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SPECTROPHOTOMETRIC DETERMINATION OF NICRADIPINE AND ISRADIPINE IN PHARMACEUTICAL FORMULATIONS

A sensitive spectrophotometric method was developed for the determination of some 1,4-dihydropyridine compounds namely, nicardipine and isradipine either in pure form or in pharmaceutical preparations. The method is based on the reduction of nicardipine and isradipine with zinc powder and calcium chloride followed by further reduction with sodium pentacyanoaminoferrate (II) to give violet and red products having the absorbance maximum at 546 and 539 nm with nicardipine and isradipine, respectively. Beer's law was obeyed over the concentration range 8.0-180 μg/ml with the detection limit of 1.67 μg/ml for nicardipine and 8.0-110 μg/ml with the detection limit of 1.748 μg/ml for isradipine. The analytical parameters and their effects on the reported methods were investigated. The molar absorptivity, quantization limit, standard deviation of intercept (S_a), standard deviation of slope (S_b) and standard deviation of the residuals ($S_{y/x}$) were calculated. The composition of the result compounds were found 1:1 for nicardipine and 1:2 for isradipine by Job's method and the conditional stability constant (K_f) and the free energy changes (ΔG) were calculated for compounds formed. The proposed method was applied successfully for the determination of nicardipine and isradipine in their dosage forms. The results obtained were in good agreement with those obtained using the reference or official methods. A proposal of the reaction pathway was presented.

Key words: spectrophotometer; nicardipine; isradipine; pharmaceutical preparations.

Calcium antagonists block the influx of calcium ions through voltage-operated calcium channels located in the cell membrane. Among the different groups, dihydropyridines is the most numerous and includes the largest number of novel compound. They act upon the L-type channel, which has a specific dihydropyridine site in its extra cellular surface and bind more selectively to vascular calcium channels than to those in the myocardium. Newer dihydropyridines exhibit greater selectivity, with evidence for a specific vascular vessel bed binding. Each of these agents is effective in the treatment of hypertension and angina pectoris [1-3].

The therapeutic importance of dihydropyridines initiated several methods on its determination, both in formulations and in biological fluids. These methods include: spectrofluorometric [4], voltammetric [5-9], amperometric [10] thin-layer chromatographic [11], capillary gas chromatographic [12] and high-performance liquid chromatographic [13-15]. The visible spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories because of their simplicity, selectivity and sensitivity. In the literature, only few spectrophotometric methods have been reported for dihydropyridines compounds. Derivative spectrophotometric procedures have been described for the determination of amlodipine besylate based on the formation of an oxidative coupling product of the drug with 3-methyl-2benzothiazolinone hydrazone hydrochloride in the presence of ceric ammonium sulphate [16]. Another spectrophotometric method for the determination of nifedipine depends on the reduction of nitro group to hydroxylamino group which then reacted with *N*-methyl-1,4-benzoquinoneimine to form colored product [17]. A number of other extractive spectrophotometric methods [18-21] have also been reported in literature for the assay of dihydropyridines in pharmaceutical formulations. The charge transfer reaction has been used for the determi-

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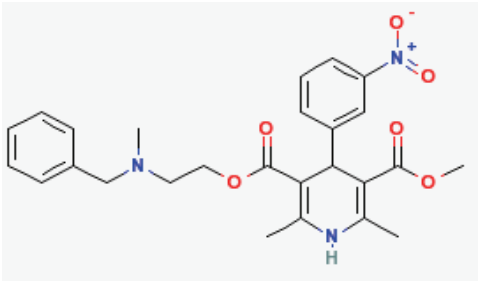
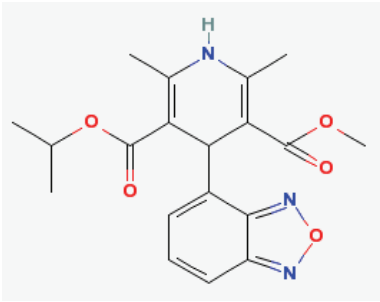
nation of amlodipine besylate with *p*-chloranilic acid in 1,4-dioxan-chloroform medium[22].

Sodium pentacyanoaminoferrate (II) (SCAF) has been used to detect aromatic nitro compounds [23].

The compounds studied were: nicardipine (NIC), 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)methyl-2-[methyl(phenyl methyl)amino]-3,5-pyridinedicarboxylic acid ethyl ester, and isradipine (ISRA), 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinecarboxylic acid methyl 1-methylethyl ester.



Nicardipine



Isradipine

The aim of this work is the development of a simple and sensitive method for the determination of NIC and ISRA in the pure and pharmaceutical preparations. The proposed method is based on the reduction of nicardipine and isradipine with zinc powder and calcium chloride followed by further reduction with sodium pentacyanoaminoferrate (II) to give color products. The results obtained were promising.

EXPERIMENTAL

Apparatus

All Absorption spectra were made M501 using UV-Visible spectrophotometer (Cambridge, UK) with a scanning speed of 200 nm/min, wavelength range 190-1100 nm and a band width of 2.0 nm, equipped with 1 cm quartz cells.

Materials and reagents

All chemicals and reagents used were of analytical grade and used without further purification. Double distilled de-ionized water was used to prepare all solutions.

Materials

Nicardipine hydrochloride was obtained from Sigma chemical co. (St. Louis, MO, USA). Isradipine was gratefully gifted by Novartis Pharma (Basle, Switzerland).

Pharmaceutical formulations

Tablets contained isradipine (Lomir tablets), labeled to contain 2.5 mg of isradipine per tablet (Novartis Pharma, AG, Basle, Switzerland). Capsules contained nicardipine (Pelcard capsules), labeled to contain 50 mg of nicardipine per capsule (Global Napi Pharmaceuticals, Egypt).

Standard solutions

Stock solutions of pure NIC and ISRA were prepared separately by dissolving 0.1 g of NIC and ISRA in methanol then completed to 100 ml with the same solvent. Working solutions of lower concentrations were prepared by the appropriate dilution with methanol.

Reagents

Sodium pentacyanoaminoferrate (II) $Na_3[Fe(CN)_5NH_3]$ (SCAF) was prepared according to Vogel [24], and used as a 0.1% solution, made fresh daily.

Calcium chloride solution, 10% (w/v), was obtained from (BDH, Poole, England).

Zinc powder was obtained from (BDH, Poole, England).

General recommended procedures

Procedure for calibration graphs

Into a series of 25 ml measuring flasks, accurately measured aliquots of standard NIC or ISRA solutions in the concentration range shown in (Table 1) were transferred. A 0.3 g of zinc powder for NIC or 0.1 g zinc powder for ISRA and 5 ml of calcium chloride solution were added for each flask. The reaction mixture was left to stand for 5 min at room temperature with occasional shaking. The mixture was filtered through a dry filter paper (Whattman No. 42) into 25 ml measuring flasks. Each residue was washed thoroughly with three 2-ml portions of ethanol and washings passed to the same flask. 5 ml of SCAF solution was added to each combined filtrate and washings, and the mixture was left to stand for 10 min at room temperature. The volume was completed to the mark with distilled water. The absorbance was measured at 546 or 539 nm for NIC or ISRA, respectively. A blank reagent without the drug was carried out simultaneously. Calibration graphs were constructed by plotting

the absorbance versus the final concentration (μg/ml) of the drugs. Alternatively, the regression equations were derived.

Procedure for pharmaceutical formulations

The contents of twenty tablets (Lomir tablets labeled to contain 2.5 mg of isradipine per tablet) or contents of ten capsules (Pelcard capsules labeled to contain 50 mg of nicardipine per capsule) were crushed, powdered or emptied. An accurate weight equivalent to 25 mg NIC or ISRA was dissolved in 20 ml of methanol with shaking for 5.0 min and filtered. The filtrate was diluted in a 25 ml measuring flask with methanol. An aliquot of the diluted drug solution was treated as described. The found content of the tablets or capsules were calculated either from the calibration graph or the regression equation.

RESULTS AND DISCUSSION

Optimization of experimental parameters

The reduction of NIC or ISRA with the zinc and calcium chloride solution and subsequently reacted with SCAF in aqueous ethanol, produces a violet or red product, respectively, having a broad absorption peak with its maximum at 546 nm for NIC and 539 nm for ISRA (Figure 1). The various experimental factors affecting the development and stability of the reaction product were studied and optimized. Such factors were changed individually, while others remained constant, which include the amount of zinc powder, the concentration of the calcium chloride solution, the concentration of SCAF solution, time of reaction, the effect of the reaction temperature, stability of the product and the effect of interferences.

Table 1. Analytical parameters for the determination of nicardipine and isradipine using the proposed method

Parameter	Compound	
	Nicardipine	Isradipine
	c / μg ml^{-1}	
	8-180	8-110
Wavelength λ_{max}, nm	546.0	539.0
Molar absorption coefficient, 10^3 l mol^{-1} cm^{-1}	2.41	2.73
Linear regression equation	$A = 0.005c + 0.002$	$A = 0.006c + 0.032$
Correlation coefficient, r	0.9960	0.9970
Detection limit, μg/ml	1.67	1.74
Quantiation limit, μg/ml	5.58	5.27
$S_{y/x}\times10^3$	6.01	5.62
$S_a\times10^3$	2.79	3.16
$S_b\times10^5$	10.2	5.1
$K_f\times10^{-3}$	104.12	0.3725
ΔG, kJ/mol	-6.27	-3.51

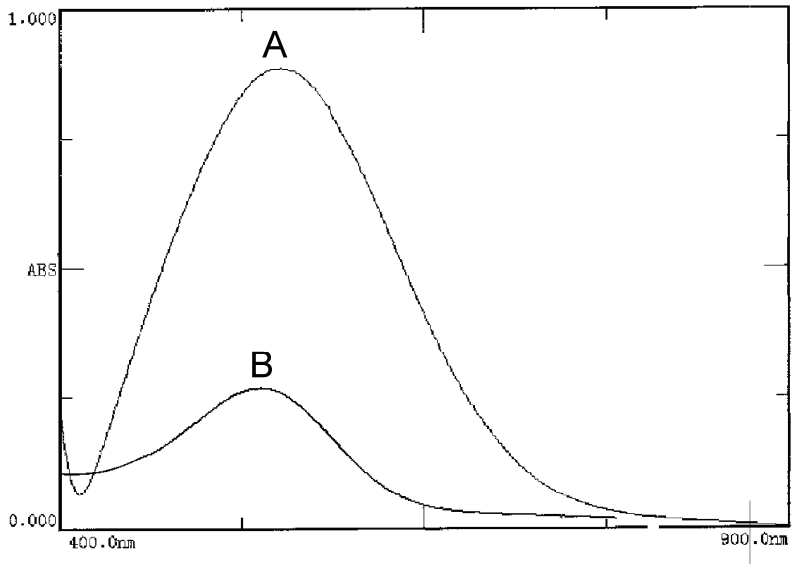


Figure 1. Absorption spectrum of the reduction product of nicardipine (160 μg/ml) (A) and isradipine (36 μg/ml) (B) with SCAF.

Effect of the amount of zinc powder

The influence of the amount of zinc powder was studied using different amounts of zinc 0.05-0.6 g. It was found that the reaction took place starting from 0.2 g upwards for NIC and hence, 0.3 g was selected as the optimum amount. The absorbance remains constant by using 0.05-0.1 g of zinc powder for ISRA, then the absorbance decreases by increasing the amount of zinc and hence, 0.1 g was selected as the optimum amount.

Effect of calcium chloride solution concentration

The reduction between the studied drugs and zinc powder takes place in the presence of calcium chloride. Different volumes of $CaCl_2$ solution ranging from 1.0-8.0 ml (10% w/v) were tested along with the amount of zinc chosen. It was noticed that 1-3 ml of 10% $CaCl_2$ was necessary without altering the completeness of reduction of NIC or ISRA.

Effect of SCAF concentration

The effect of SCAF was studied by using increasing concentrations of SCAF. It was found that 100 or 280 μg/ml of SCAF is appropriate for maximum absorbance intensity for NIC or ISRA, respecttively. Excess concentrations of SCAF (300-400 μg/ml) had little effect on the absorbance of ISRA.

Effect of reaction time

The reaction of NIC or ISRA and SCAF depends on time. Maximum absorbance intensity was observed after 8.0 min for NIC and 3.0-6.0 min for ISRA, at room temperature.

Effect of reaction temperature

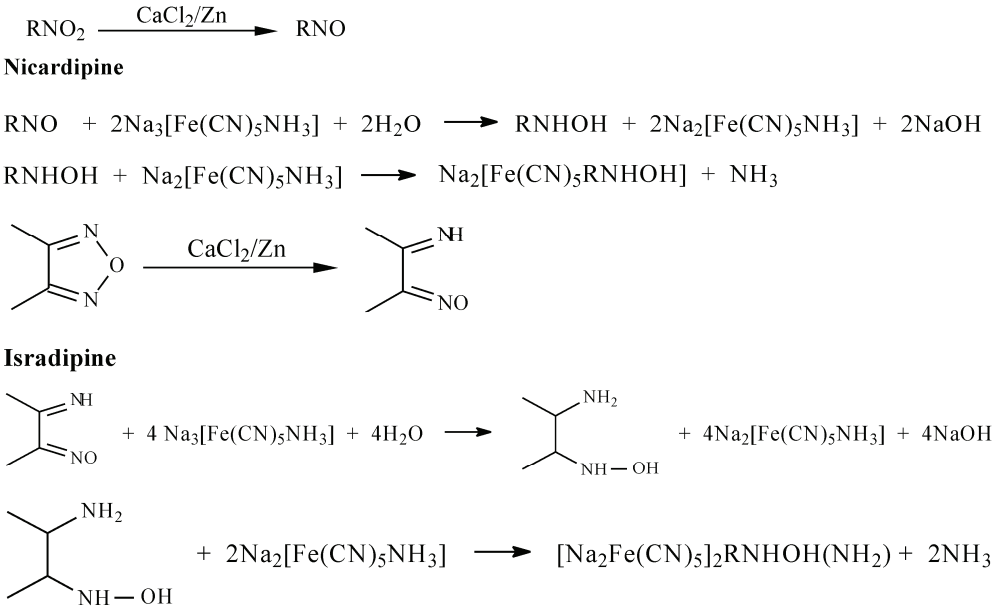
The reduction of NIC or ISRA was studied at different temperatures (25-100 °C). The values of maximum absorbance of the reduction product were almost constant from 25 to 65 °C for NIC, further temperature decreases the absorbance. For ISRA, it was observed that raising the temperature up to 30 °C has no effect on the absorbance of the product, whereas above 30 °C, the absorbance starts to decay. Therefore, working at room temperature of about 25 °C was recommended in the procedure.

Stability of the product

The stability of the reduction product was evaluated and constant absorbance readings were obtained after more than 5 h of standing at room temperature without any change in color intensity.

Stoichiometric relationship

Job's method of continuous variation [25] of equimolar solutions was employed: 2.7×10^{-3} M standard solution of drugs and 2.7×10^{-3} M solution of SCAF was used. A series solution was prepared in which the total volume of drug and reagent was kept at 10 ml. The absorbance was measured at the optimum wavelength. The molar ratio of the reagent (drug: SCAF) in the product was determined by the method continuous variations (Job's method).The results indicate that (1:1) for (NIC:SCAF) and (1:2) for (ISRA:SCAF) are formed. The suggested mechanism for the reaction products are given in (Scheme 1).



Scheme 1. Proposed pathway of the reduction of NIC and ISRA.

Conditional stability constants (K_f)

The conditional stability constants (K_f) of the reduction products were calculated from continuous variation data using Mollard method [26]. Using this equation, the stability constants were found to be as shown in Table 1. These values obtained indicate very stable reaction products. The standard free energy changes of production (ΔG) were calculated from the association constants (Table 1) by the following equation [27]:

$$\Delta G = -2.303RT\log K_f$$

Where ΔG is the free energy change of the product (kJ mol^{-1}), R the gas constant (1.987 cal mol^{-1} K^{-1}), T the temperature in Kelvin (273 + t (°C)), and K_f is the association constant of drug-reagent reduction products (l mol^{-1}). The negative values of ΔG point out to the spontaneous nature of the reactions.

Effect of interferences

In order to evaluate the selectivity of the proposed method for the analysis of pharmaceutical formulations, the effects of the presence of excipients and additives which can occur in real samples were investigated. It was found that the presence of common excipients of tablets and capsules such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate did not interfere with the determination of the studied drugs at the levels normally found in dosage forms.

Method validation

Linearity

Standard calibration curves were constructed by plotting absorbance versus concentration (μg/ml) as follows:

For NIC concentration range (8.0-180 μg/ml):

$A = 0.005c + 0.002, \quad r = 0.9960$

For ISRA concentration range (8.0-110 μg/ml):

$A = 0.006c + 0.032, \quad r = 0.9970$

The statistical parameters were given in the regression equation calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation residuals ($S_{y/x}$).

The linearity of calibration graphs was proved by high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The apparent molar absorptivities of the resulting colored reduction products and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in Table 1.

Sensitivity

The detection limits (LOD) for the proposed methods were calculated using the following equation [28]:

$$LOD = \frac{3s}{k}$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 1.67 μg/ml for NIC and 1.74 μg/ml for ISRA.

The limits of quantization, LOQ, are defined as [28]:

$$LOQ = \frac{10s}{k}$$

According to this equation, the limits of quantization were found to be 5.58 μg/ml for NIC and 5.27 μg/ml for ISRA (Table 1).

Specificity, precision, and accuracy

The specificity of the reduction reaction and selective determination of NIC and ISRA could be possible. Percentage relative standard deviation (*RSD, %*) as precision and percentage relative error (*Er, %*) as accuracy of the suggested method was calculated. The precision was carried out by three determinations at 13 different concentrations in this spectrophotometric method. The percentage relative error was calculated using the following equation:

$$Er = 100\frac{Found - Added}{Added}$$

The precision and accuracy results are shown in Table 2. These results of accuracy and precision show that the proposed method has good repeatability and reproducibility.

Analysis of pharmaceutical formulations

The proposed method has been successfully applied to the determination of NIC and ISRA in pure samples. The results obtained were compared with those given by the reference RP-HPLC method [29] and the official method [30]. The reference method [29] adopted for the quantization of nicardipine hydrochloride in capsules. The RP-HPLC involved the employment of μ-Bondapak-C_{18} column and the mobile phase consisting of 80% (v/v) CH_3CN and 20% (0.01 M) sodium acetate buffer adjusted to pH 3.5 with ace-

tic acid, the flow rate was 1.5 ml/min. The chromatographic procedure official method [30] for the determination of isradipine was carried out using a stainless steel column (10 cm×4.6 mm) packed with the stationary phase C (5 µm) (Brownlee Spheri ODS 5µ is suitable), and the mobile phase consisting of a mixture of 125 volumes of acetonitrile, 270 volumes of tetrahydrofuran and 625 volumes of water with a flow rate of 1.2 ml per min and a detection wavelength of 230 nm.

Statistical analysis [28] of the results obtained from both the methods revealed no significant difference between the performance of the two methods regarding accuracy and precision as revealed by Student's *t*-test and variance ratio, *F*-test, (Table 2).

The proposed method was further applied to commercial tablets and capsules containing NIC or ISRA. The results obtained are shown in Table 3. The average percent recoveries were quantitative, indicating good accuracy of the method and the value of *t*-test and *F*-value revealed no significant difference between the performances of the two methods.

The accuracy and precision (intra-day precision or repeatability and inter-day or intermediate preci-

Table 2. Application of the proposed spectrophotometric method for the determination of nicardipine and isradipine in pure forms

Compound	Amount added µg/ml	Amount found µg/ml	Recovery[a], %	*RSD*, %	*Er*, %	Amount added µg/ml	Reference and official methods [29,30]
Nicardipine	8	7.97	99.59	0.88	-0.41	0.02	101.60
	12	12.07	100.55	0.73	0.55	0.04	99.75
	16	15.84	98.98	0.33	-1.02	0.08	100.93
	24	24.03	100.14	0.45	0.14	0.10	101.62
	32	31.25	97.64	1.12	-2.36	0.12	99.28
	36	36.33	100.91	0.64	0.91	-	-
	60	60.68	101.14	0.40	1.14	-	-
	80	79.63	99.54	0.77	-0.46	-	-
	100	100.16	100.16	1.35	0.16	-	-
	120	121.21	101.01	0.98	1.01	-	-
	140	140.68	100.49	0.76	0.49	-	-
	160	158.58	99.11	0.81	-0.89	-	-
	180	181.74	100.96	0.95	0.96	-	-
Mean±*SD*			100.02±1.02			-	100.64±1.08 (*n* = 5)
RSD, %			1.1979			-	-
F			1.12 (5.14)			-	-
t			1.14 (2.31)			-	-
Isradipine	8	8.08	101.03	0.55	1.03	0.02	97.53
	12	12.00	100.00	0.78	0.00	0.04	101.45
	16	16.12	100.77	1.02	0.77	0.06	102.85
	20	19.94	99.69	0.99	-0.31	0.08	96.53
	28	28.39	101.40	0.32	1.40	0.10	98.38
	36	35.92	99.77	0.65	-0.23	0.12	102.55
	50	49.82	99.64	0.44	-0.4	-	-
	60	58.82	98.03	0.71	-2.0	-	-
	70	68.82	98.31	0.39	-1.7	-	-
	80	80.62	100.78	0.86	0.8	-	-
	90	88.62	98.47	1.22	-1.5	-	-
	100	100.42	100.42	0.89	0.4	-	-
	110	108.02	98.20	0.40	-1.8	-	-
Mean±*SD*			99.73±1.16			-	99.88±2.74 (*n* = 6)
RSD, %			1.1631			-	-
F[b]			5.60 (5.79)			-	-
t[b]			0.31 (2.37)			-	-

[a]The average of three trials; [b]the figures in parentheses are the theoretical values of *t* and *F* values at 95% confidence limit

Table 3. Application of the proposed spectrophotometric method for the determination of nicardipine and isradipine in their pharmaceutical formulations

Preparation	Amount taken µg/ml	Amount found µg/ml	Recovery[a], %	Amount added µg/ml	Reference and official methods [30,31]
Pelcard capsules labeled to contain 50 mg of nicerdipine per capsule[b]	32	31.62	98.84	0.04	101.12
	40	39.96	99.91	0.10	100.52
	48	48.11	100.23	0.12	100.32
	56	55.52	99.14	-	-
	64	64.22	100.35	-	-
	72	71.26	98.97	-	-
	80	79.04	98.80	-	-
Mean±*SD*			99.46±0.68		100.65±0.42
F			2.62 (8.89)		
t			0.20 (2.31)		
Lomir tablets labeled to contain 2.5 mg of isradipine per tablet[c]	16	15.87	99.19	0.04	99.84
	24	24.02	100.08	0.10	101.11
	32	31.79	99.36	0.12	101.34
	40	39.94	99.86	-	-
	60	59.39	98.98	-	-
	80	79.20	99.00	-	-
Mean±*SD*			99.41±0.46		100.76±0.80
F			3.02 (4.76)		
t			0.38 (2.37)		

[a]The average of three trials; [b]Global Napi Pharmaceuticals, Egypt; [c]Novartis Pharma, AG, Basle, Switzerland

sion) of this method, evaluated by assaying three different concentrations of NIC and ISRA, are summarized in Table 4. The differences of the mean value measured from the concentration prepared, expressed in percentages, were only -0.21, 0.13 and -3.88% at 40, 60 and 80 µg/ml of NIC and 0.34, 1.23 and -3.41 µg/ml at 40, 60 and 80 µg/ml of ISRA, which confirmed the accuracy of the method. The range of percentage of relative standard deviation (*RSD*) was 1.9--3.6% and 2.7-3.7% for NIC and 1.6-5.1% and 1.6-4.6% for ISRA for within-day and between-day analyses, respectively. The RSD values obtained allow us to conclude that the method has an acceptable precision.

A systematic study of the effects of excipients was performed by adding a known amount of the excipient to 80 µg/ml NIC or ISRA, filtering off the insoluble excipient, washing the residue, diluting in a volumetric flask and analyzing following the recommended procedure. The results revealed that no significant interference was observed from excipients, such as starch, talc, gelatin, glucose and magnesium stearate.

CONCLUSION

The present method has the advantages of high sensitivity (detection limit = 1.67 for NIC and 1.74 µg/ml for ISRA). On the other hand, the proposed method is low cost, selective, accurate and precise (intra-day and inter-day precision) as indicated by the good results of the drugs. Furthermore, the proposed method does not require the elaboration of the procedures which is usually associated with chromatogra-

Table 4. Precision of the method for the determination of NIC and ISRA in pharmaceutical formulations, expressed as relative standard deviation (RSD)

Compound	Concentration, µg/mL	Accuracy, %	Intra-day precision *RSD*, %	Inter-day precision *RSD*, %
Nicardipine	40	-0.21	3.6	2.7
	60	0.13	1.9	3.7
	80	-3.88	2.7	3.5
Isradipine	40	0.34	1.6	1.9
	60	1.23	3.5	4.6
	80	-3.41	5.1	2.8

phic methods. The disadvantage of this method is the tediousness of filtration. The proposed method could be applied successfully for the determination of nicerdipine and isradipine in pure forms, as well as dosage forms, with no interferences from tablets or capsules excipients.

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