

Multicomponent Spectrophotometric Method for Simultaneous Analysis of Delapril and Indapamide in tablets

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A multicomponent ultraviolet (UV) spectrophotometric method was developed and validated for simultaneous determination of delapril (DEL) and indapamide (IND) in tablets employing the partial least squares regression (PLSR) approach. The PLSR method was developed by a multilevel factorial design using 25 synthetic mixtures of drugs and a significant predict model ($p < 0.05$) were obtained at 225 nm for DEL ($R^2 = 0.9992$) and 243 nm for IND ($R^2 = 0.9997$). Validation parameters such as the specificity, linearity, precision, accuracy and robustness were evaluated in accordance with the ICH requirements, giving satisfactory results within the acceptable range. The proposed PLSR method was successfully applied for simultaneous determination of DEL and IND in fixed dose combinations and can be used as simple alternative to separation techniques.

Keywords: Delapril; Indapamide; Multicomponent spectrophotometry; Validation.

Introduction

Hypertension is one of the most important risk factors of cardiovascular diseases and thus to achieve blood pressure targets as soon as possible is imperative. Combination therapy for antihypertensive treatment is being increasingly recommended once monotherapy is not the adequate control of blood pressure in most patients (1,2).

A well-established antihypertensive combination can be observed for delapril (DEL) and indapamide (IND), an angiotensin-converting enzyme inhibitor and a thiazide diuretic, respectively. Due to the additive and synergistic mechanisms of actions of agents from these two drug classes, a low dose was required for effective antihypertensive effect. DEL 30 mg and IND 2.5 mg have been combined in a fixed tablet, offering the convenience and good tolerability for most patients (3,4). The chemical structures of drugs are shown in Figure 1.

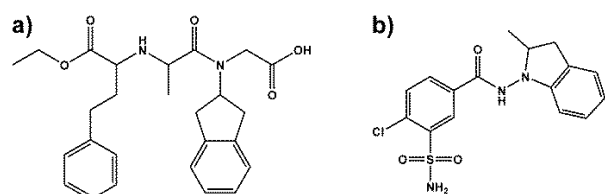


Figure 1 Chemical structures of DEL (a) and IND (b).

The tablet pharmaceutical dosage form is commercially available, but at the moment, there are no published methods for simultaneous analysis of DEL and IND as raw material or finished product. However, the current literature reveals a few number of

publications related of drugs in single dosage form or in association with others drugs. For DEL determination, these studies were performed by liquid chromatography (5), liquid chromatography-tandem mass spectrometry (6), capillary electrophoresis (7), and derivative spectrophotometry (8). On the other hand, British Pharmacopoeia, European Pharmacopoeia and United States Pharmacopoeia present official methods by liquid chromatography and spectrophotometry for IND assay in tablets and raw materials. Many of these methods use expensive and hazardous chemicals, require expertise, making the process a challenge for the environment, time consuming and complex.

The use of spectrophotometric methods provides practical and economic advantages over other instrumental techniques and could be a useful alternative for quality control routine. The development of a more convenient, simple, less time-consuming and environmentally friendly spectrophotometric method is desirable. However, the simultaneous determination of two or more active components in different pharmaceutical preparations without previous chemical separation is a common analytical problem (9,10).

The application of multivariate calibration approaches in spectrophotometry offers advantage in complex systems resolution and mixtures analysis. Among the chemometric techniques applied to multicomponent analysis, the partial least squares regression (PLSR) has been successfully used in different quantitative assays of pharmaceutical formulations (11-14).

Considering the absence of analytical methods for the simultaneous determination of DEL and IND in tablets, we decided to develop and validate a simple, fast and cost-effective multicomponent UV

spectrophotometric method employing the PLSR approach, contributing to research of green and practical alternatives methods for quality control and assuring the therapeutic efficacy of products.

Experimental

Chemicals

DEL and IND reference substances were kindly donated by Chiesi Farmaceutici (Parma, Italy) and Prati Donaduzzi (Toledo, Brazil), with a purity of 99.6% and 99.4%, respectively. Delapride® tablets (containing 30 mg and 2.5 mg of DEL of IND) were obtained from commercial source and used within the expiration date. The dosage form excipients (lactose monohydrate, magnesium stearate, hydroxypropyl cellulose and low substituted hydroxypropyl cellulose) were all pharmaceutical grades and acquired from different suppliers. Analytical grade ethanol was obtained from Vetec Química Fina Ltda (Rio de Janeiro, RJ, Brazil) and Êxodo Científica (Hortolândia, SP, Brazil). For all the analyses, ultrapure water (Milli-Q® Direct Water Purification System, Merck Millipore Corporation, Darmstadt, Germany) was used.

Analytical Solutions

The reference solutions were prepared by weighing the equivalent of 30 mg of DEL and 2.5 mg of IND chemical standards and diluted to volume with ethanol, obtaining the concentration of 300 $\mu\text{g mL}^{-1}$ and 25 $\mu\text{g mL}^{-1}$ for DEL and IND, respectively. To prepare the sample solutions, tablets containing 30 mg of DEL and 2.5 mg of IND were accurately weighed and crushed to fine powder. An appropriate amount was transferred into an individual 100 mL volumetric flask, diluted to volume with ethanol, kept in vortex for 5 min, sonicated for 15 min and filtered through a 0.45 μm membrane filter (Millipore), obtaining theoretical concentrations of 300 $\mu\text{g mL}^{-1}$ and 25 $\mu\text{g mL}^{-1}$ for DEL and IND, respectively. The reference solutions were stored at 2-8 °C, protected from light and daily diluted to an appropriate concentration in ethanol daily.

UV Apparatus

A double beam UV-Visible spectrophotometer (PerkinElmer, Singapore), model Lambda 35, was used in this study. The instrument was equipped with 1 cm quartz cells, with PerkinElmer UV WinLab software (version v5) for instrument control, data acquisition and analysis. All spectra were recorded in the range 200–300 nm with 0.1 nm intervals, at scanning speed of 960 nm min^{-1} , with a fixed slit to lead to a spectral resolution of 1 nm.

UV Procedure

The multivariate method was performed by the partial least squares regression (PLSR) mode. The PLSR experiment, using 5 concentrations of the isolated and combined drugs, was performed using a multilevel factorial design with 2 factors and 5 combinations, as shown in Table 1. The resulted spectral data matrix consisted of 25 rows representing different samples at the concentration range of 0-108 $\mu\text{g mL}^{-1}$ for DEL and 0-9.0 $\mu\text{g mL}^{-1}$ for IND. The PLSR analyses were performed using the software Minitab 17 (Minitab Inc, State College, PA, USA). All UV measurements were recorded using ethanol as blank solution.

Table 1 Selected multilevel factorial design of the calibration set mixtures for PLSR method.

Run order	Standard order	DEL ($\mu\text{g mL}^{-1}$)	IND ($\mu\text{g mL}^{-1}$)	Absorbance 225 (nm)	Absorbance 243 (nm)
1	8	36	5	0.411	0.289
2	20	84	9	0.832	0.528
3	7	36	3	0.339	0.186
4	14	60	7	0.626	0.407
5	11	60	0	0.378	0.045
6	3	0	5	0.192	0.260
7	12	60	3	0.475	0.198
8	5	0	9	0.330	0.465
9	23	108	5	0.827	0.332
10	16	84	0	0.504	0.055
11	18	84	5	0.689	0.318
12	6	36	0	0.220	0.026
13	13	60	5	0.547	0.298
14	15	60	9	0.694	0.507
15	22	108	3	0.747	0.225
16	1	0	0	0.007	0.001
17	24	108	7	0.912	0.434
18	17	84	3	0.622	0.213
19	21	108	0	0.654	0.070
20	10	36	9	0.547	0.494
21	2	0	3	0.119	0.157
22	4	0	7	0.284	0.365
23	9	36	7	0.481	0.387
24	25	108	9	1.303	0.548
25	19	84	7	0.766	0.416

Validation Procedure

The method was validated in accordance with the International Conference on Harmonisation (ICH) requirements, involving the specificity, linearity, precision, accuracy, robustness and limits of detection (LD) and quantitation (LQ) parameters (15,16).

Results and Discussion

Method Development

In order to establish the assay parameters, different solvents (water, ethanol and hydroethanolic mixture) were investigated to develop a suitable spectrophotometric method for the simultaneous routine analysis of DEL and IND in tablets. Due to capability to dissolve both drugs, low toxicity and absorbance response, ethanol was selected for the sample preparation and used as final diluent.

The UV spectra of DEL ($60 \mu\text{g mL}^{-1}$) and IND ($5 \mu\text{g mL}^{-1}$) reference solutions are shown in Figure 2. DEL spectrum has a high absorption band in the region from 200 to 230 nm. On the other hand, the IND spectrum has shown two broad bands from 200 to 220 nm and 230 to 260 nm. The wavelengths regions can be suitable for a quantitative determination of the single drugs. However, the spectral overlapping of drugs precludes the use of direct UV spectrophotometry or univariate calibration for their simultaneous determination, being required another approach or analytical instrument.

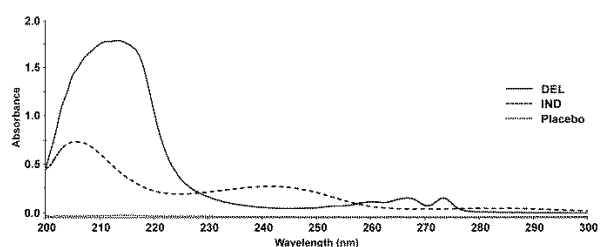


Figure 2 Overlay UV spectra of DEL reference standard ($60 \mu\text{g mL}^{-1}$), IND reference standard ($5 \mu\text{g mL}^{-1}$) and placebo.

The method development turned to the application of a multicomponent chemometric mode by PLSR. During the optimization, the compositions of binary mixtures of reference solutions were chosen randomly to avoid correlation between the concentrations of two substances. Twenty-five solutions of drugs were randomly selected and used for calibration model. The concentration ranges from $0.0\text{--}108 \mu\text{g mL}^{-1}$ (DEL) and $0.0\text{--}9.0 \mu\text{g mL}^{-1}$ (IND) were analyzed at wavelengths of 222, 225, 228, 240, 243 and 246 nm (the wavelengths contemplate the regions of best spectral intensity for both drugs). The data obtained were plotted in Minitab giving statistical graphs (Figure 3).

The multicomponent mode was adequate for all verified wavelengths, showing the best determination coefficient value in 225 nm ($R^2 = 0.9992$) and 243 nm ($R^2 = 0.9997$) for the determination of DEL and IND, respectively. Moreover, the significance of the predict model ($p < 0.05$) were observed by one-way variance analysis (ANOVA) and the standardized residuals graphs show homoscedastic behavior, confirming the applicability of the multicomponent analysis method for the mixing drugs concentrations.

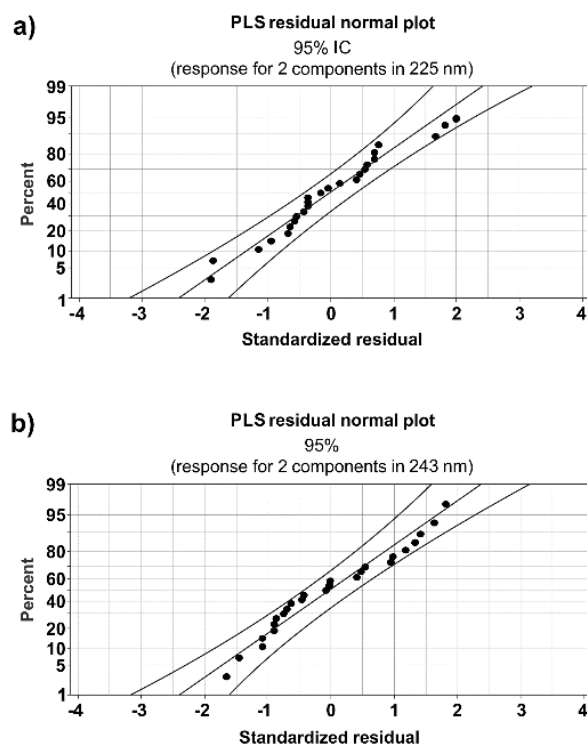


Figure 3 Results obtained from the PLSR analysis of the mixture of different concentrations of DEL and IND at wavelengths of 225 nm (a) and 243 nm (b).

Method Validation

Specificity

As previously mentioned, an overlap between UV spectra of DEL and IND was observed. Thus, the UV specificity was accessed using the PLSR approach by comparing the obtained spectra from the synthetic mixture of standard drugs and placebo solutions containing the same excipients of the commercial products.

All the solutions were scanned in the selected wavelength (225 nm for DEL and 243 nm for IND). No spectral interference was observed at specificity tests, demonstrating the ability of the method to quantify the analytes even in the coexistence of both in the pharmaceutical formulation.

Linearity

The analytical curves were obtained with seven concentrations of reference solution in the concentration range of $42\text{--}78 \mu\text{g mL}^{-1}$ (42, 48, 54, 60, 66, 72 and $78 \mu\text{g mL}^{-1}$) for DEL and $3.5\text{--}6.5 \mu\text{g mL}^{-1}$ (3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and $6.5 \mu\text{g mL}^{-1}$) for IND. Each solution was prepared in triplicate and analyzed by the developed UV method. The results were evaluated by the least square regression and by ANOVA.

The linearity was proved by the representative linear equations for DEL ($y = 0.0097x + 0.0345$; $R^2 = 0.9965$) and IND ($y = 0.0709x + 0.0095$; $R^2 = 0.9990$).

Moreover, the ANOVA was performed to verify the good fitting of the linear method and the results showed significant linear regression ($p < 0.05$) and no deviation from linearity for drugs ($p > 0.05$).

LD/LQ

The LD/LQ parameters are not a requirement for drug assay, however, it is important to demonstrate the method sensitivity. The limits were calculated based on the standard deviation of the response (y-intercepts of regression lines) and the slope using three independent analytical curves, as defined by ICH (15). The LQ/LD were 1.61/5.35 $\mu\text{g mL}^{-1}$ for DEL and 0.32/1.07 $\mu\text{g mL}^{-1}$ for IND.

Precision

The UV method precision was determined by studying the intra-day (repeatability) and inter-day precision (intermediate precision). Repeatability was evaluated assaying six determinations at the same concentration (60 $\mu\text{g mL}^{-1}$ and 5 $\mu\text{g mL}^{-1}$ for DEL and IND, respectively), during the same day, under the same experimental conditions. The intermediate precision was analyzed by comparing the results obtained on three different days.

The experimental results were expressed as relative standard deviation (RSD) and are presents in Table 2. The method variability were within the acceptable range with RSD values of less than 2.0% for DEL and IND determinations, and thus, indicating that the analytical method has excellent precision.

Table 2 Intra-day and inter-day precision data of UV method for DEL and IND in tablets.

Day	DEL		IND	
	Assay ¹ (%)	RSD ² (%)	Assay ¹ (%)	RSD ² (%)
1	99.56	1.39	99.18	0.95
2	98.77	1.96	100.18	2.12
3	101.16	1.86	99.04	1.83
Mean ³	99.83	1.94	99.47	1.69

¹Mean of six replicates, ²RSD (Relative standard deviation),

³Mean of eighteen replicates.

Accuracy

This parameter was determined by the recovery test that consisted on adding known amounts of reference solution to the placebo solution (prepared according to the specificity test). Aliquots of the initial reference solutions (300 $\mu\text{g mL}^{-1}$ of DEL and 25 $\mu\text{g mL}^{-1}$ of IND) were accordingly transferred to the placebo solutions obtaining the theoretical concentrations of 48, 60 and 72 $\mu\text{g mL}^{-1}$ of DEL and 4, 5 and 6 $\mu\text{g mL}^{-1}$ of IND. The samples corresponding to 80%, 100% and 120% of work solutions.

The analysis was performed on three subsequent days and the results were expressed as the recoveries percentage of DEL and IND from the comparison between theoretical and experimental concentrations (Table 3). A satisfactory mean percentage data of DEL (100.68%) and IND (101.60%) were obtained, evidencing the accuracy of the method.

Table 3 Recovery test for DEL and IND by UV method.

Drug	Theoretical ($\mu\text{g mL}^{-1}$)	Experimental ¹ ($\mu\text{g mL}^{-1}$)	Accuracy ² (%)	RSD ³ (%)
DEL	48	49.42	102.95	1.21
	60	60.29	100.49	
	72	73.15	101.60	
IND	4	4.11	102.78	1.10
	5	5.03	100.56	
	6	6.09	101.47	

¹Mean of three replicates, ²Percentage data recoveries, ³RSD (Relative standard deviation).

Robustness

The robustness was performed by analyzing the samples under different modifications in the methods settings, including the wavelength (nm) ethanol supplier and the sample preparation. Only the stirring time was evaluated, maintaining the same sonication period. The results (percentages of DEL and IND in the commercial tablets) and the experimental range of the selected variables evaluated are shown in Table 4. No significant changes (RSD) in simultaneous drugs determination were observed under the variations in the wavelength (0.48% for DEL and 0.91% for IND), ethanol supplier (0.78% for DEL and 1.48% for IND) and stirring time (0.93% for DEL and 1.16% for IND), thus showing the robustness of methods.

Additionally, the stability of both reference and sample solution used during analysis were evaluated. They were placed at 2-8 °C and also maintained at room temperature for 48 h and the assay results remained almost unchanged and no significant alteration relative to freshly prepared samples was observed (data not shown), showing the stability of the solutions within the indicated period, which was sufficient for the whole analytical process.

Table 4 Robustness testing of UV method for DEL and IND assay.

Factor	Levels	Assay ¹ (%)		
		DEL	Levels	IND
Wavelength (nm)	223	99.88	241	100.84
	225	100.84	243	101.13
	227	100.41	242	99.43
	RSD ²	0.48	RSD ²	0.91
Stirring time (min)	4	101.00	4	100.75
	5	99.56	5	99.18
	6	99.88	6	101.40
	RSD ²	0.76	RSD ²	1.14
Ethanol supplier	Ethanol 1	101.84	Ethanol 1	101.00
	Ethanol 2	100.52	Ethanol 2	100.77
	RSD ²	0.93	RSD ²	0.16

¹Mean of three replicates, ²RSD (Relative standard deviation).

Conclusions

This study presents a multicomponent spectrophotometric method for the determination of DEL and IND in commercial formulations. The PLSR mode was successfully developed and proved to be able to resolving binary mixtures, presenting as is a safe alternative for the simultaneous analysis of drugs in their combination formulation. The method was validated according to ICH guideline, with adequate results of specificity, linearity, precision, accuracy, and robustness, showing over chromatographic methods due to its simplicity, rapidity, lower cost and dangerous residues. Moreover, the method can be used without any prior separation of drugs and tablet excipients, and can be conveniently used for the routine quality control. Thus, this work contributes to technical and scientific development of pharmaceutical sciences, providing an innovative analytical methodology for DEL and IND analysis.

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