UDC 615.074:543.544.05.068:615.272
DOI: 10.15587/2519-4852.2023.274469

APPLICATION OF SALTS OF CHAOTROPIC ANIONS IN THE DEVELOPMENT OF HPLC METHODS FOR THE DETERMINATION OF MELDONIUM IN DOSAGE FORMS

Mariana Horyn, Marjan Piponski, Tetiana Zaremba, Tetyana Kucher, Svetlana Krstevska Balkanov, Tanja Bakovska Stoimenova, Dmytro Korobko, Nataliia Potikha, Liubomyr Kryskiw, Liliya Logoyda

The aim of the work *was to create an approach for the development of HPLC methods for the determination of meldonium in dosage forms with the usage of salts of chaotropic anions in mobile phases.*
Material and methods. *Analytical equipment: Shimadzu UPLC system LC-40 PDA; Shimadzu Nexera-i LC-2040C 3D-Plus, controlled by software Lab Solution version 5.97, electronic laboratory balance RAD WAG AS 200/C, pH-meter I-160MI. Meldonium dihydrate (purity 99.3 %) was purchased from Sigma-Aldrich (Switzerland), and Vasopro capsules 500 mg were purchased from a local pharmacy. Chromatographic conditions: Agilent Zorbax C-18 SB 150×4.6 mm×3.5 μm column was used (Agilent Technologies, USA). Mobile phases:*
1) 0.25 % KPF6 w/v – 0.1 % v/v 85 % H_3PO_4 95 % – 5 % ACN;
2) 0.3 % bis-(trifluoromethane)sulfonimide lithium salt 97 % w/v – 0.1 % v/v 85 % H_3PO_4 80 % – 20 % acetonitrile. Flow rate – 1 mL/min, T=32 °C, detection UV=at 4 channels – 190 nm, 195 nm, 200 nm, 205 nm.
Results and discussion. *We have proposed two approaches using two different salts of chaotropic anions – potassium hexafluorophosphate and bis-(trifluoromethane)sulfonimide lithium salt – for the HPLC method development. The chaotropic effects of these anions toward meldonium strongly influenced the analyte migratory behaviour. Both mobile phases involved, in addition to the use of a chaotrope, also the use of acetonitrile and pH adjustment with 0.1 % v/v 85 % H_3PO_4 solution. The detection wavelength (190 nm, 195 nm, 200 nm, 205 nm) was selected experimentally. The results were obtained for 8 concepts. Parameters of the chromatographic system confirm the conclusions and results of this investigation for the influence of chaotropic salts on N-containing molecules in an acidic pH medium, by increasing their retentivity and improving peak shape and uniformity homogeneity, even on the column without end-capping and base-deactivating. Validation of the analytical method was carried out following the requirements of SPhU.*
Conclusions. *HPLC methods for the determination of meldonium in dosage forms have been developed, using positive impacts of chaotropic salts on the molecules containing N-atoms in their molecule on their retentions and peak symmetries on the chromatogram. The validation of the analytical methods showed their suitability for pharmaceutical analysis*
Keywords: *chaotropic anions, dosage forms, HPLC, meldonium, spectrophotometry, validation*

How to cite:
Horyn, M., Piponski, M., Zaremba, T., Kucher, T., Krstevska Balkanov, S., Bakovska Stoimenova, T., Korobko, D., Potikha, N., Kryskiw, L., Logoyda, L. (2023). Application of salts of chaotropic anions in the development of hplc methods for the determination of meldonium in dosage forms. ScienceRise: Pharmaceutical Science, 1 (41), 14–22. doi: http://doi.org/10.15587/2519-4852.2023.274469

© The Author(s) 2023
This is an open access article under the Creative Commons CC BY license hydrate

1. Introduction

Meldonium (Mildronate) is a metabolic, cardioprotective drug, a structural analogue of gammabutyrobethaine (precursor of carnitine), which is used for treating cardiovascular complications and for improving the functioning of the brain. The mechanism of action of meldonium consists in the accumulation of gammabutyrobetaine, which leads to a decrease in carnitine biosynthesis. Optimized oxygen consumption and glucose metabolism in cells, oxidation of fatty acids is inhibited, and cell membrane damage is prevented. It has also been proven that meldonium induces NO (nitric oxide), which helps to reduce peripheral resistance and improve the rheological properties of blood. Due to these properties, meldonium is used for the treatment of myocardial infarction, heart failure, ischemic cerebral diseases, and arrhythmia. In 2016, World Anti-Doping Agency (WADA) added meldonium to the list of suppressed substances for athletes to use because they enhance performance and endurance [1–3]. Meldonium dihydrate is described in the European Pharmacopoeia [4, 5]; for assay of meldonium, European Pharmacopoeia proposes acidimetric non-aqueous titration. By surveying the literature review, it was found that numerous methods have been reported for the determination of meldonium in biological fluids [6–21] and food products [22], but only a few methods have been reported for the determination of medonium in dosage forms medicines [23–26]. Meldonium dehydrates (Fig. 1), 3-(2,2,2-trimethylhydrazin-2-ium-1-yl)propanoate dihydrate, is a water soluble molecule (20.2 mg/mL) with log*P*=–2.6, pKa (strongest acidic)=4.14 [27].

Only one spectrophotometric method of determination of meldonium in dosage form has been described in the scientific literature [23]. Donchenko A. et al. [23] have been developed the spectrophotometric method based on a reac-

tion with p-chloranil for the determination of meldonium in dosage forms. Toxic DMF as a medium was used to form the coloured reaction product with an absorption maximum at 556 nm. Three chromatographic methods (HPLC, HILIC) have been developed for the determination of meldonium and related substances in dosage forms [24–26]. All of them have the same disadvantages due to the time when these methods have been developed (2008, 2005, 2006 years). Nowadays, approaches to the development of chromatographic methods have been changed. New chromatographic columns have been put into practice by manufacturers, and other approaches to the selection of mobile phases and chromatographic conditions are used. Investigations and experiments of salts for chaotropic anions are quite new and promising in developing HPLC methods [28–30]. The main idea for testing the chaotropic effect in the development HPLC method came from the experiments and data described in the book of Kazakievic «HPLC for Practicing Scientists» [31].

Therefore, **the aim of our work** was to create an approach for the development of HPLC methods for the determination of meldonium in dosage forms with the usage of salts of chaotropic anions in mobile phases. Since the presence of two N-atomes in the molecular structure of meldonium, one secondary and one tertiary, chaotropic testing on the peak of meldonium as analyte will be very useful and applicative in concept for HPLC method development using alkyl reversed phase bonded columns, with C18 as the primary choice.

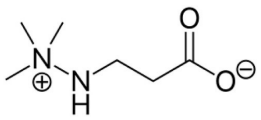

Fig. 1. Chemical structure of meldonium

2. Planning of the research

Methodology of research of development and validation of HPLC method for the determination of meldonium in dosage form includes:

1. Study of the monograph of the European Pharmacopoeia 10 and 11 edition, analysis of scientific articles.

2. Selection of chromatographic conditions (choice of detector, chromatographic column, mobile phase, optimal wavelength and injection, flow rate) and sample preparation.

3. Creation of two approaches using two different salts – potassium hexafluorophosphate and bis-(trifluoromethane)sulfonimide lithium salt.

4. Validation of the spectrophotometric method for determination of meldonium in dosage forms and study of the greenness profile assessment of the proposed method (analytical GREEnness).

3. Materials and methods

Objects of study, solvents and equipment.

Meldonium dihydrate (purity 99.3 %) was purchased from Sigma-Aldrich (Switzerland), Vasopro capsules 500 mg was purchased from a local pharmacy.

The following reagents and solvents were used in work: acetonitrile (ACN) (Honeywell), phosphoric acid (H_3PO_4) (Merck Darmstad, Germany), potassium hexafluorophosphate (KPF_6) (Merck Darmstad, Germany), bis-(trifluoromethane)sulfonimide lithium salt (Sigma-Aldrich (Switzerland).

Analytical equipment: Shimadzu UPLC system LC-40 PDA; Shimadzu Nexera-i LC-2040C 3D-Plus, controlled by software Lab Solution version 5.97, electronic laboratory balance RAD WAG AS 200/C, pH-meter I-160MI.

Chromatographic conditions: Agilent Zorbax C-18 SB 150×4.6 mm×3.5 μm column was used (Agilent Technologies, USA). Mobile phases:

1) 0.25 % KPF_6 w/v – 0.1 % v/v 85 % H_3PO_4 95 % – 5 % ACN;

2) 0.3 % bis-(trifluoromethane)sulfonimide lithium salt 97 % w/v – 0.1 % v/v 85 % H_3PO_4 80 % – 20 % acetonitrile.

Flow rate – 1 mL/min, *T*=32 °C, detection *UV*=at 4 channels – 190 nm, 195 nm, 200 nm, 205 nm.

Preparation of standard solution: 60 mg meldonium (standard sample) was put in a 10 mL measuring flask and dissolved in 5 mL freshly prepared in ultrapure water, treated on ultrasound for 5 minutes and shaken for 10 minutes using a mechanical shaker. After that, the measuring flask was filled up to mark with diluent and filtered through 0.2 μm RC syringe filter before injection. Final concentrations of 0.60 mg/mL of meldonium was obtained. After filtration, 2 μL were injected on the column.

Twelve tablets of capsules were studied to obtain statistically significant results. The granulate from the capsule, which corresponds to about 6mg/ml meldonium was put in 10 mL measuring flask and dissolved in 5 mL freshly prepared in ultrapure water, treated on ultrasound for 2 minutes and shaken for 15 minutes using a mechanical shaker. After that, the measuring flask was filled to mark with diluent and filtered through 0.2 μm RC syringe filter before injection. Final concentrations of 0.6 mg/mL of meldonium was obtained. After filtration, 2 μL were injected on the column.

4. Results

4. 1. Selection of chromatographic conditions

At the beginning of the conceptualization HPLC method development for the determination of meldonium in dosage forms, main challenges were evoked. As we mentioned before, meldonium is not a simple substance to analyze due to low molecular mass and high polarity. Understanding the facts described above, we began the development of the first approach, which involved the use of chaotropic anion salts. We started our method development by using high retentive, short, well-end-capped column Agilent Zorbax C-18 SB ((RP18, ODS, Octadecyl), Pore Size: 80 Å, Particle Size: 3.5 μ, Inner Diameter: 4.6 mm, Length: 150 mm, Carbon Load: 1 %, USP Number: L1, Category: Reversed Phase (RP)) and a first mobile phase consisting of 0.25 % KPF_6 w/v – 0.1 % v/v 85 % H_3PO_4 95 % – 5 % ACN. The use of salts of chaotropic anions in the mobile phase has shown certain advantages in the development of HPLC methods, such as increasing the column retentions of N-containing molecules, especially in acidic pH environments, and improving the peak symmetries [28]. Hexafluorophosphate chaotropic anion is the strongest in the Hofmeister series of chaotropic anions [29]. The obtained chromatogram is illustrated in Fig. 2.

As can be seen from the chromatograms on Fig. 2, the elution profile of this column and mobile phase showed good retention. To choose optimal wavelengths, we have tried four different wavelengths (190, 195, 200, 205 nm) (Fig. 3). Analyzing Fig. 3, we can conclude that all wavelengths are suitable. Full 3-D chromatogram of the standard of meldonium is illustrated on Fig. 4. To check the selectivity of the method, we have performed chromatograms on Fig. 5 and concluded that there is no interference of other components (mobile phase, excipients). To summarize all research experiments with usage of the first mobile phase, we performed overlaid 4-channels simultaneously monitored sample and standard with the first mobile phase, even though only one channel of UV wavelength appears to be sufficient. Analyzing the facts described above regarding the first approach, it is possible to conclude about the suitability of the proposed chromatographic column Agilent Zorbax C-18 SB (150×4.6 mm×3.5 µm) and mobile phase using one salt of the chaotropic anion (KPF_6) for the development of an express HPLC method for the determination of meldonium in dosage forms.

Overlaid chromatograms with 4-channels simultaneously monitored sample and standard with the first mobile phase demonstrated on Fig. 6.

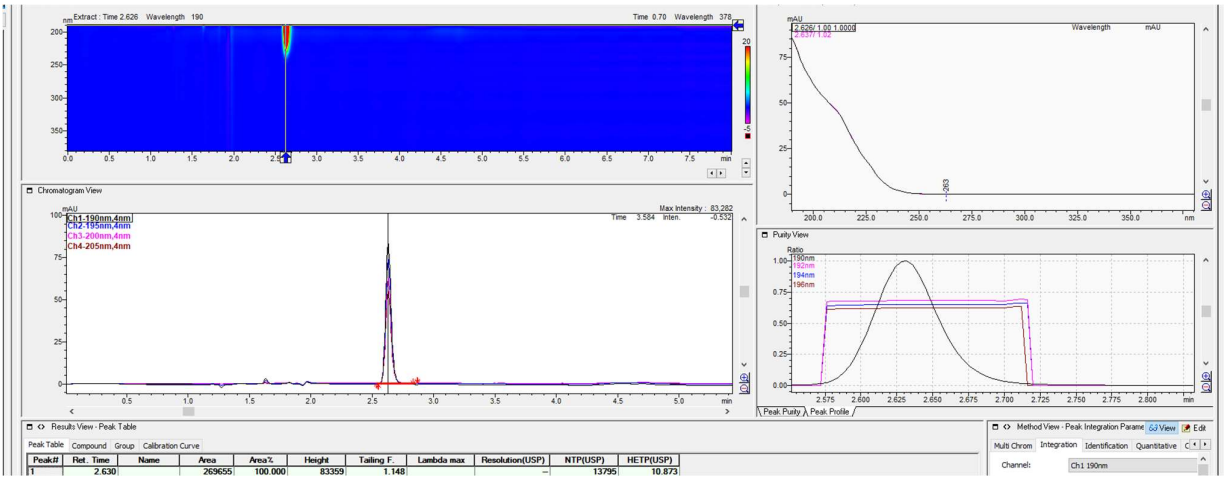

Fig. 2. Chromatogram of standard meldonium 5 mg/mL at full PDA record with contour diagram at top, chromatogram extracted at 4 channels with 4 different wavelengths, on the right side are illustrated peak UV spectrum and graphic purity calculation with first mobile phase and 2 µL injection

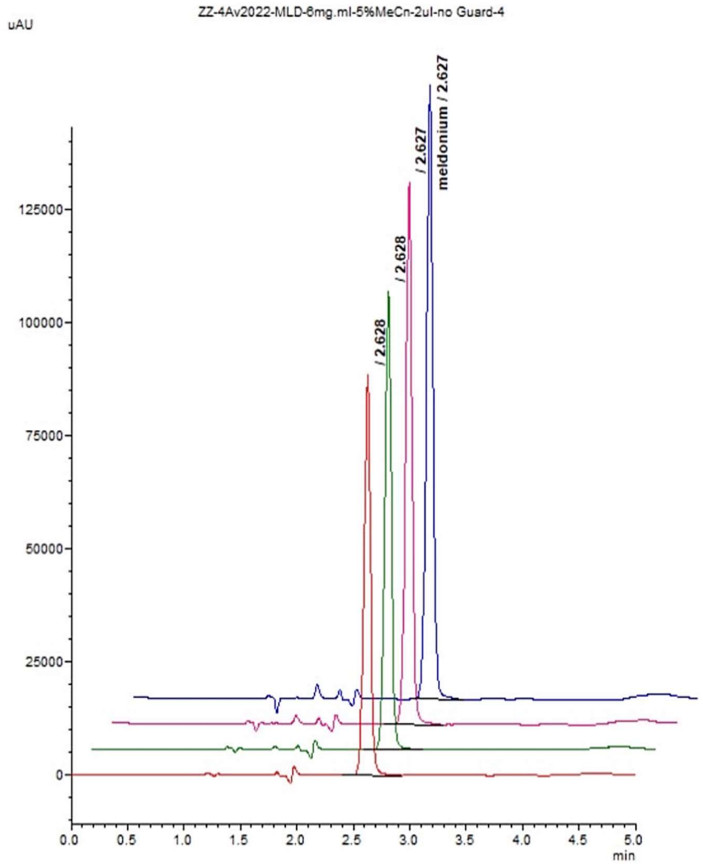

Fig. 3. Comparison of peak size of meldonium standard with the first mobile phase at 4 UV wavelengths: blue colour – 190 nm; pink – 195 nm; green – 200 nm; red – 205 nm

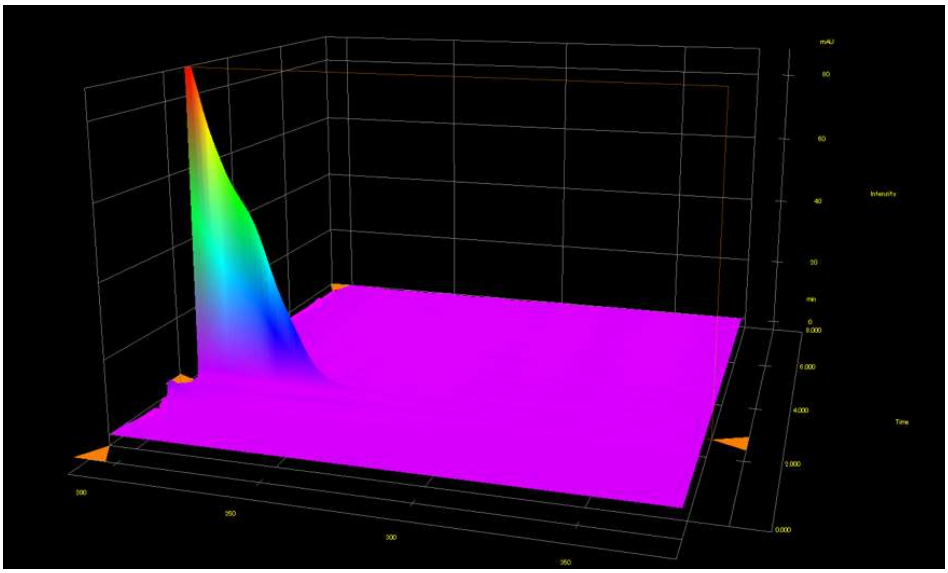

Fig. 4. Full 3-D chromatogram of the standard of meldonium

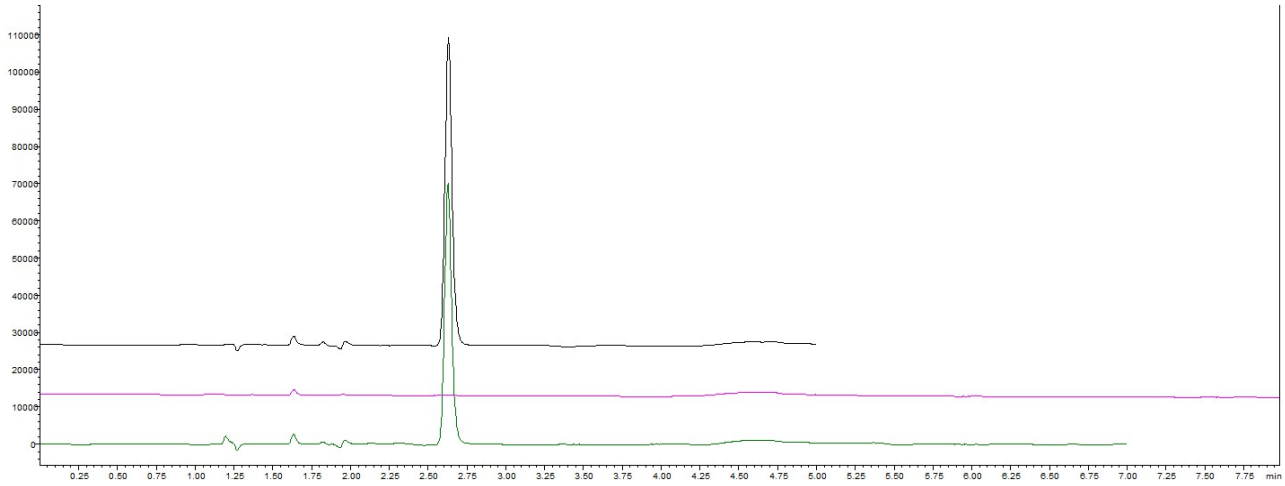

Fig. 5. Chromatograms illustrating the selectivity of the method (injected standard, medium – mobile phase, top – sample from capsule Meldonium)

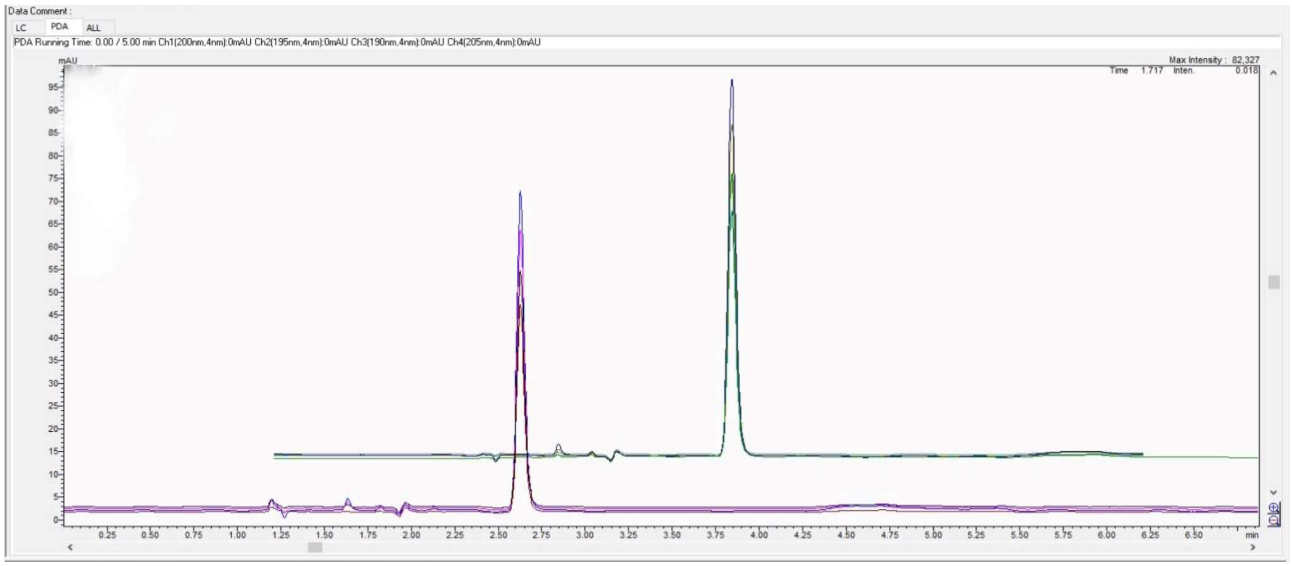

Fig. 6. Overlaid chromatograms with 4-channels simultaneously monitored sample and standard with the first mobile phase

Considering the positive results obtained in the first approach, we continued the search with the possibility of using other salts of chaotropic anions in the mobile phase. Based on viscosity data, Pandey et al. [30] discov-

ered that bis(trifluoromethanesulfonyl)imide is a stronger chaotropic ion than hexafluorophosphate. Therefore, we moved to the second approach, which involved the use of the same column as in the first concept Agilent Zorbax C-18 SB (150×4.6 mm×3.5 µm) and a mobile phase consisting 0.3 % bis-(trifluoromethane)sulfonimide lithium salt 97 % w/v – 0.1 % v/v 85 % H_3PO_4 80 % – 20 % acetonitrile. Comparison of retention increasing time of meldonium according to chaotropic power of the two strongest chaotropic salts, with the first mobile phase with KPF_6 and 5 % acetonitrile eluting at about 2.65 min, and the strongest described substance bis-(trifluoromethane)sulfonimide lithium salt is illustrated on Fig. 7.

As can be seen from the chromatograms on Fig. 7, the use of a salt of the chaotropic anion (bis-(trifluoromethane)sulfonimide lithium salt) extended the retention of meldonium to 4.4 min and made it possible to obtain an excellent chromatogram with satisfactory parameters of the chromatographic system. We performed overlaid 4-channels simultaneously monitored sample and standard with the second mobile phase (Fig. 9). Parameters of the chromatographic system which is presented on Fig. 8 confirm the conclusions and results of this investigation for the influence of chaotropic salts on N-containing molecule, by increasing their retentivity, and improving peak shape and homogeneity, even on the column without end-capping and base-deactivating. Therefore, the described second approach can also be used to develop the HPLC method for determining meldonium in dosage forms.

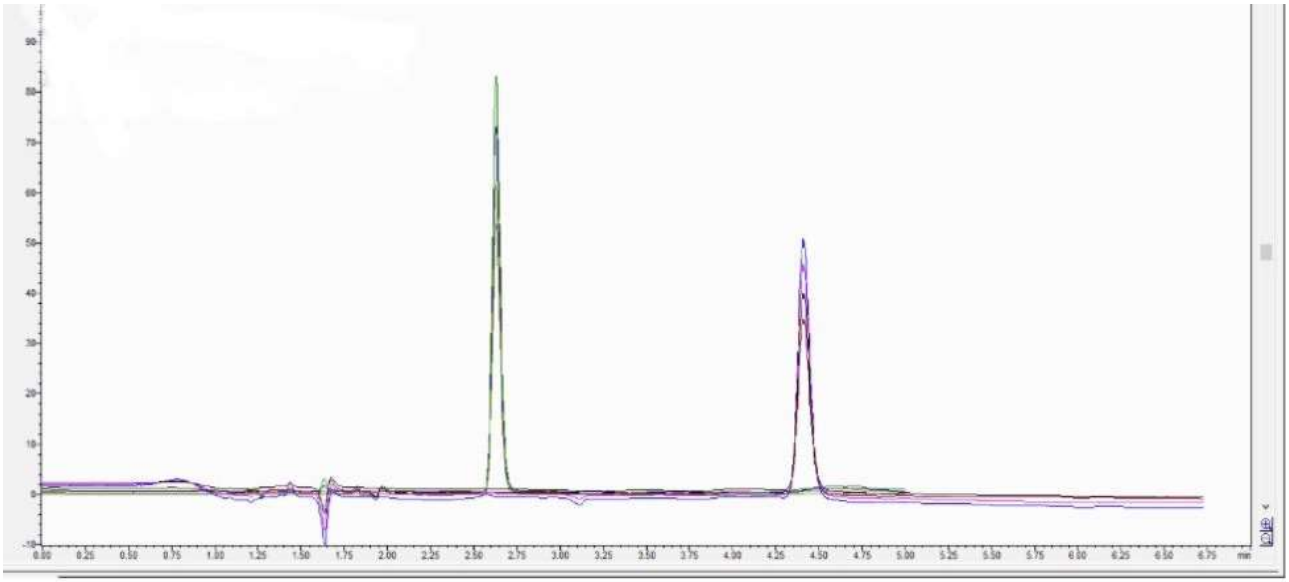

Fig. 7. Chromatograms with the comparison of retention increasing time of meldonium according to chaotropic power of the two strongest chaotropic salts, with first mobile phase with KPF_6 and 5 % acetonitrile eluting at about 2.65 min, and the strongest described substance bis-(trifluoromethane)sulfonimide lithium salt

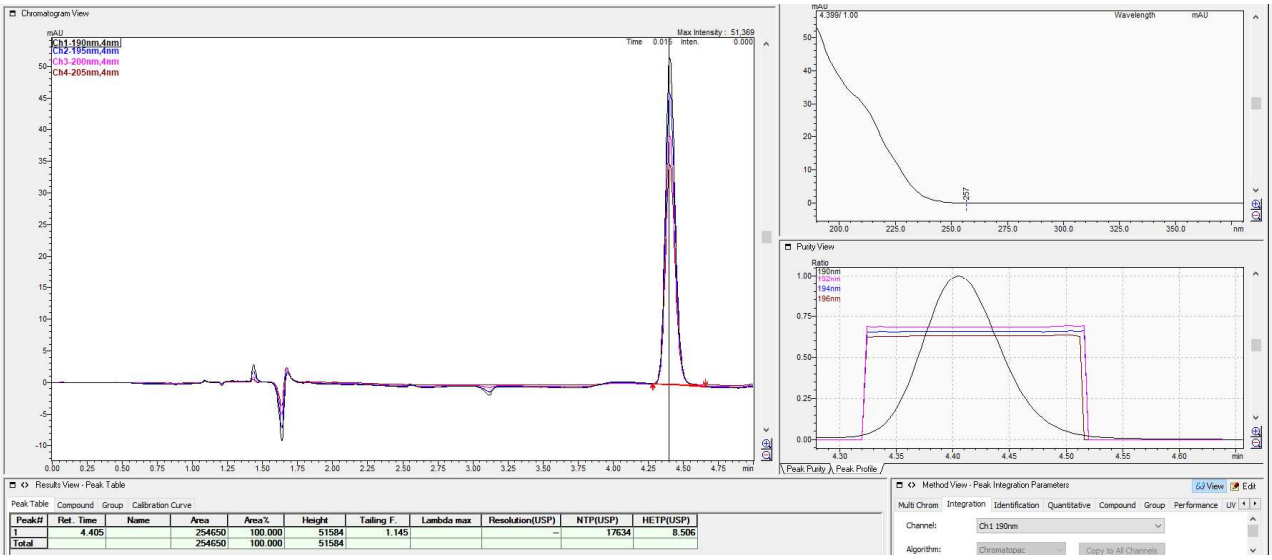

Fig. 8. Full PDA chromatogram of the standard of meldonium with second mobile phase, with strongest described chaotrop, with monitored UV-spectrum, peak homogeneity. The figure illustrates the 4-channels chromatogram overlay, UV-absorption spectrum of meldonium and peak purity-homogeneity suggested by software calculations

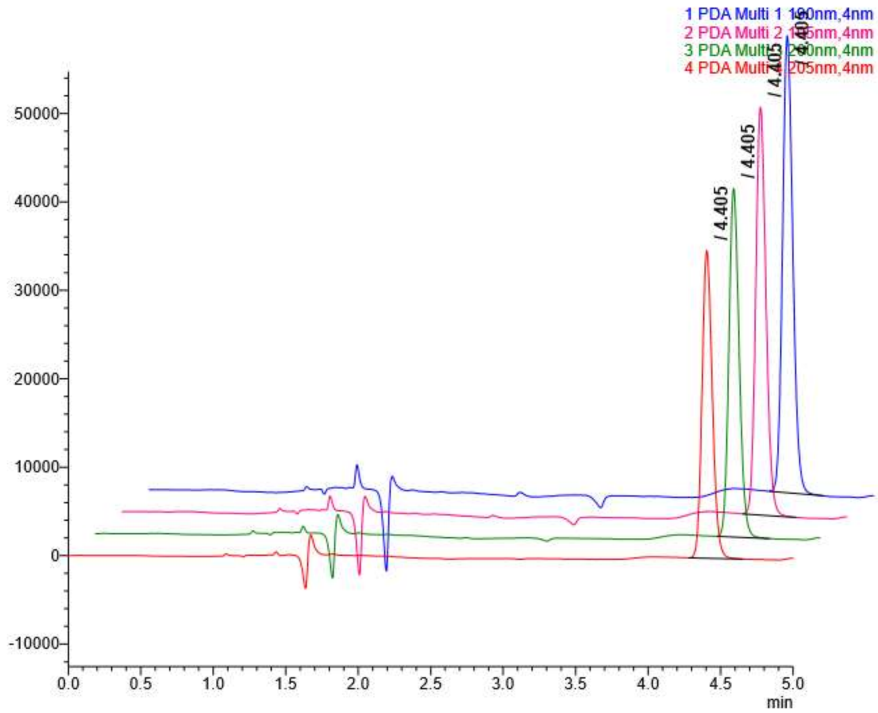

Fig. 9. Overlaid chromatograms with 4-channels simultaneously monitored sample and standard with second mobile phase with 0.3 % bis-(trifluoromethane)sulfonimide lithium salt buffer for comparison of peak heights and method sensitivity: blue colour – 190 nm; pink – 195 nm; green – 200 nm; red – 205 nm

4. 2. Determination of validation characteristics and greenness profile assessment

Validation of the analytical method was carried out in accordance with the requirements of SPhU [32]. The calculation of the assessment of the impact of the analytical technique on the environment was carried out using the AGREE (Analytical GREEnness) method (software developed by the Gdansk University of Technology, Poland)) [33]. Comparison between the proposed HPLC concepts presented in Table 1, intra-and Inter-day accuracy and precision results – in Table 2. The linearity results showed an excellent relationship between the obtained peak areas and the used concentrations of meldonium, and also indicated the high sensitivity of the proposed HPLC concepts. Analyzing results, presented in Tables 1, 2 we conclude that all proposed concepts are environmentally friendly. In the very poor available data about getting an idea and usable information about the development of the method for determination of meldonium, we can suggest our work with offered detailed and successful solutions for the mentioned aim with less sophisticated equipment for quality control lab for routine manufacturing control.

Table 1

Comparison between the proposed HPLC concepts

Concept	I	II	III	IV	V	VI	VII	VIII
1	2	3	4	5	6	7	8	9
Stationary phase	Agilent Zorbax C-18 SB (150×4.6 mm×3.5 µm)							
Mobile phase	0.25 % KPF_6 w/v – 0.1 % v/v 85 %H_3PO_4 95 % – 5 % ACN	0.25 % KPF_6 w/v – 0.1 % v/v 85 %H_3PO_4 95 % – 5 % ACN	0.25 % KPF_6 w/v – 0.1 % v/v 85 %H_3PO_4 95 % – 5 % ACN	0.25 % KPF_6 w/v – 0.1 % v/v 85 %H_3PO_4 95 % – 5 % ACN	0.3 % bis-(trifluoromethane) sulfonimide lithium salt 97 % w/v – 0.1 %v/v 85 % H_3PO_4 80 % – 20 % acetonitrile	0.3 % bis-(trifluoromethane) sulfonimide lithium salt 97 % w/v – 0.1 %v/v 85 % H_3PO_4 80 % – 20 % acetonitrile	0.3 % bis-(trifluoromethane) sulfonimide lithium salt 97 % w/v – 0.1 %v/v 85 % H_3PO_4 80 % – 20 % acetonitrile	0.3 % bis-(trifluoromethane) sulfonimide lithium salt 97 % w/v – 0.1 %v/v 85 % H_3PO_4 80 % – 20 % acetonitrile
Flow rate	1 mL/min							
Column temperature	32 °C							

Continuation of Table 1

1	2	3	4	5	6	7	8	9
Wavelengths, nm	190	195	200	205	190	195	200	205
Retention time, min	2.63	2.63	2.63	2.63	4.05	4.05	4.05	4.05
LOD/LOQ, mg/mL	0.021 0.055	0.024 0.062	0.028 0.074	0.031 0.080	0.028 0.81	0.033 0.101	0.039 0.121	0.0554 0.182
Linearity regression, R^2	1	1	1	0.9999	1	1	1	0.9999
Analytical GREEnness (AGREE)	0.76							

Table 2

Intra- and inter-day accuracy and precision results and greenness profile assessment

Concept	I	II	III	IV	V	VI	VII	VII
Mean, % (RSD, %)								
Intra-day accuracy and precision results	99.81 (0.38)	98.27 (0.89)	98.78 (0.56)	99.32 (0.17)	98.77 (0.90)	99.28 (0.49)	98.78 (0.99)	99.85 (0.11)
	99.36 (0.09)	99.46 (0.78)	99.11 (0.13)	99.56 (0.38)	98.84 (0.69)	99.86 (0.10)	99.05 (0.85)	99.74 (0.72)
	99.12 (0.56)	99.56 (0.26)	98.99 (0.78)	99.19 (0.49)	99.81 (0.18)	99.53 (0.10)	98.97 (0.93)	99.64 (0.26)
Inter-day accuracy and precision results	98.95 (0.67)	99.11 (0.99)	99.56 (0.36)	98.90 (0.95)	98.70 (0.48)	99.85 (0.58)	99.28 (0.30)	98.95 (0.94)
	99.36 (0.56)	98.54 (0.67)	99.91 (0.25)	99.75 (0.40)	99.56 (0.17)	98.67 (0.14)	99.11 (0.74)	99.01 (0.70)
	99.62 (0.37)	99.35 (0.68)	98.92 (0.47)	98.70 (0.92)	99.26 (0.20)	99.93 (0.11)	98.98 (0.94)	99.16 (0.47)

5. Discussion of research results

Given the limited information in scientific publications over the past 10 years regarding the development of an HPLC method for the determination of meldonium in dosage forms, we set ourselves the goal of proposing an approach and developing an HPLC method. In the process of developing the newest scientific approaches to the development of HPLC methods and from our own experience, we drew attention to the possibility of using salts of chaotropic anion in the mobile phase. Considering the characteristics of the column, we chose for our research the Agilent Zorbax C-18 SB 150×4.6 mm×3.5 µm chromatographic column. Considering the significant advantages of introducing salts of chaotropic anions into the mobile phase, we proposed two approaches using two different salts – potassium hexafluorophosphate and bis-(trifluoromethane)sulfonimide lithium salt. In addition to the use of a chaotrope, both mobile phases involved, acetonitrile and pH adjustment with 0.1 % v/v 85 % H_3PO_4 solution. The detection wavelength (190 nm, 195 nm, 200 nm, 205 nm) was selected experimentally. Summing up, the results were obtained for 8 concepts (Tables 1, 2). The validation of the analytical methods showed their suitability for the purposes of pharmaceutical analysis. The environmental friendliness of all proposed concepts is also proven. Considering the fact that only one spectrophotometric method for the determination of meldonium [23] is described in the scientific literature, which has a number of disadvantages, and three chromatographic methods, which have lost relevance over time [24–26], proposed two scientific approaches have practical importance for chemists.

Study limitations. The proposed HPLC methods can not be used to determine meldonium in blood plasma.

Prospects for further research. The presented paper describes approaches for the development of HPLC method for the determination of meldonium in dosage forms. The next research stage is planned to develop approaches for the simultaneous determination of meldonium in combination with other API in dosage forms.

6. Conclusions

We have proposed two approaches using two different salts of chaotropic anions – potassium hexafluorophosphate and bis-(trifluoromethane)sulfonimide lithium salt – for the HPLC method development. The chaotropic effects of these anions toward meldonium strongly influenced the analyte migratory behaviour. Both mobile phases involved, in addition to the use of a chaotrope, also the use of acetonitrile and pH adjustment with 0.1 % v/v 85 % H_3PO_4 solution. The detection wavelength (190 nm, 195 nm, 200 nm, 205 nm) was selected experimentally. The results were obtained for 8 concepts. HPLC methods for the determination of meldonium in dosage forms have been developed, using positive impacts of chaotropic salts on the molecules

containing N-atoms in their molecule on their retentions and peak symmetries on the chromatogram. The validation of the analytical methods showed their suitability for pharmaceutical analysis.

Conflict of interests

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

Financing

The study was performed without financial support.

Availability of data

Data will be provided upon reasonable request.

Acknowledgement

The authors from Ukraine would like to thank all the brave defenders of Ukraine who made the finalization of this article possible.

References

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

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

3. Liepinsh, E., Vilskersts, R., Loca, D., Kirjanova, O., Pugovichs, O., Kalvinsh, I., Dambrova, M. (2006). Mildronate, an Inhibitor of Carnitine Biosynthesis, Induces an Increase in Gamma-Butyrobetaine Contents and Cardioprotection in Isolated Rat Heart Infarction. Journal of Cardiovascular Pharmacology, 48 (6), 314–319. doi: https://doi.org/10.1097/01.fjc.0000250077.07702.23

4. European Pharmacopoeia. 10th edn. (2020). Available at: https://cymitquimica.com/products/41-PUB200379/european-pharmacopoeia-10th-edition-103-104-105-electronic-version-bilingual/

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

6. 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. doi: https://doi.org/10.1016/j.jchromb.2006.12.031

7. 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. doi: https://doi.org/10.1016/j.jchromb.2009.12.030

8. 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. doi: https://doi.org/10.1002/bmc.1703

9. 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. doi: https://doi.org/10.1002/dta.1788

10. 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. doi: https://doi.org/10.3897/pharmacia.67.e50397

11. 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. doi: https://doi.org/10.1016/j.jpba.2020.113870

12. Sahartova, O., Shatz, V., Kalvinš, I. (1993). HPLC analysis of mildronate and its analogues in plasma. Journal of Pharmaceutical and Biomedical Analysis, 11 (10), 1045–1047. doi: https://doi.org/10.1016/0731-7085(93)80068-c

13. Azarian, A. A., Temerdashev, A. Z., Dmitrieva, E. V. (2017). Opredelenie meldoniia v moche cheloveka metodom VE-ZhKh s tandemnym mass-spektrometricheskim detektirovaniem. Zhurnal analiticheskoi khimii, 72 (10), 885–889. doi: https://doi.org/10.7868/s0044450217100048

14. 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. doi: https://doi.org/10.1016/j.jpba.2017.02.011

15. 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. doi: https://doi.org/10.5478/MSL.2017.8.2.39

16. 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. doi: https://doi.org/10.1016/j.trac.2021.116517

17. 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. doi: https://doi.org/10.1007/s10337-010-1839-8

18. 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. doi: https://doi.org/10.1055/s-0036-1582317

19. 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. doi: https://doi.org/10.1002/dta.2521

20. 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. doi: https://doi.org/10.1016/j.jpba.2018.08.053

21. 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. doi: https://doi.org/10.1080/00032719.2020.1748043

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

23. 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. doi: https://doi.org/10.15587/2519-4852.2018.141397

24. Hmelnickis, J., Pugovičs, O., Kažoka, H., Viksna, A., Susinskis, I., Kokums, K. (2008). Application of hydrophilic interaction chromatography for simultaneous separation of six impurities of mildronate substance. Journal of Pharmaceutical and Biomedical Analysis, 48 (3), 649–656. doi: https://doi.org/10.1016/j.jpba.2008.06.011

25. Cao, G., Lu, R., Hu, X. (2005). Determination of mildronate and related substances by HPLC-ELSD. Chinese pharmaceutical journal, 40 (11), 864.

26. Lü, R. F., Cao, G. Y., Hu, X., Zhang, J. R. (2006). HPLC Assay and determination of related substance for mildronate injection. Chinese Journal of Pharmaceutical Analysis, 26 (3), 358–360.

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

28. Kazakevich, Y. V., LoBrutto, R., Vivilecchia, R. (2005). Reversed-phase high-performance liquid chromatography behavior of chaotropic counteranions. Journal of Chromatography A, 1064 (1), 9–18. doi: https://doi.org/10.1016/j.chroma.2004.11.104

29. Flieger, J. (2006). The effect of chaotropic mobile phase additives on the separation of selected alkaloids in reversed-phase high-performance liquid chromatography. Journal of Chromatography A, 1113 (1-2), 37–44. doi: https://doi.org/10.1016/j.chroma.2006.01.090

30. Pandey, S., Fletcher, K. A., Baker, S. N., Baker, G. A. (2004). Correlation between the fluorescent response of microfluidity probes and the water content and viscosity of ionic liquid and water mixtures. The Analyst, 129 (7), 569. doi: https://doi.org/10.1039/b402145m

31. Kazakevich, Y. V., Lobrutto, R. (Eds.) (2007). HPLC for pharmaceutical scientists. John Wiley & Sons, 1104. doi: https://doi.org/10.1002/0470087951

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

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

Received date 06.12.2022
Accepted date 21.02.2023
Published date 28.02.2023

Mariana Horyn, Assistant**,** Department of Pharmaceutical Chemistry**,** I. Horbachevsky Ternopil National Medical University**,** Voli ave., 1, Ternopil, Ukraine, 46001

Marjan Piponski, PhD, Head of Department, Instrumental analysis, Quality Control Department, Replek Farm Ltd. Company for Pharmaceutical-Chemical Products, Kozle str., 188, 1000 Skopje, Republic of North Macedonia

Tetiana Zaremba, Department of Pharmaceutical Chemistry, I. Horbachevsky Ternopil National Medical University, Voli ave., 1, Ternopil, Ukraine, 46001

Tetyana Kucher, PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** I. Horbachevsky Ternopil National Medical University**,** Voli ave., 1, Ternopil, Ukraine, 46001

Svetlana Krstevska Balkanov, PhD**,** Clinic for Haematology**,** University Clinic at Medical Faculty Skopje**,** Ss. Cyril and Methodius, 1000, Skopje, Republic of North Macedonia

Tanja Bakovska Stoimenova, PhD, Specialist**,** Instrumental analysis, Quality Control Department**,** Replek Farm Ltd. Company for pharmaceutical-chemical products**,** Kozle str., 188, 1000 Skopje, Republic of North Macedonia

Dmytro Korobko, PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** I. Horbachevsky Ternopil National Medical University**,** Voli ave., 1, Ternopil, Ukraine, 46001

Nataliia Potikha, PhD, Associate Professor**,** Department of Functional and Laboratory Diagnostics**,** I. Horbachevsky Ternopil National Medical University**,** Voli ave., 1, Ternopil, Ukraine, 46001

Liubomyr Kryskiw, PhD, Associate Professor**,** Department of Pharmaceutical Chemistry**,** I. Horbachevsky Ternopil National Medical University**,** Voli ave., 1, Ternopil, Ukraine, 46001

Liliya Logoyda*, Doctor of Pharmaceutical Sciences, Professor, Head of Department, Department of Pharmaceutical Chemistry, I. Horbachevsky Ternopil National Medical University, Voli ave., 1, Ternopil, Ukraine, 46001

***Corresponding author:** *Liliya Logoyda, e-mail: logojda@tdmu.edu.ua*