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DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR THE DETERMINATION OF GLUCOSAMINE HYDROCHLORIDE IN A MEDICINAL PRODUCT IN THE FORM OF A CREAM

Nataliia Bevz, Yaroslav Studenyak, Olena Ivaniuk, Olena Ruban

The development of medicinal products requires the use of selective and reproducible analytical methods that ensure reliable determination of active ingredients. The analysis of glucosamine hydrochloride as a medicinal product in the form of a cream has specific challenges due to its high polarity and lack of a chromophore; therefore, its determination within the dosage form requires a specially adapted analytical approach.

The aim. *To develop and validate a liquid chromatographic method for the determination of glucosamine hydrochloride in a medicinal product in the form of a cream in accordance with the requirements of ICH Q2(R2), the State Pharmacopoeia of Ukraine (SPhU), and the European Pharmacopoeia (EP).*

Materials and methods. *The object of the study was an experimental batch of a cream containing glucosamine hydrochloride (1%). The analysis was performed using a KNAUER Smartline chromatograph equipped with a UV detector and a Zorbax SB-C8 column (150 × 4.6 mm, 5 μm). Phenyl isothiocyanate was used for derivatization, resulting in the formation of a UV-active glucosamine derivative. Validation was carried out with respect to specificity, linearity, accuracy, precision, system suitability, and robustness.*

Results*. Optimal chromatographic conditions were established (mobile phase: acetonitrile–water, 40:60, acidified with phosphoric acid to pH 3.0; detection wavelength 230 nm; flow rate 1.0 mL/min; column thermostat temperature 25°C), ensuring complete separation of the glucosamine derivative (retention time 2.21 min) from sodium benzoate (retention time 3.55 min) and other excipients in the cream formulation. The minimum required concentration of the derivatization reagent was determined to be 0.008–0.01 g/mL. Evaluation of validation parameters confirmed the specificity of the method, its linearity within the 80–120% range ($r = 0.9991$), precision ($\Delta Z = 0.61\%$), accuracy ($\delta = 0.31\%$), repeatability ($\leq 2\%$), and robustness (analytical solutions stable for 1 hour). Metrological assessment ($n = 6$) demonstrated that the systematic error was statistically insignificant, while the relative uncertainty of a single determination was 7.88% at a confidence level of $P = 0.95$. The detection limit of glucosamine hydrochloride is 0.23 μg/ml.*

Conclusions*. An HPLC method for the determination of glucosamine hydrochloride in a cream formulation after derivatization with phenyl isothiocyanate has been proposed and experimentally substantiated. The method complies with the requirements of international guidelines and can be applied in quality control for the identification and quantitative determination of the active pharmaceutical ingredient in the investigated medicinal product*

Keywords*: glucosamine hydrochloride, cream, derivatization, HPLC, validation, quality control*

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1. Introduction

To ensure the population's timely access to high-quality medical and pharmaceutical care, new medicinal products appear on the global pharmaceutical market every year [1]. Their development requires not only the creation of appropriate dosage forms, but also assurance of quality, safety, and efficacy at all stages of the product life cycle – from the active substance to the finished medicinal product [2].

Therefore, the approach to medicinal product development involves comprehensive analytical support at each stage, including confirmation of composition, assessment of purity, evaluation of stability, and monitoring of transformation processes during storage and use [3]. Such studies necessitate the implementation of the principles of Quality by Design, as well as the development, validation, and practical application of reliable analytical methods [4, 5].

In the case of combination medicinal products containing several active ingredients and excipients, it is necessary to ensure selective and accurate quantitative determination of each component in the presence of others, as well as to take into account the influence of the dosage form matrix (density, viscosity, distribution of active substances) on the analytical results [6, 7].

The object of the study is a promising medicinal product in the form of a cream containing the active substance glucosamine hydrochloride. The medicinal product is characterized by pronounced moisturizing and emollient properties, promotes increased skin elasticity, and

accelerates skin regeneration. The product is intended for use under occlusive dressings to improve the functional condition of the stump after amputation, which determines the relevance of its development in the context of the current medical and social situation in Ukraine [8]. For registration and implementation of the medicinal product into medical practice, a mandatory requirement is the development and validation of quality control methods in accordance with the requirements of current Ukrainian legislation, international regulatory documents (ICH), and the provisions of the State Pharmacopoeia of Ukraine and the European Pharmacopoeia [9–11].

The identification of glucosamine hydrochloride in the substance, according to pharmacopoeial monographs, is carried out based on spectral characteristics (IR spectroscopy, UV spectroscopy) as well as using qualitative reactions [11, 12].

For the development of a methodology for the identification and quantitative determination of the active substance – glucosamine hydrochloride – in the cream, a liquid chromatography method was proposed as the most effective and sensitive for the separation of organic compounds of different structures [13].

2. Planning (methodology) of the research

1. The research methodology was built on a systematic approach to pharmaceutical quality control of combined medicinal products in accordance with international requirements (ICH) and the standards of the State Pharmacopoeia of Ukraine (SPhU) and the European Pharmacopoeia. The study was conducted in the following stages:

2. To analyze the feasibility of using liquid chromatography for the identification and quantitative determination of glucosamine hydrochloride in a semi-solid dosage form, taking into account the presence of excipients.

3. To select optimal chromatographic separation conditions: type of chromatographic column, composition and pH of the mobile phase, elution mode, flow rate, temperature, and detection wavelength.

4. To perform derivatization of glucosamine hydrochloride with phenylisothiocyanate to form a phenylthiohydantoin derivative, aiming to enhance UV detection sensitivity and ensure effective chromatographic separation in the presence of other matrix components.

5. To perform identification of the active substance by comparing the retention time of the test solution peak with the corresponding standard sample, as well as to assess the specificity of the method by comparing the chromatograms of reference solutions, the test solution, and placebo.

6. To validate the developed method in accordance with the requirements of ICH Q2(R2), SPhU, and PA/PH/OMCL recommendations based on the following parameters: system suitability, specificity, linearity, accuracy, precision (repeatability), and robustness.

7. To determine the acceptance criteria for analytical results and perform statistical processing of the study data.

3. Materials and methods of the research

The object of the study was an experimental batch of a medicinal product in cream form, which included the active substance – glucosamine hydrochloride (1%) – and excipients: glycerin, grape seed oil, gum, Emulpharma 1000, dimethicone 350, sodium benzoate, and purified water.

Experimental studies were carried out throughout 2025. The research was conducted using a KNAUER Smartline liquid chromatograph equipped with a UV detector (Knauer Smartline 2500, KNAUER GmbH, Germany); analytical balances – AXIS (Poland); a Zorbax SB-C8 chromatographic column, 150 × 4.6 mm with 5 μm particle size; and a pH meter (pH-150 MI). Sample preparation was performed using Class A volumetric glassware and reagents meeting EP/SPhU requirements.

The analysis was carried out using samples of glucosamine hydrochloride (batch 020520, Zhejiang Candorly Pharmaceutical Co., Ltd., China), sodium benzoate (batch 20241024 A, Wuhan Youji Industries Co., Ltd., China), glycerol (batch 5602/286/0824, TD "Agrokhimprom"), and grapeseed oil (batch 16731020, LLC "Scientific-Production Company Vilarus", Ukraine).

Testing was performed using liquid chromatography (SPhU*, 2.2.29, 2.2.46).

Test solution. A 0.400 g sample of the product is placed into a 25.0 ml volumetric flask, 5 ml of phosphate buffer solution (pH 8.4) and 10 ml of 0.1% methanolic solution of phenylisothiocyanate are added, and the resulting mixture is heated for 20 minutes in a water bath at 80°C. Then, 1.0 ml of 0.32 M phosphoric acid solution is added, and the mixture is again heated for 20 minutes in the water bath at the same temperature. After cooling, the volume of the solution is adjusted to the mark with HPLC-grade water and mixed thoroughly.

Comparison solution (a). Approximately 100.0 mg (exact weighed amount) of glucosamine hydrochloride is placed into a 25.0 ml volumetric flask, 10 ml of chromatography-grade water (*P*) is added, and the mixture is gently stirred until the sample dissolves. The volume is then brought up to the mark with the same solvent and mixed again.

Comparison solution (b). Approximately 30.0 mg (exact weighed amount) of sodium benzoate is placed into a 25.0 ml volumetric flask, 10 ml of chromatography-grade water (P) is added, and the mixture is stirred until the sample dissolves. The volume is then brought up to the mark with the same solvent and mixed again.

*Comparison solution (***c***).* 1.0 ml of reference solution A and 1.0 ml of reference solution B are placed into a 25.0 ml volumetric flask, 5 ml of phosphate buffer solution at pH 8.4 and 10 ml of 0.1% methanolic solution of phenylisothiocyanate are added, and the resulting mixture is heated for 20 minutes in a water bath at 80°C. Then, 1.0 ml of 0.32 mM phosphoric acid solution is added, and the mixture is heated again for 20 minutes at the same temperature. The mixture is cooled, the volume is brought up to the mark with chromatography-grade water (P), and mixed.

Prior to chromatography, the solutions are filtered through a membrane filter with a pore size not exceeding 0.45 μm.

Placebo solution. The amount of glycerin (batch 5602/286/0824, TD «Agrokhimprom»), grape seed oil (batch 16731020, LLC «Scientific-Production Company «Vilarus», Ukraine), gum, Emulpharma 1000, dimethicone 350, and purified water equivalent to the excipients in 400 mg of the preparation is mixed with 12.0 ml of phosphate buffer solution at pH 8.4 and brought to 25.0 ml with methanol. 5.0 ml of the resulting solution is diluted with the mobile phase (63% methanol, adjusted with phosphoric acid to pH 2.7) to a final volume of 25.0 ml.

Chromatography is carried out on a liquid chromatograph with a UV detector under the following conditions:

– chromatographic column Zorbax SB-C8, 150 × 4.6 mm with 5 µm particle size, or an equivalent column meeting system suitability requirements;

– mobile phase flow rate – 1.0 mL/min;

– column thermostat temperature – 25°C;

– injection volume – 20 µL;

– detection wavelength – 230 nm;

– mobile phase: acetonitrile for chromatography R – water for chromatography in a ratio of 40:60, acidified with phosphoric acid to pH 3.0.

The content of the active pharmaceutical ingredient (glucosamine hydrochloride) in 100 g of the finished product, in grams, is calculated using the formula

$$X, r = \frac{S_i \cdot m_0 \cdot 25.0 \cdot 1.0 \cdot 100 \cdot P}{S_0 \cdot m_i \cdot 25.0 \cdot 25.0 \cdot 100},$$

where S_i – the mean peak area of glucosamine hydrochloride calculated from the chromatograms of the test solution;

S_0 – the mean peak area of glucosamine hydrochloride calculated from the chromatograms of the reference solution;

m_0 – mass of the standard sample (glucosamine hydrochloride), in grams;

m_i – mass of the sample, in grams;

P – content of the active substance in the standard sample (glucosamine hydrochloride), in percent.

The content of glucosamine hydrochloride in 100 grams of the medicinal product should be between 0.95 g and 1.05 g at the time of release and during storage.

Statistical processing of the data was carried out in accordance with the requirements of SPhU 5.3.N.1 "Statistical analysis of the results of a chemical experiment" using Microsoft Excel software. For data processing, the mean value of three determinations, the standard deviation of the mean result, and the relative standard deviation (RSD) were calculated. No additional statistical methods were applied, as the study was of an analytical nature.

4. Research results

Since glucosamine hydrochloride (Fig. 1, 2-amino-2-deoxy-D-glucopyranose hydrochloride, M.W. 215.6) itself weakly absorbs for direct UV detection, phenyl isothiocyanate was chosen to enhance the UV detector response. It reacts with the amino group of glucosamine in an alkaline medium to form a thiourea derivative [14].

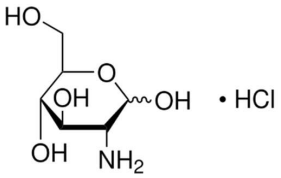

Fig. 1. Structural formula of glucosamine hydrochloride

The amount of phenyl isothiocyanate required for the reaction was determined experimentally within a reagent concentration range of 0.001–0.01 g/mL. It was found that after adding 0.008–0.01 g/mL of the reagent in methanolic solution, the chromatographic peak area remained virtually unchanged.

To study the validation characteristics, the linearity, accuracy, and precision of the method were evaluated using the "added-found" approach. The allowable concentration of glucosamine hydrochloride and sodium benzoate at release is within ± 5% of the nominal values; for the study of linearity, accuracy, and precision, the concentration range was selected from 80% to 120% with 5% increments. The acceptance criterion $B = 5\%$ indicates that the maximum analytical uncertainty should not exceed 1.6%.

The specificity of the method was confirmed by comparing the chromatograms of the test solution (Fig. 2), comparison solution (*a*) (Fig. 3), comparison solution (*b*) (Fig. 4), comparison solution (*c*) (Fig. 5), and placebo solution (Fig. 6).

In the presented chromatograms (Fig. 2–6), the peaks of the glucosamine derivative and sodium benzoate are clearly separated, with retention times matching those of the respective standard samples (2.21 min and 3.55 min, respectively). Moreover, no peaks in the placebo solution chromatogram corresponded to the retention times of either the glucosamine derivative or sodium benzoate, confirming the specificity of the proposed method.

The reproducibility of the results does not exceed ±2%, indicating the feasibility of simultaneous identification and quantification of the active pharmaceutical ingredient in the cream.

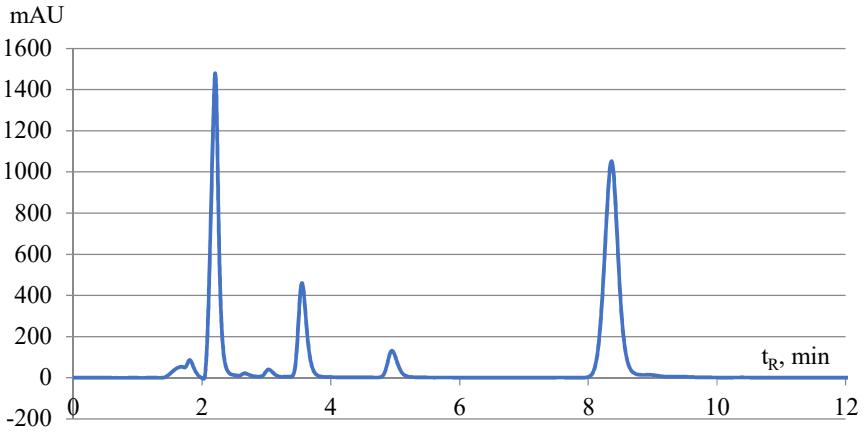

Fig. 2. Typical chromatogram of the test solution

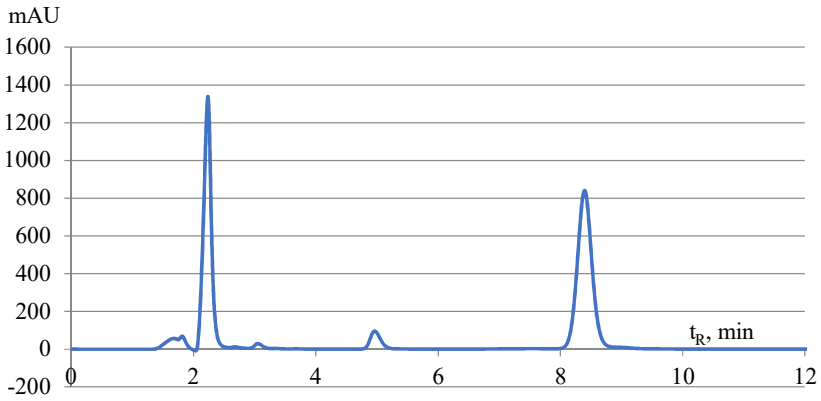

Fig. 3. Typical chromatogram of the reference solution (*a*)

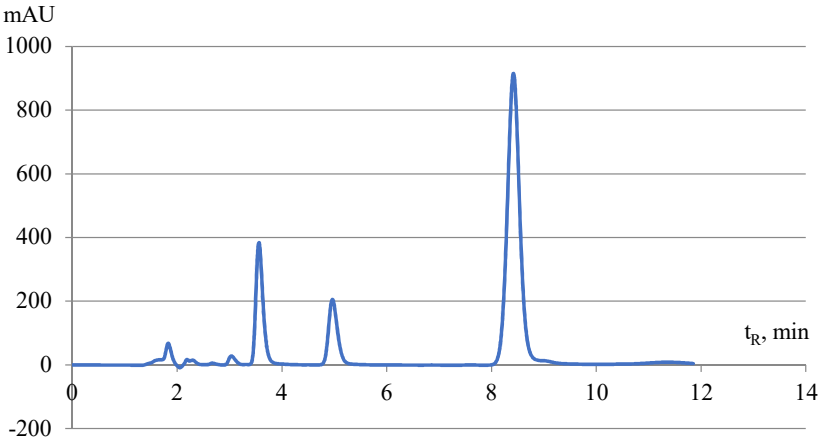

Fig. 4. Typical chromatogram of the reference solution (*b*)

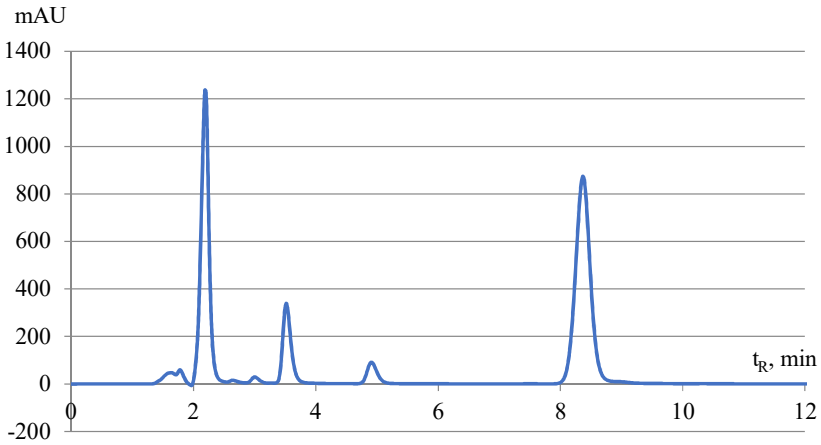

Fig. 5. Typical chromatogram of the reference solution (*c*)

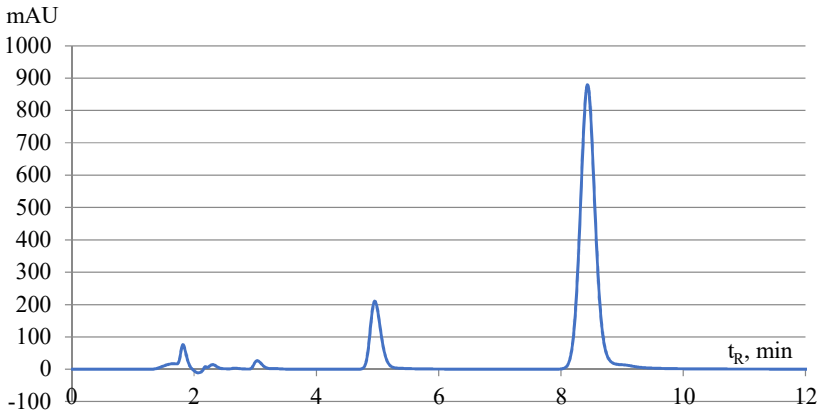

Fig. 6. Typical chromatogram of the placebo solution

The chromatograms obtained during the study meet the specified criteria for evaluating the suitability of the chromatographic system (Table 1).

Table 1

Results of the test "Suitability of the chromatographic system"

Criterion	Requirements	Peak results
Zorbax SB-C8		
Chromatographic column efficiency	> 2000	3984
Symmetry coefficient	from 0.8 to 1.5	1.00
Relative standard deviation, %	< 1.0	0.59
Resolution coefficient	≥ 1.5	1.50

When studying robustness, we investigated the stability of solutions over time (Table 2). The criterion of non-significance against the maximum permissible uncertainty of the analysis results (Δ_{AS} = 1.6%) is met within 1 hour, i.e. the solutions must be used freshly prepared [15].

As a result of the linearity study, it was found that the value of the correlation coefficients r = 0.9991 satisfies the requirements of the acceptance criterion (r > 0.9981) (Table 3). Therefore, in the entire concentration range from 80% to 120% of the nominal amount of the component in the cream, the method is linear.

Fig. 7 shows the dependence of the analytical signal on the actual concentration of glucosamine hydrochloride solutions; in normalized coordinates, the relationship is linear.

It has been demonstrated that the method exhibits sufficient precision and accuracy across the entire range of tested concentrations (Table 3). The found ΔZ value for glucosamine hydrochloride is 0.61%, which is below the critical value for precision (1.6%), thus meeting the acceptance criteria for the validation parameter "Precision." The systematic error of the method, δ = 0.31%, satisfies the requirements of the validation parameter "Accuracy" according to both criteria – statistical insignificance (≤ 0.37) and practical insignificance (≤ 0.51).

All calculated validation parameters meet the established criteria, confirming that the method is reliable and can be used for the quantitative determination of the active pharmaceutical ingredient in the studied semi-solid dosage form.

Table 2

Results of determination of solution stability

–	0 minutes	After 1 hour	Changes, in%	After 6 hours	Changes, in%
rso	10581	10533	0.46	10343	2.25
test	10320	10281	0.38	10038	2.73
Δ_{ave}		–	0.42	–	2.49

Table 3

Results of the evaluation of validation parameters for the quantitative determination of glucosamine hydrochloride

Parameter	Requirements, %	Obtained value, %	Compliance with criterion
Δ_{AS}%	≤ 1.6	1.29	Meet
$\lvert a \rvert$	≤ 2.6	0.94	Meet
$S_0 \le 0.84$		0.75	Meet
r	> 0.9981	0.9991	Meet
$\lvert \bar{Z} - 100 \rvert$	≤ 0.37	0.31	Meet according to two criteria
	≤ 0.51		
ΔZ	≤ 1.6	0.61	Meet
Δ_{intra}	≤ 1.6	0.93	Meet

The quantitative determination of glucosamine hydrochloride in the tested formulation was carried out on six samples. The metrological characteristics of the method for determining the quantitative content of the analyte are presented in Table 4.

Thus, the relative uncertainty of the mean determination with a 95 % confidence level is 3.22 %, and the true content of glucosamine hydrochloride in the cream can be considered 0.100 g ± 0.032 g.

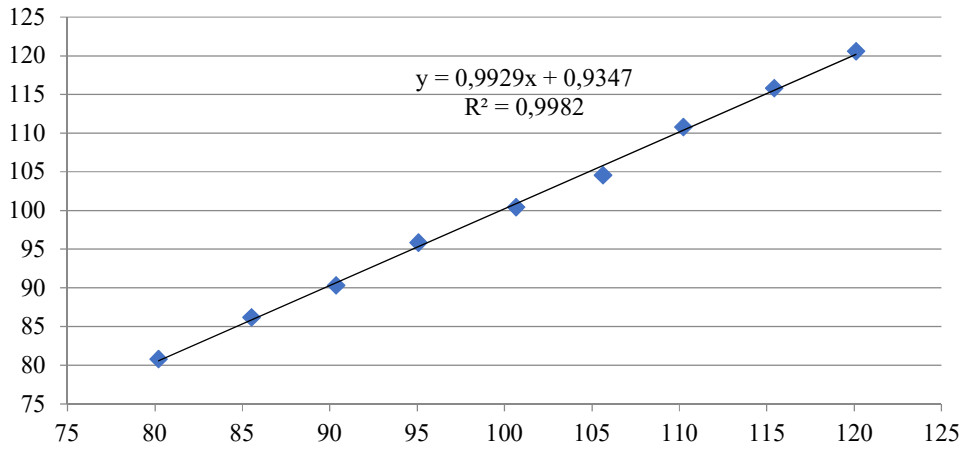

Fig. 7. Graphs of the linear dependence of the analytical signal on the actual concentration of glucosamine hydrochloride solutions, plotted in normalized coordinates

Table 4

Metrological characteristics of the quantitative content of glucosamine hydrochloride

m	ν	X_i, g	X_{ave}, g	S^2	S_x	P	$t(P, \nu)$	Δx	$\bar{\varepsilon}$, %
6	5	0.999	1.002	0.0009	0.0307	0.95	2.5706	0.0789	3.22
		1.015							
		0.961							
		1.018							
		1.044							
		0.973							

5. Discussion of research results

According to the literature, for the analysis of glucosamine hydrochloride in pharmaceutical formulations, liquid chromatography with pre-column derivatization is commonly used, followed by reversed-phase HPLC [16, 17]. Methods for the direct determination of glucosamine have also been described; however, these require the use of non-standard detectors (e.g., ELSD – evaporative light scattering detector) or specific chromatographic conditions (HILIC for polar amines) [18, 19], or chromatographic columns with different particle sizes [20]. These approaches allow avoiding the additional derivatization step but necessitate the use of guard columns, non-standard detectors, or specialized settings for selective separation and quantitative determination.

During the study, a liquid chromatography method was developed and tested for the identification and quantitative determination of glucosamine hydrochloride in a cream containing a complex matrix of excipients. Considering that the glucosamine molecule lacks a chromophore, which limits direct UV detection, the use of phenylisothiocyanate as a derivatization reagent allowed the formation of a stable and intense derivative with enhanced absorption at 230 nm. This provided a clear analytical signal and sufficient selectivity for determination in the presence of the cream base components.

The selected chromatographic conditions – C8 column, mobile phase acetonitrile/water (40:60) with pH adjusted to 3.0, temperature 25°C, and injection volume of 20 µL – provided optimal separation of glucosamine hydrochloride peaks from excipients, including sodium benzoate, and eliminated any potential influence of the cream matrix on the API signal. Comparison of the chromatograms of the test solution, standard sample, and placebo confirmed the specificity of the method, absence of interference from excipients, and correct identification of the active ingredient peak based on retention time.

Practical significance. The proposed method can be recommended for use in pre-industrial studies, during the development and registration of pharmaceutical products containing glucosamine hydrochloride, both as mono- and multi-component formulations.

Study limitations. The assessment of the cream base's influence was conducted only on the excipients included in the devel-

oped formulation; in case of any changes to the composition of the dosage form, the specificity of the method will require revalidation.

Prospects for further research. The method can be adapted for subsequent stability control of the pharmaceutical product throughout its shelf life and can be used in the development of the specification for the registration dossier.

6. Conclusions

1. Phenylisothiocyanate was proposed as a derivatization reagent, which ensured the formation of a stable derivative with distinct UV absorption, enabling the determination of glucosamine hydrochloride by liquid chromatography and increasing the selectivity of the analysis.

2. The use of a C8 column, acetonitrile/water (40:60) mobile phase at pH 3.0, column temperature of 25°C, and an injection volume of 20 μL provided clear separation of glucosamine hydrochloride and sodium benzoate peaks, as well as the absence of interference from the cream matrix.

3. The studied validation characteristics, in accordance with ICH Q2(R2) and SPhU requirements, confirmed specificity, linearity ($r \geq 0.9991$), accuracy, precision (RSD ≤ 2%), and robustness. This demonstrates the suitability of the developed method for quality control of the investigated pharmaceutical product.

4. Based on the obtained results, the proposed method can be recommended for use in pre-industrial studies, during the development and registration of pharmaceutical products, as well as a standard quality control procedure in pharmaceutical manufacturing. The method allows effective analysis of multi-component creams and can be adapted for other topical dosage forms containing glucosamine hydrochloride.

Conflict of interests

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

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Data availability

Data will be made available on reasonable request

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors’ contributions

Nataliia Bevz: Conceptualization, Methodology, Validation, Supervision, Resources, Writing – review & editing; **Yaroslav Studenyak:** Software, Investigation, Formal analysis, Visualization; **Olena Ivaniuk:** Software, Writing – original draft; **Olena Ruban:** Resources, Data curation, Project administration, Funding acquisition.

References

1. Shareef, U., Altaf, A., Ahmed, M., Akhtar, N., Almuhayawi, M. S., Al Jaouni, S. K. et al. (2024). A comprehensive review of discovery and development of drugs discovered from 2020–2022. Saudi Pharmaceutical Journal, 32 (1), 101913. https://doi.org/10.1016/j.jsps.2023.101913

2. Rudakova, O., Gubar, S., Smielova, N., Lytkin, D., Briukhanova, T., Bezchasnyuk, E. et al. (2021). Study of compatibility of components of a new combined drug for treatment of alcoholic intoxication and its hepatoprotective effect on a model of alcoholic liver injury. ScienceRise: Pharmaceutical Science, 6 (34), 91–100. https://doi.org/10.15587/2519-4852.2021.249880

3. Rudakova, O., Gubar, S., Smielova, N., Yaremenko, M., Bevz, N., Georgiyants, V. (2022). Development of a unified approach to the method of identification, quantitative determination of active substances and accompanying impurities in a combined drug by HPLC method. ScienceRise: Pharmaceutical Science, 2(36), 81–89. https://doi.org/10.15587/2519-4852.2022.255851

4. Kumar, D., Kumar, A., Kumar, V., Raj, A., Rai, R. R. M., Baliyan, V., Kumar, N. (2023). A Comprehensive Review on Analytical Method Development using RP-HPLC and Recent Advances in Pharmaceutical Applications. Journal for Research in Applied Sciences and Biotechnology, 2 (2), 53–60. https://doi.org/10.55544/jrasb.2.2.9

5. Ankit, J., Vijayraj, S. (2024). Advancements in Analytical Development and Validation: A Comprehensive Review in Pharmaceutical Sciences. Open Access Journal of Pharmaceutical Research, 8 (3). https://doi.org/10.23880/oajpr-16000317

6. Chaudhary, T., Kumar, A., Raj, N. D., Sarma, G., Dawange, S., Singh, D. (2024). An Exhaustive Review on Recent Trends in Analytical Methods: Development Strategies and Recent Applications. Current Indian Science, 2. https://doi.org/10.2174/012210299x24646823111505171

7. Savchenko, L., Uminska, K., Bevz, N., Georgiyants, V. (2018). Selection and verification of the method for phynelefrine hydrochloride assay in Simanovsky ointment. ScienceRise: Pharmaceutical Science, 1 (11), 26–31. https://doi.org/10.15587/2519-4852.2018.122094

8. Ruban, O., Slipchenko, H., Osolodchenko, T., Kovalevska, I., Khokhlenkova, N. (2025). Research on the antimicrobial preservative selection for the cream containing glucosamine hydrochloride and miramistin. Annals of Mechnikov Institute, 3, 85–91. https://doi.org/10.5281/zenodo.17105302

9. ICH Q14 – Analytical Procedure Development (2023). International Council for Harmonisation (ICH). Available at: https://www.ema.europa.eu/en/ich-q14-analytical-procedure-development-scientific-guideline

10. Derzhavna Farmakopeia Ukrainy. Dopovnennia 8 (2025). Kharkiv: Derzhavne pidpryiemstvo «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 452.

11. European Pharmacopoeia (EP). (n.d.). European Directorate for the Quality of Medicines.

12. The British Pharmacopoeia. (2023). Available at: https://www.pharmacopoeia.com/

13. Derzhavna Farmakopeia Ukrainy. Dopovnennia 5. (2021). Kharkiv: DP «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 424.

14. Gaonkar, P., Khanvilkar, V., Shettigar, R., Gadgoli, C. (2006). Spectrophotometric method for determination of glucosamine in tablets. Indian Journal of Pharmaceutical Sciences, 68, 83–84.

15. Derzhavna Farmakopeia Ukrainy. Dopovnennia 7. Vol. 2 (2024). Kharkiv: DP «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 424.

16. El-Saharty, Y. S., Bary, A. A. (2002). High-performance liquid chromatographic determination of neutraceuticals, glucosamine sulphate and chitosan, in raw materials and dosage forms. Analytica Chimica Acta, 462 (1), 125–131. https://doi.org/10.1016/s0003-2670(02)00279-9

17. Alcázar Magaña, A., Wrobel, K., Corrales Escobosa, A. R., Wrobel, K. (2015). Determinación rápida de glucosamina en formulaciones farmacéuticas mediante cromatografía líquida de alta resolución sin derivatización pre-columna. Acta Universitaria, 24, 16–22. https://doi.org/10.15174/au.2014.717

18. Pazourek, J. (2018). Determination of glucosamine and monitoring of its mutarotation by hydrophilic interaction liquid chromatography with evaporative light scattering detector. Biomedical Chromatography, 32 (12). https://doi.org/10.1002/bmc.4368

19. Asthana, C., Peterson, G. M., Shastri, M., & Patel, R. P. (2019). Development and validation of a novel high performance liquid chromatography-coupled with Corona charged aerosol detector method for quantification of glucosamine in dietary supplements. PLoS One, 14(5), e0216039. https://doi.org/10.1371/journal.pone.0216039

20. Šulc, M. (2024). Chitin quantitation (as glucosamine) in food raw materials by HPLC C18-DAD with off-line derivatization. MethodsX, 12, 102729. https://doi.org/10.1016/j.mex.2024.102729

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