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THE METROLOGICAL PARAMETERS OF DIOSMIN QUANTITATION IN PHARMACEUTICAL DOSAGE FORMS

A.V. Voronin, M.N. Kachalkin, A.V. Karpov

Samara State Medical University, Samara, Russia

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▪ ***Introduction.*** Pharmaceutical dosage forms with diosmin are allowed for medical use in Russian Federation. Simple and informative methods of diosmin quantitation for drug quality control are needed.

Aim: determination of metrological parameters of diosmin quantitation in pharmaceutical dosage forms by UV-spectrophotometry.

Matherials and methods. The study subjects Venarus®, Detralex® (tablets, suspension), Phlebopha®. Diosmin were quantified by UV-spectrophotometry. The reference-specific absorbance values of diosmin at wavelengths of 268 and 370 nm by the parameters of calibration were determined. Statistical data processing was carried out by the methods of variation statistics, correlation, one-way analysis of variance using computer programs ChemMetr 1.0, ChemMetr Evaluation 1.0, Statistica 6.0 (Statsoft Inc., USA).

Results. The range of diosmin quantitation by UV-spectrophotometry was revealed for the wavelength of 268 nm — 0,0001-0,001%, 370 nm — 0,0002-0,002%. The reference-specific absorbance values for diosmin at the wavelength of 268 and 370 nm in a sodium hydroxide solution 0,02M were 463,0 ± 24,6 and 259,0 ± 9,9 respectively. The mean errors of diosmin concentrations in pharmaceutical dosage forms were revealed for the wavelength of 268 nm — 8-12% and for 370 nm — 6-8%. Prognostic calculation of the sample preparation error (extraction) for diosmin was performed using the example of Detralex® tablets. The sample preparation error was 8%.

Conclusion. The values components of error for reference-specific absorbance value and sample preparation error for diosmin quantitation were determined (as exemplified by the study of Detralex® tablets). Calculation algorithms can be used for error estimation of sample preparation for other multicomponent samples in drug quality control.

▪ **Keywords:** diosmin; UV-spectrophotometry; metrological parameters; quantitation; reference-specific absorbance value.

МЕТРОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ДИОСМИНА В ЛЕКАРСТВЕННЫХ ПРЕПАРАТАХ

А.В. Воронин, М.Н. Качалкин, А.В. Карпов

Федеральное государственное бюджетное образовательное учреждение высшего образования «Самарский государственный медицинский университет» Министерства здравоохранения Российской Федерации, Самара

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▪ ***Введение.*** В РФ зарегистрировано ряд лекарственных препаратов на основе фармацевтической субстанции диосмина. Для контроля качества лекарственных препаратов необходимы простые и информативные методы анализа.

Цель исследования — определение метрологических характеристик методики количественного определения диосмина методом спектрофотометрии в некоторых лекарственных препаратах.

Материалы и методы. Объектами исследования были лекарственные препараты на основе фармацевтической субстанции диосмина: «Венарус», «Детралекс» (таблетки, суспензия), «Флебофа». Для количественного определения использовали метод спектрофотометрии в УФ-диапазоне. Значения удельных показателей поглощения диосмина при длинах волн 268 и 370 нм определяли по параметрам градуировочных зависимостей. Статистическую обработку аналитических данных осуществляли методами вариационной статистики, корреляционного, однофакторного дисперсионного анализа с применением компьютерных программ ChemMetr 1.0, ChemMetr Evaluation 1.0, Statistica 6.0 (Statsoft Inc., USA).

Результаты. Рабочий диапазон методики спектрофотометрического определения диосмина составил для аналитических длин волн: 268 нм — 0,0001–0,001 %, 370 нм — 0,0002–0,002 %. Значения удельных показателей поглощения диосмина при длинах волн 268 и 370 нм в растворе натрия гидроксида концентрации 0,02 моль/л составили 463,0 ± 24,6 и 259,0 ± 9,9 соответственно. Величина относительной ошибки при определении среднего значения содержания диосмина в лекарственных препаратах находилась в диапазонах: 8–12 % — для аналитической длины волны 268 нм и 6–8 % — для 370 нм. На примере анализа таблеток «Детралекс» нами был выполнен прогностический расчет относительной ошибки (погрешности) степени извлечения диосмина (пробоподготовки), она составила 8 %.

Заключение. Установлены величины вкладов в относительную ошибку методики количественного определения удельных показателей поглощения димосмина, а также степени извлечения диосмина из анализируемых лекарственных форм (на примере анализа таблеток «Детралекс»). Расчетные алгоритмы могут использованы для теоретической оценки погрешности пробоподготовки для других многокомпонентных объектов анализа в контроле качества лекарственных средств.

▪ **Ключевые слова:** диосмин; УФ-спектрофотометрия; метрологические характеристики; количественное определение; удельный показатель поглощения.

Introduction

Several drugs based on the pharmaceutical substance diosmin (3′,5,7-trihydroxy-4′-methoxy flavone-7-rutinoside) have been registered in the Russian Federation [1]. The corresponding pharmacopoeial monographs propose a quantitative determination of the main active ingredient and specific impurities using high-performance liquid chromatography to control their quality.

An urgent issue is the analytical method development using simple methods of analysis that are available in routine practice, with acceptable metrological characteristics [2]. Spectrophotometry is used as an alternative variant of the quantitative analysis of diosmin [3].

This study aimed to determine the metrological characteristics of the method for the quantitative determination of diosmin by spectrophotometry in some drugs.

Materials and methods

The objects of the study were drugs based on the pharmaceutical substance diosmin, namely Venarus, Detralex (tablets and suspension), and Phlebopha.

A pharmacopoeial standard sample of diosmin (Hyderabad, India) was used as a comparison sample.

Sample preparation of the analyzed drugs was performed by extracting sodium hydroxide solution at a concentration of 0.02 mol/L. Ultraviolet spectrophotometry was used for the quantitative determination of diosmin. The values of specific absorption indices at wavelengths of 268 and 370 nm were determined based on the corresponding equation parameters of the calibration dependences.

Statistical processing of analytical data was performed using the methods of variation statistics, correlation, one-way analysis of variance using computer programs ChemMetr 1.0, ChemMetr Evaluation 1.0 [4, 5], and Statistica 6.0 (Statsoft Inc., USA) [6].

The theoretical value of the convergence limit *RSD_r* of the investigated quantitative determination methods was calculated according to the Horwitz equation [7]:

$$RSD\_r = 0.67 \cdot RSD\_R; \quad RSD\_R = 2^{1 - 0.5 \cdot \log C};$$
$$C = w/100;$$

where w is the concentration of the analyte in the sample, % (wt.); *RSD_R* is the reproducibility limit, %; and C is the concentration factor.

The theoretical value calculation of the standard deviation S_{av} and the relative error of the mean ε_{av}, based on the convergence limit *RSD_r* of the method was performed according to the equations:

$$S_{av} = (RSD\_r \cdot x_{av}) \cdot 100;$$
$$\Delta x = S_{av} \cdot t(P, f);$$
$$\varepsilon_{av} = (\Delta x / x_{av}) \cdot 100,$$

where x_{av} is the average value of the analyte content in the sample; $t(P, f)$ is the Student's t-test; and Δx is the half-width of the confidence interval of the analyte content mean value.

The calculation of the relative error ε_{av} of the method for quantitative analysis of diosmin was performed by the equation:

$$\varepsilon = \sqrt{\left(\frac{\sigma_{x1}}{x_1}\right)^2 + \left(\frac{\sigma_{x2}}{x_2}\right)^2 + \ldots + \left(\frac{\sigma_{xn}}{x_n}\right)^2},$$

where σ_{x1}, σ_{x2}, and σ_{xn} are the values of the absolute measurement errors of the individual stages of the quantitative determination procedure; x_1, x_2, and x_n are the values of the measured quantities during the implementation of the quantitative determination method.

Results and discussion

The choice of the extractant for sample preparation of the analyzed drugs was determined by the solubility of diosmin. Diosmin is practically insoluble in water and ethyl alcohol. The solubility of diosmin in sodium hydroxide solutions is due to the ionization of phenolic hydroxyl groups (formation of phenolates). Therefore, a sodium hydroxide solution at a concentration of 0.02 mol/L was used for the preparation of standard sample solutions of diosmin and its extraction from the analyzed drugs.

The technique working range with the linear nature of the optical density depending on the diosmin concentration was 0.0001%–0.001% and 0.0002%–0.002% for analytical wavelengths of 268 and 370 nm, respectively.

The specific absorption indices values of diosmin at wavelengths of 268 and 370 nm in a sodium hydroxide solution at a concentration of 0.02 mol/L were 463.0 ± 24.6 and 259.0 ± 9.9, respectively.

The relative errors of the above absorption indices, which were determined in five series of parallel determinations and affected the overall error of the determination results, were 5.3% and 3.8%.

The metrological characteristics of the method for the quantitative determination of diosmin by spectrophotometry are presented in Table 1.

Table 1 / Таблица 1

The metrological parameters of diosmin quantitation in pharmaceutical dosage form by means of UV-spectrophotometry
Метрологические характеристики методики количественного определения диосмина методом спектрофотометрии в некоторых лекарственных препаратах

Drug/Wavelength	μ, mg%	f	x_{av}, mg%	S^2	S	P	$t(P, f)$	Δx	ε, %	ε_{av}, %	δ, %
Detralex susp./268 nm	0.9	9	0.92	0.021	0.15	0.95	2.26	0.33	36.06	11.40	1.79
Detralex susp./370 nm	1.8	9	1.69	0.022	0.15	0.95	2.26	0.34	19.82	6.27	5.82
Venarus/268 nm	0.9	9	0.86	0.01	0.098	0.95	2.26	0.22	25.80	8.16	4.48
Venarus/370 nm	0.9	9	0.89	0.011	0.10	0.95	2.26	0.24	26.38	8.34	1.10
Detralex tabl./268 nm	0.9	9	0.92	0.015	0.12	0.95	2.26	0.28	30.06	9.51	2.09
Detralex tabl./370 nm	0.9	9	0.86	0.006	0.076	0.95	2.26	0.17	20.09	6.35	5.04
Phlebopha/268 nm	0.6	9	0.60	0.011	0.10	0.95	2.26	0.24	38.99	12.33	0.29
Phlebopha/370 nm	1.2	9	1.20	0.02	0.14	0.95	2.26	0.32	26.31	8.32	0.32

N o t e. μ: true (accepted reference) level of diosmin concentration in the pharmaceutical dosage form; x_{av}: average diosmin concentration in pharmaceutical dosage form (experimental value); f: degrees of freedom; S^2: dispersion; S: standard deviation; P: confidence level; Δx: confidence interval half-width of the average diosmin concentration; ε: relative single measurement error, %; ε_{av}: relative average value measurement error; δ: relative mean value bias of diosmin concentration in pharmaceutical dosage form from the true value of the concentration.

П р и м е ч а н и е. μ — истинное значение содержания диосмина в лекарственном препарате (принятое опорное значение); $x_{сред}$ — среднее значение содержания диосмина в лекарственном препарате, определенное экспериментальным путем; f — число степеней свободы; S^2 — дисперсия; S — стандартное отклонение; P — уровень доверительной вероятности; Δx — полуширина доверительного интервала среднего значения; ε — относительная ошибка (погрешность) единичного определения; $\varepsilon_{сред}$ — относительная ошибка (погрешность) среднего значения; δ — относительное отклонение среднего значения содержания диосмина в лекарственном препарате от истинного значения содержания (систематическая ошибка).

Table 2 / Таблица 2

Findings of variance analysis for comparison of the relative mean error and bias to quantitate diosmin in the drugs by UV-spectrophotometry

Результаты дисперсионного анализа для сравнения относительной ошибки среднего значения и систематической ошибки при определении диосмина в лекарственных препаратах методом спектрофотометрии

Parameters	Total sample variance SS_{tot}	Effect variance MS_{ef}	Intergroup variance SS	Error variance MS_{err}	F-criterion	Significance level p
$\varepsilon_{av\ 268\ nm} / \varepsilon_{av\ 370\ nm}$	18.36	18.36	14.60	2.43	7.54	0.033
$\delta_{268\ nm} / \delta_{370\ nm}$	1.65	1.65	31.91	5.32	0.31	0.60

N o t e. ε_{av}: relative error of average value; δ: relative deviation (bias) of diosmin average concentration in pharmaceutical dosage form from the true value of the concentration.

П р и м е ч а н и е. $\varepsilon_{сред}$ — относительная ошибка (погрешность) среднего значения; δ — относительное отклонение среднего значения содержания диосмина в лекарственном препарате от истинного значения содержания (систематическая ошибка).

A significant difference was noted in the relative error of the mean value at two analytical wavelengths for all analyzed drugs, so that the value of the determination error at a wavelength of 268 nm was statistically significantly greater than that of 370 nm, except for the Venarus.

The relative error in determining the average value of the diosmin concentration was within 8%–12% for the analytical wavelength of 268 nm and 6%–8% for 370 nm.

The results of one-way analysis of variance confirm the presence of statistically significant differences for the parameter "relative error of the mean value of the diosmin concentration in the pharmaceutical dosage form," and the significance level of the F-criterion was <0.05. The bias of determinations did not significantly differ at analytical wavelengths of 268 nm and 370 nm (Table 2).

The analysis of the correlation dependences "relative error of the mean" and "systematic error" in the sample of results combined for two analytical wavelengths show the presence of a high relationship, as the value of the correlation coefficient r is −0.73.

The above dependencies, calculated for analytical wavelengths of 268 nm and 370 nm, are characterized by a very high relationship, with the values of the correlation coefficients r of −0.94

Table 3 / Таблица 3

The error evaluation of diosmin quantitation in Detralex® tablets using UV-spectrophotometry

Метрологическая оценка ошибки методики количественного определения диосмина в таблетках «Детралекс» методом спектрофотометрии

No.	Analysis stage	Absolute error σ_x	Relative error $(\sigma_x/x) \cdot 100$, %
1	Taking an accurate sample of 1.0 g	0.0002 g	0.020
2	Measuring the volume of 250 ml (volumetric flask)	0.3 ml	0.12
3	Dispensing a 1 ml aliquot (pipette)	0.01 ml	1.00
4	Measuring the volume of 500 ml (volumetric flask)	0.5 ml	0.10
5	Measurement of optical density at 268 nm (spectrophotometer SF-2000)	0.004	0.93
6	Error of the specific absorption index value, 268 nm	–	5.30
7	Calculated diosmin recovery: ChemMetr Evaluation 1.0 program/Horwitz equation	–	7.95/16.60
Calculated relative error ε_{av}, % (excluding the sample preparation relative error, ChemMetr Evaluation 1.0 program)			5.2
Calculated convergence limit *RSD_r*, % (Horwitz equation)			7.7
Calculated relative error ε_{av}, % (Horwitz equation)			17.4
Relative error ε_{av}, %, determined during the experiment			9.5

and –0.98, respectively. Therefore, an increased relative error of the diosmin content mean value in drugs is associated with an increased contribution of random errors, including errors in sample preparation (varying the degree of diosmin extraction). The magnitude of the systematic determination error does not depend on the wavelength used by the analyst.

A predictive calculation of the relative error of diosmin extraction degree (sample preparation) was performed using the example of the analysis of Detralex tablets, which was based on the following principles:

- considering the minimum levels of errors at each stage of the analysis, except for the extraction of diosmin from a weighed sample of tablets;
- availability of experimental data on the value of the total relative error in determining diosmin;
- square additivity of the relative errors of individual stages of the analysis (Table 3).

The relative sample preparation stage error, namely the degree of extraction of diosmin from Detralex tablets was 8%. The variant of calculating the total relative error of analysis according to the algorithm based on the Horwitz equation reached 17%.

Regardless of the used prognostic calculation variant, the stage of sample preparation makes the greatest contribution to the error of the method of spectrophotometric determination of diosmin in Detralex tablets.

Conclusion

Therefore, as a research result, the metrological characteristics of the method for the quantitative determination of diosmin by spectrophotometry in a number of drugs were determined. The contribution values to the relative error of the specific absorption technique indices of diosmin at analytical wavelengths of 268 and 370 nm, as well as the extraction degree of diosmin from the analyzed dosage forms (for example, Detralex tablets analysis), have been established. The proposed computational algorithms can be implemented for the theoretical assessment of the sample preparation error for other multi-component objects of analysis in the quality control of medicines.

The authors declare no conflict of interest.

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Information about the authors

Alexander V. Voronin — Candidate of Pharmaceuticals Sciences, Associate Professor, Head of the Department of Chemistry of Pharmaceutical Faculty. Samara State Medical University, Samara, Russia. E-mail: dimmu2000@mail.ru.

Maksim N. Kachalkin — Assistant of the Department of Chemistry of Pharmaceutical Faculty. Samara State Medical University, Samara, Russia. E-mail: maxroso@yandex.ru.

Alexander V. Karpov — Student of Pharmaceutical Faculty. Samara State Medical University, Samara, Russia. E-mail: karpov.sasha111@gmail.com.

Информация об авторах

Александр Васильевич Воронин — кандидат фармацевтических наук, доцент, заведующий кафедрой химии фармацевтического факультета. ФГБОУ ВО «Самарский государственный медицинский университет» Минздрава России, Самара. E-mail: dimmu2000@mail.ru.

Максим Николаевич Качалкин — ассистент кафедры химии фармацевтического факультета. ФГБОУ ВО «Самарский государственный медицинский университет» Минздрава России, Самара. E-mail: maxroso@yandex.ru.

Александр Вячеславович Карпов — студент фармацевтического факультета. ФГБОУ ВО «Самарский государственный медицинский университет» Минздрава России, Самара. E-mail: karpov.sasha111@gmail.com.