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FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC AND HPLC METHODS FOR DETERMINATION OF MOEXIPRIL HYDROCHLORIDE IN THE PURE FORM, PHARMECEUTICAL FORMULATIONS AND EVALUATION OF ITS STABILITY

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Abstract: A rapid, linear (over a concentration range of 0.00012–0.0012% and with correlation coefficient r = 0.999), accurate (an average recovery of 100%), precise (an average standard deviation < 1.5%) and economical first derivative UV spectrophotometric assay method (λ_{max} = 238 nm) was developed for the determination of moexipril hydrochloride (MOXL) in a pharmaceutical formulation. The method was investigated for its utility for the determination of MOXL in commercially available tablets and as a stability-indicative assay in solid state. The results obtained by means of the investigated method were statistically compared (*t*-Student test and *F*-Snedecor test) with the results obtained by means of the reference method – HPLC, which evidenced that both methods are equally precise and accurate. It was finally concluded that first derivative UV spectrophotometry is selective with reference to excipients used for the tablets' formulation, however, it is not selective with reference to MOXL degradation products.

Keywords: moexipril hydrochloride (MOXL), HPLC method, derivative UV spectroscopic method, validation

Hypertension is currently considered to be one of the most serious chronic illnesses. The class of drugs of considerable clinical importance in terms of cardiovascular diseases management are angiotensin converting enzyme inhibitors. The physiological effect of these agents on the renin-angiotensin-aldosterone system is the competitive inhibition of angiotensin-converting enzyme and resulting inhibition of the conversion of the relatively inactive angiotensin I to the highly potent vasoconstrictor – angiotensin II. This finally leads to the reduction of arterial blood pressure (1–3).

A new, orally active, non-sulfhydryl containing representative – moexipril hydrochloride, has been chosen as a subject to our study. Chemically it is (3*S*)-2-[(2*S*)-2-{[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino}-1-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (3).

This drug is usually used in the treatment of hypertension at a typical daily dose of 7.5–15 mg and congestive heart failure and its administration regimen includes monotherapy or coadministration with other antihypertensives or diuretics (4, 5).

Quality assurance in pharmaceutical industry, in accordance to ICH guidelines, plays a crucial role in further efficiency and safety of a therapy. Purity or stability of active ingredients and medicinal products is a basic aspect of a drug quality control. Therefore, the development and further validation of sensitive, rapid, simple, inexpensive and reliable methods for stability and purity assessment is of great importance and interest (6, 7).

MOXL it is not listed in any pharmacopeia thus there is no officially approved method for its analysis in its dosage forms nor in biological material. A literature survey revealed several analytical procedures that have been described for MOXL determination. These include: derivative spectrophotometric method and isocratic RP-HPLC method, which have been reported for the simultaneous determination of MOXL and hydrochlorothiazide in its co-formulated form (8), three simple spectrophotometric methods described for the assay of MOXL either in bulk form or in the presence of its degradation products, which have been based on ion pair complex formation between MOXL and bromocresol purple, bromophenol blue and bro-

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Table 1. λ_{maks} and λ_{min} of UV (A, ^{1}D, ^{2}D, ^{3}D) spectra of MOXL in methanol solution.

Method	λ_{maks} [nm]	λ_{min} [nm]	λ_{maks} [nm]
A	204.4	–	–
^{1}D	210.7	227.2	237.9
^{2}D	214.4	–	–
^{3}D	215.3	219.2	–

mothymol blue (9). A stability-indicating RP-HPLC method has also been developed for the determination and kinetic studies of MOXL (10, 11). Furthemore, another HPLC-Electrospray Ionization MS method (12) and a gas chromatographic-mass, spectrometric method have been established for the determination of MOXL in the presence of its degradation product in biological samples (13–15). Recently, also electrochemical differential pulse voltammetric method based on the enhancement effect of sodium dodecyl sulfate for the determination of MOXL in tablets has been reported (16).

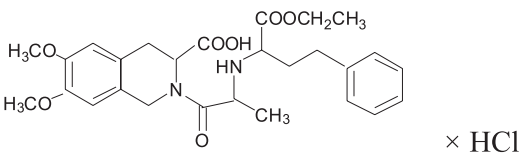

Figure 1. Chemical structure of moexipril hydrochloride

The above referenced methods (HPLC-MS, GC-MS), despite their high accuracy and precision, are inapplicable for stability-indicative assessment since they are time-consuming and require a sophisticated and expensive instrumentation.

For all these reasons, among various methods available for the determination of the drug, HPLC and UV spectrophotometry seem to be the most appropriate for a routine analysis of large number of samples, due to their simplicity, reasonable sensitivity and a significant economical advantage, which is highly beneficial in terms of kinetic studies (7, 17). In this context, an additional advantage of UV derivative spectrophotometry over HPLC is a short time of analysis, which enables a great number of measurements to be taken for stability evaluation needs.

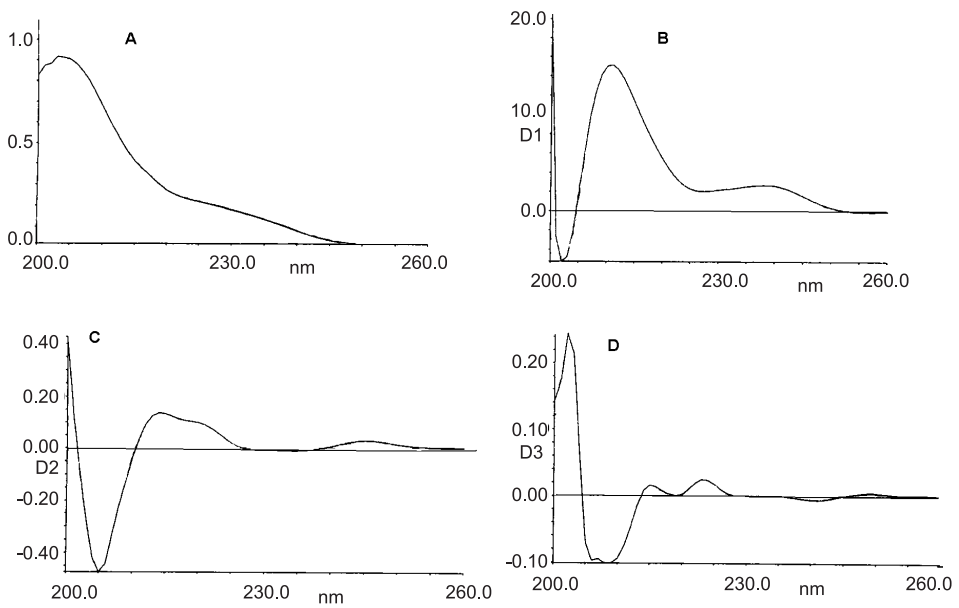

Figure 2. Zero-order absorption (**A**), first- (**B**), second- (**C**) and third-derivative (**D**) absorption spectra of MOXL in a range 200–260 nm (obtained using 1 cm quartz cell)

This is why visible spectrophotometric methods are the instrumental methods of choice that are commonly used in industrial laboratories. In the available literature, however, there is a lack of information regarding the use of UV spectrophotometry for kinetic studies of MOXL in solid state.

Therefore, the main objective of this study was to develop and validate a new, uncomplicated, fast, inexpensive, sensitive and accurate UV spectrophotometric method, which could be applied for a stability analysis of MOXL in pure state and in pharmaceutical dosage forms.

Since MOXL molecule contains an uncharacteristic phenyl chromophore and a direct spectrophotometric assay is susceptible to interference from formulation excipients and chemical reagents, for its spectrophotometric assay the differentiation of a primary spectrum is essential. This results in sharpening, shift and separation of overlaping absorption peaks which are unclear in the primary

Table 2. Validation parameters of HPLC and first order derivative spectrophotometric methods.

Parameters	HPLC (reference method)	Spectrophotometric method First order derivative
Range [%]	0.002–0.040	0.00012–0.00120
LOD [%]	0.001	0.00004
LOQ [%]	0.02	0.00010
Regression equation	y = ax + b	
a ± Δa	52.14 ± 2.54	12375 ± 518
s_a	1.03	224
b ± Δb	0.0573 ± 0.064[1]	0.304 ± 0.122 [2]
s_b	0.0262	0.529
Regression equation	y = ax	
a ± Δa	54.16 ± 2.17	12734 ± 496
s_a	0.961	219
r	0.998	0.999
Precision RSD [%] Intra-day [3] Inter-day [4]	 0.94; 0.95; 1.05 0.76	 1.01; 1.12; 1.29 1.16
Accuracy, recovery	Amount found x ± Δx (%); RSD (%)	
model mixture I	98.65 ± 0.71; RSD = 0.888	98.85 ± 0.68; RSD = 0.828
model mixture II	99.72 ± 0.94; RSD = 1.02	99.62 ± 1.04; RSD = 1.24
model mixture III	99.93 ± 0.39; RSD = 0.561	99.83 ± 0.59; RSD = 0.712
Cardiotensin 7.5 analysis [mg] (label claim 7.5 mg MOXL)		
	Amount found mg	
x ± Δx [mg] SD RSD (%)	7.551 ± 0.06 0.084 1.12	7.560 ± 0.05 0.069 0.915
Statistical analysis of the results obtained by means of first derivative UV spectrophotometry and HPLC (reference method)		
[1]D ↔ HPLC	$1.491 < F_{\alpha,f}$	$0.128 < t_{\alpha,f}$
Theoretical F-value (ν = 7, 7) and t-value (ν = 14) at 95% confidence level are 3.79 and 2.145, respectively		

[1] intercept b statistically insignificant (Student *t*-test, a = 0.05); [2] intercept b statistically significant (Student *t*-test, a = 0.05); [3] concentrations of MOXL 0.00012, 0.0006, 0.0012% for spectrophotometric methods and for HPLC method 0.004, 0.016, 0.04%; [4] concentrations of MOXL 0.0006% for spectrophotometric methods and for HPLC method 0.016%. S_a – standard deviation of the calibration line; S_b – standard deviation of the intercept b

spectrum. As a result, derivative UV spectrophotometry represents a more sensitive and selective tool in comparison with direct UV absorption measurements.

EXPERIMENTAL

Material, reagents and apparatus

Moexipril hydrochloride was supplied by Schwarz Pharma AG (batch: 28057), each tablet contained 7.5 mg equivalent of moexipril hydrochloride (Cardiotensin 7.5).

Methanol and acetonitrile were of HPLC grade (Merck KGaA, Darmstadt, Germany). Potassium phosphate monobasic was purchased from POCh S.A., Gliwice, Poland. All other chemicals were of analytical reagent grade. Water used was freshly bidistilled.

Spectrophotometric measurements were performed on a Perkin-Elmer Lambda-6 spectrophotometer equipped with UV WinLab Version 2.70.01 software. The UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 240 nm/min, response time 0.5 s and fixed slit width of 3 nm. The concentrations of MOXL in its solutions in methanol were determined within wavelength range of 210–400 nm.

For HPLC assay a Shimadzu liquid chromatograph equipped in: Rheodyne 7125 injector with a fixed loop of 25 μL capability, UV-VIS SPD-6AV detector, LC-6A pump and C-R**6**A chromatopac integrator was used.

High performance liquid chromatography was performed on a RP-Hypersil MOS 100 C8 (size 10 μm, 250 mm × 4 mm I.D., Merck) column. The detector wavelength was set at 210 nm and mobile phase was pumped isocratically at a flow rate of 1.1 mL/min. The injection volume was 20 μL. All determinations were performed at ambient temperature.

Preparation of standard solutions

Spectrophotometric method: a standard solution (0.0012%) of MOXL was prepared by dissolving an appropriate amount of MOXL in methanol.

HPLC method: a standard solution (0.04%) of MOXL was prepared by dissolving an appropriate amount of MOXL in methanol.

Stored at 6^{o}C in the dark, standard solutions were stable during the period of study.

Analysis of tablets

Twenty tablets (Cardiotensin 7.5) were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (7.5 mg of MOXL) was weighed and put with addition of 25.0 mL of methanol into 50 mL volumetric flask. The prepared mixture was sonicated for 15 min and filtered (solution A).

Spectrophotometric method: 1.0 mL of solution A was diluted to 25.0 mL with methanol (solution A_1). The UV spectra D_1 (first order derivative spectra) were recorded against methanol.

HPLC method (reference method): mixture of 1.0 mL of the solution A and 1.0 mL of internal standard (I.S.) – xylomethazoline hydrochloride (solution R) was analyzed by HPLC. Samples (20 μL) to be analyzed were injected into the HPLC column and the emerging signals were recorded.

Preparation of model mixtures for evaluation of methods' accuracy

Model mixtures were prepared using Cardiotensin 7.5 tablets.

Ten tablets of Cardiotensin 7.5 were grounded in a mortar with addition of: 25 mg of MOXL (Model mixture I), 50 mg of MOXL (Model mixture II) and 75 mg of MOXL (Model mixture III).

Each mixture was grounded with a hand pistle for 20 min. The recovery was defined as percentage of the determined concentration of the constituent under examination with respect to the weighed amount.

Preparation of mobile phase

The mobile phase used in HPLC method was aqueous phosphate buffer at pH 2.0 (0.001 mol/L) and acetonitrile (1:1, v/v). The preparation of the former included: the dissolution of 0.0681 g of KH_2PO_4 (M = 136.09 g/mol) in 400 mL of water in 500 mL volumetric flask, the adjustment to the desired pH value using 80% orthophosphoric acid and final filling up the volume with water. The prepared mobile phase was filtered and degassed prior to use.

Procedure for MOXL kinetic study

Exactly weighed amounts of MOXL (0.0100 g) in opened, 5 mL glass vials were used for the determination of stability in the presence of relative humidity RH = 76.4%. The samples were stored in dessicator in an automatically controlled heat chamber at 363 K. After fixed time intervals, the samples of the investigated material were removed from the heat chamber, quantitatively transferred into 25 mL measuring flasks and dissolved up to volume with methanol (solution S).

Spectrophotometric method: 0.5 mL of the solution S was diluted to 25.0 mL with methanol. UV spectra D_1 were recorded against methanol.

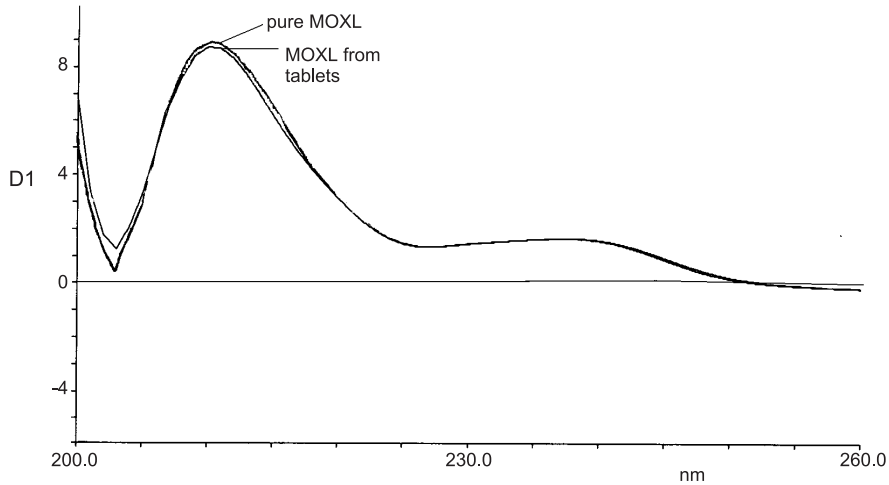

Figure 3. First-derivative absorption spectra for pure MOXL in a 0.00078% methanolic solution and for a methanolic extract from MOXL tablets (MOXL concentration of 0.00078%)

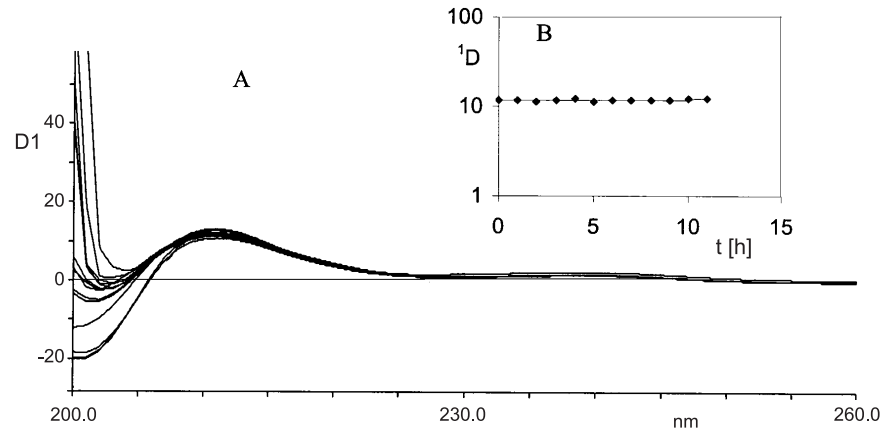

Figure 4. First-derivative absorption spectra for the MOXL degradation in a solid state (**A**), and a semi-logarythmic plot of first-derivative *versus* time for the degradation reaction in a solid state (**B**)

HPLC method (reference method): 1.0 mL of the solution S was mixed with 1.0 mL of the internal standard solution. The obtained mixture was analyzed by HPLC method. The chromatograms were interpreted using the following expression: $P_i/P_{I.S.} = f(t)$, where P_i stands for the area of MOXL signal, and $P_{I.S.}$- represents the area of I.S. (xylomethazoline hydrochloride).

RESULTS AND DISCUSSION

Spectrophotometric analysis

The first stage of this study included the selection of an analytical wavelength. For this, primary (A), first (D_1), second (D_2) and third (D_3) derivative absorption spectra were recorded in 1 cm quartz cells over a wavelength range of 200–300 nm (Table 1, Fig. 2). All the spectra represented a smooth line, however, only D_1 spectrum was characterized by a satisfactory analytical wavelength with the maximum of the absorption at λ_{max} = 238 nm. Absorption maxima in A, D_2 and D_3 spectra were below 220 nm, which was the reason for their rejection since λ_{max} < 220 nm is inapplicable for a qualitative pharmaceutical analysis. Then, the validation process of the selected D_1 UV spectrophotometric method (λ_{max} = 238) was performed and sensitivity, linearity, precision and accuracy were evaluated. The equations of

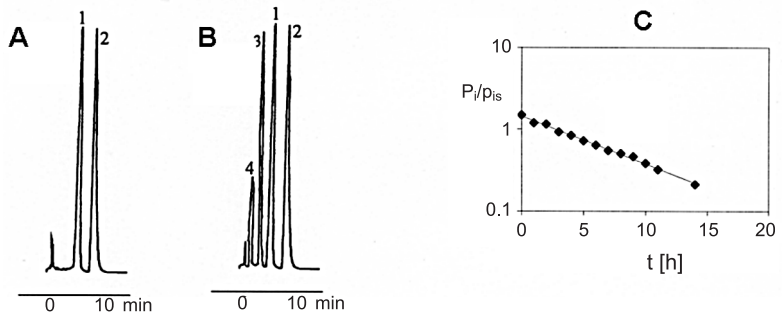

Figure 5. Stability-indicative assay of MOXL by HPLC. Chromatograms obtained for MOXL in methanol in t = 0 (**A**), and after forced degradation test (t = 8 h, RH = 76.4%, T = 363 K) (**B**) and a semi-logarythmic plot of P_I/P_{IS} *versus* time for a degradation reaction in a solid state (**C**).

the calibration curves were obtained from a linear regression analysis of the derivative amplitudes *versus* corresponding drug concentrations. The linearity of the calibration graph in a range of 0.00012–0.00120% was validated by the high value of correlation coefficient and intercept value, which was statistically insignificant (Table 2). The regression equation was calculated as Y = aC + b, where "C" stands for MOXL concentrations and the value of the intercept "b" is not statistically different from zero.

In order to assess the accuracy of the investigated method, the recovery study was conducted by analyzing the synthetic model mixtures of MOXL in three different ratios according to the procedure described above. The results are presented in Table 2. High (ca. 100.0%) recovery and low standard deviation was observed for first derivative UV spectroscopic method.

The precision of the proposed method was evaluated by replicate determination of eight samples at three different concentrations: low (0.00012%), medium (0.0006%) and high (0.0012%) (Table 2). To assess the repeatability (intra-day), ten samples were determined for MOXL concentration of 0.0006%. CV(%) value was < 1.5% indicating a good precision of this measurement (Table 2).

The validated first derivative UV spectrophotometric method was subsequently evaluated for its utility for analysis of MOXL in tablets (Fig. 3). The absorption spectrum of Cardiotensin 7.5 mg tablets methanolic extract was recorded and compared with the pure MOXL absorption spectrum (Fig. 3). No spectral interferences were observed between excipients used for the tablets' formulation and the active substance, which evidences the method's applicability for the determination of MOXL in tablets.

Additionally, the results of the determination of MOXL in tablets obtained by means of the proposed method were statistically compared with the results obtained by means of the reference – HPLC method using Student *t*- and -Snedecor *F*- tests (Table 2). It was evidenced that there is no significant difference between the two methods with respect to mean values and standard deviations indicating that first derivative UV spectrophotometric method is equally precise and accurate as HPLC method and can be recommended for routine analyses of MOXL in tablets.

The proposed method was finally tested as a stability-indicative assay for MOXL in solid state according to the above referenced procedure. It was observed that the UV spectra of MOXL methanol solutions subjected to the kinetic studies (over conditions described in Procedure for MOXL kinetic study) do not differ from those not subjected to decomposition study (Fig. 4). Changes in MOXL concentration during time *t* were observed only in HPLC method. The results obtained by means of HPLC evidenced that the MOXL decomposition proceeds according to the first order reaction model and the decomposition rate constant is $k \pm Dk = (3.67 \pm 0.13) \times 10^{-5}$ s^{-1}. The results are presented in Fig. 5.

CONCLUSION

This is the first study investigating the utility of first derivative UV spectrophotometric method as a stability-indicative assay for MOXL stability evaluation. The proposed method is accurate (the recovery about 100%), linear (r = 0.999), precise (SD <

1.5%), selective with reference to excipients used in the pharmaceutical formulation (Fig. 3), sensitive, rapid (only few minutes are required for the analysis), economical and can be used alternatively with HPLC reference method for the routine analysis and quality control of MOXL in its solid dosage formulations.

However, the MOXL decomposition in solid state was observed only in HPLC method while the first derivative UV spectrophotometric method turned out to be inappropriate for kinetic studies as a stability-indicative assay (Fig. 4) due to its low selectivity of MOXL decomposition products.

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