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Development of A New Colorimetric-Flow System Approach for The Determination of Cefotaxime Sodium in Pharmaceutical Formulations

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Abstract

A new colorimetric-flow injection method has been developed and validated for the detection of Cefotaxime sodium in pharmaceutical formulations. This method stands out for its rapid and sensitive nature. The formation of a brown-colored complex between Cefotaxime sodium and the Biuret reagent in a highly alkaline environment serves as the basis for the detection. The intensity of this colored complex is measured using a custom-built Continuous Flow Injection Analyzer, enabling accurate quantification of Cefotaxime sodium. Optimization studies of the chemical and physical parameters such as dilution of Biuret reagent, effect of the medium basicity, flow rate, sample loop and others have been investigated. The calibration graph was linear in the range of 10-650 $\mu g.ml^{-1}$ for each blue & green light source, with correlation coefficient r = 0.9509 & 0.9991 for blue & green respectively. The limit of detection was 5 $\mu g.ml^{-1}$ for diluting the lowest concentration in the calibration graph. The RSD% was less than 0.7% for 50 and 100 $\mu g.ml^{-1}$ (n=6) concentration of Cefotaxime sodium in each light source. Cefotaxime sodium was successfully determined using the proposed approach in two pharmaceutical products. the conventional approach (UV-spectrophotometry at wavelength 388 nm) and the newly devised method analyses were compared using the conventional add approach and the t-test at a 95% confidence level revealed that there was no discernible difference between the two procedures.

Keywords: Cefotaxime sodium, flow injection analysis, Biuret reagent, spectrophotometry.

تطوير طريقة جديدة لنظام الحقن الجرياني–اللوني لتقدير سيفوتاكسيم الصوديوم في المستحضرات الصيدلانية

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الخلاصة

تم تطوير والتحقق من طريقة حقن تدفق لونيمترية جديدة للكشف عن سيفوتاكسيم الصوديوم في المستحضرات الصيدلانية. تتميز هذه الطريقة بسرعتها وحساسيتها. يشكل سيفوتاكسيم الصوديوم مع كاشف البيوريت معقدًا بني اللون في وسط قلوي مرتفع، وهذا التفاعل هو أساس الكشف. يتم قياس شدة هذا المعقد الملون باستخدام جهاز تحليل الحقن التدفقي المستمر المُصمم خصيصًا، مما يتيح تحديدًا دقيقًا لكمية

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سيفوتاكسيم الصوديوم. تم إجراء دراسة وتحسين العوامل الكيميائية والفيزيائية للنظام المستخدم. وكان مدى منحني المعايرة الخطي من 10–650 مايكروغرام / مل لكل من المصدر الضوئي للون الأزرق واللون الأخضر، مع معامل الارتباط 0.9509 و0.9991 للأزرق والأخضر على التوالي. وكان حد الكشف 5 مايكروغرام/مل لتخفيف أدنى تركيز على الرسم البياني للمعايرة. معامل الانحراف القياسي RSD٪ أقل من 0.7٪ لتركيز 50 و 100 ميكروغرام / مل (العدد = 6) من سيفوتاكسيم الصوديوم لمصادر الضوئية المستخدمة (الأزرق والاخضر). تم تقدير سيفوتاكسيم الصوديوم بنجاح باستخدام هذه الطريقة في اثنين من المستحضرات الصيدلانية. تمت مقارنة الطريقة التقليدية (قياس الطيف الضوئي للأشعة فوق البنفسجية عند الطول الموجي 388 نانومتر) والتحليل بالطريقة المبتكرة باستخدام طريقة الإضافة القياسية واختبار t. وعند حدود ثقة بنسبة 95٪ أنه لا يوجد فرق جوهري بين الطريقتين.

Introduction

The chemical name for Cefotaxime sodium (CFTS) (Figure 1) is sodium (6R,7R)-3-[(acetyloxy)methyl]-7-[[(2Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [1]. Figure 1 presents the intricate molecular architecture of Cefotaxime, a prominent third-generation cephalosporin antibiotic. This remarkable drug is widely employed to combat a diverse range of severe infections caused by resilient bacteria that have developed resistance to other antibiotics. Since some of these antibiotics can cross the blood-brain barrier, they are effective against a broader variety of gram-negative bacteria, *Citrobacter youngae, Staphylococcus aureus*, and other bacteria that may resist third-generation antibiotics. The common name of cefotaxime sodium is Claforan, an antibiotic medicine used to treat various bacterial infections [2]. Cefotaxime sodium is the most effective medication for treating severe infections that pneumococcal strains may cause.

Cefotaxime sodium, an antibiotic, has more excellent than countless antimicrobial doings, a broad-spectrum antibacterial nature activity, extremely heightened resistance to the effects of blactamase, as well as a high adverse effect index [3,4]. As a result, Among the many infections, it has successfully treated various diseases including meningitis, peritonitis, septicemia, urinary tract infections, lung infections, and infections of the limbs, muscles, and skin. In the literature, there are several analytical methods for the spectrophotometric analysis of CFTS [5], HPLC-UV [6], Voltammetry [7], atomic absorption spectrometric [8], and Capillary zone electrophoresis [9]. While the flow injection analysis is used for the determination of some other species (ions and different drugs) via homemade instruments [10-18]. In the present study, we developed a novel analytical technique for the sensitive and rapid determination of cefotaxime sodium. The method utilizes flow injection analysis coupled with a colorimetric detection approach (FIA-colorimetry). It exploits the reaction between cefotaxime sodium and Biuret reagent to generate an intensely colored brown complex.

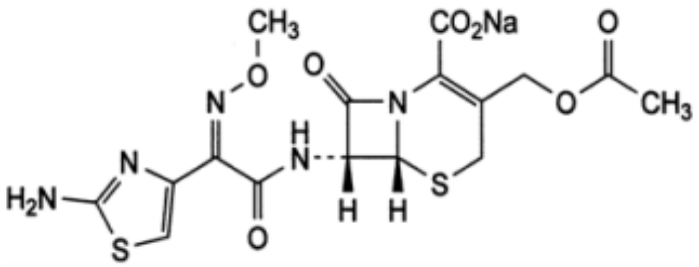


Figure 1: The chemical structure of Cefotaxime sodium [3]

Experimental

Analytical reagent-grade chemicals were used in this study. Cefotaxime sodium (CFTS) $C_{16}H_{16}N_5NaO_7S_2$ (477.447 g.mol^{-1}, SDI) was dissolved in 0.0999g in 100 ml of distilled water to provide a standard solution (0.002094 mol.L^{-1}). Biuret reagent (10%) which consists of mixed Cupric Sulfate and Sodium-Potassium tartrate and sodium hydroxide. The Cupric sulfate $CuSO_4.5H_2O$ (0.024 mol.L^{-1}) (249.69 g.mol^{-1}, H & W (H.W. Sands Corp)) was prepared by dissolving 1.4981g in distilled water, while Sodium-Potassium tartrate $KNaC_4H_4O_6.4H_2O$ (0.085 mol.L^{-1}) (282.23 g.mol^{-1}, M&B (May & Baker was a British chemical company)) was made by dissolving 5.9974 g in distilled water, and Sodium hydroxide (NaOH) 40 g was dissolved in distilled water to made a stock solution (1 mol.L^{-1}, 40 g.mol^{-1}, BDH). A stock acid solution of sulfuric acid H_2SO_4 (96% w/w, 1.84 g.ml^{-1}, BDH, 0.1 mol.L^{-1}, hydrochloric acid HCl (35% w/w, 1.18 g.mL^{-1}, 0.1 mol.L^{-1}), and acetic acid CH_3COOH (99.5% w/w, 1.05 g.mL^{-1}, BDH, 0.1 mol.L^{-1}). Pipetting 4.4 mL, 2.724 mL, and 2.88 mL respectively, of concentrated acids and completing the volume with distilled water to 500 mL volumetric flasks to produce 0.1 mol.L^{-1}. Na_2CO_3 standard solution (BDH, 105.99 g.mol^{-1}, 0.1 mol.L^{-1}) was used to standardize each acid.

Apparatus

The flow injection analysis system used for the determination of cefotaxime sodium is depicted schematically in Figure 2. At the heart of the system was a two-channel variable speed peristaltic pump manufactured by Standard Peristaltic Pump located in Lone, Zhangqiu District, Jinan City, Shandong, China. This pump served to propel the steady flow of carrier and reagent streams throughout the analysis. Another critical component was an injection valve equipped with a sample loop for injecting the cefotaxime sodium sample into the flowing carrier stream. This injection valve was a six-port medium pressure model obtained from IDEX Corporation based in the United States. Finally, the various components of the system were interconnected and fluids transported using Teflon tubing measuring 0.7 mm in internal diameter and varying lengths. This fully automated flow injection analysis setup allowed for reproducible introduction of the cefotaxime sodium sample into the continuous fluidic paths for subsequent colorimetric reaction and detection of the analyte downstream. The readout of the system is composed of (Laptop-computers, Dell). The CFTS spectrum was scanned using a digital double-beam UV-1800 spectrophotometer from Shimadzu, Japan with a 1 cm quartz cell.

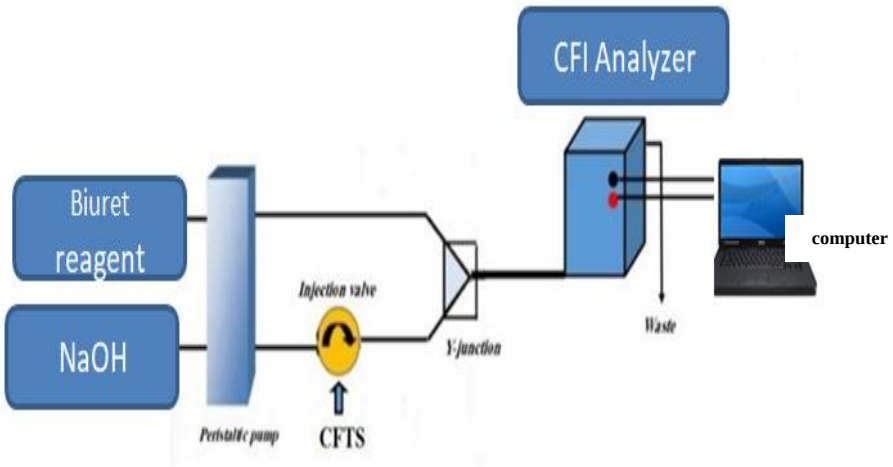
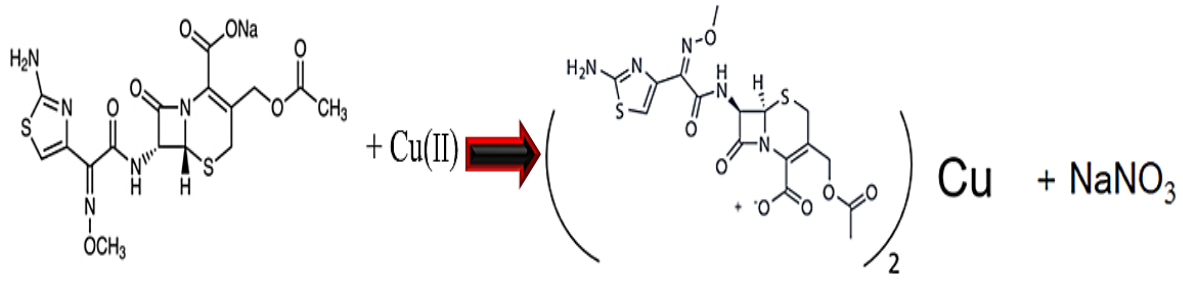


Figure 2: A schematic of the Flow Injection Analysis Manifold for Quantifying Cefotaxime Sodium with Biuret Reagent

Methodology

The flow injection analysis (FIA) manifold depicted in Figure 2 was employed for the determination of cefotaxime sodium concentrations in pharmaceutical formulations. The analytical procedure capitalized on the reaction between cefotaxime sodium and Biuret reagent under basic conditions to generate a colored ion pair complex. Specifically, the flow manifold consisted of two independent fluidic lines. The first line delivered an alkaline carrier stream of 0.1 mol/L sodium hydroxide solution at a flow rate of 3 mL/min. Meanwhile, the second line transported the colorimetric Biuret reagent, which was prepared as a 10% (v/v) solution. Upon injection into the carrier stream via the injection valve, the cefotaxime sodium samples reacted with Biuret reagent in the basic sodium hydroxide medium. This resulted in the formation of a purplish-violet ion pair complex, the absorbance of which could then be measured for quantitative analysis of cefotaxime sodium. Thus, this optimized FIA setup provided an efficient means of analyte determination through chromatographic reaction and detection. The CFTS standard (157 μL) was injected in the carrier stream via an injection valve that led to meet with the colorimetric reagent in Y-junction to form a purplish-violet ion-pair complex which was monitored in the homemade photometer. A predicted reaction equation has been postulated for this reaction [19-21], as illustrated in Scheme 1.



Scheme 1: Proposal reaction equation for ion-pair complex for the CFTS–Biuret to form a purplish-violet

Optimization of Variable

The chemicals and physical parameters such as Biuret reagent concentration, basicity medium effect, carrier and reagent flow rate, cefotaxime injected volume, and reaction coil have been investigated to prove the optimum parameters of ion-pair reaction.

Chemical Variables

Effect of Biuret Reagent Concentration

A series of diluted solutions of 10% Biuret reagent ranging from (5 to 50 ml) (Reagent: D.W (v/v%)) and sample volumes of 157μ L were used to explore the effects of different Biuret concentrations. Two lines manifold system was used at a flow rate of 3 ml.min^{-1} for the carrier stream (NaOH) and regent line (Biuret reagent). Studies were conducted to determine the optimal concentration of Biuret reagent for complex formation and maximum absorbance. It was observed that increasing the Biuret concentration from 0 to 10% (v/v) led to a rise in the reaction producing the colored species. However, beyond 10% (v/v) Biuret, the absorbance began to decrease. This downward trend can be attributed to inter-filter effects as higher reagent concentrations likely resulted in non-specific background color generation. Based on these findings, the most suitable Biuret concentration was identified as 10% (v/v) for yielding the analyte-Biuret complex with strongest light absorption. This concentration of 10% Biuret reagent in deionized water was then used in all subsequent experiments to ensure highest sensitivity of detection across different optical systems evaluated. The data were

summarized in the average of three successive readings, with a relative standard deviation and a 95% confidence interval for the average response, which are summarized in Table 1. The plot of the data from the CFIA microphotometer is shown in Figure 3.

Table 1: Variation of Biuret concentration on the absorbance of color species

Type of light source	[Biuret] R:DW v/v%	Average of analyzer response n=3	Standard Deviation (SD) $SD = \sqrt{\frac{\sum(x-\bar{x})}{n-1}}$	%RSD $\%RSD = \frac{SD}{\bar{x}}$	Confidence interval $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
Blue light	5:45	823.96	2.51	0.30	823.96 ± 6.23
	10:40	988.00	1.00	0.10	998.00 ± 2.48
	15:35	984.56	3.36	0.34	984.56 ± 8.35
	25:25	808.66	2.51	0.31	808.66 ± 6.25
	50	612.53	1.59	0.26	612.53 ± 3.96
Green light	5:45	935.03	2.31	0.24	935.03 ± 5.7
	10:40	1055.33	1.52	0.14	1055.33 ± 3.79
	15:35	957.83	4.50	0.47	957.83 ± 11.19
	25:25	830.33	2.51	0.30	830.33 ±6.25
	50	623.36	1.80	0.28	623.36 ±4.47

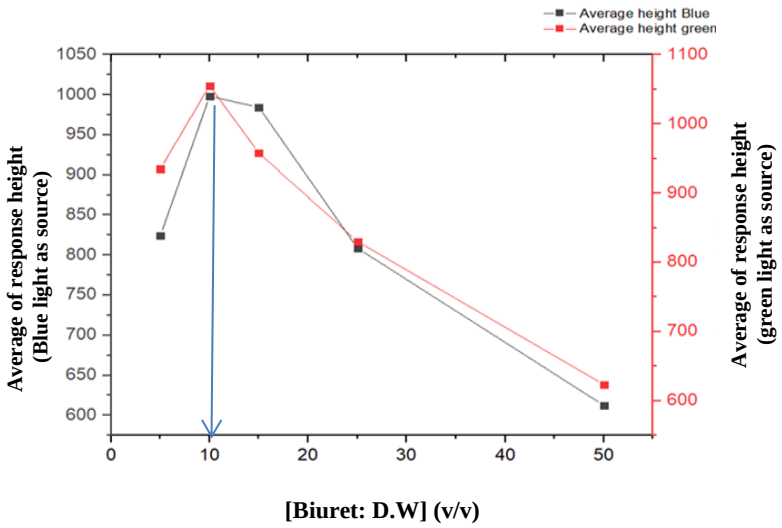


Figure 3: Variation effect of Biuret concentration on microphotometer response using CFTS (500µg/ml)

Effect of Basic Medium on the Reaction

Different bases were used at variable concentrations (NaOH 0.01, 0.05, 0.1 mol.L^{-1}), Na_2CO_3 (0.05, 0.01, 0.1 mol.L^{-1}) & $NaHCO_3$ (0.05, 0.01, 0.1 mol.L^{-1}) in addition to the carrier stream of H_2O at 3 ml.min^{-1}. Biuret reagent (10% v/v) was utilized in a 157µ L sample volume. Table 2 displays the results that the effect of (Na_2CO_3 and $NaHCO_3$) on the decline in absorbance was not particularly significant, which could be attributable to the dissociation of some colored species, the NaOH at 0.1 mol.L^{-1} led to an increase in absorbance. Therefore, NaOH (0.1 mol.L^{-1}) was chosen as the optimum carrier stream for each light source (i.e., blue & green), which provided adequate sensitivity.

Table 2: Effects of the basic medium's variation as a carrier stream on the species' absorption of color

Type of light source	Type of media as carrier	Average of analyzer response n=3	Standard Deviation (SD) $SD = \sqrt{\frac{\sum(x - \bar{x})}{n-1}}$	%RSD $\%RSD = \frac{SD}{\bar{x}}$	Confidence interval $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
Blue Light	H_2O	998.00	1.00	0.10	998.00 ± 2.48
	NaOH (0.01)	1038.43	2.51	0.24	1038.43 ± 6.24
	NaOH (0.05)	1108.56	1.75	0.15	1108.56 ± 4.34
	NaOH (0.1)	1429.66	4.50	0.31	1429.66 ± 11.20
	$NaHCO_3$ (0.01)	946.40	2.95	0.31	946.40 ± 7.33
	$NaHCO_3$ (0.05)	1000.73	2.00	0.20	1000.73± 4.97
	$NaHCO_3$ (0.1)	1025.64	1.86	0.18	1025.64 ± 6.24
	Na_2CO_3 (0.01)	998.00	1.00	0.10	998.00 ± 2.48
	Na_2CO_3 (0.05)	956.40	2.90	0.30	956.40 ± 7.21
	Na_2CO_3 (0.1)	942.36	2.25	0.23	942.36 ± 5.23
Green Light	H_2O	1055.33	1.52	0.14	1055.33 ± 3.79
	NaOH (0.01)	1012.86	1.85	0.18	1012.86 ± 4.61
	NaOH (0.05)	1107.60	2.16	0.19	1107.60 ± 5.37
	NaOH (0.1)	1400.53	1.59	0.11	1400.53 ± 3.96
	$NaHCO_3$ (0.01)	1088.73	2.00	0.18	1088.73 ± 4.97
	$NaHCO_3$ (0.05)	1059.06	2.57	0.24	10.59.06 ± 6.38
	$NaHCO_3$ (0.1)	1021.34	2.42	0.23	1021.34 ± 8.42
	Na_2CO_3 (0.01)	1055.33	1.52	0.14	1055.33 ± 3.79
	Na_2CO_3 (0.05)	1351.00	2.00	0.15	1351.00 ± 4.96
	Na_2CO_3 (0.1)	1376.43	1.82	0.13	1376.43 ± 5.63

Physical Parameters

The effect of Flow Rate Effect on developed method

The flow rate ranged from 2-5 $ml.min^{-1}$ for each line i.e., (the carrier stream and the reagent Biuret). To find the ideal flow rate that would be employed throughout this study, the flow rate for the determination was examined. The optimum concentration (10% V/V) of Biuret, using 500µg/ml of cefotaxime sodium, with 157µL as a sample volume, while NaOH(0.1mol/L) carrier stream was employed. Figure (4) presents the results that were obtained. It was noticed that an increase of absorbance with an increase of flow rate up to $3ml.min^{-1}$for each line and each light source. But a high flow rate (i.e., more than 3 $ml.min^{-1}$) led to a reduction in the colored species absorbance at the measuring flow cell. The 3 $ml.min^{-1}$ was determined to be the optimal flow rate for each the carrier stream, and the Biuret, respectively for both blue and green light of source.

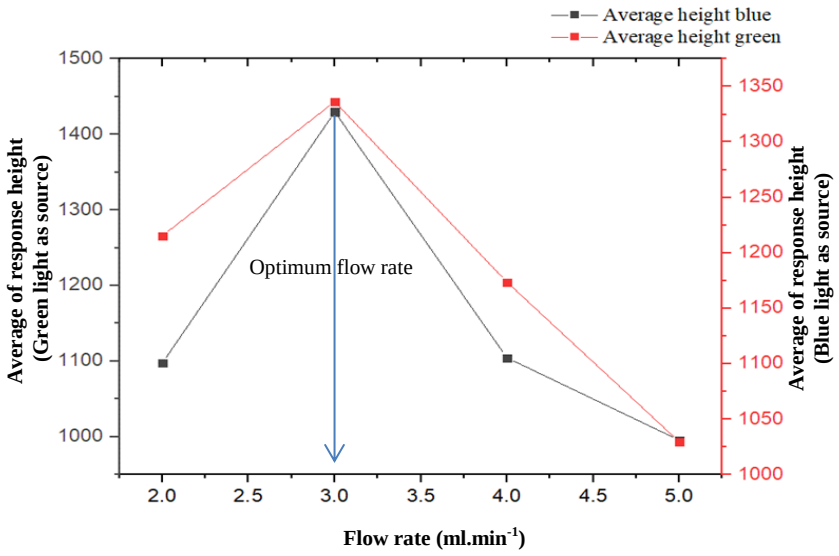


Figure 4: CFTS (500 g/ml)-Biuret (10% V/V)-NaOH (0.1 mol/L) system: Variation effect of flow rate on analyzer response

The effect of Sample Volume

In this study, variable lengths of Teflon tubes ranging from 5 cm to 40 cm with 0.5 mm internal diameter were used to vary the sample volumes from 39.25 to 314 µL. The perivenous optimum parameters were kept constant such as 500 µg.ml^{-1} of CFTS, 10:40 v/v of Biuret: D.W., 0.1 mol.L^{-1} of NaOH, flow rate of 3.0 ml.min^{-1}. The results show an increase in the sample volume resulted in a significant rise in the detector signal until reaching 157 µL as shown in Table (4). However, when using sample volumes exceeding 157 µL, there was a slight decrease in response height, which was most likely caused by a continuous, protracted colour segment in front of the detector which caused the inner filter effect. Therefore, the 157 µL was the optimum sample segment for the determination of each light source (blue & green light sources).

Table 4: Variation in the energy response of the transducer as a function of injected sample volume

Type of light source	**Sample volume (cm)**	**Sample volume (µL)**	**Average of analyzer response n=3**	**Standard Deviation (SD)** $SD = \sqrt{\frac{\sum(x - \bar{x})}{n-1}}$	**%RSD** $\%RSD = \frac{SD}{\bar{x}}$	**Confidence interval** $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
Blue Light	5	39.25	615.33	2.31	0.37	615.33 ± 5.75
	10	785	68.73	3.40	0.49	68.73 ± 8.46
	20	157	1448.06	1.90	0.13	1448.06 ± 4.72
	30	235.5	1205.00	1.52	0.17	1205.00 ± 3.79
	40	314	1172.66	2.51	0.21	1172.66 ± 6.25
Green Light	5	39.25	875.36	1.80	0.20	875.36 ± 4.47
	10	785	662.10	1.90	0.29	662.10 ± 4.72
	20	157	1336.30	3.80	0.28	1336.30 ± 9.46
	30	235.5	1280.00	1.00	0.11	1280.00 ± 2.48
	40	314	1206.20	1.70	0.14	1206.20 ± 4.24

Effect of Reaction Coil Length

Using (500 μg.ml^{-1}) CFTS - (10:40 v/v) Biuret: D.W - (0.1 mol.L^{-1}) NaOH system. The study was carried out to study the impact of the reaction coil. The homogeneity and completion of a chemical reaction are significantly influenced by the reaction coil length. Different coil lengths (10-40 cm) were employed in this study. Table (5) shows the reaction coil is not apparated to complete the reaction of CFTS with Biuret in NaOH as a medium, and the obtained result shows the response is complete without the reaction coil. It was found that using a reaction coil leads to dispersion of the colour species.

Table 5: Effect of reaction coil on the absorbance of colour species using CFTS (500 μg.ml^{-1}) – Biuret: D.W (10:40 v/v)-NaOH (0.1 mol.L^{-1})

Type of light source	Reaction coil (cm)	Average of analyzer response n=3	Standard Deviation (SD) $SD = \sqrt{\frac{\sum(x - \bar{x})}{n-1}}$	%RSD $\%RSD = \frac{SD}{\bar{x}}$	Confidence interval $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
Blue Light	Without coil	1448.06	1.90	0.13	1448.06 ± 4.72
	10	1035.40	1.44	0.14	1035.40 ± 3.58
	20	1050.06	1.90	0.18	1050.06 ± 4.72
	30	890.23	3.21	0.36	890.23 ± 7.98
	40	894.53	2.05	0.23	894.53 ± 5.11
Green Light	Without coil	1336.30	3.80	0.28	1336.30 ± 9.45
	10	1022.90	2.32	0.22	1022.90 ± 5.77
	20	1018.00	1.00	0.09	1018.00 ± 2.48
	30	884.13	1.80	0.20	884.13 ± 4.48
	40	891.23	1.90	0.21	891.23 ± 4.74

Calibration Graph

Utilizing the optimum physical and chemical parameters attained in the previous studies in this research, several options for determination ranging from CFTS drug (10-1000 μg.ml^{-1}) were organized. Each concentration in the calibration curve was measured and repeated three times. The average of the three measurements was plotted versus the CFTS concentration, a linear graph. Figure (5) shows the linearity from 10-650 μg.ml^{-1} of CFTS obtained for each light source (blue & green). Above 650 μg.ml^{-1} the value of the correlation coefficient will likely decline and diverge from linearity as coloured species increase due to the inner filter effect of highly intense coloured species, which a weakening of transmitted light may bring on. The outcome data are summed up in the linear equation of the range 10-650 μg.ml^{-1} of each light source (blue & green) as a form of:

$\bar{y}i=173.545\pm48.232+2.468\pm0.592$[CFTS] μg.ml^{-1},
$r=0.9509$ & $r^2\%=90.42\%$ (blue light source).

$\bar{y}i=9.5898\pm0.0983+2.7072\pm0.2352$[CFTS]μg.ml^{-1},
$r=0.9991$& $r^2\%=99.81\%$ (green light source).

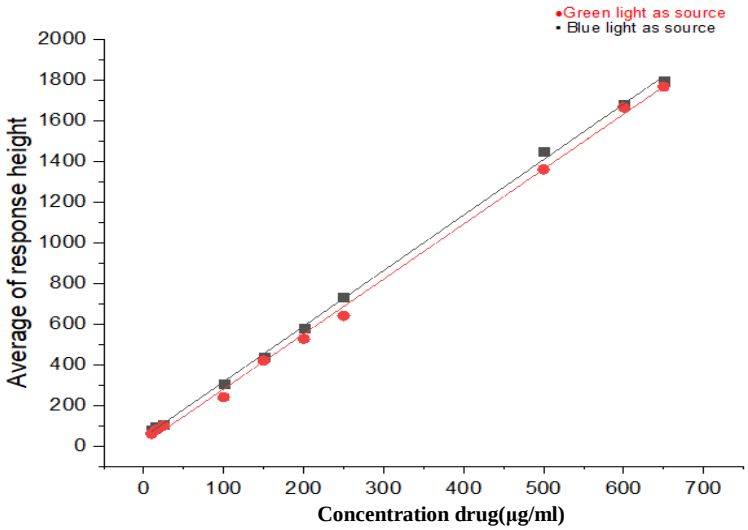


Figure 5: Calibration curve for determination of CFTS using proposed flow injection method depending on formation of ion-pair complex with Biuret reagent in alkaline medium

Limit of detection (L.O.D.)

The limit of detection of the proposed method was experimentally determined. Using the optimized solution system containing CFTS-Biuret reagent (10:40 v/v) in 0.1 mol/L sodium hydroxide, the lowest calibration standard concentration of 5 µg/mL was sequentially diluted. The absorbance readings were plotted against concentration and a linear regression analysis was performed. Based on this calculation, the limit of detection was estimated to be 1.495 µg/mL. This value was further validated by progressive dilution of the lowest calibration standard to the point where the analyte concentration could no longer be reliably distinguished from the blank measurement.

Repeatability

Using the ideal conditions, the repeatability of measurement and the effectiveness of the CFIA microphotometer were examined at fixed CFTS concentrations, specifically 50 and 100 g/ml. Repeated measurements for six subsequent injections (Figure 6) reveal a response-time profile with an RSD% of less than 0.7% for the used concentrations.

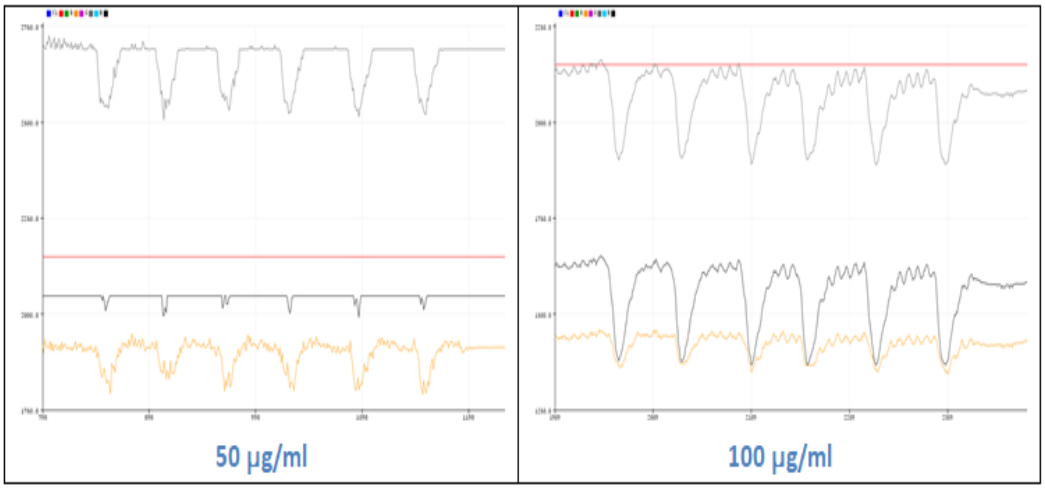


Figure 6: Repeatability studied of CFTS reaction with Biuret regent in basic medium using homemade photometer

Calibration Graph for The Conventional Approach

To make a comparison of the obtained results from the proposed method, the same reaction of CFTS with Biuret was carried out, and absorbance measuring using a conventional spectrophotometer (Shimadzu-1800, Japan) to determine the concentration of cefotaxime sodium in pure form and pharmaceutical preparations. The obtained results for the traditional spectrophotometer show the linearity of calibration carve from 5-300 $\mu g.ml^{-1}$ as shown in Figure (7), after fixing the optimum of [Biuret : D.W], which was 10:40 (v/v). A first-degree equation in the following format was used to represent the outcomes:

$\bar{y}i=0.0283\pm0.00132+0.00533\pm0.00083[CFTS]\mu g.ml^{-1}$,
$r= 0.9967$ & $r^2\%=99.34\%$

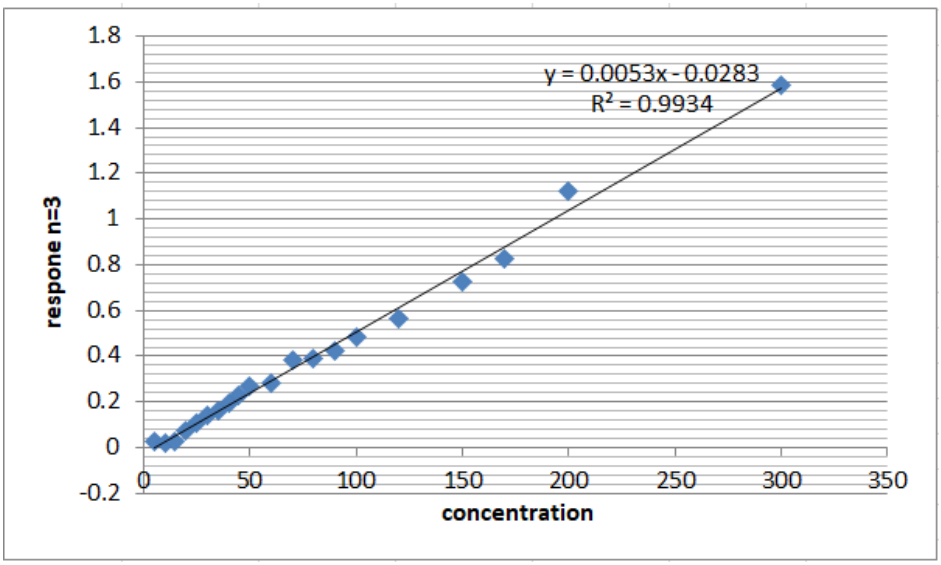


Figure 7: Calibration graph of the same reaction CFTS-Biuret-NaOH that measured in traditional spectrophotometer (UV-spectrophotometric)

Pharmaceutical Preparations Analysis

Two distinct pharmaceutical companies, (Cefotaxime, LDP, Spain-500 mg & cefotaxime sodium, Abbott India-1000 mg) injected on NaOH as a carrier stream employing a microphotometer-CFIA and developed manifold design technique was used in the analysis of preparations. The obtained results were displayed in Table 6 (developed method and traditional method), and in which show found concentration of medications and recovery; in addition to a t-test (Table. 7) between two methods.

Table 6: Determination of CFTS in different samples using different methods (microphotometric -CFIA method & traditional methods)

Type of company & content of the drug (mg)	Amount of CFTS in injection (mg) by microphotometric-CFIA method	Recovery % $\%\mathbf{Rec.} = \frac{\mathbf{Found}}{\mathbf{Taken}} * \mathbf{100}$	Amount of CFTS in tablet (mg) by UV-VIS Spectrophotometric	Recovery % $\%\mathbf{Rec.} = \frac{\mathbf{Found}}{\mathbf{Taken}} * \mathbf{100}$
	Blue light source			
	Blue light source			
1) LDP, Spain-500mg	503.32	100.66	495.22	99.04
	499 .00	99.8		
2) Abbott India-1000 mg	1014.00	101.4	996.23	99.62
	997.00	99.7		

Statistical Treatment (t-test)

Two commercial medicines from distinct manufacturing processes were compared using a t-test at α=0.05. A comparison between the developed approach and UV-VIS Spectrophotometric was done. Table 7 (Column 6) summarizes the findings of the comparison using 500 mg of LDP, Spain. The results show that there were no significant differences at = 0.05 (95% confidence level) between the developed method and the traditional method (classical method) for determining CFTS in pharmaceutical drugs.

Table 7: Comparison of the results for the determination of CFTS in LDP, Spain-500mg of drug using different methods (microphotometric -CFIA method & reference methods) by paired t-test

CFTS (µg/ml) prepared sample	CFTS pure (µg/ml) (add)	Found CFTS (µg/ml) by Developed method		Found CFTS (µg/ml) by UV-VIS method	Paired t-test Compared between two methods
		Blue L.S.*	Green L.S.*		Blue L.S.*
					Green L.S.*
50	25	76.34	77.00	74.00	**$t_{tab.}$ (3.182)> $t_{cal.}$ 3.182 > / - 0.475 /**
50	50	102.34	101.88	98.56	
50	100	149.00	148.45	148.98	**$t_{tab.}$ (3.182)> $t_{cal.}$ 3.182 > 2.42**
50	150	198.94	197.22	197.11	

*Light Source, $t_{0.05/2,}$= 3.182 for n-1

So, the results of the comparison of both methods showed that the suggested technique was more sensitive. and have a more linear range than the classical method.

Conclusion

In this work, a novel flow injection analysis method was developed and optimized for the quantitative determination of cefotaxime sodium in pharmaceutical formulations. The assay is based on the colored ion pair complex formation between cefotaxime sodium and Biuret reagent under alkaline conditions. Key findings demonstrate that the proposed FIA method offers several advantages in terms of simplicity, speed, and sensitivity compared to more traditional analytical techniques. Validation experiments confirmed the assay's linear dynamic range, accuracy, and precision for cefotaxime sodium quantification. Additionally, comparison of results to a reference method via t-test analysis showed no significant differences, highlighting the reliability of the newly developed approach. Overall, the proposed FIA technique fulfills the requirement of being an alternative method for routine quality control analysis of cefotaxime sodium in pharmaceutical preparations.

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