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Normal and Reverse Flow Injection Analysis Methods for Estimation of Mesalazine in Pharmaceutical Dosage Forms

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Abstract

Determining the actual amounts of active ingredients in various pharmaceutical commercial forms is still receiving a lot of attention. Two flow injection analysis (FIA) methods were suggested for the determination of mesalazine (MES) in pharmaceutical forms. Normal and reverse FIA systems (nFIA and rFIA) combined with UV-Vis spectrophotometric techniques were used for the analysis. The methods involved in using two mods of FIA systems for measuring a colored product result from the coupling of MES with (DHP) after being oxidized with sodium periodate in alkaline medium. The absorbance of the red-colored dye was measured at a maximum wavelength of 500 nm. The calibration graphs for MES were linear in the ranges of 2.5–200 µg/mL and 0.5–60 µg/mL, with an RSD of better than 3% for both methods, respectively. Also, the limits of detection were 1.2 and 0.2 µg/mL and the limits of quantitation were 3.6 and 0.7 µg/mL of MSL for the nFIA and rFIA systems, respectively. All physical and chemical conditions of flow systems, such as flow rate, reaction coil length, and reagent concentrations, were carefully studied. The proposed methods were applied for determining MES in four pharmaceutical preparations (tablets) without any interference.

Keywords: Mesalazine; normal and reverse FIA; pharmaceutical forms; 2,2'-dihydroxybiphenyl

1. Introduction

Mesalazine (chemically known as 5-amino- 2- hydroxybenzoic acid) is used to treat inflammatory bowel diseases, especially non-specific ulcerative colitis and Crohn's disease. MES is metabolized in vivo by acetylating enzymes that produce N-acetylmesalazine [1], and it helps remove oxygen-derived free radicals, which are often generated in patients with inflammatory bowel disease [2]. MES is a first-line treatment for many patients with ulcerative colitis. It is thought to have a beneficial anti-inflammatory action via increased expression of peroxisome proliferator-activated receptors in gastrointestinal epithelial cells [3].

Different analytical techniques were reported in the literature for the MES determination, including spectrofluorometry [4], high-pressure liquid chromatography (HPLC) [5], microfluidic device-based liquid phase microextraction-HPLC [6], cyclic voltammetry [7], liquid chromatography-mass spectroscopy [8], FIA [9], and spectrophotometry [10–15].

Due to their simplicity, excellent reproducibility, and inexpensive instrumentation costs, flow-injection analysis (FIA) approaches have attracted a lot of interest and are widely used. The FIA technique is extensively used for the analysis of a wide spectrum of organic and inorganic compounds [16]. The normal and reverse modes of the various FIA procedures are given a lot of consideration [17, 18]. The normal flow injection analysis method (nFIA) entails injecting a small volume of sample into a reagent carrier stream that travels through a thin bore tube to a spectrophotometer, where the derivative is determined. The reverse flow injection analysis (rFIA), on the other hand, involves injecting a small amount of reagent solution into the carrier and sample streams. The present work included two simple and rapid FIA-spectrophotometric methods (normal and reverse) for estimation of MES in pharmaceutical forms using 2, 2'-dihydroxybiphenyl (DHP) as a colorimetric reagent. The immediate formation of a red-colored product was allowed to be applied using FIA techniques and detected spectrophotometrically. The methods are applied for the assay of MES in pharmaceutical samples. The method is simple, fast, and efficient.

2. Experimental

2.1. Instruments and FIA manifolds

A single-beam UV-visible spectrophotometer (Shimadzu 1240) was used for measuring absorbance, equipped with a flow quartz cell (50 μL and 1-cm path length). Flow was controlled in addition to the reagent and sample solutions introduced, respectively, using a six-channel peristaltic pump (Ismatec, Switzerland) and injection valve (Rheodyne, USA). Polytetrafluoroethylene tubes (0.8 mm i.d.) were utilized for the transport lines and connected the components of the FIA manifold, while Teflon tubes (0.5 mm i.d.) were employed to create varied lengths of reaction coils (RC). Two types of three channels—FIA manifolds (normal and reverse)—were used for the analysis of the target drug compound (Fig. 1). For nFIA (method A), a solution of MES was injected through the injection valve into the stream of solution produced by the combination of sodium periodate and sodium hydroxide solutions at the Y-link, which then

met with the stream of DHP and mixed together inside the reaction coil. For rFIA (method B), a solution of DHP reagent was reversely injected through the injection valve into the stream of solution created by a combination of sodium periodate and sodium hydroxide solutions at Y-link, which was then met and mixed with the stream of drug in the reaction coil. For both FIA manifolds and through a peristaltic pump, the solutions have been pumped at a flow rate of 6 and 4.8 mL/min for the nFIA and rFIA methods, respectively, and the red product's absorbance was measured at a maximum wavelength of 500 nm at the end of the manifold.

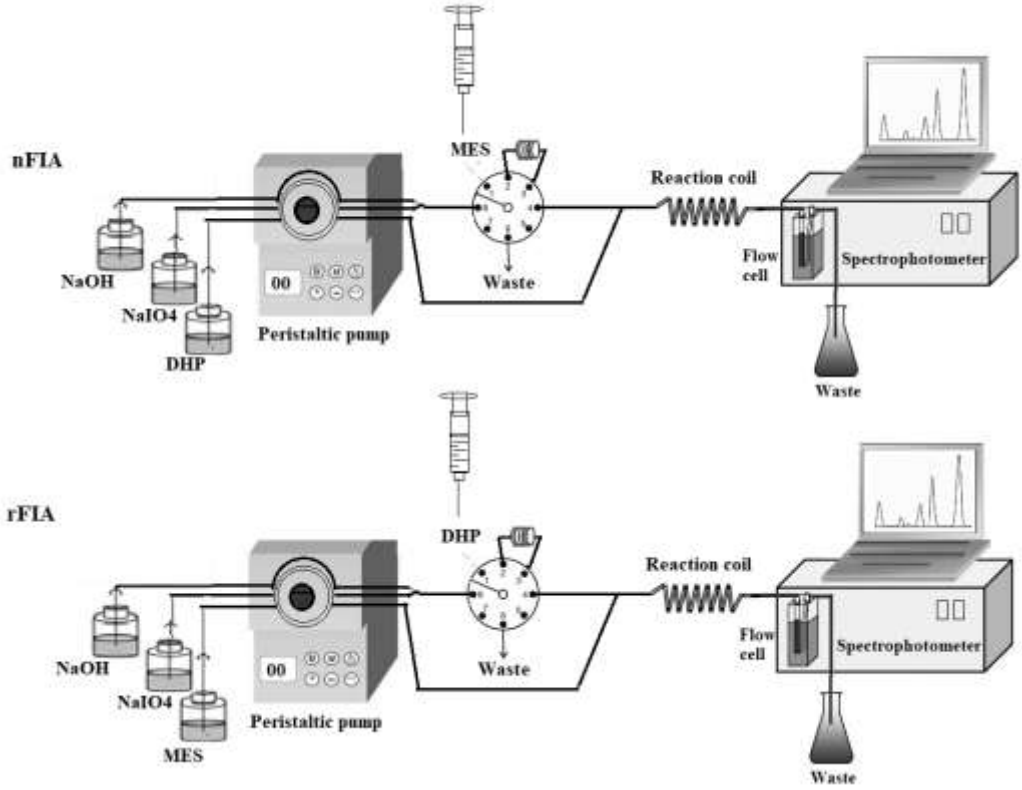


Figure 1. Normal and reverse three channels-FIA manifolds for determination of MES.

2.2. Reagents and solutions

All of the reagents employed were analytical reagent grade, and distilled water was utilized to make all of the solutions. Samarra Pharmaceutical Manufacturing Company (Iraq) provided MES standards (99.9% w/w). MES tablets containing the active ingredient (Pentasa® tablets, 500 mg, Ferring/Germany; Pentasa® tablets, 500 mg, Ferring/Istanbul; Pentasa tablets, 500 mg, Ferring/Milano; MESACOL® tablets, 400 mg, UNIPHARMA/Syria) were purchased locally. 2, 2'-Dihydroxy-biphenyl (British Drug Houses, UK), sodium periodate (Fluka, Buchs, Switzerland), and sodium hydroxide (BDH) were purchased from local pharmacies. A 500 µg/mL stock standard solution of MES was prepared in a 100 mL volumetric flask by dissolving 50 mg of MES in 25 mL ethanol and completing to the mark with distilled water. More diluted solutions of the drug

were obtained by simply diluting them with distilled water. A 0.4655 g of DHP was dissolved in 25 mL of ethanol, transferred to a 250 mL volumetric flask, and completed to the mark with distilled water to prepare 0.01 M of DHP solution, which was then kept in a brown bottle. Working standard solutions were made by serially diluting the standard stock solution by the required volumes with distilled water. Stock solutions of 0.1 M sodium hydroxide and 0.01M sodium periodate were prepared by dissolving 1.0 g and 0.5347 g of sodium hydroxide and sodium periodate, respectively, in 250 ml of distilled water.

2.2.1. Preparation of the solution of pharmaceutical applications

Twenty tablets of commercial pharmaceutical forms were accurately weighed and finely crushed. Powdered tablets weighing 0.6960 g that said they contained 400 mg of active ingredient and 0.7500 g that said they contained 500 mg of active ingredient (equal to 50 mg of MES) were put into a 100 mL volumetric flask and mixed with 25 mL of ethanol for five minutes. Then it was diluted with distilled water and filtered through filter paper. The filtrate was diluted with distilled water to provide the necessary diluted solutions. Finally, the MES assay was carried out in accordance with the recommended FIA procedures.

2.3. Procedure

2.3.1. Procedure of normal FIA

A sequence of standard solutions of MES (2.5–200 μg/mL) was prepared. Through the injection valve of the nFIA manifold (three channels), a volume of MES solution (100 μL) was injected into the stream of solution created at the Y-link by the combination of 3 mM sodium periodate and 1 mM sodium hydroxide solutions. The resultant solution was next combined with a stream of 5 mM DHP and mixed inside the reaction coil (25 cm) at a flow rate of 6 mL/min.

2.3.2. Procedure for Reverse FIA

The reverse type of FIA was carried out by injecting 100 μL of a solution containing 8 mM of DHP into a stream of solution resulting from combining 8 mM sodium periodate and 10 mM sodium hydroxide solutions. The solution is then mixed with MES solution (ranging from 0.5 to 60μg/mL) in a 25-cm reaction coil at a 4.8 mL/min flow rate. For both methods, the spectrophotometric measurements of red dye were made at 500 nm at the end of FIA manifolds. During optimization of all variables of FIA systems, 50 μg/mL of MES was used.

3. Results and discussion

Experimental tests showed that when the MES molecule was oxidized and then coupled with DHP in a basic medium, a sensitive red dye was made. When the reaction was carried out manually, the product was generated directly (within a few seconds) and remained stable for at least two hours. These distinctive qualities meet the criteria for the suggested completely automated and sensitive normal and reverse FIA methods for the estimation of MES. The red product's absorption spectrum, tested in comparison to the reagent blank, revealed a distinctive wavelength value of 500 nm (**Figure 2**). MES has a phenolic ring substituted by an amino group. Under oxidation

conditions, the presence of these groups increased the opportunity for the compound's oxidation. The Ar-OH group of the DHP molecule, which is definitely transformed into a reactive state (phenoxide) in a basic medium, reacts with oxidized MES via the amino group. In order to analyze the stoichiometry of the MES:DHP utilizing equimolar quantities of both drug and reagent, Job's method for continuous variations was applied, and the 1:1 mole ratio was achieved. A possible reaction pathway is shown in **Scheme 1**.

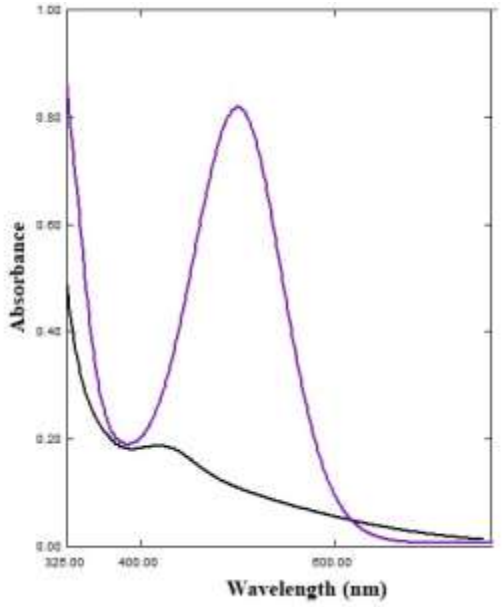
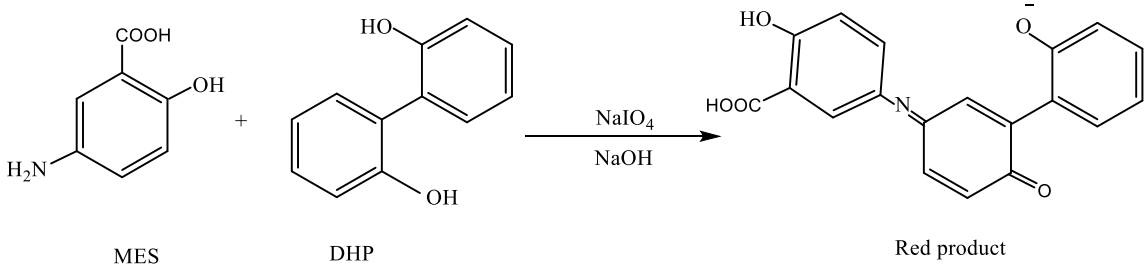


Figure 2. Absorption spectra of the red dye formed by reacting 50 µg/mL of MES with DHP measured versus the blank, and the blank versus distilled water.



Scheme 1: Proposed reaction pathway

3.1. Optimization of Flow Injection Parameters

By changing one variable at a time while leaving the rest constant, the chemical and physical factors that were most influencing the development of the red dye product and the stability of analytical signals for both FIA systems were thoroughly analyzed.

3.1. Study of the manifold design

The main components of the reaction adopted for the assay of MES are the reagent, the oxidant and the reaction medium. So, various designs for three-channel manifolds were investigated for both normal and reverse FIA methods to carry out various reaction routes. The results indicated that the manifold C shown in **Figure3** provided maximum absorbance intensity and good precision for nFIA and rFIA and was selected for next use. Furthermore, the manifold arrangement of nFIA (**Figure2**) with the suggested reaction's pathway, which included the oxidation of the MES molecule followed by coupling with the reagent in a basic medium.

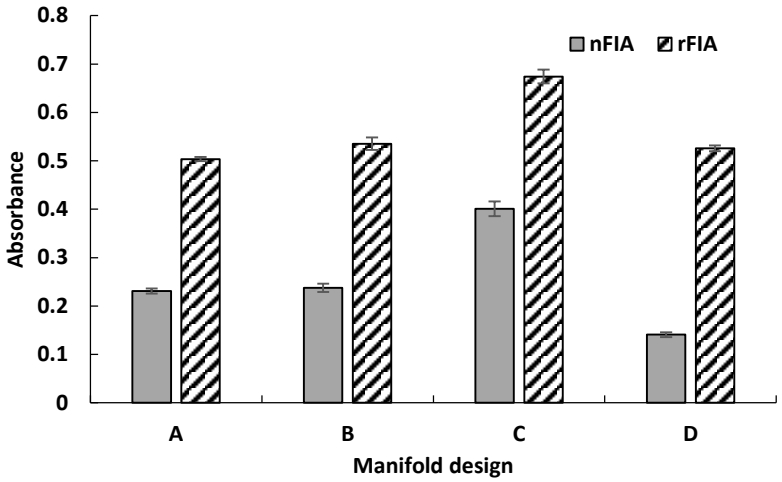


Figure 3. Effect of manifold design (A: nFIA (Y(DHP+NaOH)+(Inj. MES)+$NaIO_4$) & rFIA (Y(MES+NaOH)+(Inj. DHP)+$NaIO_4$); B: nFIA(Y(DHP+$NaIO_4$)+(Inj. MES)+NaOH) & rFIA: Y(MES+ $NaIO_4$)+(Inj. DHP)+NaOH); C: nFIA(Y($NaIO_4$+NaOH)+(Inj. MES)+DHP) & rFIA: (Y($NaIO_4$+NaOH)+(Inj. DHP)+ MES); D: nFIA (NaOH+(Inj. MES)+Y(DHP+$NaIO_4$) & rFIA (NaOH+(Inj. DHP)+Y(MES + $NaIO_4$). 'Y' means a junction point combined two stream of solutions.

3.2.1. Optimization of the chemical parameters

3.1.3.1. Influence of DHP concentration

For normal and reverse systems, the effects of different DHP concentrations in the range of 1–7 and 2–10 mM, respectively, were examined. The concentrations of 5 and 8 mM, which produced the maximum absorbance for the nFIA and rFIA methods, respectively, were chosen as optimum concentrations (**Figurt 4A**).

3.1.3.2. Influence of base species

Previous studies have shown that confirmation in an alkaline medium is required for the development of the coupling reaction, specifically the transformation of the Ar-OH group of DHP to the reactive phenoxide group; therefore, the effects of various types of bases were examined. The results showed that sodium hydroxide was given the best analytical signal and high precision for both methods, so it was selected for further use (**Figure 4B**).

3.1.3.3. Influence of NaOH concentration

The concentrations of NaOH were investigated between 3–30 and 0.5–30 mM for the nFIA and rFIA methods, respectively, and the highest absorbance intensities were achieved at 10 mM for normal and 1 mM for reverse FIA systems (**Figure 4C**).

3.1.3.4. Influence of sodium periodate concentration

Oxidant concentration was also studied in the ranges of 1-7 and 3-10 mM for normal and reverse FIA respectively. The results indicated that 3 and 8 mM gave maximum intensity and were chosen as optimum concentrations for both methods, respectively (**Figure 4D**).

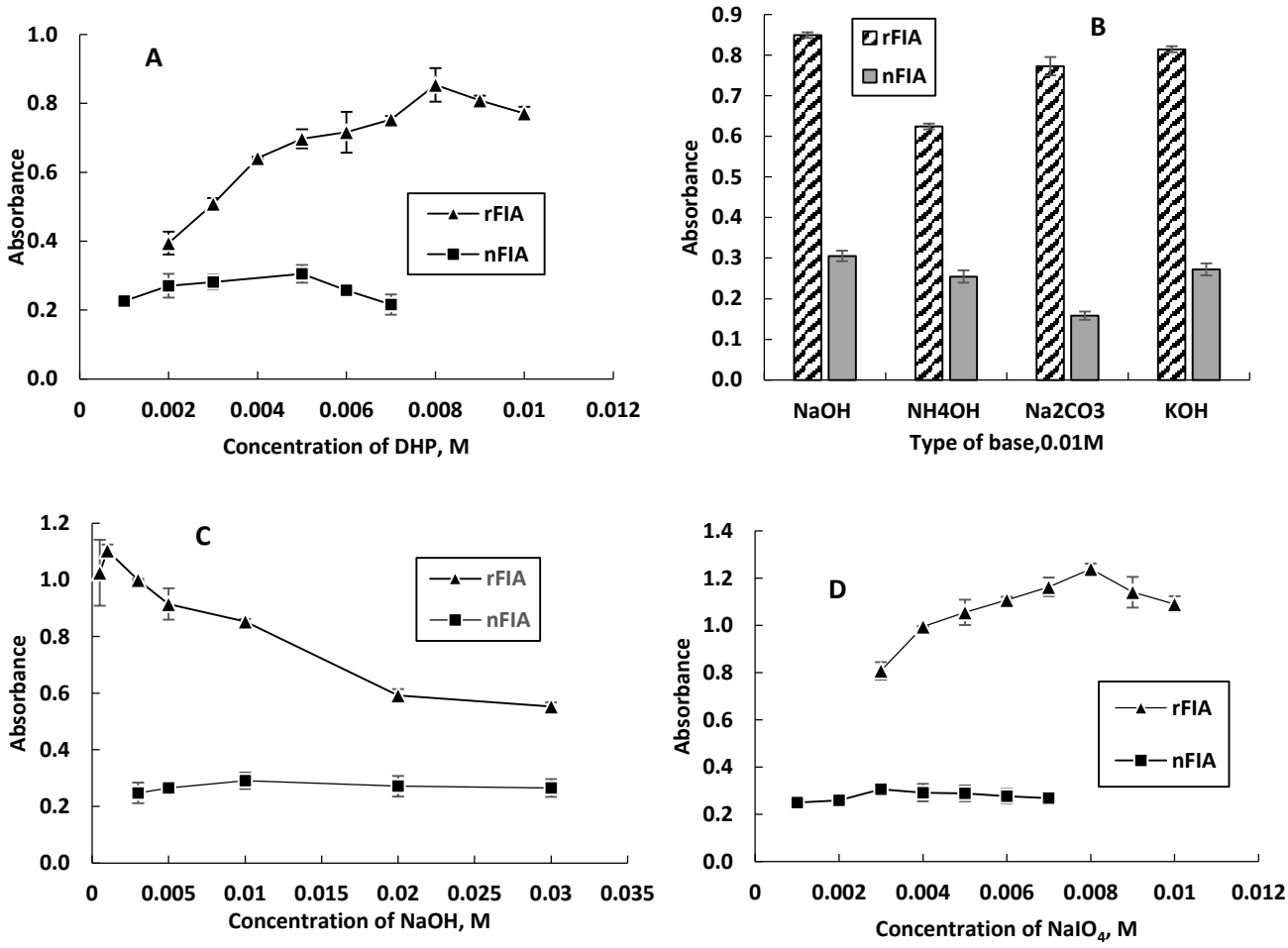


Figure 4: showed the effect of (A) DHP concentration, (B) type base, (C) NaOH concentration, and (D) NaIO4 Concentration.

3.2.2. Optimization of the physical parameters

3.1.3.1. Influence of total flow rate

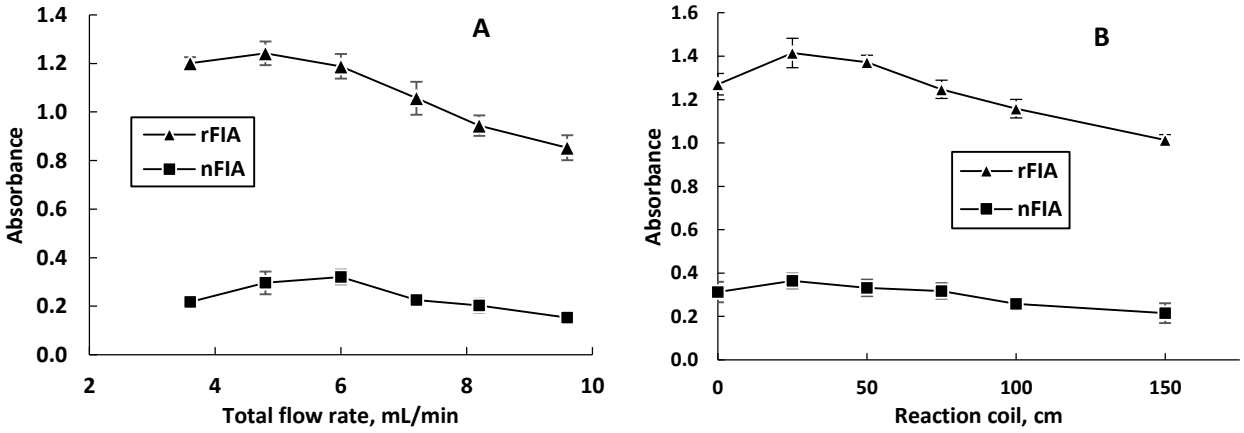
Along with the sample frequency, flow rate is a significant factor that mostly determines the product's sensitivity. So, under ideal conditions for both the normal and reverse flow methods, this variable was investigated in the ranges of 2.32-9.6 mL/min for both systems. As shown in Fig. 5A, the analytical signal increased with an increased flow rate up to 6 and 4.8 mL/min for nFIA and rFIA, respectively, before gradually decreasing. Reduced residence time, which was needed to get the colored product to its maximum values, as well as the dispersion effect, may be responsible for the decreased analytical signal. Therefore, the optimal values for nFIA and rFIA were selected to be 6 and 4.8 mL/min, respectively.

3.1.3.2. Influence of mixing coil

Different reaction coil lengths in the range of 0-150 cm were investigated in order to analyze the influence of reaction coil length. For both approaches, the analytical signal reached its maximum value at 25 cm and then steadily fell when the coil length was increased due to an increase in dispersion. Therefore, 25 cm was the ideal length for the further studies, as shown in **Figure 5B**.

3.1.3.3. Influence of injected volume

The amount of analyte or reagent injected through the injection valve into the normal or reverse FIA manifolds was optimized. For this study, several loop lengths connected to the injection valve provided a range of volumes between 50 and 150 µL were used. The findings (**Figure 5C**) demonstrated that 100 µL of injected volume provided the greatest absorbance with good precision for both methods, and it was subsequently chosen for further uses. Beyond 100 µL, the analytical signal faded, which was attributed to a high sample-to-reagent ratio or dispersion. Table 1 contains an overview of the optimal values for the investigated FIA variables.



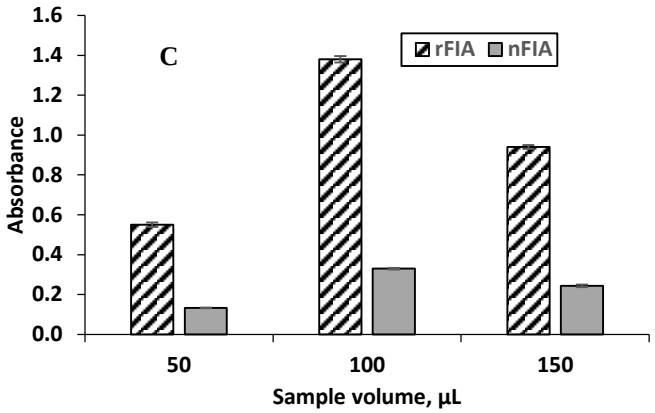


Figure 5: showed the effect of **(A)** total flow rate, **(B)** reaction coil length, **(C)** and (C) injected volume.

Table 1. Selected FIA factors for the assay of MES using normal and reverse FIA methods.

FIA factors	Range of study		Optimum value	
	nFIA	rFIA	nFIA	rFIA
Chemical factors				
Conc. of DHP (mM)	1-7	2-10	5	8
Conc. of $NaIO_4$ (mM)	1-7	3-10	3	8
Alkaline medium type	NaOH, KOH, NH_4OH, Na_2CO_3		NaOH	NaOH
Conc. of NaOH (mM)	3-30	0.5-30	10	1
Physical factors				
Total flow rate (mL/min)	2.32 - 9.6		6	4.8
Length of mixing coil (cm)	0-150		25	25
Sample volume (µL)	50-150		100	100

3.2. Validation of the Suggested Methods

The calibration curves for the estimation of MES using both FIA systems were obtained under ideal conditions after analyzing all the physical and chemical parameters of both FIA systems (Fig. 6A, B). In order to observe the linearity of the calibration graphs, a number of standard MES solutions were injected or propelled. **Table 2** lists the regression equations, correlation coefficient, slope, and molar absorptivity values, in addition to some statistical values. The analytical findings indicated adequate precision, good linearity, and high sensitivity for the MES assay. The linearity of the proposed methods was in the ranges of 2.5–200 µg/mL (LOD 1.18 µg/mL, % RSD <2.87, n = 5) for the nFIA method and 0.5–60 µg/mL (LOD 0.24 µg/mL, %RSD<1.48, n = 5) for the rFIA method.

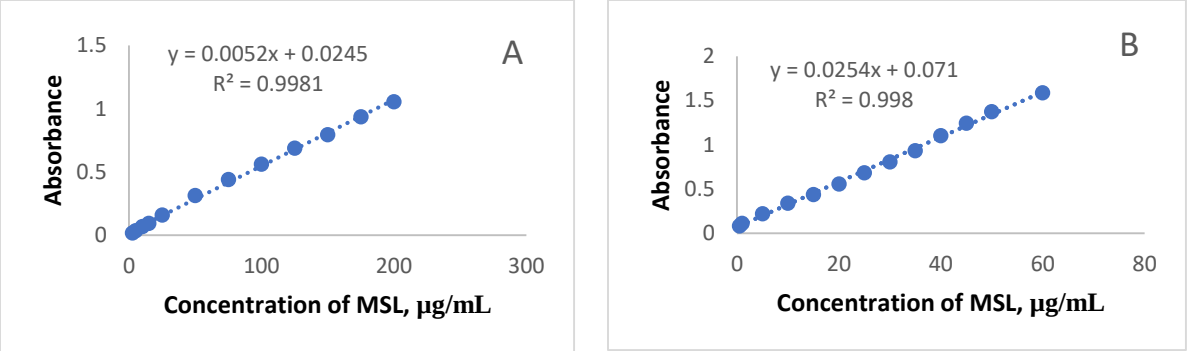


Figure 6. Calibration curves of MES (A) nFIA; and (B) rFIA.

Table 2: Analytical characteristic of the suggested methods

Parameter	Value	
	nFIA	rFIA
Regression equation	y = 0.0052x + 0.0245	y = 0.0254x + 0.0710
Linear range (µg/mL)	2.5-200	0.5-60
Correlation coefficient, r	0.9991	0.9990
Detection limit (S/N=3) (µg/mL)	1.177	0.242
Limit of quantification (µg/mL)	3. 569	0.733
Molar absorptivity, ε (L/mol cm)	0.79×10^{3}	0.39×10^{4}
Sandell's sensitivity, S (µg/cm^2)	0.190	3.94×10^{-2}
Reproducibility, %	<2.87	<1.48
Recovery,%	99.41	98.47
Slope, b (mL/µg)	0.0052	0.0254
Intercept, a	0.0245	0.0710
$S_{y/x}$	1.73×10^{-2}	2.33×10^{-2}
S_b	7.35×10^{-5}	3.47×10^{-4}
S_a	7.59×10^{-3}	1.11×10^{-2}
Through-put (hr^{-1})	82	65

3.3. Reproducibility and Accuracy

The precision and accuracy of normal and reverse FIA methods were investigated. Three different concentrations of mesalazine solutions were assayed (5 replicates) on the same day and over six consecutive days (intra- and inter-day variation, respectively). For both approaches, the findings shown in Table 3 demonstrated high precision (low values of RSD 1.0–3.6 and 1.1–1.5%, respectively) and tolerable accuracy (recovery values within the range of 98.7–100.7 and 97.6–99.4%, respectively).

Table 3: Intra and inter-day accuracy and precision for assay of MES for nFIA and rFIA

Method	Taken conc. (µg/mL)	Intra-day (n=5)				Inter-day (n=15)			
		Found conc. (µg/mL)	Relative error (%)	Recovery (%)	RSD (%)	Taken conc. (µg/mL)	Relative error (%)	Recovery (%)	RSD (%)
nFIA	25	25.06	0.24	100.24	3.58	24.63	-1.48	98.52	2.66
	125	123.33	-1.34	98.66	1.45	122.75	-1.80	98.20	1.44
	175	176.25	0.71	100.71	1.01	174.56	-0.25	99.75	1.56
rFIA	10	9.84	-1.60	98.40	1.49	9.74	-2.60	97.40	1.97
	25	24.41	-2.36	97.64	1.45	24.13	-3.48	96.52	1.96
	40	39.76	-0.60	99.40	1.09	39.35	-1.63	98.37	1.41

3.6. Influence of the additives in pharmaceutical forms

The impact of a few likely interfering substances (additives) that are typically added to active ingredients in tablets was investigated. The testing was achieved by spiking 50 µg/mL of mesalazine with a twenty-fold excess concentration of some excipients such as glucose, lactose, poly vinyl pyrrolidone (PVP), starch, and magnesium stearate. **Table 4** shows acceptable recovery values were attained, representing insignificant interference with the present method.

Table 4. Analysis of MES in the presence of common interferences using nFIA.

Additive (1000 µg/mL)	Amount of MES (µg/ mL)		(Recovery ± SD) % (n=5)
	Added	Found	
Glucose	50	49.39	98.78±1.7
Lactose		50.89	101.78±0.7
PVP		49.64	99.28±0.7
Starch		50.32	100.64±1.2
Mg stearate		50.65	101.30±0.5

3.7. Assay of MES in pharmaceutical forms

Four different kinds of commercial pharmaceutical MES tablets were analyzed in order to determine the applicability of the recommended FI approaches. The results that were obtained showed excellent agreement between the taken and founded amounts with minimal values of percentage error. Recovery values for both FI approaches were contrasted with those attained using the UV method [19]. The proposed and reference procedures were statistically compared using the F and t-tests [20, 21], and the computed values were lower than the theoretical ones, pointing to no significant variance between the two methodologies in terms of accuracy and precision (**Table 5).**

Table 5: Estimation of MES in tablets using nFIA and rFIA methods.

Pharmaceutical form	Proposed methods										UV method				
	nFIA method					rFIA method									
	Added conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[a]	Mean Rec. (%)	RSD (%)[a]	Added conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[a]	Mean Rec. (%)	RSD (%)[a]	Added conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[b]	Mean Rec. (%)	RSD (%)[b]
MESACOL Syria PENTASA® Istanbul PENTASA Milano PENTASA® Germany Pure MES t (2.306)[c] F (9.605)[c]	50 75 100 50 75 100 50 75 100 50 75 100	48.47 73.51 96.35 49.16 73.40 99.28 49.21 74.56 97.11 48.64 75.95 100.73	96.94 98.01 96.35 98.32 97.87 99.28 98.42 99.41 97.11 97.28 101.27 100.73	97.10 98.49 98.31 99.76 99.87	2.223 3.342 2.203 3.542 2.502 2.352 2.012 3.192 2.683 3.011 1.541 1.161	20 30 40 20 30 40 20 30 40	19.54 29.41 39.78 19.80 29.55 40.01 19.70 29.40 39.87 19.73 29.80 40.02	97.70 98.03 99.45 99.00 98.50 100.03 98.50 98.00 99.68 98.65 99.33 100.05	98.39 99.18 98.98 73 99.34 98.48	0.911 1.461 1.161 1.050 0.901 1.561 1.761 1.301 1.332 2.931 1.741 1.881	20 40 20 40 20 40 20 40	19.55 39.69 19.93 40.34 20.26 39.55 20.07 40.29	97.75 99.23 99.65 100.85 101.30 98.88 100.35 100.73	98.49 100.25 100.09 100.54	1.311.062.963.052.542.503.021.56
		99.06													
		1.522 1.729					1.993 4.288				$(n_1-1)=4$, $(n_2-1)=4$, $(n_1+n_2-2)=8$				

a, (n=5); b, (n=5); c, Theoretical value; RSD, relative standard deviation; Conc., concentration

3.8. Conclusion

The current continuous flow injection spectrophotometric methods have the observable advantages of rapid estimation of the active ingredient (mesalazine) in pharmaceutical tablets with very small amounts of sample (100 microliters) and lower waste production, with high sampling rates for nFIA and rFIA, respectively. The FIA methods were discovered to be sensitive, inexpensive, and interference-free. With good precision, there was no need for any pre-extraction or heating. The procedures show that rFIA analysis could greatly improve the sensitivity and

precision for determining MES higher than the nFIA method. The procedure was applied successfully to the determination of MES in commercial pharmaceutical tablets.

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