Continuous Flow Injection Spectrophotometric Determination Of Mesalazine In Pharmaceutical Forms

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ABSTRACT

Simple and sensitive continuous flow injection spectrophotometric method for the assay of mesalazine (MES) in commercial dosage forms was adopted. Using manifold with three channels, MES was determined by oxidative coupling reaction with o-coumaric acid after oxidation with sodium periodate in an alkaline medium. A blue color product was formed immediately at room temperature and measured maximally at λmax 659 nm. All the chemical and physical conditions of the flow system were studied and optimized to obtain high sensitivity and repeatability. Using optimum conditions, the Beer's low obeyed over the range from 5-150 µg/mL MES with limits of detection and quantification of 1.48 and 4.94 µg/mL MES respectively. Minimum values of percentage error (-1.4%) and relative standard deviation (1.8%) indicated the accuracy and precision of the method. Also, good sampling frequency of 42 h-1 for the FI procedure was obtained. The developed method satisfactory used for rabid assay of MES in pharmaceutical forms.

INTRODUCTION

Mesalazine (MES), chemically named 5-amino- 2-hydroxybenzoic acid, is an anti-inflammatory drug (Fig. 1). It is used for the treatment of inflammation of the digestive tract. Due to its predominant actions, MES is metabolized in the gut with some side effects [1]. The pharmacokinetic action of MES is not yet known, but it may be considered to be local rather than systemic [2]. MES plays a helpful role in the removal of oxygen-derived free radicals which are formed extensively in patients with inflammatory bowel disease [3].
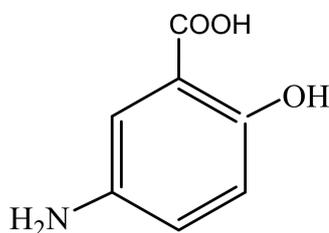
Fig. 1: Mesalazine

The literature survey involved different analytical methods for assay of MES, included spectrophotometric [4-9], spectrofluorometric [10], voltammetric [11, 12], HPLC [13, 14], HPLC-ESI-MS/MS [15], electrochemical sensing [16, 17], and other methods. Flow-injection analysis (FIA) methods have received great attention and extensive application because of simplicity, high reproducibility, and low instrumentation cost. FIA widely used for routine analysis of different organic and inorganic compounds [18, 19]. This technique is also used for unstable reactions or even does not reach equilibrium [20]. Until now the literature contains no FIA method for the determination of MES. This work involved a simple and rapid FIA-spectrophotometric method for assay of MES in pharmaceutical forms using o-coumaric acid (CA) as a new reagent in the presence of sodium periodate. A blue color product was formed immediately and measured spectrophotometrically at 659 nm. The method is applied for the assay of MES in pure and dosage forms.

Experimental
Equipment and flow injection setup

A single beam spectrophotometer (Shimadzu UV-Visible 1240) was used for the measurement of absorbance by connecting with FI manifold. The flow injection manifold composed of flow cell (50 µL), Ismatec CH–8152-Switzerland peristaltic pump, and 6 ports injection valve (Rheodyne, Altex 210, Supelco-USA) used for injection MES solutions. The solution of reagent, oxidant, and base were pumped through a flexible vinyl tubing (0.5 mm i.d.), which then mixed inside the reaction coil (RC) made from Teflon tubes (0.5 mm i.d.). A manifold with three channels (Fig. 2) was used for the continuous flow estimation of the MES compound. Through the injection valve, a solution of MES was injected into the stream of the sodium periodate which later meet with the solution results from combined CA and sodium hydroxide solutions at Y-link and finally mixed inside the reaction coil. The peristaltic pump with a rate of 2.27 mL/min has propelled the solution throughout the manifold. The absorbance of the blue product was reported at the end of the manifold at λmax 659 nm.
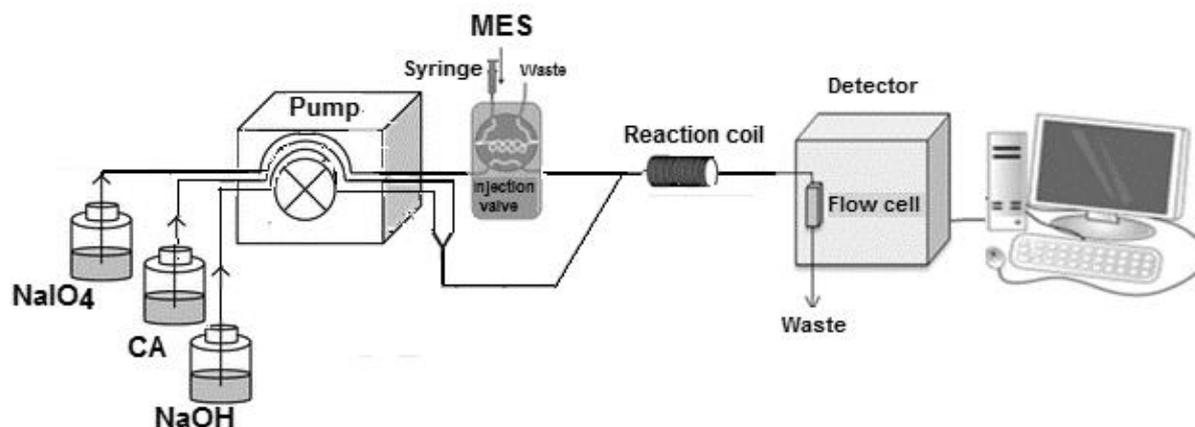
Fig. 2: Continuous flow manifold designed for MES

Materials and reagents

All the reagents used were of analytical reagent grade and all the solutions were prepared using bidistilled water. The standard MES (99.9% pure) was kindly donated by the Iraqi Pharmaceutical Manufacturing Company (SDI-Samarra/Iraq). Pharmaceutical applications of MES (Pentasa® tablets 500 mg/Ferring pharmaceutical/UK-Awasalazin-SR® tablets 500 mg/Awamedica/Iraq) were purchased from the local market. A Stock solution of MES, 500 µg/mL, was prepared by dissolving 50 mg of MES in 25 mL ethanol in a 100 mL volumetric flask and then diluted with bidistilled water. The working concentrations were obtained from simple diluting with bidistilled water. An aqueous solution of o-coumaric acid (5 mM) was freshly prepared by dissolving 0.082 g of CA in a 100 mL bidistilled water. Solutions of (0.1 M) NaOH and (0.01 M) $NaIO_4$ were prepared by dissolving a known amount of these materials in 250 and 100 mL bidistilled water respectively.

Analysis of MES tablets

Twenty Penatsan or Awasalazin retard tablets (declared 500 mg MES) were weighed and finely powdered. In 100 mL volumetric flask, an amount of powdered tablets (equivalent to 50 mg of MES) was weighed, transferred, and shaken for 5 min with 25 mL of ethanol and diluted with bidistilled water. The solution was then filtered using filter paper. Working solutions have been prepared by dilution of filtrate with bidistilled water. Assay of MES was performed by following the recommended FIA procedure.

FIA procedure

A three-lined manifold was designed as depicted in Fig.2. A 100 µL sample of standard MEL solution (range of 5-150 µg/mL) was injected via a plastic syringe through an injection valve into the stream of 0.1 M $NaIO_4$ solution. The solution later was met with the stream resulting from combined 0.01 M of CA and 0.3 M NaOH solution and mixed in the 25 cm reaction coil. A peristaltic pump has propelled the solutions (total flow rate of 2.27 mL/min) and the blue dye absorbance was reported at 659 nm. A 50 µg/mL of MES was used in all experiments for estimation of the best conditions of the FIA system.

RESULTS AND DISCUSSION

Preliminary investigations showed that a sensitive blue dye product was formed after oxidation of MES drug and then coupling with CA in an alkaline medium. Manually the reaction product was formed immediately (within 2s) and the was stable for at least two hours with high molar absorptivity. These unique characteristics encounter with the requirements for the suggested a fully automated and highly sensitive continuous FIA method for estimation of MES. The blue product's absorption spectrum against blank showed a characteristic wavelength value at 659 nm (Fig. 3).
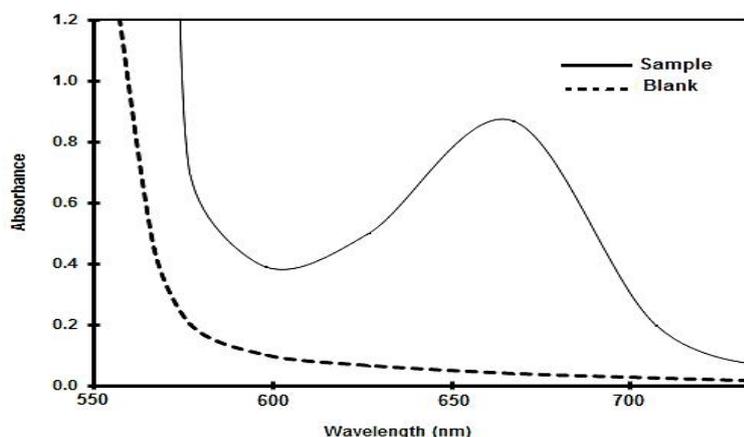
Fig. 3: Absorption spectra of 50 µg/mL of MES reacted with CA/$NaIO_4$/NaOH assessed against the blank and the blank against distilled water

MES contains a primary amino group and phenolic ring, and the existence of these groups accelerated oxidation of this compound under oxidation conditions. The phenolic reagent (CA), which is easily converted to a more reactive form (phenoxide) in an alkaline medium, is then reacted with oxidized MES through the amino group. The stoichiometry of the MES: CA dye was considered via Job's method with equimolar concentrations (5×10^{-3} M) of both drug and reagent. A mole ratio of 1:1 MES: CA was founded. As a result, the probable reaction mechanism maybe takes place as shown in Scheme 1.
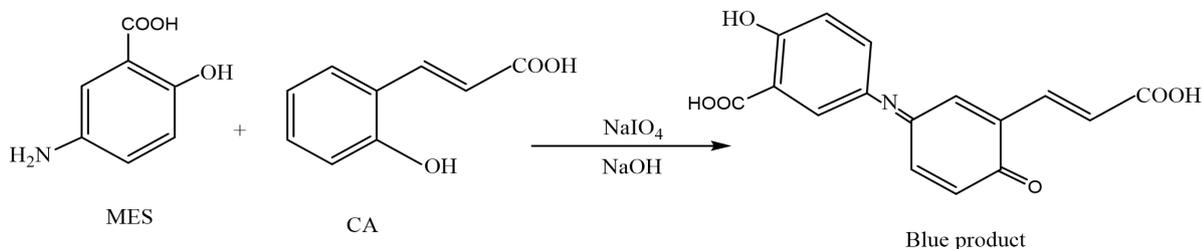
Scheme 1: Suggested mechanism for the reaction.

The physical and chemical factors that could affect the sensitivity and reproducibility of the reaction, and subsequently the assay of MES drug were studied. The optimization of variables was carried out using the univariate method, in which a variable is changed while keeping the others at constant values.

Selected FI manifold design

The automated reaction consists mainly of reagent, oxidant, the medium of reaction, and analyte. As a result, several three-channels manifolds design were studied to perform different reaction paths. According to the results in Table 1, the manifold No.1 was chosen, which offered a maximum absorbance intensity and high precision. Also, the manifold arrangement was agreeing with the proposed mechanism of the reaction involved oxidation of the drug then coupling with the reagent in an alkaline medium. A 100 µL of MES has been injected into the sodium periodate stream and then met the result of combined solutions (NaOH and CA) by "Y" connecting tube which finally mixing in a reaction coil.

Table 1: Effect of manifold design.

No.	Manifold design	Peak height
1	$NaIO_4$+(Inj. MES)+Y(CA+ NaOH)	0.201
2	CA+(Inj. MES)+Y($NaIO_4$+NaOH)	0.142
3	Y($NaIO_4$+NaOH)+ (Inj. MES)+CA	0.114
4	NaOH+(Inj. MES)+Y(CA+ $NaIO_4$)	0.037

Effect of flow rate and reaction coil length

Influence of the total flow rate on analytical signal and sample frequency was examined using rates between 1.47 to 3.56 mL/min. High but broad peaks were obtained at low flow rates up to 2.27 mL/min. Increase the flow rate more than 2.27 mL/min led to a decreased in peaks height but increase sampling frequency, this due to dilution and dispersion and decreased the residence time. In FIA systems, the reaction coil is usually used for mixing the reactants and increases the residence time and as a result enhance the sensitivity. The influence of coil length on absorbance was studied in the range of 0-75 cm. A coil length of 25 cm gave a maximum absorbance (peak height), this may be belonging to a rapid coupling reaction between MES and CA. As a result, this length was selected as optimal and used in further investigations (Fig. 4b).
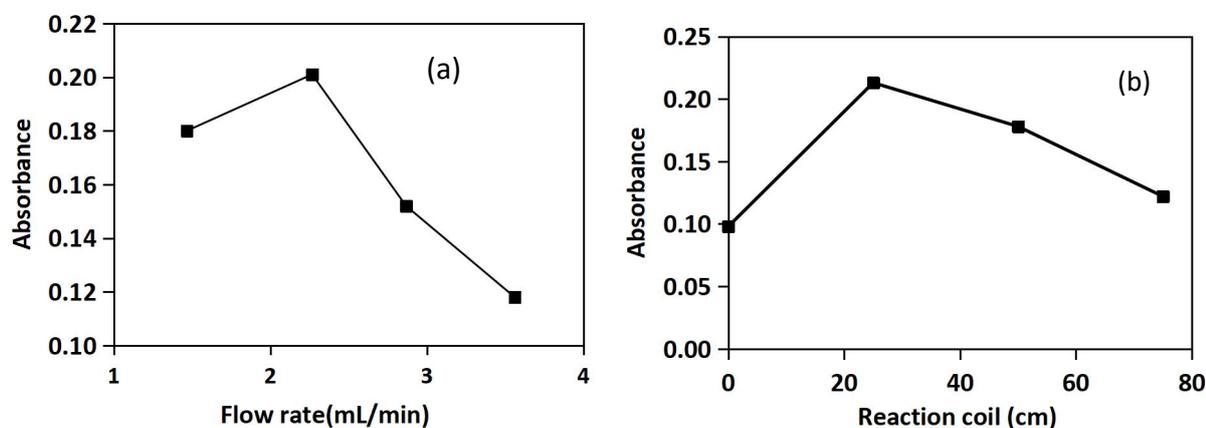
Fig. 4: Influence of (a) total flow rate and (b) reaction coil length.

Effect of injected sample volume

The influence of injected sample volume was investigated by injection different volumes (75, 100 and 150 μL) of 50 μg/mL of MES while the other conditions were constant (25 cm coil length, 2.27 mL/min flow rate, 0.2 M NaOH, 0.01 M CA, 0.1 NaIO₄). The results in Figure 5 showed that 100 μL gave maximum peak height with good repeatability and so used throughout.
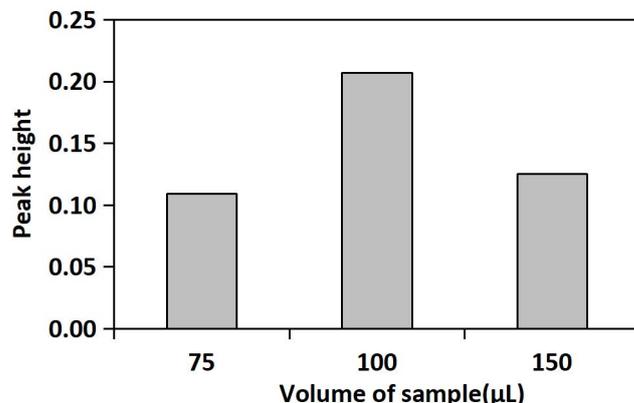
Fig. 5: Study the volume of sample

3 Effects of CA concentration, base species and its concentration

The effect of varying CA concentration on the reaction progress was considered in the range 0.005 to 0.015 M. The results (Fig. 6a) indicated that the peak height was progressively increased with increased CA concentration up to 0.01 M and then decreased slightly. Thus, the concentration of 0.01 M CA was selected to obtain maximum sensitivity. The previous investigations indicated that the development of the coupling reaction needs to confirm in an alkaline medium especially for conversion of the hydroxyl group of CA to a more reactive phenoxide group. Different bases solutions were examined such as ammonium hydroxide, sodium carbonate, and sodium hydroxide, but only the later gave a good response. The effect of NaOH concentration (studied range 0.15-0.35 M) on the analytical signal is shown in Figure 4b. The maximum response was obtained using 0.3 M sodium hydroxide (Fig. 6b) which was considered as optimum concentration. The optimum concentration of sodium periodate was also investigated using all the previous optimum conditions. Absorbance was increased up to 0.1 M concentration of sodium periodate then decreased (Fig. 6c), hence, 0.1 M concentration was chosen in further experiments. All the previous optimum variables are documented in Table 2.
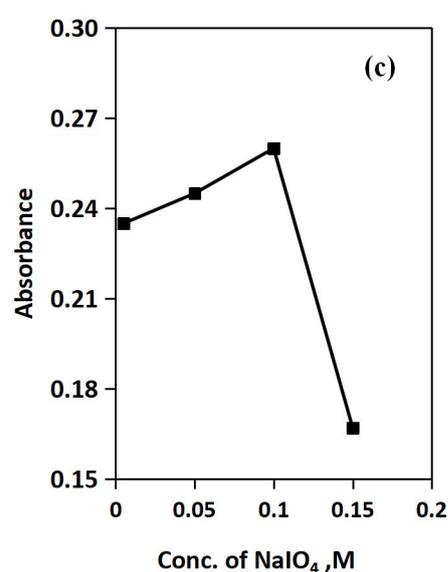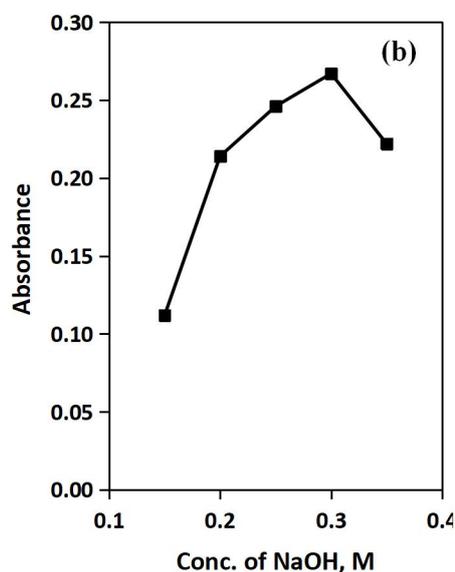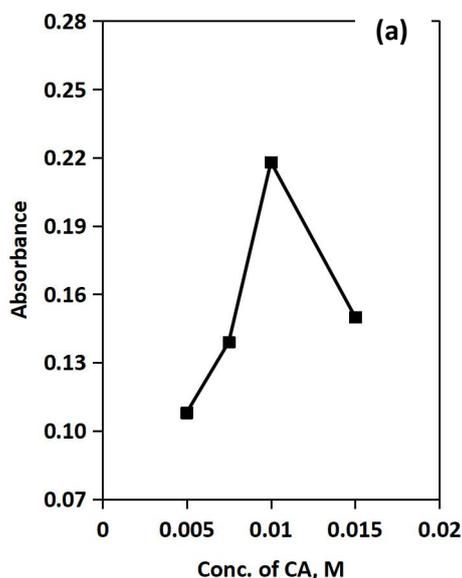
Variable	Studied range	Selected value
Chemical variables		
Concentration of CA (M)	0.005 - 0.015	0.01
Concentration of NaIO₄ (M)	0.005 - 0.15	0.1
Type of base	NaOH, Na₂CO₃, NH₄OH	NaOH
Concentration of NaOH (M)	0.15-0.35	0.3
Physical variables		
Total flow rate (mL/min)	1.47- 3.56	2.27

| Reaction coil (cm) | 0-75 | 25 |
| Sample volume (µL) | 75-150 | 100 |

The figure of merits for the suggested method was summarized in Table 3. The sampling frequency was 42 sample/h.

Table 3: Analytical characteristic of the suggested method

Parameter	value
Regression equation	A= 0.0032[MES] + 0.1117
Linear range (µg/mL)	5-150
Correlation coefficient, r	0.9996
Molar absorptivity, ε (L/mol cm)	0.49×10^3
Sandell's sensitivity, S (µg/cm^2)	0.31
Limit of detection (s/n=3) (µg/mL)	1.48
Limit of quantification (µg/mL)	4.94
Reproducibility, %	1.8
Through-put (hr^{-1})	42
Average of recovery,%	98.72
Slope, b (mL/µg)	0.0032
Intercept, a	0.1117
$S_{y/x}$	5.50×10^{-3}
S_b	4.05×10^{-5}
S_a	2.92×10^{-3}

Accuracy and precision

The accuracy and intra/inter-day precision of the proposed method has been expressed in terms of percentage recovery and relative standard deviation (RSD) of the signal respectively. A 25 and 50 µg/mL of MES solutions were analyzed in five replicates on the same day (intra-day), and over six successive days (inter-day variation). The obtainable recoveries values and small RSD% showed in Table 4, proved the method's high accuracy and reproducibility.

Table 4: Accuracy and intra/inter-day precision for assay of MES

Added conc. (µg/mL)	Intra-day (n=5)				Inter-day (n=15)			
	Found conc. (µg/mL)	Relative error (%)	Recovery (%)	RSD (%)	Found conc. (µg/mL)	Relative error (%)	Recovery (%)	RSD (%)
25	24.66	-1.36	98.64	1.42	24.43	-2.28	97.72	2.02
50	49.41	-1.18	98.82	1.84	49.31	-1.38	98.62	1.98

Effect of interferences

The effect of some probable interferences (additives) that are usually combined with MES in tablets was studied. A recovery testing was performed by spiking 20 µg/mL of MES with 10-fold of each additive individually. Table 5 shows acceptable recoveries values (97.05-100.25%) were obtained indicating insignificant interfering with the suggested method.

Table 5: Study the effect of some additives

Additive (200 µg/mL)	Amount of MES, µg/ mL		(Recovery ± SD) % (n=5)
	Present	Found	
Lactose	20	19.41	97.05±0.09
Starch		20.05	100.25±0.12
Polyvinyl pyrrolidone		19.67	98.35±0.32
Mg-stearate		19.53	97.65±0.66

Analysis of Pharmaceutical forms

The applicability of the suggested FI method was examined by analyzing two types of commercial pharmaceutical MES tablets. The obtainable results referred to good agreement between the added and found concentrations with small values of percentage error. The recoveries for the FI method were compared to those obtained by employing the standard BP method [21]. A statistical comparison between the suggested and reference methods was carried out using F and t-tests [22], and the calculated values were less than the theoretical ones indicated no major difference in accuracy and precision between the two approaches (Table 6).

Table 6: Assay of MES in tablets using FIA and reference methods

Dosage form	Proposed method				Standard method[21]			
	Taken conc. (μg/mL)	Found conc. (μg/mL)	Rec. (%)a	RSD (%)a	Taken conc. (μg/mL)	Found conc. (μg/mL)	Rec. (%)b	RSD (%)b
Pentasa® tablets	25	24.69	98.76	2.23	5	4.88	97.60	1.34
	50	50.03	100.06	1.76	15	14.98	99.87	0.98
	100	99.72	99.72	1.97	20	20.13	100.65	0.71
Awasalazin-SR® tablets	25	24.66	98.64	1.56	5	4.91	98.20	1.77
	50	49.23	98.46	0.96	15	14.71	98.07	0.56
	100	98.21	98.21	0.77	20	19.67	98.35	0.23
t (2.776)c	0.679							
F (19.000)c	1.744							

CONCLUSION

The present method offers an obvious advantage of fast estimation of MES in dosage forms with consumed small amounts of sample (< 100 microliters), lower production of waste and high sample throughput of 42 h^{-1}. The method was found to be sensitive, inexpensive, and free of interferences. The method did not require any chemical pretreatment, temperature, or pH control and extraction step. The satisfactory results indicated the possibility of using the present FI method in routine analysis of MES.

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