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UV DERIVATIVE SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR DETERMINATION OF IMIDAPRIL HYDROCHLORIDE IN TABLETS AND FOR ITS STABILITY ASSESSMENT IN SOLID STATE

BEATA STANISZ*, KATARZYNA REGULSKA and KINGA KOLASA

Department of Pharmaceutical Chemistry, University of Medical Science,
6 Grunwaldzka St., 60-780 Poznań, Poland

Abstract: Two methods for determination of imidapril hydrochloride (IMD) in the form of tablets were developed and the stability-indicative determination of IMD in solid state formulations by means of the proposed methods was investigated. IMD is not a pharmacopeial raw material, therefore there is no official method for its determination and purity assessment. The following analytical techniques were adopted for IMD determination: reverse-phase high performance liquid chromatography (RP-HPLC) and first derivative (^{1}D) ultraviolet spectrophotometry. RP-HPLC analysis was performed with the use of LiChrosfer RP-18 column as a stationary phase and acetonitrile-methanol-phosphate buffer pH 2.0 (60:10:30 v/v/v) as a mobile phase. The proposed method showed good linearity (in a range 40.0 – 400.0 μg/mL), accuracy, precision and selectivity for: IMD, its degradation product, and for oxymetazoline as an internal standard (IS). Additionally, different spectrophotometric methods were tested, and the first derivative spectrophotometry was accepted for further research. This method showed good linearity (in a range 4.0 – 40.0 μg/mL), precision and accuracy. The proposed methods were successfully applied to the pharmaceutical dosage form containing the investigated compound without any interference from the excipients. Finally, the results of the suggested methods were statistically compared using *t*-Student and *F*-Snedecor tests in the assessment for their equivalence.

Keywords: imidapril hydrochloride, RP-HPLC analysis, derivative UV, validation

Imidapril hydrochloride (IMD; (–)-(4*S*)-3-[(2*S*)-2-[[(1*S*)-1-ethoxycarbonyl-3-phenylpropyl] amino]-propionyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid hydrochloride) is a prodrug-type inhibitor of angiotensin converting enzyme (ACE-I). Its active, *in-vivo* de-estrified metabolite – imidaprilat, shows strong ACE inhibitory action. ACE is a key enzyme of rennin-angiotensin-aldosterone system. It catalyses the conversion of angiotensin I into its biologically active form – angiotensin II and thus controls serum levels of this octapeptide. Angiotensin II – a potent vasoconstrictor, acts as a hypertensive agent and plays an important role in the development and extent of essential hypertension. For this reason, angiotensin converting enzyme inhibitors are widely used as first-choice drugs in the treatment of cardiovascular system diseases. Imidapril hydrochloride is administered orally at a dose of 2.5–10.0 mg/day. The pharmacokinetic parameters of imidapril after oral administration of 10 mg are: c_{max}: 39.3 ± 10.1 ng/mL, T_{max}: ca. 2 h, and $t_{1/2}$: 1.8 ± 0.9 h (1).

IMD is not an official pharmacopeial raw material and therefore there is no officially approved method neither for its determination in the pharmaceutical dosage forms nor for its stability or purity assessment: in solid state formulations, as a raw material or in body fluids. Many methods, however, have been already tested including: bioanalytical techniques i.e., radioimmunoassay (2–5), radioenzymatic (6, 7) or fluoroenzymatic (8) reactions; HPLC assays with UV-Vis (9–11, 36), fluorescence (12–15), electrochemical (16, 17) and voltammetric (18, 19) detection; GC (20); various chromatographic techniques coupled with MS i.e., GC-MS (21–23) and LC-MS (24–29) and spectrophotometric assays (30). There are also papers on the application of thin-layer radiochromatography (31), and capillary electrophoresis with laser-induced fluorescence detection (32), potentiometry (33, 34) and densitometry (35). Bioanalytical techniques were rather expensive due to radiolabelled compounds used and the achievement of desirable precision required sometimes a triple sample analysis. Similarly,

* Corresponding author: e-mail: bstanisz@ump.edu.pl

HPLC techniques required a complicated sample pretreatment and time-consuming chromatographic separation and their sensitivity was usually insufficient for the pharmacokinetic studies. Also GC- and LC-MS-coupled techniques were inappropriate for stability-indicative assay due to sophisticated and expensive instrumentation needed for analysis and in case of GC-MS – complicated derivatization process.

For this reason, new analytical methods characterized by good selectivity/stereoselectivity, sensitivity, precision and accuracy need to be developed. From the practical point of view, it is also essential to optimize two aspects concerning the investigated methods i.e., time and costs of the analysis since they are both key factors in the process of method selection for kinetic studies in which a great number of measurements is usually taken.

The main idea of this study was to develop and investigate the utility of high performance liquid chromatography (HPLC) and various techniques of UV spectrophotometry i.e., A, ^{1}D, ^{2}D, ^{3}D for analytical determination of IMD in the form of tablets as well as for its stability-indicative determination in a solid state.

The results obtained by means of the above mentioned methods were subsequently statistically compared. The analytical procedure for both methods comprised the following stages: 1) validation; 2) evaluation of the key parameters (i.e., linearity, accuracy and precision); 3) assay of IMD content in the pharmaceutical preparation; 4) determination of the kinetic mechanism and estimation of disintegration rate constant for IMD under the following environmental conditions: temperature 363 K and elevated relative humidity RH = 76.4%.

EXPERIMENTAL

Chemicals

Imidapril hydrochloride (IMD) was supplied by Jelfa Pharmaceutical S.A. Jelenia Góra, Poland. Sodium chloride and oxymetazoline hydrochloride were purchased from Sigma-Aldrich. Methanol and acetonitrile were of HPLC grade (Merck, Darmstadt, Germany). Potassium phosphate monobasic was purchased from POCh, Gliwice, Poland. Water used was freshly bidistilled. All other chemicals were of analytical reagent grade.

Pharmaceutical preparation

A commercial pharmaceutical preparation: Tanatril tablets Jelfa Pharmaceutical S.A., Jelenia Góra, whose declared active ingredient's content was 10 mg of IMD.

Apparatus

UV-VIS spectrophotometer, Lambda-6 UV WinLab Version 2.70.01, Perkin Elmer with spectral bandwidth: 1.0 nm, scanning speed: 240 nm/min and wavelength increment ($\Delta\lambda$): 1.0 nm.

High performance liquid chromatograph Shimadzu (LC – 6A pump, Shimadzu, UV-VIS detector, 6AV, Shimadzu, integrator C – RGA Chromatopac, Shimadzu injector loop Rheodyne Berkley, California 7125, USA).

Procedure for spectrophotometric method

Solutions

IMD stock solution was prepared by dissolving the investigated compound in methanol to the concentration of 200 μg/mL. Its stability indication was undertaken after ten days of storage under temperature of 5^{o}C and no degradation products were detected. The determined content was 99.98 %.

A series of IMD standard solutions in a concentration range of 4.0 and 40.0 μg/mL was prepared by diluting the stock solution.

Assay procedure for the tablets

The accurate amount of powdered tablets (the equivalent to 10.0 mg of IMD) was weighed and 10 mL of methanol was added. After 15 min of shaking, the obtained solution was completed to a final volume of 25.0 mL with the same solvent and filtered through Whatman No. 42 filter paper (solution A). One mL of solution A was diluted with methanol to 10.0 mL. Absorption spectra in a range of 220–280 nm were recorded with a solution layer of 1 cm. The measurement parameters were identified at the analytical wavelength using "zero-crossing" technique.

Procedure for kinetic study

Samples of IMD (0.0100 g) were weighted in 5 mL glass vials for stability determination. The samples were placed in a desiccator filled with saturated solution of mineral salt (NaCl, RH = 76.4%) and then transferred into a thermal chamber heated to the temperature of 363^{o}K. The samples were taken out of the thermal chamber within time intervals depending on the decomposition rate, and cooled to the ambient temperature.

The solution for kinetic study

The content of each vial was subsequently quantitatively transferred into volumetric flasks

using methanol as a solvent, shaken for 10 min and completed with the same solvent to 25.0 mL (solution A). The amount of 0.5 mL of solution A was then completed with methanol to 10.0 mL. The absorption spectra in the range 200–300 nm were recorded as a time function with a solution layer of 1 cm.

The procedure for high performance liquid chromatography

The preparation of phosphate buffer at pH 2.0

The exact amount of 0.0681 g of potassium dihydrogen phosphate (KH_2PO_4) was weighed and transferred into a 500 mL volumetric flask; 400 mL of water was added and the flask was shaken till the salt dissolution. The solution was adjusted to pH 2.0 by adding 85% ortophosphoric (V) acid and then it was completed with water to the volume of 500.0 mL.

The chromatographic separation was performed at ambient temperature on a LiChrospher RP-18 (5 µm, 25 cm × 4 mm) column. IMD was separated isocratically with a mobile phase of acetonitrile, methanol and phosphate buffer at pH 2.0 (60:10:30 v/v/v). The mobile phase was degassed in an ultrasonic bath prior to use. The injection volume was 20 µL.

All the solutions used in the HPLC method were degassed and filtered through 0.45 µm micropore filter.

Solutions

The accurate amount of 0.020 g of IMD was weighed, dissolved in methanol and completed with the same solvent to 50.0 mL (stock solution). The aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 mL of stock solution were measured and transferred to a series of volumetric flasks. Each flask was completed with methanol to 10.0 mL to reach the concentration range of 40–400 µg/mL.

Analytical procedure for tablets

The portions of powdered tablets (pharmaceutical preparation – Tanatril 10 mg), equivalent to 0.0050 g of IMD were weighed and 5 mL of methanol was added. After 15 min of shaking, the solution was completed with the same solvent to 10.0 mL and then it was mixed and filtered.

The internal standard (IS) solution

The exact amount of 0.020 g of oxymetazoline hydrochloride was weighed and dissolved in 100.0 mL of methanol (solution IS with a concentration of 200 µg/mL).

The procedure for kinetic study

The stability of IMD under elevated relative humidity conditions RH = 76.4% and temperature of 363 K was studied. The conditions of kinetic study were described in a section: *Procedure for spectrophotometric method.*

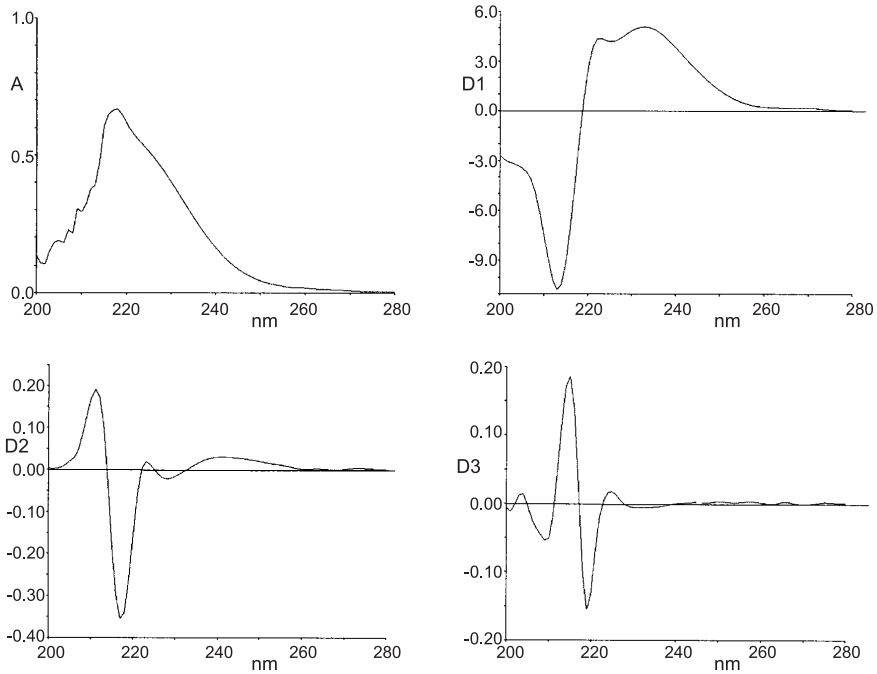

Figure 1. A, ^{1}D, ^{2}D, ^{3}D absorption spectra of IMD in methanol as a function of wavelength

Table 1. Values of λ_{max} and λ_{min} of UV spectra (A, ^{1}D, ^{2}D, ^{3}D) of IMD in methanol.

Method	λ_{max} [nm]	λ_{min} [nm]	λ_{max} [nm]
A	218	–	–
^{1}D	234	213	222
^{2}D	211	217	223
^{3}D	215	219	224

Table 2. Statistical analysis of validation results obtained for RP-HPLC and first derivative UV spectrophotometric method and the estimation of their utility for IMD determination in tablets.

Parameters	RP-HPLC	First derivative spectrophotometry
Range (μg/mL)	40.0 – 400.0	4.0 – 40.0
Detection limits (μg/mL)	24.0	3.0
Determination limits (mg/mL)	450.0	50.0
Regression equation (Y)[a]		
Slope a ± Δa	0.00319 ± 0.00019	0.119 ± 0.0064
S.D. on slope (S_a)	0.000091	0.0028
Intercept[b] b ± Δb	0.026 ± 0.0049	0.089 ± 0,016
S.D. on slope (S_b)	0.0228	0.0696
Statistical analysis of intercept *b*	$t = b/S_b = 1.12$[b]	$t = b/S_b = 1.29$[b]
Correlation coefficient (r)	0.998	0.998
Precision		
RSD(%)	0.693	0.921
Commercial tablets		
Tanatril 10 mg IMD per tablet	(10.38 ± 0.079) mg	(10.35 ± 0.081) mg
RSD(%)	1.06	1.09
Statistical analysis of method		
t	0.379	
F	1.024	

Theoretical values at 95% confidence limits $F = 3.18$ and $t = 2.26$ [a] Y = a*C* + b where c is concentration in mg/mL and Y in absorbance units (first derivative spectrophotometry); [b] value $t = b/S_b$ is less than critical value $t_{\alpha,f} = 2.228$ at significance level $\alpha = 0.05$, which indicates the value b = 0 (statistically insignificant).

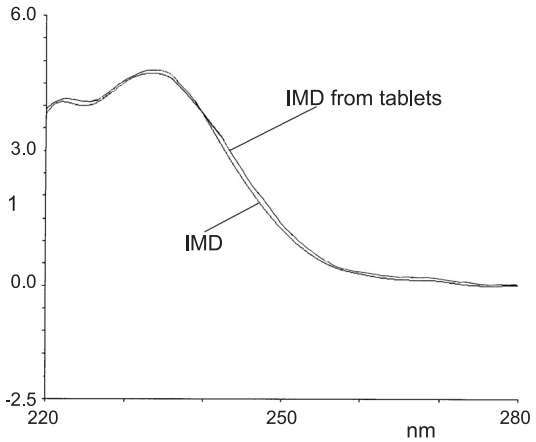

Figure 2. The comparison of first derivative UV spectrum obtained from powdered commercial tablets (concentration of IMD 0.04 μg/mL) and first derivative UV spectrum of pure IMD (concentration of IMD 0.04 μg/mL)

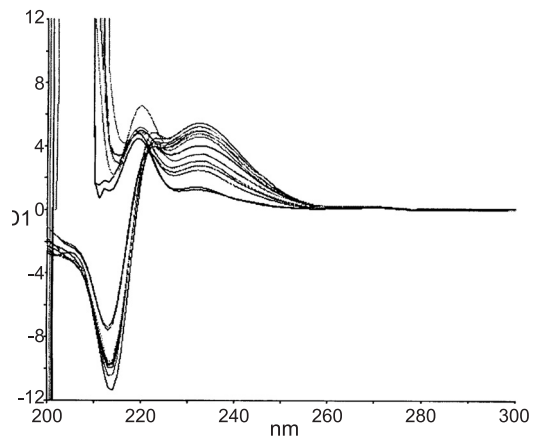

Figure 3. HPLC chromatogram of methanol solution of IMD after heating for 6 days at 363 K, RH = 76.4%. Peak IMD: imidapril hydrochloride, peak P: decomposition product, and peak IS: internal standard

The solution for kinetic study

The content of the vials was quantitatively transferred into volumetric flasks using methanol as a solvent; each flask was shaken for 10 min and completed with the same solvent to 25.0 mL (solution A). One milliliter of IS (methanol solution of oxymetazoline hydrochloride with the concentration of 200 μg/mL) was added to 1.0 mL of solution A. The obtained solutions were injected (in the amount of 20 μL) into a column and chromatograms were recorded.

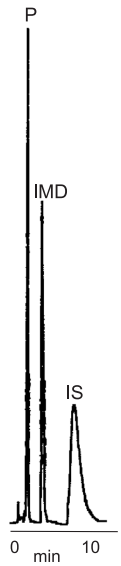

Figure 4. Absorption spectra $^1D = f(\lambda)$ for decreasing concentrations of IMD in solid state (RH = 76.4, T = 363K) in time

RESULTS AND DISCUSSION

Discussion of the validation process

UV spectrophotometry method

The proposed UV spectrophotometric method provided a rapid and uncomplicated procedure for quality control analysis of IMD tablet dosage forms.

The analysis of A, ^1D, ^2D and ^3D spectra (Fig. 1., Table 1) proved that first-derivative (^1D) UV spectrophotometry was the most appropriate for the determination of IMD in tablets due to its the most selective wavelength (λ_{max} = 234 nm). From the analytical point of view, the absorption measurement at the wavelength λ_{max} = 218 nm (in case of A spectrometry) is not optimal due to its low specificity for the investigated compound. Second and third derivative UV spectrophotometry showed low sensitivity and unsatisfactory analytical wavelengths, λ_{max} and λ_{min} below 225 nm. In further validation of ^1D UV spectrophotometric method, calibration graphs were constructed by plotting Δ^1D value (selected amplitudes from ^1D spectrum) *versus* drug concentrations and linear relationship was observed. The adequate regression equation was computed and found to be the following: $y = ac = (0.119 \pm 0.0064)\ c$; standard deviation of the slope of the linear regression was $S_a = 0.0028$ while intercept *b* was statistically insignificant (Tab. 2). A

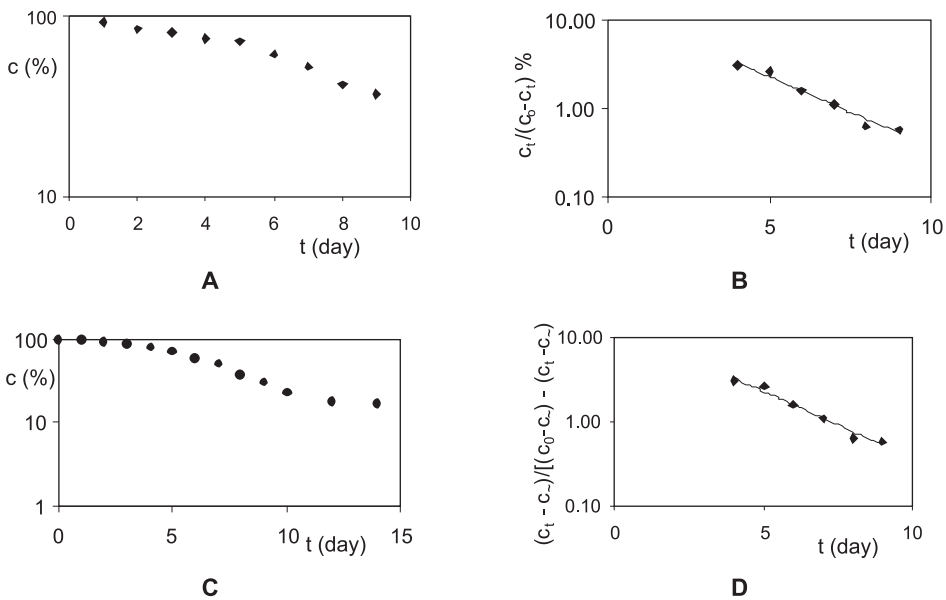

Figure 5. Semi-logarithmic plots c = f(t); [**A** – HPLC, **C** – ^1D UV spectrophotometic method], $c_t/(c_o-c_t) = f(t)$; [**B** – HPLC] and $(c_t-c_\infty)/[(c_o-c_\infty) - (c_t-c_\infty)] = f(t)$; [**D** – ^1D UV spectrophotometic method] for the degradation of IMD in solid phase (T = 363 K, RH = 76.4%)

high value of correlation coefficient (r = 0.998) indicated a strong linear relationship between measured value ^1D and corresponding concentration of active ingredient in a sample. The precision of the proposed analytical method was considered at three levels: high, average and low, and it was found to be optimal since the variability ratio (RSD%) on each level was less than 1.10%. The values of LOQ and LOD were 50.0 and 3.0 μg/mL, respectively. The proposed method was applied to the pure investigated substance as well as to the commercial pharmaceutical dosage form. No interference between IMD and the excipients was detected, which was evidenced by the absence of significant differences between the recorded spectra (Fig. 2). The content of IMD in tablets, determined by means of first derivative UV spectrophotometry was (10.35 ± 0.081) mg.

High performance liquid chromatography

A simple, inexpensive and time-sparing (a chromatogram recording took less than 10 min) high performance liquid chromatographic method was developed for the determination of IMD. Its selectivity for IMD (t_R = 6 min), its degradation product (t_R = 4 min), and IS (t_R = 8.5 min), was confirmed (Fig. 3). The validation evidenced that the method was linear in the range of tested concentrations, precise and sensitive. The internal standard – oxymetazoline hydrochloride was used with the view to eliminate possible errors caused by the environmental temperature influence or the volume differences between the samples injected.

The ratio of IMD/IS peak area was taken into consideration. The robustness of the HPLC method was investigated by analysis of the samples under variety of experimental conditions such as small quantitative changes in the mobile phase (5–7% of acetonitrile, 1–2% of methanol) and flow rate changes (1.0–1.2 mL/min). The effect on peak shape and retention time was studied. It was evidenced that the changes of these conditions did not influence IMD or IS peak shape, however, they modified the retention times, but only to a limited extent ± 2%. The correlation coefficient value was r = 0.998 which confirmed a strong linearity and good correlation between the variables. The linearity was confirmed by plotting the measured peak area *versus* the corresponding concentrations. The regression equation was found to be: $y = ac = (0.00319 \pm 0.00019)\ c$; (Tab. 2). The precision and the waste synthetic model mixtures recovery were satisfactory. The summary of the method characteristics is demonstrated in Table 2.

It was finally concluded that the method can be successfully applied to the assessment of IMD in tablets. The determined content of the active substance corresponds to the one claimed by the manufacturer and equals (10.38 ± 0.079) mg.

The precision of both methods was statistically compared by the use of *F*-Snedecor and *t*-Student's tests. The calculated *F*- and *t*-values were found to be less than the corresponding critical ones indicating comparable precision of both methods. The detailed summary of the statistical analysis for both methods is demonstrated in Table 2.

The validated, spectrophotometric and HPLC methods were used for the stability-indicating assessment of IMD in solid state formulations.

In the spectrophotometric method, the variations of the measured first derivatives values, taken from UV spectra, were used for the determination of IMD disintegration constants. For this purpose, the IMD samples were exposed to the accelerated degradation test (363K/76.4% RH) prior to the analysis. The results showed that the measured ^1D values gradually decreased starting from $^1D_0$ to reach a constant value $^1D_\infty > 0$ (Fig. 4). The first derivatives variations analysis at λ = 234 nm did not demonstrate any shifts in their amplitudes maxima, however, the amplitudes variations occurred at λ = 218 nm. The disintegration of IMD progressed according to the second-order autocatalytic reaction model (Fig. 5C). The disintegration constants under the experimental conditions were calculated using the Prout-Tompkins equation:
$\ln (c_t - c_8) / [(c_0 - c_\infty) - (c_t - c_\infty)] = C - k \times t$, after previous use of "subtraction technique" (Fig. 5D), where: c_0, c_∞ and c_t – stand for the concentration of IMD in the time t_0, t_∞ and t_t, respectively, C = induction period and k = disintegration rate constant (s^{-1}).

In case of the proposed HPLC method, conducted under the same kinetics conditions as the UV first-derivative analysis, the disintegration rate constants were calculated by the use of the following equation (Fig 5A,B): $\ln c_t/(c_0 - c_t) = C - k \times t$.

The calculated disintegration rate constants by means of the investigated methods were the following: $k \pm \Delta k = (4.2099 \pm 0.9559)\ 10^{-6}\ s^{-1}$ (HPLC) and $k \pm \Delta k = (4.2134 \pm 0.8509)\ 10^{-6}\ s^{-1}$ (^1D UV spectrophotometry).

The test for parallelism of the slopes of straight lines was performed in order to confirm the equivalence of both methods in the IMD qualitative estimation. The calculated values of t_0 were less than the critical value $t_{0.05}$, which evidences that both methods can be used alternatively for IMD stability-indicative assay.

CONCLUSION

The objective of this study was to develop a standard procedure for IMD qualitative analysis and its stability-indicative assay in solid state formulations. With this aim RP-HPLC and ^{1}D UV spectrophotometric methods have been validated and their sensitivity, specificity and linearity have been proven. Their equivalence has been confirmed by statistical analysis (*F*-Snedecor and *t*-Student, the test for the parallelism of slopes of the lines) and therefore both methods are recommended for routine and quality control analysis of IMD as well as for its stability assessment.

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