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Research article / Оригинальная статья

Development, Validation and Approbation Analytical Method for the Quantitative Determination of Taurine by HPLC-UV Method in the Test of Comparative Dissolution Kinetics

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Abstract

Introduction. Taurine is a non-proteinogenic amino acid. The molecule is involved in lipid metabolism, adsorbs fat-soluble vitamins, and its conjugates with bile acids contribute to the emulsification of fats in the intestine. Drugs, which include a taurine molecule, have anti-cataract, cardiotonic, metabolic effects, and also stimulate regeneration. Among the dosage forms, where taurine acts as an active substance, there is a solid dosage form – film-coated tablets. One of the methods for assessing the quality of solid dosage forms is a comparative dissolution kinetics test. High-performance chromatography with ultraviolet detection is a widely used method for quantification within the dissolution test, however, for taurine, which does not contain chromophore groups in its structure, this method is not directly applicable. To solve this problem, one can apply the method of pre-column derivatization, because of which an fragment is introduced into the structure, providing a bathochromic shift in the UV spectrum of the starting compound.

Aim. Development, validation and approbation analytical method for the quantitative determination of taurine by high-performance chromatography with ultraviolet detection as part of a test comparative kinetics dissolution of taurine tablets with a dosage of 250 and 500 mg.

Materials and methods. The following preparations were used for the analysis: taurine tablets, film-coated 250 mg and 500 mg, domestic production with a valid expiration date. The comparative dissolution kinetics test was carried out on a DT 126 Light instrument for the "Dissolution" test (ERWEKA GmbH, Germany). Chromatographic separation and detection were performed on a Nexera-i LC-2040 high-performance liquid chromatograph (Shimadzu Corporation, Japan) equipped with a column and sample thermostat, a degasser, an autosampler, and an ultraviolet detector. Detection was carried out at a wavelength of 254 nm after derivatization of the taurine molecule with 4-toluenesulfonyl chloride. Were used a Shim-pack Velox C18 5 μm 4.6 × 150 mm column (Shimadzu Corporation, Japan) and a Shim-pack Velox C18 EXP Guard Column Cartridge 5 μm 4.6 × 5 mm (Shimadzu Corporation, Japan). Primary data were processed using LabSolutions Single LC software (Shimadzu Corporation, Japan).

Results and discussion. The optimal conditions for taurine derivatization have been selected, and a method for the quantitative determination of taurine by HPLC-UV in test comparative kinetics dissolution in three dissolution media: 0.1M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as in the quality control medium – purified water has been developed and validated. During the validation of the developed methodology, it was found that the validation characteristics are within the acceptance criteria in 4 dissolution media. The analytical range of the method was 0.05–1.2 mg/mL and allows the developed method to be used for the quantitative determination of tablets with a dosage of 250 mg and 500 mg as part of the test comparative kinetics dissolution. The method was tested in 4 dissolution media, in all media, there was a complete release in both dosages (more than 85 % by 30 minutes).

Conclusion. The method was tested in three dissolution media: 0.1 M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as in the quality control medium – purified water. In all media, there was a complete release in both dosages (more than 85 % by 30 minutes).

Keywords: comparative dissolution kinetics test, HPLC-UV, taurine, 4-toluenesulfonyl chloride, derivatization, validation

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Andrey M. Poluyanov and Igor E. Shohin invented and designed the experiment. Andrey M. Poluyanov, Adrian Kochug andLidia S. Mitrofanova analyze on HPLC-UV. Andrey M. Poluyanov, Adrian Kochug and Lidia S. Mitrofanova performed a comparative dissolution kinetics test. Andrey M. Poluyanov, Oleg Yu. Vergasov, Ilya D. Nikitin participated in data processing. Andrey M. Poluyanov, Ilya D. Nikitin and Oleg Yu. Vergasov participated in writing the text of the article. Elizaveta N. Fisher was the leader of this study. All authors participated in discussion of the results.

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Разработка, валидация и апробация аналитической методики количественного определения таурина методом ВЭЖХ-УФ в рамках проведения теста сравнительной кинетики растворения

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Резюме

Введение. Таурин является непротеиногенной аминокислотой. Молекула участвует в липидном обмене, адсорбирует жирорастворимые витамины, а его конъюгаты с желчными кислотами способствуют эмульгированию жиров в кишечнике. Лекарственные препараты, в состав которых входит молекула таурина обладают антикатарактным, кардиотоническим, метаболическим действием, а также стимулируют регенерацию. Среди лекарственных форм, где в качестве действующего вещества выступает таурин есть твердая лекарственная форма – таблетки, покрытые пленочной оболочкой. Одним из методов оценки качества твердых лекарственных форм является тест сравнительной кинетики растворения. Широко распространенным методом количественно определения в рамках теста растворения является высокоэффективная хроматография с ультрафиолетовым детектированием, однако для таурина, не содержащего хромофорных групп в своей структуре, этот метод на прямую не применим. Для решения данной проблемы можно применить метод предколоночной дериватизации, в результате которой в структуру вводится фрагмент, обеспечивающий батохромный сдвиг в УФ-спектре исходного соединения.

Цель. Разработка, валидация и апробация аналитической методики количественного определения таурина методом высокоэффективной хроматографии с ультрафиолетовым детектированием в рамках проведения теста сравнительной кинетики растворения таблеток таурина дозировкой 250 и 500 мг.

Материалы и методы. Для анализа использовались препараты: таурин таблетки, покрытые пленочной оболочкой 250 мг и 500 мг, отечественного производства с действующим сроком годности. Тест сравнительной кинетики растворения проводили на приборе для теста «Растворение» DT 126 Light (ERWEKA GmbH, Германия). Хроматографическое разделение и детектирование проводили на высокоэффективном жидкостном хроматографе Nexera-i LC-2040 (Shimadzu Corporation, Япония), оснащенном термостатом колонок и образцов, дегазатором, автосамплером и ультрафиолетовым детектором. Детектирование проводилось при длине волны 254 нм после дериватизации молекулы таурина 4-толуолсульфонилхлоридом. Использовали колонку Shim-pack Velox C18 5 μm 4.6 × 150 мм (Shimadzu Corporation, Япония) и предколонку Shim-pack Velox C18 EXP Guard Column Cartridge 5 μm 4.6 × 5 мм (Shimadzu Corporation, Япония). Обработку первичных данных проводили при помощи программного обеспечения LabSolutions Single LC (Shimadzu Corporation, Япония).

Результаты и обсуждение. Подобраны оптимальные условия дериватизации таурина, разработана и валидирована методика количественного определения таурина методом ВЭЖХ-УФ в рамках теста сравнительной кинетики растворения в трёх средах растворения: 0,1М раствор хлористоводородной кислоты с pH 1,2, ацетатный буферный раствор с pH 4,5, фосфатный буферный раствор с pH 6,8, а также в среде контроля качества – воде очищенной. При проведении валидации разработанной методики установлено, что валидационные характеристики находятся в пределах критериев приемлемости во всех средах растворения. Аналитический диапазон методики составил 0,05–1,2 мг/мл и позволяет применять разработанную методику для количественного определения в рамках теста сравнительной кинетики растворения таблеток с дозировкой 250 мг и 500 мг.

Заключение. Методика была апробирована в трех средах растворения: 0,1 М раствор хлористоводородной кислоты с pH 1,2, ацетатный буферный раствор с pH 4,5, фосфатный буферный раствор с pH 6,8, а также в среде контроля качества – воде очищенной. Во всех средах наблюдалось полное высвобождение у обеих дозировок (более 85 % к 30 минуте).

Ключевые слова: тест сравнительной кинетики растворения, ВЭЖХ-УФ, таурин, 4-толуолсульфонилхлорид, дериватизация, валидация

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. А. М. Полуянов и И. Е. Шохин придумали и разработали эксперимент. А. М. Полуянов, А. Кочуг и Л. С. Митрофанова провели исследование методом ВЭЖХ-УФ. А. М. Полуянов, А. Кочуг и Л. С. Митрофанова провели тест сравнительной кинетики растворения. А. М Полуянов, О. Ю. Вергасов, И. Д. Никитин участвовали в обработке данных. А. М. Полуянов, И. Д. Никитин и О. Ю. Вергасов участвовали в написании текста статьи. Е. Н. Фишер была руководителем данного исследования. В обсуждении результатов участвовали все авторы.

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INTRODUCTION

Currently, drugs based on amino acids and their derivatives are rather common, which is related to their wide range of pharmacological activity. In clinical practice, drugs of this group are prescribed for the treatment of atherosclerosis (histidine), cirrhosis of the liver (methionine), myocardial infarction and stroke (L-arginine), Parkinson's disease (bensarazide, L-DOPA) and a number of other pathological conditions[1].

One of the representatives of this class is taurine – β-aminosulfonic acid (Figure 1). This substance is involved in a number of physiological processes in the human body: from the regulation of digestion to the transmission of nerve impulses. Taurine is involved in lipid metabolism, adsorbs fat-soluble vitamins and is part of paired bile acids (taurocholic, taurodeoxycholic), which contribute to the emulsification of fats in the intestine [1]. The effect of taurine on the nervous system was found. As a glycine agonist, taurine reduces convulsive activity by binding to glycine receptors, i.e. it is a potential anticonvulsant [2]. During brain development, taurine influences cell migration, modulates neurotransmission at synapses and can accelerate brain development, in contrast to glutamic acid, GABA, and aspartic acid, elevated levels of which slow down brain development [3, 4]. Nowadays, the discussed aminosulfonic acid is used in the treatment of type 1 and type 2 diabetes mellitus, heart failure, cataracts and other diseases.

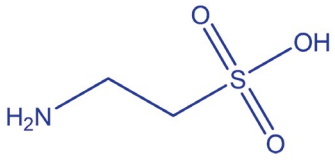


Figure 1. Structural formula of taurine

On the Russian market, there are drugs in five dosage forms, the active ingredient of which is taurine. One of them is a solid dosage form: film-coated tablets. Among the quality control tests of this dosage form is the comparative dissolution kinetics test (CDKT), which allows to evaluate the quality of the produced drug batches. In accordance with the regulatory documentation[2], taurine substance is quantified by alkalimetric titration, but this method is not applicable to CDKT for a number of reasons. First, dissolution is carried out in media, pH value of which is shifted from neutral. Secondly, titration cannot be automated and scaled in industrial environments.

HPLC-UV is a modern method for quantification of small molecules, but UV detection is only possible for molecules containing chromophore groups. Taurine is not one of them, but this problem can be solved by derivatizing the starting substance. For molecules containing an amino group, a tosylation reaction is possible, in which an arylsulfone fragment is introduced into the structure, providing a batochromic shift in the UV spectrum of the parent compound (Figure 2).

The aim of the study is to develop, validate and test an analytical method for the quantification of taurine by HPLC-UV as part of the comparative dissolution kinetics test of taurine tablets.

MATERIALS AND METHODS

Reagents and solutions

During the study, the following reagents were used: concentrated hydrochloric acid, chemically pure (Sigma Tech LLC, Russia); glacial acetic acid, RFE, USP, BP, Ph. Eur. (PanReac AppliChem, USA); concentrated orthophosphoric acid, chemically pure (Component-Reaktiv LLC, Russia); sodium hydroxide, AR grade (Component-Reaktiv LLC, Russia); sodium acetate trihydrate, chemically pure (JSC LenReaktiv, Russia); acetonitrile, HPLC-S gradient grade (Biosolve Chimie, France); anhydrous potassium dihydrogen phosphate, AR grade (Component-Reaktiv LLC, Russia); sodium dihydrogen phosphate monohydrate, chemically pure (Scharlab, Spain); anhydrous sodium hydrogen phosphate, AR grade (JSC LenReaktiv, Russia); boric acid, chemically pure (JSC LenReaktiv, Russia).

Excipients for the preparation of placebo solution were provided by the tablet manufacturer.

The objects of analysis were drugs: taurine film-coated tablets 250 mg and 500 mg, nationally produced with a valid shelf life.

Taurine, a powder substance was used as a reference sample, taurine content 99.3 %, Russia, batch XP19053171, valid until 07.05.2022.

The derivator was 4-toluosulfonyl chloride, powder substance, content of 4-toluo-sulfonyl chloride 99.9% (MNPK Biotiki LLC, Russia, batch S7240326, valid until 10.10.2023).

Dissolution media were prepared according to the Pharmacopoeia of the Eurasian Economic Union. Purified

[1] State Drug Register. Available at: https://grls.rosminzdrav.ru/ Accessed: 21.02.2023.

[2] State Pharmacopoeia of the Russian Federation, XIV edition, pharmacopeial monograph 2.1.0039.15 Taurine. Available at: https://docs.rucml.ru/feml/pharma/v14/vol3/1593/ Accessed: 21.02.2023.

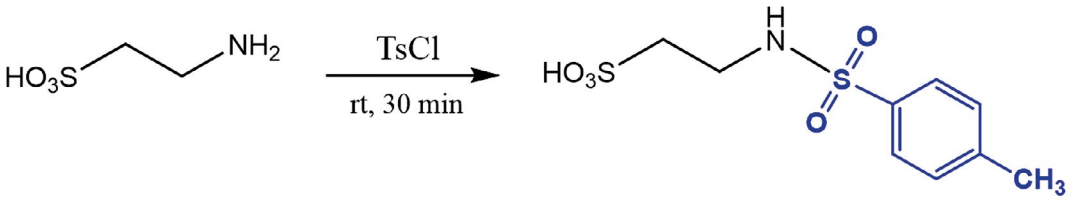


Figure 2. **Scheme of taurine derivatization with tosyl chloride**

water was used as a quality control medium (QC). All resulting dissolution media were filtered using a vacuum filtration system for liquids and degassed using a vacuum pump.

Equipment

The comparative dissolution kinetics test was carried out on the device for the dissolution test DT 126 Light (ERWEKA GmbH, Germany).

Chromatographic separation and detection were carried out on a high-performance liquid chromatograph Nexera-i LC-2040 (Shimadzu Corporation, Japan), equipped with a column and sample thermostat, a degasser, an autosampler and an ultraviolet detector. The Shim-pack Velox C18 5 µm 4.6 × 150 mm column (Shimadzu Corporation, Japan) and the Shim-pack Velox C18 EXP Guard Column Cartridge 5 µm 4.6 × 5 mm (Shimadzu Corporation, Japan) were used.

Source data were processed using LabSolutions Single LC software (Shimadzu Corporation, Japan).

Conditions of chromatographic separation and detection

Chromatographic column: Shim-pack Velox C18 5 µm 4.6 × 150 mm (Shimadzu Corporation, Japan).

Pre-column: Shim-pack Velox C18 EXP Guard Column Cartridge 5 µm 4.6 × 5 mm (Shimadzu Corporation, Japan).

Thermostat temperature: 40 °C.

Mobile phase: buffer solution pH 5.5 (eluent A); acetonitrile (eluent B).

Flow rate of the mobile phase: 1.0 ml/min.

Gradient of the composition of the mobile medium is shown in Figure 3.

Sample volume: 5 µl.

Retention time of taurine derivative: 3.3 min.

Run time: 7 minutes.

Detection: UV detector with an absorption wavelength of 254 ± 2 nm.

RESULTS AND DISCUSSION

Sample preparation

Substances interacting with amino acids to form chromophore-containing products were considered as potential derivators. These include 4-toluenesulfonyl chloride, o-phthalaldehyde and ninhydrin (Figure 4). The use of o-phthalaldehyde also requires the presence of 3-mercapto-propionic acid and expensive fluorenylmethoxycarbonyl chloride as reagents [5]. The key disadvantage of using ninhydrin as a derivative is the duration of the process. In addition, this reaction is carried out when heated [6]. In this study, 4-toluenesulfonyl chloride was selected as a derivator due to its high reactivity, low price and ability to form stable complexes with the taurine molecule [7].

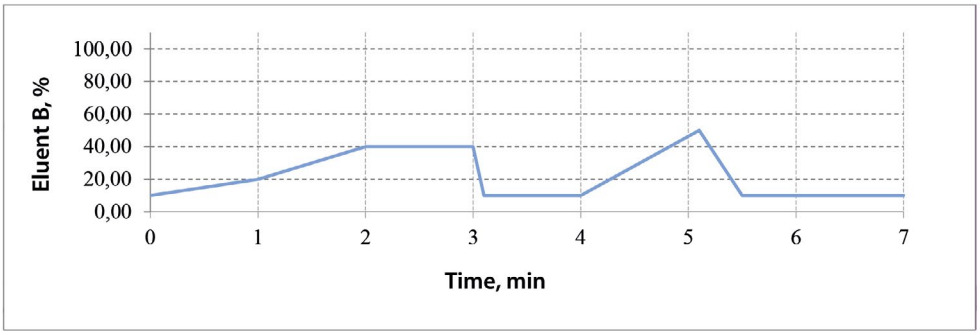


Figure 3. **Gradient of the composition of the mobile phase**

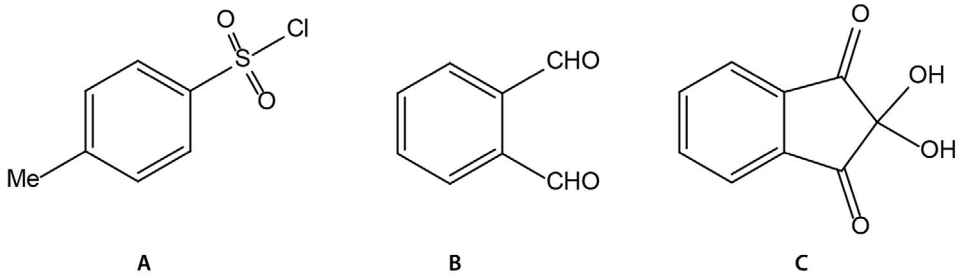


Figure 4. Structural formulas of derivatizers.
A – 4-toluenesulfonyl chloride; B – o-phthalaldehyde; C – ninhydrin

To maintain the optimal pH value, a borate buffer solution with a pH of 9.0 was used. It was experimentally confirmed that this buffer retained the pH value after adding each of the dissolution media used.

A 42.5 % solution of phosphoric acid was selected as a stop reagent, and the optimal volume added to the sample was chosen [8]. The concentration of the solution was selected in such a way that the shift of the pH value to the acidic direction to the pH value of 2.0 occurred when adding to the mixture: borate buffer solution – any of the dissolution media used.

Time and rate of solution centrifugation were determined after the addition of borate buffer solution, afer the addition of the derivator, as well as, after addition of the stop-reagent [9].

Development of a chromatographic method

Identity test and quantification were carried out with the method of high-performance liquid chromatography.

To detect the test substance, an ultraviolet detector with preliminary pre-column derivatization was used, which made it possible to detect the peaks of the taurine derivative due to the presence of chromophore groups in the structure of the derivator.

While the method was developed, based on the physicochemical properties of the substances to be determined, the chromatographic column Shim-pack Velox C18 5 μm 150 × 4.6 mm was selected (Table 1).

The conditions of chromatographic separation were selected experimentally, based on the articles [10], then elaborated to meet the necessary requirements, due to a number of particularities: low resolution between the peaks of the derivator and the derivative due to the close lipophilicity value, similar retention times of the substance to be determined and the non-sorbable components of the excipients of the dosage form, as well as the low elimination strength of the mobile phase in the isocratic mode, leading to gradual accumulation of the substance in the chromatographic column, which had a negative impact on the sensitivity and accuracy in the routine analysis of a large number of samples in CDKT.

Table 1. Physical and chemical properties

Properties	Values
Solubility in water, mg/ml	105
Log P (water – octanol)	–3,36
pKa (the most pronounced acid center)	1,5
pKa (the most pronounced main center)	9,34
pI (isoelectric point in solution)	5,12
The pH value of an aqueous solution of taurine	4,8–6,0

Method validation

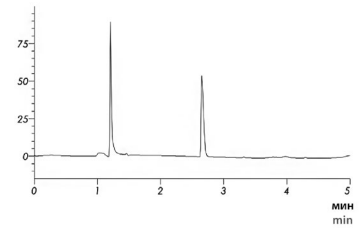
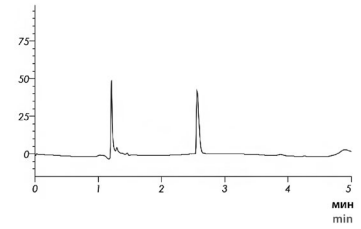
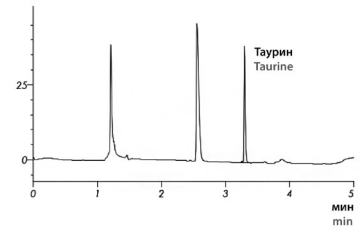
The method was validated in accordance with the Decision of the Council of the Eurasian Economic Commission dated 03.11.2016 № 85 "On approval of the Rules for conducting drug bioequivalence studies in the Eurasian Economic Union" Appendix 7, section 2, paragraph 20, validation of CDKT methods by to the following parameters: specificity, linearity, accuracy and precision, robustness, stability; as well as the Decision of the Council of the Eurasian Economic Commission dated July 17, 2018 № 113 “On Approval of the Guidelines for the Validation of Analytical Methods for Drug Testing”.

Specificity

To evaluate the specificity, model solutions, placebo solution, derivator solution, stop reagent solution, and taurine API solution were investigated. For each dissolution medium, the listed solutions were prepared.

It was established that on the chromatograms of solutions: placebo, dissolution media, stop reagent, derivator and their mixture, as well as a solution of taurine reference sample, there are no peaks with the retention time of the taurine derivatization product, and as a result, the method is specific. The obtained chromatograms are presented in Table 2.

Table 2. Type of chromatogram of standard samples indicating the retention time of components

Sample	Chromatogramm
Solution of placebo, derivatizer and stop reagent	
Solution of derivatizer and stop reagent	
Test solution with added derivatizer and stop reagent	

Linearity

Calibration and test solutions were prepared in three dissolution media: 0.1 M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as in a quality control medium – purified water, in three repetitions for 9 calibration levels, covering the range for two dosages of drugs at the level of 10, 20, 40, 100 and 120 % of the nominal taurine content in a tablet with a dosage of 250 and 500 mg.

A borate buffer solution was added to each solution, the samples were centrifuged for 3 minutes at a rate of 3000 rpm, then a derivator was introduced in the amount indicated in Table 3. At regular intervals after the addition of the derivator, a stop-reagent solution was added to each sample to stop the reaction.

During the validation, 4 analytical cycles were performed. The analytical cycle included calibration solutions and test solutions. A linear dependence was plotted for each of them (Figure 5).

The relative error (E, %) was calculated with the validated software for the work on a high-performance liquid chromatograph Shimadzu LC-2040C Plus with a UV detector (Shimadzu OpenLab, Japan). The relative error (E, %) of the calibration solution concentrations should be in the range from –15 to +15 % at the lower level, in the range from -10 to +10% for the remaining points. The data obtained are presented in Table 4.

In addition, the concentrations of taurine in calibration solutions were calculated using Excel tables, equations of linear dependences between the areas of the taurine peak and the concentration of its solu-

Table 3. Preparation of calibration solutions and test solutions of taurine

Calibration level	1	2	3	4	5	6	7	8	9
$V_{ref.}$, ml	1	2	4	8	10	12	16	20	24
Concentration, mg/ml	0,05	0,1	0,2	0,4	0,5	0,6	0,8	1	1,2
Concentration, % of nominal dosage 250 mg	10	20	40	80	100	120	–		
Concentration, % of nominal dosage 500 mg	–	10	20	40	50	60	80	100	120
Volume of borate buffer pH 9.0, µl	900								
Volume of modifying solution, µl	30								
Stop reagent volume, µl	15								
Placebo weight, mg	10	–		20	10/20	10	–		20
№ test solution	1	–		2	3/4	5	–		6

Table 4. **Calibration solutions for linearity estimation**

Calibration level	% of nominal dosage 250 mg	% of nominal dosage 500 mg	Nominal concentration, mg/ml	Calculated concentration, mg/ml				*E*, %				Acceptability criteria
				KK (QC)	pH 1,2	pH 4,5	pH 6,8	KK (QC)	pH 1,2	pH 4,5	pH 6,8	
1	10	–	0,05	0,049	0,045	0,047	0,05	–2,7	–9,7	–5,7	–0,2	No more than 15 %
2	20	10	0,1	0,10	0,10	0,10	0,10	–2,8	0,3	3,6	–4,1	
3	40	20	0,2	0,20	0,21	0,20	0,20	1	2,9	1,6	–4,8	
4	80	40	0,4	0,42	0,41	0,41	0,41	4,7	2	2,7	3,4	
5	100	–	0,5	0,48	0,51	0,52	0,51	–4,8	2	4,1	1,7	No more than 10 %
6	120	–	0,6	0,62	0,58	0,56	0,57	4	–3,6	–0,5	–4,5	
7	–	80	0,8	0,79	0,82	0,78	0,83	–1,1	2,3	–2,9	3,3	
8	–	100	1	0,98	0,96	0,98	1,02	–3,3	–4,1	–2	1,7	
9	–	120	1,2	1,23	1,23	1,22	1,18	2,1	2,1	1,8	–2	No more than 15 %

Note. *E*, % – relative error of determination. *E*, % – relative error of determination.

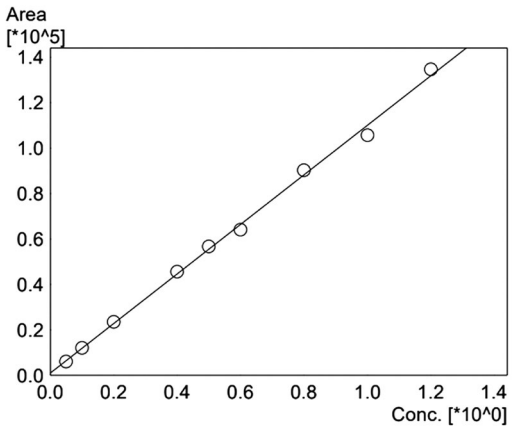


Figure 5. **Calibration graph of the ratio of the area of the taurine peak to the concentration**

tions were constructed, the values of the correlation coefficients of these dependencies, the tangent of the angle of inclination of the calibration curve, the length of Y-intercept, as well as the deviations of the concentration values calculated according to the equations from those introduced into these solutions, the calculations are shown in Table 5.

For all the studied cycles, the method linearity is satisfactory, since the value of the correlation coefficient of the obtained dependencies is greater than 0.99, and the amounts of taurine calculated according to the equations differ from those introduced by less than 10 % (less than 15 % for the lower limit of linearity).

Accuracy and precision

The method accuracy was evaluated based on the results of the analysis of test solutions containing certain (administered) amounts of taurine and placebo of the drug.

The same amount of placebo was added to each test solution, based on the nominal concentration of taurine in 1 tablet. Placebo tablets were prepared in accordance with the composition of the drug, grinding a mixture of auxiliary components in the ratio indicated in Table 6 in a porcelain mortar.

Three series of test solutions were prepared for each dosage, sequentially using one of the 4 dissolution media as a solvent.

The analysis of validation samples was carried out within 4 sequences of 6 samples for each level. Accuracy and precision were evaluated within the cycle (sequence 1) and between two cycles (sequences 1 and 2). For the obtained values of concentrations, the values of the relative standard deviation (RSD, %) and the average percentage of the method accuracy (Z_{mean}, %) given in Table 7 were calculated.

Table 5. **Linearity estimation**

Dissolution medium		Correlation coefficient (R)	Tangent of the slope of the calibration curve (k)	The length of the segment along the y-axis (b)	Linear equation $y = kx + b$
QC	Average value	0,9987	96121	5377	$y = 96121x + 5377$
	Calibration curve № 1	0,9986	95557	5595	$y = 95557x + 5595$
	Calibration curve № 2	0,9985	96425	5107	$y = 96425x + 5107$
	Calibration curve № 3	0,9985	96380	5429	$y = 96380x + 5429$
pH 1,2	Average value	0,9988	102339	–476	$y = 102339x - 476$
	Calibration curve № 1	0,9970	101448	–441	$y = 101448x - 476$
	Calibration curve № 2	0,9994	100992	–211	$y = 100992x - 211$
	Calibration curve № 3	0,9983	104577	–777	$y = 102339x - 777$
pH 4,5	Average value	0,9997	89172	3946	$y = 89172x + 3946$
	Calibration curve № 1	0,9994	88570	4351	$y = 88570x + 4351$
	Calibration curve № 2	0,9998	89017	3868	$y = 89017x + 3868$
	Calibration curve № 3	0,9996	89931	3618	$y = 89931x + 3618$
pH 6,8	Average value	0,9992	99698	4438	$y = 99698x + 4438$
	Calibration curve № 1	0,9989	99140	4483	$y = 99140x + 4483$
	Calibration curve № 2	0,9991	100008	4540	$y = 100008x + 4540$
	Calibration curve № 3	0,9994	99945	4291	$y = 99945x + 4291$

Table 6. **Composition of taurine tablets**

Component name	Quantity, mg	
	For 1 tablet with a dosage of 250 mg	For 1 tablet with a dosage of 500 mg
Taurine	250	500
Cellulose microcrystalline	23	46
Potato starch	18	36
Gelatin	6	12
Silicon dioxide colloidal (aerosil)	0,3	0,6
calcium stearate	2,7	5,4
Total, mg	300	600
Total, mg placebo	50	100

The results obtained suggest that the precision and accuracy of the analysis method is satisfactory, since the relative standard deviation for each of the studied taurine concentrations does not exceed the established value of 4 %, and the accuracy of the taurine derivative content does not exceed the range of 98.0–102.0 %.

Robustness

The robustness of the analytical method is evaluated by comparing solutions of known concentration analyzed with a deliberate small variation of three different analysis parameters. Table 8 shows the calculation of the effect of fluctuations in parameters such as: flow rate, column thermostat temperature and wavelength of the UV detector.

According to the results, the robustness of the analysis method is satisfactory, since the relative standard deviation for each of the studied parameters does not exceed the set value of 4 %.

Table 7. **Accuracy and precision of the methodology (inter-day, intra-day)**

Introduced mg/ml	Found (mg/ml), mean		$Z_{av.}$, %		
	inter-day	intra-day		inter-day	intra-day
Quality control medium (purified water)					
0,05	0,05	0,05	97,87	0,90	0,83
0,40	0,40	0,38	98,42	1,31	1,64
0,50	0,51	0,51	102,13	1,14	0,55
0,50	0,51	0,51	101,93	0,88	0,43
0,60	0,58	0,58	97,33	0,43	1,64
1,20	1,19	1,15	99,36	1,73	2,01
Hydrochloric acid solution pH 1.2					
0,05	0,05	0,05	102,00	0,73	2,90
0,40	0,41	0,42	101,92	0,90	2,03
0,50	0,51	0,51	102,07	0,54	0,24
0,50	0,49	0,47	97,80	0,65	1,85
0,60	0,59	0,58	98,39	2,03	1,81
1,20	1,20	1,19	100,28	0,80	1,54
Acetate buffer pH 4.5					
0,05	0,05	0,05	100,00	0,91	0,15
0,40	0,41	0,39	101,92	2,10	3,53
0,50	0,52	0,50	103,73	2,14	1,34
0,50	0,53	0,49	104,27	2,23	1,05
0,60	0,61	0,59	102,50	1,31	1,57
1,20	1,21	1,20	100,83	1,94	0,98
Phosphate buffer solution pH 6.8					
0,05	0,05	0,05	96,67	1,18	2,01
0,40	0,39	0,38	96,92	1,31	1,60
0,50	0,49	0,50	97,40	1,78	1,97
0,50	0,49	0,49	96,93	1,00	1,22
0,60	0,60	0,59	100,28	0,79	0,73
1,20	1,20	1,20	100,11	0,94	0,30

Note. $Z_{av.}$, % – openness. RSD, % – relative standard deviation.

Table 8. **Calculation of robustness**

Analysis parameters		Concentration, mg/ml			
		pH 1,2	pH 4,5	pH 6,8	KK
Flow rate ml/min	0,9	0,511	0,518	0,509	0,510
	1	0,501	0,503	0,507	0,502
	1,1	0,507	0,502	0,509	0,501
RSD, %		0,99	1,77	0,23	0,98
Column oven temperature, °C	38	0,503	0,506	0,501	0,499
	40	0,507	0,514	0,507	0,502
	41	0,519	0,518	0,509	0,511
RSD, %		1,63	1,19	0,82	1,24
Detector wavelength, nm	253	0,511	0,510	0,502	0,502
	254	0,507	0,503	0,501	0,506
	255	0,506	0,502	0,507	0,501
RSD, %		0,52	0,86	0,64	0,53

Stability

The stability of solutions was evaluated by the change in the concentrations of the analyzed solutions (or the corresponding peak areas), using for comparison samples of 100 % concentration level of the nominal value for a dosage of 250 mg 24 hours after their preparation at room temperature and when heated at 37 °C in a drying oven, which simulates the temperature control conditions of beakers in the dissolution tester. The results are presented in Table 9.

The relative standard deviation for the solutions did not exceed 5 %. According to the results obtained, the test solutions are stable for 24 hours after their preparation at room temperature and at temperature of 37 °C in a drying oven, which simulates heating in a dissolution tester.

The results of the validation confirm the suitability of the method for the analytical tasks set, namely: carrying out the CDKT in three dissolution media: 0.1 M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as purified water in a quality control medium. The analytical range of the method was 0.05–1.2 mg/ml.

Use of the developed method

On the basis of the developed method, CDKT of taurine tablets with a dosage of 250 and 500 mg was carried out. The resulting profiles are presented in figures 6–9.

Table 9. **Stability calculation**

Storage terms	Concentration, mg/ml	RSD, %
Dissolution medium purified water (quality control medium)		
Storage at 37 °C	0,515	0,4
	0,515	
Storage at room temperature	0,504	0,6
	0,505	
Dissolution medium 0.1 M hydrochloric acid pH 1.2		
Storage at 37 °C	0,518	0,7
	0,507	
Storage at room temperature	0,501	1,0
	0,499	
Dissolution medium Acetate buffer pH 4.5		
Storage at 37 °C	0,493	2,2
	0,499	
Storage at room temperature	0,501	1,6
	0,504	
Dissolution medium Phosphate buffer pH 6.8		
Storage at 37 °C	0,501	2,2
	0,507	
Storage at room temperature	0,489	1,4
	0,49	

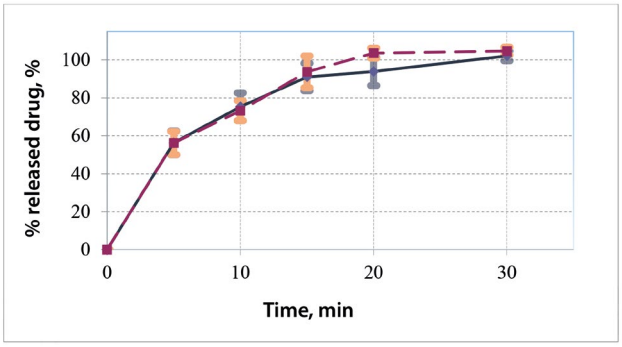


Figure 6. **Averaged profiles of Taurine dissolution in drug "Taurine, tablets, 250 mg" and "Taurine, tablets, 500 mg" in purified water (quality control medium).**

The graph displays the standard deviations (SD) of the DV release values at each time point. Blue color – dosage of 250 mg, red color – dosage of 500 mg

In all media, a complete release was observed at each dosage (greater than 85 % by 45 minutes). The profiles for dosages of 500 mg and 250 mg were of the same form.

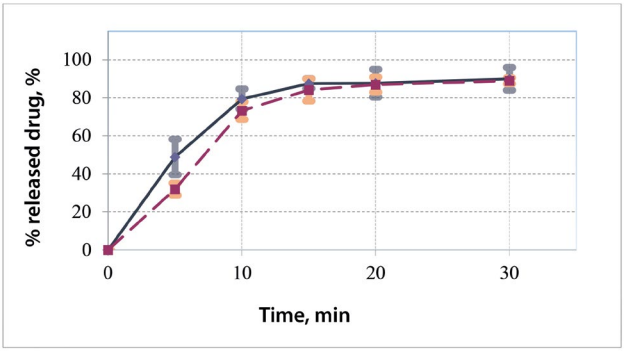


Figure 7. **Averaged profiles of Taurine dissolution in drug "Taurine, tablets, 250 mg" and "Taurine, tablets, 500 mg" in the medium of hydrochloric acid solution pH 1,2.**

The graph displays the standard deviations (SD) of the DV release values at each time point. Blue color – dosage of 250 mg, red color – dosage of 500 mg

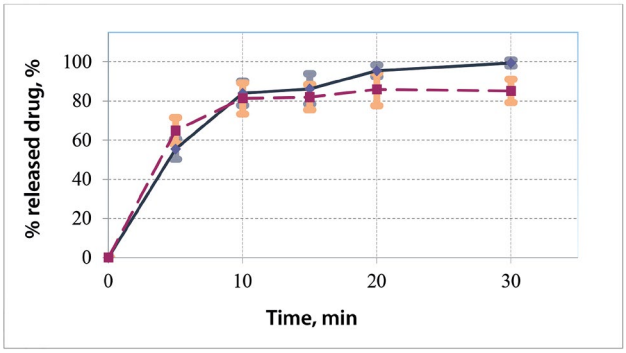


Figure 8. **Averaged profiles of Taurine dissolution in drug "Taurine, tablets, 250 mg" and "Taurine, tablets, 500 mg" in an acetate buffer solution pH 4.5.**

The graph displays the standard deviations (SD) of the DV release values at each time point. Blue color – dosage of 250 mg, red color – dosage of 500 mg

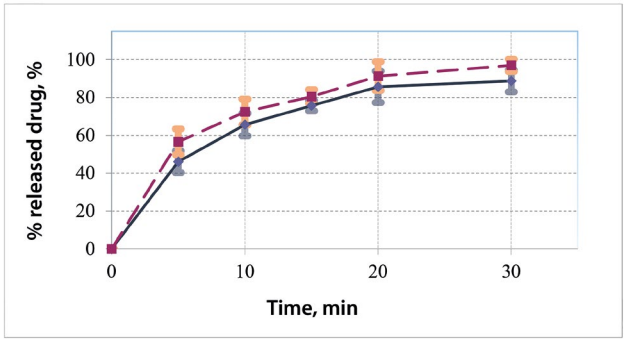


Figure 9. **Averaged profiles of Taurine dissolution in drug "Taurine, tablets, 250 mg" and "Taurine, tablets, 500 mg" in a medium of phosphate buffer solution pH 6.8.**

The graph displays the standard deviations (SD) of the DV release values at each time point. Blue color – dosage of 250 mg, red color – dosage of 500 mg

CONCLUSION

The optimal conditions for the derivatization of taurine were selected, a method for the quantification of taurine by HPLC-UV was developed and validated in CDKT in three dissolution media: 0.1 M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as in a quality control medium – purified water. During the validation of the developed method, it was found that the validation characteristics were within the acceptance criteria in three dissolution media: 0.1 M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as purified water in a quality control medium. The analytical range of the method is 0.05–1.2 mg/ml and allows the use of the developed method for the quantification of tablets with a dosage of 250 and 500 mg as part of CDKT.

The method was tested in CDKT in three dissolution media: 0.1 M hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5, phosphate buffer solution with pH 6.8, as well as in a quality control medium – purified water, complete release was observed in both dosages (more than 85 % by 30 minutes).

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