta 47:471–478
22. The United State pharmacopoeia 24. National Formulary 19. U.S. Pharmacopoeia Convention. Rockville
23. The British Pharmacopoeia (1993) Her Majesty Stationary Office, London
24. Umoh OE (2022) Adegoke O A and Effiong D E (2022) Potentials of N-(2, 4-dinitro-1-naphthyl)-p-toluenesulphonamide and 2, 4-dinitro-1-naphthol as novel charge transfer acceptors in pharmaceutical analysis of some quinolones and cephalosporins. Future J Pharm Sci 8:18. https://doi.org/10.1186/s43094-022-00407-7
25. Job P (1928) Job's method of continuous variation. J Ann Chim 10(9):113
26. ICH guidelines Q2(R1) Validation of Analytical procedures: Text & Methodology. http://www.ich.org/fileadmin/publicwebsite/ICHproducts/guidelines/quality/Q2R1/step4/Q2R1Guideline
27. United States Pharmacopoeia (USP 24/NF 19, USP 29/NF 24). United States Pharmacopoeia Convention, USA
28. Geffken D, Salem H (2006) Spectrofluorimetric study of the charge-transfer complexation of certain fluoroquinolones with 2, 3, 5, 6-tetrafluoro-p-benzoquinone. Am J Appl Sci 3(8):1952–1960
29. Alfred M, James S, Arthur C, Chun AHC (1993) Physical pharmacy. Lea and Febiger, Philadelphia, pp 343–360
30. Benesi HA, Hildebrand JH (1949) Spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. J Am Chem Soc 71:2703–2707
31. Tsubomura H, Lang R (1961) Molecular complexes and their spectra. XIII. Complexes of iodine with amides, diethyl sulphide and diethyl disulfides. J Am Chem Soc 83:2085–2092
32. Aloisi G, Pignatoro S (1973) Molecular complexes of substituted thiophens with σ and π acceptors. J Chem Soc Faraday Trans 1(69):534
33. Briegleb G (1961) Elektron-donator-acceptor komplexe. Springer, Berlin
34. McConnell HM, Ham JJ, Platt JR (1953) Regularities in the spectra of molecular complexes. J Chem Phys 21:66–70
35. Foster R (1969) Organic charge transfer complexes. Academic Press, London, pp 623–755
36. Du LM, Yang YQ, Wang QM (2004) Spectrofluorometric determination of certain quinolone through charge transfer complex formation. Anal Chim Acta 516(1–2):237–243
37. Banfi and Patiny (2008)
38. Al-Attas AS, Habeeb MM, Al-Raimi DS (2009) Synthesis, and spectroscopic studies of charge transfer complexes. J Mol Struct 928:158–170
39. Miyan L, Khan IM, Ahmed A (2015) Synthesis and spectroscopic studies of charge transfer complex of 1, 2-dimethylimiazole as an electron donor with π-acceptor 2, 4-dinitro-1-naphthol in different polar solvents. Spectrochim Acta Part A Mol Biomol Spectrosc 146:240–248
40. Eman YF, Gehad GM (2010) Electronic, infrared, mass, 1 HNMR spectral studies of the charge-transfer complexes of sulphonamides drugs with π-acceptor in acetonitrile. J Mol Struct 979:46–55
41. Chatterjee NR, Deshpande AD, Baheti KG (2004) Degradation of β-lactam antibiotics. Curr Sci 87(12):1684–1695

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ► Open access: articles freely available online
- ► High visibility within the field
- ► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com