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Computer–Assisted Optimization and Validation of LC Analysis of Trimetazidine Dihydrochloride and Its Impurities

Mirjana B. Medenica[1,*], Darko P. Ivanovic[2], Igor B. Popovic[3], Andjelija M. Malenovic[2], and **Biljana S. Jancic[2]**

[1]Faculty of Pharmacy, Department of Physical Chemistry, Vojvode Stepe 450, 11000 Belgrade, Serbia and Montenegro; [2]Faculty of Pharmacy, Department of Drug Analysis, Vojvode Stepe 450, 11000 Belgrade, Serbia and Montenegro; [3]Medicines and Medical Devices Agency of Serbia, Vojvode Stepe 458, 11000 Belgrade, Serbia and Montenegro

Abstract

Trimetazidine dihydrochloride is an anti-anginal drug, which possesses protective properties against ischemia inducing heart damage. In this paper, a new procedure for liquid chromatographic analysis was successfully developed, optimized, and applied in assessment of trimetazidine dihydrochloride content and its impurities, Y-145, Y-235, and Y-234 at most 1.0%, 0.2%, and 0.2%, respectively, in commercially available pharmaceutical preparation containing 35 mg of trimetazidine dihydrochloride. The retention behavior of trimetazidine dihydrochloride and its impurities is investigated by using several stationary and mobile phases to settle a simple, sensitive, and precise RP-HPLC method. The separation conditions are optimized by DryLab 2000 Plus Chromatography Optimization Software version 3.5.00. Separations are performed on PurospherSTAR RP18 endcapped (150 × 4.6 mm, 5 µm particle size) column at 20°C with UV detection at 210 nm. The mobile phase composition is acetonitrile–aqueous phase (10 mmol/L disodium hydrogenphosphate and 2 mmol/L sodium dihydrogen phosphate, pH 7.6) (30:70 v/v). Afterwards, the method is validated; the important statistical parameters for selectivity/specificity, linearity, precision, limit of detection, and quantitation are defined. The recovery value of the trimetazidine dihydrochloride is 98.06%, and the content of impurities is 0.23% for Y-145, less than 0.02% for Y-235, and less than 0.01% for Y-234. In addition, this method is used for analyzing trimetazidine dihydrochloride and its impurities in pharmaceuticals and bulk drug.

Introduction

Trimetazidine dihydrochloride, an anti–ischemic metabolic agent, improves myocardial glucose utilization through the inhibition of fatty acid oxidation, and it can be used as a vasodilator in angina pectoris or ischemic heart disease (1,2).

The analyzed tablets contain 35 mg of trimetazidine dihydrochloride (1–[(2,3,4–trimethoxyphenyl)methyl]piperazine dihydrochloride) and not more than 1.0% of Y-145 (4-(2,3,4-Trimethoxybenzyl)-1-piperazinecarbaldehyde hydrochloride), not more than 0.2% of Y-235 (2,3,4-trimethoxybenzylalcohol), and not more than 0.2% of Y-234 (2,3,4-trimethoxybenzaldehyde) impurities, calculated to the content of trimetazidine dihydrochloride. Structures of trimetazidine dihydrochloride and its impurities are shown in Figure 1.

In the present literature, there are no references dealing with analysis of trimetazidine and its impurities. Spectrophotometric determination of trimetazidine dihydrochloride in bulk drug and solid dosage forms was developed (3), as well as first-derivative

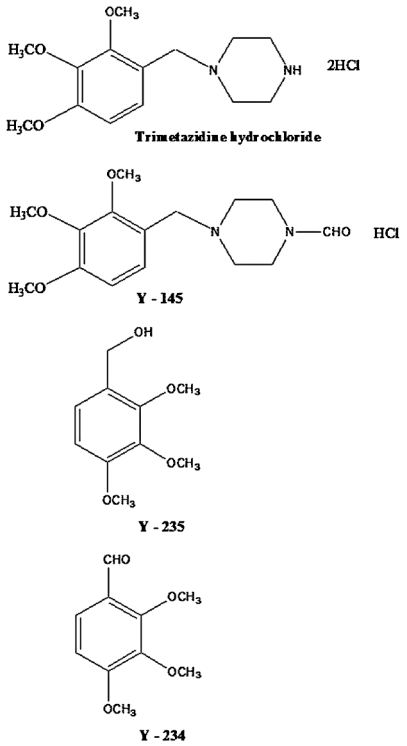

Figure 1. Structures of trimetazidine dihydrochloride and its impurities (Y-145, Y-234, and Y-235)

* Author to whom correspondence should be addressed: email medenica@pharmacy.bg.ac.yu

spectrophotometric method (4). Densitometric high-performance thin-layer chromatographic method for analysis of trimetazidine dihydrochloride, both as a bulk drug, and in appropriate formulations was reported (5). Analysis of trimetazidine in presence of its degradation products inducted by different agents like acid, hydrogeny peroxydi, etc. was acheived using a liquid chromatography (LC) method (6). In addition, stripping square-wave voltammetry at a glassy carbon electrode was also successfully applied to an assay of trimetazidine dihydrochloride in the tablet dosage form (7). Many methods have been sought for carrying out pharmacokinetic studies and for assessing trimetazidine bioequivalences of pharmaceutical formulations. The plasma concentration of trimetazidine dihydrochloride was estimated by both reversed-phase LC (8,9) and LC–mass spectrometry (MS)–MS method (10,11).

The aim of this investigation was to define the optimal chromatographic conditions using software to optimize the simultaneous determination of trimetazidine and its impurities. In this paper, computer simulation software (DryLab) was employed in developing an LC method for separation of trimetazidine and its three impurities. Then, the method was validated and linearity, precision, limits of detection, and limit of quantitation were settled. Finally, the optimized and validated method was applied to assaying a pharmaceutical dosage form.

Experimental

Reagents and samples

All reagents used were of an analytical grade. Acetonitrile-gradient grade (Lab Scan, Ireland), water (HPLC grade,) disodiumhydrogenphosphate (Merck, Darmstadt, Germany), and sodium-dihydrogenphosphate (Merck) were used to prepare the mobile phase. The tablets (Preductal MR, batch number: 3M3003) produced Les Laboratories Servier Industrie, France. Working standards of trimetazidine dihydrochloride, Y-145, Y-235, and Y-234 were obtained from the manufacturer.

Equipment and software

The chromatographic system, Hewlett Packard 1100 (Agilent, Technologies, Germany), consisted of an HP 1100 pump (serial: DE23912441), HP 1100 UV–vis detector (serial JP24019419), HP ChemStation integrator, and HP autosampler 1100 (serial DE 23909987). The columns: Purospher® STAR C_{18} 150 mm × 4.6 mm; LiChroCART C_{18} 5 µm, 250 × 4.6 mm; Zorbax SB $C_{18,}$ 5 µm, 150 × 4.6 mm and Zorbax Eclipse XDB C_8 5 µm, 150 × 4.6 mm were used in the chromatographic investigation and separation. The statistical software DryLab 2000 Plus Chromatography Optimization Software version 3.5.00 (Molnar-Institut für angewandte Chromatographie, Schneeglöckchenstrasse 47, D-10407 Berlin, Germany) was employed for the method optimization.

Standard solutions for linearity testing

The stock solutions were prepared by dissolving the working standard substances in the mobile phase to obtain the concentration of 0.1 mg/mL for trimetazidine dihydrochloride, 1.0 µg/mL for impurity Y-145, 0.2 µg/mL for impurities Y-234 and Y-235. For the calibration curves, a series of seven solutions were prepared in the concentration range from 0.01 mg/mL to 0.1 mg/mL for trimetazidine dihydrochloride; from 0.1 µg/mL to 1.0 µg/mL for Y-145, and from 0.02 µg/mL to 0.2 µg/mL for Y-235 and Y-234.

Laboratory mixtures

In order to justify the validity of the proposed method, the solutions of trimetazidine, Y-145, Y-234, and Y-235 were made in the concentration ratio corresponded to the content in tablets. For quantitative analysis, three series with ten solutions for each concentration were prepared (0.05 mg/mL, 0.06 mg/mL, and 0.07 mg/mL for trimetazidine dihydrochloride; 0.5 µg/mL, 0.6 µg/mL and 0.7 µg/mL for Y-145; 0.1 µg/mL, 0.12 µg/mL and 0.14

Table I. Chromatographic Parameters for Trimetazidine Dihydrochloride and its Impurities*

pH of the mobile phase	6.9			7.4			7.6			8.8		
Parameters / Compound	k'	α	Rs	k'	α	Rs	k'	α	Rs	k'	α	Rs
Obtained with Dry Lab software												
Trimetazidine dihydrochloride	0.6	3.50	12.51	0.6	3.01	11.47	0.8	2.42	9.80	2.0	1.21	2.83
Y-235	2.0	1.45	5.64	2.0	1.47	5.91	2.0	1.5	6.22	2.4	1.25	3.65
Y-145	2.8	2.77	18.89	2.9	2.73	18.67	2.9	2.67	18.34	3.0	2.66	18.33
Y-234	7.9			7.8			7.8			7.9		
Experimental Results												
Trimetazidine dihydrochloride	1.35	2.61	12.66	1.53	2.30	10.43	1.78	1.99	8.90	3.55	1.18	1.78
Y-235	3.52	1.37	5.97	3.53	1.40	6.55	3.54	1.43	7.31	4.19	1.22	2.34
Y-145	4.81	2.60	20.64	4.95	2.54	20.30	5.05	2.49	21.24	5.10	2.48	20.88
Y-234	12.53			12.57			12.55			12.66		

* Abbreviations: k' = Retention factor, α = Selectivity factor, and Rs = Resolution factor.

µg/mL for Y-234 and Y-235) and injected into the column.

Sample solutions

Ten solutions in concentration of 0.06 mg/mL calculated to trimetazidine dihydrochloride were prepared from homogenous tablet mass according to the following procedure. The powered tablet mass corresponds to 15 mg trimetazidine dihydrochloride dissolved in volumetric flask of 25 mL with mobile phase and placed into the ultrasonic bath for 40 min. After that, the solution was centrifuged at 4000 rpm (5 min) and then diluted with mobile phase in ratio 1:10. The obtained solutions were injected in the column using an autosampler.

Chromatographic conditions

Separations were performed on the Purospher STAR C_{18} (150 mm × 4.6 mm, 5 µm particle size) column at 20°C. The samples were introduced using autosampler. Separation and simultaneous determination of the trimetazidine dihydrochloride and impurities Y-145, Y-235, and Y-234 were performed using the mobile phase, which consisted of 300 mL of acetonitrile and 700 mL of aqueous phase, which contained 10 mmol/L disodium hydrogenphosphate and 2 mmol/L sodium dihydrogenphosphate. The mobile phase was filtered through a 0.2 µm Millipore filter. The flow rate was 1.0 mL/min, temperature of column 20°C, and injection volume 20 µL with UV detection at 210 nm.

Results and Discussion

In the recent years, development of any chromatographic method has always been followed by method optimization because all the proposed methods should be simple and cheap as much as possible and less time consuming. The general goal in any optimization is to discover the conditions that produce the best outputs. Method optimization could be done using the experimental design (12–16), chemometrics (17–19), or different kinds of software like the DryLab (20–28). Computer–assisted optimization employing specific software, such as DryLab 2000 Plus Chromatography Optimization Software version 3.5.00, proved to be quite a useful tool for optimization in different types of chromatographic analysis. Either chemometrics or software is used for method optimization. The most important part, and at the same time the starting point is the preliminary investigation. It helps to recognize and select the chromatographic factors that affect separation the most. In the conventional separations, the retention of analytes is related to their hydrophobicity. For that reason, the starting point in the method development was the analysis of chemical structures of the investigated substances, because the retention of the investigated substances could be predicted. Trimetazidine and Y-145 as molecules possessing piperazine have a basic character, and a bad peak shape in reverse-phase chromatographic analysis was expected. Difference in structure between them (Y-145 has a formyl group on piperazine part) resulted in a different lipophilicity of molecules, so in reversed-phase mode a longer retention of Y-145 was expected. The other two impurities, Y-234 and Y-235, do not have a basic character because of the absence of the piperazine part. The difference between them is at position 1, Y-235 has a formyl group and Y-234 has hydroxy–methyl group. According to the structure and knowledge of the separation in the reverse-phase separation, Y-235 as more lipophilic compound will have longer retention. So, this analytical problem presents resolution of two structurally similar pairs of substances, trimetazidine/Y-145 and Y-234/Y-235. This consideration leads to a selection of non-polar columns (C_8 and C_{18}) for preliminary experiments. Chromatographic behavior of trimetazidine and its impurities was analyzed using a different mobile and stationary phases.

Separation on C_8 (Zorbax Eclipse XDB C_8 5 µm, 150 × 4.6 mm) column with mobile phases containing different ratio of acetonitrile at pH lower than 5.0, resulted in coelution of trimetazidine dihydrochloride with a peak of mobile phase. There was no separation in pH range between 5.0 and 8.0. The usage of the mobile phase with pH greater than 8.0 gave a bad peak shape of the active compound. The addition of triethylamine (TEA) had no influence on a peak symmetry of trimetazidine dihydrochloride. Under these chromatographic conditions, the resolution of the pair trimetazidine dihydrochloride/Y-145 had values less than 1 as well. Furthermore, the separation on the C_{18} (LiChroCART C_{18} 5 µm, 250 × 4.6 mm and Zorbax SB $C_{18,}$ 5 µm, 150 × 4.6 mm) was investigated. The acetonitrile content in the mobile phase varied from 20% to 40% with or without addition of TEA. The separation was not achieved with these mobile phases. On C_{18} (PurospherSTAR RP18 endcapped, 150 × 4.6 mm, 5 µm), the separation was performed with acetonitrile–aqueous (from 20:80 v/v to 40:60 v/v) as a mobile phases. Addition of sodium-dodecil sulphate in different concentrations resulted in formation of ion pair with trimetazidine dihydrochloride, and it led to the separation of the investigated substances. However, a chromatographic peak for trimetazidine dihydrochloride was asymmetrycal, and the retention time was very long and unreproducible. The addition of TEA resulted in better separation of the investigated substances and the run time was significantly reduced. Better results were not obtained with other types of ion pair reagents, such as heptan-1-sulfonic acid and octan-1-sulfonic acid.

Table II. The Important Parameters for the Calibration Curves*

Parameter	Trimetazidine dihydrochloride (mg/mL)	Y-145 (mg/mL)	Y-235 (mg/mL)	Y-234 (mg/mL)
Concentration range	0.01 – 0.1	0.1 – 1.0	0.02 – 0.2	0.02 – 0.2
$y = ax + b$	$94261x + 42.48$	$151.66x - 0.0598$	$177.35x + 0.299$	$88.4x - 0.26$
r	0.9998	0.9999	0.9997	0.9994
S_b	57.94	0.62	0.27	0.20
t_{tab}	0.931	0.123	1.399	1.696

* Abbreviations: r = Correlation Coefficient; S_b = Standard Deviation of the Intercept; t_{tab} = 1.943 (p = 0.05).

Variations of column temperature (from 25°C to 45°C) had no influence on the separation. Since the best results were achieved on this column, it was used for further investigations. Separation and peak symmetry were significantly improved with mobile phases containing acetonitrile and different ratio of disodium hydrogenphosphate and sodium dihydrogenphosphate in the aqueous phase. Retention times of the investigated substances were shortened, but non-retention behavior of trimetazidine was noticeable at lower values of pH. When an analyte is ionized, its retention is strongly influenced with pH of the mobile phase. Considering a trimetazidine basic character, it is in ionized form in acidic solutions, so pH of the mobile phase is the most important chromatographic factor which could affect its behavior. Structurally similar Y-145 impurity is less basic because of the presence of formyl group, which decreases the basic character of piperazine part, and at the same time it is more lipophilic and its retention is longer. According to the previously mentioned, the pH interval from 6.0 to 8.0 was studied. In that pH range, trimetazidine is in a molecular form, so its retention must be longer than at lower values of pH.

In the method optimization, one factor was changed and the other chromatographic factors were kept on constant level. In this way, for optimization, a few experiments only are enough for predicting a chromatographic behavior in a wide range of the investigated factors. The pH of the aqueous phase was the factor, which was extracted for analysis by the software as the most important factor for chromatographic separation of the investigated mixture. Moreover, in order to find the best conditions for the separation, four different pH levels (6.9, 7.4, 7.6, and 8.8) of aqueous phase were chosen as the most important factor, while the other chromatographic factors (content of acetonitrile 30% v/v, aqueous phase 10 mmol/L disodium hydrogenphosphate, and 2 mmol/L sodium dihydrogenphosphate, temperature 20°C and flow rate 1 mL/min) were constant. Resulting retention times and peak areas are introduced in DryLab. The computer-assisted optimization performed by the software using the obtained experimental data as well as the column parameters (diameter, particle size and pore diameter), the instrument parameters (void volume: 1 mL, extra column volume and temperature), a flow rate, and the composition of phosphate buffer. According to the input parameters, the software gave a resolution map for the most critical pair in the separation (Y-235/Y-145) shown in Figure 2 as well as predicted values for chromatographic parameters (Table I).

The resolution map indicates that the proposed method is robust within the pH range 7.6 ± 0.2 which was enabled by the phosphate buffer in the mobile phase. The chromatographic parameters obtained from DryLab, as well as the experimental results (Table I), indicated that the optimal values for the retention time, selectivities, and resolutions would be gained with the mobile phase containing acetonitrile–aqueous phase (10 mmol/L disodium hydrogenphosphate and 2 mmol/L sodium dihydrogenphosphate, pH 7.6) (30:70).

Comparing the predicted results and experimental ones for selectivity and resolution, a good agreement of corresponding chromatographic parameters can be seen. Significant difference between predicted values and values calculated from experimental data for the retention factor is caused by a difference in software calculation of t_0 and experimental t_0 value. Because of that, the software is using resolution to produce resolution maps for further preditions and optimal conditions setting.

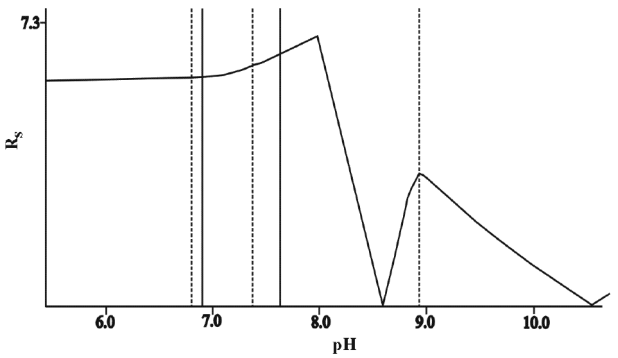

Figure 2. Resolution map for resolution between Y-235 and Y-145 obtained by DryLab 2000 Plus Chromatography Optimization Software version 3.5.00.

Table III. LOD and LOQ

Compound	LOQ* (mg/mL)	LOD* (mg/mL)
Trimetazidine dihydrochloride	0.07	0.020
Y-145	0.02	0.005
Y-235	0.02	0.007
Y-234	0.04	0.001

* Experimentally determined values.

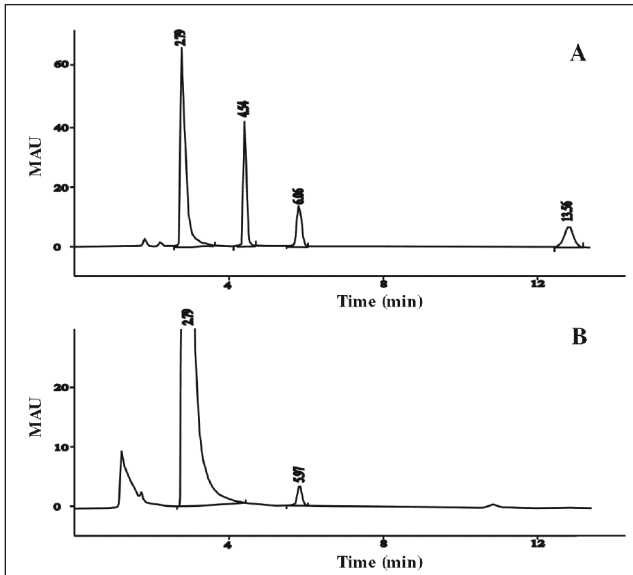

Figure 3. The chromatogram of trimetazidine dihydrochloride (t_R = 2.792 min) and its impurities: Y-235 (t_R = 4.54 min), Y-145 (t_R = 6.05 min), and Y-234 (t_R = 13.56 min) in: A, laboratory mixture; B, tablets; (mobile phase: acetonitrile–aqueous (10 mmol/L disodium hydrogenphosphate and 2 mmol/L sodium dihydrogenphosphate, pH 7.6) (30:70 v/v); flow rate, 1.0 mL/min; UV detection, 210 nm).

After establishing the optimal conditions, the method was submitted to the method validation (e.g., selectivity, linearity, precision, limit of quantification and limit of detection were determined). Furthermore, the assay was selective, and no significant interfering peaks of the excipients were observed at the retention time of trimetazidine dihydrochloride and its impurities. All the excipients were eluted at different times and they did not interfere with analyzed compounds. The representative chromatograms of the laboratory mixture and the corresponding tablets are given in Figure 3.

In the concentration range from 0.01 mg/mL to 0.1 mg/mL for trimetazidine dihydrochloride, from 0.1 µg/mL to 1.0 µg/mL for Y-145, and from 0.02 µg/mL to 0.2 µg/mL for Y-234 and Y-235, a linear relationship was obtained and the regression parameters are given in Table II.

The linear relationship of the peak over the mentioned concentration range for trimetazidine dihydrochloride, Y-145, Y-235, and Y-234 was obtained. Since the correlation coefficient for the calibration curves of trimetazidine dihydrochloride and its three investigated impurities was greater than 0.9994 and a statistical parameter (t_b) calculated using Statistica 5 software was lower than tabular value (t_{tab}), it could be concluded that the calibration curves were within the linearity acceptance criteria.

For the quantitative analysis, it was important to define the values of a limit of detection (LOD) and a limit of quantification (LOQ) (Table III). The signal-to-noise ratio of 3:1 and 10:1 were taken as LOD and LOQ, respectively and further confirmed by taking dilutions from the secondary stock solution till the peak area obtained has 3 (for LOD) and 10 (for LOQ) fold then the standard deviation of six determination.

For the evaluation of method precision the important statistical values, such as standard deviation (S) and coefficient of variation (CV), as well as good recoveries, were calculated. Standard deviation and coefficient of variation have the small-required values (Table IV).

For the evaluation of the method precision, the important statistical values such as standard deviation (S) and coefficient of variation (CV), as well as good recoveries were calculated. The standard deviation and the coefficient of variation had small, required values.

After the evaluation, the proposed method was applied for the determination of the content of trimetazidine dihydrochloride, Y-145, Y-234 and Y-235 in the Preductal tablets available on the pharmaceutical market. The content of trimetazidine dihydrochloride was 98.06%, and the level of impurities met the given requirements well (e.g., Y-145 0.23%, level of Y-235 and Y-234 was under the LOD).

The results of the assay for trimetazidine dihydrochloride and its impurities in tablets are given in Table V.

Table IV. Precision of the Proposed LC Method

Compound	Injected	Found	CV (%)	R (%)
Trimetazidine dihydrochloride (mg/mL)	0.05	0.0515 ± 0.0003*	0.66	103.1
	0.06	0.0603 ± 0.0002	0.37	100.5
	0.07	0.0701 ± 0.0003	0.42	100.2
Y-145 (mg/mL)	0.5	0.494 ± 0.005	1.03	98.87
	0.6	0.594 ± 0.003	0.45	98.98
	0.7	0.699 ± 0.002	0.35	99.90
Y-235 (mg/mL)	0.10	0.098 ± 0.001	1.12	98.29
	0.12	0.1182 ± 0.0008	0.64	98.49
	0.14	0.1392 ± 0.0007	0.50	98.40
Y-234 (mg/mL)	0.10	0.099± 0.002	1.95	99.66
	0.12	0.118 ± 0.001	0.74	98.79
	0.14	0.139 ± 0.001	0.85	99.06

* Standard Deviation (n = 10).

Table V. Quantitative Analyses

Compound	Taken (mg/mL)	Found (mg/mL)	Found (mg/tbl)	CV (%)	R (%)
Trimetazidine hydrochloride	0.06	0.0588 ± 0.0005*	34.32	0.8	98.06
Compound	**MAC† (mg/mL)**	**Found (mg/mL)**	**Found (%)**	**CV (%)**	
Y-145	0.6	0.227 ± 0.006	0.23	2.52	
Y-235	0.12	Less than LOD‡		–	
Y-234	0.12	Less than LOD‡		–	

* Standard Deviation, S (n = 10).
† MAC: Maximum allowed content.
‡ LOD: Limit of Detection of impurity (see Table III).

Conclusion

The computer–assisted optimization employing the specific software was used in order to obtain the most appropriate chromatographic conditions for the analysis of trimetazidine dihydrochloride and its impurities. This paper presents a useful improvement in drug analysis offering a simpler and quicker way to obtain the optimal separation conditions. The evaluation of the proposed method proved its selectivity, precision and accuracy, and so its applicability to the qualitative and quantitative analysis of the investigated substances in the corresponding tablets in the presence of excipients. The proposed method was rapid and sensitive, and represents a significant improve-ment in chromatographic analysis and application of this technique for a drug analysis purposes.

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