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Spectrophotometric Method for the Determination of Some Drugs Using Fast Red B Salt

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Abstract: A simple spectrophorometric method for the determination of secnidazole, niclosamide, nifuroxazide and sulphasalzine is described. The method is based on reduction of the nitro group present in secnidazole and niclosamide molecule using zinc powder and dilute hydrochloric acid followed by reaction with fast red B salt in presence of ammonium chloride and sodium hydroxide, while in case of nifuroxazide and sulphasalazine the reaction takes place directly without any prior reduction between the phenolic group present in each drug and fast red B salt in presence of sodium hydroxide. Beer's law is valid in the concentration ranges 2.5-15, 1.25-10, 2.5-15, and 2.5-13.75 $\mu g.mL^{-1}$ for secnidazole, niclosamide, nifuroxazide and sulphasalazine respectively. The proposed method is applied successfully for the estimation of the mentioned drugs either in pure form or in their pharmaceutical formulations.

Keywords: Spectrometric method**,** Secnidazole, Niclosamide, Nifuroxazide and Sulphasalzine

Introduction

Secnidazole, 1-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol[1], is an antiprotzoal agent used in treatment of amoebiasis and has also been tried in trichomonasis[2]. The drug is not yet official in any pharmacopoeia. Spectrophotometric methods[3-7] were reported for the determination of secnidazole. Polarographic[8,9] and high performance liquid chromatography (HPLC) [10, 11] methods have been also proposed.

Niclosamide, 5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxy benzamide[12], is used as anthelmentic drug[2]. Spectrophotometric methods based on reaction with metol and chromium(VI)[13], coupling with 4-amino antipyrine[14], 3-methylbenzothiazolin-2-one hydrazone hydrochloride[15]. Ammonium reineckate[16], *p*-benzoquinone[17] and difference spectroscopy[18] were applied for the estimation of niclosamide as well as HPLC[19,20] and voltametric[21] methods.

Sulphasalazine, 2-hydroxy-5-[[4-[[(pyridin-2-yl) amino] sulphonyl] phenyl]azo]benzoic acid, is used in treatment of ulcerative colitis[2]. Few spectrophotometric methods were reported for the determination of secnidazole including near infra red spectroscopy[21], NMR-MS[22], HPLC[23-29] and voltametric [30-31] methods were also applied for its determination

Nifuroxazide,2´-(5-Nitrofurfurylidene)-4-hydroxybenzohydrazide[1], is an antibacterial used in the treatment of colitis and diarrhoea[2]. Spectrophotometric[33-38,] Polarographic[33,34,39], voltametric[40] and chromatographic methods[38,41,42] were used for its determination.

2-Methoxy-4-nitrobenzenediazonium 1,5-naphthalene disulfonate[43] (fast red B salt) is a commercially available solid diazonium salt, it was used as a spraying agent for the visual detection of metabolic products of tetrachlorobenzene, which is mainly tetrachloro phenol in the biological fluids of rates[44] and as spraying agent for the visual detection of some phenols on TLC silica gel plates[45-47]. Also it was used for the quantitative colorimetric determination of mefanamic acid in biological materials[48]. In the present investigation, we report the development of accurate, reproducible and sensitive spectrophotometric method based on the formation of azo dye from the coupling of a diazonium salt (fast red B salt) with either the amino group (resulted from the reduction of the nitro group) of secnidazole and niclosamide or the phenolic group of nifuroxazide and sulphasalzine. The proposed methods have been successfully applied for estimation of the cited drugs in pure form and in pharmaceutical formulations.

Experimental

Apparatus

SHIMADZU uv-1201 UV-Vis spectrophotometer equipped with 10 mm quartz cell was used for all spectral measurements.

Material and Reagents

All solvents and chemicals used were of analytical grade quality. Fast Red B salt (Merk, Germany) was prepared (0.05%) in methanol, filtered, kept in ice bath, protected from light and used within one hour. Sodium hydroxide 1% and 5% was prepared in distilled water. Ammonium chloride solution (5%) also prepared in distilled water.

Secnidazole (EPICO) stock solution was prepared (0.1%) by dissolved in distilled water. Niclosamide (Misr Co.) stock solution (0.1%) was prepared in ethanol. Nifuroxazide (AMOUN Co.) 0.025% solution was prepared by dissolved first in 5 mL dimethyle formamide and made up to 100 mL with methanol. Sulphasalzine (El Kahira pharmaceutica and Chemical Industries) solution (0.0125%) was prepared by dissolved first in 5 mL dimethyle formamide and made up to 100 mL with methanol.

Dosage forms

Secnidazole tablets (EPICO, Egypt) labeled to contain 500 mg of secnidazole per tablet. Niclosan tablets (Misr CO.) labeled to contain 500 mg of niclosamide per tablet. Antinal capsules (AMOUN Co.) labeled to contain 200 mg of nifuroxazide per capsule. Slazopyrine tablets (El Kahira pharmaceutica and Chemical Industries) labeled to contain 500 mg of sulphasalazine per tablet.

Procedures

Procedure for secnidazole and niclosamide

A known volume of stock drug solution containing 25 mg of secnidazole or 12.5 mg of niclosamide was transferred into 100 mL Erlenmeyer flasks. 10 mL 1 N hydrochloric acid and 2 g of zinc dust was added followed by occasional shaking. The flask was allowed to stand

for 1 h at room temperature. The reaction mixture was filltered into a 100 mL volumetric flask through a filter paper. The filter paper was washed twice with the appropriate solvent (water and ethanol for secnidazole and niclosamide respectively) and then maed up to complete volume with the appropriate solvent.

An accurately measured volume of the reduced drug solution (0.1-0.6 mL and 0.1-0.8 mL of secnidazole and niclosamide respectively) was pipetted out into a set of 10 mL volumetric flasks, then appropriate volume of fast red B salt solution (1 mL for secnidazole and 1.5 mL for niclosamide) was added followed by 1 mL ammonium chloride and sodium hydroxide (0.5 mL of 1% solution for secnidazole and 1 mL of 5% solution for niclosamide). The solution was made up to10 mL with distilled water. The absorbance was measured at 505 nm and 530 nm (for secnidazole and niclosamide respectively) against a blank reagent prepared simultaneously without drug (Table 1).

Procedure for nifuroxazide and sulphasalazine

An accurately measured volume of the standard drug solution (0.1 –0.6 mL and 0.2 –1.1 mL for nifuroxazide and sulphasalazine respectively) was pipetted out in a set of 10 mL volumetric flasks, then 1 mL of fast red B salt solution followed by 1% sodium hydroxide solution (1 mL for nifuroxazide and 1.5 mL for sulphasalazine) were added. The volume was diluted to 10 mL with distilled water. The absorbance was measured at 450 and 460 nm for nifuroxazide and sulphasalazine respectively against a blank reagent prepared simultaneously (Table 1).

Procedure for dosage forms

Procedure for secnidazole and niclosine

The content of 10 tablets under investigation was thoroughly powdered and mixed. An accurately weighed amount of the powder equivalent to 100 mg of secnidazole or niclosamide was dissolved in 50 mL in the proper solvent and filtered through a filter paper, washed with the proper solvent. The clear filtrate was diluted to 100 mL with proper solvent in 100 mL measuring flask. The appropriate volumes of each drug were pipetted out into a 100 mL Erlenmayer flask and preceded as in general procedure.

Procedure for antinal and salazopyrine

The content of 10 capsules or tablets of each under investigation was thoroughly powdered and mixed. An accurately weighed amount of the powder equivelent to 25 mg of nifuroxazide or 12.5 mg of sulphasalzine was dissolved in 50 mL of the proper solvent, and filtered through a filter paper, washed with the proper solvent. The clear filtrate was diluted to 100 mL with proper solvent in 100 mL measuring flask. The proper volumes of each drug were pipetted out into 10 mL volumetric flasks and preceded as in general procedure.

Results and Discussion

The most generally applicable method for the determination of phenol and primary aromatic amine is coupling with diazotized aromatic amine or diazonium salts, yielding a colored azo dye. The azo compounds formed are intensely colored because the diazenedyl linkage –N=N– brings two aromatic rings in conjugation. This gives an extended system of delocalized– electrons and allows absorption of light in the visible region (*i.e.* bathochromic shift).

Colorimetric determination of phenol and amines *via* coupling with diazonium salts can be used quantitvely. But as a rule, diazonium salts were unstable and reagent must be usually being prepared immediately prior to use. To overcome this latter problem, diazonium salts

in the solid state may be used[49]. The most stable solid diazonium salts are the fluroborates and double salts of diazonium chloride and metal salt such as zinc chloride. A large variety of these compounds are now commercially available. In this investigation, stabilized diazonium salt, 2- methoxy-4-nitrobenzene diazonium 1,5-naphthalene disulfonate which present in the solid state and can be prepared shortly before use. Fast red B salt is applied for the colorimetric determination of phenolic and amino drugs in pure form as well as pharmaceutical dosage forms.

Fast red B salt reacts with nifuroxazide and sulphasalazine (phenolic drugs) in faintly basic medium and with secnidazole and niclosamide (reduced drugs) in faintly acidic medium but in case of the reduced drug coupling will not occur if the acidity of the medium is too high. It is so obvious that neutralizing the high acidity of both HCl and SO_3H group of the diazonium salt will bring about coupling and this can be achieved by addition of sodium hydroxide. Coupling of diazonium salt with phenols or amines takes place at *p*-position but since the *p*-position is blocked in all drugs so coupling will takes place in *o*- position.

Fast red B salt was used for the determination of nifuroxazide and sulphasalazine and determination of secnidazole and niclosamide after reduction of the nitro group present in the drug moiety to amino group using hydrochloric acid and zinc dust. The colored compounds developed in alkaline medium were measured at 505, 530, 450 and 460 nm for secnidazole, niclosamide, nifuroxazide and sulphasalazine respectively (Figure 1).

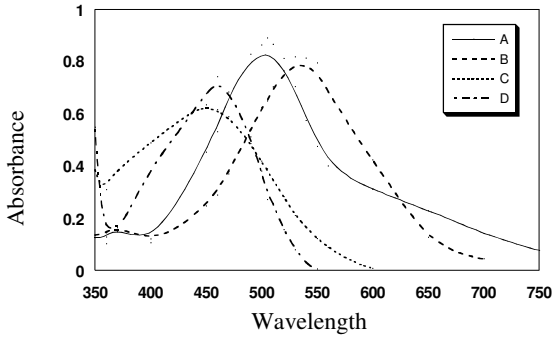


Figure 1. Abrorption spectra of the reaction between fast red B salt solution and (A) secnidazole 15 µg.mL^{-1}, (B) niclosamide 8.75 µg.mL^{-1} (C) nifuroxazide 10 µg.mL^{-1}and (D) sulphasalazine 11.25 µg.mL^{-1}.

Effect of reagent volume

When various volumes of fast red B salt were added to a fixed concentration of each studied drug, 1-1.5 mL of 0.05% reagent solution were found to be sufficient for the production of maximum and reproducible color intensity. Larger volumes lead to constant or decreased color intensity (Figure 2). The most favorable sequence is drug- reagent- sodium hydroxide for the highest color intensity and least developing time to reach maximum absorbance.

Effect of alkalinity

In order to establish the optimum alkaline condition for each azo dye formed, reagent under consideration was allowed to react with each studied drug in different concentrations of different types of alkalis. The highest absorbance values were obtained on using 5% sodium hydroxide for niclosamide and 1% sodium hydroxide for others. Furthermore, the amount of sodium hydroxide used varied from 0.5-1.5 mL according to the drug studied (Figure 3).

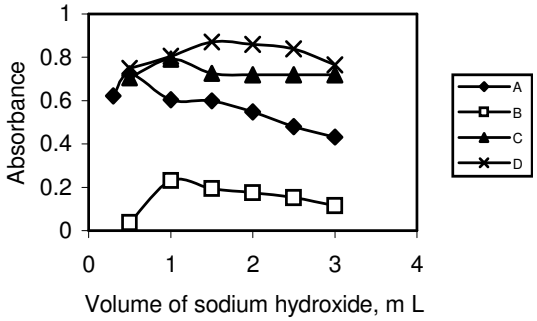


Figure 2. Effect of the volume of fast red B salt solution on its reaction with (A) secnidazole 12.5 µg.mL^{-1}, (B) niclosamide 6.25 µg.mL^{-1} (C) nifuroxazide 12.5 µg.mL^{-1} and (D) sulphasalazine 12.5 µg.mL^{-1}.

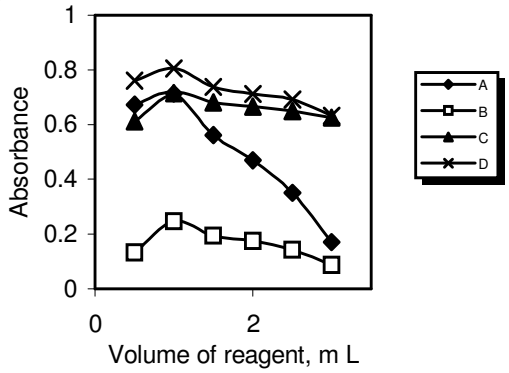


Figure 3. Effect of the volume of sodium hydroxide solution on the reaction between fast red B salt and (A) secnidazole 12.5 µg.mL^{-1}, (B) niclosamide 6.25 µg.mL^{-1} (C) nifuroxazide 12.5 µg.mL^{-1} and (D) sulphasalazine 12.5 µg.mL^{-1}.

Effect of ammonium chloride

In case of secnidazole and niclosamide, the use of excess sodium hydroxide is required to dissolve the precipitate of zinc hydroxide resulted from the reaction between sodium hydroxide and zinc chloride resulted from the reduction process but this leads to decrease in the absorbance so ammonium chloride is used prior to the addition of sodium hydroxide to prevent the precipitation of zinc hydroxide. The proper volume of ammonium chloride was found to be 1 mL of 5% solution for both drugs (Figure 4).

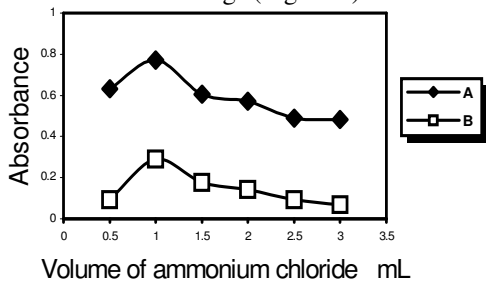


Figure 4. Effect of the volume of ammonium chloride solution on the reaction between fast red B salt and (A) secnidazole 12.5 µg.mL^{-1} and (B) niclosamide 6.25 µg.mL^{-1}.

Stoichiometric relationship

The stoichiometry of azo dye formed was determined by the molar ratio method using equimolar solutions (1×10^{-3} M). Fixed volume of each drug was used (0.2 mL for sulphasalazine and 0.5 mL for other drugs) and different volumes of fast red B salt were added to the drug. In all cases the plots reach maximum value at the point of equal volumes of drug and reagent, indicating the formation of 1: 1 drug to reagent ratio (Figure 5).

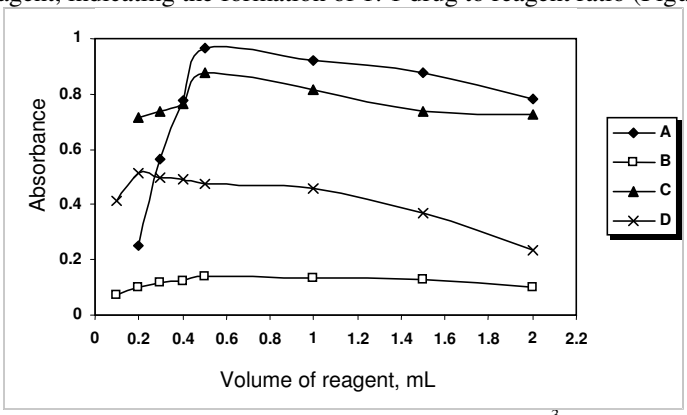


Figure 5. The molar ratio method for the reaction between 1×10^{-3} M fast red B salt and (A) 0.5 mL of 1×10^{-3} M secnidazole, (B) 0.5 mL of 1×10^{-3}M niclosamide (C) 0.5 mL of 1×10^{-3} M nifuroxazide and (D)0.2 mL of 1×10^{-3} M sulphasalazine.

Analytical data

A linear correlation was found between absorbance and concentration in the ranges given in Table 2. The correlation coefficients, intercepts and slopes for the calibration data for each system are calculated. The reproducibility of the methods was determined by running six replicate samples. The mean molar absorptivity and sandell's sensitivity as calculated from Beer's law are represented in Table 2. For more accurate results, Ringbom optimum concentration range was calculated and presented in Table 1.

Accuracy and precision

Applying proposed method on pure form of the studied drugs resulted in excellent recovery as proved by statistical comparison of the results obtained by those of the official or reference methods using *t*-test and *F*-test as presented in Table 3.

Table 1. Analytical parameters for the determination of the cited drugs using fast red B salt

Parameters	Secnidazole	Niclosamide	Nifuroxazide	Sulphasalazine
Volume or fast red B salt, mL	1	1.5	1	1
Volume of sodium hydroxide, mL	0.5,1% solution	1,5% solution	1,1% solution	1.5,1% solution
Volume of 5% NH_4Cl, mL	1	1	-----	------
λ_{max}	505	530	450	460
Beer's law limits, µg. mL^{-1}	2.5-15	1.25-10	2.5 -15	2.5-13.75
Ringbom concentration range, µg. mL^{-1}	3.06-10.59	1.49- 7.34	2.74- 12.64	3.34-11.56
Regression equation*				
Intercept, a	0.0004	0.0015	0.0011	-0.0015
Slope, b	0.0585	0.0902	0.0612	0.0677
Correlation coefficient, r	0.9999	0.9999	0.9999	0.9999

*$A = a + b\,C$, where C = concentration of drug in µg mL^{-1}, A = absorbance, a = intercept, b = slop.

Table 2. Results of the analysis for determination of the cited drugs using fast red B salt

Parameters	Secnidazole			Niclosamide			Nifuroxazide			Sulphasalazine		
	Taken $\mu g.mL^{-1}$	Found $\mu g.mL^{-1}$	Recovery %	Taken $\mu g.mL^{-1}$	Found $\mu g.mL^{-1}$	Recovery %	Taken $\mu g.mL^{-1}$	Found $\mu g.mL^{-1}$	Recovery %	Taken $\mu g.mL^{-1}$	Found $\mu g.mL^{-1}$	Recovery %
	2.5	2.48	99.55	1.25	1.24	98.89	2.5	2.48	99.28	2.50	2.51	100.73
	5.0	5.01	100.37	2.50	2.52	100.88	5.0	5.03	100.62	3.75	3.73	99.45
	7.5	7.48	99.73	3.75	3.77	100.66	7.5	7.53	100.41	5.00	4.94	98.81
	10.0	10.06	100.61	5.0	4.97	99.44	10.0	9.998	99.98	6.25	6.25	100.79
	12.5	12.54	100.32	6.25	6.22	99.60	12.5	12.49	99.98	7.50	7.55	100.73
	15.0	14.98	99.89	7.5	7.52	100.29	15.0	15.03	100.21	8.75	8.75	100.02
	-	-	-	8.75	8.78	100.41	-	-	-	10.0	10.02	100.22
	-	-	-	10.0	9.98	99.83	-	-	-	11.25	11.29	100.37
	-	-	-	-	-	-	-	-	-	12.5	12.51	100.14
	-	-	-	-	-	-	-	-	-	13.75	13.72	99.85
Mean	100.07			100			100.08			100.11		
±SD	0.415			0.675			0.463			0.624		
±RSD	0.414			0.675			0.463			0.624		
V	0.172			0.456			0.215			0.39		
±SE	0.169			0.238			0.189			0.197		
N	6			8			6			10		
Molar absorbitivity $L\ mol^{-1}cm^{-1}$	1.087×10^4			3.008×10^4			1.686×10^4			2.691×10^4		
Sandell sensitivity $\mu g.ml^{-1}$	0.017			0.0108			0.0163			0.0148		

Table 3. Statistical analysis of results obtained by the proposed method compared with official [12] and reference methods [5, 38]

Para-meters	Secnidazole		Niclosamide		Nifuroxazide		Sulphasalzine	
	Proposed method	Reference Method[5]	Proposed method	Official method[12]	Proposed method	Reference method[38]	Proposed method	Official method[12]
Mean*	100.07	100.08	100.0	99.40	100.08	99.83	100.41	99.94
N	6	6	8	5	6	9	10	6
V	0.172	0.221	0.456	0.44	0.215	0.653	0.39	0.703
±S.D.	0.415	0.47	0.675	0.663	0.463	0.808	0.624	0.838
±R.S.D.	0.414	0.47	0.675	0.667	0.463	0.81	0.624	0.839
±S.E.	0.169	0.192	0.238	0.296	0.189	0.27	0.197	0.342
t	0.019(2.23)[a]		1.57 (2.2) [a]		0.678(2.16)[a]		0.464(2.14)[a]	
f	1.07(5.05)[b]		1.036 (4.12) [b]		3.03(3.69)[b]		1.802(3.48) [b]	

**Average of 6 determinations. a and b are the Theoretical t-values and f-ratios at p_0.05.*

Table 4. Statistical analysis of results obtained by the proposed method for the studied drugs in their pharmaceutical preparations applying the standard addition technique.

Preparation	Taken µg.mL^{-1}	Added µg.mL^{-1}	Recovered	Recovery %	N	Mean	± SD	±RSD	±SE	V
Secnidazole tablets	2.5	2.5	2.52	101.07	5	100.17	0.654	0.652	0.292	0.427
		5.0	4.96	99.23						
		7.5	7.518	100.24						
		10.0	10.022	100.22						
		12.5	12.509	100.07						
Niclosan tablets	2.5	1.25	1.23	98.62	6	99.94	0.795	0.795	0.324	0.632
		2.5	2.52	100.89						
		3.75	3.75	100.19						
		5.0	4.98	99.62						
		6.25	6.28	100.51						
		7.5	7.48	99.78						
Antinal capsules	2.5	2.5	2.485	99.403	5	99.95	0.402	0.402	0.18	0.162
		5.0	4.99	99.93						
		7.5	7.54	100.53						
		10.0	10.004	100.04						
		12.5	12.48	99.88						
Salazopyrine tablets	2.5	2.5	2.51	100.41	7	100.10	0.421	0.421	0.159	0.177
		3.75	3.723	99.28						
		5.0	5.01	100.207						
		6.25	6.28	100.52						
		7.5	7.52	100.33						
		8.75	8.73	99.86						
		10.0	10.01	100.10						

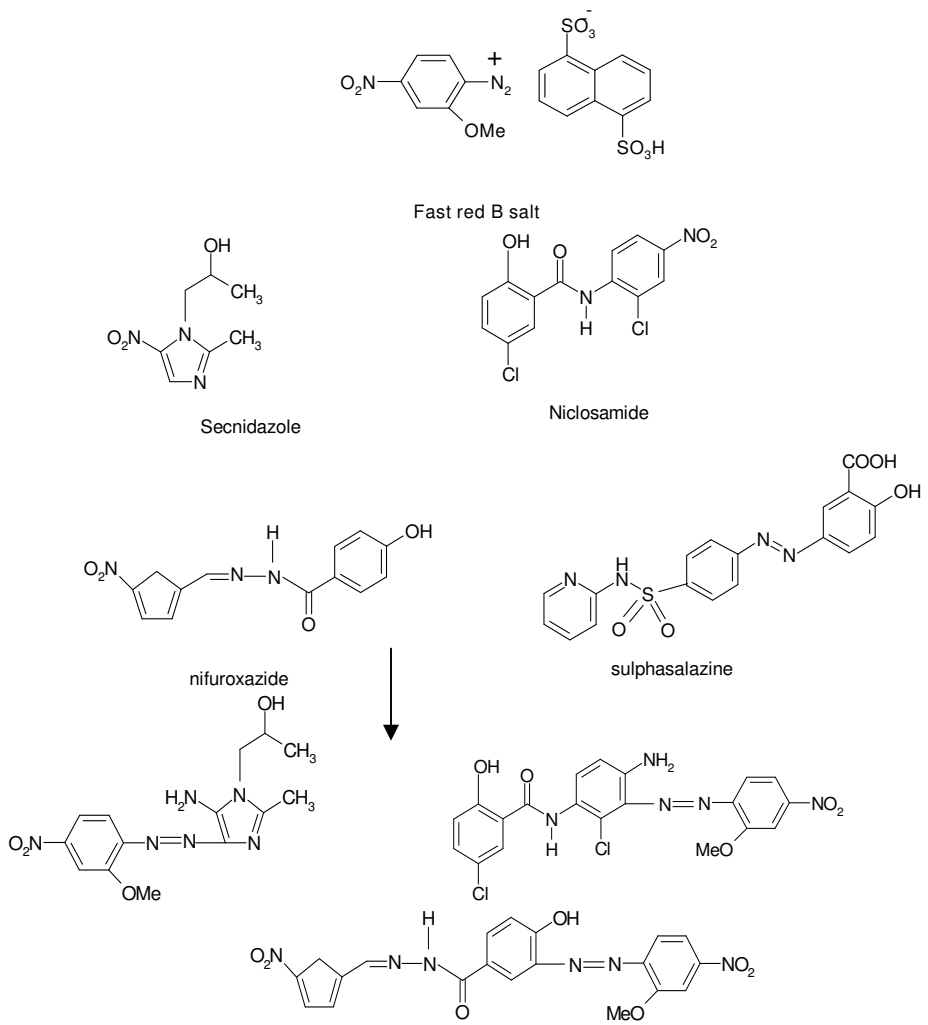
Analytical application

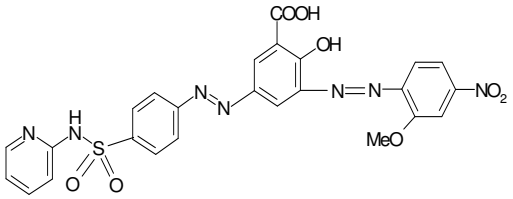
The validity of the proposed procedures are tested to determine the studied drugs in their dosage forms applying the standard addition technique and proved its suitability without interference from additives. The results obtained are given in Table 4.

Conclusion

The proposed method was successfully utilized for the determination of the studied drugs in pure form and in pharmaceutical formulations and proved to be highly sensitive, simple, accurate, precise and less time consuming. Student *t*- and *f*-values gave lower values than the theoretical ones indicating high accuracy and precision compared to the official one.

Suggested mechanism for the coupling reaction:





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