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DEVELOPMENT OF CONTINUOUS FLOW INJECTION ANALYSIS METHOD FOR DETERMINATION OF OXYMETAZOLINE AND VANCOMYCIN HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS

Bayan Hassan and Hind Hadi*

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

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ABSTRACT. Continuous flow injection (CFI)-spectrophotometric method has been developed for oxymetazoline (OXY) and vancomycin hydrochloride (VAN) determination in pure and dosage forms. Sulfadimidine drug was used as a safe chromogenic reagent by diazotization coupling with studied drugs to produce sensitive azo dyes with maximal wavelengths of 498 and 441 nm, respectively, for OXY and VAN drugs. The effect of various chemical and physical conditions on the signal response has been studied. Under optimum conditions, Beer's law was linear over the concentration range of 5–200 and 6–200 µg/mL with limits of detection of 2.19 and 1.79 µg/mL for OXY and VAN, respectively. The CFI systems gave a sample throughput of more than 120 samples per hour for 150 µL injection volume for both drugs. The proposed methods were used for the estimation of microgram quantities of both drugs in their different dosage forms with great effectiveness. The method's validity and applicability were thoroughly investigated and the recoveries values were compared with those of standard pharmacopeia methods. The specified approaches were used without the excipients interfering in pharmaceutical applications.

KEY WORDS: Oxymetazoline hydrochloride, Vancomycin Hydrochloride, Flow injection, spectrophotometry, Pharmaceutical formulations

INTRODUCTION

Oxymetazoline hydrochloride (OXY) is a non-selective adrenergic drug that has been used as eye and nasal drops to cause significant vasospasm and an elevation in blood pressure by acting on adrenergic receptors [1]. Numerous analytical approaches have been used for the assay of OXY [2-8]. However, most of these procedures necessitate the use of sophisticated instruments, costly reagents, and many manipulation steps. Vancomycin hydrochloride (VAN) is a glycopeptide antibiotic used to treat a variety of Gram-positive bacteria [9]. It's also the antibiotic of choice for treating methicillin-resistant staphylococci and drug-resistant enterococcus species nowadays [10]. Several assessment techniques have been employed for determining VAN in different samples including spectrophotometry [11], high performance liquid chromatography (HPLC) [12, 13], capillary electrophoresis [14], ultra-performance liquid chromatography-tandem mass spectrometry [15], mass spectrometry [16, 17], and spectrofluorimetry [18]. Until now, spectrophotometric approaches have been the most popular method for routine analytical work in the field of pharmaceutical preparation analysis. For regular assay of various chemicals (organic and inorganic components), FIA is frequently employed [19, 20]. FIA is a quick and easy technique that can be utilized for reactions that are unstable or do not reach equilibrium. The motivation of this study was to present a rapid and simple spectrophotometric technique alongside with continuous flow injection technique for quantitative assays of OXY and VAN, due to their ease of use and adequate sensitivity, as well as significant cost savings. Diazotization coupling reaction using sulfadimidine drug as safe reagent was selected for this purpose. The reaction product has been spectrophotometrically measured at 498 and 441 nm for OXY and VAN drugs, respectively.

*Corresponding author. E-mail: hind.h@sc.uobaghdad.edu.iq

EXPERIMENTAL

Chemical and reagents

All of the chemical reagents were analytical grade and were employed without further purification. Distilled water was used to make the solutions. The Iraqi Pharmaceutical Manufacturing Company (Samarra/Iraq) generously provided the standard OXY and VAN (99.9%) and sulfadimidine sodium was supplied as a standard solution (333 mg/mL) from uniPharma co./Malaysia. The researched drugs' pharmaceutical applications were obtained from the local market. A standard stock solution of 500 µg/mL OXY and/or VAN was prepared by dissolving 0.050 g of the pure drugs in 100 mL distilled water. Working solutions were obtained from serial dilutions with distilled water.

The solution of 0.01 and 0.02 M of diazotized reagent solution was prepared by transferring 0.9 and 1.8 mL of the standard solution of sulfadimidine sodium (333 mg/mL) to 100 mL volumetric flask followed by 3 mL of 1M HCl in an ice–bath. After 5 min, 0.069 and 0.1380 g of sodium nitrite ($NaNO_2$, Merck) was added to the solutions, shaked well, and completed to the mark with distilled water. Potassium hydroxide (KOH, BDH), 2M solution was prepared by dissolving accurate weights of the base in distilled water. 1 M of ammonium hydroxide (NH_4OH, Merck) solution was prepared by appropriate dilution of the concentrated solution (25%w/w, Merck) with distilled water in volumetric flasks.

Equipment

A Shimadzu UV– Vis 1240, digital single beam recording spectrophotometer equipped with 50 µL quartz flow cell, was used for all spectrum and absorbance measurements connecting with CFI system. The flow injection approach consisted of a peristaltic pump (Ismatec CH–8152-Switzerland) connected to 6 ports injection valve (Rheodyne, Supelco-USA) used for injection of the studied drugs solutions. The reagent and base solutions were pumped through a flexible vinyl tubing (0.5 mm i.d.), which then met with sample solution and mixed through a reaction coil made from Teflon tubes (0.5 mm i.d.).

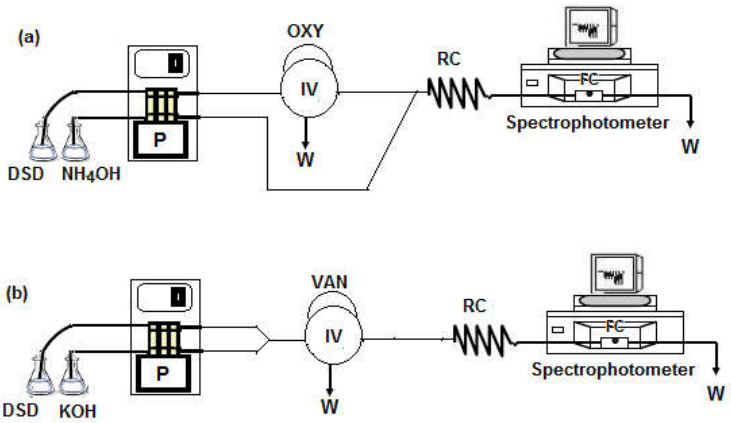


Figure 1. A diagram of the CFI manifolds for (a) OXY and (b) VAN; IV, injection valve; P, peristaltic pump; FC, flow cell; RC, reaction coil; W, waste.

General procedures for CFI method

Flow injection setup

Two-channel CFI manifolds, which offered a maximum absorbance intensity were used for assay both drugs (Figure 1). OXY was injected into a stream of diazotized sulfadimidine (DSD), which was then mixed with ammonium hydroxide using a mixing coil. Also, two-channel manifold was employed for the estimation of the VAN drug. One of these two channels was used to transport DSD and the other to transport potassium hydroxide. VAN drug was injected through an injection valve inside the stream of both reagents solution, which then mixed inside the reaction coil. The absorbance was measured at 498 and 441 nm for OXY and VAN, respectively.

A series of standard solutions of OXY in the range of 5-200 µg/mL were prepared from stock solutions. A 150 µL of the drug solution was injected into a stream of 0.02 M DSD, which was then coupled with a stream of 0.5 M ammonium hydroxide and mixed together in a reaction coil (75 cm) with a total flow rate of 2.87 mL/min (Figure 1a). The resulting absorbance of the colored dye was measured at λmax 498 nm at the end of the manifold.

From the stock solution, working standard solutions of VAN ranging from 6-200 µg/mL were prepared, and a 150 µL aliquot was injected using an injection valve. At a total flow rate of 2.87 mL/min, the streams of 0.01M DSD and 0.1M potassium hydroxide solution were pumped and combined at T-link, then merged with injected sample and mixed in 75 cm reaction coil (Figure 1b). The resulting absorbance of the colored dye was measured at λ_{max} 441 nm at the end of the manifold.

Pharmaceuticals sample preparation

Solution of OXY dosage forms (100 µg/mL). The contents of five containers of commercial OXY nasal drops of pharmaceuticals OXYMET® (0.05%, PHARAONIA–Egypt) and Alerjon® (0.025%, Edol- Portugal) were accurately mixed then 10 and 20 mL of the resulting mixture were diluted respectively to 50 mL with distilled water in a volumetric flask.

Solution of VAN dosage forms (100 µg/mL). Pharmaceutical dosage forms of VAN (Vancomycin hydrochloride® (1 g, EuroVanc, India), and VOXIN® (1 g, VIANEX S.A., Greece)) were obtained from commercial sources. The contents of five vials from each preparation were mixed, and an accurately weighed amount equivalent to 10 mg was transferred into a 100 mL volumetric flask, dissolved, and diluted to volume with distilled water. Further diluted solutions for pharmaceutical preparations for both studied drugs were made by using distilled water.

RESULTS AND DISCUSSION

Manual preliminary investigations showed that after adding an aqueous solution of OXY and/or VAN to DSD solution in alkaline medium, sensitive orange and yellow azo dyes were produced immediately and remained stable for 1 hour at least. The absorption spectra of the colored dyes obtained by diazotization reactions and the reagent blank were measured over the range of 350–800 nm using a spectrophotometer. Figure 2 shows that the highest absorption of the azo dyes products is at 498 and 441 nm for OXY and VAN, respectively.

Instead of harmful and expensive reagents, sulfadimidine drug was used as a safe and green development color reagent. In the presence of nitrous acid, the aromatic amino group in the sulfadimidine molecule is diazotized [21]. The phenolic groups in the OXY and VAN structures facilitate coupling with diazonium salts, especially after they have been converted to phenoxide under alkaline circumstances. The drug-to-reagent ratio was determined by the continuous variation method (job's method) and was found to be 1:1, and according to these results, the

diazonium salt product can be associated with OXY and VAN at room temperature from a single position. Scheme 1 depicts the strong coupling ability of DSD with OXY and VAN for the creation of colorful products.

Optimization of continuous flow injection systems

The proposed flow systems were investigated for the development of CFI procedures for OXY and/or VAN determination based on the diazotization reaction between the studied drugs and the sulfadimidine drug. The best experimental conditions were determined using the univariate method. A solution of 50 µg/mL of the drug (OXY and/or VAN) was injected three times in CFI manifolds for parameters optimization, and the absorbance was measured against a reagent blank at 498 nm and 441 nm, respectively. The influence of various chemical and physical CFI conditions on the intensity of the colored products was studied as follows.

Effect of acid and sulfadimidine reagent concentration. The effect of varying quantities of hydrochloric acid (2-5 mL of 1 M) employed for the diazotization process of the sulfadimidine drug was examined. A 3 mL of 1 M HCl was found to be the most optimal amount for obtaining maximal absorbance for both drugs (Figure 3) and was chosen for further use. Different DSD concentrations in the range of 0.005-0.03 M were also investigated for both drugs. The greatest intensity was attained when 0.02 and 0.01 M of DSD were used for OXY and VAN, respectively.

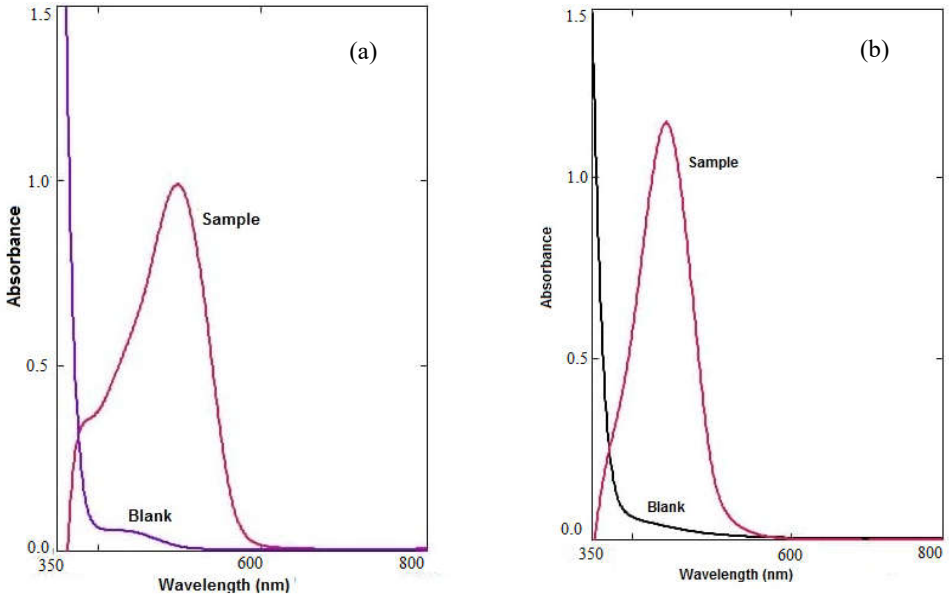
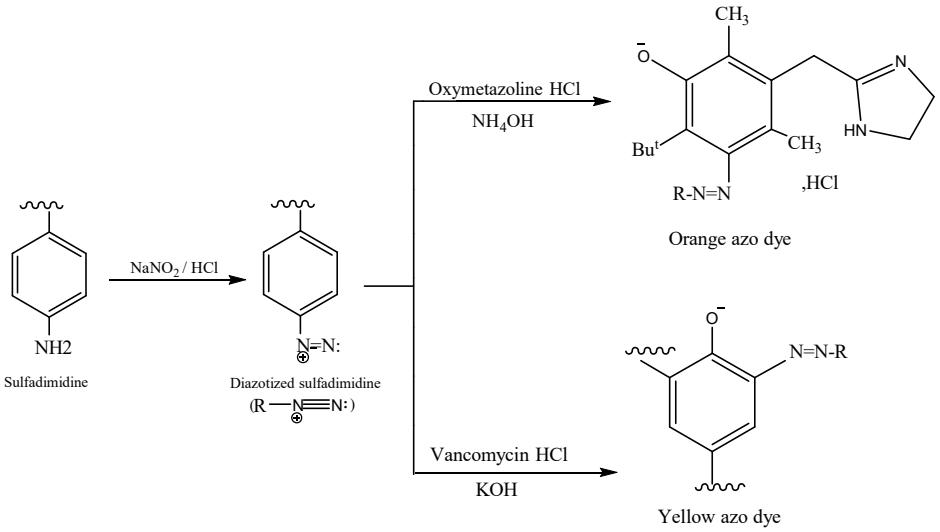
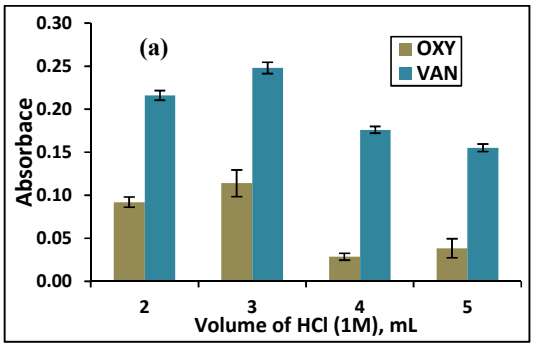


Figure 2. Absorption spectra of azo dyes formed by reacted (a) OXY and (b) VAN with DSD in alkaline medium versus reagent blank.



Scheme1. Proposed mechanism of the reaction between OXY and/or VAN with DSD.



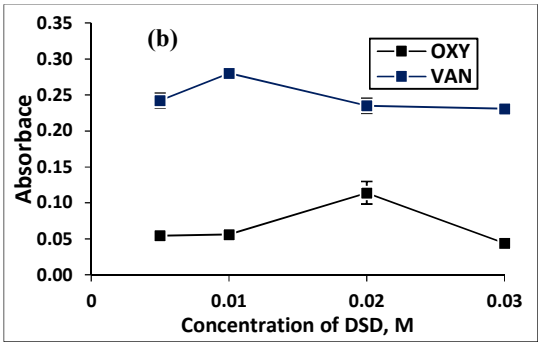


Figure 3. Study of the optimum (a) volume of HCl and (b) concentration of DSD.

Effect of type and concentration of the base. An initial examination revealed that an alkaline medium is required for the appearance and development of the azo dyes, which could be attributed to the conversion of phenolic drugs to more reactive phenoxide molecules. So different bases were examined for both drugs. The results showed that ammonium hydroxide and potassium hydroxide produced the best analytical responses for OXY and VAN, respectively, and were employed in further studies (Figure 4a).

In addition, various concentrations of both bases were investigated in a range of concentrations (0.1-1.0 and 0.05-1 M for OXY and VAN, respectively). The optimal concentrations of ammonium hydroxide and potassium hydroxide were found to be 0.5 and 0.1 M, respectively (Figure 4b).

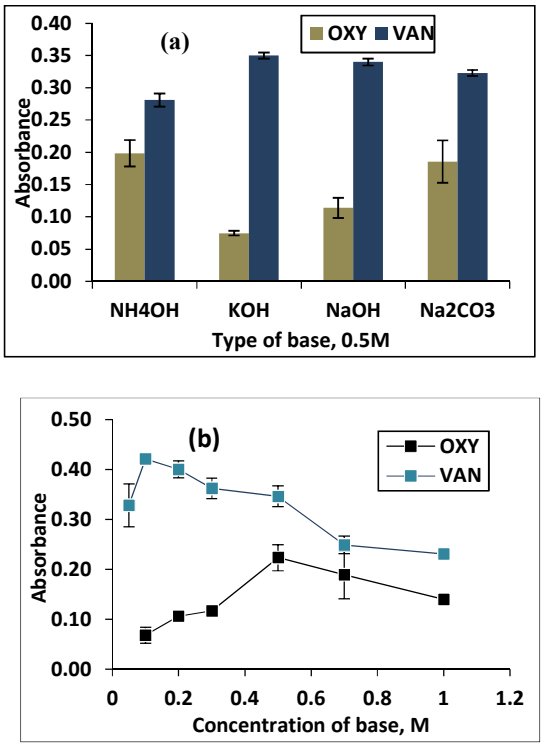


Figure 4. Study of (a) type of base, (b) concentration of NH_4OH and KOH.

Effect of mixing coil length, injected volume, and reagent flow rate. Under the optimum reagent concentrations, the effect of physical variables such as flow rate, injected sample volume, and mixing coil length was examined. In the range of 1.47-5.27 mL/min, the influence of total flow rate on the sensitivity of colored reaction products was investigated for both active ingredients. As demonstrated in Figure 5a, a total flow rate of 2.87 mL/min yielded the greatest absorbance for both medicines, which then decreased because of dilution and increase the dispersion. So, this rate was employed in all subsequent tests. The influence mixing coil length was tested in the range of 25–150 cm, as it is an important characteristic that affects the sensitivity of the colored reaction product. As demonstrated in Figure 5b, a coil length of 75 cm produced the maximum absorbance for both drugs, and this length was selected as the optimum length of the coil. Different micro

liters of both drugs (75-200 µL) were injected into the sample loop to determine the optimum injected sample volume. The results showed that a 150 µL injected sample provided the best absorbance for both drugs (Figure 5c). Due to the high sample-to-reagent volume ratio and increase dispersion, absorbance drops over 150 µL.

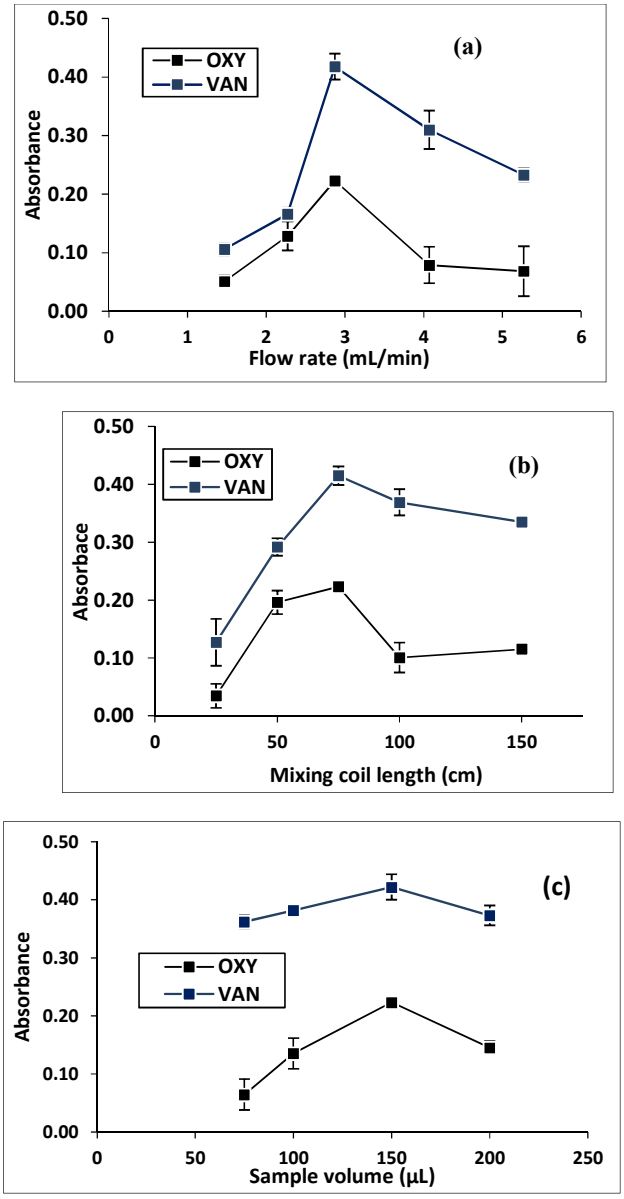


Figure 5. Effect of the (a) total flow rate, (b) mixing coil length and (c) the injection sample volume.

Selected chemical and physical variables

Table 1 lists all of the chemical and physical variables that were investigated and found to be the most ideal in terms of sensitivity and reproducibility.

Table 1. Parameters range studied and optimum values for determination of OXY and VAN.

Parameter	Studied range	Selected value	
		OXY	VAN
Volume of 1 M HCl (mL)	2-5	3	3
Concentration of DSD (M)	0.005-0.03	0.02	0.01
Type of base	NH_4OH, NaOH, KOH, Na_2CO_3	NH_4OH	KOH
Concentration of NH_4OH (M)	0.1-1	0.5	---
Concentration of KOH (M)	0.05-1	---	0.1
Injected sample volume (µL)	75-200	150	150
Length of mixing coil (cm)	25-150	75	75
Flow rate (mL/min)	1.47-5.27	2.87	2.87

Analytical characteristics

Calibration graphs. Using CFI systems and a series of OXY and VAN standard solutions, linear calibration graphs for both drugs were obtained based on optimum variables listed previously. Each concentration level was tested in five replicate. Table 2 summarized all analytical characteristics, including linearity, reproducibility, limit of detection (LOD), and limit of quantification (LOQ). The LOD was defined as the concentration of analyte that produced a signal that differed from the blank by three times the blank signal's standard deviation. The LOQ is defined as the analyte giving a signal that is at least 10 times the standard deviation of the blank signal and the LOD and LOQ values for the studied drugs are listed in Table 2. For the calibration data, the slope, intercept, molar absorptivity, and correlation coefficient were provided. CFI methods showed good linearity within the range 5-200 and 6-200 µg/mL for OXY and VAN drugs, respectively with correlation coefficients of best than 0.999.

Reproducibility and accuracy. The suggested methods' intra-day precision and accuracy were investigated at three concentrations levels for each medication, with five replicate determinations through the same day. Also, the inter-day precision and accuracy were investigated by examining the same three concentrations with five replicate measurements performed within three days. The associated regression equations were used to compute recovered concentrations, which were found to be satisfactory (Table 3). The results show that the proposed methods for estimating the researched drugs have a high degree of precision and accuracy.

Interferences

Tablets, capsules, and medical drops are examples of commercial pharmaceutical formulations that incorporate additives. To assess the selectivity and applicability of proposed methods, the influence of some common excipients on drugs analysis was studied. OXY's medical forms are eye and nasal drops, therefore the interference of some additives that may be added to the OXY drug for manufacturing needs was evaluated. To the 50 µg/mL of OXY solution, 100 folds of each interference (NaCl, lactose, polyvinylpyrolidone, Na_2HPO_4, and EDTA) were added and evaluated separately utilizing CFI technique. Drug additives had minimal to no effect on recovery (99-103%), indicating that these methods could be employed for high-accuracy and precision drug quality control.

Table 2. Analytical features of the CFI methods for both active ingredients

Variables	Value	
	OXY	VAN
Regression equation	y = 0.0037x + 0.0317	y = 0.0061x + 0.0736
Linear range (µg/mL)	5-200	6-200
Correlation coefficient, r	0.9992	0.9995
Limit of detection (s/n = 3) (µg/mL)	2.19	1.79
Limit of quantification (µg/mL)	6.62	5.42
Molar absorptivity ε (L/mol cm)	1.09×10^{-3}	9.07×10^{-3}
Through-put (h^{-1})	138	138
Slope, b	0.0037	0.0061
Intercept, a	0.0317	0.0736
$S_{y/x}$	1.26×10^{-2}	1.49×10^{-2}
S_b	5.66×10^{-5}	6.75×10^{-5}
S_a	6.10×10^{-3}	7.27×10^{-3}
Repeatability (intra-day)	0.42-1.67	0.39-1.01
Intermediate precision (inter-day)	0.65-1.67	0.55-0.89
Average recovery (%)	100.4	100.5

Table 3. Reproducibility and accuracy for proposed CFI methods

Drug	Conc. taken (µg/mL)	Intra-day[a]			Inter-day[b]		
		Conc. found (µg/mL)	RE (%)	% Recovery ± RSD	Conc. Found (µg/mL)	RE (%)	% Recovery ± RSD
OXY	50	48.73	-2.54	97.46 ± 0.89	49.15	-1.70	98.30 ± 1.67
	75	75.89	1.19	101.2 ± 1.7	75.55	0.73	100.7 ± 1.4
	100	102.9	2.89	102.9 ± 0.4	103.2	3.24	103.2 ± 0.7
VAN	75	76.81	2.41	102.4 ± 1.0	76.45	1.93	101.9 ± 0.8
	100	99.44	-0.56	99.44 ± 0.39	98.85	-1.15	98.85 ± 0.89
	150	149.5	-0.32	99.68 ± 0.60	149.1	-0.57	99.43 ± 0.55

a (n = 5), b (n = 15), RE; relative error, Conc.; concentration, RSD; relative standard deviation.

Analysis of OXY and VAN in pharmaceutical formulations

The findings of the OXY and VAN assays in pharmaceutical formulations were obtained using CFI procedures as shown in Table 4. The obtained results closely match the claimed content. The proposed methods were evaluated using recovery studies for both drugs, and the results revealed that both approaches had excellent recoveries values ranging from 99.8% to 100.4%. The findings of the two techniques were compared to the results provided by the official method [22] for both medicines. There are no significant differences between recommended and standard procedures for the assay of medicines in dosage forms, according to the t and F-test results at the 95% confidence limit [23].

CONCLUSION

The suggested CFI methods have been applied successfully for the determination of OXY and VAN in pharmaceutical samples, with relative standard deviation (%RSD) ranging from 0.58 to 3.17% and percentage recoveries of best than 96.9 for both drugs. Furthermore, using a sulfadimidine drug as a diazotization agent for drugs assay using CFI device is simple and inexpensive, and it is highly precise (RSD of 1.67%), sensitive (LOD of 2.19 and 1.79 g/mL for OXY and VAN, respectively), and fast, with a sample throughput of better than 120 per hour for both drugs. Finally, the proposed CFI systems consumed a little amount of chemicals and reagents solutions (less than 250 mL/h), resulting in less waste than the batch technique.

Table 4. Application of the proposed methods in assay of studied drugs in pharmaceutical samples.

Dosage form	CFI method						Official method					Calculated t and F-test (theor.)
	Taken conc. (µg/mL)	Spiked conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[a]	Mean (%Rec. ± SD)	RSD (%)[a]	Taken conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[b]	Mean (%Rec. ± SD)	RSD (%)[b]	
OXYMET®	25	25	49.45	98.90	100.1 ± 1.0	0.66	10	10.06	100.60	100.7 ± 0.1	1.29	t = 0.71 (2.78) F = 15.11 (19.00)
		50	74.17	98.89		2.33						
		75	100.29	100.29		1.85						
	50	25	75.98	101.31		2.88	20	20.15	100.75		0.76	
		50	100.77	100.77		0.47						
		75	125.69	100.55		0.78						
Alerjon®	25	25	50.35	100.70	100.3 ± 1.8	3.17	10	9.91	99.10	99.18 ± 0.11	1.67	
		50	76.10	101.47		1.30						
		75	99.93	99.93		1.10						
	50	25	72.70	96.93		2.57	20	19.85	99.25		1.54	
		50	101.69	101.69		1.34						
		75	126.43	101.14		1.38						
EuroVanc®	25	25	48.15	96.30	99.82 ± 2.02	1.40	10	10.02	100.20	100.5 ± 0.5	0.97	t = 0.39 (2.78) F = 1.16 (19.00)
		50	75.71	100.95		1.25						
		75	101.35	101.35		0.85						
	50	25	74.13	98.84		1.09	20	20.17	100.85		1.25	
		50	101.66	101.66		0.86						
		75	124.74	99.79		0.74						
VOXIN®	25	25	50.14	100.28	100.4 ± 0.4	0.93	10	9.98	99.80	99.73 ± 0.11	1.49	
		50	75.12	100.16		0.72						
		75	100.93	100.93		0.58						
	50	25	74.86	99.81		1.06	20	19.93	99.65		1.26	
		50	100.53	100.53		0.60						
		75	125.95	100.76		1.71						

a, Average of five determinations; b, Average of five determinations; c, Theoretical value; Rec.: recovery.

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