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Indirect Flow-Injection Spectrophotometric Determination of Some β-Lactam Antibiotics

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ABSTRACT

A simple and sensitive indirect spectrophotometric flow injection analysis (FIA) method for the determination of cefixime (CF), ceftriaxone (CFTR), cefotaxime (CFX) and cefuroxime (CFU) in pharmaceutical formulations and biological fluids has been suggested. Drugs under investigation are firstly oxidized by excess N-bromosuccinimide (NBS) in acidic medium, then the excess NBS is reacted with Rhodamine B (RB) to bleach its pinkish red color. Chemical variables and flow injection variables are all optimized to enhance reproducibility and sensitivity. The suggested procedure followed Beer's law over concentration ranges of 5 – 30 µg.ml-1 for all tested drugs. The method is successfully applied to the determinations of the drugs under investigation in different pharmaceutical preparations. Results obtained by the suggested method were in excellent agreement with those obtained by the formal HPLC methods.

Keywords: Cephalosporins, FIA, HPLC, Pharmaceutical Products, Human Plasma and Urine.

INTRODUCTION

The genus *Stereocaulon* Hoffm. (Stereocaulaceae, Lecanorales, Ascomycota) is an interesting genus from lichen which is found throughout the world. The morphology of the *Stereocaulon* genus consists of the crustose type primary thallus and fruticose type secondary thallus. The primary thallus in most species of *Stereocaulon* is disappeared at a very early stage of development. In the secondary thallus, there are several important parts such as pseudopodetia which show persistent phyllocladia (or phyllocladioid branchlets), apothecia as a sexual organ that contains spores, and in most species cephalodia which contain cyanobacteria (Nostoc, Rhizonema or Stigonema)[1].

Cefixime, ceftriaxone, cefotaxime and cefuroxime are kinds of cephalosporin antibacterial drugs. They are the second major group of semi-synthetic β- lactam antibiotics used in clinical medicine [1, 2]. They are used to treat infections induced by both gram-negative and gram-positive bacteria and interfere with the synthesis of vital structural parts of bacterial cell wall [3, 4].

Several analytical techniques for the determination of the drugs under investigation in biological and pharmaceutical preparations are described in literature. These include spectrophotometric [5-10], spectrofluorometric [11,12], chromatographic [13-16], capillary electrophoresis, electrochemical, potentiometry, voltammetry and flow injection analysis [17-20]. Chromatographic procedures are well-known and specific; however, they are time consuming and require sophisticated instruments. Equally, most batch spectrophotometric procedures require prior extraction of the colored product and take long reaction time for complete color intensity. Overcoming these drawbacks and automating the procedure are of current interest for the analysis of pharmaceutical compounds.

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Because of the ability of the Flow Injection Analysis (FIA) method to conduct quick, accurate and precise analysis, it is of concern to evaluate its applicability in pharmaceutical products, plasma and urine samples. The automatic nature of FIA reduces the need for skilled and well- trained analysts to conduct the chemical analysis.

Experimental

Reagents and solutions

All chemicals used are in a pure grade and used without further purification as they have been obtained. During this entire work, distilled water is used. Hydrochloric acid solution (0.1 M) is prepared by diluting the calculated quantity of concentrated HCl with distilled water. Sodium hydroxide solution is prepared by dissolving the proper amount of NaOH in a total volume of 1 L with distilled water. N-Bromosuccinimide (NBS) solution ($1.0x10^{-3}$ M) is prepared daily by dissolving 0.0445 g of NBS (Merck) in a total volume of 250 mL with 0.1 M HCl. Rhodamine B (RB) solution ($2.0x10^{-5}$ M) is prepared by dissolving 0.0024 g of RB in a total volume of 250 mL with 0.1 M HCl.

Standard solutions

Standard solutions of the active ingredients were prepared by dissolving 0.01 g of the drug under investigation in a total volume of 100 mL of 0.05M NaOH. Standard solutions for linearity study are prepared by diluting the calculated volumes of the stock solution with 0.05 M NaOH.

Tablet, Capsule and Powders

The content of one capsule of the commercial product Suraxim (200 mg cefixime/capsule) is emptied in a 500 mL volumetric flask and hydrolyzed with 5 mL of 0.05 M NaOH for 30 min at 80°C. The concentration of cefixime in this solution is supposed to be 400 $\mu g.mL^{-1}$. The solution is used to prepare different concentrations within the linearity range by proper dilution with 0.05M NaOH. For tablets analysis, the content of one tablet of the commercial product Oraxim (250 mg cefuroxime /tablet) is emptied in a 500 mL volumetric flask and hydrolyzed with 5 mL of 0.05 M NaOH for 30 min at 80°C. The concentration of cefuroxime in this solution is supposed to be 500 $\mu g.mL^{-1}$. The solution is used to prepare different concentrations within the linearity range by proper dilution with 0.05 M NaOH. For powders, 0.01 g of the commercial product Ceftax powder (1000 mg of cefotaxime) and commercial product Samixon powder (1000 mg of ceftriaxone) is accurately weighed and transferred to 100 mL volumetric flask and then the procedure is continued as above.

Plasma and urine samples

A 500 µL of the drug under investigation (2000-ppm stock solution dissolved in water) is added to 1 mL drug-free plasma. After that, 0.5 mL of the 10% Trichloroacetic acid (TCA) is added. The mixture is vigorously mixed in a tube for 1 min and then centrifuged at 3000 rpm for 30 min to ensure complete protein precipitation. Next, 100-400 µL of the mixture is added to 10-mL volumetric flask and hydrolyzed with 5 mL of 0.05 M of NaOH for 30 min at 80°C. Urine samples are treated by the same procedure.

Apparatus

The suggested configuration of the FI system is shown in Figure 1. It's made up of two channels. A Varian DMS-100 UV-Visible spectrophotometer is used to perform all absorption measurements. Teflon tubing of 0.51 mm i.d. is utilized to build up the system. The sample solution is injected via a Rheodyne 6-way injection valve and combined with the carrier ($1.0x10^{-3}$ M NBS) in the first mixing coil (RC1). A home-made confluence point was used to ensure quick mixing of sample mixture with the reagent ($2.0x10^{-5}$ M RB) in the second reaction coil (RC2). A sample injection volume of 50 µL is used. The absorption of the color generated is tracked at 555 nm.

Procedure

A volume of 50 µL of the prepared sample solution is injected by a syringe into the carrier stream ($1.0x10^{-3}$ M NBS), pumped at a flow rate of 0.40 mL/min. The reagent ($2.0x10^{-5}$ M RB) was introduced downstream to ensure fast and sufficient mixing at a flow rate of 0.40 mL/min. After injection, when the maximum absorbance is reached, the valve is returned to the load position. Upon reaching the baseline, another sample slug is injected. The height of the absorbance peak is used for calibration.

Results and Discussion

Development of the Methods

The suggested FIA technique enables the target compounds to be quantified quickly and economically in pharmaceutical formulations without the need for time-consuming sample preparation steps. NBS have been extensively used as an analytical reagent [21-24]. In this study, it is found that NBS can oxidize the target drugs in acidic medium. In addition, it reacts Instantly with Rhodamine B (RB) in an acidic medium to fade out its color. Therefore, after the oxidation of drugs by NBS, the excess NBS is reacted with the RB. Figure 2 shows the absorption spectra of the reagents used and the reaction products. The decrease in the RB absorption (ΔA) is proportional to the drug concentration. Thus, the various parameters influencing the oxidation reaction, and hence the subsequent determination are optimized.

Influence of chemical variables

The effect of the acidity on the analytical signal, is considered over acidic and basic pH ranges. Various buffer solutions are being screened (acetate, citrate, borate and phosphate). No critical impact for the buffer type on the analytical signal is observed. All findings, however, stated that the reaction is better performed in acidic medium. Different concentrations of HCl are used as a solvent for both NBS and RB solutions. The maximum analytical signal is achieved when the carrier solution was 0.1 M HCl, which is used in all subsequent experiments (Figure 3).

The influence of changing RB concentration on the analytical signal is studied at different concentration of NBS as shown in Figure 4. Fixed volume (80 µL) of cefotaxime is used and injected into the NBS stream. For all NBS and RB combinations tested, best results are obtained when the NBS-to-RB concentration ratio was minimal. For instant, maximum signal was obtained at [NBS] = 0.2 mM and [RB] = 0.04 mM (NBS/RB = 5). However, the concentration of NBS must be high enough to react with both the drug and the RB. Therefore, a 0.7 mM NBS is selected to make sure that we have enough NBS to react completely with samples that contain high levels of the active ingredient (drugs under investigation). In addition, the base line stability is much better at 0.7 mM NBS. Based on these results, the concentrations of NBS and RB selected throughout this work are 0.7 mM and 0.04 mM, respectively.

Influence of FIA variables

The effect of the reagents flow rate is studied keeping other conditions constant, over the range 0.2 – 2.0 mL/min. In all cases, the same flow rate is used in both channels. The highest signals are obtained when the flow rate is 0.4 mL/min for each line (Figure 5). At higher flow rates, considerable decrease in the analytical signal is observed. This is because the reaction time will be lowered at greater pumping rates and therefore the reaction would not proceed to completion. At lower flow rates, the dispersion of the reaction product zone will increase leading to increased peak broadening and analysis time. Therefore, as a compromise between peak broadening, sensitivity, and sampling time, a total flow rate of 0.8 ml/min (0.4 ml/min for each line) is selected. At this pumping rate, the time for one injection is 45 seconds, and therefore the sample throughput is about 80 samples/hr.

The suggested FIA setup uses two reaction coils (Figure 2). The first coil (RC1), where the drug was oxidized by NBS, was changed over the 30 to 120 cm

range. When the length of the coil was increased to 50 cm, a significant rise in the analytical signal was noted and then began to decline (Figure 5). Longer coils led to peak broadening and a longer time to return to the baseline. The length of the first reaction coil (RC1) was therefore chosen to be 50 cm in order to ensure high sensitivity and high measuring rates. Similarly, when the second reaction coil (RC2) is changed from 30 to 120 cm, a substantial shift in the analytical signal is noted. Maximum analytical signals are acquired when RC2 is 60 cm (Figure 5).

Different lengths of the sample loop are mounted on the injector and tested to assess the impact of the injected sample volume on the analytical signal. As expected, an increase in the volume of the injected sample results in a peak height increase. As a result, measurement sensitivity could be enhanced by increasing the sample volume. However, by increasing the sample volume, the peak width and time for the signal to return to the base line are increased. A volume of 80µl, which ensure a reasonable sensitivity and sampling rate, is selected.

Evaluation of the method

Under the optimum conditions, the calibration curves for determining the studied drugs are constructed. Absorbance versus concentrations plots are straight lines. The linearity is excellent and Beer's law is followed for the drugs being investigated. Typical calibration data for drugs tested using the suggested FI technique are shown in Table 1. The precision of the measurements is high as reflected by the low RSD values (RSD is 1.15 % , n = 6). The detection limit (LOD) is determined as the analyte concentration resulting in a signal that is three times the blank standard deviation. Similarly, the limits of quantification (LOQ) is determined as the concentration of the analyte resulting in a signal that is ten times the blank standard deviation. The intra-day (within-day) precision is evaluated by the replicate analysis of two different concentrations of drugs within the linearity range at different time intervals. The inter-day (different days) precision is similarly evaluated on several days up to 3 days. Every day, a new calibration graph is constructed. The results in both cases indicated high precision, as the percent RSD did not exceed 3%.

Table (1): Data for the calibration graphs (n = 6) using the proposed FIA method

Parameter	Cefixime	Ceftriaxone	Cefotaxime	Cefuroxime
Linearity range ($mg.L^{-1}$)	5 - 30	5 - 30	5 - 30	5 - 30
Intercept (b)*	7.10×10^{-3}	1.60×10^{-2}	5.00×10^{-4}	1.01×10^{-2}
Slope (a)*	2.43×10^{-2}	2.82×10^{-2}	2.82×10^{-2}	2.55×10^{-2}
Corr. Coeff.(r^2)	0.999	0.997	0.994	0.997
LOD ($mg.L^{-1}$)	0.12	0.09	0.10	0.10
LOQ ($mg.L^{-1}$)	0.40	0.30	0.33	0.35

Applicability of the proposed FIA

The impact of prevalent excipients usually used in pharmaceutical formulations is studied to examine the applicability of the suggested FI technique to routine pharmaceutical evaluation. Synthetic mixtures containing different concentrations of drugs in the presence of more than 100 folds of common additives are prepared. The insoluble material is filtered off before injection. No interference from the additives usually present in commercially available products is observed and

recoveries between 103.9 and 99.3 % are achieved. An additional assessment of the suggested FI technique in pharmaceutical analysis is carried out by conducting recovery experiments from commercial formulations (Table 2). As shown in Table 2, the recoveries are excellent (100.3 – 108.8%) for all of the drugs tested, proving the potential of this method in pharmaceutical analysis.

Table 2: FIA and HPLC results for the analysis cefixime, ceftriaxone, cefotaxime and cefuroxime in pharmaceutical preparations.

Drug	Trade Name & Labeled Claim	Taken ($\mu g.ml^{-1}$)	%Recovery ± RSD, (n = 6)	
			FIA	HPLC
Cefixime	Suraxim (200mg/Capsule)	10	100.3 ± 1.3	101.4 ± 1.2
		20	103.0 ± 0.7	103.1 ± 1.7
		25	100.7 ± 0.5	102.3 ± 0.9
Ceftriaxone	Samixon (1000mg/ powder)	10	100.5 ± 1.2	100.0 ± 1.2
		20	105.3 ± 0.3	101.9 ± 1.8
		25	104.2 ± 0.6	103.6 ± 0.9
Cefotaxime	Ceftax (1000mg/ powder)	10	103.1 ± 0.5	102.9 ± 3.1
		20	102.9 ± 0.7	104.5 ± 0.6
		25	108.8 ± 0.4	106.9 ± 1.0
Cefuroxime	Oraxim (250mg/tablet)	10	100.9 ± 1.1	102.7 ± 5.2
		15	101.9 ± 2.9	102.6 ± 3.7
		20	104.8 ± 0.5	104.3 ± 4.2

Furthermore, the outcomes acquired by the FIA procedure are also compared with the outcomes acquired by the HPLC reference method [25] for the same sample set by means of 99% confidence level t- and F-tests. There are no significant differences between the two methods ' outcomes. obtained by the FIA method are statistically compared with those obtained by the official HPLC method. No significant differences in precision and accuracy between the outcomes of the two methods. It can therefore be concluded that, for routine assessment, the FIA technique has the benefit of being easier, faster and more practical.

Conclusion

A simple, sensitive and accurate FIA method for the analysis of Cefixime, ceftriaxone, cefotaxime and cefuroxime is proposed. The suggested method demonstrates excellent linearity, accuracy and reproducibility and is effectively implemented without interferences for the evaluation of the target drugs in pharmaceutical forms and biological fluids. The results

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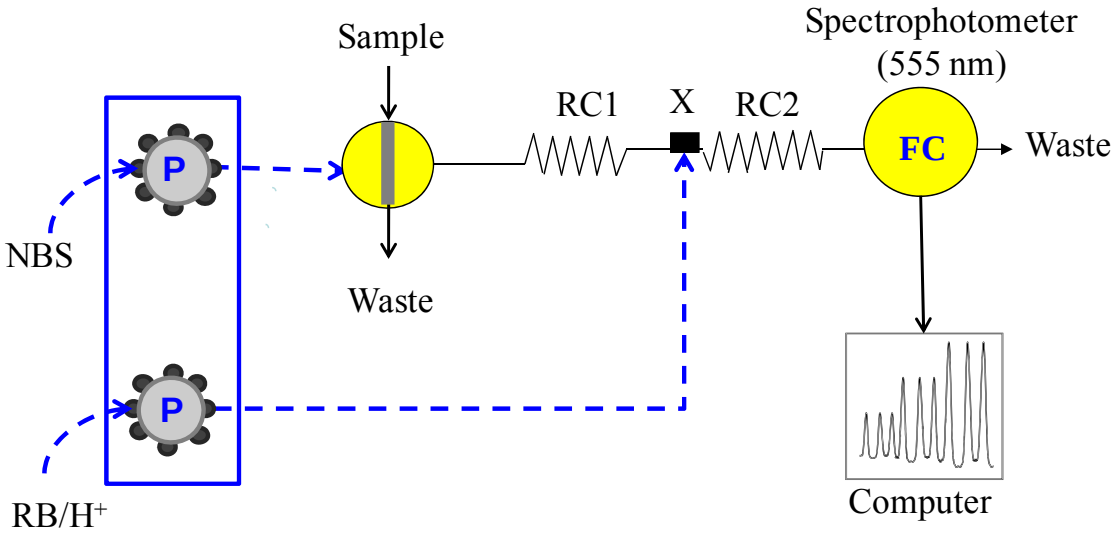

Figure 1: Schematic diagram of the proposed FIA system. P, peristaltic pump; RC1 and RC2 are the reaction coils; X, confluence point; FC, flow cell.

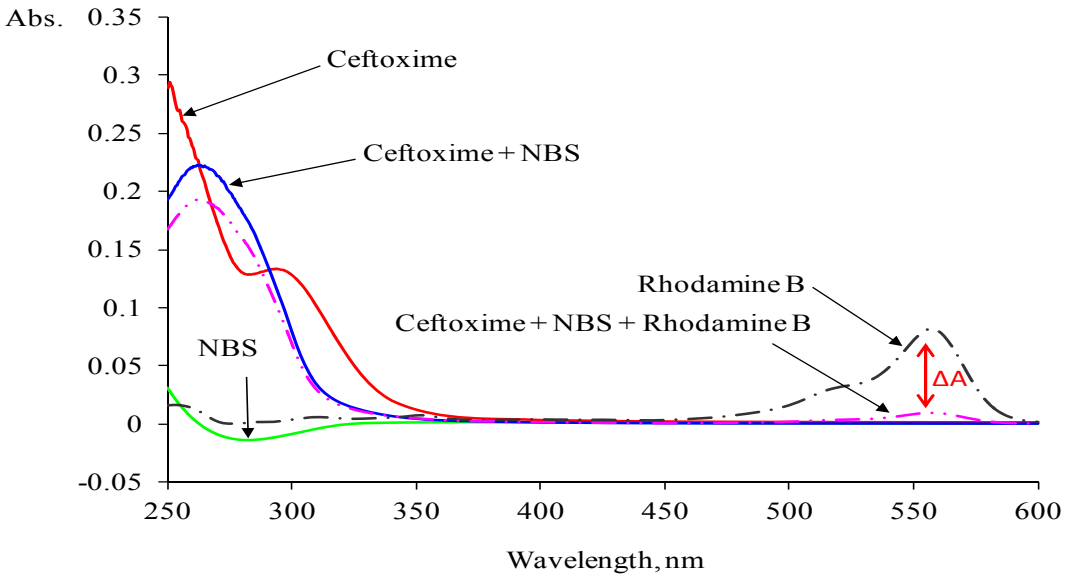

Figure 2: UV-Vis spectra for ceftoxime, NBS, RB, mixture of ceftoxime + NBS and mixture of ceftoxime + NBS + RB.

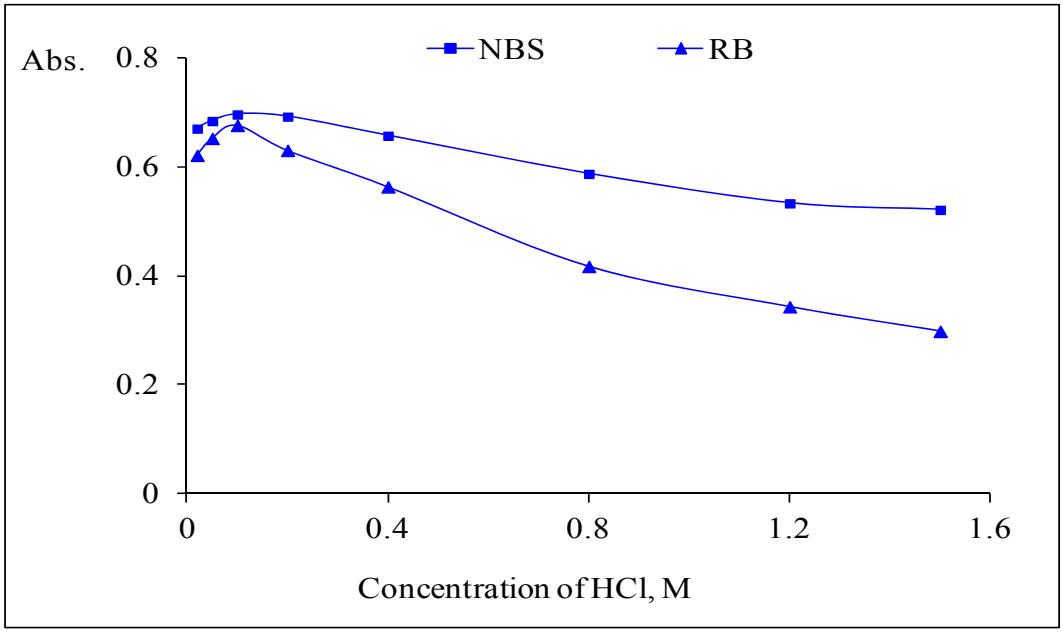

Figure 3: Effect of the reagents (NBS and RB) acidity on the analytical signal.

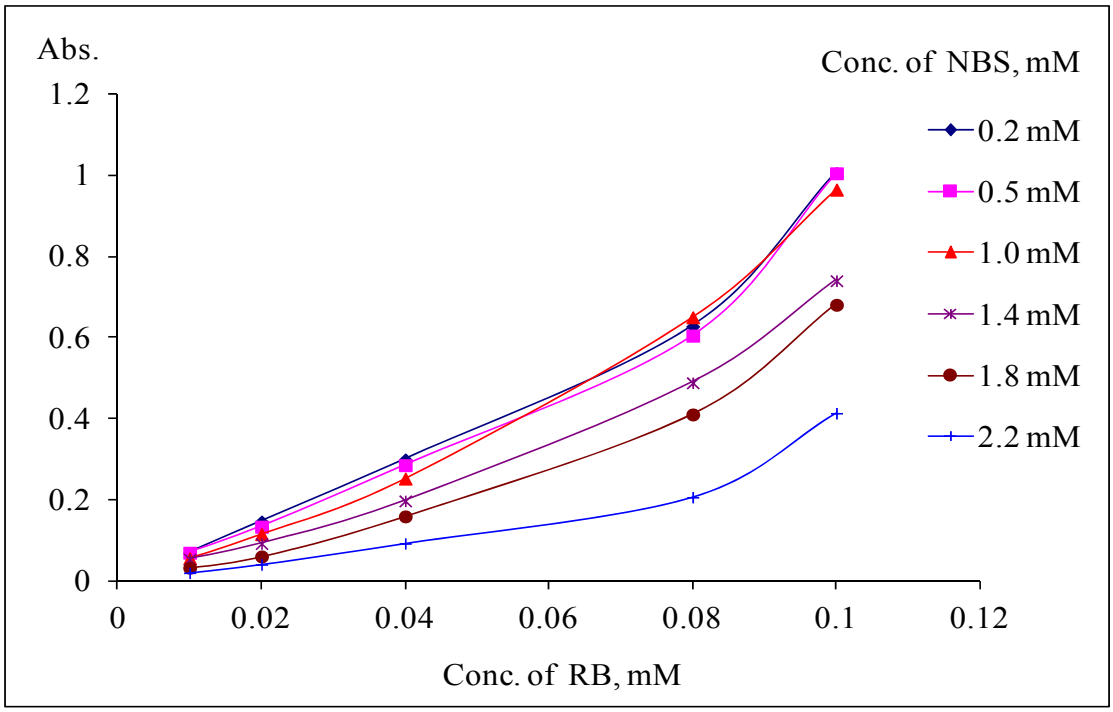

Figure 4: Effect of NBS and RB concentrations on the analytical signals.

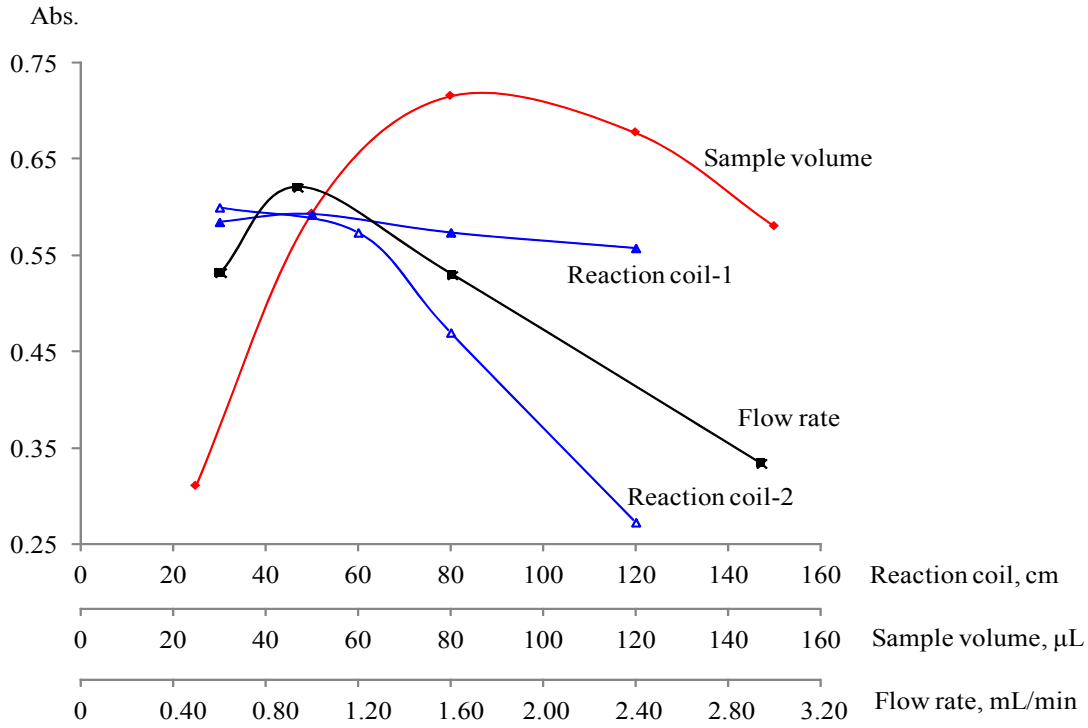

Figure 5: Effect of the flow rate, reaction coils (RC1 And RC2), and sample volume on the analytical signals.

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التحديد الطيفي غير المباشر لبعض مضادات بيتا لاكتام الحيوية بطريقة الحقن الجرياني

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ملخص

تم اقتراح طريقة جديدة بسيطة وحساسة لتقدير بعض مضادات بيتا لاكتام الحيويه في المستحضرات الصيدلانية والسوئل البيولوجية بطريقة الحقن الجرياني. تعتمد الطريقة الجديدة على اكسدة الدواء بكمية زائدة من **N-bromosuccinimide (NBS)** في الوسط الحمضي ثم مفاعلة ما تبقى مع صبغة **Rhodamine B (RB)** حيث يتسبب ذلك بقصر لون **RB** القرمزي. تم قياس الانخفاض في شدة اللون القرمزي عند الطول الموجي **555** نانوميتر. تم ضبط العديد من المتغيرات للحصول على اقصى قدر من الحساسية واعلى سرعة في التحليل. تم تطبيق الطريقة الجديدة بنجاح في تحديد الأدوية في المستحضرات الصيدلانية المختلفة. مقارنة النتائج التي تم الحصول عليها بالطريقة الجديدة بنتائج التحليل باستخدام الكروموتوغرافيا السائلة **HPLC** لنفس العينات اظهرت النتائج عدم وجود فروق معنوية ذات دلالة احصائيه بين نتائج الطريقتين.

الكلمات الدالة: مضادات بيتا لاكتام، التحليل بالحقن الجرياني، الكروماتوغرافيا السائلة، المنتجات الصيدلانية، بلازما الدم.

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