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New FI-Spectrophotometric Methods for Determination of Olsalazine in Pure and Pharmaceutical Preparations *via* Complexation with Quinalizarin Reagent

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A new, rapid and sensitive flow injection analysis (FIA)-spectrophotometric methods for the determination of trace amounts of olsalazine in aqueous solution and in pharmaceutical preparations are described. The methods are based on the charge transfer reaction between olsalazine and quinalizarin in methanol to form an intense reddish orange, methanol-soluble product that is stable and has a maximum absorption at 570 nm. Beer's law was obeyed over the concentration range of 0.5-45 and 10-150 μg mL^{-1} with the detection limits of 0.125 and 2.480 μg mL^{-1} for batch and flow injection methods, respectively. The optimum conditions (chemical and physical) experimental parameters affecting on the sensitivity and stability of the coloured product are carefully investigated. The optimized flow injection analysis system is able to determine olsalazine through put 52 h^{-1}. Common exicpients used as additives in drugs formulations do not interfered in the proposed methods. The methods were applied successfully to the determination of olsalazine in dosage forms. The results were compared statistically with the British pharmacopoeia method.

Keywords: Olsalazine, Flow injection-spectrophotometric method, Quinalizarin, Charge-transfer complex.

INTRODUCTION

Olsalazine ($C_{14}H_{10}N_2O_6$, m.w. = 302.239, Fig. 1), chemically is 5,5'-azobis(salicylic acid) or 3,3'-azobis(6-hydroxybenzoic acid), a yellow crystalline powder which melts with decomposition at 240 °C. Olsalazine has acceptable stability under acidic or basic conditions[1].

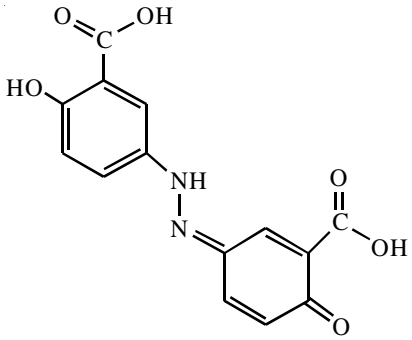


Fig. 1. Chemical structure of olsalazine

Olsalazine is an anti-inflammmatory activity drug used in the treatment of inflammatory bowel disease such as ulcerative colitis. Olsalazine is a derivative of salicylic acid[2], inactive by itself (it's a prodrug). It is converted by the bacteria in the colon to mesalamine. Mesalamine works as an anti-inflammatory agent in treating inflammatory diseases of the intestines[3].

Olsalazine was given to eight health volunteers as a 10 mg i.v. bolus dose and as a 1 g oral dose with and without food. Food intake did not influence systemic a vailability of olsalazine[2,4]. It does not cure ulcerative colitis, but it may decrease symptoms such as stomach pain, diarrhea and reacted bleeding caused by irritating smell of the colon rectum. Olsalazine is used to increase the amount of time between attacks[5]. In this perspective, the wide applications of olsalazine in both clinical and experimental medicine have prompted extensive interest in its determination. The literature reported several analytical methods for the determination of olsalazine in pharmaceutical preparations they include; HPLC with electro-chemical[6], differential pulse and square wave voltammetry using glassy carbon disc electrode in different buffer systems[7], spectrophotometric[8,9], capillary electrophoresis[10], British pharmacopeia[11].

A literature survey has not revealed any flow-injection spectrophotometric methods for determination of the drug in pure or pharmaceutical formulation. The formation of charge transfer complex can be rapidly assessed for its validity as a simple quantitative analytical method for many drug substances which can wet as an electron donor. Quinalizarin (π-acceptor)[12] has been investigated spectrophotometrically and have been

successfully utilized in the determination of variety of electron-donating basic compounds.

The aim of this work was to develope a simple and efficient FI-spectrophotometric method for determination of olsalazine in pharmaceutical formulations, through its charge transfer reaction with quinalizarin.

EXPERIMENTAL

A UV-visible double beam spectrophotometer (9200, Biotech Engineering management Co. Ltd. (UK) was used as detector, supplied with a flow cell of a 100 µL internal volume and 10 mm optical path length. The instrument was set at 570 nm for all absorbance measurements. Two channel flow injection analysis manifold and peristaltic pump (A Reglo analytic MS-216, type 160, Germany) was employed to transport the carrier and quinalizarin solutions with flexible PVC tubes (1.5 mm i.d.) was used to propel all solutions and sample loop was made of Teflon (0.5 mm i.d.). Injection valve (a Rheodyne 5041 six-3 ways) was employed to inject the drug sample into the system. Reaction coil (RC) was made of glass with internal diameter of 1 mm. The manifold was built up with plastic connections.

A two channels manifold (Fig. 2), channel (A) was employed to transport quinalizarin reagent and channel (B) to transport methanol solution. The sample was injected into the stream of methanol solution through the injection valve and then mixed with the stream of quinalizarine solution at the same flow rate of 2.0 mL min^{-1} for propel solutions using peristaltic pump and the response was recorded using recorder (Semins, Germany) either absorbance or peak height (cm) measured at λ_{max} = 570 nm.

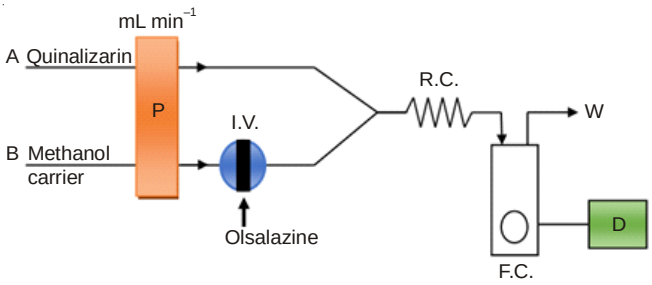


Fig. 2. Flow injection analysis manifold for the spectrophotometric determination of olsalazine by charge transfer reaction with quinalzarine in methanol. Where R.C.; reaction coil, F.C.; Flow cell, I.V.; Injection valve, P; Peristaltic pump, D; Deterctor, w; waste.

All solvents (acetone, methanol, acetonitrile, dimethyl sulfoxide and ethanol) and chemicals were of analytical grade and supplied by Pride Solvents & Chemical Co., New York, USA. Quinalizarin (1,2,5,8-tetrahydroxyanthraquinone $C_{14}H_8O_6$, m.w. 274 g mol^{-1}) solution 500 µg mL^{-1} was prepared by dissolving 25 mg of the reagent, procured by Tedia (Columbus Chemical Industries, Inc.) in 25 mL of DMSO and transferred to a 50 mL calibration flask and make the volume up to the mark with DMSO. This solution was stable for 1 week only.

Standard solution of olsalazine: Stock solution of olsalazine (500 µg mL^{-1} = 1.65 × 10^{-3} M) was provided from SDI Co., Iraq. This solution was prepared by dissolving 0.0500 g of olsalazine in 10 methanol, transferred to a 100 mL volumetric flask and diluted to the mark with the same solvent.

Analysis of commercial dosage forms: Olsalazine preparations were obtained from commercial sources.

• Dipentum tablets or capsule (UCB Pharma, Belgium), (colour; Beige); the active ingredient is (olsalazine sodium), 250 mg capsules and tables 500 mg.

• Olsalazinum tablets (SDI, Iraq) 0.5 g olsalazine tablet was purchased from local pharmacy market.

• Olsalazina oral syrup (SDI, Iraq); 0.5 olsalazine / 100 mL; 20 mL was taken from container 500 mg of olsalazine in 100 mL, dissolved with 5 mL methanol, transferred into 100 mL volumetric flask and diluted up to the mark with the same solvent to obtain 100 µg mL^{-1}.

Tablets solutions (500 µg mL^{-1} = 1.44 × 10^{-3} M): Thirteen tablets or capsules were selected randomly from different packets or strips for 250, 500 mg drug dose. The tablets were weighted, crushed and grinded to fine dust then followed accurately by weighing an amount equivalent to 0.005 and 0.02 active ingredient (50, 100 µg mL^{-1}) for 0.25 g and 0.5 g drug dose. The powder was then dissolved in 10 mL methanol (16 % v/v), filtered to remove any undissolved residue that affecting on the signal. The filtrate was completed to 100 mL with the same solvent to the mark. Further appropriate solutions of pharmaceutical for batch and flow injection analysis procedures were made by using distilled water.

General procedure for calibration

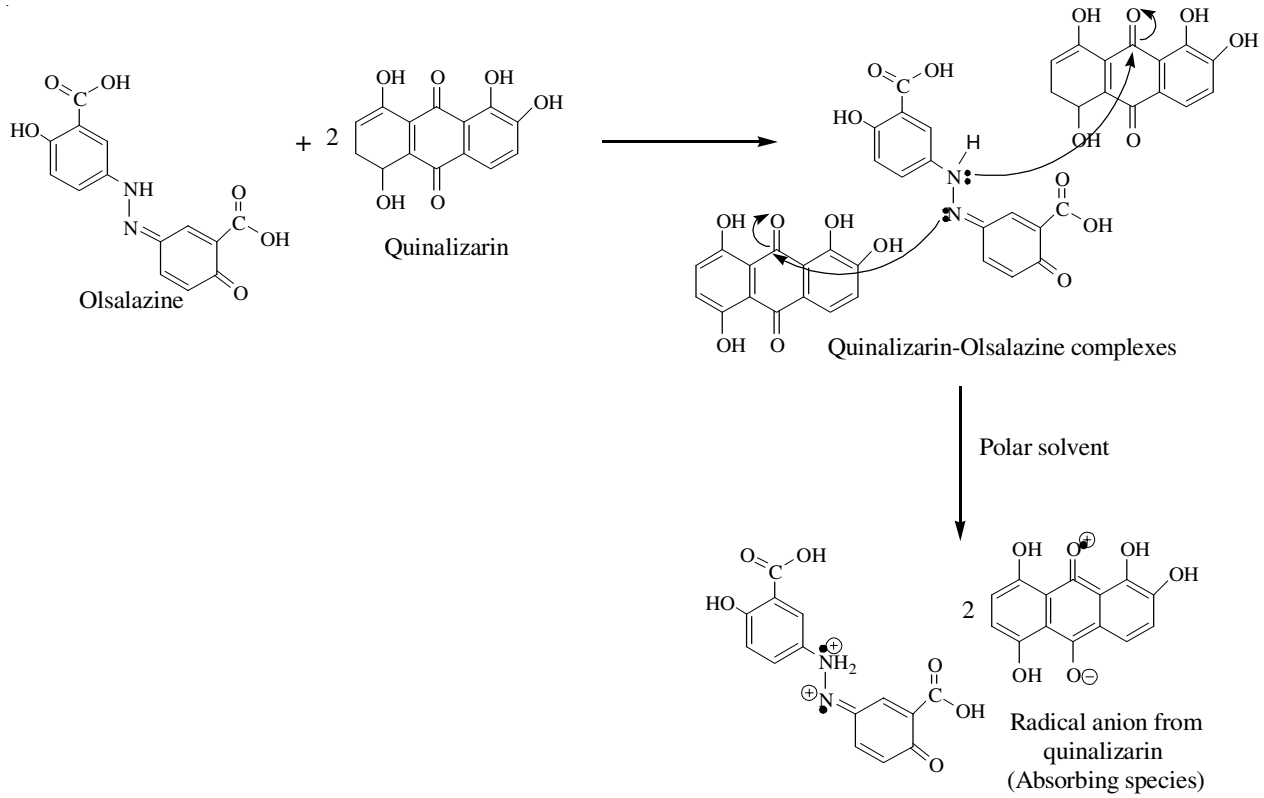
Batch procedure: A 1.5 mL of 1.84 × 10^{-4} M quinalizarin was transferred into a series of 25 mL volumetric flasks containing an aliquot volumes of 1 mL standard solution [500 µg mL^{-1} (1.65 × 10^{-3} M)] of olsalazine, then 2 mL of 0.5 M (16 % conc.) methanol was transferred into the series of 25 mL volumetric flasks, then were diluted to the mark with methanol. After 15 min, the absorbance of the coloured product was measured at 570 nm against the corresponding reagent blank.

Flow injection analysis procedure: Mixtures of olsalazine and quinalizarin in the range of 10-150 µg mL^{-1} were prepared from the working solution of 500 µg mL^{-1} (1.65 × 10^{-3} M). A 100 mL sample volume of olsalazine was injected into the carrier of the methanol (0.5 M, 16 % (v/v)), charge transfer complexes were formed at T-link with a flow rate of 2 mL min^{-1} of each channel. The resulting absorbance as peak height of the reddish orange product was measured at 570 nm and a calibration graph was constructed. Optimization of conditions were performed on 500 µg mL^{-1} of olsalazine.

RESULTS AND DISCUSSION

Charge transfer reactions have been widely employed in the development of spectrophotometric analytical methodologies applies to several drugs[13,14], however no work has been reported in the literature regarding the determination of olsalazine using flow injection analysis.

Reaction mechanism: Olsalazine reacts with quinalizarin through charge-transfer complex (n→π^* transition) to form a reddish-orange coloured product which has a maximum absorption at wavelength of 570 nm and with a molar absorption coefficient of 1.32 × 10^4 L mol^{-1} cm^{-1} (Fig. 3). The suggested reaction mechanism is given in **Scheme-I**.



Scheme-I: Proposed reaction mechanism

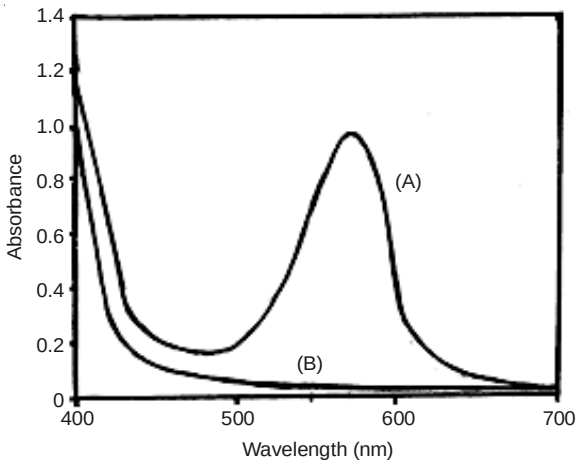


Fig. 3. Absorption spectra of the charge transfer complex (20 μg mL^{-1}) of olsalazine against reagent blank (A) and blank against methanol (B)

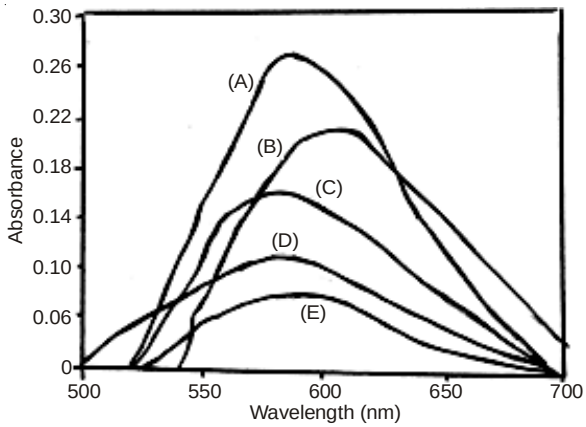


Fig. 4. Absorption spectra of charge-transfer complex at different solvents (A) methanol, (B) DMSO, (C) acetonitrile, (D) acetone, (E) ethanol

Batch spectrophotometric determination

Effect of solvent nature: The influence of solvents plays an important role in some charge transfer reactions, as they able to facilitate the total charge transfer and allow the complex disintegration and stabilization of the radical anion formed, which is the absorbing species. According to the literature, solvents with high dielectric constant are more active[15]. Because of insolubility of quinalizarin in water, the reaction was performed in ethanol, acetonitrile, acetone, DMSO and methanol. The best sensitivity was achieved with methanol (Fig. 4), and perhaps because of the capacity of this solvent to form stable hydrogen bonds with the radical anion. Maximum absorbance of the solutions was observed at 570 nm in methanol with highest sensitivity.

Effect of order of addition: Drug-reagent-solvent was selected as the favourable sequence of addition for the complete colour development and highest absorbance at the recommended wavelength and used in all subsequent experiments.

Effect of time and temperature: Experiment results revealed that the colour intensity reached maximum after the addition of the reagent (quinalizarin) to drug (olsalazine) in an methanol for 10-15 min. Therefore, 15 min development time was suggested at the optimum reaction time and remains stable for 120 min. The effect of temperature on the colour intensity of the product was also studied. In practice, high absorbance was obtained when the colour was developed at room temperature (25 °C).

Stoichiometry of the reaction: The stoichiometry of the reaction between olsalazine and quinalizarin was investigated

using the continuous variation Job's method[16]. As shown in Fig. 5, the product gave maximum absorbance was 0.32, indicating that it reacts with quinalizarin in a proportion of 1:2 and confirming the assumption raised before when the effect of quinalizarin concentration was studied. In view of this result, a reaction mechanism was proposed considering the transfer of two free electrons of two nitrogen atoms (one electron of each nitrogen) present in one molecule of olsalazine to the charge-deficient center of two molecules of quinalizarin (**Scheme-I**). The stability constant of the product in methanol under the described experimental conditions was 3.247×10^4 L mol^{-1}.

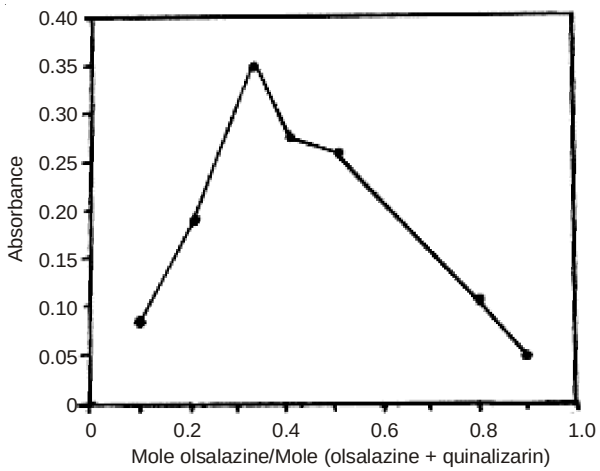


Fig. 5. Job's method for olsalazine sodium

In order to assess the possible analytical applications of the proposed method, the effects of some common excipients frequently found with olsalazine drugs in pharmaceutical forms were studied. A synthetic sample solutions containing 20 µg mL^{-1} of olsalazine and excess amounts (10-fold excess) of each excipients (200 µg mL^{-1}) was analyzed. None of these substances interferred seriously (Table-1).

TABLE-1
DETERMINATION OF 20 µg mL^{-1} OF OLSALAZINE IN THE PRESENCE OF SOME COMMON EXCIPIENTS

Excipients	Olsalazine ($\bar{x}$)	$E_{rel.}$ (%)	Rec. (%)
Polyvinyl pirrolidome	20.35	1.75	101.75
Talc	20.186	0.93	100.93
Starch	20.08	0.40	100.40
Mg-Stearate	19.87	-0.65	99.35
Lactose	20.175	0.875	100.88

Optimization chemical variables

Effect of quinalizarin concentration: The reagent concentration in solution is an important parameter to be investigated, since the maximum conversion of the analyte into absorbing species rely on the amount of the reagent available in the solution for reaction. To achieve this objective, an experiment conducted by varying the quinalizarin concentrations in the range (3.7×10^{-5} - 5.5×10^{-4} M) while the quinalizarin concentration was maintained constant at 10 µg mL^{-1}. As can be seen from Table-2, a remarkable increase of the absorbance at 570 nm was observed up to 1.84×10^{-4} M quinalizarin concentration and after this point, the absorbance was decreased express as peak height. This probably occurred because at 1.84×10^{-4} M, there was already sufficient excess of the reagent to consume all olsalazine present in the medium. So, quinalizarin concentration at 1.84×10^{-4} M was established for the method to obtain a satisfactory sensitivity.

TABLE-2
EFFECT OF QUINALIZARIN CONCENTRATION

Concentration of quinalizarin (M)	Peak height (cm)
3.70×10^{-5}	4.4
7.40×10^{-5}	5.2
1.84×10^{-4}	6.4
2.90×10^{-4}	4.3
3.60×10^{-4}	3.4
5.50×10^{-4}	3.7

Effect of methanol concentration: It was found that methanol media gave a best sensitivity for the reaction between drug and reagent, the effect of various percentage of methanol (m.w. = 32.04, BDH, sp (%) = 1.328, purity 95 %) were studied. The results showed that 16 % of CH_3OH was efficient for accurate reproducible volumes used. The absorbance as peak height of the coloured product increased and became more stable in 16 % of methanol concentration (Table-3).

TABLE-3
EFFECT OF CH_3OH CONCENTRATION

Concentration of CH_3OH (v/v %)	Peak height (cm)
16	7.2
33	6.6
50	4.5
69	3.2
83	2.8

Optimization of physical variables

Effect of flow rate: The effect of total flow rate on the analytical response was studied over the range of 0.8-4.5 mL min^{-1}. The results obtained showed that a total flow rate of 2 mL min^{-1} gave the highest response for olsalazine as shown in Fig. 6 and was used in all subsequent experiments.

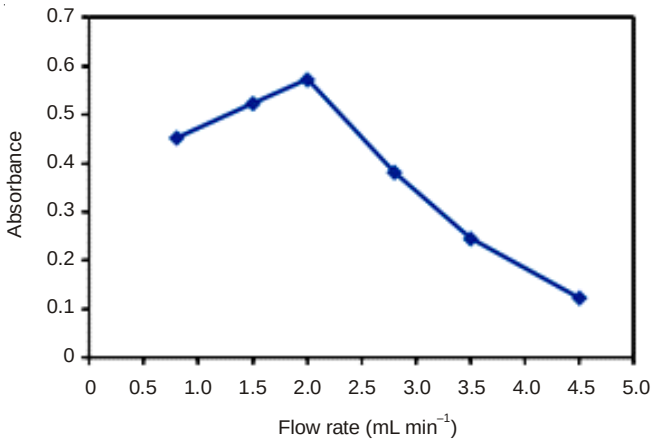


Fig. 6. Effect of total flow rate

Effect of injection sample volume: The effect of injection sample volume was investigated in the range (50-300) µL using different length of sample loop. The results obtained showed

that an injection sample of 100 µL gave the best absorbance for olsalazine drug as shown in Fig. 7 and was used in all subsequent experiments.

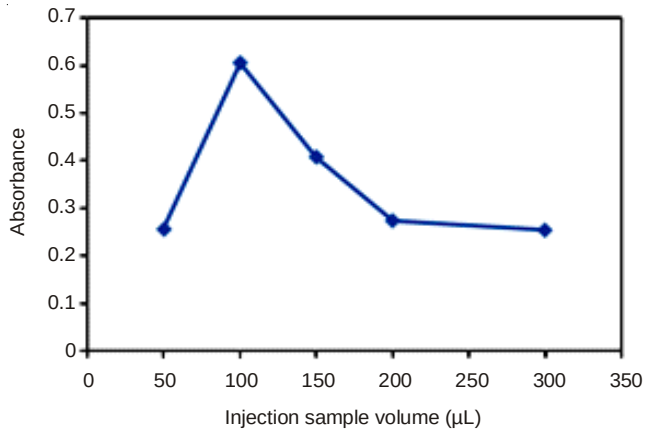


Fig. 7. Effect of injected sample volume for determination of olsalazine using FI procedure

Effect of reaction coil length: The reaction coil length can affect on the sensitivity of the colour reaction product and was investigated in the range of 50-200 cm. The results showed that a reaction coil length of 50 cm gave the highest peak height for olsalazine (Table-4, Fig. 8). By using longer reaction coil, the signal was decreased, because a large dispersion occurred and broad peak was obtained. Thus, a reaction coil of 50 cm was chosen.

TABLE-4
EFFECT OF REACTION COIL LENGTH ON THE PRODUCT

Length coil (cm)	Peak height (cm)			Abs.
50	8.2	8.2	8.2	0.645
100	8.2	8.3	8.2	0.524
150	7.2	7.4	7.4	0.225
200	6.4	6.5	6.4	0.095

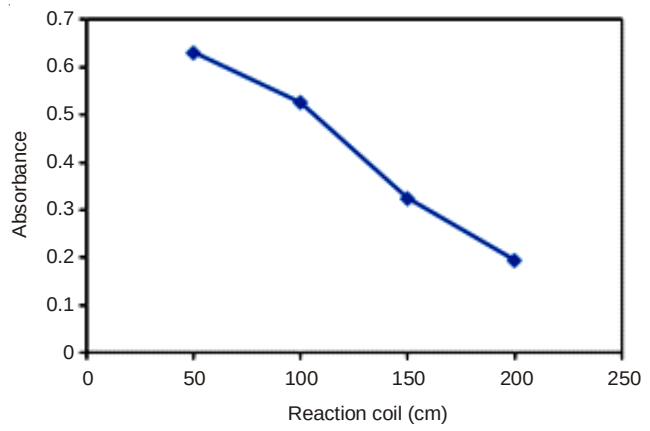


Fig. 8. Effect of reaction coil (cm) for determination of olsalazine

Calibration graph for the determination of olsalazine drug: Analytical characteristics for both batch and FI methods were obtained. The calibration graph for both procedures were determined using a series of standard solutions analyzed in triplicate. The intercept (a), slope (b), correlation coefficient (r) and correlation of determination (r^2) were evaluated by least squares regression method (Table-5). Statistical evaluation[17,18] of the regression line gave the values of standard deviations for residuals ($S_{y/x}$), intercept (S_a) and slope (S_b) at 95 % confidence limit for (n = 2). These small figures point out to refer a high precision of the developed methods.

TABLE-5
ANALYTICAL FEATURES OF THE DEVELOPED METHODS FOR OLSALAZINE

Parameter	FI method	Batch method
Slope (b) (mL μg^{-1})	0.0018	0.0169
Intercept (a)	0.0137	0.0063
Linearity range (µg mL^{-1})	10-150	0.5-45
Correlation coefficient (r)	0.9989	0.9997
r^2	0.9978	0.9994
Limit of detection (µg mL^{-1})	2.480	0.125
Limit of quantification (µg mL^{-1})	3.847	0.245
$S_{y/x}$	0.0049	0.0052
S_a	0.0038	0.0014
S_b	2.34×10^{-5}	1.53×10^{-4}
$E_{rel.}$ (%)*	1.174***	-0.135**
RSD (%)*	1.457***	1.256**
Average of recovery (%)	100.44	99.03
Sample throughput (h^{-1})	52	4

*Average of six determinations; **For 20 µg mL^{-1} of olsalazine; *** For 50 µg mL^{-1} of olsalazine

Accuracy and precision of FI and batch spectrophotometric methods: The accuracy and precision of two methods were studied by analyzing six replicate samples of olsalazine by FI and batch spectrophtometric methods. The low values of percentage errors (E %) and relative standard deviation (RSD %) are summarized in Table-5, indicated the high accuracy and precision of two methods.

Pharmaceutical applications: Flow injection analysis method offers rapid and accurate determination because of its speed by sample throughout (62 injection h^{-1}) and wider linear range of calibration graph. The proposed methods were successfully applied to the analysis of different formulations containing olsalazine and the results are summarized in Table-6.

For all formulations examined, the assay results of both methods were in good agreement with the declared content. The results obtained by two proposed methods were compared with those obtained by the official spectrophotometric method using quinalizarin reagent[11] across applying F-test and t-test at 95 % confidence limits for (n = 2). The calculated valued for F-test were 2.845 and 3.247, t-test value were 2.158 and 0.847 for the batch and flow injection analysis methods respectively, did not exceed the critical values of F-test for a one-tailed = 6.388 and t-test = 2.773 ($n_1 + n_2$-2=4). It reveals that there are no significant differences between the proposed method and British Pharmacopoeia method with respect to precision and accuracy in the determination of olsalazine in pharmaceutical preparations.

Conclusion

Two sensitive and accurate, simple batch and FI spectrophotometric methods have been developed for the determination of olsalazine in pharmaceutical preparations. The developed procedures based on the charge transfer complex reaction between drug with quinalizarine in the presence of

TABLE-6
PHARMACEUTICAL APPLICATIONS FOR OLSALAZINE USING THE PROPOSED METHODS

Method	Dosage forms	[Olsalazine] ($\mu g\ mL^{-1}$) presence	Found ($\bar{x}$)	$E_{re.}$ (%)	*Rec. (%)`	*RSD (%)	British Pharmacopoeia
Flow injection analysis	Dipentum capsules (olsalazine sodium)	25	24.857	-0.572	99.43	1.253	100.27
		100	102.824	2.824	102.83	2.948	
	Olsalazina oral syrup	25	23.878	-4.488	95.51	1.326	
		100	101.235	-1.235	98.765	2.547	
	Olsalazinum tablets	25	25.243	0.972	99.028	1.438	
		100	100.534	0.534	99.466	1.642	
Batch	Dipentum (capsules)	8	7.892	-1.35	98.65	1.542	101.32
		20	20.235	1.175	101.18	3.249	
		40	39.426	-1.435	98.57	1.872	
	Olsalazina oral syrup	8	8.247	3.088	103.09	3.453	
		20	19.857	-0.715	99.29	1.437	
		40	38.873	-2.818	97.18	1.223	
	Olsalazinum tablets	8	7.944	-0.700	99.30	0.858	
		20	20.088	0.443	100.44	1.459	
		40	40.345	0.863	100.86	2.523	

*Average of six determinations

methanol. Concerning the reported UV methods[8,9,19], which needed expensive materials and reagents or high temperature and the necessary that pre-treatment procedures involving extraction of the active ingredient to avoid interference from tablet additives. However, FI-spectrophotometric method is no need for solvent extraction or separation steps before the analysis. The proposed methods, don't needs neither temperature nor pH control. In addition, the wide applicability of the new method for routine quality control is well established by analyzing the assay of olsalazine at concentration of trace level (ppm) in dosage forms. There is no significant difference in accuracy and precision between the proposed method and BP method for determination of olsalazine in pharmaceutical preparations.

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