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DEVELOPMENT OF THE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MELDONIUM IN CAPSULES BY USING ALIZARINE

Mariana Horyn, Marjan Piponski, Olha Poliak, Nataliia Shulyak, Marta Sulyma, Liliya Logoyda

The aim of the work *was to develop a simple, rapid, economic spectrophotometric method for the determination of meldonium in capsules based on the reaction with alizarin.*

Materials and methods. *Analytical equipment: double-beam UV-visible spectrophotometer Shimadzu UV 1800 (Japan), a pair of 1 cm matched quartz cells, software UV-Probe 2.62, laboratory electronic balance RAD WAG AS 200/C, pH-meter I-160MI. Pharmacopoeial standard sample (CRS) of meldonium dihydrate (Sigma-Aldrich, (≥98 %, HPLC)), alizarin (Synbias), capsules Metamax (Darnytsia) 250 mg, Vasopro (Farmak) 500 mg, Mildronate (Grindex) 500 mg, dimethylformamide (“Honeywell Riedel-de Haen”).*

Results and discussion. *A spectrophotometric method for determining meldonium in capsules by reaction with alizarine has been developed. The absorption maximum of the formed complex in dimethylformamide was at a wavelength of 517 nm. Stoichiometric ratios of reactive components «meldonium- alizarin» were 1:1. Validation of the developed analytical method for the determination of meldonium in medicines was carried out in accordance with the requirements of the SPhU. The optimal conditions for performing the quantitative determination of meldonium have been established: concentration of alizarin solution – 0.8 %, volume 0.8 % alizarin solution – 0.5 ml, heating time – 20 min, temperature – 95±2 °C. Linearity has been in the concentration range of 0.0402–0.1073 mg/mL, the limit of detection – 2.84 μg/mL, and the limit of quantification – 8.59 μg/mL. The eco-friendliness of the developed analytical method was carried out using the analytical eco-scale, AGREE, and GAPI methods.*

Conclusions. *The developed method can be used as an arbitration method for the routine analysis of meldonium capsules*

Keywords: *alizarin, meldonium, spectrophotometry, validation, quantitative determination, capsules*

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1. Introduction

Meldonium is a metabolic medicine which is often used to treat various pathological conditions in which organ ischemia is observed. Often, as a result of hypertension and coronary heart disease, can develop myocardial infarction, which is accompanied by ischemia of the heart. The chemical structure of meldonium is 3-(2,2,2-trimethylhydrazin-2-ium-l-yl)propanoate dihydrate, a precursor of carnitine synthesis. Blocking the formation of carnitine leads to a decrease in the permeability of fatty acids inside the cell, where their beta oxidation actually occurs. The pathway of energy generation through fatty acid oxidation is very oxygen-consuming, so it can increase tissue ischemia. The optimal way for ischemia is the supply of energy due to the oxidation of glucose. Meldonium is a partial inhibitor of fatty acid oxidation and increases the efficiency of oxygen consumption by the ischemic organ through glucose oxidation. In addition, meldonium, in combination with other antianginal drugs, allows an increase in the effectiveness of traditional therapy and improves the quality of life of patients [1–4].

The State Pharmacopoeia of Ukraine (SPhU) does not have a monograph on the substance meldonium. The European Pharmacopoeia (Ph. Eur.) has a monograph on the substance meldonium dihydrate. Ph. Eur. regulates the identification of meldonium dihydrate by absorption spectrophotometry in the IR range and quantitative determination by acidimetric in a non-aqueous medium with potentiometric fixation of the titration point [5]. After conducting a review of scientific literature regarding the existing methods of meldonium analysis, it has been shown that there is only one spectrophotometric method [6] and numerous chromatographic methods [7–21] for the determination of meldonium in substances, pharmaceuticals and biological fluids. The spectrophotometric determination of meldonium in medicines had been proposed by a scientific group from the ZSMU under the leadership of prof. S. O. Vasyuk was based on the formation of a coloured reaction product of meldonium with p-chloranil in dimethylformamide (DMF) medium with an absorption maximum at a wavelength of 556 nm. The sample preparation of the initial solutions included dissolving a portion of meldonium in water and DMF and p-chloranil in DMF. The formed complex of meldonium with p-chloranil and the compensating solution (p-chloranil in DMF) were heated for 20 min in a water bath at a temperature of 95 °C before measurement. The method was linear in the concentration range of 8.00–20.00 mg/100 mL [6].

Ukrainian scientists Pidpruzhnykov Y. V. et al. developed a UHPLC-MS/MS method for the determination of meldonium to study the bioequivalence of oral drugs using a column Waters Acquity UPLCW BEH HILIC (50×2.1 mm, 1.7 μm) and a mobile phase: water, acetonitrile and 200 mM formic acid (adjusted to pH 3.0 12.5 % ammonium hydroxide solution) (25:70:5). Flow rate was 0.3 mL/min, run time of chromatography was 1.4 min. The developed method was linear in the concentration range of 10–6000 ng/mL. The proposed UHPLC/MS/MS method for the determination of meldonium can be used to study the bioequivalence of oral drugs [7].

The methods of analysis of the substance meldonium dihydrate described in the Ph. Eur. are not relevant for the determination of meldonium in the drugs. In addition, in scientific sources, there are no sufficiently simple and fast methods for the quantitative determination of meldonium in medicines. This is also necessary for arbitration control. Therefore, there was a need to develop a spectrophotometric method for the determination of meldonium in capsules for routine analysis.

Therefore, the aim of our work was to develop a simple, rapid, economic spectrophotometric method for the determination of meldonium in capsules based on the reaction with alizarin.

2. Planning of the research

Methodology of research of development and validation of the spectrophotometric methods for the determination of meldonium in pharmaceutics includes:

1. Analysis of the scientific literature.

2. Study of the SPhU and Ph. Eur. Monographs.

3. Selection of reaction conditions between meldonium and alizarine (choice of solvent, concentration reagent, temperature conditions, optimal wavelength, detection of stoichiometric coefficients).

4. Development and validation of the spectrophotometric method for determination of meldonium in capsules.

5. Study of the greenness profile assessment of the developed method (eco-scale, analytical GREEnness, GAPI).

3. Materials and methods

Objects of study, solvents and equipment,

Analytical equipment: double-beam UV-visible spectrophotometer Shimadzu UV 1800 (Japan), a pair of 1 cm matched quartz cells, software UV-Probe 2.62, laboratory electronic balance RAD WAG AS 200/C, pH-meter I-160MI. Pharmacopoeial standard sample (CRS) of meldonium dihydrate (Sigma-Aldrich, (≥98 %, HPLC)), alizarin (Synbias), capsules Metamax (Darnytsia) 250 mg, Vasopro (Farmak) 500 mg, Mildronate (Grindex) 500 mg, DMF (Honeywell Riedel-de Haen).

Proposed procedure for the determination of meldonium with alizarin.

33.53 mg of CRS meldonium dihydrate was transferred into a 25.00 mL volumetric flask with 2.5 mL distilled water. The mixture was shaken and diluted to volume with DMF. Aliquot 0.5 mL was added to 0.5 mL of 0.8 % alizarin in DMF. The volume 10.00 mL was made up to the mark by adding DMF. The absorbance of the resulting solution was measured against the background of the compensating solution (a solution containing all components except the analyte) at a wavelength of 517 nm.

Procedure for capsules for the determination of meldonium with alizarin.

Twenty capsules were accurately weighed. A quantity of granulated capsules containing 33.53 mg of meldonium dihydrate was transferred into a 25.00 mL volumetric flask with 2.5 mL distilled water. The mixture was shaken for 15 min, diluted to volume with DMF and then filtered. Aliquot 0.5 mL was added to 0.5 mL of 0.8 % alizarin in DMF. The volume 10.00 mL was made up to the mark by adding DMF. The absorbance of the resulting solution was measured against the background of the compensating solution (a solution containing all components except the analyte) at a wavelength of 517 nm.

4. Results

4. 1. Selection of reaction conditions

Meldonium – a polar molecule with a low molecular weight, without any chromophores. The described facts complicate the development of analytical methods. We tested an alkaline solution of copper (II) sulfate and ninhydrin as potential reagents for the development of spectrophotometric methods for the determination of meldonium. However, these reagents did not interact with meldonium. Sulfophthalein dye (bromphenol blue) gave a reaction with meldonium with the formation of a reaction product with an absorption maximum at a wavelength of 595 nm; however in further, there were problems with the linearity of the analytical method. Only one spectrophotometric method for the determination of meldonium in dosage forms by reaction with p-chloranil has been described in the scientific literature [6]. However, the proposed method has some disadvantages, including labour-intensive (heating) and the use of a toxic solvent (DMF). We repeated this analytical method for the determination of meldonium by reaction with p-chloranil. We found that the maximum absorption of the formed product was observed at wavelengths 535 nm (not at 556 nm). However, this method is a confirmation that quinones can be potential reagents for the development of spectrophotometric methods for the determination of meldonium in pharmaceuticals. Alizarin is a dihydroxyanthraquinone, which is an anthracene-9,10-dione in which two hydroxy groups are located in the 1 and 2 positions [22]. Taking into consideration the physicochemical properties of alizarin, DMF was chosen as the solvent. Further research was conducted to study the conditions for the interaction of meldonium with alizarin (choice of reagent concentration, heating time and temperature, detection of stoichiometric coefficients) for the development of a spectrophotometric method. The complex of meldonium with alizarin has a maximum absorbance at wavelength 517 nm. The spectra of absorbance of the reaction product of meldonium with alizarin are shown in Fig. 1.

To choose an optimal concentration of reagent, we tested concentrations from 0.1 to 1 % of a solution of

alizarin in DMF. The largest increase in the amount of absorbance was observed when 0.8 % alizarin solution was added to meldonium. Therefore, in further experiments to select the normal conditions for the reaction, the choice was stopped on 0.8 % solution of alizarin in DMF (Fig. 2).

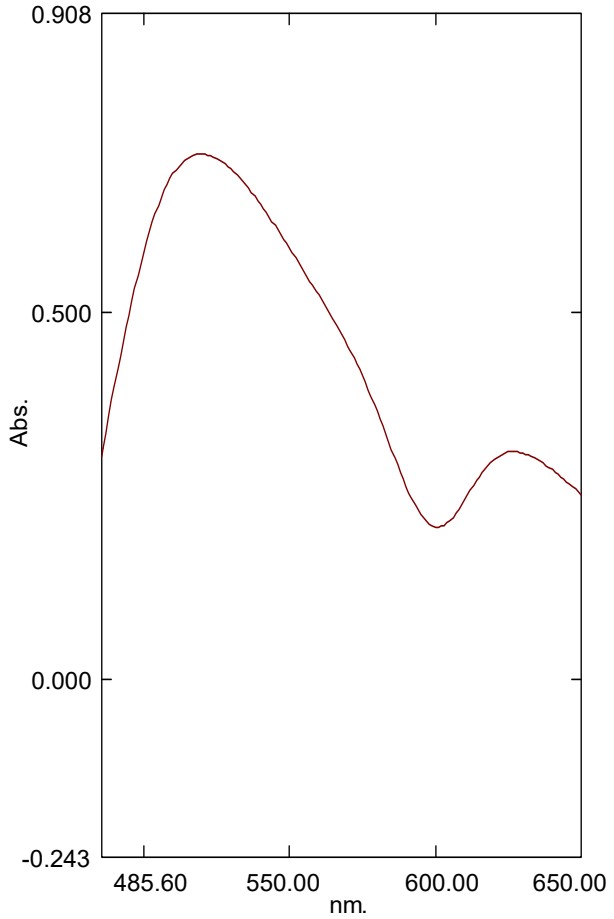

Fig. 1. Absorbance spectrum of the reaction product of meldonium (C=6.25×10^{-3} M) with 0.8 % solution of alizarine

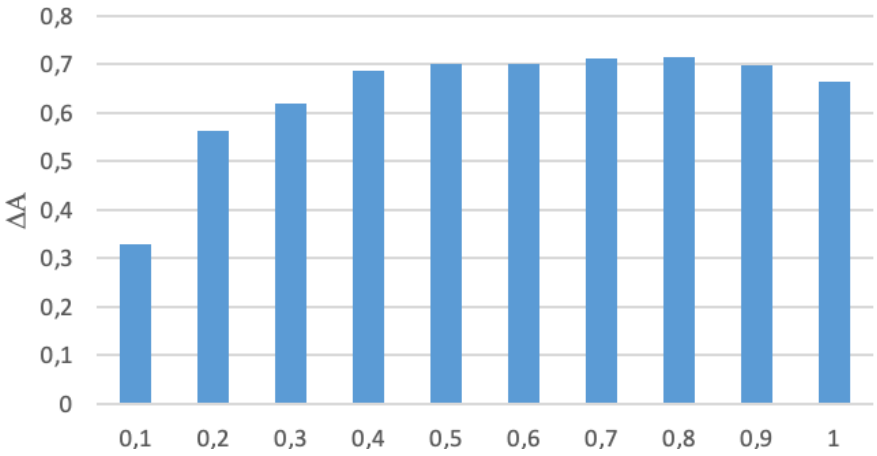

Fig. 2. The graph of dependence of the amount of absorbance depending on the concentration of the alizarine solution

The sample preparation of the initial solutions included dissolving a portion of meldonium in water and DMF. The formed complex of meldonium with alizarin and the compensating solution (alizarin in DMF) were heated. An important condition for the reaction was the heating time and temperature. Experimentally, it was established that the heating time was 20 minutes (Fig. 3), and the temperature was (95±2) °C (Fig. 4).

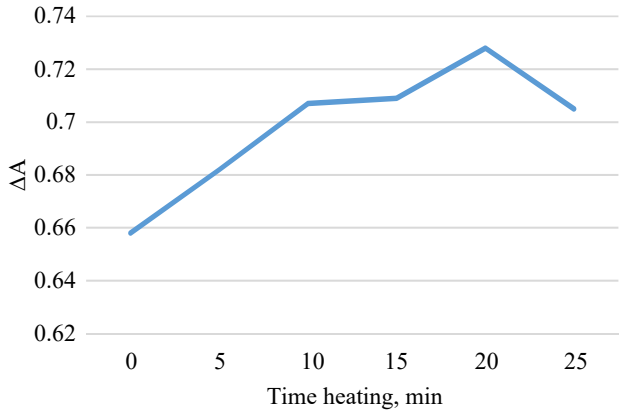

Fig. 3. The graph of dependence of the amount of absorbance of the reaction product of meldonium with alizarin depending on heating time

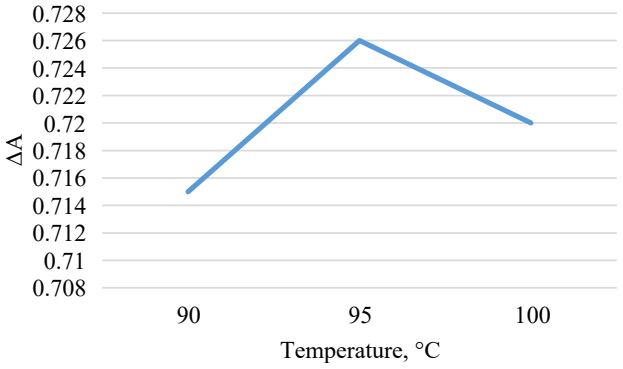

Fig. 4. The graph of dependence of the amount of absorbance of the reaction product of meldonium with alizarin depending on temperature

The stability of the determined solutions over time is one of the important aspects that is taken into consideration when developing an analytical method. Using a buffer solution to stabilize pH or other additional operations slows the sample preparation of the method will have a negative impact on the «greeneess». It was established that the solutions are stable for 45 min (Fig. 5).

We calculated the sensitivity parameters of the reaction between meldonium and alizarin. The molar absorption (ε) was 1.38×10^{3}, the specific absorption (a) was 7.58×10^{-2}, and the Sendel coefficient (Ws) was 0.14. From the above, it can be concluded that the developed method of spectrophotometric determination of meldonium in medicines is highly sensitive.

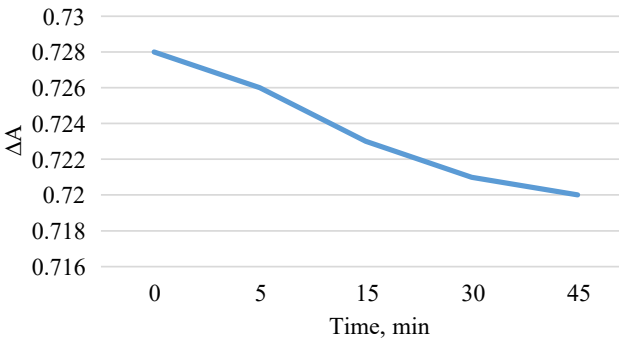

Fig. 5. Graph of the dependence of the absorbance of the reaction product of meldonium with alizarin depending on time

The stoichiometric coefficients of the reacting components (meldonium-alizarin) were determined by the method of continuous changes (Job's method) and the saturation method (by the method of molar ratios). The graph of the dependence of the amount of absorbance on the ratio of the volumes of the components of the isomolar series at a wavelength of 517 nm (Job's method) is presented in Fig. 6. Saturation curves are demonstrated in Fig. 7 (by the method of saturation).

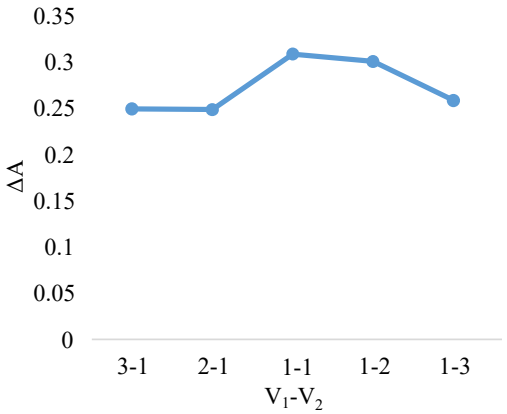

Fig. 6. Graph of the dependence of absorbance on the composition of the isomolar solution:
V_1 – volume 7.0×10^{-3} M meldonium solution;
V_2 – volume 7.0×10^{-3} M solution alizarin) at 517 nm

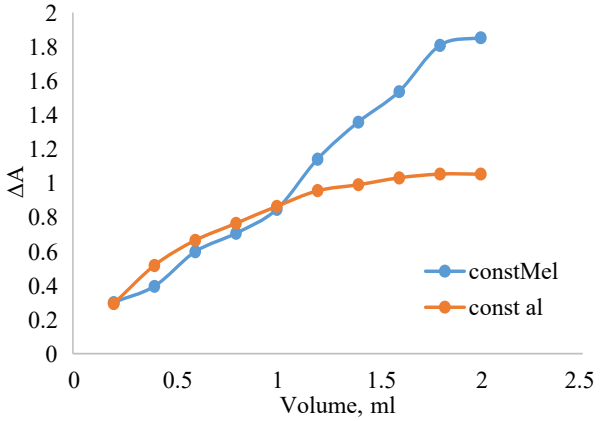

Fig. 7. Saturation curves: meldonium solution at a constant concentration of reagent (1.00 mL of 7.0×10^{-3} M solution), alizarin solution at a constant concentration of meldonium (1.00 mL of 7.0×10^{-3} M solution)

According to the results obtained in Fig. 6, 7 the stoichiometric ration of the reactive components of meldonium – alizarin corresponds 1: 1.

4. 2. Determination of validation characteristics

The spectrophotometric method for the determination of meldonium in capsules has been validated in accordance with the requirements of SPhU [23] for the selected parameters: specificity, linearity, range of application, robustness, accuracy and precision.

4. 2. 1. Specificity

To define the specificity of the analytical method for the determination of meldonium in capsules, a solution of auxiliary substances («placebo») was prepared. The results of the specificity study of the analytical method for the determination of meldonium in capsules are presented in Table 1. The obtained values from Table 1 indicate that the absorbance of auxiliary substances is insignificant (the found value of δ_{noise} is 0.29 %) and does not exceed the acceptance criterion.

Table 1

The results of the study of the specificity

Absorbance of placebo ($A_{placebo}$)	The absorbance of a solution of impurities ($A_{impurities}$)	The absorbance of the compensating solution (A_{st})	Value δ_{noise}, %	Criteria
0.001	–	0.348	0.29	≥ 0.5 %

4. 2. 2. Linearity and range of application

The linearity of the spectrophotometric method for the determination of meldonium by reaction with alizarin was studied in the concentration range of 0.0402–0.1073 mg/mL, using model solutions, in accordance with the requirements of the SPhU (the method of least squares). Analytical parameters are given in Table 2.

Table 2

Analytical parameters

Indicator	Value	Criteria	Conclusion
$b\pm(S_b)$	1.5230±(0.0435)	–	–
$a\pm(S_a)$	0.2478±(0.0034)	$\|a\|\leq2.6$	Corresponds
R^2	0.9995	>0.9924	Corresponds
LOD, μg/mL	2.84	–	–
LOQ, μg/mL	8.59	–	–

The limit of detection (LOD), limit of quantification (LOQ) were calculated to be 2.84 μg/mL and 8.59 μg/mL, correlation coefficient R^2=0.9995.

4. 2. 3. Robustness

The study of the robustness of the analytical method was performed at the stage of development of the spectrophotometric method for the determination of meldonium by reaction with alizarin during the establishment of optimal conditions of reaction between meldonium and alizarin (stability of solutions over time,

the amount of added alizarin). It was determined that the analyzed solutions were stable for 45 min (Fig. 5), and vacillation in the amount of the added reagent (alizarin solution) within ±10 % does not significantly affect the value of the absorbance (Table 3).

Table 3

Effect of the amount of added alizarine solution on the absorbance

Volume of 0.8 % alizarine solution, mL	ΔA
0.1	0.262
0.2	0.286
0.3	0.305
0.4	0.327
0.5	0.346

4. 2. 4. Accuracy and precision

The accuracy and precision of the analytical method were determined by preparing model solutions with a definitely known concentration of the meldonium with content 80–120 % of the nominal. The acquired results of the calculations are demonstrated in Table 4.

The method of determining meldonium by reaction with alizarin is characterized by sufficient precision. The obtained value of the relative confidence interval of the value Δz (0.04 %) is less than the critical value for the convergence of the results (1.6 %).

Table 4

The results of the analysis of model mixtures and their statistical processing for quantitative determination

Model solutions	Content, %		The ratio of found to added, $Z_i=(Y_i/X_i)\cdot 100$ %
	Added, $X_i=(C_i/C_{rx})$ 100 %	Found, $Y_i=(C_i/C_{rx})$ 100 %	
M_1	80.44	80.41	99.96
M_2	84.98	85.00	100.02
M_3	90.04	90.01	99.96
M_4	95.02	94.98	99.96
M_5	100.21	100.23	100.02
M_6	104.99	105.07	100.08
M_7	109.98	109.97	99.99
M_8	115.13	115.06	100.03
M_9	119.89	119.91	100.02
Average value, Z, %			100.01
Standard deviation, S_z, %			0.04
Relative confidence interval $\Delta z=t(95\ \%, 8)\cdot S_z=2.3060\ S_z$, %			0.09
Critical value for the convergence of results $\Delta z \le \max\Delta_{As}=1.6$ %			Corresponds (0.09<1.60)
Systematic error $\delta=\lvert Z-100\rvert$, %			0.01
The criterion of uncertainty of systematic error $\delta \le \max \delta$, %			Corresponds (0.01<0.51)
General conclusion			Correct

The systematic error was 0.01 % and was practically insignificant, which confirms the sufficient accuracy method in the entire range of concentrations from 80 to 120 %.

The determination of intra-laboratory precision was carried out on six samples of the same series of capsules by different analysts and on different days (3 days) by evaluating the value of the relative confidence interval, which should be less than the maximum permissible uncertainty of the analysis results: $\Delta z \le 1.6$ (at B=5 %) (Table 5).

Table 5

Results of intra-laboratory precision study

No. solution	Value Z_i, %		
	1 experiment	2 experiment	3 experiment
1	100.18	100.25	99.95
2	100.05	100.17	99.98
3	99.91	100.11	100.18
4	99.87	100.21	99.97
5	100.05	99.98	100.10
6	100.01	100.14	100.09
Average Z (%)	100.01	100.14	100.05
RSD_x, %	0.11	0.09	0.09
Relative standard deviation, RSD_Z (%)	0.10		
Relative confidence interval, $\Delta_{\overline{Z}}$	0.09≤1.6		
The critical value of the convergence of results, Δ_{As}, %	1.6		

The value of the relative confidence interval for six parallel determinations of one series of drugs meets the acceptance criterion (≤1.6 %), which indicates confirmation of the intra-laboratory precision (Table 6).

The results of the quantitative determination of meldonium dihydrate in capsules are shown in Table 6.

Table 6

The results of quantitative determination of meldonium dihydrate in capsules

Drug	Found, g	Metrological characteristics
Capsules «Metamax» 250 mg	0.2491	$\overline{m}=0.2505$ g
	0.2509	$S=1.43\times10^{-3}$
	0.2485	$t=2.57$
	0.2515	$\Delta x=1.50\times10^{-3}$
	0.2510	RDS=0.57
	0.2522	ε=0.60 %
Capsules «Vasopro» 500 mg	0.5082	$\overline{m}=0.5032$ g
	0.5029	$S=3.33\times10^{-3}$
	0.5055	$t=2.57$
	0.4992	$\Delta x=3.49\times10^{-3}$
	0.5034	RDS=0.66
	0.5002	ε=0.69 %

4. 3. Assessment of the impact of analytical methods on the environment

Greenness was an important aspect in the development of spectrophotometric methods for the determination of meldonium in drugs. Meldonium is a difficult analyte to analyze due to its low molecular weight and

high polarity. The optimal conditions for quantitative determination were the use of DMF as a solvent and the heating of the obtained solution. These parameters significantly reduce the greenness of the developed method. For calculating the impact of analytical methods on the environment, the following tools were used: analytical eco-scale, AGREE tool (Analytical GREEnness) and GAPI. Software for calculating greenness using methods AGREE tool (Analytical GREEnness) and GAPI developed by scientists from the Gdańsk University of Technology [24–27]. The score of the analytical eco-scale was 81 (Table 7). This result indicates excellent «green» analysis. The analytical eco-scale method is somewhat outdated (does not evaluate all indicators), so the AGREE and GAPI methods remain relevant. A pictogram of the analytical method using the AGREE tool and GAPI is shown in Fig. 8, 9, respectively.

Table 7

Analytical eco-scale for assessing the «greenness» of the proposed analytical method

Parameters	Penalty points
Reagents alizarin	2
DMF	4
Occupational hazard	5
Waste	8
Total number of penalty points	19
Ball of analytical eco-scale	81
Conclusion	Excellent «green» analysis

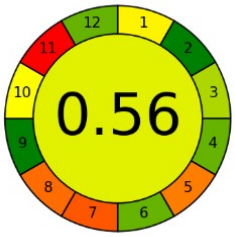

Fig. 8. Pictogram of an analytical method using AGREE tool

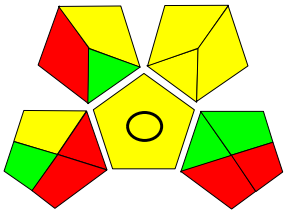

Fig. 9. Pictogram of analytical method using GAPI

5. Discussion of research results

We have proposed a developed method of spectrophotometric determination of meldonium dihydrate by reaction with alizarin at maximum absorption of the reaction product at a wavelength of 517 nm. The optimal conditions for quantitative determination were established: DMF solvent, concentration of alizarin solution – 0.8 %, volume of 0.8 % alizarin solution – 0.5 ml, heating time – 20 min, temperature (95±2) °C. The stoichiometric ratios of the reactive components (meldonium- alizarin) as 1:1 were defined by the methods of continuous changes and the saturation method. The spectrophotometric method for the determination of capsules has been validated in accordance with the requirements of SPhU. The analytical method was linear in the concentration range of 0.0402–0.1073 mg/mL. LOD and LOQ were calculated to be 2.84 μg/mL and 8.59 μg/mL, with correlation coefficient R^2=0.9995. Analyzed solutions were stable for 45 min (Fig. 5), and vacillation in the amount of the added reagent (alizarin solution) within ±10 % do not significantly affect the value of the absorbance (Table 4). The results of the study of greenness by the methods of AGREE and GAPI indicate that the developed method is not green enough, but it can be used as an arbitration method in the routine analysis of drugs containing meldonium.

Study limitations. The proposed analytical method can not be used to determine meldonium dihydrate in the presence of other antihypertensive and metabolic API in medicines.

Prospects for further research. The next stage of research is planned to develop and validate the spectrophotometric method for determination of meldonium in capsules based on the reaction with other quinones.

6. Conclusions

A spectrophotometric method for the determination of meldonium in capsules by reaction with alizarin in DMF medium has been developed. Stoichiometric ratios of reactive components (meldonium – alizarin) were determined, which were 1:1. The developed spectrophotometric method for the determination of meldonium in capsules has been validated in accordance with the requirements of SPhU. The analytical method was linear in the concentration range of 0.0402–0.1073 mg/mL. LOD and LOQ were to be 2.84 μg/mL and 8.59 μg/mL respectively. In summary, the developed method can be used as an arbitration method for the routine analysis of meldonium capsules.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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References

1. Meldonium. Available at: https://go.drugbank.com/drugs/DB13723

2. Dambrova, M. (2002). Mildronate Cardioprotective Action through Carnitine-Lowering Effect. Trends in Cardiovascular Medicine, 12 (6), 275–279. https://doi.org/10.1016/s1050-1738(02)00175-5

3. Sjakste, N., Kalvinsh, I. (2006). Mildronate: an antiischemic drug with multiple indications. Pharmacologyonline, 1, 1–18.

4. Volynskyi, D., Vakaliuk, I. (2019). Use of meldonium in the treatment of patients with coronary artery disease and concomitant arterial hypertension. EUREKA: Health Sciences, 6, 9–14. https://doi.org/10.21303/2504-5679.2019.001018

5. European Pharmacopoeia. 11 ed. (2021). Available at: https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition

6. Donchenko, A., Nahorna, N., Vasyuk, S. (2018). Development and validation of spectrophotometric method for the determination of meldonium dihydrate in dosage forms. ScienceRise: Pharmaceutical Science, 4 (14), 23–27. https://doi.org/10.15587/2519-4852.2018.141397

7. Pidpruzhnykov, Y. V., Sabko, V. E., Iurchenko, V. V., Zupanets, I. A. (2011). UPLC-MS/MS method for bioequivalence study of oral drugs of meldonium. Biomedical Chromatography, 26 (5), 599–605. https://doi.org/10.1002/bmc.1703

8. Lv, Y.-F., Hu, X., Bi, K.-S. (2007). Determination of mildronate in human plasma and urine by liquid chromatography–tandem mass spectrometry. Journal of Chromatography B, 852 (1-2), 35–39. https://doi.org/10.1016/j.jchromb.2006.12.031

9. Peng, Y., Yang, J., Wang, Z., Wang, J., Liu, Y., Luo, Z., Wen, A. (2010). Determination of mildronate by LC–MS/MS and its application to a pharmacokinetic study in healthy Chinese volunteers. Journal of Chromatography B, 878 (5-6), 551–556. https://doi.org/10.1016/j.jchromb.2009.12.030

10. Görgens, C., Guddat, S., Dib, J., Geyer, H., Schänzer, W., Thevis, M. (2015). Mildronate (Meldonium) in professional sports – monitoring doping control urine samples using hydrophilic interaction liquid chromatography – high resolution/high accuracy mass spectrometry. Drug Testing and Analysis, 7 (11-12), 973–979. Portico. https://doi.org/10.1002/dta.1788

11. Horyn, M., Logoyda, L. (2020). Bioanalytical method development and validation for the determination of metoprolol and meldonium in human plasma. Pharmacia, 67 (2), 39–48. https://doi.org/10.3897/pharmacia.67.e50397

12. Oliveira, D., de Araújo, A., Ribeiro, W., Silva, D., Duarte, A. C., de Sousa, V., Pereira, H. G. (2021). Screening method of mildronate and over 300 doping agents by reversed-phase liquid chromatography-high resolution mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 195, 113870. https://doi.org/10.1016/j.jpba.2020.113870

13. Kim, Y., Jeong, D., Min, H., Sung, C., Park, J. H., Son, J., Kim, K. H. (2017). Method for screening and confirming meldonium in human urine by high-resolution mass spectrometry and identification of endogenous interferences for anti-doping testing. Mass Spectrometry Letters, 8 (2), 39–43. https://doi.org/10.5478/MSL.2017.8.2.39

14. Parr, M. K., Botrè, F. (2022). Supercritical fluid chromatography mass spectrometry as an emerging technique in doping control analysis. TrAC Trends in Analytical Chemistry, 147, 116517. https://doi.org/10.1016/j.trac.2021.116517

15. Görgens, C., Guddat, S., Bosse, C., Geyer, H., Pop, V., Schänzer, W., Thevis, M. (2017). The atypical excretion profile of meldonium: Comparison of urinary detection windows after single- and multiple-dose application in healthy volunteers. Journal of Pharmaceutical and Biomedical Analysis, 138, 175–179. https://doi.org/10.1016/j.jpba.2017.02.011

16. Cai, L.-J., Zhang, J., Peng, W.-X., Zhu, R.-H., Yang, J., Cheng, G., Wang, X.-M. (2011). Determination of Mildronate in Human Plasma and Urine by UPLC–Positive Ion Electrospray Tandem Mass Spectrometry. Chromatographia, 73 (7-8), 659–665. https://doi.org/10.1007/s10337-010-1839-8

17. Tretzel, L., Görgens, C., Geyer, H., Thomas, A., Dib, J., Guddat, S. et al. (2016). Analyses of Meldonium (Mildronate) from Blood, Dried Blood Spots (DBS), and Urine Suggest Drug Incorporation into Erythrocytes. International Journal of Sports Medicine, 37 (6), 500–502. https://doi.org/10.1055/s-0036-1582317

18. Rabin, O., Uiba, V., Miroshnikova, Y., Zabelin, M., Samoylov, A., Karkischenko, V. et al. (2018). Meldonium long-term excretion period and pharmacokinetics in blood and urine of healthy athlete volunteers. Drug Testing and Analysis, 11 (4), 554–566. https://doi.org/10.1002/dta.2521

19. Forsdahl, G., Jančić-Stojanović, B., Anđelković, M., Dikić, N., Geisendorfer, T., Jeitler, V., Gmeiner, G. (2018). Urinary excretion studies of meldonium after multidose parenteral application. Journal of Pharmaceutical and Biomedical Analysis, 161, 289–295. https://doi.org/10.1016/j.jpba.2018.08.053

20. Rusu, L. D., Bratu, I., Mărușoiu, C., Moldovan, Z., Rada, M. (2020). Analytical methods for meldonium determination in urine samples. Analytical Letters, 54 (1-2), 233–241. https://doi.org/10.1080/00032719.2020.1748043

21. Temerdashev, A., Azaryan, A., Dmitrieva, E. (2020). Meldonium determination in milk and meat through UHPLC-HRMS. Heliyon, 6 (8), e04771. https://doi.org/10.1016/j.heliyon.2020.e04771

22. Alizarin. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Alizarin Last accessed: 10.05.2023

23. State Pharmacopoeia of Ukraine. Vol. 1 (2015). Kharkiv: SE "Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines, 11148.

24. Gałuszka, A., Migaszewski, Z. M., Konieczka, P., Namieśnik, J. (2012). Analytical Eco-Scale for assessing the greenness of analytical procedures. TrAC Trends in Analytical Chemistry, 37, 61–72. https://doi.org/10.1016/j.trac.2012.03.013

25. Pena-Pereira, F., Wojnowski, W., Tobiszewski, M. (2020). AGREE –Analytical GREEnness Metric Approach and Software. Analytical Chemistry, 92 (14), 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887

26. Płotka-Wasylka, J. (2018). A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta, 181, 204–209. https://doi.org/10.1016/j.talanta.2018.01.013

27. Płotka-Wasylka, J., Wojnowski, W. (2021). Complementary green analytical procedure index (ComplexGAPI) and software. Green Chemistry, 23 (21), 8657–8665. https://doi.org/10.1039/d1gc02318g

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