Tonkie Khimicheskie Tekhnologii = Fine Chemical Technologies.
2025;20(3):276–288 ISSN 2686-7575 (Online)

Analytical methods in chemistry and chemical technology
Аналитические методы в химии и химической технологии

UDC 615.072
https://doi.org/10.32362/2410-6593-2025-20-3-276-288
EDN DNWEEB

RESEARCH ARTICLE

Quantitative determination of 8-methoxypsoralene in mild dosage form by high-performance liquid chromatography

Adnan Alsayed, Anastasiya A. Prezhedromirskaya✉, Elizaveta A. Shnyak, Stanislav A. Kedik

MIREA – Russian Technological University (M.V. Lomonosov Institute of Fine Chemical Technologies), Moscow, 119454 Russia

✉ *Corresponding author, e-mail: a.a.pregedromirskaya@ipt.ru.com*

Abstract

Objectives. To develop and validate a method for the quantitative determination of 8-methoxypsoralen in a soft dosage form in accordance with the requirements of the State Pharmacopoeia of the Russian Federation, 15th edition, and the Pharmacopoeia of the Eurasian Economic Union.

Methods. Quantitative determination of 8-methoxypsoralen was performed by high-performance liquid chromatography on a Chromaster 5000 (*Hitachi*, Japan) with a diode array detector. Chromatography was performed on a Kromasil EternityXT-5-C18, 5 μm, 250 × 4.6 mm column in isocratic mode with a mobile phase of acetonitrile/water in a ratio of 50 : 50% (*v*/*v*). The flow rate was 1.0 mL/min, while the detection wavelength was 250 nm.

Results. The optimal condition for the extraction of 8-methoxypsoralen was found to be ultrasonic gel extraction at 40°C for 15 min using acetonitrile. The best peak resolution of 8-methoxypsoralen was achieved during gel analysis at 250 nm using a reversed-phase sorbent with an octadecyl phase (C18) grafted onto silica gel. The acetonitrile/water mixture was used as a mobile phase in a volume ratio of 50 : 50% to minimize chromatography time while maintaining optimal resolution. From the validation procedures, it was confirmed that the method is specific, linear ($R^2 > 0.997$) and reproducible (relative standard deviation was ≤3.0%). The accuracy of the analytical method was from 98.26% to 101.02%, while the values of the detection and quantitative determination limits were 0.006 and 0.020 μg/mL, respectively. The developed quantitative determination method demonstrated its stability when varying as the column temperature and flow rate by ±5%.

Conclusions. As effectively implemented using the high-performance liquid chromatography method, the method for quantitative determination of 8-methoxypsoralen has a number of advantages over the previously described methods, including reduced analysis time, as well as increased sensitivity and effectiveness, which makes it possible to apply the developed method in assessing the quantitative content of 8-methoxypsoralen in a soft dosage form—gel for the treatment of psoriasis.

Keywords

8-methoxypsoralen, methoxalen, 8-MOP, mild dosage form, gel, quantitative determination, high-performance liquid chromatography, validation

Submitted: 31.10.2024
Revised: 15.01.2025
Accepted: 03.04.2025

For citation

Alsayed A., Prezhedromirskaya A.A., Shnyak E.A., Kedik S.A. Quantitative determination of 8-methoxypsoralene in mild dosage form by high-performance liquid chromatography. *Tonk. Khim. Tekhnol. = Fine Chem. Technol.* 2025;20(3):276–288. https://doi.org/10.32362/2410-6593-2025-20-3-276-288

НАУЧНАЯ СТАТЬЯ

Количественное определение 8-метоксипсоралена в мягкой лекарственной форме методом высокоэффективной жидкостной хроматографии

А. Алсайед, А.А. Прежедромирская✉, Е.А. Шняк, С.А. Кедик

МИРЭА – Российский технологический университет (Институт тонких химических технологий им. М.В. Ломоносова), Москва, 119454 Россия

✉ *Автор для переписки, e-mail: a.a.pregedromirskaya@ipt.ru.com*

Аннотация

Цель. Разработка и валидация методики количественного определения 8-метоксипсоралена в мягкой лекарственной форме в соответствии с требованиями Государственной Фармакопеи Российской Федерации XV издания и Фармакопеи Евразийского экономического союза.

Методы. Количественное определение 8-метоксипсоралена проводили методом высокоэффективной жидкостной хроматографии на приборе «Chromaster 5000» (*Hitachi*, Япония) с диодно-матричным детектором. Хроматографирование выполняли на колонке Kromasil EternityXT-5-C18, 5 мкм, 250 × 4.6 мм в изократическом режиме с подвижной фазой ацетонитрил/вода в соотношении 50 : 50% (об/об). Скорость потока составляла 1.0 мл/мин, длина волны детектирования — 250 нм.

Результаты. Установлено, что экстракция активного вещества из геля под действием ультразвука при температуре 40°C в течение 15 мин с использованием ацетонитрила является наиболее оптимальным условием для извлечения 8-метоксипсоралена. Наилучшее пиковое разрешение 8-метоксипсоралена было достигнуто при анализе геля на длине волны 250 нм с помощью обращенно-фазового сорбента с октадецильной фазой (C_{18}), привитой к силикагелю. Использование в качестве подвижной фазы смеси ацетонитрил/вода в объемном соотношении 50 : 50% позволило обеспечить минимальное время хроматографирования при сохранении оптимального разрешения. По данным валидационных процедур уставлено, что методика специфична, линейна ($R^2 > 0.997$) и воспроизводима (относительное стандартное отклонение составило ≤ 3.0%). Точность аналитической методики составила от 98.26% до 101.02%, а значения пределов обнаружения и количественного определения — 0.006 и 0.020 мкг/мл соответственно. Разработанная методика количественного определения показала свою устойчивость при варьировании как температуры колонки, так и скорости потока на ±5%.

Выводы. Методика количественного определения 8-метоксипсоралена была эффективно реализована с использованием метода высокоэффективной жидкостной хроматографии и обладает рядом преимуществ по сравнению с ранее описанными методиками. Эти преимущества заключаются в сокращении времени анализа, увеличении чувствительности и эффективности, что позволяет применять разработанную методику для оценки количественного содержания 8-метоксипсоралена в мягкой лекарственной форме — геле при лечении псориаза.

Ключевые слова

8-метоксипсорален, метоксален, 8-МОП, мягкая лекарственная форма, гель, количественное определение, высокоэффективная жидкостная хроматография, валидация

Поступила: 31.10.2024
Доработана: 15.01.2025
Принята в печать: 03.04.2025

Для цитирования

Алсайед А., Прежедромирская А.А., Шняк Е.А., Кедик С.А. Количественное определение 8-метоксипсоралена в мягкой лекарственной форме методом высокоэффективной жидкостной хроматографии. *Тонкие химические технологии*. 2025;20(3):276–288. https://doi.org/10.32362/2410-6593-2025-20-3-276-288

INTRODUCTION

Psoralenes are natural furanocoumarins found in medicinal plants such as *Psoralea corylifolia* L., *Ficus carica* L. and *Ficus petiolaris* L., *Ammi majus* L., and *Heracleum sosnowskyi* L. They have found wide application in photochemotherapy (Psoralen UltraViolet A (PUVA)), which uses psoralen as a photosensitizer combined with ultraviolet radiation in the 320–400 nm wavelength region [1]. Indications for phototherapy include epidermal diseases such as atopic dermatitis [2], psoriasis [3], vitiligo [4], photodermatoses, mycosis fungoides [5], and diseases due to deep cutaneous lesions (e.g., scleroderma).

The most commonly used photosensitizer when taking this approach is 8-methoxypsoralen (8-MOP). Considered one of the best generators of singlet oxygen and superoxide radicals among psoralens [6], 8-MOP is activated by ultraviolet radiation (UV) to form pyrimidine compounds inside cells (Fig. 1). After intercalating one psoralen molecule into the DNA double strand, one photon of light is absorbed under UV irradiation, followed by binding of a thymine base and absorption of an additional photon of light, binding of another thymine base, and so on. DNA-psoralen cross-linking inhibits DNA replication and causes cell cycle arrest [7]. This induces a number of antipolyferative, antiangiogenic, apoptotic and immunosuppressive effects [8].

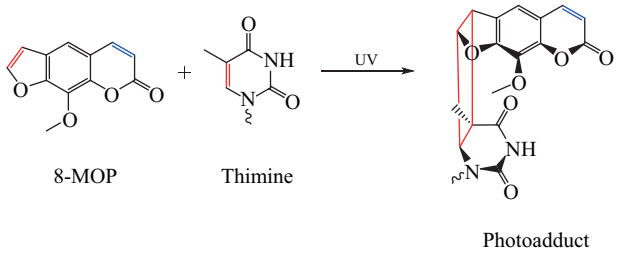

Fig. 1. Chemical structure of 8-MOP and its photoaddition to DNA

In studies comparing the efficacy of treatment methods, oral administration of PUVA solution was found to be more effective than parenteral administration [9]. However, gastrointestinal side effects, psychiatric disorders, optic nerve damage, and increased risk of melanoma and squamous cell cancer are possible [10]. Furthermore, 8-MOP is virtually insoluble in water and thus exhibits uneven absorption from the gastrointestinal tract, including inter-subject variability in plasma concentration [11]. Hence, the proposed topical therapy of 8-MOP is a more effective approach to enhance the bioavailability of the drug.

Various delivery systems designed to ensure the required level of resorption of 8-MOP, such as niosomes [12], nanoemulsions [13], microemulsions [14], and solid lipid nanoparticles [15], have been previously prepared and investigated to improve the transdermal penetration of 8-MOP. Conventional dosage forms such as ointments, creams and gels can be used as a carrier for 8-MOP nanosystems for topical application.

In addition to the necessity for research on the selection of drug delivery systems, it is important to develop and validate analytical techniques for detecting and quantifying 8-MOP content in soft dosage forms. High performance liquid chromatography (HPLC) equipped with a spectrophotometric detector, such as the Diode Array Detector (DAD), is one of the most commonly used quantification methods due to its versatility and ease of use [16, 17].

HPLC, namely its reversed-phase variation, is the simplest and most sensitive method for the quantification of 8-MOP, based on the peculiarities of its structure and physicochemical properties. Various parameters for instrumental analysis of 8-MOP have been described in the literature. Pitzanti *et al.* used a chromatograph with fluorescence detector at wavelengths of 317 and 445 nm. The analysis was an isocratic elution on an X Terra RP18 column (3.5 µm, 4.6 × 100 mm, *Waters*, USA). The mobile phase used was water, methanol and acetonitrile in a volume ratio of 40 : 40 : 20. Mahmoud *et al.* performed detection using UV detector to determine the 8-MOP content [18]. The researchers selected the conditions for the determination of 8-MOP on ACE® C18 column (5 µm, 4.6 × 150 mm, *Advanced Chromatography Technologies*, United Kingdom) in isocratic elution mode with methanol/water mobile phase in the volume ratio of 60 : 40. Detection was performed at a wavelength of 300 nm. Ageev *et al.* proposed a technique using a spectrophotometric detector [19]. This approach used a Symmetry Shield C18-RP column (5 µm, 250 × 4.6 mm, *Waters*, USA) with a mobile phase consisting of phosphate buffer with pH 5.6 and acetonitrile in a volume ratio of 50 : 50, and detection was performed at a wavelength of 285 nm. Kulikov *et al*. modified the previously described technique and used an acetonitrile/water system in a volume ratio of 50 : 50 as the mobile phase [20]. Barradas *et al*. used a chromatograph with UV detector and a NovaPak C18 column (150 × 3.9 mm, *Waters*, USA) [13]. The mobile phase was water and methanol in the ratio of 65 : 35. Detection was performed at a wavelength of 300 nm.

These methods have a number of disadvantages: the use of salt buffers in the mobile phase can lead to an increase in the working pressure of the equipment

and, accordingly, additional efforts to maintain the performance of the device. An additional disadvantage pertains to the use of methanol, which belongs to the group of particularly dangerous poisons and is under strict control and accounting. For this reason, the development of new analytical techniques for the determination of 8-MOP is still an urgent task[1].

Thus, the aim of the present study the development and validation of a new, more accurate, reproducible, selective, stable, highly sensitive methodology for the determination of the quantitative content of 8-MOP in a soft dosage form of the gel used in psoriasis therapy. In this case, the analytical methodology and validation procedures was carried out in accordance with the guidelines of good manufacturing practice and in compliance with the recommendations of the rules of production and quality control of medicines[2] and the 15th Edition of the State Pharmacopoeia of the Russian Federation[3].

MATERIALS AND METHODS

Reagents and materials

In the study to determine the quantitative content of 8-MOP in soft dosage form, 8-MOP substance (*Henan Tianfu Chemical Co.*, China) was used, as well as the following reagents: acetonitrile (highest purity, *Cryochrom*, Russia), water for chromatography (deionized water with electrical conductivity >0.18 mOhm/m).

The object of the study was a laboratory sample in the form of a gel with 8-MOP (hereinafter, "the gel"), whose composition is presented in Table 1.

Equipment

For sample preparation, Pioneer PA214C electronic analytical scales (China) were used. Preparation of water for chromatography was carried out using a Vodoley-M water deionizer (*Khimelektronika*, Russia). The studied samples were prepared using an ultrasonic bath (*Sapfir*, Russia). The study was carried out using a Chromaster 5000 chromatograph (*Hitachi*, Japan), equipped with a PUMP Chromaster 5160 universal pump module, 5430 Diode Array detector, 5310 Column Oven column thermostat and automatic dosing device, 5260 Autosampler. Control and data processing were carried out using the MultiChrome version 3.4 software[4].

Statistical processing of the results was carried out in accordance with General Pharmacopoeia Article (GPA) 1.1.0013.15 "Statistical processing of chemical experiment results"[5] using Microsoft Office Excel 2016 software.

Chromatography conditions

The chromatographic analysis conditions and chromatographic system suitability requirements are presented in Table 2.

Table 1. Composition of gel with 8-MOP

Components	Manufacturer	Concentration, wt %/wt
8-Methoxypsoralen	*Henan Tianfu Chemical Co*., China	0.67
Clove oil	*Naturalnye masla*, Russia	7.95
Pluronic F68	*Sigma-Aldrich*, USA	1.06
Hydroxyethyl cellulose 250 HHX	Natrosol™ 250 G PHARM, *Ashland*, USA	1.4
Purified water (PA.2.2.0020)	–	88.92

1 https://regulation.eaeunion.org/upload/iblock/4ec/jsw9jphfi1xvwlf9vt4otsb8y2lz5322/ria_30062017_mdoc.pdf/. Accessed March 11, 2025.

2 https://meganorm.ru/Data2/1/4293828/4293828749.pdf/. Accessed March 11, 2025.

3 https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/validatsiya-analiticheskikh-metodik/. Accessed March 11, 2025.

4 https://multichrom.ru/documentation/manuals/. Accessed March 11, 2025.

5 https://pharmacopoeia.ru/wp-content/uploads/2016/11/OFS.1.1.0013.15-Statisticheskaya-obrabotka-rezultatov-eksperimenta.pdf. Accessed March 11, 2025.

Table 2. Chromatographic parameters

Parameter	Value
Column	Kromasil EternityXT-5-C18, 5 μm, 250 × 4.6 mm, (*Nouryon*, No. X05CLA25)
Elution	Isocratic
Mobile phase	Acetonitrile : water (50 : 50% *v/v)*
Flow rate	1.0 mL/min
Temperature of column	25°C
Detection wavelength	250 nm
Injection volume	20 μL
Run time	10 min
System suitability requirements Number of theoretical plates (*N*) Relative standard deviation (RSD) Asymmetry Factor (As)	 At least 5000 At least 3.0% 0.8 < As < 1.5

Sample preparation methods

8-MOP reference standard sample solution (0.67 mg/mL)

67.0 mg (exact weighing) of 8-MOP substance was placed in a 100-mL volumetric flask, dissolved in acetonitrile, brought to the mark with the same solvent, and mixed.

8-MOP standard sample solution (0.067 mg/mL)

1.0 mL of the initial solution of 8-MOP standard sample was taken into a 10-mL volumetric flask, dissolved in acetonitrile, brought to the mark with the same solvent, and mixed. The resulting standard sample solution was filtered through a 33-mm Millipore Millex-HN Nylon 0.45 μm syringe filter (*Merck Millipore*, Germany) and transferred to a chromatographic vial.

Gel solution (10 mg/mL)

1.0 g (exact weighing) of gel was placed in a 100-mL measuring flask, 85 mL of acetonitrile was added; following treatment with ultrasound for 30 min, the volume of the solution was brought to the mark with acetonitrile. The resulting solution was filtered using a 33-mm Millipore Millex-HN Nylon 0.45 μm syringe filter and transferred to a chromatography vial.

Course of analysis

To determine the quantitative content of 8-MOP in the gel, sequential chromatographic analysis of the working solution of 8-MOP standard sample (at least 5 times) and the gel solution injected in triplicate was carried out.

Calculations

The content of 8-MOP ($C_{8\text{-MOP}}$, mg/g) in the soft dosage form was determined according to formula (1).

$$C_{8\text{-MOP}} = \frac{S_{gel} \cdot a_{8\text{-MOP}} \cdot V_{st.s.} \cdot 100 \cdot P}{S_{8\text{-MOP}} \cdot 100 \cdot 10 \cdot a_{gel} \cdot 100} = \frac{S_{gel} \cdot a_{8\text{-MOP}} \cdot 1 \cdot 100 \cdot P}{S_{8\text{-MOP}} \cdot 100 \cdot 10 \cdot a_{gel} \cdot 100} = \frac{S_{gel} \cdot a_{8\text{-MOP}} \cdot P}{S_{8\text{-MOP}} \cdot a_{gel} \cdot 1000}, \quad (1)$$

where $S_{8\text{-MOP}}$ and S_{gel} are average values of 8-MOP peak areas on chromatograms of 8-MOP standard sample solution and gel solution, respectively; $a_{8\text{-MOP}}$ is the 8-MOP standard sample weight, which was used to prepare a stock solution of 8-MOP standard sample, mg; $V_{st.s.}$ is the aliquot of the stock solution of 8-MOP standard sample used for final dilution, mL; a_{gel} is gel weight, g; P is a content of the main substance in the standard sample, %.

Method validation

Specificity

The specificity of the methodology for the quantification of 8-MOP in the gel was proved by comparing the chromatograms obtained by analyzing the solvent (acetonitrile), 8-MOP standard sample solution and gel solution.

Linearity and analytical domain

The linearity and analytical range of the methodology for the quantification of 8-MOP was established using 8-MOP standard sample solutions with concentration levels of 80%, 90%, 100%, 110%, and 120% of the nominal loading. The solutions were prepared by diluting the stock solution with a concentration of 0.67 mg/mL. The analysis was carried out in threefold repetition. According to the obtained results, a graph of the dependence of the peak area of 8-MOP on concentration was plotted. Using the mathematical dependence, the linear regression was calculated, and the correlation coefficient was determined (R^2).

Limit of detection and limit of quantification

As recommended by the State Pharmacopoeia, the limit of detection (LOD) and limit of quantification (LOQ) were determined by the ratio of analytical signal height to noise level. These parameters are determined using equations (2) and (3), respectively:

$$\mathrm{LOD} = \frac{3 \cdot h}{H} \cdot C, \quad (2)$$

$$\mathrm{LOQ} = \frac{10 \cdot h}{H} \cdot C, \quad (3)$$

where h is the background noise level, H is the 8-MOP peak height, C is the 8-MOP solution concentration.

Correctness

The correctness of the method was evaluated by the additive method by analyzing 9 individually prepared solutions of 8-MOP standard sample with concentration levels of 80%, 100%, 120% of the nominal loading in three repetitions each. The solutions were prepared by diluting the stock solution (concentration 0.67 mg/mL).

According to the results of the analysis, the response factor (RF) was calculated by formula (4).

$$\mathrm{RF} = \frac{\text{experimental value}}{\text{real value}} \cdot 100\%. \quad (4)$$

Based on the nine calculated values of the response factor, the relative standard deviation (RSD) and confidence interval were determined using equations (5) and (6), respectively.

$$\mathrm{RSD} = \frac{s}{x_{\mathrm{av}}} \cdot 100\%, \quad (5)$$

where s is the standard deviation of the measurement series and x_{av} is the average value of the variable being changed.

$$\Delta x = \frac{t(P, f) \cdot s}{\sqrt{n}}, \quad (6)$$

where $t(P, f)$ is the tabular value of Student's criterion at P (confidence level) = 95%, f (number of degrees of freedom) = 8; s is the standard deviation of the measurement series; n is the number of measurements.

Precision (repeatability)

To assess the precision (repeatability) of the methodology, we used a variant in which we prepared six solutions of 8-MOP standard sample with a concentration level of 100% of the nominal loading (the preparation procedure is described in the *Linearity* section).

According to the results of measurements, the relative standard deviation of peak areas of 8-MOP was calculated by formula (5).

Intra-laboratory (intermediate) precision

To assess the intra-laboratory precision of the methodology, six solutions of 8-MOP standard sample were prepared each with a concentration level of 100% of the nominal loading (the preparation procedure is described in the *Linearity* section). Two chemists analyzed the prepared solutions through a complete analytical procedure from sample preparation to results on different days.

From the measurements of each of the two analytical sessions, the relative standard deviation of the 8-MOP peak areas was calculated using formula (5). Fisher's criterion (F) for two analytical sessions was calculated by formula (7).

$$F = \frac{s_1^2}{s_2^2}, \quad (7)$$

where s^2 are dispersions of the first and second series of measurements.

Stability

To assess stability, a solution of 8-MOP standard sample was prepared with a concentration level of 100% of the nominal loading (the preparation procedure is described in the *Linearity* section). The solution was chromatographically analyzed at column temperatures differing by ±5% from the temperature stated

in the methodology, i.e., at 23.5 and 26.5°C, respectively. The flow rate was also varied by ±5% from the value stated in the method.

The relative standard deviation of the peak areas of 8-MOP at different chromatography conditions was calculated from the results of measurements according to formula (5).

RESULTS AND DISCUSSION

Development of a quantitative determination methodology

Despite the many techniques already developed and validated for the quantification of 8-MOP, previous studies have not provided evidence of the reproducibility and performance of these approaches during routine laboratory analysis.

When developing the methodology for quantitative determination of 8-MOP in soft dosage form by HPLC, several variants of chromatographic columns differing in the type of filler were used. The low solubility of the detected substance in aqueous media was taken into account during the selection of the stationary phase to indicates the preferable use of sorbent with octadecyl phase (C_{18}) grafted to silica gel [21]. Since the properties of the stationary phase can change over time during use or simply storage [22], two columns with similar stationary phase characteristics were used in the development process: a Luna C18(2) 5 μm, 250 × 4.6 mm column (*Phenomenex*, USA) and a Kromasil Eternity XT-5-C18, 5 μm, 250 × 4.6 mm column. From the evaluation of the suitability of the chromatographic system, the efficiency of the Luna C18(2) column was found to be 1400 theoretical plates, which did not meet the suitability requirements for the chromatographic system. Since the Kromasil EternityXT-5-C18 column demonstrated higher efficiency (15000 theoretical plates) under otherwise identical conditions, it was selected as the primary column for the analytical methodology for the quantification of 8-MOPs.

Further, the effect of introducing acetonitrile and methanol as an organic modifier into the mobile phase was evaluated. The use of acetonitrile resulted in optimal resolution in a short period of time (less than 10 min), while the use of methanol as a component of the mobile phase caused a change in the asymmetry of the peak of the detected substance, which does not meet the requirements of the suitability of the chromatographic system. In the course of further studies, we varied the volume content of acetonitrile in water for chromatography in the range of 20–50% due to the insufficient dissociation of the lower water content in the silanol groups [23]. Eluents with higher water content (more than 50%) cause dehydration of the stationary phase [24]. Under these conditions, a hydrocarbon film formed due to dispersion interactions between the alkyl groups of the stationary phase becomes stronger than when interacting with the eluent, which actually blocks the interaction of the synol groups with 8-MOP. Optimal sensitivity and resolution were achieved at an acetonitrile/water ratio of 50 : 50% (*v*/*v*) and a mobile phase flow rate of 1.0 mL/min.

Evaluation of the electronic absorption spectrum of 8-MOP (Fig. 2) using DAD, showed that the maximum response is observed at absorption of radiation with a wavelength of 250 nm.

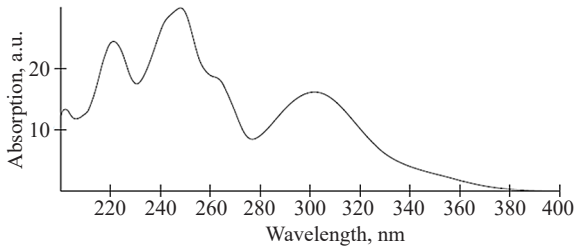

Fig. 2. Spectral analysis of 8-MOP

Method validation

Validation of the method was performed in accordance with GPA.1.1.0012.15 "Validation of Analytical Methods"[6] and the document "Guide for Validation of Analytical Methods"[7] for the following characteristics: specificity, LOD, LOQ, linearity, analytical range, correctness, repeatability (convergence), intra-laboratory (intermediate) precision.

Specificity

To assess the specificity of the methodology for the quantification of 8-MOP, the following model samples were analyzed: solvent (acetonitrile), 8-MOP standard sample solution and gel solution.

The chromatograms of the solvent (Fig. 3), 8-MOP standard sample solution (Fig. 4), and gel solution (Fig. 5) are as presented below.

In the chromatogram of the blank sample (solvent), there are no peaks with retention times corresponding

6 https://pharmacopoeia.ru/ofs-1-1-0012-15-validatsiya-analiticheskih-metodik/. Accessed March 11, 2025.

7 https://regulation.eaeunion.org/upload/iblock/4ec/jsw9jphfi1xvwlf9vt4otsb8y2lz5322/ria_30062017_mdoc.pdf/. Accessed March 11, 2025.

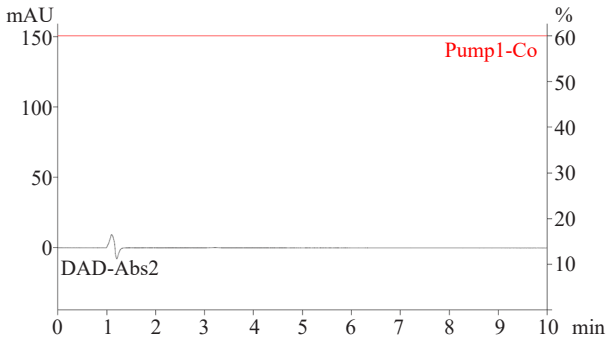

Fig. 3. Chromatogram of solvent

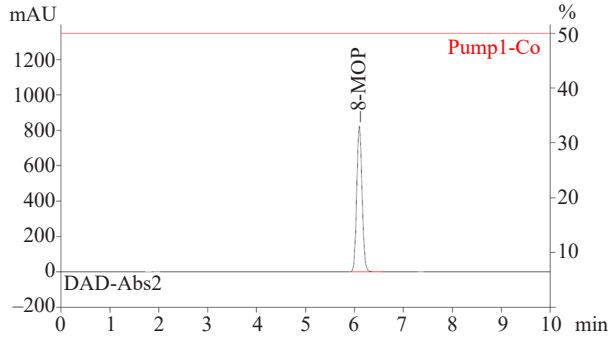

Fig. 4. Chromatogram of reference standard 8-MOP solution (0.067 mg/mL)

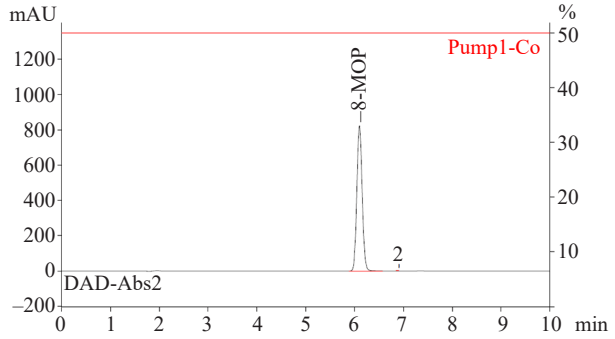

Fig. 5. Chromatogram of gel solution (10 mg/mL)

to a retention time of 8-MOP that could interfere with the determination of the analyte. The retention time of 8-MOP on the chromatogram of the standard sample solution (Fig. 4) is identical to that of the analyte peak on the chromatogram of the gel solution (Fig. 5). Thus, it is experimentally confirmed that the presence of accompanying components and impurities does not affect the analytical result and the technique is specific.

LOD and LOQ

The LOD of 8-MOP was 0.006 μg/mL and the LOQ was 0.02 μg/mL, allowing qualitative and quantitative compositional evaluation of samples with low analyte content.

Linearity and analytical domain

To confirm the linearity of the developed methodology, chromatography of 8-MOP standard sample solutions was performed with concentration levels of 80%, 90%, 100%, 110%, and 120% of the nominal value of 8-MOP concentration in the standard sample solution (Table 3). Solutions of each concentration level were analyzed in triplicate.

Table 3. Linearity parameters

Concentration, %	Concentration of 8-MOP, mg/mL	Peak area, mAU·s
80	5.36	2732.56
	5.36	2712.03
	5.36	2756.31
90	6.03	3074.36
	6.03	3098.33
	6.03	3058.87
100	6.7	3436.12
	6.7	3415.33
	6.7	3485.13
110	7.37	3757.21
	7.37	3788.99
	7.37	3741.22
120	1.2	4098.22
	1.2	4134.12
	1.2	4107.84
Slope		514.15
Segment cut off by a straight line on the *y* axis		−18.364
Linear correlation (R^2)		0.9979

Based on the results obtained, a calibration plot of the dependence of the peak area of 8-MOP on the concentration of 8-MOP in the standard sample solutions was constructed (Fig. 6). Linear regression was calculated using the mathematical dependence. The correlation coefficient was 0.9979, which indicates a linear relationship between concentrations and peak area values of 8-MOP.

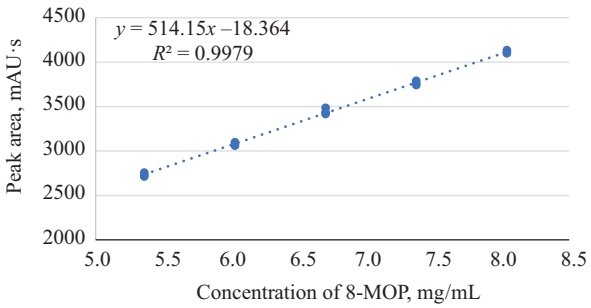

Fig. 6. Linear calibration curve

Correctness

To confirm the correctness of the methodology, nine solutions were analyzed: three solutions having a concentration level of 100% of the nominal value of 8-MOP concentration in the standard sample solution and three solutions each with a concentration level limiting the linear range of the methodology, i.e., 80% and 120% of the nominal value of 8-MOP concentration in the standard sample solution, respectively (Table 4).

The average value of the method opening parameter used to evaluate the correspondence between the results obtained with this analytical technique and the value taken as true was 99.42%. All values of the response factor are in the range of 95–105%, corresponding to the limits required in the 15th Edition of the State Pharmacopoeia of the Russian Federation.

Precision (repeatability)

The intra-laboratory precision of the 8-MOP quantification technique was determined by analyzing six similar solutions of 8-MOP standard sample at the same concentration but on different days and by two chemists (Table 5).

Precision was assessed by processing the experimental data obtained during two analytical sessions by calculating the relative standard deviation of the concentrations found. The relative standard deviation of the 8-MOP peak area for each analytical session conducted on different days, by different chemists, was ≤3.0%. The statistical equivalence of the results obtained from the two analytical sessions of the results was assessed by calculating Fisher's criterion

Table 4. Accuracy parameters

Concentration, %	Amount taken, mg/mL	Peak area, mAU·s	Amount found, mg/mL	Response, %
80	5.36	2732.56	5.31	99.01
80	5.36	2712.03	5.27	98.26
80	5.36	2756.31	5.35	99.87
100	6.7	3436.12	6.67	99.60
100	6.7	3415.33	6.63	99.00
100	6.7	3485.13	6.77	101.02
120	8.04	4098.22	7.96	98.99
120	8.04	4134.12	8.03	99.86
120	8.04	4107.84	7.98	99.22

Statistical characteristics	Results	Eligibility criteria
Average, %	99.42	95–105
RSD, %	0.79	≤3.0
The upper limit of the confidence interval (P = 95%), %	101.02	100
The lower limit of the confidence interval (P = 95%), %	98.26	

Table 5. Precision parameters

No.	Chemist 1		Chemist 2	
	Peak area, mAU·s	Found concentration, mg/mL	Peak area, mAU·s	Found concentration, mg/mL
1	3426.24	6.65	3455.36	6.71
2	3355.69	6.52	3498.33	6.79
3	3512.67	6.82	3412.85	6.63
4	3478.98	6.76	3512.65	6.82
5	3504.22	6.80	3459.36	6.72
6	3400.25	6.60	3425.22	6.65
RSD, %		1.81	1.13	
Fisher's criterion *F* (95; 5; 5)			2.54	

(*F*-test). The value of Fisher's criterion is less than the tabulated value of Fisher's criterion *F* (95, 5, 5) = 99.01, indicating an insignificant difference between the results of the two analytic sessions at 95% confidence level. Thus, the conducted validation studies demonstrate that the methodology provides comparable results under the influence of additional random factors.

Stability

To assess the stability of the methodology for quantitative determination of 8-MOP, the standard sample solution was chromatographed by varying the column temperature by ±5% from the temperature stated in the methodology, i.e., at 23.5 and 26.5°C, respectively. The deviation from the eluent flow rate stated in the method was ±5% (Table 6).

Table 6. Robustness parameters

Column temperature, °C	Flow rate, mL/min	Peak area, mAU·s
23.5	1.0	3456.51
		3524.12
		3489.22
25.0	1.0	3512.36
		3497.36
		3524.98
26.5	1.0	3552.14
		3547.56
		3485.22
25.0	0.95	3458.69
		3541.22
		3567.54
25.0	1.05	3478.29
		3500.27
		3466.88
RSD, %		1.01

Changes in flow rate and column temperature values by ±5% do not significantly affect the obtained results. The relative standard deviation of the 8-MOP peak area on the chromatograms of the standard sample solution of less than 3.0% meets the requirements of the suitability of the chromatographic system.

CONCLUSIONS

The described technique for the quantitative determination of 8-MOP by HPLC offers a number of significant advantages over most of the previously described methods. The use of acetonitrile instead of methanol as an organic modifier of the mobile phase allows working in acetonitrile/water mixture at a wavelength corresponding to the maximum response of the detector at absorption of radiation by the substance. Elution of the analyte in the presence of acetonitrile occurs earlier than in the presence of methanol in the mobile phase. This significantly reduces the analysis time, which is the most important factor for routine analysis. Unlike existing techniques, no salt buffers were used in the mobile phase; such approaches are undesirable due to increased working pressure and the considerable effort required to maintain instrument performance.

The technique was validated according to the guidelines set forth in the 15th Edition of the State Pharmacopoeia of the Russian Federation, which confirms its accuracy, precision, selectivity, and reliability. Considering the sensitivity of the methodology, its efficiency and ability to meet all validity parameters, it represents a reliable platform for the quantification of 8-MOP in finished dosage form.

Authors' contribution

All the authors took an active part in the discussion, analysis, and development of the experiment, processing the results, writing the text of the article and discussing it.

The authors declare no conflicts of interest.

REFERENCES

1. Bishnoi A., Parsad D. Phototherapy for vitiligo: A narrative review on the clinical and molecular aspects, and recent literature. *Photoderm. Photoimm. Photomed*. 2024;40(3):12968. https://doi.org/10.1111/phpp.12968
2. Molla A. A Comprehensive Review of Phototherapy in Atopic Dermatitis: Mechanisms, Modalities, and Clinical Efficacy. *Cureus.* 2024;16(3):e56890. https://doi.org/10.7759/cureus.56890
3. Barros N., Sbroglio L., Buffara M., Baka J., Pessoa A., Azulay-Abulafia L. Phototherapy. *An. Bras. Dermatol*. 2021;96(4):397–407. https://doi.org/10.1016/j.abd.2021.03.001
4. Wełniak A., Białczyk A., Kamińska B., Czajkowski R. Phototherapy in the management of vitiligo – an updated narrative review. *Eur. J. Clin. Exp. Med*. 2024;22(3):668–676. https://doi.org/10.15584/ejcem.2024.3.26
5. Zengarini C., Baruffaldi G., Piraccini B.M., Bardazzi F., Mussi M., Stancic B., Pileri A. Nb-UVB and PUVA therapy in treating early stages of Mycosis Fungoides: A single-center cross-sectional study. *Photoderm. Photoimm. Photomed*. 2023;39(5):435–440. https://doi.org/10.1111/phpp.12873
6. Serrano-Pérez J.J., González-Luque R., Merchán M., *et al*. The family of furocoumarins: Looking for the best photosensitizer for phototherapy. *J. Photochem. Photobiol. A: Chem.* 2008;199(1): 34–41. https://doi.org/10.1016/j.jphotochem.2008.04.013
7. Diekmann J., Theves I., Thom K.A., Gilch P. Tracing the Photoaddition of Pharmaceutical Psoralens to DNA. *Molecules.* 2020;25(22):5242. https://doi.org/10.3390/molecules25225242
8. Vieyra-Garcia P., Wolf P. A deep dive into UV-based phototherapy: Mechanisms of action and emerging molecular targets in inflammation and cancer. *Pharmacol. Ther*. 2021;222:107784. https://doi.org/10.1016/j.pharmthera.2020.107784
9. Schalla W., Schaefer H., Kammerau B., Zesch A. Pharmacokinetics of 8-methoxypsoralen (8-MOP) after oral and local application. *J. Invest. Dermatol*. 1976;66:258–259.
10. Lapolla W., Yentzer B.A., Bagel J., Halvorson C.R., Feldman S.R. A review of phototherapy protocols for psoriasis treatment. *J. Am. Acad. Dermatol.* 2011;64(5):936–949. https://doi.org/10.1016/j.jaad.2009.12.054
11. Hönigsmann H., Szeimies R., Knobler R. Chapter 238. Photochemotherapy and Photodynamic Therapy. In: Goldsmith L.A., Katz S.I., Gilchrest B.A., Paller A.S., Leffell D.J., Wolff K. (Eds.). *Fitzpatrick's Dermatology in General Medicine*. 8th Edition. TMcGraw-Hill Companies; 2012.
12. Kassem A.A., Abd El-Alim S.H., Asfour M.H. Enhancement of 8-methoxypsoralen topical delivery via nanosized niosomal vesicles: formulation development, *in vitro* and *in vivo* evaluation of skin deposition. *Int. J. Pharm*. 2017; 517(1–2):256–268. https://doi.org/10.1016/j.ijpharm.2016.12.018
13. Barradas T.N., Senna J.P., Cardoso S.A., de Holanda e Silva K.G., Mansur C.R.E. Formulation characterization and *in vitro* drug release of hydrogel-thickened nanoemulsions for topical delivery of 8-methoxypsoralen. *Mater. Sci. Eng. C Mater. Biol. Appl*. 2018;92:245–253. https://doi.org/10.1016/j.msec.2018.06.049
14. Wu J.Y., Li Y.J., Liu T.T., *et al.* Microemulsions vs chitosan derivative-coated microemulsions for dermal delivery of 8-methoxypsoralen. *Int. J. Nanomed*. 2019;14:2327–2340. https://doi.org/10.2147/IJN.S191940
15. Pitzanti G., Rosa A., Nieddu M., *et al.* Transcutol® P containing SLNs for improving 8-methoxypsoralen skin delivery. *Pharmaceutics.* 2020;12(10):973. https://doi.org/10.3390/pharmaceutics12100973
16. Chew Y.L., Khor M.A., Lim Y.Y. Choices of Chromatographic Methods as Stability Indicating Assays for Pharmaceutical Products: A Review. *Heliyon*. 2021;7(3):e06553. https://doi.org/10.1016/j.heliyon.2021.e06553
17. Araujo P. Key Aspects of Analytical Method Validation and Linearity Evaluation. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* 2009;877(23):2224–2234. https://doi.org/10.1016/j.jchromb.2008.09.030
18. Mahmoud D., ElMeshad A., Fadel M., Tawfik A., Ramez S. Photodynamic therapy fortified with topical oleyl alcohol-based transethosomal 8-methoxypsoralen for ameliorating vitiligo: Optimization and clinical study. *Int. J. Pharm*. 2022;614:121459. https://doi.org/10.1016/j.ijpharm.2022.121459

19. Ageev V.P., Shlyapkina V.I., Kulikov O.A., Zaborovskiy A.V., Tararina L.A. Qualitative and quantitative analysis of the main psoralen derivatives in the juice of Sosnovsky's hogweed. *Farmatsiya = Pharmacy*. 2022;71(3):10–17 (in Russ.). https://doi.org/10.29296/25419218-2022-03-02
20. Kulikov O., Ageev V., Brodovskaya E., Shlyapkina V., Petrov P., Zharkov M., Yakobson D., Maev I., Sukhorukov G., Pyataev N. Evaluation of photocytotoxicity liposomal form of furanocoumarins Sosnowsky's hogweed. *Chem. Biol. Interact*. 2022;357:109880. https://doi.org/10.1016/j.cbi.2022.109880
21. Ahmed A., Skinley K., Zhang H. Column technology for liquid chromatography. In: Fanali S., Haddad P.R., Poole C., Lloyd D.K. (Eds.). *Liquid Chromatography: Fundamentals and Instrumentation*. Volume 1 in *Handbooks in Separation Science*. 2023. P. 37–60. https://doi.org/10.1016/B978-0-323-99968-7.00007-2
22. Bachhav H., Shirsath G., Badhe S., Kurne D., Sonawane S. AI-driven data analysis for identification of impurities in HPLC chromatograms & artificial intelligent system for HPLC column selection and method development. *World J. Pharmac. Res*. 2024;13(13)238–263.
23. García-Alvarez-Coque M., Torres-Lapasió J., Ruiz-Angel M., Navarro-Huerta J. Secondary chemical equilibria in reversed-phase liquid chromatography. In: Fanali S., Haddad P.R., Poole C., Lloyd D.K. (Eds.). *Liquid Chromatography: Fundamentals and Instrumentation*. Volume 1 in *Handbooks in Separation Science*. 2023. P. 121–143. https://doi.org/10.1016/B978-0-323-99968-7.00012-6
24. Žuvela P., Skoczylas M., Jay Liu J., Bączek T., Kaliszan R., Wong M.W., Buszewski B. Column Characterization and Selection Systems in Reversed-Phase High-Performance Liquid Chromatography. *Chem. Rev*. 2019;119(6):3674–3729. https://doi.org/10.1021/acs.chemrev.8b00246

About the Authors

Adnan Alsayed, Process Engineer, Department of Biotechnology and Industrial Pharmacy, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (78, Vernadskogo pr., Moscow, 119454, Russia). E-mail: Adnanalsayed00@gmail.com. https://orcid.org/0009-0004-8265-934X

Anastasiya A. Prezhedromirskaya, Engineer, Department of Biotechnology and Industrial Pharmacy, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (78, Vernadskogo pr., Moscow, 119454, Russia). E-mail: a.a.pregedromirskaya@ipt.ru.com. https://orcid.org/0009-0001-5985-3900

Elizaveta A. Shnyak, Can. Sci. (Pharm.), Associate Professor, Department of Biotechnology and Industrial Pharmacy, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (78, Vernadskogo pr., Moscow, 119454, Russia). E-mail: elizaweta__@mail.ru. ResearcherID H-9402-2013, RSCI SPIN-code 7112-7197, https://orcid.org/0000-0001-8560-7060

Stanislav A. Kedik, Dr. Sci. (Eng.), Professor, Head of the Department of Biotechnology and Industrial Pharmacy, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (78, Vernadskogo pr., Moscow, 119454, Russia). E-mail: doctorkedik@yandex.ru. Scopus Author ID 7801632547, https://orcid.org/0000-0003-2610-8493

Об авторах

Алсайед Аднан, инженер-технолог, кафедра биотехнологии и промышленной фармации, Институт тонких химических технологий им. М.В. Ломоносова, ФГБОУ ВО «МИРЭА – Российский технологический университет» (119454, Москва, пр-т Вернадского, д. 78). E-mail: Adnanalsayed00@gmail.com. https://orcid.org/0009-0004-8265-934X

Прежедромирская Анастасия Александровна, инженер, кафедра биотехнологии и промышленной фармации, Институт тонких химических технологий им. М.В. Ломоносова, ФГБОУ ВО «МИРЭА – Российский технологический университет» (119454, Москва, пр-т Вернадского, д. 78). E-mail: a.a.pregedromirskaya@ipt.ru.com. https://orcid.org/0009-0001-5985-3900

Шняк Елизавета Александровна, к.фарм.н., доцент, кафедра биотехнологии и промышленной фармации, Институт тонких химических технологий им. М.В. Ломоносова, ФГБОУ ВО «МИРЭА – Российский технологический университет» (119454, Москва, пр-т Вернадского, д. 78). E-mail: elizaweta__@mail.ru. ResearcherID H-9402-2013, SPIN-код РИНЦ 7112-7197, https://orcid.org/0000-0001-8560-7060

Кедик Станислав Анатольевич, д.т.н., профессор, заведующий кафедрой биотехнологии и промышленной фармации, Институт тонких химических технологий им. М.В. Ломоносова, ФГБОУ ВО «МИРЭА – Российский технологический университет» (119454, Россия, Москва, пр-т Вернадского, д. 78). E-mail: doctorkedik@yandex.ru. Scopus Author ID 7801632547, https://orcid.org/0000-0003-2610-8493

Translated from Russian into English by H. Moshkov
Edited for English language and spelling by Thomas A. Beavitt