*Scientific paper*

# First-Order Derivative UV Spectrophotometric Method for Simultaneous Measurement of Delapril and Manidipine in Tablets

**Vítor Todeschini,* Amanda Thomas Barden, Leticia Lenz Sfair, Maximiliano da Silva Sangoi and Nadia Maria Volpato**

**Postgraduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Rio Grande do Sul, 90610-000 Porto Alegre-RS, Brazil*

** Corresponding author: E-mail: vitor_todeschini@hotmail.com; Tel.: + 55 51 33085214*

*Received: 18-01-2013*

## Abstract

A first-order derivative spectrophotometric ($^1$D-UV) method was developed and validated for simultaneous determination of delapril (DEL) and manidipine (MAN) in tablets. The $^1$D-UV spectra were obtained using $\Delta\lambda$ = 4.0 nm and wavelength set at 228 nm for DEL and 246 nm for MAN. The method was validated in accordance with the ICH requirements, involving the specificity, linearity, precision, accuracy, robustness and limits of detection and quantitation. The method showed high specificity in the presence of two drugs and formulation excipients and was linear over the concentration range of 18–54 μg mL$^{-1}$ ($r^2$ = 0.9994) for DEL and 6–18 μg mL$^{-1}$ ($r^2$ = 0.9981) for MAN with adequate results for the precision (≤ 1.47%) and accuracy (98.98% for DEL and 100.50% for MAN). Moreover, the method proved to be robust by a Plackett-Burman experimental design evaluation. The proposed $^1$D-UV method was successfully applied for simultaneous analysis of DEL and MAN in tablets and can be used as alternative green method to separation techniques. The results were compared with the validated liquid chromatography, capillary electrophoresis and liquid chromatography-tandem mass spectrometry methods, showing non-significant difference.

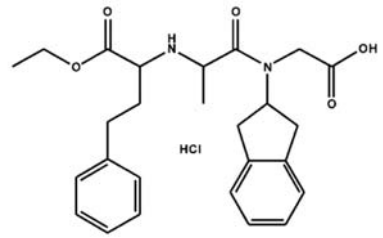
**Keywords:** Delapril, derivative UV spectrophotometry, green method, manidipine, validation.

## 1. Introduction

High blood pressure is the major cardiovascular risk factor and one of main cause of death around the world. Control of blood pressure reduces the high mortality associated with hypertension and is a necessary step to reduce global cardiovascular risk and for management of their complications.[1,2]

Combination therapy of the antihypertensive agents is frequently required to achieve blood pressure targets in a large proportion of patients.[3] Due to their complementary mechanisms of action, delapril hydrochloride (DEL), an angiotensin-converting enzyme inhibitor, and manidipine dihydrochloride (MAN), a dihydropyridine calcium antagonist, can be considered a rational drugs combination and an optimal antihypertensive drug treatment for mild to moderate essential hypertension.[2,4] The chemical structures of both drugs are shown in Figure 1.

a)




b)

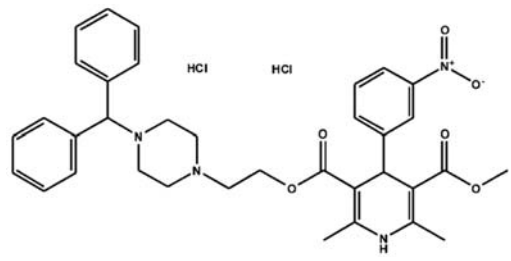



**Figure 1.** Chemical structures of delapril (a) and manidipine (b).

The literature reveals a few number of publications related to DEL and MAN determination in biological matrices using liquid chromatography (LC) with differents detectors and applied to pharmacokinetic studies or enantiomeric analysis.[5–11] However, for the analysis of both drugs in pharmaceutical formulation, some analytical methods have been developed by our research group. Recently, two stability-indicating methods performed by LC,[12] and by capillary electrophoresis, using micellar electrokinetic chromatography (MEKC) mode,[13] were validated for simultaneous analysis of DEL and MAN in commercial tablets. Additionally, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was also developed with the same purpose.[14] Many of these methods have to use expensive and hazardous chemicals, require expertise, making the process a challenge for the environment, time consuming and complex.

Spectrophotometric techniques provides practical and economic advantages over other methods and could be an useful alternative for quality control routine.[15–17] Multi-component analysis has been proved to be useful for resolving analytes mixtures in drug analysis. The derivative technique in UV spectrophotometry tends to emphasize subtle spectral features by presenting them in a new and visually more accessible way, allowing the resolution of overlapping spectra, and reducing the effect of spectral background interferences.[18] Besides that, compared with conventional spectrophotometric analysis, derivative spectrophotometry allows to eliminate the interference from excipients and co-formulated drugs.[18]

There is no publication concerning the UV spectrophotometric determinations of DEL and MAN as finished product in the current literature. Thus, the aim of this study was to develop and validate a simple, fast and cost-effective first-order derivative spectrophotometric ($^1$D-UV) method for simultaneous measurements of DEL and MAN in their combined dosage form. Additionally, the responses obtained by the proposed method were compared to those LC, MEKC and LC-MS/MS methods, contributing to research of green and practical alternatives methods for quality control and assuring the therapeutic efficacy of products.

# 2. Experimental

## 2. 1. Chemicals and Reagents

Delapril hydrochloride and manidipine dihydrochloride reference substances were kindly donated by Chiesi Farmaceutici (Parma, Italy). Hipertil® (Chiesi Farmacêutica Ltda, SP, Brazil) tablets, containing 30 mg of DEL and 10 mg of MAN, were obtained from commercial source and used within their shelf life period. The excipients contained in the dosage form (lactose monohydrate, magnesium stearate, hydroxypropyl cellulose, low substituted hydroxypropyl cellulose, riboflavin and aluminum lake) were all pharmaceutical grades and acquired from different suppliers. LC-grade methanol was obtained from Tedia Company Inc (Fairfield, OH, USA). For all the analyses, ultrapure water was used (Milli Q Gradient System, Millipore Corp, Bedford, MA, USA).

## 2. 2. Instrumentation

### 2. 2. 1. UV

The UV-Visible spectrophotometer was a double-beam (Shimadzu, Kyoto, Japan), model UV-1800, equipped with 1 cm quartz cells, with a spectral band width (1 ± 0.2 nm) and wavelength accuracy of ± 0.1 nm (with automatic wavelength correction). UV Probe software version 2.33 (Shimadzu) was used for instrument control, data acquisition and analysis.

### 2. 2. 2. LC

The LC apparatus was a Shimadzu LC system (Shimadzu Corp, Kyoto, Japan) equipped with a SCL-10A$_{VP}$ system controller, a LC-10AD$_{VP}$ binary pump, a SIL-10AD$_{VP}$ autosampler, a CTO-10ACvp column oven, and a SPD-M10A$_{VP}$ photodiode array (PDA) detector. The peak areas were automatically integrated using the Class VP (v 6.12) computer software.

### 2. 2. 3. MEKC

The MEKC experiments were performed on an Agilent $^{3D}$CE apparatus (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, a PDA detector and power supply able to deliver up to 30 kV. CE ChemStation software was used for instrument control, data acquisition and analysis.

### 2. 2. 4. LC-MS/MS

The LC-MS/MS method was performed on a Shimadzu LC system equipped with a SCL-10A$_{VP}$ system controller, LC-10AD$_{VP}$ quaternary pump, DGU-14A degasser, a SIL-10AD$_{VP}$ autosampler, and a CTO-10AD$_{VP}$ column oven. The triple quadrupole mass spectrometer (Micromass UK Ltd., Manchester, UK), model Quattro LC, was equipped with an ESI source in positive mode. Data acquisition and analysis were performed using the Masslynx (v 3.5) software. A syringe pump (Bioanalytical Systems Inc, West Lafayette, IN, USA) was used to infuse the solutions.

## 2. 3. Preparation of Reference Solutions

The stock solutions were prepared by weighing accurately, 15 mg of DEL (purity 99.6%) and 5 mg of MAN (purity 99.7%) reference standards, with ± 0.01 mg of precision, and diluted to volume with methanol, obtaining the

concentration of 1.5 mg $mL^{-1}$ and 0.5 mg $mL^{-1}$ for DEL and MAN, respectively. The stock solution was stored, for not more than seven days, at 2–8 °C, protected from light and daily diluted to an appropriate concentration in water.

## 2. 4. Preparation of Sample Solutions

To prepare the extraction sample solutions, tablets containing 30 mg of DEL and 10 mg of MAN were daily weighed and crushed to fine powder. An appropriate amount was transferred into an individual 20 mL volumetric flask, diluted to volume with methanol, kept in vortex for 5 min, sonicated for 10 min and filtered through a 0.45 μm membrane filter (Millipore Corp), obtaining theoretical concentrations of 1.5 mg $mL^{-1}$ for DEL and 0.5 mg $mL^{-1}$ for MAN. Working sample solutions were prepared by diluting the extraction solution to an appropriate concentration with water.

## 2. 5. UV Procedure

The experiments on UV method were performed using the $^1$D-UV mode. The solutions were recorded at a fast scan speed with a fixed slit to lead to a spectral resolution of 1 nm. The spectra were obtained by instrumental electronic differentiation using a wavelength interval ($\Delta\lambda$) of 4 nm in the range of 200–300 nm. The analysis were carried out at 228 nm ($^1$D228) for DEL and 246 nm ($^1$D246) for MAN, with working sample containing 36 μg $mL^{-1}$ and 12 μg $mL^{-1}$ of DEL and MAN, respectively. The analytical responses obtained were multiplied by 10 (scaling factor of 10). The spectrophotometric measurements were recorded using water as blank solution.

## 2. 6. LC, MEKC and LC-MS/MS Procedures

The LC, MEKC and LC-MS/MS methods applied for the simultaneous analysis of DEL and MAN in the pharmaceutical dosage form were previously developed and validated by our research group and the data were compared with obtained results by the proposed $^1$D-UV method.

The LC experiments were performed on an analytical column Shim-pack $C_8$ (Shimadzu, Tokyo, Japan) maintained at 35 °C. The mobile-phase consisted of acetonitrile and a solution of triethylamine 0.3% adjusted to pH 3.0 with phosphoric acid (55:45; v/v), run at a flow-rate of 1.2 mL $min^{-1}$, with detection at 220 nm using PDA detector. The injection volume was 20 μL of the solutions containing 30 μg $mL^{-1}$ and 10 μg $mL^{-1}$ of DEL and MAN, respectively.[12]

For MEKC method, the experiments were carried out on an uncoated fused-silica capillary with 50 μm i.d. and 72 cm of effective length, thermostatized at 35 °C. The buffer solution consisted of 50 mM of borate and 5 mM of SDS at pH 9.0. A constant voltage of 25 kV (current about 15 μA) was applied during the analysis, with detection at 208 nm using a PDA detector. Samples containing 60 μg $mL^{-1}$ of DEL and 20 μg $mL^{-1}$ of MAN were injected using the hydrodinamic mode at 50 mbar for 5 s. Salicilic acid was used as internal standard (IS).[13]

The LC-MS/MS method was achieved on an analytical column Luna $C_8$ (Phenomenex, Torrance, USA), maintained at 45 °C, with a mobile-phase consisted of methanol and 10 mM ammonium acetate (90 : 10, v/v), run at a flow rate of 0.25 mL $min^{-1}$. The injection volume was 10 μL of the solutions containing 450 ng $mL^{-1}$ of DEL and 150 ng $mL^{-1}$ of MAN. The mass spectrometry method was performed employing positive electrospray ionization operating in multiple reaction mode, monitoring the transitions of 453.1>234.1 for DEL, 611.1>167.0 for MAN and 412.2>223.0 for fesoterodine (IS).[14]

## 2. 7. Validation of the 1D-UV Method

The aim of an analytical procedure validation is to demonstrate that it is suitable for its intended purpose. The method was validated by determining the parameters such as: specificity, linearity, precision, accuracy, LoD, LoQ and robustness following the International Conference on Harmonisation (ICH) guidelines.[19]

## 2. 8. Sample Analysis

For quantitative determination of DEL and MAN in the tablet formulation, the respective working sample solutions were diluted to appropriate concentrations (as described above for each analytical technique) and the assay (%) of the drugs was calculated against the reference substances. The results were converted to milligrams (mg) for statistical comparison.

# 3. Results and Discussion

## 3. 1. Optimization of the Spectrophotometric Conditions

During method development, several tests were performed in order to establish the assay parameters. In this context, different solvents were investigated to develop a suitable spectrophotometric method for the simultaneous analysis of DEL and MAN in tablets. For diluents selection, the employed criteria were the drugs solubility, the sample preparation easiness and the method sensitivity. Due to great capability to dissolve both drugs, methanol was selected for the first step of sample preparation. However, aiming the reduction of the organic solvent consumption, water was used as final diluent. Water is the better solvent considering toxicological risks and the absence of demanding residue storage, maintaining the stability of the drugs. Moreover, the use of methanol as final

diluent changes the drugs absorption spectra, making this method unfeasible.

The overlay zero-order UV spectra of DEL, MAN and placebo (which was prepared with the tablet excipients) are represented in Figure 2a. The maximum absorption wavelengths in water were around 210 nm and 230 nm for DEL and MAN, respectively. However, significant overlapping of the three spectra can be observed, which prevents the simultaneous determination of the two compounds by direct absorbance measurements, being required another tool or analytical instrument.

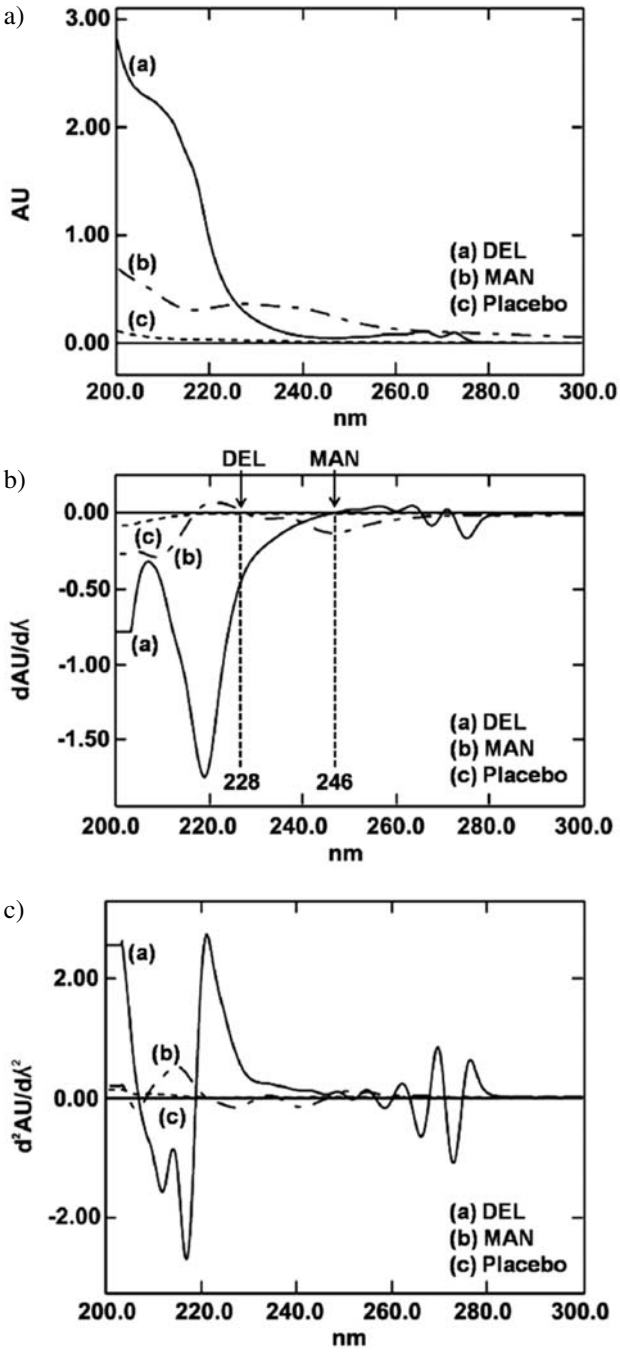



**Figure 2.** Overlay UV spectra (a), first-order derivative spectra (b) and second-order derivative spectra (c), of delapril reference standard (36 μg mL$^{-1}$), manidipine reference standard (12 μg mL$^{-1}$) and placebo.

A suitable technique for overcoming this problem is the derivative spectrophotometry.[20] Due to the overlapping spectra, the $^1$D-UV method was considered appropriate for resolving mixtures of both elements and eliminates the interference from excipients absorption over DEL and MAN signal. This approach, based on zero-crossing measurements, involves analysis of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectrum of another component.[20]

As observed in Figure 2b, the zero crossing wavelengths at 246 nm for DEL and 228 nm for MAN did not suffer any mutual interference of drugs and pharmaceutical excipients. Therefore, these points were selected as optimum regions to simultaneous determination of drugs (228 nm for DEL and 246 nm for MAN).

On the other hand, the use of second derivative was also possible, but it was discarded because did not present analytical advantages. The second-order spectra of DEL, MAN and placebo are showed in Figure 2c.

Additionally, beyond the different orders tested, various smoothing and scaling factors were analyzed in order to optimize the derivative method. The smoothing factor of $\Delta\lambda = 4$ and a scaling factor of 10 showed an adequate signal-to-noise ratio, high spectral resolution and were suitable to enlarge the drugs signals, facilitating its measurement and decreasing the signal reading errors. With $\Delta\lambda$ increase, the signal-to-noise ratio also improves and the fluctuation in a derivative spectrum decreases. However, if the value of $\Delta\lambda$ is too large, the spectral intensity signal deteriorates.

## 3. 2. $^1$D-UV Method Validation

### 3. 2. 1. Specificity

Specificity is a procedure to determine the analyte in presence of others components such as different analytes, sample matrix, degradation products and impurities. The method specificity was assessed by comparing the obtained spectra from the commercial formulations and the synthetic mixture of drugs standard and placebo solutions. On both determinations, the absorption spectra were similar (Figure 3a; Figure 3b). Moreover, in $^1$D-UV spectra analysis (Figure 2b), no spectral interference of two compounds and placebo were observed at the both selected wavelength (228 nm for DEL and 246 nm for MAN). Therefore, the proposed method is specific for the simultaneous analysis of DEL and MAN.

### 3. 2. 2. Linearity

The linearity was determined by constructing three independent analytical curves, each one with seven con-

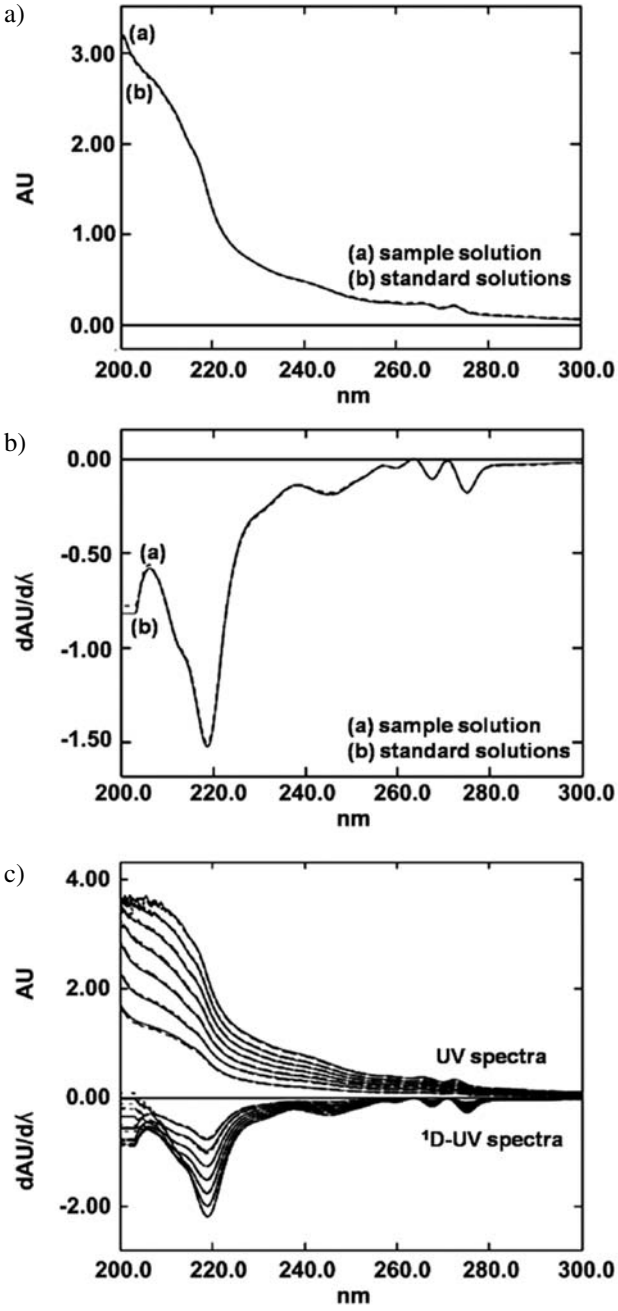



**Figure 3.** Overlay UV spectra of delapril (DEL) and manidipine (MAN) reference standards and sample of pharmaceutical formulation (a); Overlay first-order derivative spectra of DEL and MAN reference standards and sample of pharmaceutical formulation (b); UV spectra and first-order derivative spectra of DEL at concentration range of 18–54 μg $mL^{-1}$ and MAN reference standards at concentration range of 6–18 μg $mL^{-1}$ (c).

centrations of both drugs, in the range of 18–54 μg $mL^{-1}$ (18; 24; 30; 36; 42; 48; 54 μg $mL^{-1}$) for DEL and 6–18 μg $mL^{-1}$ (6; 8; 10; 12; 14; 16; 18 μg $mL^{-1}$) for MAN. The Figure 3c shows the zero and first order derivative spectra containing increasing amounts of a mixture of DEL and MAN. When analyzed in zero order, the absorption of both compounds in the selected wavelengths remained in the adequate range for the instrument, showing the acceptable concentration range of the method.

The $^1$D-UV absorbance of both analytes, against the respective reference concentrations, was used for plotting the graphs, and the linearity was evaluated by the least square regression analysis and by analysis of variance (ANOVA). The value of the determination coefficient calculated ($r^2$) was 0.9994 ($y = 0.0090x + 0.0015$) for DEL and 0.9981 ($y = 0.0177x + 0.0331$) for MAN. Moreover, the ANOVA ($\alpha = 0.05$) 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 DEL ($F_{calculated} = 1.77 < F_{critical} = 2.96$) and for MAN ($F_{calculated} = 1.25 < F_{critical} = 2.96$).

### 3. 2. 3. Precision

The precision of the analytical method was verified through repeatability and intermediate precision measurements. Repeatability was examined assaying six independent sample preparations in the same day (intra-day), at the same concentration and under the same experimental conditions. The intermediate precision was assessed by comparing the results obtained on three different days (inter-day). The samples were prepared from extraction solutions of DEL (1.5 mg $mL^{-1}$) and MAN (0.5 mg $mL^{-1}$), whose final concentration of DEL (36 μg $mL^{-1}$) and MAN (12 μg $mL^{-1}$) was achieved by dilution in water. The results were expressed as relative standard deviation (*RSD*).

For repeatability (6 experiments), the *RSD* value obtained was 1.47% (mean assay = 100.30%) for DEL and 1.26% (mean assay = 99.80%) for MAN. The RSD values obtained for inter-day precision (total of 18 experiments) were 1.31% (mean assay = 100.37%) for DEL and 1.03% (mean assay = 100.18%) for MAN. These results can be observed in Figure 4. The variability of the results was low, with *RSD* values within the acceptable range (< 2%), indicating the precision of the proposed method.

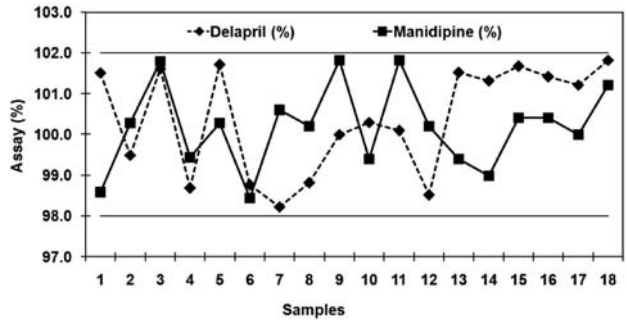



**Figure 4.** Representative graph of assay data for intra and inter-day precision (n=18) obtained by $^1$D-UV method for delapril (36 μg $mL^{-1}$) and manidipine (12 μg $mL^{-1}$) analysis.

### 3. 2. 4. Accuracy

The accuracy of a method is expressed as the closeness of agreement between the found result and the value that is accepted as reference one. It was evaluated by the determination of the analytes in solutions prepared by the standard addition method and expressed in terms of percentage recoveries of added DEL and MAN from the real samples. Suitable volumes of the standard solutions of DEL (1.5 mg $mL^{-1}$) and MAN (0.5 mg $mL^{-1}$), equivalent to 16.67, 33.34 and 50% of the nominal analytical concentration of the drugs (work concentration of 36 μg $mL^{-1}$ for DEL and 12 μg $mL^{-1}$ for MAN) were added to the samples of tablets to obtain solutions at concentrations of 42, 48 and 54 μg $mL^{-1}$ for DEL and 14, 16 and 18 μg $mL^{-1}$ for MAN (all concentrations in the linearity range). The results were expressed as the percentage of DEL and MAN reference substances recovered from the sample and are shown in Table 1. The mean recovery data, comprising the three levels added, were 98.98% (*RSD* = 0.92%) for DEL and 100.50% (*RSD* = 1.49%) for MAN, demonstrating that the method is accurate within the desired range.

### 3. 2. 5. LoD and LoQ

The LoD/LoQ parameters are not a requirement for drug assay, however, it is useful to show the sensitivity of the method and that the analysis is being conducted in a suitable concentrations range. The limits were calculated from the slope and the standard deviation of the intercepts of three analytical curves, according to ICH guidelines.[19] The results for LoD and LoQ were 0.13 and 0.42 μg $mL^{-1}$ for DEL and 0.08 and 0.26 μg $mL^{-1}$ for MAN.

### 3. 2. 6. Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for the routine analysis. In order to analyze the simultaneous variations of the factors on the considered responses, a multivariate approach using design of experiments is recommended in robustness testing.[21] The robustness was investigated using the Plackett-Burman design and was performed by the selection of three factors, studied at two levels (high and low): Δλ (8–2), scalling factor (12–8) and stirring time during the sample preparation (6–4 min) in 12 experiments. The data were the percentages of DEL and MAN in the commercial tablets (relative to their label claimed concentration) obtained in relation to the standard solutions response in each experiment. The experimental plan and the assays (%) are summarized in Table 2. The statistical evaluation of data was performed by the MINITAB 14 (Minitab Inc, State College, PA, USA) data analysis software.

**Table 1:** Experimental values obtained in the recovery test for delapril (DEL) and manidipine (MAN) by using the $^{1}$D-UV method.

| Analyte | Added level (μg $mL^{-1}$) | Nominal concentration (μg $mL^{-1}$) | Mean concentration found[a] (μg $mL^{-1}$) | Accuracy (%) | *RSD*[b] (%) |
|---|---|---|---|---|---|
| DEL | 6 | 42 | 42.53 | 100.01 | |
| | 12 | 48 | 48.37 | 98.68 | 0.92 |
| | 18 | 54 | 54.21 | 98.25 | |
| MAN | 2 | 14 | 13.92 | 101.96 | |
| | 4 | 16 | 15.90 | 100.56 | 1.49 |
| | 6 | 18 | 17.82 | 98.97 | |

[a] Mean of three replicates [b] *RSD* = Relative standard deviation

**Table 2:** Selected Plackett–Burman design for the robustness testing of delapril (DEL) and manidipine (MAN).

| Experiment | Δλ | Scaling factor | Stirring time | DEL Assay (%) | MAN Assay (%) |
|---|---|---|---|---|---|
| 1 | 8 | 8 | 6 | 101.59 | 99.24 |
| 2 | 8 | 8 | 4 | 102.02 | 98.85 |
| 3 | 8 | 12 | 6 | 101.92 | 99.24 |
| 4 | 8 | 12 | 4 | 101.98 | 97.70 |
| 5 | 2 | 12 | 6 | 101.77 | 100.45 |
| 6 | 2 | 12 | 6 | 100.96 | 100.46 |
| 7 | 2 | 12 | 4 | 99.87 | 98.16 |
| 8 | 8 | 8 | 6 | 100.96 | 99.24 |
| 9 | 2 | 8 | 4 | 101.89 | 100.93 |
| 10 | 2 | 8 | 6 | 101.89 | 100.23 |
| 11 | 2 | 8 | 4 | 100.76 | 98.62 |
| 12 | 8 | 12 | 4 | 100.42 | 98.96 |

After the calculation of the effects for each parameter, the statistical analysis through the *t*-test allowed to define what is significant and what is not. A chart of bars was used to evaluate the significance of the analyzed factors (Figure 5). The magnitude of each effect is represented by a horizontal column. This plot will also include a vertical line to indicate the $P = 0.05$ threshold for statistical significance. Effects in which the bars are smaller than the critical *t*-value were not considered significant and did not affect the response variables.[21,22] At the studied ranges, the effects of the factors were not statistically significant ($P > 0.05$) for the response studied (assay (%)). So, there were no significant changes in the assay regarding the percentage of drugs contents under the modifications made in the experimental conditions, showing the robustness of the developed method.

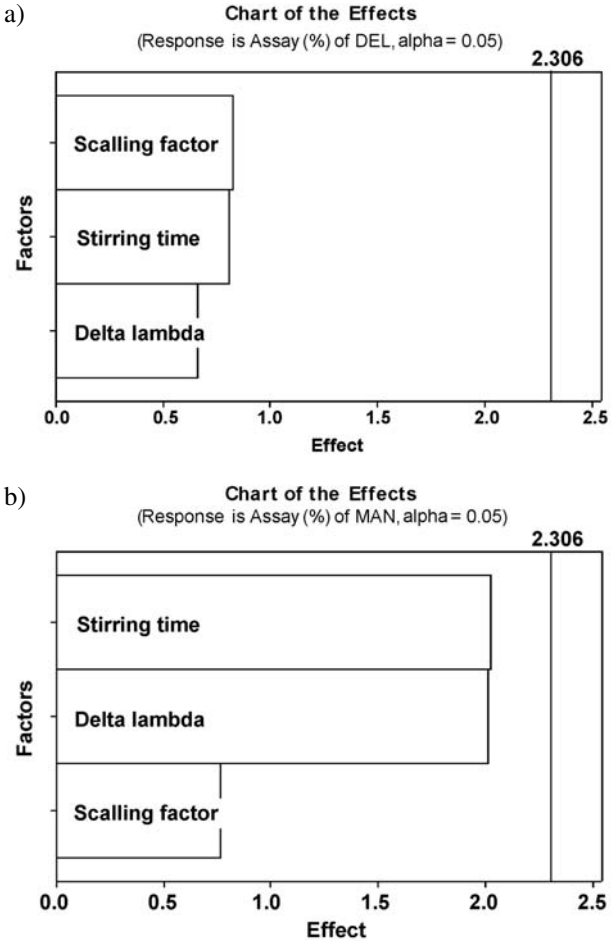



**Figure 5.** Bars charts representing the effects of the factors on quantitative determination (assay) of delapril (a) and manidipine (b) in Placket-Burman experimental design (n = 12). The $t_{critical}$ is represented by the vertical line.

### 3. 2. 7. Solution Stability

In order to demonstrate the stability of both reference solution and tablet sample solution during analysis, they were placed at 2–8 °C and also maintained at room temperature for 48 h. The assay results for DEL and MAN 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.

As previously mentioned, two stability-indicating methods performed by LC and MEKC for DEL and MAN evaluation were developed by our research group. The different stress conditions that the drugs were submitted during the stability studies demonstrated the susceptibility of DEL and MAN under alkaline and photolytic conditions, respectively. Some degradation products formed could be visualized by PDA detector for both drugs, and lots of them presented very similar spectrum. Therefore, an UV method, even with the derivative tool, would be difficult to evaluate both drugs and theirs degradations products simultaneously and, thus, its application in stability studies becomes limited when compared with separation techniques.

## 3. 3. DEL and MAN Assay in Commercial Tablets

The validated $^1$D-UV method was applied for the simultaneous measurements of DEL and MAN in tablets, and the results compared to those obtained using the previously validated LC, LC-MS/MS and MEKC methods. The experimental values of the four methods (in milligrams per tablet) were compared statistically by ANOVA

**Table 3:** Assay results obtained by $^1$D-UV, LC-UV, MEKC and LC-MS/MS methods for delapril (DEL) and manidipine (MAN) determination in the pharmaceutical formulation and *P-value* furnished by ANOVA analysis.

| | Experimental amount[a] | | | |
|---|---|---|---|---|
| | DEL | | MAN | |
| **Methods** | **Assay[b] (mg)** | ***RSD*[c] (%)** | **Assay[b] (mg)** | ***RSD*[c] (%)** |
| $^1$D-UV | 30.18 | 1.40 | 10.01 | 1.19 |
| LC-UV | 30.18 | 0.65 | 10.00 | 0.85 |
| MEKC | 30.13 | 1.17 | 9.99 | 1.17 |
| LC-MS/MS | 30.11 | 0.76 | 9.98 | 0.90 |
| *P-value* (*P*>0.05) | 0.978 | | 0.979 | |

[a] Tablets containing 30 mg of DEL and 10 mg of MAN [b] Mean of five replicates [c] *RSD* = Relative standard deviation

showing non-significant difference ($P > 0.05$) for content of DEL and MAN, as shown in Table 3. Hence, the $^1$D-UV method can be applied to routine analysis of both drugs in pharmaceutical formulation in the specific wavelengths, with benefit of short analysis time and low organic solvents consumption when compared with the others.

## 4. Conclusions

The first-order derivative spectrophotometric method was successfully validated and demonstrated to be simple, specific, reproducible, rapid and cost effective. Moreover, the proposed method can be used without any prior separation of drugs and tablet excipients and, when the assay results were compared, non-significant difference between the previously validated LC-UV, LC-MS/MS and CE methods was found. Considering that the chromatographic and electrophoretic methods are more expensive, time consuming, and need more steps, the proposed method is adequate for routine analysis. It is a safe alternative for the simultaneous analysis of DEL and MAN in their combination formulation and can be conveniently used for the routine quality control.

## 5. Acknowledgements

The authors wish to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support and Chiesi Group for the provision of delapril and manidipine reference standards.

## 6. References

1. A. D. Lopez, C. D. Mathers, M. Ezzati, D. T. Jamison, C. J. L. Murra, *Lancet*. **2006**, *367*, 1747–1757.
2. M. L. Otero, *Vas. Health. Risk Manag*. **2007**, *3*, 255–263.
3. K. K. Gaddam, A. Verma, M. Thompson, R. Amin, H. Ventura, *Med. Clin. North Am*. **2009**, *93*, 527–786.
4. P. L. McCormack, G. M. Keating, *Drugs*. **2006**, *66*, 961–969.
5. T. Miyabayashi, K. Yamashita, I. Aoki, M. Motohashi, T. Yashiki, K. Yatani, *J. Chromatogr. B*. **1989**, *494*, 209–217.
6. T. Ohkubo, T. Uno, K. Sugawara, *J. Chromatogr. B*. **1996**, *687*, 413–418.
7. J. Jing, W. Ren, X. Chen, H. He, W. Zhou, X. Zhu, Y. Sun, G. Wang, *Biom. Chrom*. **2007**, *21*, 836–840.
8. M. Yamaguchi, K. Yamashita, I. Aoki, T. Tabata, S. Hirai, T. Yashiki, *J. Chromatogr. B*. **1992**, *575*, 123–129.
9. U. Tsukasa, O. Tadashi, S. Kazunobu, *J. Liq. Chrom.* Relat. *Tech*. **1999**, *22*, 1117–1128.
10. A. Stockis, S. De Bruyn, C. Gengler, F. Goethals, S. Lens, G. Poli, D. Acerbi, *Arzneim. Forsch. Drug. Res*. **2003**, 53, 554–561.
11. A. Stockis, C. Gengler, F. Goethals, B. Jeanbaptiste, S. Lens, G. Poli, D. Acerbi, *Arzneim. Forsch. Drug. Res*. **2003**, *53*, 627–634.
12. V. Todeschini, A. S. Meira, M. S. Sangoi, A. B. Barth, C. S. Paim, N. M. Volpato, *J. Liq. Chromatogr. Related Technol.* **2012**, *35*, 603–620.
13. V. Todeschini, A. S. Meira, M. S. Sangoi, D. Miron, A. D. Lange, N. M. Volpato, *J. AOAC*. (article accepted for publication).
14. V. Todeschini, M. S. Sangoi, N. M. Volpato, *Eur. J. Mass Spectrom*. **2011**, *17*, 287–296.
15. C. V. Garcia, J. Sippel, M. Steppe, E. E. S. Schapoval, *Anal. Lett*. **2006**, *39*, 341–348.
16. M. D. Malesuik, C. S. Paim, E. E. S. Schapoval, M. Steppe, *Quim. Nova*, **2010**, *33*, 739–742.
17. M. S. Sangoi, V. Todeschini, M. Steppe, *Acta Chim. Slov.* **2012,** *59,* 136–143.
18. C. Ojeda, F. Rojas, *Anal. Chim. Acta*. **2004**, *518*, 1–24.
19. ICH Guideline Q2(R1) on "Validation of Analytical Procedures: Text and Methodology", International Conference on Harmonization, **2005**, Geneva.
20. A. A. El-Sayed M. M. Hamed, S. A. El-Reefy, *J. Anal. Chem*. **2010**, *65*, 1113–1117.
21. Y. V. Heyden, A. Nijhuis, J. Smeyers-Verbeke, B. G. M. Vandeginste, D. L. Massart, *J. Pharm. Biomed. Anal*. **2001**, *24*, 723–753.
22. K. Vanaja, R. H. S. Rani, *Clin. Res. Regul. Aff.* **2007**, *24*, 1–23.

## Povzetek

Razvili in validirali smo derivativno spektrofotometrično metodo prvega reda (1D-UV) za hkratno določitev delaprila (DEL) in manidipina (MAN) v tabletah. 1D-UV spektre smo pridobili pri $\Delta\lambda$ = 4,0 nm in valovni dolžini 228 nm za DEL ter 246 nm za MAN. Metodo smo validirali v skladu z ICH zahtevami: specifičnost, linearnost, natančnost, točnost, robustnost, meje zaznave in določanja. Metoda je bila visoko specifična v prisotnosti dveh učinkovin in pomožnih snovi. Bila je linearna v koncentracijskem območju 18–54 μg mL$^{-1}$ ($r^2$ =0.9994) za DEL ter 6–18 μg mL$^{-1}$ ($r^2$ = 0.9981) za MAN. Dobili smo primerne rezultate za natančnost (≤ 1,47%) ter točnost (98,98 % za DEL in 100,50 % za MAN). Glede na Plackett-Burmanovo evaluacijo eksperimentalnega načrta se je metoda izkazala za robustno. Predlagano 1D-UV metodo smo uspešno uporabili za hkratno analizo DEL in MAN v tabletah in jo lahko uporabimo kot alternativno zeleno metodo separacijskim tehnikam. Rezultate smo primerjali z validirano tekočinsko kromatografsko, kapilarno elektroforezno in tekočinsko kromatografsko - tandemsko masnospektrometrično metodo, razlike so bile nesignifikantne.