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Spectrophotometric Determination of Five Commercial Drugs in Pure Form and Pharmaceutical Formulations by Ion-Pair Complexation with Alizarin Red S

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Abstract

Simple, accurate, selective and rapid spectrophotometric methods have been developed for the determination of itopride, midodrine, diclofenac, mesalamine and sumatriptan in their pure as well as in pharmaceutical dosage forms. The method was based on ion-pair complex formation between the drugs and anionic dye, alizarin red S in an acidic medium (pH 2.0-4.0). The complexes formed were quantitatively extracted into chloroform and measured at 284, 287, 279, 285 and 280 nm wavelength for itopride, midodrine, diclofenac, mesalamine and sumatriptan respectively. Beer's law was obeyed in the concentration range of 3.5-35 µg mL^{-1} for itopride and diclofenac, 2.5-180 µg mL^{-1} for midodrine, 3.0-80 µg mL^{-1} for mesalamine and 4.0-200 µg mL^{-1} for sumatriptan. The stoichiometry of the complexes formed between the drugs and the dye was 1:1 as determined by Job's method of continuous variation. The association constant (K_{IP}) of the ion-pair complexes formed was evaluated using Benesi-Hildebrand equation. Limit of detection, limit of quantification and Sandell's sensitivity of the methods were also estimated. The proposed methods were successfully employed for the determination of these drugs in their pharmaceutical dosage forms.

Keywords:

Drugs, Alizarin Red S, Ion-pair complex, Spectrophotometry

1. Introduction

Methods based on spectrophotometry have been successfully used for the analysis of pharmaceuticals, especially for bulk drugs and in the quality control of marketed product as they are much simpler, cost-effective and less time consuming compared to other methods. These methods provide accurate and precise results and find widespread use, especially where expensive equipments like HPLC, HPTLC, GC and electrophoresis are not easily available [1-3]. Determination of drugs by spectrophotometry is accomplished either by direct measurement of UV light (zero order or derivative spectra) or through some chemical reactions like complex formation (ion-pair, charge-transfer) with specific reagents, oxidation-reduction process and catalysis [1].

Itopride (ITO) hydrochloride, a benzamide derivative is novel gastroprokinetic agent possessing acetylcholine esterase inhibitory and dopamine D2 receptor antagonist effects. It is

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useful for the symptomatic treatment of various gastrointestinal disorders, including functional dyspepsia and gastroesophageal reflux disease [4]. It acts by increasing acetyl choline concentrations which lowers the esophageal sphincter pressure, stimulates gastric motility, accelerates gastric emptying, and improves gastro-duodenal coordination [5].

Midodrine (MID) hydrochloride is a vasopressor and a prodrug which is indicated for the treatment of symptomatic orthostatic hypotension. Its active metabolite desglymidodrine is an alpha 1-adrenoceptor agonist and exerts its actions via activation of the alpha-adrenergic receptors of the arteriolar and venous vasculature, producing an increase in vascular tone and elevation of blood pressure [6, 7].

Diclofenac (DIC) sodium is a well known and widely used as an analgesic, antipyretic and anti-inflammatory agent. The primary mode of action is thought to be by prevention of prostaglandin synthesis by inhibition of cyclooxygenase. Further, it is reported to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis [8].

Mesalamine (MES) belongs to a class of drugs known as aminosalicylates. It is an anti-inflammatory drug used to treat inflammatory bowel disease, such as ulcerative colitis and as first-line therapy for mild-to-moderate Crohn's disease. It helps to reduce symptoms of ulcerative colitis such as diarrhea, rectal bleeding, and stomach pain [9, 10].

Sumatriptan (SUM) succinate belongs to a group of tryptamine-based drugs used in the acute treatment of migraine headaches and was the first antimigraine agent approved by US FDA in 1991 [11]. Triptans acts by selectively binding to serotonin type-1D receptors (serotonin agonist). They block vasoconstriction and transmission of signals to the trigeminal nucleus and thus prevent peripheral sensitization [12].

Literature reveals several analytical methods for the determination of ITO [13-20], MID [21-24], DIC [25-30], MES [31-39] and SUM [40-48] in pharmaceutical preparations and biological samples. These methods have employed different analytical techniques like spectrophotometry [13-15, 21, 22, 25, 26, 31-38, 43-48], spectrofluorometry [18], ion-selective electrodes [23, 29], ICP-AES [19], FT-Raman [28], HPTLC [17, 27, 40, 41], HPLC-UV [16, 21, 39], HPLC-fluorescence [24, 42] and LC-MS/MS [30].

The aim of the present work was to develop selective, rapid and cost-effective methods for the determination of selected drugs (ITO, MID, DIC, MES and SUM) in pure form and in tablet formulation by UV spectrophotometry. The chemical structures of the studied drugs are shown in Fig. 1. The validated methods utilize an anionic dye, Alizarin red S to form ion-pair complexes with these drugs under acidic conditions, which were extracted into chloroform. Besides the advantage of speed and simplicity, all the methods gave accurate and precise results and can be readily applied in pharmaceutical industries for industrial quality control.

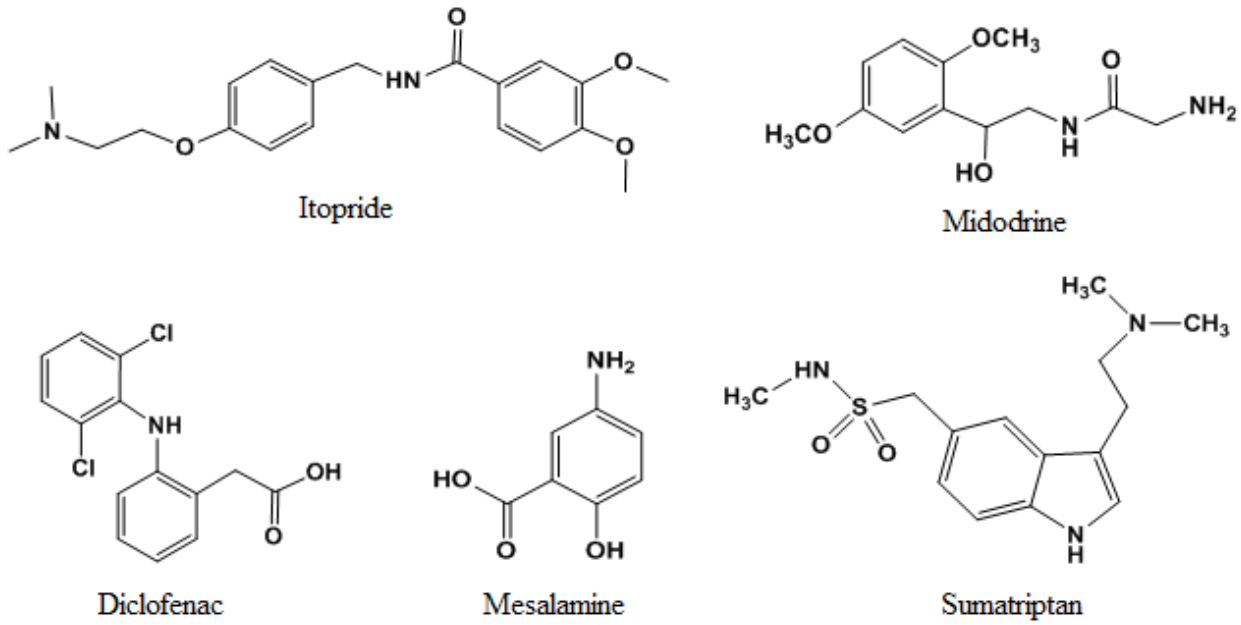

Fig. 1: Chemical structures of the drugs

2. Experimental

2.1. Equipments

A Jasco V-570 double beam UV-Visible spectrophotometer from Jasco International Co. Ltd. (Tokyo, Japan) with matched 10 mm quartz cells was used for spectral measurements. The wavelength accuracy was within ± 0.5 nm and a bandwidth of 1 nm was kept for all the methods. Data processing was done with Spectra manager software version 1.53.01. A digital LI 127 Elico pH meter from Elico Ltd. (Hyderabad, India) was employed for pH measurements. Weighing of substances was carried out on Sartorius GD503 (Bradford, MA, USA) analytical balance, having a readability of 0.0001 g. Varivol II micropipettes used for accurate and precise transfer of solutions were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) with a varying volume capacity of 0.5 to 5.0 mL.

2.2. Reagents and materials

Reference standards of itopride hydrochloride (99.33 %), midodrine hydrochloride (99.13 %), diclofenac sodium (98.69 %), mesalamine (99.60 %) and sumatriptan succinate (98.77 %), were purchased from Clearsynth Labs Pvt. Ltd. (Mumbai, India). Spectroscopic grade chloroform for extraction was obtained from Mallinckrodt Baker, S.A.de C.V. (Estado de Mexico, Mexico). Analytical grade alizarin red S (ARS) was procured from S. D. Fine Chem. (Mumbai, India) and used without further purification. Water was purified using Milli-Q water purification system from Millipore (Bangalore, India). Pharmaceutical formulations, Ganaton® (Itopride hydro chloride) 50 mg tablet, Abbott Japan Co. (Tokyo, Japan), ProAmatine® (midodrine hydro chloride)10 mg tablets, Shire US Inc. (Newport, USA), Voltaren® (diclofenac sodium) 50 mg tablets, Novartis Pharmaceuticals Australia Pty Ltd. (NSW, Australia), Walasa® (mesalamine) 400 mg tablet, Wallace Pharmaceuticals Ltd. (Goa, India) and Sumitrx® (sumatriptan succinate) 50 mg tablets, Sun Pharmaceutical Industries Ltd. (Mumbai, India) were obtained from commercial sources.

2.3. Solution preparation

Standard stock solutions of the drugs (1.0 mg mL^{-1}) were prepared in deionized water. Their working solutions in the calibration concentration range were prepared by appropriate

dilution of the stock solutions with deionized water. The stock solution of ARS solution (0.10%) was prepared in deionized water.

2.4. General extraction procedure

Into a series of glass tubes, 1.0 mL of working solutions of the drugs in the concentration range, [ITO (17.5-175 μg mL^{-1}), MID (12.5-900 μg mL^{-1}), DIC (17.5-175 μg mL^{-1}), MES (15.0-400 μg mL^{-1}), and SUM (20.0-1000 μg mL^{-1})] was transferred. The pH of the solution was set with 0.1 M HCl [pH 2.0 for ITO, MID and DIC; pH 3.0 for MES and pH 4.0 for SUM], followed by addition of 1.0 mL, 0.1 % ARS solution. The volume in each tube was adjusted to 5.0 mL with deionized water and extracted with 5.0 mL chloroform in a vortex mixer for 2.0 min. After complete extraction, the layers were allowed to separate and the chloroform layer containing the ion-pair complex of the drugs was measured against their respective reagent blank solutions. The reagent blank solution was prepared in a similar manner without the drug. All extractions were carried out at room temperature (25 ± 0.5 ºC).

2.5. Sample preparation for formulations

Twenty tablets of each drug (ITO, MID, DIC, MES and SUM) were separately weighed and grounded into fine powered. An amount equivalent to one tablet (50 mg for ITO, DIC and SUM, 10 mg for MID and 400 mg for MES) was accurately weighed and transferred to 100 mL (500 mL for MES) volumetric flasks containing 50 mL deionized water (250 mL for MES). The contents of the flasks were sonicated for 15 min to dissolve the drugs and the volume was completed with deionized water. The resulting solutions were filtered with 0.45μ membrane filter and diluted appropriately with water before measurements.

3. Results and Discussion

Several extractive spectrophotometric methods based on ion-pair complex formation are reported in literature for the determination of drugs in their pharmaceutical preparations. An ion-pair complex is formed as a result of association of two oppositely charged species at a suitable pH. By using different anionic dyes and organic solvents to extract the ion-pair complex from aqueous medium, quantitation of several pharmaceutical compounds that possess basic moieties (primary, secondary or tertiary amino group) can be done by ion-pair extractive spectrophotometry. Reported procedures have utilized different dyes like methyl orange for ITO [15], ninhydrin for MID [22], bromocresol green and bromocresol purple for MES [31], and naphthalene blue 12 BR and methylene blue for SUM [45]. As shown in Fig. 1, the selected drugs have primary, secondary or tertiary amino groups which can be readily protonated under acidic conditions. Ion-pairs formed with the anionic dye, alizarin red S (ARS) can be readily used for the determination of these drugs in bulk and pharmaceutical dosage forms. In the present work, spectrophotometric methods have been developed based on the extraction of ion-pairs of ITO, MID, DIC, MES and SUM with ARS into chloroform from acidic solutions. Further, conditions like wavelength for measurement, pH, organic diluents, ARS concentration, and shaking time were optimized for quantitative formation of ion-pair complexes with maximum stability. The absorption spectra of the drugs and ARS are shown in Fig. 2. The absorption maxima for ITO, MID, DIC, MES, SUM and ARS were observed at 257, 291, 276, 301, 283 and 423 nm respectively.

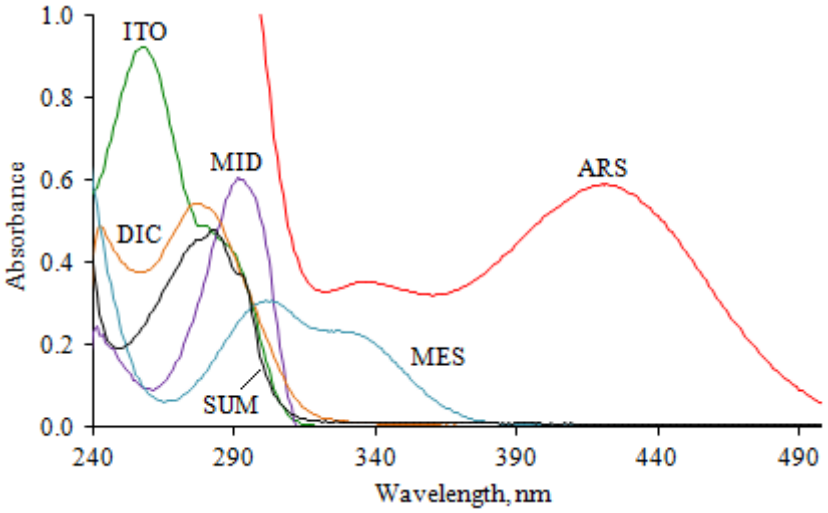

Fig. 2: Representative absorption spectra of studied drugs [ITO (20 µg mL^{-1}), MID (120 µg mL^{-1}), DIC (20 µg mL^{-1}), MES (30 µg mL^{-1}), and SUM (100 µg mL^{-1})] and ARS (0.05 %) in aqueous medium

Their ion-pair complexes with ARS in chloroform are shown in Fig. 3a-e. The selected wavelengths for measurements were 284, 287, 279, 285 and 280 nm for ITO, MID, DIC, MES and SUM respectively. The response obtained at these wavelengths was reproducible and linear.

3.1. Optimization of extraction conditions

3.1.1. Selection of extraction solvent

The effect of nature of extraction solvent on the ion-pair complexes was evaluated using different organic diluents. Chloroform, carbon tetrachloride, dichloromethane and ethyl acetate were studied for the extraction of complexes. It was found that chloroform provided better efficiency as well as selective extraction compared to other solvents and hence was selected in the present work. Moreover, one extraction was adequate to achieve quantitative recovery for all the drugs.

3.1.2. Effect of pH

The effect of pH on the formation of ion-pairs was examined by varying the pH from 2.0-6.0 using 0.1 M HCl. Above pH 6.0 there was substantial decrease in the absorbance and the results were not reproducible. Under the optimized conditions, maximum absorbance of the complexes was found at pH 2.0 for ITO, MID and DIC respectively; pH 3.0 for MES and pH 4.0 for SUM.

3.1.3. Effect of ARS concentration

The effect of ARS concentration on the colour intensity of the complex at the selected wavelength was tested by varying the concentration from 0.01 to 0.20 % at selected wavelengths. Best results were obtained with 0.1% ARS for all the complexes. However, excess dye did not affect the color of the complex as well the absorbance.

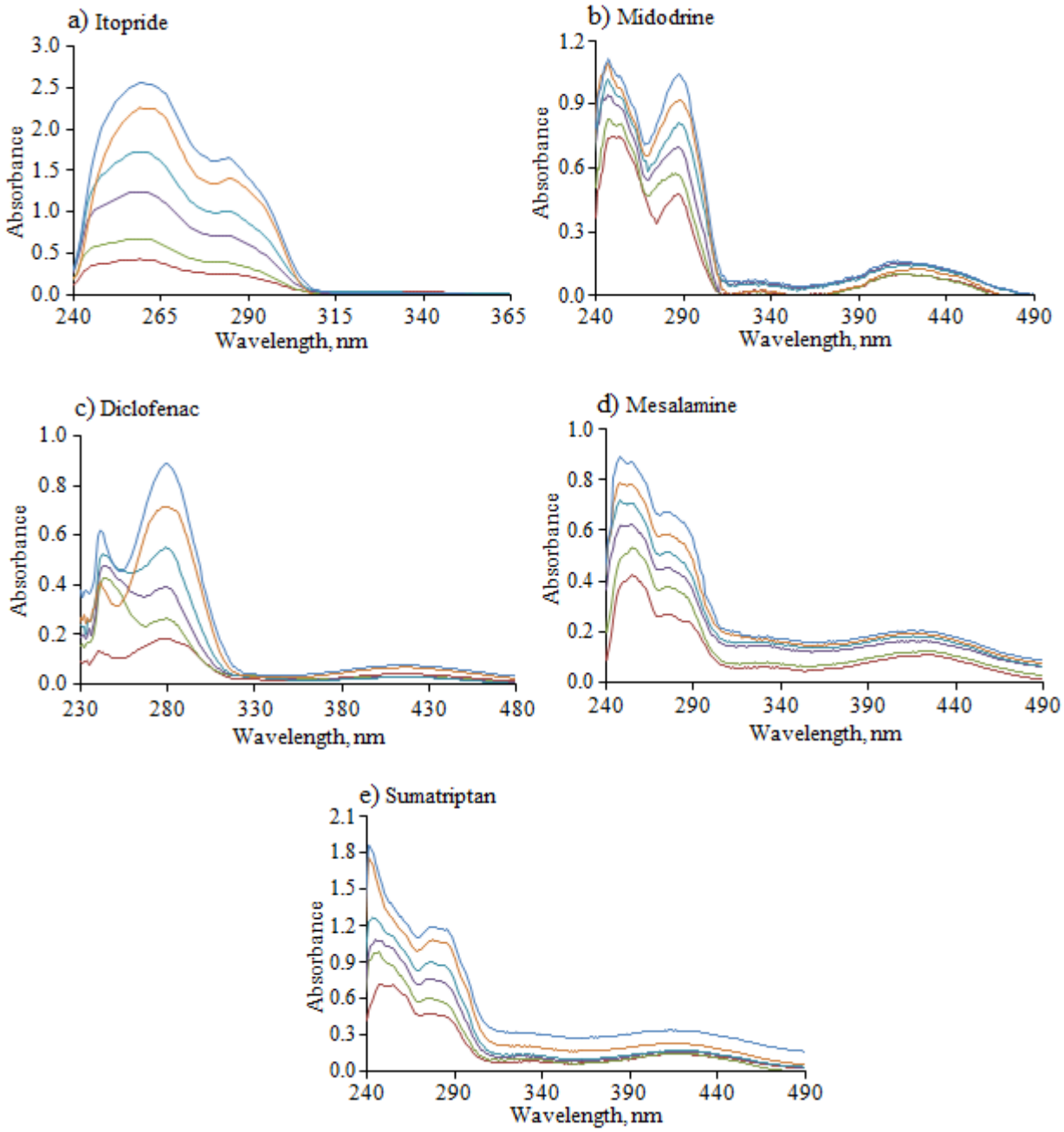

Fig. 3: Ion-pair complex formation of ARS with (a) ITO (3.5-35 µg mL^{-1}), (b) MID (2.5-180 µg mL^{-1}), (c) DIC (3.5-35 µg mL^{-1}), (d) MES (3.0-80 µg mL^{-1}), and (e) SUM (4.0-200 µg mL^{-1})] in chloroform

The suggested mechanism of drugs-ARS ion-pair complex formation is depicted in Fig. 4.

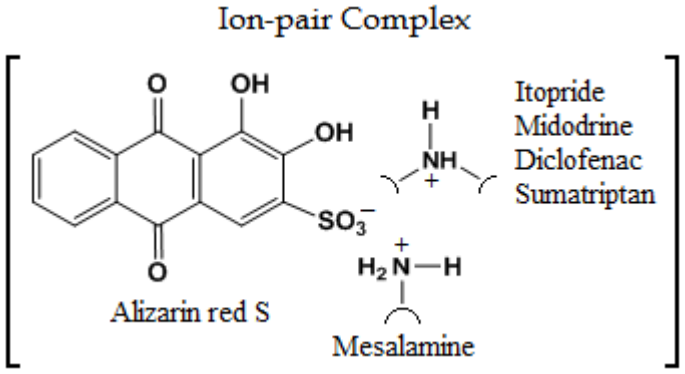

Fig. 4: Plausible mechanism of ion-pair complex formation between the drugs and the anionic dye, alizarin red S

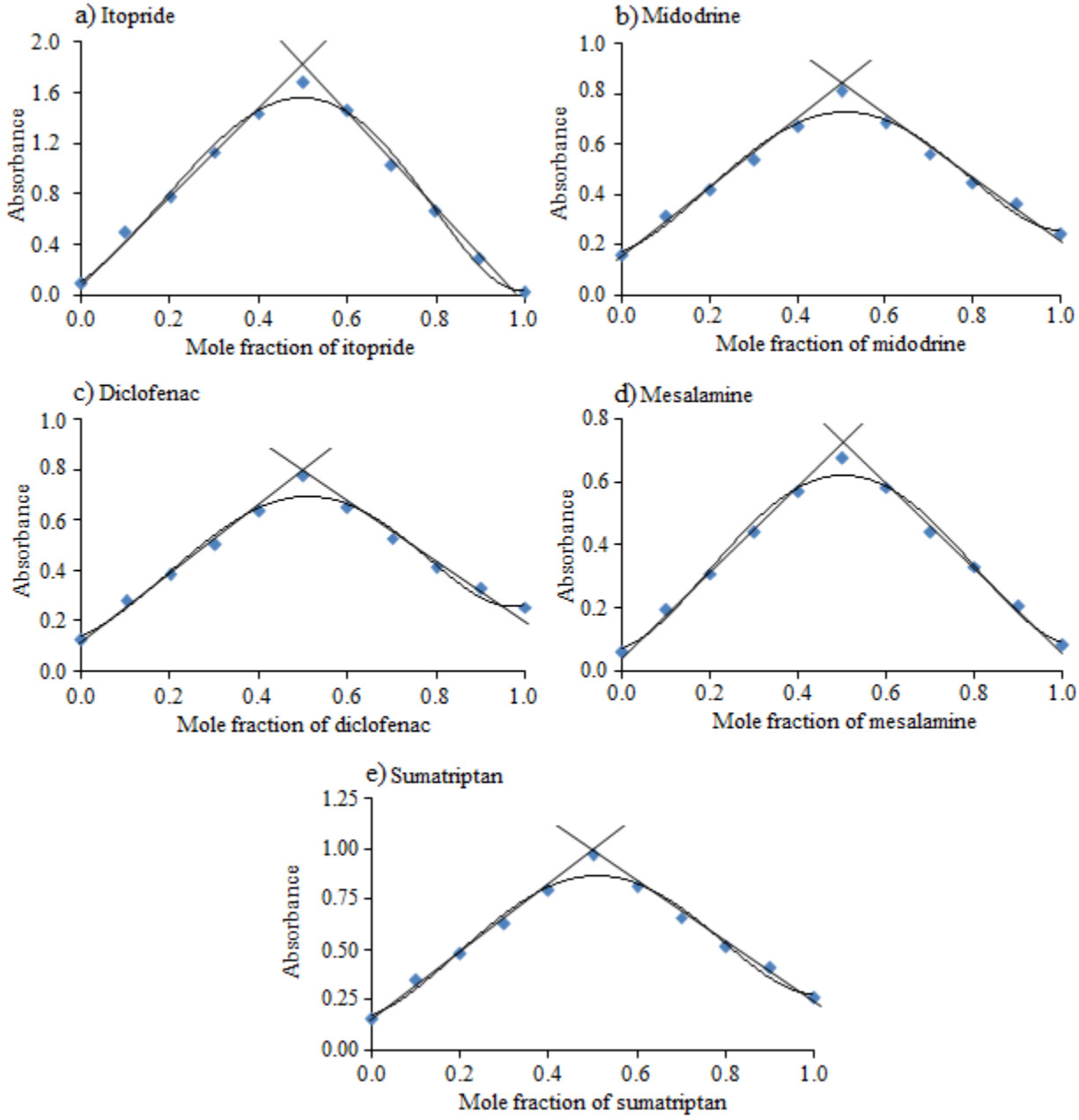

Fig. 5: Job's plot for ion-pair complexes of ARS with (a) ITO, (b) MID, (c) DIC (d) MES and (e) SUM in chloroform (2.0×10^{-3} M)

3.1.4. Effect of shaking mode and time

The impact of different approaches for extracting the ion-pair complexes was assessed by using a magnetic stirrer, a vortex mixture and a separating funnel. Although quantitative results were obtained in all three modes of extraction, slightly higher absorbance values were found during vortex mixing. Further, shaking times ranging from 1.0 to 5.0 min was studied and the optimum time for maximum extraction was 2.0 min for all ion-pair complexes.

3.1.5. Effect of temperature and stability of the ion-pair complexes

The effect of temperature on ion-pair complexes was studied at 25, 30 and 35 ºC. It was observed that the complexes were stable up to 30 ºC with negligible change in the absorbance values. Above this temperature there was a slight increase (~ 2-3 %) in the absorbance of the complexes. This is due to the volatile nature of the solvent. Nevertheless, best results were found at 25 ºC and all the complexes were stable for a minimum period of 24 h.

3.1.6. Stoichiometry of ion-pair complexes

In order to determine the composition of the ion-pair complexes, Job's method of continuous variation was employed [49]. For this experiment, solutions of identical concentrations (2.0×10^{-3} M) of the drugs and ARS were prepared and mixed in varying volume ratios such that the total volume of each mixture was constant. The absorbance of the complexes formed was measured at 284, 287, 279, 285 and 280 nm for ITO, MID, DIC, MES and SUM respectively. The values of absorbance were plotted against the mole fraction of the drugs. The stoichiometry observed for each drug-dye ion-pair complex was 1:1 as evident from the results in Fig. 5.

3.1.7. Association constants of ion-pair complexes

The association constants for the ion-pair complexes formed between the drugs and the dye were evaluated using the Benesi-Hildebrand equation [50] as shown below,

$$\frac{[\mathrm{ARS}]}{\mathrm{A_C}} = \frac{1}{\varepsilon_C} + \frac{1}{K_{IP}\varepsilon_C} \times \frac{1}{[\mathrm{Drug}]}$$

where, [ARS] and [Drug] are the total concentration of alizarin red S and drug respectively, A_C is the absorbance of the complex, ε_C is the molar absorptivity of the ion-pair complex and K_{IP} is the association constant of drug-dye ion-pair complex. The values for K_{IP} were obtained from the slope of lines obtained by plotting [ARS]/A_C against 1/[Drug] for all the drugs. All the plots were linear as shown in Fig. 6.

Gibbs free energy (ΔG^0) values were also computed for the ion-pair complexes according to the relationship:

$$\Delta \mathrm{G}^{O} = -RT \ln K_{\mathrm{IP}}$$

where, K_{IP} is the association constant of drug-dye ion-pair complex, R is the gas constant (8.314 J/mol K), and T is the temperature in Kelvin. The negative values of ΔG^0 indicate that the reactions were spontaneous and thermodynamically favored (Table 1).

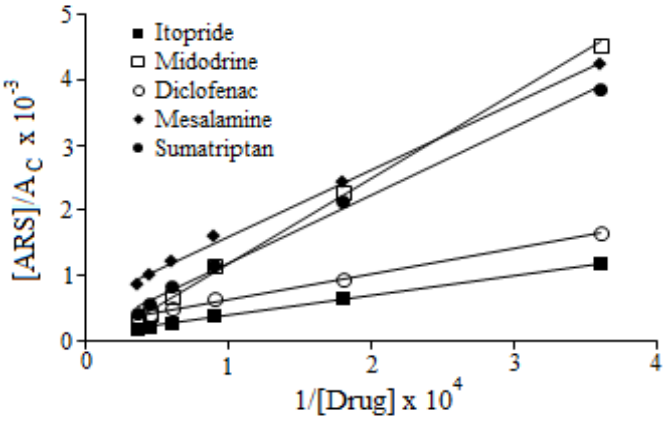

Fig. 6: Benesi-Hildebrand plots for association constants of ion-pair complexes between the drugs and ARS

Table 1: Association constants of the ion-pair complexes between the drugs and ARS

Parameters	Itopride	Midodrine	Diclofenac	Mesalamine	Sumatriptan
λ (nm)	284	287	279	285	280
K_{IP}, association constant (L mol^{-1})	1698	6267	4005	4551	5382
Correlation coefficient (r^2)	0.9965	0.9983	0.9970	0.9975	0.9959
Gibbs free energy, ΔG^0 (kJ mol^{-1})	-18.43	-21.66	-20.55	-20.87	-21.28

3.2. Method validation results

3.2.1. Linear range, limit of detection and limit of quantitation

Linearity of the methods were ascertained by preparing calibration standards at 3.5, 7.0, 14.0, 21.0, 28.0 and 35.0 µg mL^{-1} concentrations for ITO and DIC, 2.5, 15.0, 30.0, 85.0, 130 and 180 µg mL^{-1} for MID, 3.0, 6.0, 12.0, 25.0, 55.0 and 80.0 µg mL^{-1} for MES and 4.0, 8.0, 20.0, 60.0, 120 and 200 µg mL^{-1} for SUM. A linear relationship was found between the measured absorbance and the concentration range studied for each drug (Fig. 7), with correlation coefficients (r^2) ≥ 0.9971. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the expression, 3*s*/S and 10*s*/S respectively, where '*s*' is the standard deviation of the blank and 'S' is the slope of the calibration line [51,52]. LOD is defined as the lowest concentration of an analyte that can be reliably detected but not necessarily quantified, while LOQ is taken as the lowest concentration that can be determined with acceptable accuracy and precision, under the optimized experimental conditions. The Beer's law range, molar absorptivity, Sandell's sensitivity, LOD and LOQ values, slope and intercept of linear graphs for all the drugs are presented in Table 2.

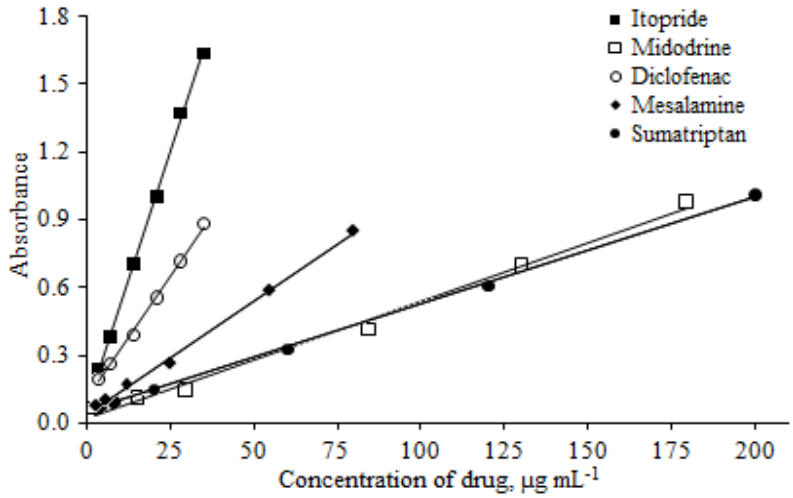

Fig. 7: Calibration curves for ITO, MID, DIC, MES and SUM at 284, 287, 279, 285 and 280 nm respectively

Table 2: Optical characteristics and analytical data for the studied drugs

Parameter	Itopride	Midodrine	Diclofenac	Mesalamine	Sumatriptan
Beer's law (µg mL-1)	3.5-35	2.5-180	3.5-35	3.0-80	4.0-200
Sandell's sensitivity (µg cm-2)	0.0206	0.1873	0.0383	0.0955	0.1978
λmax (nm)	284	287	279	285	280
LOD (µg mL-1)	0.0664	0.3318	0.1311	0.1934	0.8921
LOQ (µg mL-1)	0.2011	1.0054	0.3973	0.5861	2.7032
Molar absorptivity (L mol-1 cm-1)	1.96 × 104	1.60 × 103	8.32 × 103	2.20 × 103	1.86 × 103
Slope	0.0453	0.0052	0.0219	0.0099	0.0047
Intercept	0.0715	0.0163	0.1030	0.0374	0.0510
Correlation coefficient (r2)	0.9986	0.9980	0.9971	0.9984	0.9976

3.2.2. Accuracy and precision

To evaluate the accuracy and precision of the methods quality control (QC) samples were prepared at four concentration levels, 5.0, 10.0, 25.0, 30.0 µg mL^{-1} for ITO and DIC, 10, 75, 110, 150 µg mL^{-1} for MID, 10.0, 30.0, 45.0, 65.0 µg mL^{-1} for MES and 15.0, 80.0, 110.0, 160.0 µg mL^{-1} for SUM. Intra-day and inter-day accuracy (% relative error) and precision (% relative standard deviation, RSD) was determined at these concentration levels using six replicates and the results are shown in Table 3.

The % RSD for intra-day and inter-day varied from 0.56 to 4.75 and 0.60 to 4.91 respectively for all the drugs. The results indicate acceptable accuracy and precision of the proposed methods for the analysis of these drugs.

Table 3: Intra and inter-day accuracy and precision data for the selected drugs

Nominal conc. ($\mu g\ mL^{-1}$)	Intra-day (n=6)			Inter-day (n=6)		
	Mean conc. found ± SD	Precision (% RSD)	Relative error (%)	Mean conc. found ± SD	Precision (% RSD)	Relative error (%)
Itopride						
5.00	5.06 ± 0.14	2.76	1.27	4.97 ± 0.12	2.47	-0.52
10.00	9.93± 0.38	3.80	-0.67	9.80 ± 0.47	4.79	-2.00
25.00	25.03 ± 0.26	1.02	0.12	24.32 ± 0.53	2.16	-2.72
30.00	29.93 ± 0.49	1.63	-0.22	30.16 ± 0.80	4.79	0.53
Midodrine						
10.00	9.92 ± 0.47	4.75	-0.83	10.12 ± 0.54	4.36	1.17
75.00	74.87 ± 0.67	0.90	-0.18	75.13 ± 0.53	0.71	0.18
110.0	110.4± 1.60	1.45	0.35	109.9 ± 1.64	1.49	-0.11
150.0	144.8± 0.82	0.56	-3.47	145.2 ± 1.36	0.94	-3.18
Diclofenac						
5.00	5.02 ± 0.20	3.94	0.40	5.04 ± 0.14	2.73	0.83
10.00	9.80 ± 0.40	4.08	-2.00	10.08 ± 0.52	4.15	0.83
25.00	24.93 ± 0.61	2.44	-0.27	25.12 ± 0.82	3.27	0.60
30.00	29.72 ± 0.48	1.61	-0.94	30.02 ± 0.78	2.59	0.06
Mesalamine						
10.00	10.35 ± 0.39	3.80	3.50	9.82 ± 0.58	4.91	-1.80
30.00	30.92 ± 0.877	2.84	3.06	30.20 ± 0.62	2.05	0.67
45.00	44.83 ± 0.72	1.61	-0.37	44.08 ± 0.48	0.98	-2.04
65.00	64.83 ± 0.76	1.17	-0.26	65.22 ± 0.62	0.95	0.34
Sumatriptan						
15.00	15.48 ± 0.56	3.64	3.22	14.70 ± 0.46	3.13	-2.00
80.00	80.13 ± 1.05	1.31	0.17	79.75 ± 1.89	2.37	-0.31
110.0	109.8 ± 1.15	1.05	-0.21	110.2 ± 1.24	1.13	0.20
160.0	160.3± 0.99	0.62	0.16	160.4 ± 0.96	0.60	0.26

3.2.3. Analysis of pharmaceutical formulations

To check the accuracy of the proposed methods recovery studies were carried out using the standard addition technique. Definite amount of pre-analyzed tablets samples were spiked with pure drug at three levels and the total amount of the drug was found using the proposed methods. This study was performed with five replicates at each level. The recovery of the pure drug was quantitative with % RSD values ranging from 0.26-4.20 for all the drugs (Table 4). Interference study was also done to check the selectivity of the present method in presence of commonly used excipients in tablet formulations. This experiment was done by adding the excipients (amount equivalent to five times the upper limit of quantitation of each drug) such as corn starch, microcrystalline cellulose, magnesium stearate, lactose, silicon dioxide, titanium dioxide and iron oxide individually at two QC levels (lowest and highest concentration) for all the drugs. The results confirm that their presence did not interfere in the quantitative determination of these drugs.

Additionally, the validity of the proposed method was evaluated by comparing the results obtained using reference methods for each drug with tablet formulations. Table 5 shows that the results obtained were in close agreement and were compared statistically using Student's *t*-test for accuracy and *F*-test for precision at 95 % confidence level. The calculated '*t*' and '*F*' values were well below the tabulated values (t = 2.77 and F = 6.39, for four

degrees of freedom). This proves that the developed methods are not statistically different from the reference method with respect to accuracy and precision.

Table 4: Determination of drugs in dosage forms by standard addition method (n = 5)

Tablet formulation	Amount of taken drug (µg)	Amount of pure drug added (µg)	Total amount found (µg) (Mean ±SD)	Precision (% RSD)	Recovery of pure drug (%)
Ganaton® (itopride hydrochloride), 50 mg	10	8	17.82 ± 0.18	1.33	99.0
	10	10	19.68 ± 0.21	0.94	98.4
	10	12	21.51 ± 0.33	0.26	97.8
ProAmatine® (midodrine hydrochloride) 10 mg	60	48	107.59 ± 0.58	2.22	99.6
	60	60	119.02 ± 0.26	1.89	99.2
	60	72	131.49 ± 0.10	0.71	99.6
Voltaren® (diclofenac sodium) 50 mg	10	8	17.79 ± 0.39	3.95	98.8
	10	10	19.85 ± 0.47	0.56	99.3
	10	12	21.76 ± 0.76	1.24	98.9
Walasa® (mesalamine) 400 mg	30	24	53.91 ± 0.59	4.20	99.8
	30	30	59.19 ± 0.22	2.66	98.7
	30	36	65.89 ± 0.41	0.49	99.8
Sumitrex® (sumatriptan succinate) 50 mg	80	64	143.09 ± 0.39	1.11	99.4
	80	80	159.22 ± 0.24	0.57	99.5
	80	96	174.31 ± 0.85	2.03	99.0

Table 5: Statistical comparison of the proposed method with reference method for determination of drugs in tablet formulations (n = 5)

Tablet formulation	Label claim (mg)	Amount found (%); Mean ± SD	
		Reference method[a]	Developed method
Ganaton® (itopride hydrochloride)	50	100.27 ± 1.05	100.01 ± 1.34 t = 2.21; F = 3.27
ProAmatine® (midodrine hydrochloride)	10	99.53 ± 1.13	99.21 ± 0.94 t = 2.03; F = 3.98
Voltaren® (diclofenac sodium)	50	100.45 ± 0.78	99.79 ± 1.13 t = 1.05; F = 4.78
Walasa® (mesalamine)	400	99.14 ± 0.86	99.01 ± 0.68 t = 1.75; F = 4.05
Sumitrex® (sumatriptan succinate)	50	99.36 ± 0.91	100.38 ± 0.74 t = 2.19; F = 3.04

[a] Ref. [15] for ITO, Ref. [21] for MID, Ref. [25] for DIC, Ref. [31] for MES, Ref. [45] for SUM

4. Comparison with reported work

The extractive spectrophotometric methods developed for ITO with methyl orange [15], MES with bromocresol green and bromocresol purple [31] , are much less sensitive with an LOQ of 10 µg mL^{-1} for ITO and 20/25 µg mL^{-1} for MES. For SUM method with naphthalene blue 12 BR and methylene blue [46] the LOQ achieved (4.0 µg mL^{-1}) was comparable with

the present work. Moreover, these methods describe studies related to the analysis of drugs in pharmaceutical preparation with no information about the association constant of ion-pair complexes, their stability and stoichiometry. For MID and DIC there are a few direct spectrophotmetric methods available in the literature. In one such method, the primary amino group of MID is reacted with ninhydrin in methanol to give a purple coloured complex [22]. Similarly for DIC a direct method has been proposed by reacting the drug with conc. HNO_3 followed by measurement of the yellow coloured product [25]. Nevertheless, both these methods may not be adequately selective in presence of diverse matrix components as demonstrated in the present work.

5. Conclusion

The results obtained in the proposed methods were based on the formation of ion-pair complexes of the drugs (ITO, MID, DIC, MES and SUM) with anionic dye, ARS under the optimized experimental conditions. The positively charged primary/secondary nitrogen groups of the drugs interacted with the sulphonic acid group of ARS through electrostatic attraction. The developed methods are simple, selective, rapid and economical and the results obtained showed acceptable precision and accuracy and recovery of the drugs. Further, all the drugs were successfully estimated in pure forms as well as in their respective dosage formulations without any interference from the commonly used additives and excipients.

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Conflict of interests

The authors declare no conflict of interest.

References

1. Siddiqui MR, Alothman ZA and Rahman N (2013) Analytical techniques in pharmaceutical analysis: A review. Arab J Chem DOI: 10.1016/j.arabjc.2013.04.016.
2. Gouda AA, Kotb El-Sayed MI, Amin AS and El Sheikh R (2013) Spectrophotometric and spectrofluorometric methods for the determination of non-steroidal anti-inflammatory drugs: A review. Arab J Chem 6: 145.
3. Rojas FS and Ojeda CB (2009) Recent developments in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008: A review. Anal Chim Acta 635: 22.
4. S Yoon S, Lee H, Kim TE, Lee S, Chee DH, Cho JY, Yu KS and Jang IJ (2014) Comparative steady-state pharmacokinetic study of an extended-release formulation of itopride and its immediate-release reference formulation in healthy volunteers. Drug Des Devel Ther 8: 123.
5. Huang X, Lv B, Zhang S, Fan YH and Meng LN (2012) Itopride therapy for functional dyspepsia: A meta-analysis. World J Gastroenterol 18: 7371.
6. Izcovich A, Gonzalez Malla C, Manzotti M, Catalano HN and Guyatt G (2014) Midodrine for orthostatic hypotension and recurrent reflex syncope: a systematic review. Neurology 83: 1170.
7. Biaggooni I (2014) New developments in the management of neurogenic orthostatic hypotension. Curr Cardiol Rep 16: 542.

8. Dastidar SG, Ganguly K, Chaudhuri K and Chakrabarty AN (2000) The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. Int J Antimicrob Agents 14: 249.

9. Nanda K and Moss AC (2012) Update on the management of ulcerative colitis: treatment and maintenance approaches focused on MMX® mesalamine. Clin Pharmacol Adv App 4: 41.

10. Sandborn WJ, Feagan BG and Lichtenstein GR (2007) Medical management of mild to moderate Crohn's disease: evidence-based treatment algorithms for induction and maintenance of remission. Alimentary Pharmacol Ther 26: 987.

11. Deleu D and Hanssens Y (2000) Current and emerging second-generation triptans in acute migraine therapy: a comparative review. J Clin Pharmacol 40: 687.

12. Khoury CK and Couch JR (2010) Sumatriptan-naproxen fixed combination for acute treatment of migraine: a critical appraisal. Drug Des Devel Ther 4: 9.

13. Zate SU, Kothawade PI, Gajbe JW, Pramod AS and Boraste SS (2010) Spectrophotometric method development and validation of itopride hydrochloride in bulk and dosage form. Int J Drug Deliv 2: 340.

14. Gupta KR, Joshi RR, Chawla RB and Wadodkar SG (2010) UV spectrophotometric method for the estimation of itopride hydrochloride in pharmaceutical formulation. E-J Chem 7: S49.

15. Choudhary B, Goyel A and Khokra SL (2009) New visible spectrophotometric method for estimation of itopride hydrochloride from tablets formulations using methyl orange reagent. Int J Pharmacy Pharm Sci 1: 159.

16. Thiruvengada RV, Mohamed STs, Ramkanth S, Alagusundaram M, Ganaprakash K and Madhusudhana CC (2010) A simple RP-HPLC method for quantitation of itopride hcl in tablet dosage form. J Young Pharm 2: 410.

17. Suganthi A, John S and Ravi TK (2008) Simultaneous HPTLC determination of rabeprazole and itopride hydrochloride from their combined dosage form. Ind J Pharm Sci 70: 366.

18. Walash MI, Ibrahim F, Eid MI and Abass SAEL (2013) Stability-Indicating Spectrofluorimetric method for determination of itopride hydrochloride in raw material and pharmaceutical formulations. J Fluoresc 23: 1293.

19. Khalil S (2013) Micro-determination of itopride hydrochloride in pharmaceutical formulations and urine samples using icp-atomic emission spectrometry. Int J Pharm Bio Sci 4: 786.

20. Ptácek P, Klíma J and Macek J (2009) Optimized method for the determination of itopride in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr B 877: 842.

21. Sattar OA, Rezk MR, Badawy AM and Khattab OM (2013) Selective determination of midodrine hydrochloride in the presence of its acidic degradation product. Anal Chem Indian J 12: 182.

22. El-Gindy A, Sallam S and Abdel-Salam RA (2008) Spectrophotometric method for determination of midodrine hydrochloride using ninhydrin in pharmaceutical formulations. Anal Chem Indian J 7: 529.

23. Elzanfaly ES, Zaazaa HE and Merey HA (2013) Ion Selective phosphotungestate and β-cyclodextrin based membrane electrodes for stability-indicating determination of midodrine hydrochloride. Acta Chim Slov 60: 256.

24. Yoshida H, Ohno Y, Yoshikuni K, Todoroki K, Nohta H and Yamaguchi M (2003) Determination of midodrine in human plasma by high-performance liquid chromatography with fluorescence detection. Anal Sci 19: 317.

25. Matin AA, Farajzadeh MA and Jouyban A (2005) A simple spectrophotometric method for determination of sodium diclofenac in pharmaceutical formulations. Il Farmaco 60: 855.

26. Khaskheli AR, Sirajuddin S, Abro K, Sherazi STH, Afridi HI, Mahesar SA and Saeed M (2009) Simpler and faster spectrophotometric determination of diclofenac sodium in tablets, serum and urine samples. Pak J Anal Environ Chem 10: 53.

27. Lala LG, D'Mello PM and Naik SR (2002) HPTLC determination of diclofenac sodium from serum. J Pharm Biomed Anal 29: 539.

28. Mazurek S and Szostak R (2008) Quantitative determination of diclofenac sodium in solid dosage forms by FT-Raman spectroscopy. J Pharm Biomed Anal 48: 814.

29. Hosseinzadeh R, Maleki R and Matin AA (2007) Interaction of diclofenac with bovine serum albumin investigated by diclofenac-selective electrode. Acta Chim Slov 54: 126.

30. Abdel-Hamid ME, Novotny L and Hamza H (2001) Determination of diclofenac sodium, flufenamic acid, indomethacin and ketoprofen by LC-APCI-MS. J Pharm Biomed Anal 24: 587.

31. Darak V, Karadi AB, Arshad M and Raju SA (2011) Simple extractive spectrophotometric determination of mesalamine by acid-dye complexation methods in solid dosage form. Res J Pharmacy Tech 4: 962.

32. Chandra BS, Bhogela SS, Shaik M, Vadlamudi CS, Chappa M and Maddirala NS (2011) Simple and sensitive spectrophotometric methods for the analysis of mesalamine in bulk and tablet dosage forms. Quim. Nova 34: 1068.

33. Jejurkar L, Tapar KK, Game MD, Birari T and Ghenge G (2011) Development and Validation of Spectrophotometric Method for Mesalamine in Pure and In Tablets. Res J Pharmacy Tech 4: 589.

34. Zakaria RA (2013) Spectrophotometric Determination of mesalazine by 8-hydroxy quinoline and N-(1-naphthyl)ethylenediamine dihydrochloride reagents in bulk and capsule dosage forms. Raf J Sci 24: 146.

35. 35.Sama NS, Gurupadayya BM and Kumar A (2011) Spectrophotometric determination of mesalamine by PDAC and NQS reagents in bulk and tablet dosage form. J Pharmacy Res 4: 39.

36. 36.Hamdon EA, Zakaria SA and AL-Hamdany NM (2012) Spectrophotometric determination of mesalazine with phloroglycinol in pharmaceutical preparation. Tikrit J Pure Sci 17: 133.

37. Reddy MP, Prabhavathi K, Reddy NR and Reddy PR (2011) Two simple spectroscopic methods for the estimation of mesalamine in bulk sample and its pharmaceutical dosage forms. Global J Pharmacol 5: 101.

38. Singh RK, Patel PS and Gupta P (2010) UV spectrophotometric method for the estimation of mesalazine in bulk and its pharmaceutical dosage forms. Int J Pharm Sci Res 1: 44.

39. Jejurkar L and Jejurkar A (2012) HPLC method of detection for 5ASA in pure and in tablets. J Pharm Sci Tech 4: 792.

40. Shah CR, Suhagia BN, Shah NJ and Shah RR (2008) Development and validation of a HPTLC method for the estimation of sumatriptan in tablet dosage forms. Indian J Pharm Sci 70: 831.

41. Sharma MC (2013) Validated Densitometric Method for the quantification of mesalamine in pharmaceutical dosage form. World Appl Sci J 28: 1098.

42. Ge Z, Tessier E, Neirinck L and Zhu Z (2004) High performance liquid chromatographic method for the determination of sumatriptan with fluorescence detection in human plasma. J Chromatogr B 806: 299.

43. Ramu BK, Rupakumari G, Ramarao K and Raghubabu K (2011) Development of new visible spectrophotometric methods for quantitative determination of sumatriptan succinate based on charge-transfer complex formation. Int J Pharmacy Pharm Sci Res 2: 47.

44. Ramu BK and Raghubabu K (2011) Estimation of sumatriptan succinate in bulk and formulations by visible spectrophotometry using aromatic aldehydes. Int J Appl Bio Pharm Tech 2: 86.

45. Ramu BK and Raghubabu K (2011) Extractive visible spectrophotometric assay for sumatriptan succinate estimation based on ion-association complex formation. Res J Pharmacy Tech 4: 1212.

46. Ramu BK and Raghubabu K (2011) Ion association method for the determination of sumatriptan succinate from tablet dosage forms using tropaeolin OOO. Int J Pharmacy Pharm Sci 3: 175.

47. Prashanth KN, Basavaiah K and Raghu MS (2013) Utilization of N-bromosuccinimide as a brominating agent for the determination of sumatriptan succinate in bulk drug and tablets. Int J Anal Chem 2013: Article ID 934357.

48. Ramu BK and Raghubabu K (2010) Visible spectrophotometric determination of sumatriptan succinate in tablet dosage forms using folin reagent. Int J Pharm Biomed Sci 1: 49.

49. Job P (1928) Formation and stability of inorganic complexes in solution. Anal Chem 9: 113.

50. Benesi HA and Hildebrand JH (1949) A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. J Am Chem Soc 71: 2703.

51. Shrivastava A and Gupta VB (2011) Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron Young Sci 2: 21.

52. ICH Harmonised Tripartite Guideline (2005) Validation of analytical procedures: text and methodology- Q2(R1).