PHARMACIA

Pharmacia 67(2): 39–48
DOI 10.3897/pharmacia.67.e50397

Research Article

Bioanalytical method development and validation for the determination of metoprolol and meldonium in human plasma

Mariana Horyn[1], Liliya Logoyda[1]

1 *Department of Pharmaceutical Chemistry, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine*

Corresponding author: Liliya Logoyda (logojda@tdmu.edu.ua)

Received 22 January 2020 ♦ **Accepted** 9 March 2020 ♦ **Published** 31 July 2020

Citation: 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

Abstract

Aim. The main purpose of this study was to develop a simple, precise, rapid and accurate method for the quantification of metoprolol and meldonium in human plasma.

Materials and methods. The resolution of peaks of metoprolol was best achieved with Discovery C18, 50 × 2.1 mm, 5 µm column and meldonium - ZORBAX HILIC Plus, 50 × 2.1 mm, 3.5 µm column. Samples of metoprolol were chromatographed in a gradient mode (eluent A (acetonitrile – water – formic acid, 5 : 95 : 0.1 v/v), eluent B (acetonitrile – formic acid, 100 : 0.1 v/v)). The initial content of the eluent B is 0%, which increases linearly by 1.0 min to 100% and to 1.11 min returns to the initial 0%. The mobile phase was delivered at a flow rate of 0.400 mL/min into the mass spectrometer ESI chamber. The injection volume was 5µl. Samples of meldonium were chromatographed in a isocratic using mobile phase water – acetonitrile – ammonium formate buffer 200 мМ, 20 : 75 : 5 v/v).

Results. The total chromatographic run time was 2.0 minutes and the elution of metoprolol, meldonium and IS occurred at ~1.39 and 1.18 minutes, respectively.A linear response function was established at 2 - 200 ng/mL for metoprolol and 50 -5000 ng/mL for meldonium in human plasma. The% mean recovery for metoprolol in LQC, MQC and HQC was 99.0%, 107.5% and 96.8%, for meldonium in LQC, MQC and HQC was 94.1%, 100.2% and 93.1% respectively. The lowest concentration with the RSD <20% was taken as LLOQ and was found to be 2.31 ng/mL for metoprolol, 47.70 ng/mL for meldonium. The % accuracy of LLOQ samples prepared with the different biological matrix lots were found 115.4% for metoprolol and 95.5% for meldonium, which were found within the range of 80.00–120.00% for the seven different plasma lots. % CV for LLOQ samples was observed as 12.8% and 7.7% respectively, which are within 20.00% of the acceptance criteria.

Conclusion.A rapid method was developed for simultaneous determination of metoprolol and meldonium in human plasma. The method was strictly validated according to the ICH guidelines. Acquired results demonstrate that proposed strategy can be effortlessly and advantageously applied for routine examination of metoprolol and meldonium in human plasma.

Keywords

LC-MS/MS, Metoprolol, Meldonium, Validation, Human plasma

Introduction

Metoprolol (Fig. 1) is a beta1-selective (cardioselective) adrenoceptor blocking agent, for oral administration, available as extended-release tablets. Metoprolol succinate extended-release tablet has been formulated to provide a controlled and predictable release of metoprolol for once-daily administration. The tablets comprise a multiple unit system containing metoprolol succinate in a multitude of controlled release pellets. Each pellet acts as a separate drug delivery unit and is designed to deliver metoprolol continuously over the dosage interval. The tablets contain 23.75, 47.5, 95 and 190 mg of metoprolol succinate equivalent to 25, 50, 100 and 200 mg of metoprolol tartrate, USP, respectively. Its chemical name is (±)1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol. Metoprolol is a propanolamine that is 1-(propan-2-ylamino) propan-2-ol substituted by a 4-(2-methoxyethyl)phenoxy group at position 1. It has a role as a beta-adrenergic antagonist, an antihypertensive agent, a xenobiotic and an environmental contaminant. It is a propanolamine, an aromatic ether, a secondary alcohol and a secondary amino compound. Metoprolol is used for a number of conditions, including hypertension, angina, acute myocardial infarction, upraventricular tachycardia, ventricular tachycardia, congestive heart failure, and prevention of migraine headaches.

The State Pharmacopoeia of Ukraine (SPhU) does not have a monograph on the substance of metoprolol or on the prepared medical form (The State Pharmacopeia of Ukraine 2015). However, the United States Pharmacopoeia regulates the determination of metoprolol succinate in extended-release tablets. For identification, IR-spectroscopy and HPLC are proposed. For quantitative determination of metoprolol succinate in tablets in assay and dissolution test – HPLC, respectively. Chromatographic conditions for the determination of metoprolol succinate, tablets are given in the monograph of the United States Pharmacopoeia, which is used the chromatographic column 4-mm × 12.5-cm; 5-µm packing L7 and mobile phase consisting of acetonitrile and buffer (25:75). Mobile phase rate – 1 ml/min, detection wavelength – 280 nm, tailing factor - NMT 2.0, relative standard deviation - NMT 2.0%. The proposed method of the United States Pharmacopeia requires a long sampling.

The European Pharmacopeia has a monograph on metoprolol tartrate tablets. Identification of metoprolol tartrate of the European Pharmacopeia regulates the absorption spectrophotometry in the infrared region, UV-spectrophotometry and HPLC, quantitative determination – HPLC/UV (European Pharmacopoeia 2016). As a solvent, mixture of methanol and 0.1 M hydrochloric acid is used, mobile phase – solution of 1-pentanesulfonic acid sodium salt (monohydrate), anhydrous sodium acetate in mixture of methanol and water and adding glacial acetic acid. Mobile phase rate – 1.0 ml/min, detection wavelength – 254 nm.

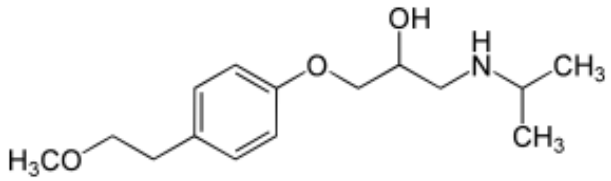

Figure 1. Chemical structure of metoprolol.

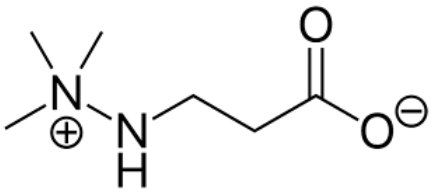

Figure 2. Chemical structureof meldonium.

Nowadays metabolic therapy is an important component of the treatment of virtually any disease of the internal organs. Drugs affecting the metabolic processes in the heart, brain, liver, muscles, are widely prescribed by general practitioners and narrow specialists. A special place among them take cardioprotectors – a group of drugs that improves metabolic processes in ischemic myocardium, increase resistance to hypoxia, eliminate cellular metabolism disorders. To the well-known and recognized by clinicians cardioprotectors also belongs meldonium Meldonium, sold under the brand name Mildronate, is a performance-enhancing drug that is the source of much debate in the world of sports doping. Initially designed in Latvia for use in animals, the World Anti-Doping Agency (WADA) has indicated that this drug is banned in Olympic sports. Meldonium is primarily manufactured by a Latvian drug company called Grindeks. It is also known as Mildronāts, Quaterine, trimethylhydrazinium propionate, and 3-(2,2,2-trimethylhydraziniumyl)propionate (Fig. 2). Meldonium is a structural analogue of gamma-butyrobetamine. At the heart of the mechanism of action of the drug is the reduction in the amount of carnitine in the body, which in the conditions of oxygen insufficiency leads to inhibition of oxidation of fatty acids. Meldonium induces biosynthesis of nitric oxide, which promotes relaxation of smooth muscle of vessels, improves microcirculation and endothelial function. The therapeutic effect of the drug is due to the variety of its pharmacological effects. Meldoniumhas been classified as a hydrazinium base of low molecular weight, and its determination is rather difficult. The State Pharmacopoeia of Ukraine (SPhU) does not have a monograph on the substance of meldonium dihydrate or on the prepared medical form (The State Pharmacopeia of Ukraine 2015). However, the substance meldoniumdihydrateis described in the European Pharmacopoeia and the State Pharmacopoeia of the Russian Federation (SPRF). Thus, the SPRF XII recommends the quantitative determination of meldoniumdihydrate by the acidimetry method

in a non-aqueous medium with a potentiometric fixation of the end point of titration.

Combination theraphy of meldonium and metoprolol is used in the treatment of various chronic cardio-vascular diseases and disorders of the cerebral circulation, as well as to improve mental and physical capacity. In the contemporary literature, some bioanalytical methods were reported for quantification of metoprolol and meldonium in human plasma (Albers et al. 2005; Braza et al. 2002; Angier et al. 2005; Jensen et al. 2008; Gowda et al. 2007; Peng et al. 2010; Zupanets et al. 2010; Kondratova et al. 2016, 2017; Liliya et al. 2016; Logoyda 2018a, b, c, 2019; Logoyda et al. 2017a, b, c, 2018a, b, c; Mykhalkiv et al 2018a, b). Therefore, it was thought desirable to develop a simple, accurate, cheap and fast procedure that could be applied for the determination of metoprolol and meldonium in human plasma, this study performed assay validations as per guidelines. While this method with validation details were economical and applied for pharmacokinetic studies of metoprolol and meldonium.

Materials and methods

Chemicals and reagents

Metoprolol (purity 100.0%), meldonium (purity 99.3%), bethanechol (Internal Standard) (purity 99.9%) (Internal Standard), bisoprolol (purity 99.9%) were purchased from Moehs Catalana, S.L., Spain, Zhejiang Huahai Pharmaceutical Co., Ltd, KHP, EDQM – Council of Europe. HPLC grade acetonitrile and methanol were purchased from CHROMASOLV, HPLC grade formic acid were purchased from Fluka. All other chemicals and reagents were of analytical grade. Microcaps® disposable micropipettes (50 µl, catalog number: 1-000-0500) were purchased from Drummond Scientific Company, USA. The control human dipotassium ethylenediaminetetraacetic acid (K_2EDTA) plasma sample was procured from Red Cross Society, Ukraine.

Instrumentation and chromatographic conditions

A Shimadzu HT (Shimadzu, Japan) LC system equipped with degasser (DGU-14A), binary pump (LC-20ADXR) along with auto-sampler (SIL-20ACHT) was used to inject 5 µl aliquots of the processed samples on DiscoveryC18, 50 × 2.1 mm, 5 µm column maintained at 25 ± 1 °C (for determination of metoprolol). Samples were chromatographed in a gradient mode (eluent A (acetonitrile – water – formic acid, 5 : 95 : 0.1 v/v), eluent B (acetonitrile – formic acid, 100 : 0.1 v/v)). The initial content of the eluent B is 0%, which increases linearly by 1.0 min to 100% and to 1.11 min returns to the initial 0%. The mobile phase was delivered at a flow rate of 0.400 mL/min into the mass spectrometer ESI chamber(Logoyda 2019).For determination of meldonium we used ZORBAX HILIC Plus, 50 × 2.1 mm, 3.5 µm column and mobile phase water –acetonitrile – ammonium formate buffer200 мM, 20 : 75 : 5 v/v). Paramatrs of electrospray ionizer and MRM parametrs are listed in Tables 1, 2. The analytical data were processed by Analyst Software (version 1.5.2).

Table 1. Parametrs of ionizer electrospray.

	Parametr	Value
1	Polarity	Positive
2	Nebulizer Gas (NEB, Gas 1)	15
3	Curtain Gas (CUR)	8
4	Collision Gas (CAD)	4
5	IonSpray Voltage (IS)	5000
6	Temperature (TEM)	400
7	Turbo IonSpray Gas	8
8	Horizontal Position	8.0
9	Lateral Position	2.0

Standard solutions

Metoprolol, meldonium and IS were weighed accurately into volumetric flasks using an analytical microbalance. Approximately 0.5 mg/mL primary stock solutions of metoprolol, 2.5 mg/ml primary stock solutions of meldonium, 1.0 mg/mL primary stock solutions of bethanechol (IS), 0.2 mg/mL primary stock solutions of bisoprolol (IS) were prepared in methanol. The stock solutions were stored at −20 °C, which were found to be stable for 1 month. The stock solutions of metoprolol and meldonium were successively diluted with methanol and water to prepare secondary stocks and working solutions. Secondary stock solutions and working solutions were used to prepare calibration curve (CC) and quality control (QC) samples. Working stock solutions were stored at 4 °C for a week. Working stocks were used to prepare plasma calibration standards. Blank human plasma was screened before spiking to ensure that it was free from endogenous interference at retention times of metoprolol, meldonium and IS, respectively. Calibration standards' samples were pre-

Table 2. Multiple reaction monitoring (MRM) parameters. Abbreviations: DP, declustering potential; FP, focusing potential; EP, entrance potential;CE, collision energy; CXP, collision cell exit potential.

ID	Parent, m/z	Daughter, m/z	Time, ms	DP, V	FP, V	EP, V	CE, V	CXP, V
Metoprolol	268.164	116.2	50	21	150	11	27	20
Bisoprolol (IS)	326.435	116,2	50	46	260	11	27	20
Meldonium	147.000	59,0	100	16	120	11	25	10
Bethanechol (IS)	161.283	102,2	100	66	370	11	19	8

pared by spiking the blank human K_2EDTA plasma with appropriate concentration of metoprolol and meldonium. Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with metoprolol and meldonium at appropriate concentrations (for metoprolol 5.94 ng/mL low QC [LQC], 64.5 ng/mL medium QC [MQC], and 145 ng/mL high QC [HQC], for meldonium 141 ng/mL low QC [LQC], 1503 ng/mL medium QC [MQC], and 3492 ng/mL high QC [HQC]) and 120 µL plasma aliquots were distributed into different tubesaccording to Guideline EMEA/CHMP and Guideline FDA/CDER. All the samples were stored at −80 °C ± 10 °C.

Sample preparation

A simple protein precipitation extraction method was followed for extraction of metoprolol and meldonium at from human plasma. From the deep freezer, the required quantities of CC standards and QC samples were withdrawn. The samples were thawed at room temperature. To an aliquot of 100 µl plasma, 20 µl of IS was added. To this mixture, 300 µl of methanol was added and vortexed for 2 minutes, followed by centrifugation at 6000 rpm for 5 minutes at 4 °C. After centrifugation, approximately 50 µl supernatant was aliquoted into, respectively, labeled autosampler vials, which were later placed in the autosampler at 15 °C ± 4 °C. 10µl of the sample was injected onto LC-MS/MS system for analysis.

Method validation

A full validation according to the ICH guidelines was performed for the assay in K_2EDTA human plasma (Guideline on Validation of Bioanalytical Methods 2009, Guidance for Industry. Bioanalytical Method Validation. Food and Drug Administration 2010).

Specificity

The specificity of the method was evaluated by analyzing human plasma samples from different lots to investigate the potential interferences at the chromatographic peak region for analytes and IS. The acceptance criterion for the experiment was that should have <20% area response to that of the LLOQ level response in the same matrix. Two lots of hemolyzed plasma samples were also analyzed to ensure specificity against potential biological interferences.

Linearity

The points CC (2–200 ng/ml for metoprolol and 50–5000 ng/ml for meldonium) were constructed by plotting the peak area ratio of each analyte: IS against the nominal concentration of calibration standards in K_2EDTA human plasma. Following the evaluation of different weighing factors, the results were fit into linear regression analysis using 1/X2 (X: Concentration) weighing factor. The CC should have a correlation coefficient (r) of 0.99 or better. The acceptance criteria for each back-calculated standard concentration were ±15% deviation from the nominal value except at LLOQ, which was set at ±20%.

Matrix effect

The effect of human plasma constituents over the ionization of metoprolol, meldonium and IS was determined by post-column infusion method to evaluate matrix effect. Briefly, an infusion pump delivers a constant amount of analyte into LC system outlet entering to mass spectrometer inlet. To follow the analyte signal, the mass spectrometer was operated in MRM mode. The human plasma constituent sample extract was injected on LC column. A steady ion response was obtained as a function of time since the analyte was infused at a constant rate. Any endogenous compound that elutes from the column which causes a variation in ESI response of the infused analyte was seen as a suppression or enhancement in the response of the infused analyte. A separate experiment was performed with metoprolol, meldonium and IS solutions, which were infused at a constant rate, and blank matrix sample injected through the LC. To evaluate matrix effect, different lots of human plasma were spiked with analyte concentration levels at LQC and HQC levels. According to guidelines, the acceptance criterion for each back-calculated concentration was ±15% deviation from the nominal value.

Precision and accuracy

The intra-assay precision and accuracy were estimated by analyzing six replicates containing metoprolol, meldonium at four different QC levels concentrations (for metoprolol 5.94 ng/mL low QC [LQC], 64.5 ng/mL medium QC [MQC], and 145 ng/mL high QC [HQC], for meldonium 141 ng/mL low QC [LQC], 1503 ng/mL medium QC [MQC], and 3492 ng/mL high QC [HQC]) in human plasma. The four-level QC samples on four different runs were performed to assess the inter assay precision. The acceptance criteria for each back-calculated standard concentration were 85–115% accuracy from the nominal value except at LLOQ, which was set at 80–120%.

Recovery

The efficiency of metoprolol, meldonium and IS extraction from human plasma was determined by comparing the responses of the analytes extracted from replicate QC samples (n = 6) with those of neat standard solutions spiked in post-extracted plasma blank sample at equivalent concentrations by protein precipitation extraction method. Recovery of metoprolol was determined at LQC (5.94 ng/mL) and HQC (145 ng/mL) concentrations, meldonium was determined at LQC (141 ng/mL) and HQC (3492 ng/mL) concentrations whereas the recovery of IS was determined at a single concentration of 20 ng/mL.

Stability experiments

Stability tests were conducted to evaluate the stability of metoprolol, meldonium in plasma samples under different conditions. 8 hrs bench top stability, processed samples stability (autosampler stability for 26 hrs at 10 °C), three cycles of freeze-thaw stability, 30 days of long-term stability at −80±10 °C were performed at LQC and HQC levels using six replicates at each level. Samples were considered stable if assay values' acceptance criterion was of accuracy (i.e., 85–115% from fresh samples) and precision (i.e., ±15% relative standard deviation [RSD]).

Results and discussion

Meldonium can be perfectly determined by HILIC chromatography and metoprolol by conventional reversed-phase HPLC. We tried to find a common method that would allow us to identify both analytes at one time - reversed-phase HPLC with an ion-pair reagent for meldonium. Alas, such a mutual compromise struck on both analytes, and failed to obtain the necessary results, despite several attempts, even when switching to a more sensitive instrument. As a result, we had to go back to the separate methods on the surface, and immediately everything turned out. Sampling remained common - methanol precipitation. In the present study, optimization and critical evaluation of mobile phase composition (gradient), flow rate, and analytical column were important to obtain good resolution of peaks of interest from the endogenous components, which in turn affect reproducibility and sensitivity of the method. Selection of chromatographic conditions for the proposed method was optimized to suit the preclinical pharmacokinetic studies. To ease the sample preparation in microtubes and to reduce the usage of solvent, the plasma volume was kept low. Initial feasibility experiments of a various mixture(s) of solvents such as acetonitrile, methanol and formic acid along with altered flow rates (in the range of 0.1–0.6 ml/min) were performed to optimize an effective chromatographic resolution of metorolol, meldonium and IS. Various analytical columns were tested to obtained good and reproducible response within short run time. The resolution of peaks of metoprolol was best achieved with DiscoveryC18, 50 × 2.1 mm, 5 µm column and meldonium - ZORBAX HILIC Plus, 50 × 2.1 mm, 3.5 µm column. Samples of metoprolol were chromatographed in a gradient mode (eluent A (acetonitrile – water – formic acid, 5 : 95 : 0.1 v/v), eluent B (acetonitrile – formic acid, 100 : 0.1 v/v)). The initial content of the eluent B is 0%, which increases linearly by 1.0 min to 100% and to 1.11 min returns to the initial 0%. The mobile phase was delivered at a flow rate of 0.400 mL/min into the mass spectrometer ESI chamber. The injection volume was 5 µl. Samples of meldonium were chromatographed in a isocratic using mobile phase water – acetonitrile – ammonium formate buffer 200 mM, 20 : 75 : 5 v/v).

The purpose of sample extraction optimization is mainly to achieve high extraction recovery with negligible or low matrix effects to improve sensitivity and reliability of LC-MS/MS analysis. A poor extraction procedure decreases method robustness due to the presence of endogenous interference in the sample extracts, which are not efficiently cleaned up due to poor extraction procedure decreases the method robustness due to the endogenous interference in the sample extracts. With time-saving advantage and simplicity, the protein precipitation extraction method was chosen as an extraction method. The attained LLOQ was sufficient to quantify metoprolol and meldonium in low-dose pharmacokinetic studies according to Guideline EMEA/CHMP and Guideline FDA/CDER.

Metoprolol and meldonium eluted at ~1.39 and 1.18minutes, respectively. During a direct infusion experiment, the mass spectra for metoprolol, meldonium and IS revealed peaks at m/z 347.128, 377.165 and 455.385, respectively as protonated molecular ions, $[M+H]^+$. Typical multiple reaction monitoring chromatograms of metoprolol, meldonium and internal standard in dipotassium ethylenediaminetetraacetic acid human blank plasma are shown in Figs 3, 4.

The total chromatographic run time was 2.0 minutes and the elution of metoprolol and meldonium occurred at ~1.39 and 1.18 minutes, respectively.

Specificity

Different lots of plasma were analysed to ensure that no endogenous interferences were present at the retention time of metoprolol and meldonium LLOQ level samples along with plasma blank from the respective plasma lots were prepared and analysed (Table 3).

In all plasma blanks, the response at the retention time of metoprolol and meldonium was less than 20% of LLOQ response and at the retention time of IS, the response was less than 5% of mean IS response in LLOQ.

Table 3. Results of specificity for metoprolol and meldonium.

S. No	Metoprolol				Meldonium			
	STD	LLOQ		% Interference	STD	LLOQ		% Interference
	BL	Area	RT		BL	Area	RT	
1	0	422	1.38	NIL	0	6671	1.18	NIL
2	0	430	1.39	NIL	0	6702	1.19	NIL
3	0	442	1.39	NIL	0	8184	1.18	NIL
4	0	456	1.38	NIL	0	8319	1.18	NIL
5	0	461	1.38	NIL	0	7001	1.18	NIL
6	0	441	1.39	NIL	0	8915	1.18	NIL
7	0	439	1.38	NIL	0	8299	1.18	NIL
8	0	434	1.38	NIL	0	8218	1.19	NIL
9	0	425	1.38	NIL	0	8144	1.18	NIL
10	0	440	1.39	NIL	0	8111	1.17	NIL

*Average of triplicate injections.

Linearity

The calibration standard curves had a reliable reproducibility over the standard concentrations across the calibra-

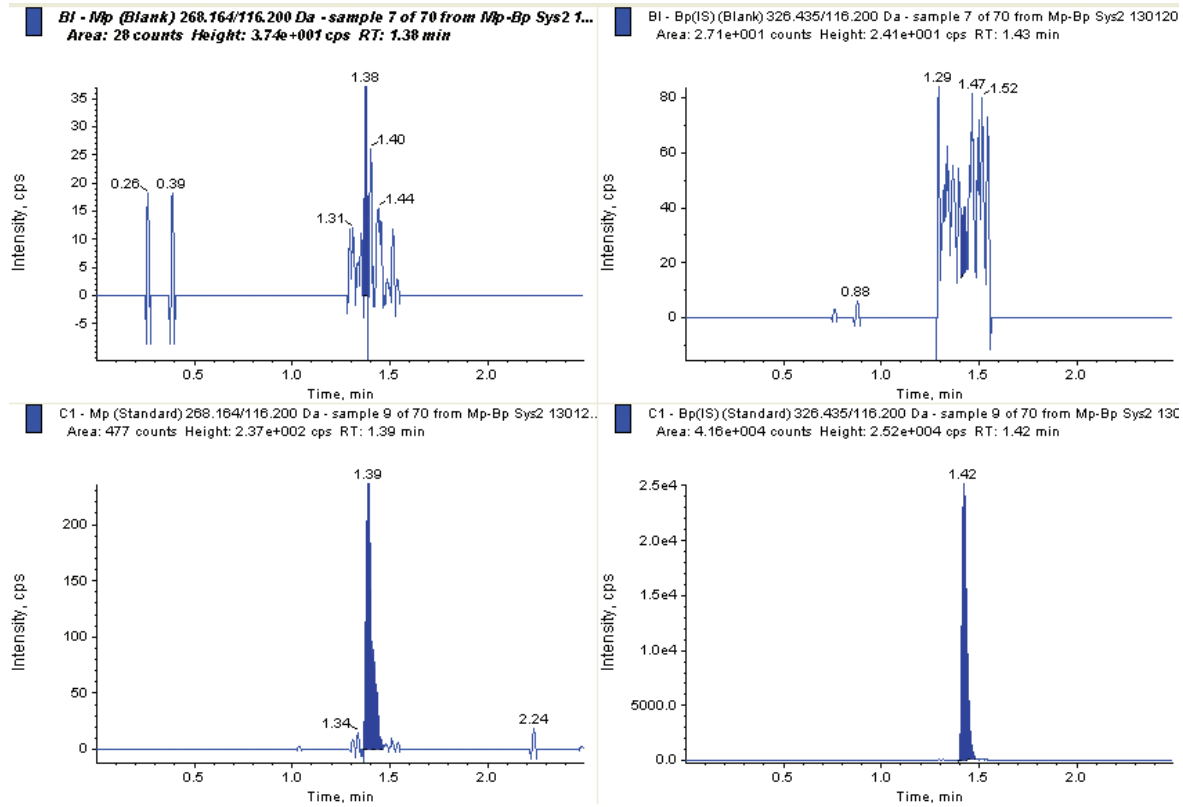

Figure 3. Typical multiple reaction monitoring chromatograms of metoprolol and internal standard in dipotassium ethylenediaminetetraacetic acid human blank plasma.

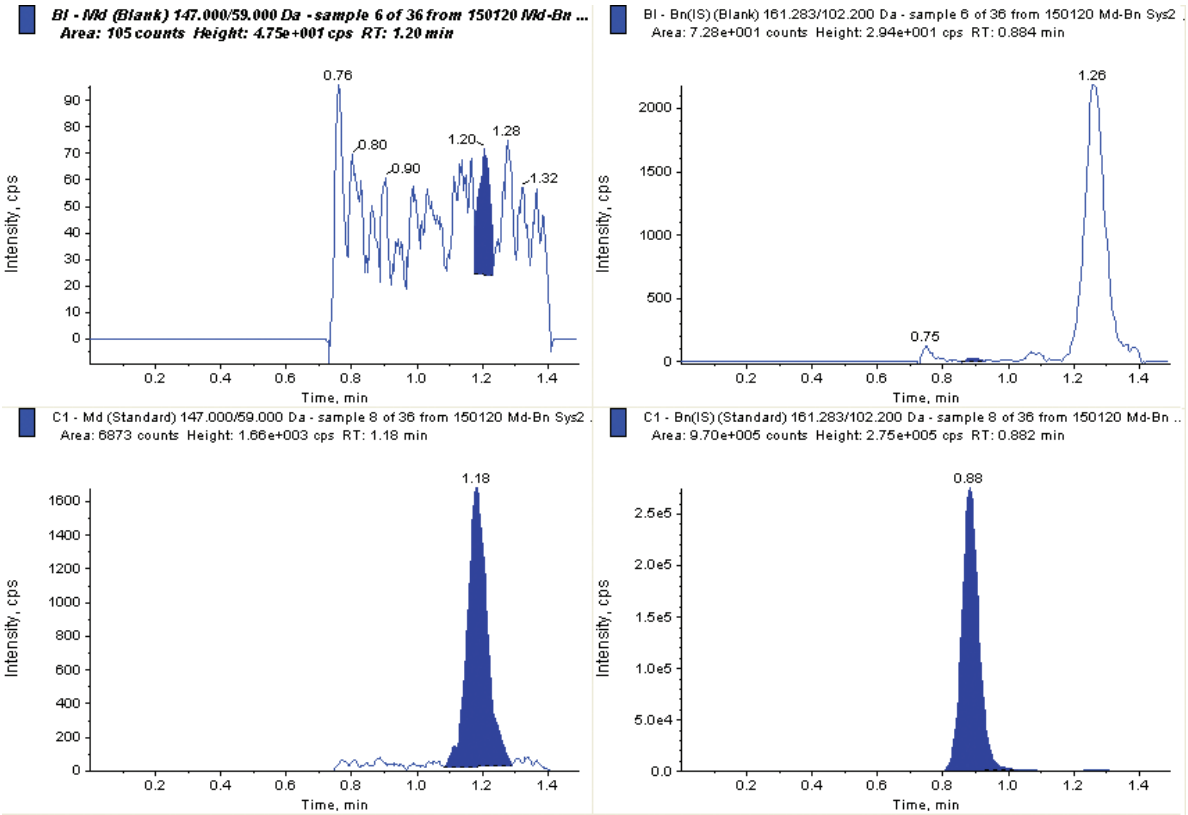

Figure 4. Typical multiple reaction monitoring chromatograms of meldonium and internal standard in dipotassium ethylenediaminetetraacetic acid human blank plasma.

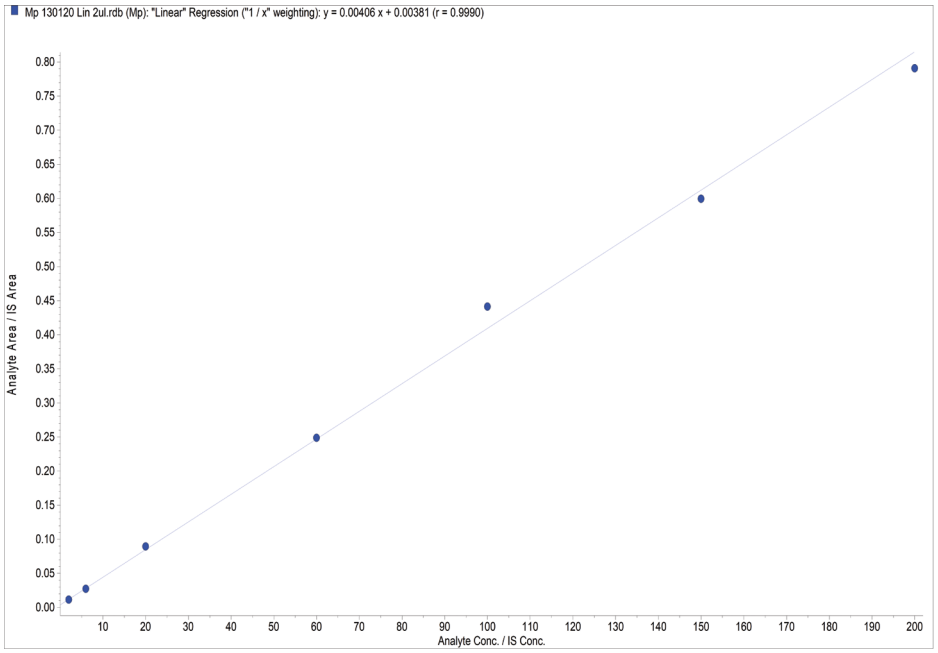

Figure 5. The calibration curve of metoprolol in human plasma.

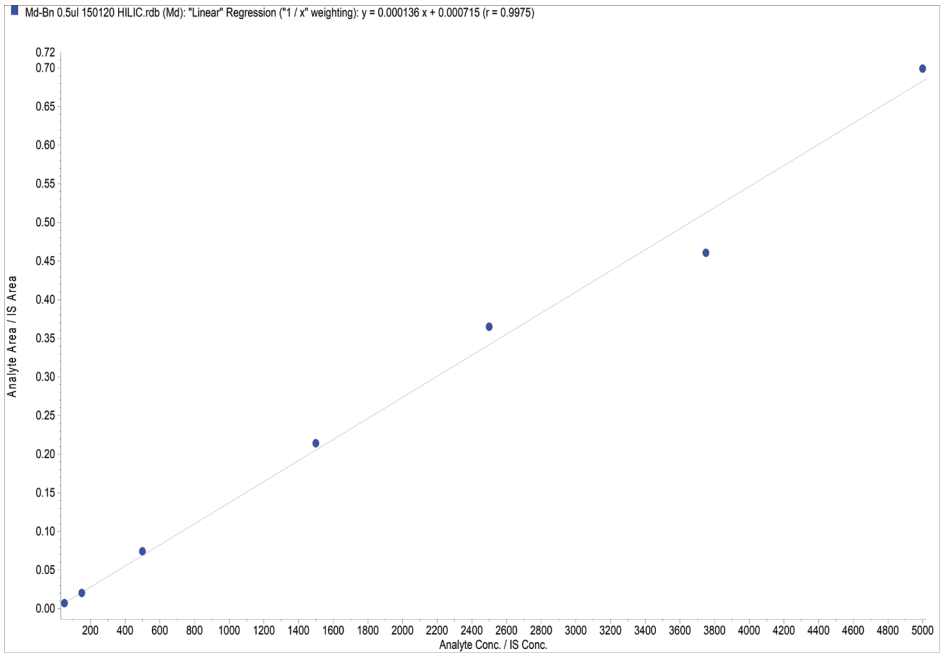

Figure 6. The calibration curve of meldonium in human plasma.

tion range. The average regression (n = 3) was found to be > 0.997 for all analytes.

The calibration curve (peak area ratio Vs Concentration) was linear over working range for metoprolol of 2 to 200.00 ng/mL with 7 point calibration used for quantification by linear regression, shown in Fig. 5. The regression equation for the analysis was

Y = 0.00406x + 0.00381 with coefficient of correction (R^2) = 0.9990.

The calibration curve (peak area ratio Vs Concentration) was linear over working range for meldonium of 50 to 5000.00 ng/mL with 7 point calibration used for quantification by linear regression, shown in Fig. 6. The regression equation for the analysis was

Y = 0.000136x + 0.000715 with coefficient of correction (R^2) = 0.9975.

Recovery

The % mean recovery for metoprolol and meldonium in LQC, MQC and HQC are listed in Tables 4, 5.

The % mean recovery for metoprolol in LQC, MQC and HQC was 99.0%, 107.5% and 96.8%, for meldonium in LQC, MQC and HQC was 94.1%, 100.2% and 93.1% respectively.

Table 4. The % mean recovery of metoprolol for LQC, MQC and HQC. Abbreviations: Lower quality control (LQC), middle quality control (MQC), higher quality control (HQC). Each value is represented as a mean±SD of 5 observations (n = 5), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria < 2.0.

No.	LQC	MQC	HQC
1	5.88	61.6	147
2	5.78	63.6	144
3	6.22	66.3	147
4	5.94	65.1	147
5	5.88	66.1	140
Mean	5.94	64.5	145
SD	0.167	1.96	3.08
% CV	2.8	3.0	2.1
% MeanRecovery	99.0	107.5	96.8

Table 5. The % mean recovery of meldonium for LQC, MQC and HQC. Abbreviations: Lower quality control (LQC), middle quality control (MQC), higher quality control (HQC). Each value is represented as a mean±SD of 5 observations (n = 5), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria < 2.0.

No.	LQC	MQC	HQC
1	139	1400	3331
2	142	1575	3690
3	148	1584	3700
4	138	1530	3377
5	139	1424	3363
Mean	141	1503	3492
SD	4.09	85.6	186
% CV	2.9	5.7	5.3
% MeanRecovery	94.1	100.2	93.1

Intraday (within run) and Inter-day (between run) precision and accuracy

The within-run coefficients of variation ranged between 0.331% and 0.619% for metoprolol. The within-run percentages of nominal concentrations ranged between 98.80% and 100.63% formetoprolol. The between-run coefficients of variation ranged between 0.332% and 0.615% for metoprolol. The between-run percentages of nominal concentrations ranged between 98.98% and 101.71% for metoprolol. Results are presented in Table 6. The assay values on both the occasions (intra- and inter-day) were found to be within the accepted limits.

The within-run coefficients of variation ranged between 0.353% and 0.719% for meldonium. The within-run

Table 6. Intra-day and Inter-day precision data of metoprolol. Each value is represented as a mean±SD of observations, SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria < 2.0.

Day	Intra-day precision		Inter-day precision	
	Mean	RSD %	Mean	RSD %
1	98.80	0.378	101.71	0.332
2	100.41	0.619	98.98	0.390
3	100.63	0.331	100.53	0.615

Table 7. Intra-day and Inter-day precision data of meldonium. *Each value is represented as a mean±SD of observations, SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria < 2.0.

Day	Intra-day precision		Inter-day precision	
	Mean	R.S.D %	Mean	R.S.D %
1	99.23	0.353	101.79	0.514
2	101.17	0.719	99.57	0.349
3	100.82	0.376	100.13	0.674

percentages of nominal concentrations ranged between 99.23% and 101.17% for meldonium. The between-run coefficients of variation ranged between 0.349% and 0.674% for meldonium. The between-run percentages of nominal concentrations ranged between 99.57% and 101.79% for meldonium. Results are presented in Table 7.

Matrix effect

The lowest concentration with the RSD < 0% was taken as LLOQand was found to be 2.31 ng/mL for metoprolol, 47.70 ng/mL for meldonium. The% accuracy of LLOQ samples prepared with the different biological matrix lots were found 115.4% for metoprolol and 95.5% for meldonium, which were found within the range of 80.00–120.00% for the seven different plasma lots.% CV for LLOQ samples was observed as 12.8% and 7.7% respectively, which are within 20.00% of the acceptance criteria. Results are presented in Tables 8, 9.

Table 8. Results of matrix effect of metoprolol. Abbreviations Lower limit of quantification (LLOQ). Eachvalueisrepresentedas a mean ± SD of 5observations (n = 5), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria < 2.0.

No.	LLQC
1	2.19
2	2.20
3	1.94
4	2.56
5	2.66
Mean	2.31
SD	0.295
% CV	12.8
% MeanRecovery	115.4

Table 9. Results of matrix effect of meldonium. Abbreviations: Lower limit of quantification (LLOQ). Each value is represented as a mean ± SD of 5observations (n = 5), SD: Standard Deviation, RSD: Relative Standard Deviation, #Acceptance criteria < 2.0.

No.	LLQC
1	43.7
2	47.0
3	52.1
4	50.9
5	45.0
Mean	47.7
SD	3.65
% CV	7.7
% MeanRecovery	95.5

Table 10. Stability data of metoprolol and meldonium at QCs in human plasma. °Back-calculated plasma concentrations; •Mean assayed concentration/mean assayed concentration at 0 hrs × 100. FT: Freeze-thaw, SD: Standard deviation, QC: Quality control.

Nominal concentration (ng/mL)	Stability	Mean±SD° (n=6)	Precision (% CV)
Metoprolol – 5.94	0 h	5.94±0.43	2.24
	7 h (bench-Top)	5.92±0.37	2.34
	22 h (in-injector)	5.91±0.39	2.46
	3 FT cycles	5.90±0.41	2.36
Meldonium – 141	0 h	141±0.33	3.09
	7 h (bench-Top)	141±0.25	2.55
	22 h (in-injector)	140±0.49	3.17
	3 FT cycles	140±0.39	2.17
Metoprolol –145	0 h	145±0.49	2.09
	7 h (bench-Top)	144±0.51	3.31
	22 h (in-injector)	144±0.42	2.31
	3 FT cycles	142±0.51	3.06
Meldonium – 3492	0 h	3492±0.51	3.12
	7 h (bench-Top)	3491±0.31	2.32
	22 h (in-injector)	3491±0.24	2.07
	3 FT cycles	3489±0.61	3.27

Stability

The predicted concentrations for metoprolol (5.94ng/mL and 145 ng/mL) and meldonium (141 ng/mL and 3492 ng/mL) deviated within ± 15% of the fresh sample concentrations in a battery of stability tests namely, in-injector (22 hrs), bench-top (7 hrs), and repeated four freeze/thaw cycles stability (Table 10).

The results were found to be within the assay variability limits during the entire process.

Conclusion

A highly sensitive, specific, reproducible, rapid and high-throughput LC-MS/MS assay was developed and validated to quantify metoprolol and meldonium in human plasma as per the regulatory guidelines. The present method involved a simple precipitation method of sample preparation, which gave consistent and reproducible recoveries.

Acquired results demonstrate that proposed strategy can be effortlessly and advantageously applied for routine examination of metoprolol and meldonium in human plasma. The combination was taken up for developing a bioanalytical method development and validation so that further it would be useful for performing pharmacokinetic studies.

Acknowledgements

Authors are grateful to the Ministry of Health of Ukraine Fund for providing scholarship for studies related to solutions fordevelopment of original combinations of antihypertensive agents, their analysis and standardization (№ 509 24.02.2020).

References

Albers S, Elshoff JP, Volker C, Richter A, Laer S (2005) HPLC quantification of metoprolol with solid-phase extraction for the drug monitoring of pediatric patients. Biomed Chromatography 19: 202–207. https://doi.org/10.1002/bmc.436

Angier MK, Lewis RJ, Chaturvedi AK, Canfield DV (2005) Gas chromatographic-mass spectrometric differentiation of atenolol, metoprolol, propranolol, and an interfering metabolite product of metoprolol. Journal of Analytical Toxicology 29: 517–521. https://doi.org/10.1093/jat/29.6.517

Braza AJ, Modamio P, Lastra CF, Marino EL (2002) Development, validation and analytical error function of two chromatographic methods with fluorometric detection for the determination of bisoprolol and metoprolol in human plasma. Biomed Chromatography 16: 517–522. https://doi.org/10.1002/bmc.195

FDA/CDER (2001) Guidance for Industry. Bioanalytical Method Validation. Food and Drug Administration/Center for Drug Evaluation and Research, 2001. http://www.fda.gov/cder/guidance/4252fnl. [accessed 28 June 2010]

EMEA/CHMP. Guideline on Validation of Bioanalytical Methods (Draft). EMEA/CHMP/EWP/192217/2009. European Medicines Agency/Committee for Medicinal Products for Human Use, 2009. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/12/WC500018062.pdf [accessed 19 November 2009]

European Pharmacopoeia (2016) European Pharmacopoeia (9th edn). https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition

Jensen BP, Sharp CF, Gardiner SJ, Begg EJ (2008) Development and validation of a stereoselective liquid chromatography tandem mass spectrometry assay for quantification of S and R-metoprolol in human plasma. Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences 865: 48–54. https://doi.org/10.1016/j.jchromb.2008.02.006

Gowda KV, Mandal U, Selvan PS, Solomon WDS, Ghosh A, Sarkar AK, et al. (2007) Liquid chromatography tandem mass spectrometry method for simultaneous determination of metoprolol tartrate and ramipril in human plasma. Journal of Chromatography. B, Analytical

technologies in the biomedical and life sciences 858: 13–21. https://doi.org/10.1016/j.jchromb.2007.07.047

Kondratova Y, Adebayo T, Logoyda L, Korobko D, Berdey I, Kuchmerovska T (2016) Development of the methodology of the chromatographic determination of amlodipine in medicines. International Journal of Applied Pharmaceutics 7(4): 32–35. https://doi.org/10.7897/2277-4343.074128

Kondratova Y, Logoyda L, Voloshko Y, Abdel-Megied A, Korobko D, Soroka Y (2017) Development and validation of HPLC-DAD method for the determination of bisoprolol in tablet dosage forms. International Journal of Applied Pharmaceutics 9(6): 54–59. https://doi.org/10.22159/ijap.2017v9i6.21616

Liliya L, Dmytro K, Olena S, Ihor B, Tamara K (2016) Development of Methodology for Identification of Captopril in Medicines. Asian Journal of Pharmaceutics 10(3): 168–171. https://www.asiapharmaceutics.info/index.php/ajp/article/view/723

Logoyda L (2018a) Bioanalytical method development and validation from the simultaneous determination of verapamil and enalapril in the present of enalaprilat by HPLC MS/MS. International Journal of Applied Pharmaceutics 10(3): 19–27. https://doi.org/10.22159/ijap.2018v10i4.24528

Logoyda L (2018b) A HPLC-MS/MS method development and validation for the simultaneous determination of nifedipine and enalapril in human plasma. International Journal of Applied Pharmaceutics 10(4): 35–42. https://doi.org/10.22159/ijap.2018v10i4.24528

Logoyda L (2018c) Bioanalytical method development and validation from the simultaneous determination of verapamil and enalapril in the present of enalaprilat by HPLC MS/MS. International Journal of Applied Pharmaceutics 10(3): 19–27. https://doi.org/10.22159/ijap.2018v10i4.24528

Logoyda L (2019) Analysis of approaches to the development and validation of the methods of analysis of some active pharmaceutical ingredients from the group of angiotensin converting enzyme inhibitors in drugs and biological liquids. International Journal of Applied Pharmaceutics 11(4): 1–7. https://doi.org/10.22159/ijap.2019v11i4.32420

Logoyda L, Kondratova Y, Korobko D, Susla O, Soroka Y, Tsytsiura R, Pidruchna S (2017a) Youden's test of the chromatographic determination of captopril in pharmaceuticals. International Journal of Green Pharmacy 11(3): 188–191. https://www.greenpharmacy.info/index.php/ijgp/article/view/1124

Logoyda L, Korobko D, Saprun S, Zarivna N (2017b) Development of methods for the chromatographic identification of active pharmaceutical ingredient from group of angiotensin-converting enzyme inhibitors in pharmaceuticals. International Journal of Green Pharmacy 11(4) Suppl.: 737–741. https://www.greenpharmacy.info/index.php/ijgp/article/view/1353

Logoyda L, Korobko D, Ivanusa I, Kovalenko S (2017c) Development of the methodology of the chromatographic determination of nifedipine in medicines. Asian J Pharm Clinical Res 10(3): 149–52. https://doi.org/10.22159/ajpcr.2017.v10i3.15841

Logoyda L, Abdel-Megied AM, Kondratova Y, Trofimenko O, Korobko D, Dakhym I (2018a) Development and validation of HPLC method for the simultaneous determination of enalapril maleate in present of their impurities: application to tablet analysis. International Journal of Applied Pharmaceutics 10(1): 98–102. https://doi.org/10.22159/ijap.2018v10i1.22805

Logoyda L, Korobko D, Oleshchuk O, Proniv T, Dmutriv M (2018b) A HPLC MS/MS method development and validation for the simultaneous determination of bisoprolol and enalapril in the present of enalaprilat in human plasma. International Journal of Applied Pharmaceutics 10(2): 31–40. https://doi.org/10.22159/ijap.2018v10i2.23195

Logoyda L, Mykhalkiv M, Polyauk O, Zarivna N, Soroka Y, Demydiak O (2018c) Ultra-high-performance liquid chromatography as assay method for the investigation of conditions of captopril extraction by organic solvents. Asian Journal of Pharmaceutics 12(1) Suppl.: 111–4. http://www.asiapharmaceutics.info/index.php/ajp/article/view/2049/0

Mykhalkiv M, Logoyda L, Polyauk O, Zarivna N, Soroka Y, Ryabokon S, Riabokon M (2018) HPLC as assay method for the investigation of conditions of bisoprolol extraction by organic solvents. International Journal of Green Pharmacy 12(1) Suppl.: 276–9. https://www.greenpharmacy.info/index.php/ijgp/article/view/1633

Mykhalkiv M, Logoyda L, Ivanusa I, Soroka Y, Yakubishyna I (2018) High-performance liquid chromatography as assay method for the investigation of conditions of enalapril maleate extraction by organic solvents. International Journal of Green Pharmacy 12(1): 62–65. https://www.greenpharmacy.info/index.php/ijgp/article/view/1525

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, Analytical technologies in the biomedical and life sciences 878, 551–556. https://doi.org/10.1016/j.jchromb.2009.12.030

The State Pharmacopeia of Ukraine [in 3 vol.] (2015) State Enterprise "Ukrainian Scientific Expert Pharmacopoeial Center of the Quality of Medicines" (2ndiss.). Kharkiv: State Enterprise "Ukrainian Scientific and Experimental Pharmacopoeial Center for the Quality of Medicinal Products", 1128 pp.

Zupanets IA, Pidpruzhnykov YV, Golovenko MJ, Bezugla NP, Borisjuk IY (2010) The study of trimethylhydrazinium propionate pharmacokinetic. Clininal Phamacy 14: 18–23. https://doi.org/10.1055/s-0029-1240873

Supplementary material 1

Supplementary data

Authors: Mariana Horyn, Liliya Logoyda

Data type: statistical data

Link: https://doi.org/10.3897/PlantSociology.67.50397.suppl1