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Comparison of high-performance thin layer chromatography/ UV-densitometry and UV-derivative spectrophotometry for the determination of trimetazidine in pharmaceutical formulations

MARCIN GACKOWSKI[1]*
MARCIN KOBA[1]
KATARZYNA MĄDRA-GACKOWSKA[2]
STEFAN KRUSZEWSKI[3]

[1] *Department of Toxicology, Faculty of Pharmacy, Collegium Medicum of Nicolaus Copernicus University Bydgoszcz, Poland*

[2] *Department of Geriatrics, Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Toruń 85-094 Bydgoszcz, Poland*

[3] *Medical Physics Division, Biophysics Department, Faculty of Pharmacy Collegium Medicum Nicolaus Copernicus University Bydgoszcz, Poland*

New methods for assaying trimetazidine dihydrochloride on the basis of thin layer chromatography and spectrophotometry are proposed and compared in the paper. In HPTLC/UV-densitometry, separation is achieved by using a mobile phase composed of ammonia-methanol (30:70, *V*/*V*) on silica gel HPTLC plates F254. Quantification using a non-linear calibration curve is accomplished by densitometric detection at 230 nm. Derivative spectrophotometric determination of trimetazidine dihydrochloride is carried out from the fourth derivative of the absorbance at 233 nm in peak-zero mode. Statistical comparison led to the conclusion that there is no significant difference between the two studied methods and, moreover, that they demonstrate satisfactory accuracy and precision for routine applications.

Keywords: trimetazidine dihydrochloride, high performance thin layer chromatography/UV-densitometry, derivative spectrophotometry, pharmaceutical formulations

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Trimetazidine (chemical name [1-(2,3,4-trimethoxybenzyl)-piperazine], TMD), is a cytoprotective drug for stable angina, which may be also used for the treatment of systolic dysfunction in cardiac failure patients (1). This anti-anginal agent was developed and marketed in the 1970s by Laboratoires Servier but its effectiveness raised a lot of controversy, to such an extent that some have gone so far as to call it a "placebo drug" (2, 3). Nevertheless, TMD is widely prescribed as a long-term treatment in Europe and Asia (4). In general, it has been treated as a highly safe and well-tolerated drug; however, in 2012, the European Medicines Agency (EMA) recommended some restrictions on TMD (3).

Only a limited number of studies reported in recent years show that high performance thin layer chromatography (HPTLC) can be successfully applied for the quantifica-

* Correspondence, e-mail: marcin.gackowski@cm.umk.pl

tion of trimetazidine in pharmaceutical dosage forms; more spectrophotometric methods were found for the analysis of TMD (5–9). Most of those methods are based on simple UV spectrophotometry or require additional reagents and procedures to obtain colored products and consequently need ample time for execution. The primary aim of this study is to suggest more convenient alternatives to the above listed methods by designing procedures with minimum equipment, chemicals and time consumption as well as to point out the most suitable method for routine control of trimetazidine. Based on our previously developed methods (10, 11), the newly established methods involve HPTLC/UV-densitometry and UV-derivative spectrophotometry, which are commonly used in pharmaceutical analysis, mainly because of their low cost, simplicity and rapidity. The secondary aim of the study is to establish a method based on the fourth-order spectra for determination of TMD and compare it with HPTLC/UV-densitometry.

EXPERIMENTAL

Chemicals

Trimetazidine dihydrochloride ([1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride], 97 %, used as standard, was purchased from Sigma-Aldrich (USA). Pharmaceutical preparation Metazydyna® (trimetazidine dihydrochloride, 20 mg) was from Polfa Pabianice S.A. (Poland). Talc was obtained from Zakład Farmaceutyczny Amara (Poland). Methanol, ethanol 96 %, 0.1 mol L^{-1} NaOH, 0.1 mol L^{-1} NaCl and ammonia 20 %, were all from Polskie Odczynniki Chemiczne S.A. (Poland). Water was prepared by means of a Milli-Q Water Purification System (Millipore, USA).

Apparatus

Instrumentation essential for HPTLC included: a precoated silica gel aluminium HPTLC plate 60 F-254 (20×20 cm, thickness 150 μm, particle size 5–7 μm; Merck, Germany), a horizontal DS-type chamber (Chromedes, Poland), a CD 60 HPTLC densitometer using ProQuant software (Desaga, Germany) for densitometric measurement and a photographic documentation system CabUV-Vis composed of Canon Power Shot G5 digital apparatus with ProViDoc 3.0 software (Desaga).

Table I. Chromatographic parameters of the HPTLC assay of TMD

Parameter	Value
R_f (retardation factor, relate-to-front) / hR_f (R_f multiplied by 100)	0.74 / 74
k' (capacity factor)	0.36
SN (separation number)[a]	10
N (theoretical plate number)	1900
HETP (height equivalent to a theoretical plate)	47 μm

[a] SN – Separation number or spot capacity (maximum number of substances completely separated between $R_f = 0$ and $R_f = 1$).

Table II. Types of calibration curves, recovery and precision of trimetazidine dihydrochloride quantification by the HPTLC/UV-densitometric method

							Determination in model mixtures					Determination in tablets			
Fitting curve	a	b	c	d	m	R^a	Model mixture (%)[b]	Recovery (%)[c]	Mean recovery (%)[d]	RSD (%)[e]	Mean RSD (%)[f]	Declared mean content (mg)[g]	Found mean content (mg)[h]	% of declared content[d]	RSD (%)[e]
							80.0	106.2		0.7					
$y = a + bx$	2079.2	70.404				0.9750	100.0	105.2	104.8	1.3	1.0		19.71	98.6	2.8
							120.0	102.8		1.0					
							80.0	99.7		1.4					
$y = a + bx + cx^2$	1124.7	127.93	−0.4006			09980	100.0	103.6	101.3	2.1	1.3		19.79	98.9	2.3
							120.0	100.6		1.6					
												20.0			
							80.0	89.9		1.2					
$y = a + bx + cx^2 + dx^3$	906.98	153.33	−0.8455	0.002		0.9986	100.0	99.1	96.6	1.9	1.5		18.85	94.2	4.5
							120.0	100.8		1.4					
							80.0	87.2		1.3					
$y = ax^m$	640.41				0.5876	0.9984	100.0	98.0	96.0	1.9	1.6		18.31	91.6	4.8
							120.0	102.9		1.7					

[a] Correlation coefficient; [b] Model mixtures contained 80.0, 100.0 and 120.0 % of TMD compared to tablets; [c,d] Percentage and mean percentage of recoveries of the data obtained for the studied compound calculated for six independent measurements; [e,f] Percentage and mean percentage of RSDs calculated for six independent measurements; [g,h] Declared and found mean contents of studied compounds in one tablet. According to the *Polish Pharmacopeia X* (2017), the drug content should be between a minimum of 85 % and maximum of 115 % of the amount declared by the manufacturer.

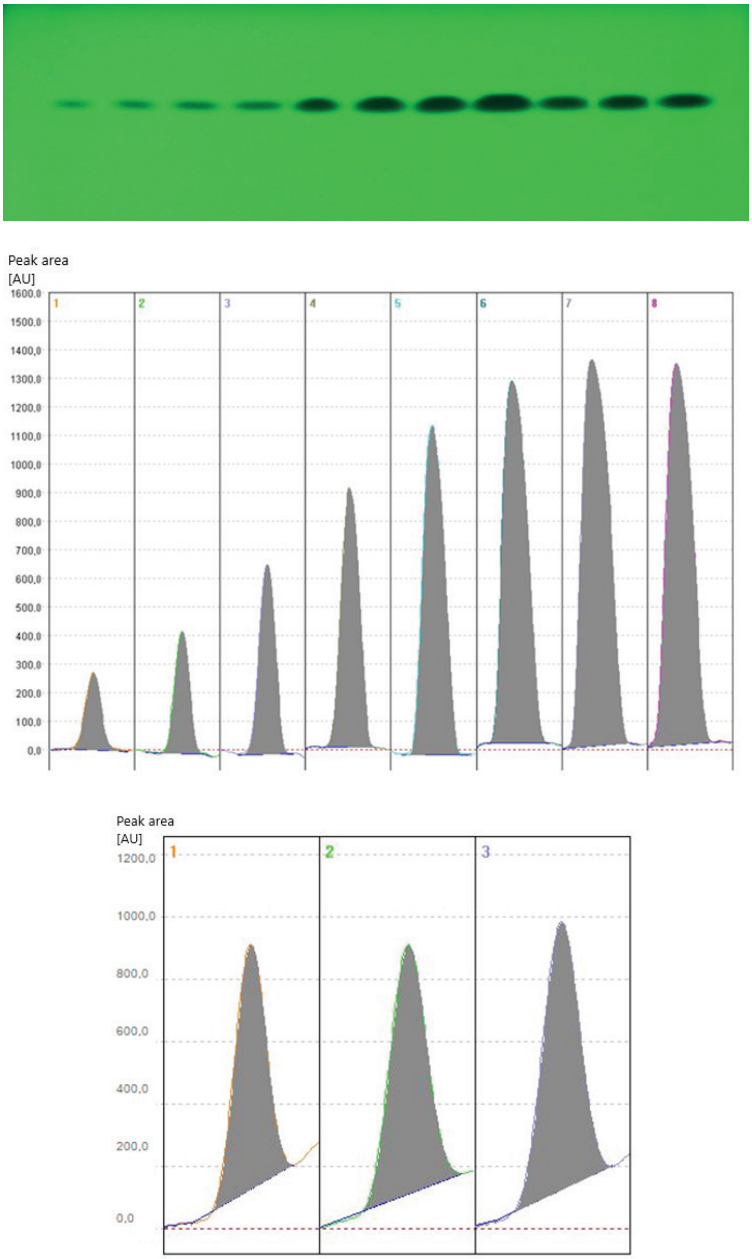

Fig. 1. a) HPTL chromatogram of trimetazidine dihydrochloride at 230 nm with standard lines 1–8 (1–0.25 μg, 2–0.50 μg, 3–0.75 μg, 4–1.00 μg, 5–1.25 μg, 6–1.50 μg, 7–1.75 μg, 8–2.00 μg TMD) and tablet extracts (lines 9–11); b) densitogram with standard lines (1–8); c) densitogram with tablet extracts (lines 1–3).

For spectrophotometric measurements, a double beam UV-Vis spectrophotometer (Hitachi U-2800, Japan) was used to record spectra over the 190–400 nm range at 25 °C in a 1-cm quartz cell. UV Solutions 2.0 software was used to calculate derivative spectra (first-, second-, third- and fourth-order) using the Savitzky-Golay method (order = 3, $\Delta\lambda$ = 6 nm) with peak-zero (P-0), zero-peak (0-P) and peak-peak techniques of measuring.

Standard solution

Selection of solvent for analyses was based on recording and investigating the spectra of TMD dissolved in water, 0.1 mol L^{-1} HCl, 0.1 mol L^{-1} NaOH, ethanol and methanol. Most of them were eliminated because of the absorption maximum below 0.3, solution color after extraction from tablets or time-consuming extraction. Methanol was chosen as the most appropriate solvent for HPTLC as well as for spectrophotometry and time of extraction was optimized at 5 min. A quantity of 100 mg of pure TMD was dissolved in methanol and diluted to 100 mL in a calibrated flask giving a stock solution (1 mg mL^{-1}) for further analyses. After appropriate dilutions, this stock solution was used for calibration.

Calibration

HPTLC/UV-densitometry. – Stock solution of TMD diluted to a concentration of 0.25 μg mL^{-1} was applied (lines 1–8, Fig. 1) onto the plate in the range of 0.25–2.00 μg per spot. After 10-min evaporation of the solvent at room temperature, a chromatogram was developed using the horizontal technique in the chamber saturated with the mobile phase composed of ammonia/methanol (30:70, *V*/*V*). Saturation time was 60 min. A chromatogram run was 9 cm and hR_f value was 74. In the next step, the separation plate was dried and after 15 minutes, densitometric analysis was performed in the absorbance mode at a wavelength of 230 nm (Figs. 1a,b). All measurements were repeated six times. Finally, calibration curves were created as the relationship between the peak area value and drug quantity per spot (Table II).

UV-derivative spectrophotometry. – Nine dilutions of the TMD stock solution in the concentration range of 0.25–10.0 μg mL^{-1} were used. Consecutive spectra were recorded by a double beam spectrophotometer against methanol (blank) (Fig. 2). All measurements were done six times for each concentration (Table III).

TMD assay in model mixtures and pharmaceutical formulation

Three laboratory prepared model mixtures were prepared by adding the TMD standard to the samples in an amount from 80 to 120 % with respect to the declared content of TMD in the pharmaceutical preparation Metazydyna® (20 mg). Trimetazidine dihydrochloride tablets Metazydyna® 20 mg were triturated and a mass equivalent to 5.00 mg of TMD was transferred into a volumetric flask (for model mixtures equivalent to 4.00, 5.00 and 6.00 mg of TMD, resp.) and dissolved in 20 mL methanol (repeated six times). After swirling, sonification, filtration and rejection of the first portion of the filtrate, six independent working solutions of TMD were prepared for analysis and applied onto a chromatographic plate in a volume of 5 μL each line, corresponding to 1.00, 1.25 (for model mixture and sample of Metazydyna®) and 1.50 μg of trimetazidine per spot (80.0, 100.0, 120.0 %). Finally, densitometric scanning was performed under the abovementioned conditions.

Table III. The best calibration curves, statistical evaluation, recovery and precision of the trimetazidine dihydrochloride assay by derivative spectrophotometry

Spectrum/ method[a]		Wave-length (nm)	R^b	$y = ax + b$		t_{Sa}[c]	t_{Sb}[d]	Coefficient of essentiality[e]			Determination in model mixtures					Determination in tablets			
				a	b			a	b	R	Model mixtures (%)[f]	Recovery (%)[g]	Mean recovery (%)[h]	RSD (%)[i]	Mean RSD (%)[i]	Declared mean content (mg)[k]	Found mean content (mg)[l]	% of declared content[g]	RSD (%)[i]
D1	peak-zero	260.0	0.9596	0.00006	−0.00002	0.00001842	0.0003629	+	−	+	80.0 100.0 120.0	124.1 121.7 104.1	116.6	5.0 5.6 5.2	5.3	20.0	19.77	98.8	6.1
		260.5	0.9974	0.00006	−0.000002	0.00001491	0.0002938	+	−	+	80.0 100.0 120.0	122.3 119.9 101.9	114.7	4.9 5.8 5.13	5.3		19.43	97.1	6.3
		261.5	0.9980	0.00005	−0.000005	0.00001219	0.0002401	+	−	+	80.0 100.0 120.0	132.2 135.0 113.1	127.0	4.8 5.8 5.4	5.3		21.71	108.5	6.9
D4	peak-zero	232.5	0.9913	0.0000002	−0.00000007	0.000000091	0.0000017861	+	−	+	80.0 100.0 120.0	113.0 113.3 96.7	107.7	2.8 2.1 2.3	2.4		21.67	108.3	2.8
		233.0	0.9939	0.0000002	−0.00000009	0.000000095	0.0000018738	+	−	+	80.0 100.0 120.0	106.7 104.3 95.3	102.1	1.9 1.7 2.2	1.9		19.87	99.3	2.8
		234.0	0.9917	0.000002	−0.0000001	0.000000102	0.0000020192	+	−	+	80.0 100.0 120.0	96.7 93.3 83.3	91.1	3.6 2.5 2.8	2.9		18.67	93.3	5.3

[a] D_1 (first-derivative), D_4 (fourth-derivative) spectrum; [b] Correlation coefficient; [c,d] Confidence of the slope (a) or of the intercept (b) at 95 % confidence level ($t_{\alpha,f} = 2.776$ for $\alpha = 0.05$ and $f = (n-2)$, where $n = 6$); [e] Coefficient of a (slope), b (intercept) and R (correlation), respectively, tested at 95 % confidence level using the point hypothesis test: +, essential; –, not essential; [f] Model mixtures containing 80.0, 100.0 and 120.0 % of the studied compound compared to the labeled tablet amount; [g,h] Percentage and mean percentage of recoveries of the data obtained for the studied compound calculated for six independent measurements; [i,j] Percentage and mean percentage of relative standard deviations calculated for six independent measurements; [k,l] Declared and found mean content of studied compounds in one tablet. According to the *Polish Pharmacopeia X* (2017) the drug content should be between a minimum of 85 % and maximum of 115 % of the amount declared by the manufacturer.

Next, for the spectrophotometric method, tablet extracts were diluted with methanol to approx. 5.00 μg $mL^{-1}$and model mixture extracts were diluted to 4.00, 5.00 and 6.00 μg mL^{-1}, resp. UV-spectra were recorded against the blank (methanol) over 190–400 nm and derivative spectra were subsequently calculated.

Validation of HPTLC and derivative spectrophotometry methods was performed in accordance with the Association of Analytical Communities (AOAC) Guidelines for Standard Method Performance Requirements (12). Accuracy of the abovementioned methods was assessed by recovery rates after addition of a standard on three levels into laboratory-prepared model mixtures, and repeatability was expressed as relative standard deviation (RSD). Other validation parameters are limit of detection (*LOD*) and quantification (*LOQ*), specificity, linearity, Horwitz ratio.

Statistical testing for significant difference between the elaborated methods was performed by means of *t*- and *F*-tests.

RESULTS AND DISCUSSION

In the present study, two independent analytical methods for assaying trimetazidine dihydrochloride in the pharmaceutical dosage form have been developed, validated and finally compared.

Initially, methanol was selected as the most suitable solvent for both analyses, ensuring good solubility, application on chromatographic plates, effective extraction and no interference with TMD in absorbance measurements.

HPTLC separation was followed by a densitometric scan in order to find the analytical wavelength for quantitative analysis; this was 230 nm (chromatographic parameters are presented in Table I). For the linear model, *R* was 0.9750, RSD 1.0 and 2.8 %, mean recovery 104.8 and 98.6 % for model mixtures and tablets, resp. Some findings showed that calibration curves for HPTLC, when the densitometer worked in UV or Vis, showed non-linear fit (13–15). On the other hand, linearity was also observed, especially after transformation in agreement with Lambert-Beer´s law (15). In accordance with our previous experience (10), relationships between the recorded peak areas and TMD quantity per spot were non-linear (Table II). In sum, the best recovery value (101.3 %) and precision (RSD 1.3 %) were achieved for a quadratic equation ($y = a + bx + cx^2$) calibration curve with $R = 0.9980$ (Table II). In addition, for quantification of TMD in tablets, the precision was also acceptable, with RSD 2.3 % and recovery 98.9 % (Fig. 1).

Next, the UV-derivative spectrophotometry method was applied to determine trimetazidine dihydrochloride in tablets. First, the zero-order spectrum (Fig. 2a) was recorded for 0.25–10.0 μg mL^{-1} of TMD with λ_{max} = 205.5 nm. First-, second-, third- and fourth-derivative spectra (first- and fourth- derivatives are shown in Figs. 2b,c) were obtained and measured by the peak-zero and peak-peak techniques. The vast majority of calculated calibration lines were characterized by satisfactory linearity ($R > 0.99$); only two were rejected. Moreover, for all curves, based on statistical testing, the intercept (b) was not significantly different from zero, which led to the calculation from equation $y = ax$. For most curves, the results were characterized by good precision (RSD 1.9–5.3 %). However, analyses of the model mixtures presented an overestimated mean recovery value for some of the proposed calibration curves. Namely, some curves were not appropriate for estimation. Only selected derivative spectra and wavelengths gave satisfactory recovery and

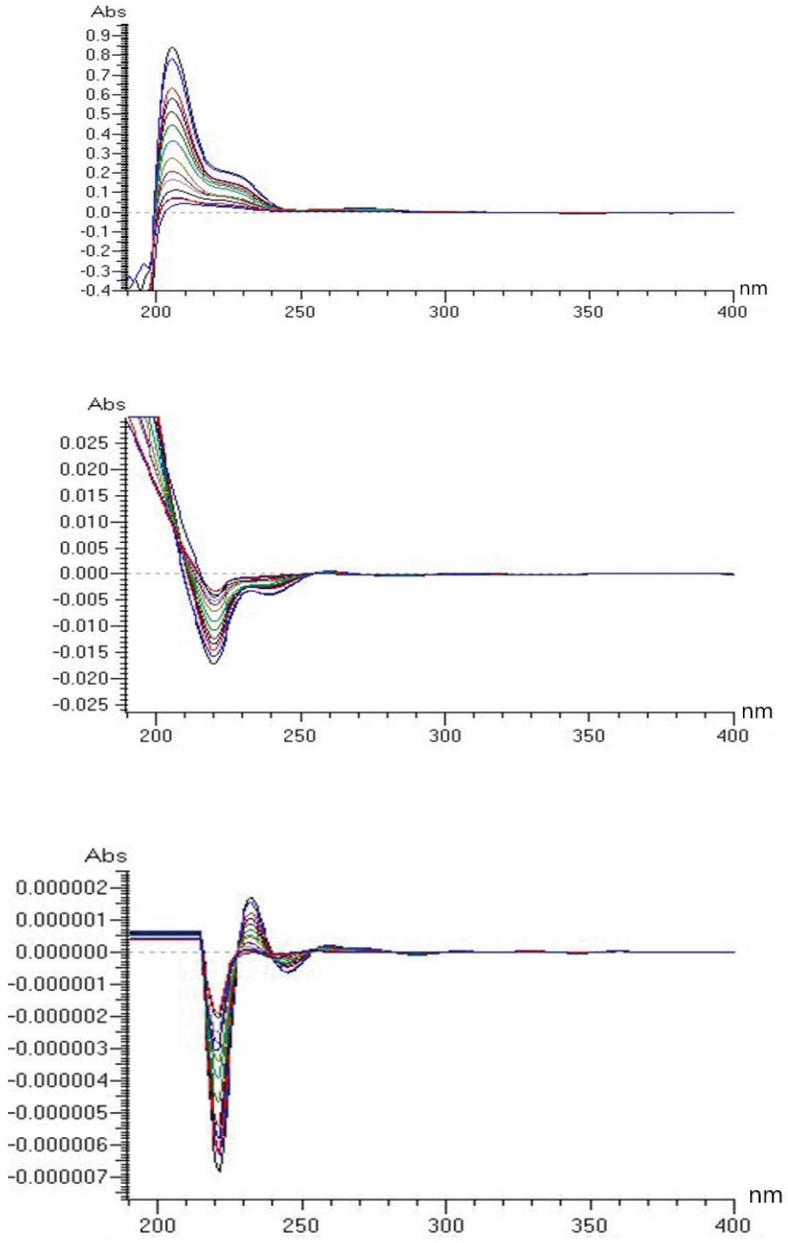

Fig. 2. a) Zero-order, b) first- and c) fourth-order derivative spectra of trimetazidine dihydrochloride in the concentration range 0.25–10.0 μg mL^{-1} in methanol.

Table IV. Statistical comparison, limits of detection and quantification of trimetazidine by the new methods

Method	TMD						
	LOD (μg mL^{-1})	*LOQ* (μg mL^{-1})	Calibration curve equation	*R*	HorRat	*t*	*F*
HPTLC/UV-densitometry	0.83	2.51	$y = 1124.7 + 127.93x - 0.4006x^2$	0.9980	0.94		
UV-derivative spectrophotometry (4th derivative mode, 233 nm)	0.15	0.46	$y = 2 \times 10^{-7}x$	0.9939	1.15	0.93	0.57

Number of determinations: $n = 6$; significance level $p = 0.05$; tabular *t*-factor and *F*-factor for the degree of freedom during determination of precision or accuracy: $t = 2.23$; $F = 5.05$. *LOD* – limit of detection, *LOQ* – limit of quantitation.

might be used to establish a quantification method. The most accurate conditions were found for the fourth-order derivative spectrum (recovery 102.1 and 99.3 % for model mixtures and tablets, resp.) with mean RSD 1.9 % for model mixtures and 2.8 % for tablets as well as for zero-order (Table III).

No interference was observed from talc during chromatographic separation, spectrophotometric measurement (190–400 nm) or densitometric measurement at 230 nm. Lower values for detection (*LOD*) and quantification limit (*LOQ*) were obtained for derivative spectrophotometry, making the spectrophotometric methods more sensitive than HPTLC/UV-densitometry (Table IV) (10, 11).

Accuracy of the elaborated methods was evaluated by recovery at three levels (80.0, 100.0, 120.0 % of the studied compound compared to the tablet label amount) and confirmed that both methods were reliable. Mean recovery for determination in model mixtures was 101.3 and 102.1 % for HPTLC and spectrophotometry, resp. Precision of the estimated methods was assessed as well, with RSD values of 1.2 and 1.9 % for HPTLC and derivative spectrophotometry, resp. It must be emphasized that there was no significant difference between the elaborated methods, since the calculated *t*- and *F*-values did not surpass theoretical values at the confidence level of 95 % (Table IV). All the obtained values of HorRat (Horwitz ratio) were between 0.3 and 1.3 (Table IV), which means that both elaborated methods can be considered acceptable for single, namely, in-house, laboratory validation (12).

CONCLUSIONS

The present study demonstrates the potential application of HPTLC and derivative spectrophotometry methods in analyses of trimetazidine dihydrochloride in its pharmaceutical formulation. On the one hand, the derivative spectrophotometry method is characterized by better sensitivity (lower *LOD* and *LOQ*) but, on the other hand, HPTLC/UV-densitometry offers better recovery and precision. Nevertheless, both methods can be successfully applied for routine quantification of TMD and are attractive alternatives to methods requiring derivatization, tedious extraction, expensive reagents or instruments.

REFERENCES

1. W. C. Stanley and M. Marzilli, Metabolic therapy in the treatment of ischaemic heart disease: The pharmacology of trimetazidine, *Fundam. Clin. Pharmacol.* **17** (2003) 133–145; https://doi.org/10.1046/j.1472-8206.2003.00154.x
2. P. Chrusciel, J. Rysz and M. Banach, Defining the role of trimetazidine in the treatment of cardiovascular disorders: some insights on its role in heart failure and peripheral artery disease, *Drugs* **74** (2014) 971–980; https://doi.org/10.1007/s40265-014-0233-5
3. European Medicines Agency, *European Medicines Agency recommends restricting use of trimetazidine-containing medicines, EMA/CHMP/417861/2012*; EMA, London 2012, https://www.ema.europa.eu/documents/press-release/european-medicines-agency-recommends-restricting-use-trimetazidine-containing-medicines_en.pdf; last access date August 31, 2018.
4. Z. Liu, J. M. Chen, H. Huang, M. Kuznicki, S. Zheng, W. Sun, N. Quan, L. Wang, H. Yang, H. M. Guo, J. Li and J. Zhuang, The protective effect of trimetazidine on myocardial ischemia/ reperfusion injury through activating AMPK and ERK signaling pathway, *Metabolism* **65** (2016) 122–130; https://doi.org/10.1016/j.metabol.2015.10.022
5. A. Onay-Besikci and S. A. Özkan, Trimetazidine revisited: A comprehensive review of the pharmacological effects and analytical techniques for the determination of trimetazidine, *Cardiovasc. Ther.* **26** (2008) 147–165; https://doi.org/10.1111/j.1527-3466.2008.00043.x
6. T. K. Murthy, G. D. Sankar and S. R. Yarraguntla, Visible spectrophotometric methods for the determination of trimetazidine dihydrochloride in pharmaceutical formulations, *Indian Drugs* **39** (2002) 230–233.
7. A. A. Chiş, F. G. Gligor, G. Cormoş, E. Curea and M. Bojiţă, Spectrophotometric method for the determination of amorolfine, *Farmacia* **58** (2010) 629–636.
8. S. O. Thoppil, R. M. Cardoza and P. D. Amin, Stability indicating HPTLC determination of trimetazidine as bulk drug and in pharmaceutical formulations, *J. Pharm. Biomed. Anal.* **25** (2001) 15–20; https://doi.org/10.1016/S0731-7085(00)00440-4
9. L. I. Bebawy, M. F. El Tarras and S. A. El Sabour, Determination of trimetazidine dihydrochloride in the presence of its acid-induced degradation products, *J. AOAC Int.* **87** (2004) 827–833.
10. M. Gackowski, M. Koba and K. Mądra-Gackowska, Determination of lormetazepam in tablets using high-performance liquid chromatography, and derivative spectrophotometry methods, *J. Planar Chromatogr. - Mod. TLC* **31** (2018) 235–242; https://doi.org/10.1556/1006.2018.31.3.9
11. M. Gackowski, M. Koba and S. Kruszewski, Comparison of UV- and derivative-spectrophotometric and HPTLC UV-densitometric methods for the determination of amrinone and milrinone in bulk drugs, *Curr. Pharm. Anal.* **14** (2018) 1–8; https://doi.org/10.2174/1573412914666180627141659
12. AOAC International, *Guidelines for Standard Method Performance Requirements (Appendix F), AOAC Off. Methods Anal*; AOAC Int. 2016, http://www.eoma.aoac.org/app_f.pdf; https://doi.org/10.13140/RG.2.2.20883.89125, access date August 31, 2018.
13. P. Rashmin, P. Mrunali, D. Nitin, D. Nidhi and P. Bharat, HPTLC method development and validation: Strategy to minimize methodological failures, *J. Food Drug Anal.* **20** (2012) 794–804; https://doi.org/10.6227/jfda.2012200408
14. M. Koba, K. Koba and T. Bączek, Determination of oxazepam in pharmaceutical formulation by HPTLC UV-densitometric and UV-derivative spectrophotometry methods, *Anal. Lett.* **42** (2009) 1831–1843; https://doi.org/10.1080/00032710903060719
15. B. Spangenberg, Does the Kubelka-Munk theory describe TLC evaluations correctly?, *J. Planar Chromatogr. – Mod. TLC* **19** (2006) 332–341; https://doi.org/10.1556/JPC.19.2006.5.1