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Original Article

Use of alizarin red S as a chromogenic agent for spectrophotometric determination of loratadine in pharmaceutical formulations

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ABSTRACT

The study involved two simple, rapid, selective and cost-effective spectrophotometric methods for the determination of loratadine in pharmaceutical formulations. Method A was based on the formation of yellow coloured ion-pair complex between loratadine and alizarin red S in acid medium which was extracted into dichloromethane and the absorbance was measured at 425 nm. Method B was based on the breaking of yellow loratadine-alizarin red S ion-pair complex in alkaline medium followed by the measurement of violet colour free dye at 550 nm. Under the optimized conditions, Beer's law was obeyed over the concentration ranges of 2.5–90.0 and 2.5–30.0 µg/ml loratadine for method A and method B, respectively. The molar absorptivity, Sandell's sensitivity, detection and quantification limits were calculated. The methods were validated for intra-day and inter-day accuracy, precision, selectivity, robustness and ruggedness. The proposed methods were applied successfully to the determination of LOR in pure drug and commercial formulations. The accuracy and reliability of the proposed methods were further established by reference method and also by recovery studies via standard addition technique.

Keywords: Loratadine; Alizarin red S; Ion-pair complexation; spectrophotometry; validation.

INTRODUCTION

Loratadine, ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidine carboxylate [1] is non-sedative, second generation H1-antihistamine drug. Its structure is shown in figure 1. It is more selective for peripheral H1-receptors as opposed to the central nervous system H1-receptors and cholinergic receptors. This selectivity significantly reduces the occurrence of adverse drug reactions, such as sedation, while still providing effective relief of allergic conditions. The reason for their peripheral selectivity is that most of these compounds are zwitterionic at physiological pH (pH ~7.4). If they are very polar, they do not cross the blood-brain barrier and act mainly outside the central nervous system, that is why they produce very little or no sedation. It is a potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis by Schering-Plough Company [2].

Various analytical methods have been reported for the quantitative determination of LOR both in dosage forms and in biological fluid forms. United States pharmacopoeia (USP) 2008 [3] has introduced chromatographic methods (High Performance Liquid Chromatography HPLC and High Performance Thin Layer Chromatography HPTLC) for separation and quantitative determination of LOR in pure and pharmaceutical dosage forms. LOR oral solution and tablets are estimated by HPTLC, using ethyl ether and diethylamine (40:1, v/v) as a developing solvent system. UV spectrophotometric methods were studied to the determination of LOR hydrochloride in tablets and suspension [4,5]. Derivative spectrophotometric method was analysed for the assay of three binary mixtures of pseudoephedrine with cetirizine, fexofenadine and LOR. This

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method was based on the use of the first derivative of the ratio spectrum [6]. Stability-indicating methods have been reported for the determination of LOR in the presence of its degradation products developed by 1D ratio spectra at 236, 262.4 and 293.2 nm and by second derivative spectrophotometry at 266 nm [7].

A sensitive gas-liquid chromatographic method (GLC) has been developed for the study of LOR and its active metabolite in human plasma, using a nitrogen phosphorus detector and a fused-silica capillary column [8]. GC/MS method for the determination of LOR and pheniramine in human serum developed [9]. Capillary gas chromatography methods were applied for the determination of residual organic solvents-diethyl ether, isopropyl alcohol, acetonitrile, 2-methylpropan-2-ol, ethyl acetate, tetrahydrofuran, cyclohexane, triethylamine, toluene in LOR samples.

A capillary column was used with temperature programmed control and DMF as the solvent [10]. Agilent capillary column was used with flame ionization detector (FID) detector and nitrogen as the carrier gas [11]. LOR was subjected to GC-chemical ionization (CI)-MS analysis with He carrier gas and temperature programming from 70ºC to 300ºC at 20ºC/min [12]. A capillary electrophoretic method has been developed for the determination of LOR in tablets using 24 mmol/L glycine as a carrier cation, 1.6 mmol/L citric acid and 84 mmol/L acetic acid as counter ions at pH 3.2 with UV detection at 240 nm [13].

To the best of our knowledge, there are few reports on the use of visible spectrophotometry for assay of LOR in pharmaceuticals. They are shown in table 1. Visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories. All the previously reported visible spectrophotometric methods are less sensitive and few methods require a rigid pH control and tedious liquid-liquid extraction steps. In the present communication, we report the development of two accurate precise spectrophotometric methods which provide rapid and economic procedures and more sensitive compared to the previously reported spectrophotometric methods.

EXPERIMENTAL

Instrument: An ELICO SL-218 double beam spectrophotometer equipped with 1 cm matched quartz cells was used for all the spectral measurements.

Materials: Pharmaceutical grade loratadine (LOR), certified to be 99.80% pure, was received from Orchid Pharmaceuticals, India. The following pharmaceutical preparations were purchased from commercial sources in the local market and subjected to analysis: Lorinol-10 (10mg) from Micro labs Ltd., South Sikkim, India and Lorfast Meltab (10mg) from Cadila Pharmaceuticals Ltd., India. All the reagents and solvents used were of analytical reagent grade and distilled water was used throughout the study. Alizarin Red S, KOH were bought from Sigma Aldrich, India and HCl (specific gravity 1.180) from Fisher Scientific Company.

Preparation of solutions

Standard stock LOR solution: A stock standard solution of 100 µg/ml of LOR was prepared by dissolving accurately weighed 10 mg of pure drug in 5ml of 0.1M HCl and diluting to the mark with water in a 100 ml standard flask.

Alizarin red S (0.05%): 0.05 g of ARS was dissolved in distilled water and made up to 100ml in 100ml calibrated flask.

Hydrochloric acid: 0.1 M in distilled water.

Potassium hydroxide: 1.0% (w/v) of KOH in methanol.

Recommended procedure

Method A (based on the study of ion-pair complex): Different aliquots (0.25, 0.5 - 9.0 ml) of a standard LOR (100 µg/ml) solution were accurately transferred into a series of 125 ml separating funnels and the total volume was adjusted to 15.00 ml by adding adequate quantity of water. To each funnel 5 ml of 0.1 M HCl was added, followed by 5 ml of 0.05% ARS solution. The content was mixed well and after 10 min, the formed ion-pair complex was extracted with 10 ml of dichloromethane after shaking for 1 min. The two phases were allowed to separate and dichloromethane layer was dried over anhydrous sodium sulphate and the absorbance of yellow LOR-ARS ion-pair complex was measured at 425 nm against a reagent blank prepared in the similar way without drug.

Method B (based on the measurement of the free form of ARS from the broken ion-pair): Into a series of 125 ml separating funnels, 5.0 ml aliquots of a standard solution (100 µg/ml) were transferred separately and the total volume in each separating funnel was adjusted to 15.0 ml by adding water. To each funnel, added 5 ml of 0.1 M HCl and 5 ml of 0.05% ARS solution. The content was mixed thoroughly and after 10 min, the ion-pair complex was extracted with 10 ml of dichloromethane by shaking for 1 min. The two layers were allowed to separate and the organic

layer of all separating funnels was passed over anhydrous sodium sulphate and then collected in a 50 ml dry standard flask. Varying aliquots (0.25–3.0 ml) of this organic layer, LOR-ARS ion-pair complex (50 µg/ml in LOR), were transferred into a series of 5 ml standard flasks and the total volume was adjusted to 3.00 ml by adding dichloromethane. To each flask, 2.0 ml of 1.0% methanolic KOH was added, the content was mixed well and the absorbance of violet colored species was measured at 550 nm against the reagent blank.

Procedure for commercial tablets: Ten tablets each containing 70 mg or 100 mg of LOR were weighed and finely powdered. An amount of the powder equivalent to 10 mg of LOR was accurately weighed, transferred to a 100 ml volumetric flask and dissolved in 5ml of 0.1M HCl. 60 ml of water was added and the content was shaken thoroughly for about 15 min. The volume was diluted to the mark with water, mixed well and filtered using Whatman number-41 filter paper. First 10 ml portion of the filtrate was rejected and a suitable aliquot of the filtrate (containing 100 µg/ml LOR) was used for the assay by the recommended procedure of method A. The ion-pair complex LOR-ARS (50 µg/ml in LOR, prepared in method A) of the tablets was used for assay by applying the procedure described in method B.

Procedure for the selectivity study: The selectivity was tested by both placebo blank analysis and recovery studies. Placebo blank, commonly employed excipients added to the formulations, consisting of 50 mg starch, 30 mg lactose, 30 mg acacia, 30 mg calcium gluconate, 40 mg talc, 50 mg magnesium stearate and 30 mg sodium alginate was prepared as described under the Section 2.4.3 and then subjected to analysis. A synthetic mixture was prepared by adding 10 mg of pure LOR to the above mentioned placebo blank and the mixture was homogenized. Following the same procedure for tablets, the synthetic mixture solution was prepared and a suitable quantity was subjected for the analysis by both the methods.

RESULTS AND DISCUSSION

Absorption spectra: The reaction between LOR and ARS in an acidic medium to form a yellow ion-pair complex was studied and extracted using dichloromethane. The absorption spectra (Figure. 2) of the formed ion-pair complex was recorded at 380-500 nm against the reagent blank solution and exhibited a maximum absorption at 425 nm (method A). In method B, this LOR-ARS ion-pair complex was treated with methanolic KOH to yield a chromogen, the dissociated form of ARS, which exhibits bathochromic shift to maximum absorbance at 550 nm which was recorded at 485-680 nm (Figure. 3).

Reaction Mechanism: LOR forms ion-pair complex with ARS due to the formation of protonated pyridine ring in acid medium. In the piperidine ring, protonation is very difficult due to steric effect. ARS is a dye of anthroquinone derivative type. So, when LOR is treated with ARS dye in acid medium, a yellow ion-pair complex extractable into dichloromethane is formed and the possible reaction pathway for the formation of ion-pair complex (method A) is given in Scheme 1. Canamares et al. (2006) [19] have shown that alizarin can exist in three different forms (neutral absorbing at 433 nm, monoionized absorbing at 526 nm and deionized absorbing at 567 nm) based on different pHs 3.4, 10.0 and 12.8, respectively. Nemodruk and Karalova (1969) [20] have stated that alizarin red S has a yellow color in acid solutions (pH < 5) which becomes a violet on increasing pH to 11. They explain that this phenomenon is due to the dissociation of the alizarin red S molecule at the two phenol groups and forming dianionic form of ARS^{2-}. So, when LOR-ARS ion-pair complex formed in method A, is treated with methanolic KOH, the ion-pair complex will break, and yellow color will change to violet color due to the formation of dianionic form of the dye (ARS^{2-}). The negative charge present near to anthroquinone group involved delocalisation with C=O group in anthroquinone type ring. The possible reaction pathway for method B is illustrated in Scheme 2.

Method development: A number of preliminary experiments created optimum conditions necessary to achieve complete reaction formation, quantitative extraction of the ion-pair complex, complete breaking of formed ion-pair complex and highest sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 425 nm.

Effect of pH on Ion-Pair Complex Formation: The effect of pH of the aqueous phase was studied by extracting yellow colored complex in the presence of either hydrochloric acid or acidic buffers of pH 2.0-4.0. It was noticed that the maximum color intensity was observed using dilute hydrochloric acid. Also, the formed LOR-ARS ion-pair complex was found to be pH independent since no remarkable changes were observed while using different concentrations of HCl such as 0.1, 0.2, 0.3, 0.4 and 0.5 M. Further, 5.0 ml of 0.1M HCl gave reproducible results and it was fixed

throughout this study. Therefore, method A requires no pH adjustment.

Effect of Reagent Concentration: The effect of ARS concentration in method A was studied by measuring the absorbance of solutions containing a fixed concentration of 20 µg/ml LOR and different volumes of 0.05% ARS at 425 nm. The study showed that 5.0ml of 0.05% ARS solution was needed as the difference between the absorbances of the blank and the measured species was maximum (Figure.4). Similarly, the effect of methanolic KOH concentration required to break the ion-pair complex and formation of the dianionic form of the dye in method B was studied by measuring the absorbance of solutions containing a fixed concentration of ion-pair complex (15 µg/ml) and different volumes of 1.0% methanolic KOH at 550 nm. The results showed that 2.0ml of 1.0% (w/v) methanolic KOH was sufficient to yield a maximum absorbance as can be seen in Figure.5.

Selection of Extracting Solvent: Three organic solvents, namely, chloroform, dichloromethane (DCM) and 1,2-dichloroethane were tested for quantitative extraction of the formed ion-pair complex. Based on the results, dichloromethane was selected as extracting solvent due to its efficiency, the greater stability of the extracted ion-pair complex (>24 h), its high sensitivity, maximum absorbance of the measured species and short time required for the separation of the two phases.

Effect of Time, Sequence of Addition and Stability: The effect of contact time between LOR and ARS in the presence of 0.1M HCl (method A) was studied in the time range of 0-30 min before extraction and it was found that 10 min was the minimum time to achieve maximum absorbance at 425 nm. Shaking times of 0.5–3 min were studied and the results showed that 1.0 min was sufficient to produce a constant absorbance. In method B, the effect of time required to break ion-pair complex was studied after the addition of methanolic KOH to the ion-pair complex and it was found that the breaking of the complex was instantaneous. There was no appreciable change in the absorbance of the measured species if the order of addition of the reactants was varied in method A. The absorbance of yellow ion-pair complex remained stable for more than 24 h at room temperature (method A) and the absorbance of the violet colour of the dianionic form of ARS (method B) was found to remain stable for 2 hr.

Effect of Volume of the Aqueous Phase: The effect of volume of aqueous phase was studied by using different volumes of aqueous phase (including LOR, ARS and HCl) such as 15, 20, 25, 30 and 35ml and extracted with 10ml of DCM where 20 µg/ml of LOR was used. From the results shown in table 2, it was found that 25ml of aqueous phase was sufficient to give maximum absorbance of measured ion-pair complex and minimum absorbance of reagent blank and hence an aqueous phase of 25ml was fixed throughout the developed method.

Effect of Number of Extractions and Volume of Organic Solvent: It was found that one extraction with 10ml of organic solvent and a second extraction with identical quantity of dichloromethane yielded negligible absorbance.

Composition of LOR-ARS Ion-Pair Complex: The composition of the ion-pair complex formed in method A between LOR and ARS were found by applying Job's method of continuous variations. In this method, 6×10^{-4}M solutions of LOR and ARS were used and mixed in varying volume ratios in such a way that the total volume of the drug and ARS was kept at 5ml in the total volume of 25ml of the aqueous phase. The absorbance of extracted ion-pair in each composition was measured and plotted against the mole fraction of the drug (Figure. 6). The plot reached a maximum value at a mole fraction of 0.5 which indicated that a 1:1 (LOR:ARS) ion-pair complex was formed through the electrostatic attraction between positive protonated LOR and ARS anion.

Method validation

The proposed methods were validated in according to the current ICH guidelines [21].

Analytical parameters: Under optimum experimental conditions for LOR determination, the standard calibration curves were studied by plotting the absorbance versus concentration. The linear regression equations were obtained by the method of least squares and Beer's law was obeyed over the concentration ranges stated in table 3. Many parameters such as intercept (b), slope (m), molar absorptivity, Sandell's sensitivity, correlation coefficient (r), standard deviation of intercept (S_b), standard deviation of slope (S_m), limits of detection and quantification for both methods are summarized in table 3. The linearity of calibration graphs was proved by the high values of the correlation coefficient (r) and the small values of the Y-intercepts of the regression equations.

Precision and accuracy: The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of LOR were analyzed in seven replicates (repeatability) during

the same day (intra-day precision) and five consecutive days (inter-day precision). From the results shown in table 4, the percentage relative standard deviation (% RSD) values were ≤ 2.13% (intra-day) and ≤ 2.06% (inter-day) indicating high precision of the proposed methods. Also, the accuracy of the proposed methods was evaluated as percentage relative error (%RE) and from the results shown in table 4, it is found that the accuracy is good (RE ≤1.90%).

Selectivity: The selectivity of the proposed methods for the analysis of LOR was analysed by placebo blank and synthetic mixture analyses as shown under the Section 2.4.4. From the placebo blank analysis, the change in the absorbance with respect to the reagent blank was caused only by loratadine. Non-interference from placebo was further confirmed by carrying out recovery study from synthetic mixture by using 50 µg/ml of LOR and the percent recoveries of LOR were 99.28 ± 2.18 and 98.92 ± 2.02 for method A and method B, respectively.

Robustness and ruggedness: The estimation of the method robustness was done by interchanging some parameters namely, volume of HCl and the contact time for method A or volume of methanolic KOH for method B and performing the analysis under the optimized experimental conditions. The effect of these changes on the absorbance reading of the coloured systems in both methods were found to be negligible confirming the robustness of the proposed methods. Ruggedness was expressed as %RSD of the same procedure applied by three analysts and also by a single analyst performing analysis on three different instruments. The results given in table 5, showed that no statistical differences between different analysts and instruments suggesting that the proposed methods were rugged.

Applications to analysis of pharmaceutical formulations: The proposed methods were successfully applied to the determination of LOR in two representative tablets Lorinol-10 and Lorfast Meltab. The results obtained are shown in table 6, were compared with reference method [22] by means of Student's t-test for accuracy and F-tests for precision at 95% confidence level. As can be seen from the table 6, the calculated t- and F-values at 95% confidence level did not exceed the tabulated values of 2.57 and 5.05, respectively, indicating that there were no significant differences between the proposed methods and the reference method. To ascertain the accuracy and validity of the proposed methods, recovery experiment was performed via the standard addition procedure. To a fixed and known amount of LOR in tablets powder (pre-analyzed), pure drug was added at three levels (75%, 100% and 125%) of the quantity present in the tablets powder) and the total was measured by the proposed methods. The determination with each concentration was repeated three times and the results of this study presented in table 7 indicated that the various excipients present in the formulations did not interfere in the assay.

CONCLUSION

The results of this study demonstrate that it is possible to use alizarin red S as an ion-pairing reagent for the spectrophotometric determination of LOR in bulk drug as well as in pharmaceutical samples. The proposed methods are highly reliable owing to the stability of the ion-pair complex and the dianionic form of the ARS which are easily measured. The methods can be performed at room temperature and make use of cheaper and readily available analytical reagent. Moreover, the procedures do not involve any critical reaction conditions and no pH-adjustment is required. As can be seen from the molar absorptivity values of both methods, the method B is more sensitive than method A. From the Student's t-test and F-test values, it is clear that the results obtained by the proposed methods are in a good agreement with reference method and indicate a high accuracy and precision. Thus, the methods are useful for the quality control and routine analysis of LOR in pharmaceutical preparations since there is no interference was observed from the common excipients that might be found in commercial formulations.

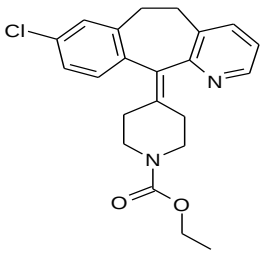


Figure 1. Structure of loratadine

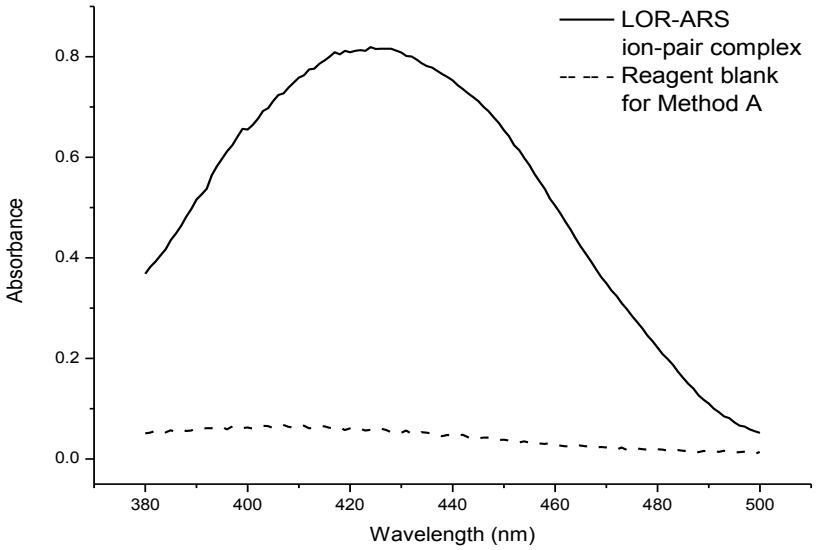


Figure 2. Absorption spectra of ion-pair complex of LOR-ARS (80 µg/ml) in method A

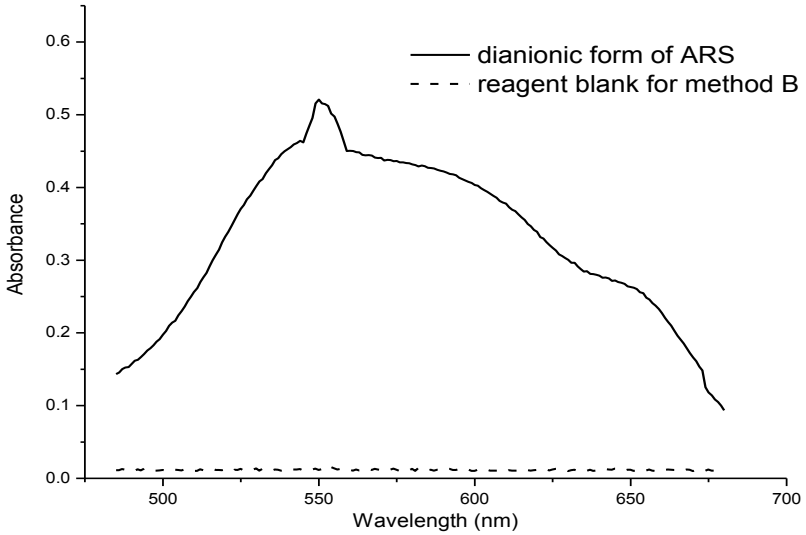


Figure 3. Absorption spectra of dianionic form of ARS (30 µg/ml) in method B

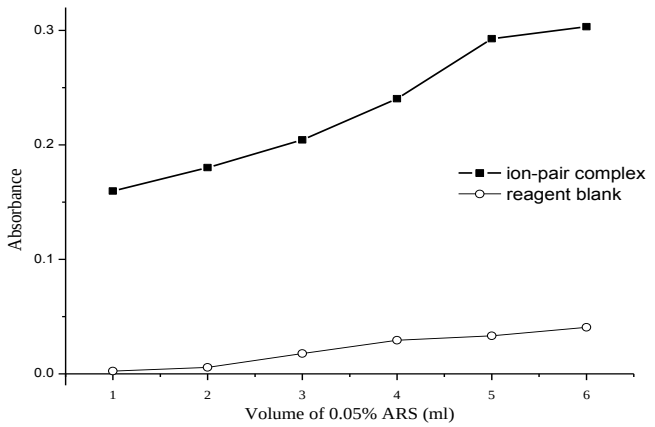


Figure 4. Effect of alizarin red S concentration on the color development (20 µg/ml LOR)

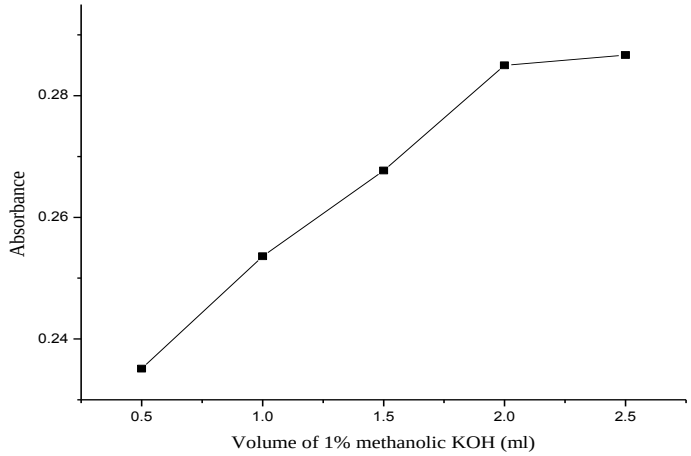


Figure 5. Effect of 1.0% methanolic KOH (15 µg/ml LOR-ARS ion-pair)

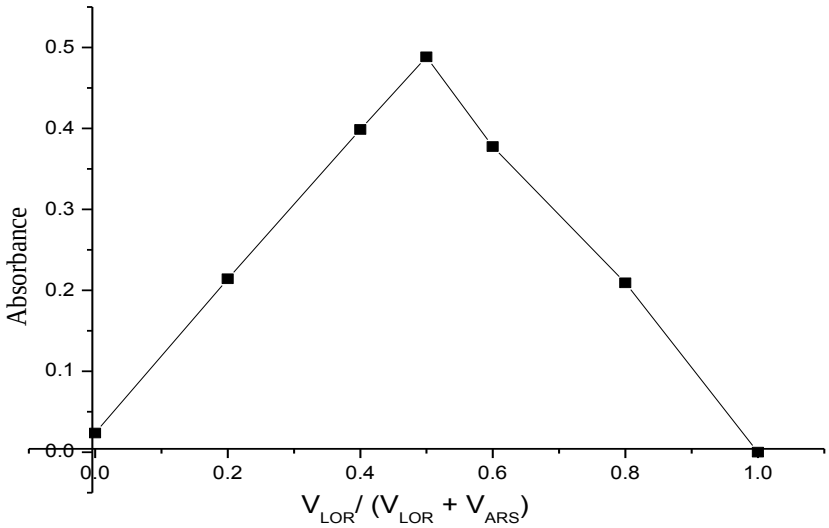


Figure 6. Job's continuous variations plot for LOR-ARS ion-pair complex

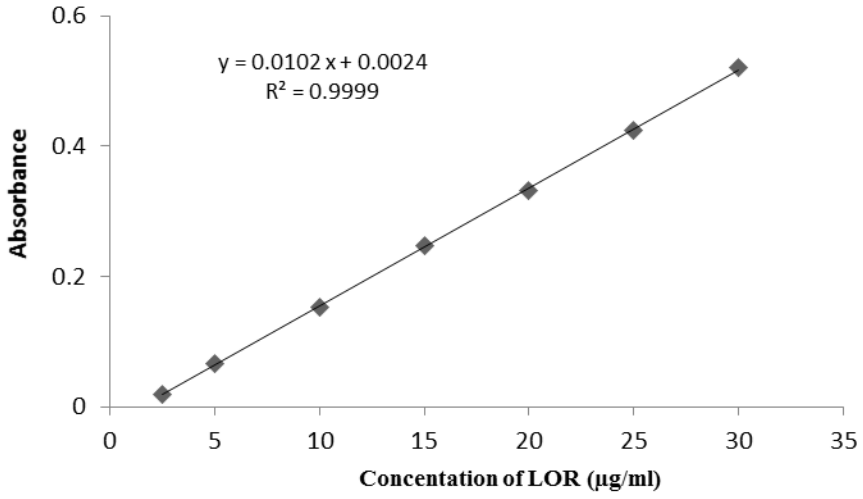


Figure 7. Calibration curve for method A

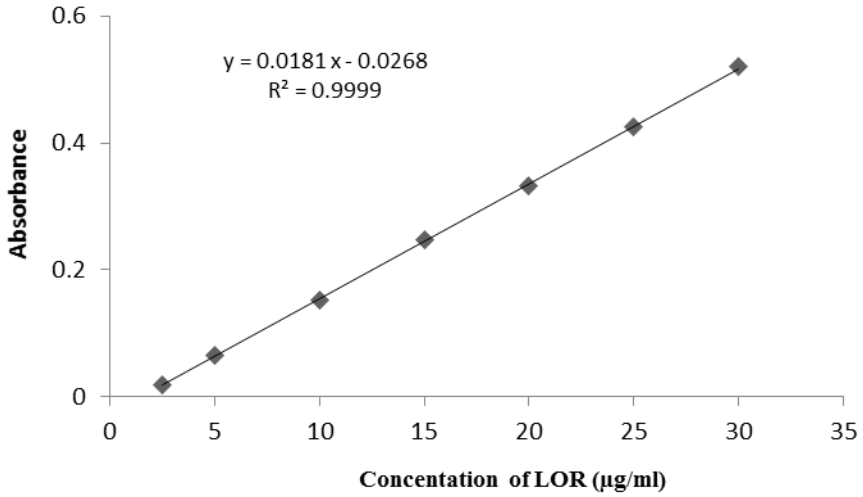
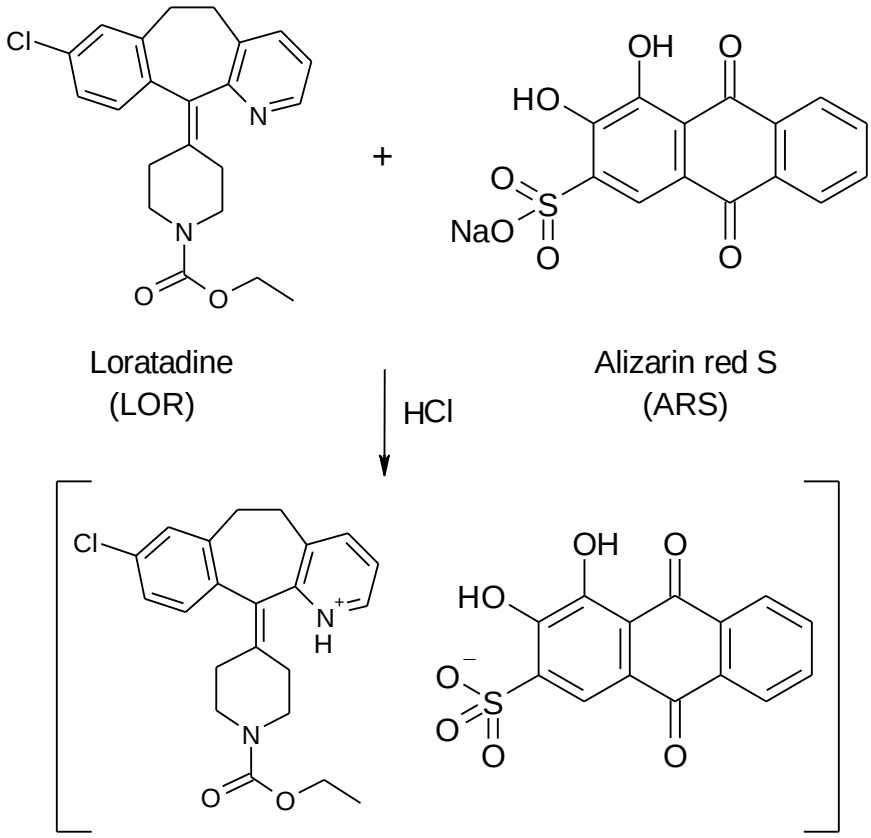
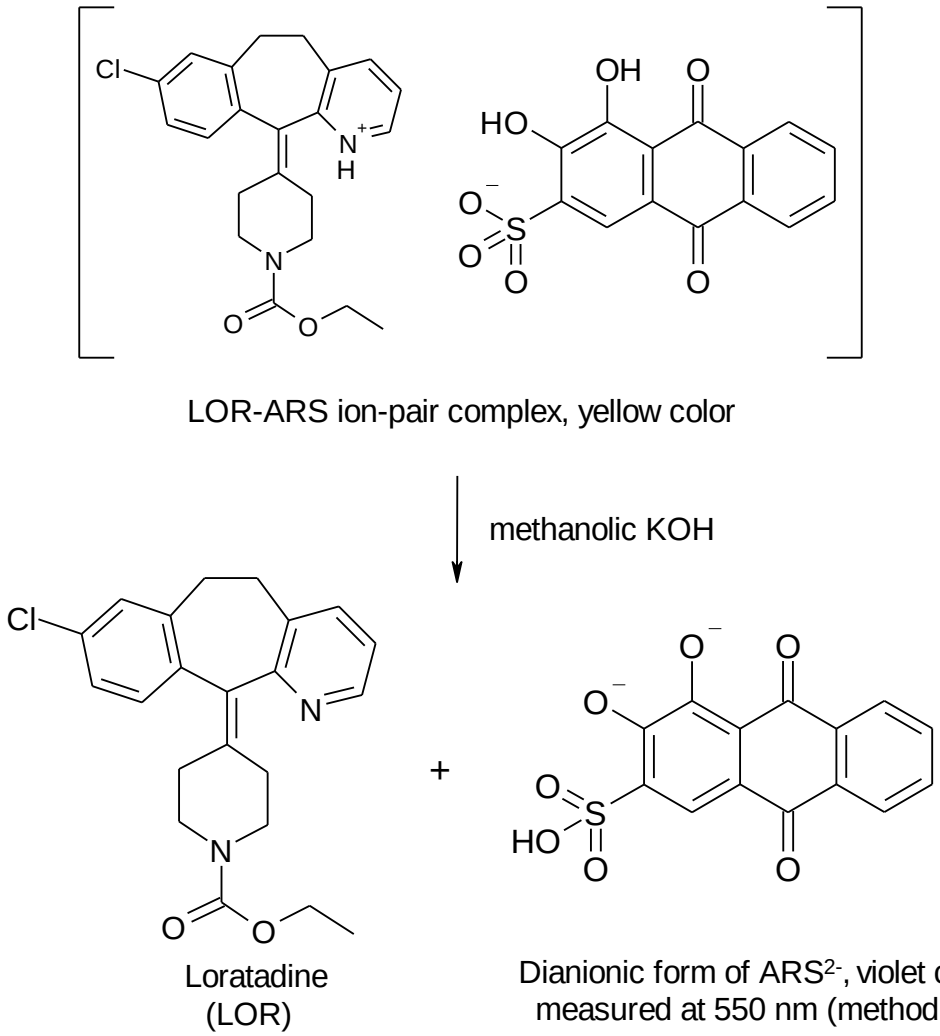


Figure 8. Calibration curve for method B



1:1 yellow ion-pair complex of LOR-ARS
measured at 425 nm (method A)

Scheme 1. The proposed mechanism for LOR-ARS ion-pair complex formation



Scheme 2. The proposed mechanism for the formation of dianionic form of ARS

TABLE 1 COMPARISON OF THE PERFORMANCE CHARACTERISTICS OF THE PROPOSED METHODS WITH THE EXISTING VISIBLE SPECTROPHOTOMETRIC METHODS

Reagent/s used	Methodology	λ_{max} (nm)	Linear range (μg/mL) (ε = L/mol/cm)	Remarks	Ref
Chloranilic acid (CAA)	Charge transfer complex measured	527	3.2 – 28.8 mg% (ε = NR)	Less sensitive	14
olybdenum thiocyanate,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ)	DCM extractable orange red ion-pair complex formed Charge transfer complex measured	469.5 588	2.5-22.5 10-80	Less sensitive, involves extraction step Less sensitive	15
erythrosine B	ion-pair complex formation	550	1-6 (ε = NR)	Less sensitive, pH adjustment required	16
romocresol purple eosin	Chloroform extractable ion pair complexation ion pair complex	409 539	20-55 ($\varepsilon = 0.64\times10^4$) 3-10 ($\varepsilon = 5.05\times10^4$)	Extraction step involved, pH adjustment required Methylcellulose solution added	17

3-methyl-2-benzothialinone hydrazone hydrochloride (MBTH) in presence of Ferric chloride Fe(III) Sodium periodate ($NaIO_4$)	oxidative coupling reaction	630 624	2-10 ($\varepsilon = 8.153 \times 10^3$) 5-25 ($\varepsilon = 9.397 \times 10^3$)	Multi-step reaction	18
Alizarin red S LOR-ARS ion pair broken with methanolic KOH	dichloromethane extractable ion-pair complex measured breaking of yellow LOR -ARS ion-pair complex and measurement of violet-colored dianionic form of ARS in alkaline medium	425 550	2.5-90 ($\varepsilon = 3.91 \times 10^4$) 2.5-30 ($\varepsilon = 6.65 \times 10^4$)	Simple, rapid, sensitive, selective and no heating step, use of single reagent, economical and no pH adjustment	Proposed methods

TABLE 2 EFFECT OF VOLUME STUDIED FOR THE AQUEOUS PHASE OF LOR-ARS ION-PAIR COMPLEX

Volume of Aqueous Phase (ml)	**Absorbance (AU) of**	
	LOR-ARS ion pair complex	**Reagent Blank**
15	0.2426	0.0234
20	0.2809	0.0238
25	0.3022	0.0184
30	0.1422	0.0285
35	0.1264	0.0218

TABLE 3 ANALYTICAL AND REGRESSION PARAMETERS

Parameter	**Method A**	**Method B**
λ_{max}, nm	425	550
Beer's law limit (µg/ml)	2.5-90	2.5-30
Concentration of reagent	0.05%	1%
Molar absorptivity (L/mol/cm)	3.91×10^4	6.65×10^4
Sandell sensitivity* (µg/cm^2)	9.8×10^{-9}	5.8×10^{-9}
Limit of detection (µg/ml)	0.8088	0.5105
Limit of quantification (µg/ml)	2.4510	1.5470
Regression equation, Y**		
Intercept (b)	0.0024	-0.0268
Slope (m)	0.0102	0.0181
Correlation coefficient (r)	0.9999	0.9999
Standard deviation of intercept (S_b)	0.0320	0.0118
Standard deviation of slope (S_m)	3.28×10^{-4}	4.70×10^{-4}

*Limit of determination as the weight in µg/ml of solution which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm

**y = mx + b, where y is the absorbance and x is the concentration in µg/ml.

TABLE 4 INTRA-DAY AND INTER-DAY PRECISION AND ACCURACY STUDIES

Method	LOR taken (µg/ml)	Intra-day (n=7)			Inter-day(n=5)		
		LOR found[a] (µg/ml)	%RSD[b]	%RE[c]	LOR found (µg/ml)	%RSD	%RE
Method A	40.0	40.38	2.13	0.95	40.56	2.06	1.40
	50.0	49.45	1.81	1.10	49.33	1.28	1.34
	60.0	60.52	2.02	0.86	60.67	1.32	1.12
Method B	10.0	10.15	1.07	1.50	10.13	1.28	1.30
	15.0	15.19	1.64	1.27	15.21	1.52	1.40
	20.0	20.38	1.36	1.90	20.26	1.44	1.30

[a]Mean value of n determinations.
[b]Relative standard deviation (%)
[c]Relative error (%).

TABLE 5 RESULTS OF ROBUSTNESS AND RUGGEDNESS

Method	LOR taken (µg/ml)	Robustness (%RSD)		Ruggedness (%RSD)	
		Parameters interchanged		Inter analysts (n=3)	Inter instruments (n=3)
		Volume of HCl/methanolic KOH[a] (ml)	Reaction time[b] (min)		
A	40	1.79	1.12	1.66	2.19
B	20	1.06	-	1.24	1.98

[a]In method A, volumes of 0.1M HCl were 4.8, 5.0 and 5.2 ml and in method B, volumes of methanolic KOH added were 1.8, 2.0 and 2.2 ml.
[b]In method A, the reaction times were 8, 10 and 12 min maintained during contact time.

TABLE 6 COMPARISON OF ASSAY RESULTS OF REFERENCE AND PROPOSED METHODS

Tablet brand name	Nominal amount, mg	Found % (of nominal amount ± SD) *		
		Reference Method	Proposed methods	
			Method A	Method B
Lorinol[a]	10	99.2 ± 0.82	100.16 ± 0.99 t = 1.86 F = 3.23	98.86 ± 1.28 t = 1.86 F = 1.27
Lorfast Meltab[b]	10	100.3 ± 1.5	100.10 ± 0.48 t = 1.83 F = 2.19	99.14 ± 1.63 t = 0.95 F = 2.34

*Mean value of five determinations
[a]Manufactured by Micro labs Ltd., South Sikkim, India
[b]Manufactured by Cadila Pharmaceuticals Ltd., India
Tabulated t-value at the 95% confidence level is 2.57
Tabulated F-value at the 95% confidence level is 5.05.

TABLE 7 RESULTS OF RECOVERY STUDY BY STANDARD ADDITION METHOD

Formulation studied	Method A				Method B			
	LOR taken (µg/ml)	Pure LOR added (µg/ml)	LOR total found (µg/ml)	Pure LOR recovered[a] (percent ± SD)	LOR taken (µg/ml)	Pure LOR added (µg/ml)	LOR total found (µg/ml)	Pure LOR recovered[a] (percent ± SD)
Lorinol (10 mg)	20.0	10.0	30.12	101.20 ± 1.06	10.0	5.0	14.93	98.60 ± 2.53
	20.0	20.0	39.69	98.45 ± 1.63	10.0	10.0	20.13	101.30 ± 1.34
	20.0	30.0	50.43	101.43 ± 0.96	10.0	15.0	24.76	98.40 ± 2.79
Lorfast Meltab (10 mg)	20.0	10.0	29.85	98.50 ± 2.02	10.0	5.0	15.05	101.0 ± 1.62
	20.0	20.0	40.12	100.60 ± 1.95	10.0	10.0	20.15	101.50 ± 1.46
	20.0	30.0	50.27	100.90 ± 1.64	10.0	15.0	24.94	99.6 ± 2.03

[a]Mean value of three determinations

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