Arh. farm. 2026; 76: 148 – 168 *Original scientific paper/Originalni naučni rad*

Optimization of Sample Preparation and Analytical Performance Evaluation for a Model Polar Compound in Hydroxypropyl Methycelulose-Based Controlled-Release Tablets

Marijana Bozhinovska[1,2*], Tina Achkoska[1], Ana Atanasova[1], Packa Antovska[1], Olga Gigopulu[2], Ana Poceva Panovska[2]

[1]Research & Development, Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

[2]Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000 Skopje, North Macedonia

*Corresponding author: Marijana Bozhinovska, e-mail: mtrendafilovik@alkaloid.com.mk

Received: 08 April 2026; Revised in revised forme: 20 May 2026; Accepted: 29 May 2026

Abstract

Controlled-release (CR) tablets based on hydroxypropyl methylcellulose (HPMC) provide numerous therapeutic advantages, but pose significant analytical challenges during the assay of polar active substances due to the polymer's high swelling capacity and gel-forming behaviour. This study aims to develop and evaluate a robust two-step sample preparation procedure that ensures complete extraction and accurate quantitation of a model polar basic compound incorporated into an HPMC matrix. Various diluents and agitation techniques were systematically compared to identify conditions enabling full recovery of the analyte. The optimized workflow involved initial extraction with acetonitrile for 15 minutes under ultrasonic agitation, followed by hydration with water for 45 minutes under magnetic stirring. The samples were analysed with High-performance liquid chromatography (HPLC) analysis, using ion-pair reversed-phase conditions. Acetonitrile effectively disrupted the HPMC network, preventing gel entrapment and yielding quantitative recovery (100 ± 0.5%) with excellent reproducibility (RSD < 1%). The analytical method demonstrated linearity (R^2 = 0.9997), accuracy (99.6 %), precision (RSD ≤ 0.5%), robustness, and solution stability consistent with ICH Q2 (R2) principles. The proposed two-step acetonitrile-water extraction provides a reliable, transferable strategy for the assay of

polar drugs in HPMC-based controlled-release formulations and can serve as a methodological framework for future compound-specific validation.

Key words: hydroxypropyl methylcellulose, controlled-release tablets, polar compound, sample preparation, acetonitrile extraction, method performance evaluation

https://doi.org/10.5937/arhfarm76-66354

Introduction

Hydrophilic matrix systems based on hydroxypropyl methylcellulose (HPMC) are among the most widely applied technologies for the development of controlled-release oral dosage forms due to their robustness, simplicity of manufacturing, and predictable drug-release kinetics (1–4). Upon contact with aqueous media, HPMC rapidly hydrates and forms a viscous gel layer that governs drug release through a combination of diffusion, polymer relaxation, and erosion mechanisms. The characteristics of this gel barrier, strongly influenced by polymer viscosity grade, hydration dynamics, and environmental conditions, are central to the performance of HPMC-based formulations and have been extensively studied in the context of drug delivery (5-7).

While the gel-forming ability of HPMC is advantageous for controlling drug release, it presents a significant challenge for analytical quantitation during quality control. Hydration of the polymer leads to the formation of a highly viscous, diffusion-limiting network capable of entrapping drug particles and restricting solvent penetration. As a result, incomplete extraction of the active pharmaceutical ingredient (API) may occur, leading to the underestimation of assay values and increased variability. This issue is particularly pronounced for highly water-soluble, polar, and ionizable compounds, whose distribution between the gel phase and the bulk solvent is governed by pH-dependent ionization equilibria and solvent polarity (8, 9).

Various approaches have been reported to improve extraction efficiency from hydrophilic matrices, including the use of hydro-organic solvent systems, surfactants, elevated temperatures, and extended agitation times (10, 11). In the literature, HPMC-based hydrophilic systems have repeatedly been used to modulate the release of BCS class III-relevant polar actives by different polymer grade/loading such as famotidine and metformin while still supporting acceptable assay determination (by using pharmacopeial based methods) (12, 13). In another example, acyclovir incorporated in HPMC matrix was assessed with an in vitro-in vivo (PK) approach, highlighting the necessity to align control-release with robust and reliable quantitative analysis (14).

However, these strategies are typically empirical and often compound-specific, lacking a systematic framework that links solvent properties, polymer behavior, and extraction performance. In particular, the role of solvent–polymer interactions in modulating HPMC swelling, gel formation, and matrix disintegration during analytical sample preparation remains insufficiently explored.

Organic solvents such as acetonitrile and methanol are known to influence the conformation and hydration behavior of cellulose derivatives by altering hydrogen-bonding interactions and reducing the dielectric constant of the medium. These effects can suppress polymer swelling, promote gel collapse, and facilitate the release of entrapped drug molecules. From a physicochemical perspective, the sequential use of organic and aqueous solvents offers the potential to decouple matrix disruption from analyte solubilization. Nevertheless, the systematic optimization of such multi-step extraction strategies, including solvent sequence, composition, and agitation conditions,

has not been comprehensively addressed in the context of HPMC-based controlled-release formulations (15, 16).

In this context, the present study aims to develop a mechanistically guided and experimentally validated sample-preparation approach for the quantitative determination of a model polar basic compound embedded in an HPMC matrix. A systematic investigation of solvent systems and agitation techniques was conducted to elucidate the factors governing extraction efficiency and reproducibility. Based on these findings, a two-step acetonitrile–water extraction procedure was established, designed to first disrupt the polymer network and subsequently enable complete analyte solubilization under controlled hydration conditions.

In parallel, the analytical performance of the associated ion-pair reversed-phase HPLC method was evaluated in accordance with ICH Q2 (R2) principles (17). The overall objective of this work is not only to ensure reliable quantification for the investigated system, but also to provide a transferable methodological framework applicable to a broader class of polar compounds in hydrophilic controlled-release matrices.

Materials and Methods

Materials and Reagents

The test formulation consisted of prolonged-release tablets containing a model polar basic compound in an HPMC matrix. The analyte identity is undisclosed for confidentiality reasons; its relevant physicochemical properties ($\log P < 1$, $pK_a \approx 8.2$) typify a highly water-soluble, weakly basic molecule. The molecule is primarily composed of carbon, hydrogen, and nitrogen atoms. It consists of guanidine groups that contribute to the basic properties and allows it to exist in protonated form under physiological conditions. In the Biopharmaceutics Classification System, the analyte is classified as BCS Class III due to its high solubility and low intestinal permeability.

Analytical grade reagents and solvents were used: acetonitrile (ACN), methanol (MeOH), ethanol (EtOH) (Merck, HPLC grade); 1-pentanesulfonic acid sodium salt ($C_5H_{11}NaO_3S$, Merck, for ion pair chromatography), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \times H_2O$, p.a), potassium dihydrogen phosphate (KH_2PO_4, Merck, p.a), ortho-phosphoric acid (85% o-H_3PO_4, p.a), sodium hydroxide (NaOH, Merck p.a), hydrochloric acid (37% HCl, Sigma, p.a) and purified water. A certified secondary reference standard of the model compound served for calibration solutions (0.05 mg mL^{-1}). The solution was prepared by dissolving the standard in diluent (10% acetonitrile: 90% water mixture).

Equipment

Sample agitation employed IKA Magnetic stirrer RT 15 Power, Elmasonic S 100H ultrasonic bath and VWR Advanced Orbital Shaker (Model 5000). Chromatographic analyses were performed on Thermo Dionex 3000 ultrahigh performance liquid

chromatography (UHPLC) system with PDA detector and calculated in chromatography data system (CDS) based on Chromeleon.

Chromatographic Conditions

Separation used a Waters SunFire C8 (150 × 4.6 mm, 5 µm) column with isocratic elution with flow of 1.0 mL min^{-1} at 25 °C. Mobile phase: (A) 0.01 M NaH_2PO_4 buffer (pH 3.0 ± 0.1) containing 1-pentanesulfonic acid (ion-pair agent); (B) acetonitrile. Detection λ = 230 nm; run time = 6 min.

Experimental Design for Sample Preparation

A systematic experimental approach was undertaken to explore the influence of solvent type, solvent composition, and agitation mode on the efficiency and reproducibility of extracting a polar compound from the HPMC matrix. The initial screening was designed to cover the full range of solvent polarities – from purely aqueous to purely organic systems. Each solvent or solvent mixture was combined with at least one mode of mechanical agitation (magnetic stirring, ultrasonic bath, or orbital shaking) and was tested over varying treatment durations to assess the extent of matrix disintegration and the completeness of extraction.

The overall design of the experimental work is outlined in Table I, which summarizes the solvent categories investigated, representative examples of each system, the applied agitation techniques, and the corresponding treatment durations.

Table I Tested solvent systems and extraction conditions

Tabela I Testirani sistemi rastvarača i uslovi ekstrakcije

Category	**Examples**	**Agitation**	**Time range**
Aqueous	Water, pH 6.8 buffer, 0.1 M HCl, 0.1 M NaOH	Magnetic/ultrasonic	30 – 60 min
Mixed	H_2O:ACN or H_2O:MeOH (10–90%)	Magnetic	60 – 180 min
Organic	EtOH, MeOH, ACN	Magnetic/ultrasonic/orbital	15 – 60 min

Extraction efficiency was calculated as the percentage recovery of the declared content, and variability as %RSD. Based on screening, parameters were adjusted to obtain the final optimized procedure.

Optimized Sample-Preparation Procedure

After comparative screening of solvents and agitation conditions, the final sample-preparation procedure was established as follows: A precisely weighed portion of ground tablet powder, equivalent to one-unit dose, was transferred into a 500 mL volumetric

flask. 100 mL of acetonitrile was added, and the mixture was sonicated for 15 minutes with occasional manual shaking to ensure complete dispersion of the powder. Subsequently, water was cautiously added to the flask to prevent uncontrolled gel formation and drug encapsulation, and the suspension was magnetically stirred for an additional 45 minutes to achieve full hydration and extraction of the active compound. The obtained solution was then diluted to volume with water and filtered through a 0.45 µm regenerated cellulose (RC) membrane to remove any residual undissolved particles. From this stock solution, working sample solutions were prepared at a concentration of 0.05 mg mL^{-1} using a 10% acetonitrile–90% water diluent prior to HPLC analysis.

Method Performance Evaluation

Following the establishment of the optimized sample preparation procedure, the chromatographic method was evaluated in accordance with the principles of the ICH Q2 (R2) guideline to verify its suitability for quantitative analysis of a model polar compound in HPMC-based matrices. The assessment covered selectivity, linearity and range, accuracy, precision (repeatability and intermediate precision), robustness, filtration study and stability of standard and sample solutions. A Plackett-Burman experimental design implemented in MODDE Go software was applied to examine robustness by varying flow rate, column temperature, acetonitrile content, buffer pH, and column lot within narrow ranges. The resulting data sets were statistically processed using Chromeleon CDS and MODDE to calculate mean values, relative standard deviations, and model parameters (R^2, Q^2). These performance studies are described in detail in Section (Method Performance Evaluation for a Model Polar Compound).

Results and Discussion

Influence of Solvent on Extraction Efficiency

The comparative data obtained from all solvent systems are summarized in Table II. A clear trend was observed: as the proportion of organic solvent increased, both the mean recovery and reproducibility improved markedly. In purely aqueous systems, average recoveries ranged between 94% and 97% with relative standard deviations (RSD) up to 4%. These results confirmed the visual observation of gel formation, where trapped powder residues inside hydrated HPMC domains restricted solvent access to the encapsulated compound.

Table II Optimized extraction conditions and mean recoveries

Tabela II Optimizovani uslovi ekstrakcije i srednje vrednosti prinosa

Solvent system	Composition / Ratio	Extraction technique	Time (min)	Key observations	Mean recovery (%) ± RSD
Aqueous systems	H_2O, buffer pH 6.8, 0.1 M HCl, 0.1 M NaOH	Magnetic / ultrasonic stirring	30 – 60	Gel bead formation; incomplete powder dissolution	94.9 ± 3.0
Hydro-organic mixtures	H_2O:ACN or H_2O:MeOH (10 – 90%)	Magnetic stirring	60 – 180	Partial polymer swelling; variable recovery	96.8 ± 2.1
Ethanol (pure)	100% EtOH	Ultrasonic bath	15 – 30	Acceptable recovery; high viscosity	97.9 ± 0.8
Methanol (pure)	100% MeOH	Ultrasonic bath	30 – 45	Good recovery; moderate matrix disruption	98.5 ± 0.7
Acetonitrile (pure)	100% ACN	Ultrasonic bath	15	Efficient matrix disruption; minimal gel formation	99.1 ± 0.5
Two-step extraction	ACN → H_2O	Ultrasonic (ACN) + magnetic stirring (H_2O)	15 + 45	Complete extraction; homogeneous solution	100.2 ± 0.5

Optimization Summary and Mechanistic Interpretation

Analysis of all experimental conditions revealed a clear improvement in recovery as solvent polarity increased from aqueous to organic systems and as the order of solvent addition was optimized. The two-step acetonitrile–water procedure consistently produced quantitative extraction (100 ± 0.5 %) with RSD < 1 %, combining the dehydrating action

of acetonitrile with the solubilizing power of water. This combination ensured complete matrix disintegration and homogeneous dissolution of the polar compound.

Mechanistically, acetonitrile penetrates the HPMC network without promoting extensive hydration. Its moderate polarity and weak hydrogen-bonding capacity dehydrate and collapse the gel structure, releasing the entrapped drug. The subsequent addition of water restores a polar medium that supports analyte dissolution without re-forming the gel. The sequential use of these solvents therefore unites two opposing effects – disruption and solubilization – achieving full recovery with minimal variability.

From a physicochemical perspective, this outcome reflects the balance between solvent dielectric constant and polymer hydrophilicity: water ($\varepsilon \approx 80$) drives swelling, while acetonitrile ($\varepsilon \approx 37$) suppresses chain relaxation. Their controlled combination prevents uncontrolled gelation and ensures efficient extraction. This behaviour was also studied in numerous studies that state that the low hydrogen-bonding capacity of acetonitrile is allowing its usage as extraction solvent in assay sample preparation of HPMC matrix tablets. Moreover, it is a known fact that HPMC swelling increases with solvent hydrogen-bonding (e.g. methanol, ethanol). Consequently, acetonitrile-rich media would minimize HPMC swelling and gel formation and thus improve recovery for assay determination (18, 19). The optimized two-step procedure thus represents a mechanistically justified, reproducible, and broadly applicable strategy for analysing polar compounds in hydrophilic controlled-release tablets. The subsequent section presents the analytical performance of the HPLC method developed in conjunction with this extraction approach.

Having established the optimal extraction conditions, the next step was to assess the analytical performance of the HPLC method coupled with this procedure. The following section presents the results of the method performance evaluation, addressing parameters such as selectivity, linearity, accuracy, precision, robustness, and stability in accordance with the ICH Q2 (R2) principles.

Method Performance Evaluation for a Model Polar Compound

Following the establishment of the optimized extraction procedure, the chromatographic method was evaluated for its analytical performance in accordance with ICH Q2 (R2). The assessment aimed to verify that the combined sample-preparation and HPLC conditions enable the accurate and reproducible quantitation of a representative polar, basic compound embedded in an HPMC matrix.

Specificity. Chromatograms of diluent, placebo, standard, and sample solutions showed no interfering peaks at the analyte retention time (~3 min), confirming that the method is specific for the analyte.

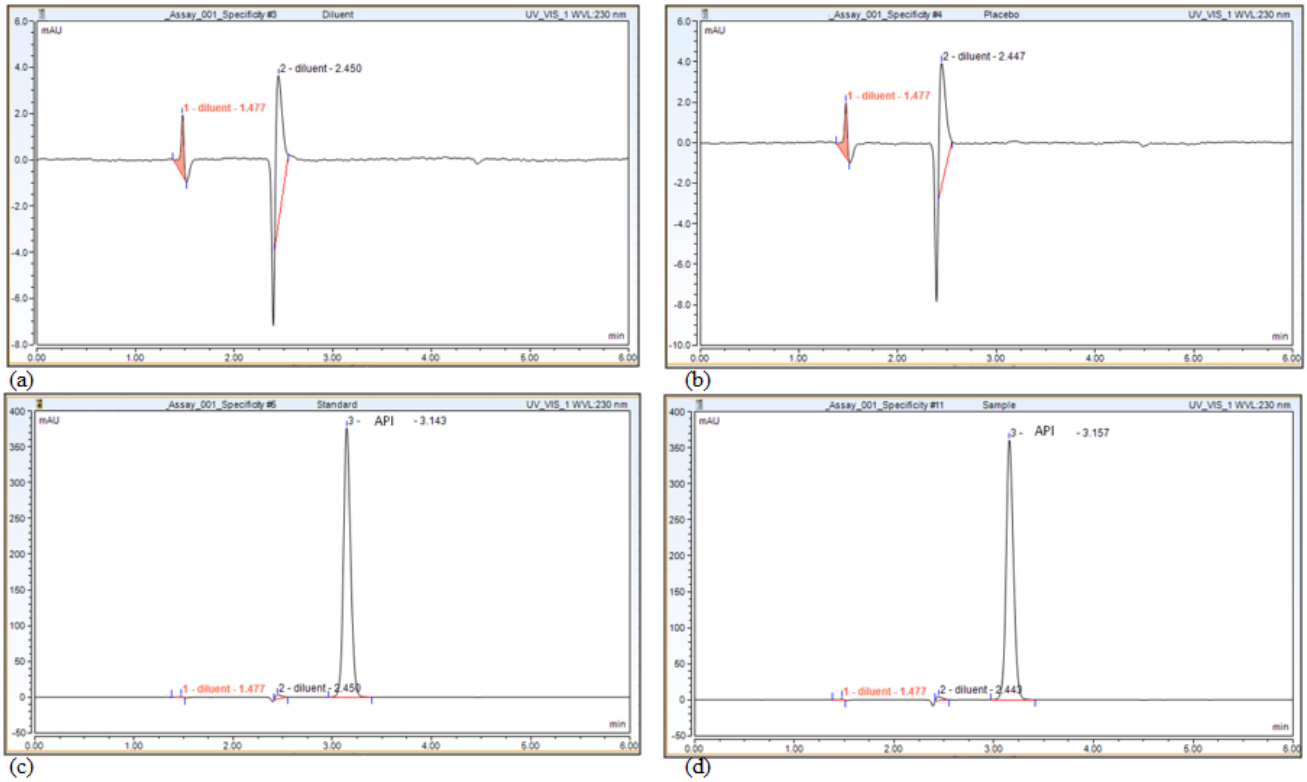

Figure 1. Validation of the method for determination of Model Polar Compound – Specificity demonstrated by the chromatograms obtained from: (a) Chromatogram for diluent; (b) Chromatogram for placebo solution; (c) Chromatogram for standard solution and (d) Chromatogram for sample solution – analyte peak (Rt ~ 3)

Slika 1. Validacija metode za određivanje model polarnog jedinjenja – Specifičnost prikazana hromatogramima dobijenim iz: (a) hromatograma rastvarača; (b) hromatograma placebo rastvora; (c) hromatograma standardnog rastvora i (d) hromatograma uzorka – pik analita (Rt ~ 3)

Linearity and range. In this study, six calibration levels were prepared from standard solutions of the model polar compound covering a concentration range of 0.02 – 0.08 mg mL^{-1}, corresponding to 40 – 160% of the nominal assay concentration (0.05 mg mL^{-1}) and injected in triplicate. Results are presented in Table III.

Table III Summary of analytical method validation results (linearity, accuracy, precision)
Tabela III Rezime rezultata validacije analitičke metode (linearnost, tačnost, preciznost)

Validation parameter	Experimental conditions / range	Results
Linearity	40 – 160% of nominal concentration (0.01998 – 0.07994 mg mL^{-1})	Calibration equation: $y = 663.01x - 1.22$; $R^2 = 0.9997$
Accuracy	50%, 100%, 150% (n = 3)	Mean recovery: 99.62%; Range: 99.41 – 100.01%; RSD ≤ 0.16%
Repeatability	6 replicate injections at 100% level	RSD: 0.1%
Method precision	6 independent sample preparations	Mean assay: 100.23%; RSD: 0.3%
Intermediate precision	Two instruments, two days	Mean assay: 100.23 – 100.98%; Overall RSD: 0.5%

These results confirm that the HPLC method provides a linear and reliable quantitative response over a broad range of concentrations. The strong correlation and reproducible response ensure the accurate quantification of the analyte, whether analysing standard solutions or test samples extracted from HPMC-based controlled-release tablets.

Accuracy. The placebo samples containing all excipients of the prolonged-release formulation were spiked with known amounts of the model polar compound at three concentration levels – 50%, 100%, and 150% of the nominal assay level (0.05 mg mL^{-1}), in triplicate. The samples were analysed by the proposed method and the amount of API recovered was calculated shown in Table III.

The quantitative recovery observed across all levels confirms that the optimized extraction step ensures complete liberation of the analyte from the HPMC matrix without loss or degradation. The consistent results at the lowest (50%) and highest (150%) levels also indicate that the method is accurate throughout the validated range and is not affected by sample concentration or matrix composition.

Precision

Repeatability. Repeatability was examined by analysing six replicate injections of the standard solution at the nominal assay concentration (0.05 mg mL^{-1}) and six independently prepared sample solutions of the model polar compound extracted from HPMC-based tablets using the optimized two-step acetonitrile–water procedure.

The relative standard deviation (RSD) of the peak areas for standard injections was 0.1%, demonstrating excellent instrument precision. These results, shown in Table III confirm that the sample-preparation and analytical steps produce highly reproducible measurements under identical conditions.

Method precision. Six sample solutions were prepared individually using a single batch of tablets as per analytical method.

The absence of significant variation, shown in Table III, among replicate preparations, confirms the homogeneity of the sample solutions and the reliability of the sample preparation procedure, which is often the most critical source of variability in analyses of controlled-release formulations.

Intermediate precision. Six sample solutions were prepared and analysed on two separate days using two different HPLC systems (Thermo Dionex Ultimate 3000 and Agilent 1260 Infinity II) operated by different analysts.

The mean assay results were 100.23% and 100.98% on Day 1 and Day 2, respectively, with individual RSD values of 0.3% and 0.2%, resulting in an overall RSD of 0.5%. This minimal inter-day variation indicates that the method is suitable for routine use as shown in Table III.

Robustness. Robustness of the method was evaluated by introducing small, deliberate variations in key chromatographic parameters and assessing their impact on critical method responses, in accordance with ICH Q2 (R2) recommendations. A Plackett-Burman design was employed to systematically investigate the influence of 5 factors on 2 levels: flow rate, column temperature, acetonitrile content in the mobile phase, buffer pH and column age. The new column was activated before the analysis while the old column was previously used and had more than 1000 injections. A total of 19 experiments were generated and analysed using software MODDE Go (Umetrics), allowing efficient screening of factor effects with a minimal number of experiments.

The selected responses included peak asymmetry, number of theoretical plates, and retention time, as these parameters are directly related to method performance and chromatographic efficiency. The evaluated ranges for each factor are summarized in Table IV, while the experimental matrix and corresponding results are presented in Table V.

Table IV List of experimental factors and levels
Tabela IV Lista eksperimentalnih faktora i nivoa

Factor	Unit	Type	Normal conditions	Lower limit	Upper limit
Flow rate	mL min^{-1}	Quantitative multilevel	1.0	0.8	1.2
Column temperature (T)	^{o}C	Quantitative multilevel	25	20	30
% of acetonitrile in the mobile phase	%	Quantitative multilevel	5	4	6
Buffer	pH	Quantitative multilevel	3.0	2.8	3.2
Column	/	Qualitative	/	Old	New

Table V Plan of experiments and experimentally obtained results
Tabela V Plan eksperimenata i eksperimentalno dobijeni rezultati

Experiment No	Flow rate (mL min^{-1})	Column temperature (°C)	Acetonitrile (%)	Buffer pH	Column	Asymmetry	Theoretical plates (N)	Retention time (min)
N1	0.8	20	4	2.8	New	1.6	8255	4.6
N2	1.2	20	4	2.8	Old	1.4	8411	3.0
N3	0.8	30	4	2.8	Old	1.3	12108	4.3
N4	1.2	30	4	2.8	New	1.5	9729	2.9
N5	0.8	20	6	2.8	Old	1.2	11975	3.5
N6	1.2	20	6	2.8	New	1.3	7994	2.3
N7	0.8	30	6	2.8	New	1.3	12776	3.4
N8	1.2	30	6	2.8	Old	1.1	11137	2.2
N9	0.8	20	4	3.2	Old	1.7	9123	4.9
N10	1.2	20	4	3.2	New	1.7	5989	3.4
N11	0.8	30	4	3.2	New	1.7	9889	4.7
N12	1.2	30	4	3.2	Old	1.3	9824	3.2
N13	0.8	20	6	3.2	New	1.3	10420	3.8
N14	1.2	20	6	3.2	Old	1.2	9388	2.5
N15	0.8	30	6	3.2	Old	1.1	13531	3.6
N16	1.2	30	6	3.2	New	1.3	10510	2.4
N17	0.8	20	4	2.8	Old	1.4	10361	4.5
N18	0.8	20	4	2.8	Old	1.5	10345	4.5
N19	0.8	20	4	2.8	Old	1.4	10356	4.5

Statistical evaluation of the data was performed by the analysis of variance (ANOVA). The obtained models demonstrated strong descriptive and predictive capabilities, with coefficient of determination (R^2) exceeding 0.95 for all responses, indicating that the models explained more than 95% of the variability of the data. $Q2 > 0.5$ confirmed good predictive power and reliability of the models of the models in estimating the effects of the investigated factors. Model validity was > 0.25 for peak asymmetry, which indicates that the model was statistically sound and free from significant lack of fit. For the number of theoretical plates and retention time, validity value was less than 0.25. According to the MODDE guideline, the model validity is derived from a lack-of-fit test that compares model residual to pure error. In the case of retention time and number of theoretical plates, the low model validity is attributed to the extremely low pure error estimated from the replicate experiments. These responses usually show very low differences between replicates under same experimental conditions that were also observed in this case. Thus, the lack-of-fit test becomes overly sensitive, resulting in artificially low validity values. The adequacy of the model is strongly supported by high

R^2 and Q^2 values, small R^2–Q^2 differences and high reproducibility. With all the above considered, the suitability of the model is confirmed for robustness assessment (19).

Finally, model reproducibility which reflects the degree of control over the experimental system, was greater than 0.9 for all the evaluated responses. This high reproducibility confirms that analytical method is stable and not significantly affected by small variations in chromatographic conditions.

In addition to the ANOVA-based evaluation, the experimental data were further analysed using Partial Least Squares (PLS) regression to assess the contribution of individual factors to the investigated responses. The summary of fit parameters for the PLS model is presented in Figure 2, where the high values of R^2 and Q^2 confirm the adequacy and predictive capability of the model.

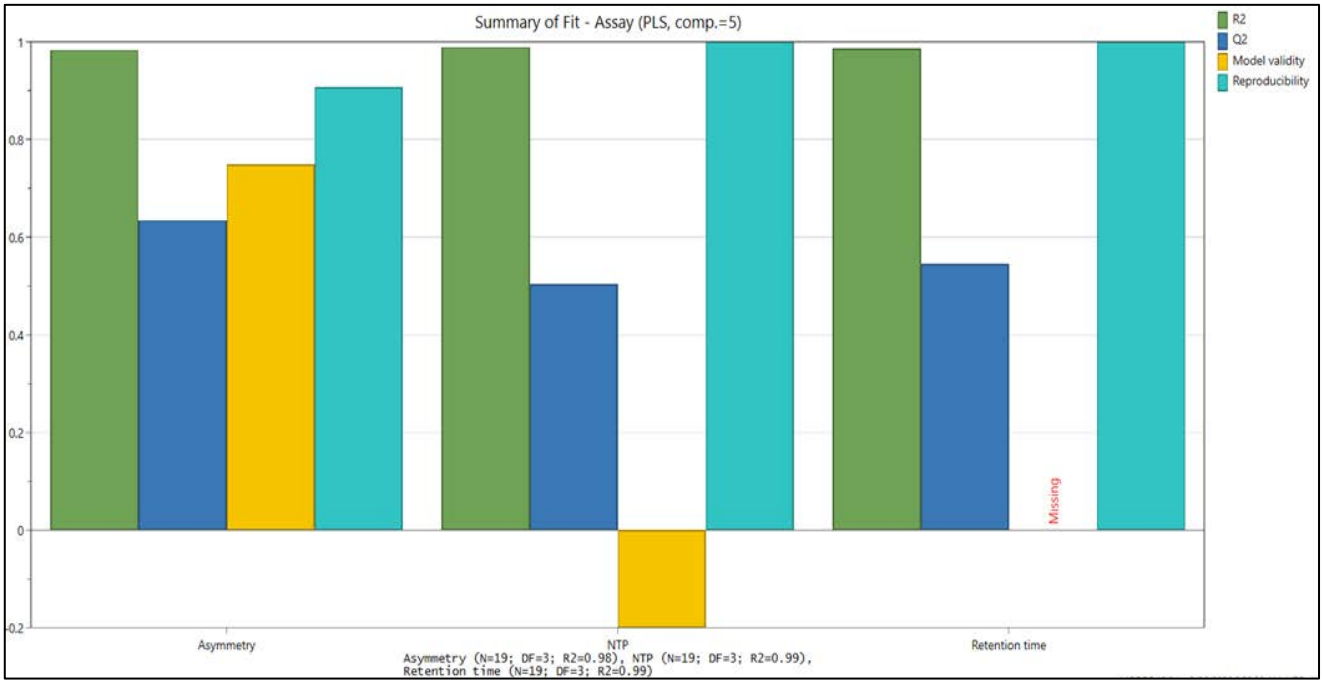

Figure 2. Summary of fit (Partial least squares regression – PLS)
Slika 2. Rezime statističke prilagođenosti (PLS regresija – parcijalna regresija najmanjih kvadrata)

The qualitative contribution of each factor on the responses was analysed using the Pareto charts, as shown in Figure 3. Pareto charts establish the critical value of the effect. A critical threshold value of 1.0 was applied to identify statistically significant effects. All calculated coefficients were below this threshold, indicating that none of the investigated factors exerted a statistically significant influence on the selected responses within the ranges studied. This finding further supports the robustness of the method, demonstrating its insensitivity to small, deliberate variations in chromatographic conditions.

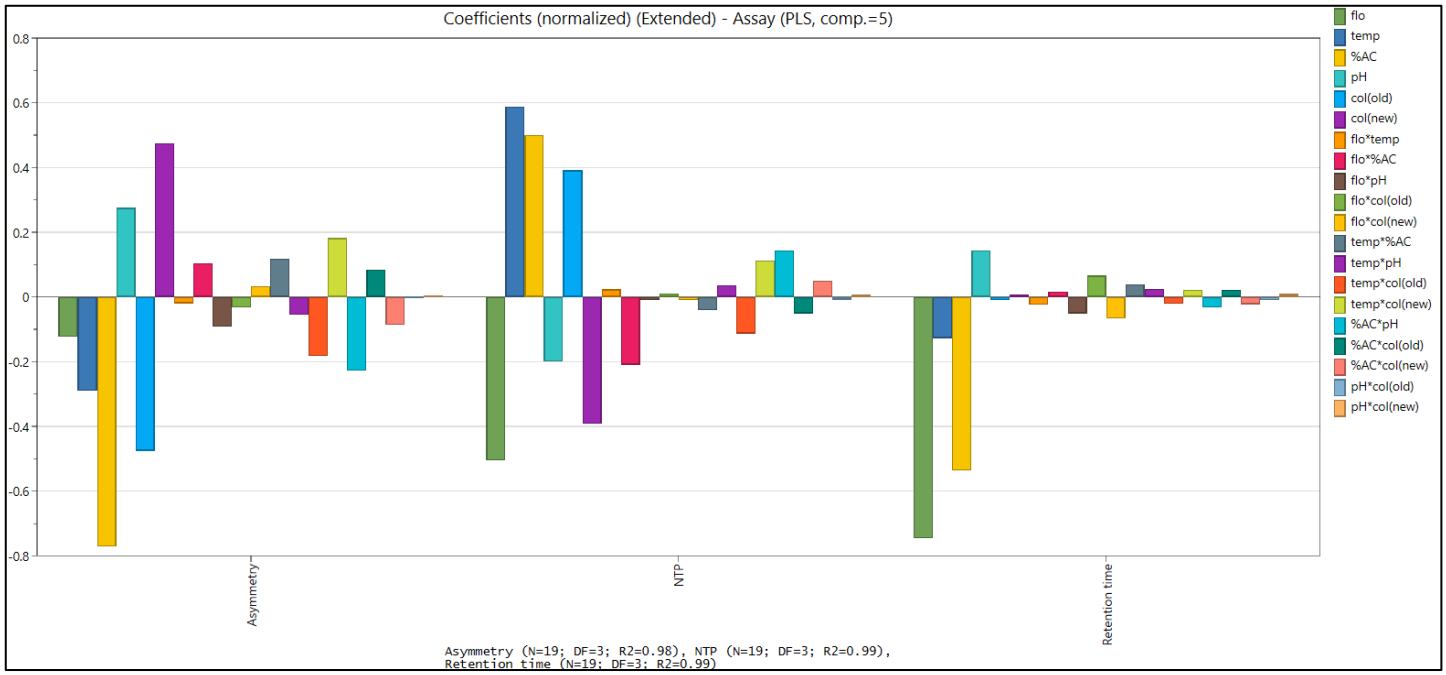

Figure 3. Pareto chart showing the significant effects based on the observation of Plackett-Burman design for the investigated responses
Slika 3. Pareto dijagram koji prikazuje značajne efekte zasnovane na rezultatima Plackett-Burman dizajna za ispitivane odgovore

To further define the operational limits of the method, a design space model (the sweet spot diagram) for all factors, was constructed and presented in Figures 4 and 5. In this approach, one factor was fixed (column), while the remaining variables were varied within the investigated ranges as previously presented in Table V. The green area represents the part of the design space where all predefined acceptance criteria for the observed responses are met (sweet spot). Following criteria were applied for assessment: Peak symmetry between 0.8 and 2.0, and number of theoretical plates ≥ 2000.

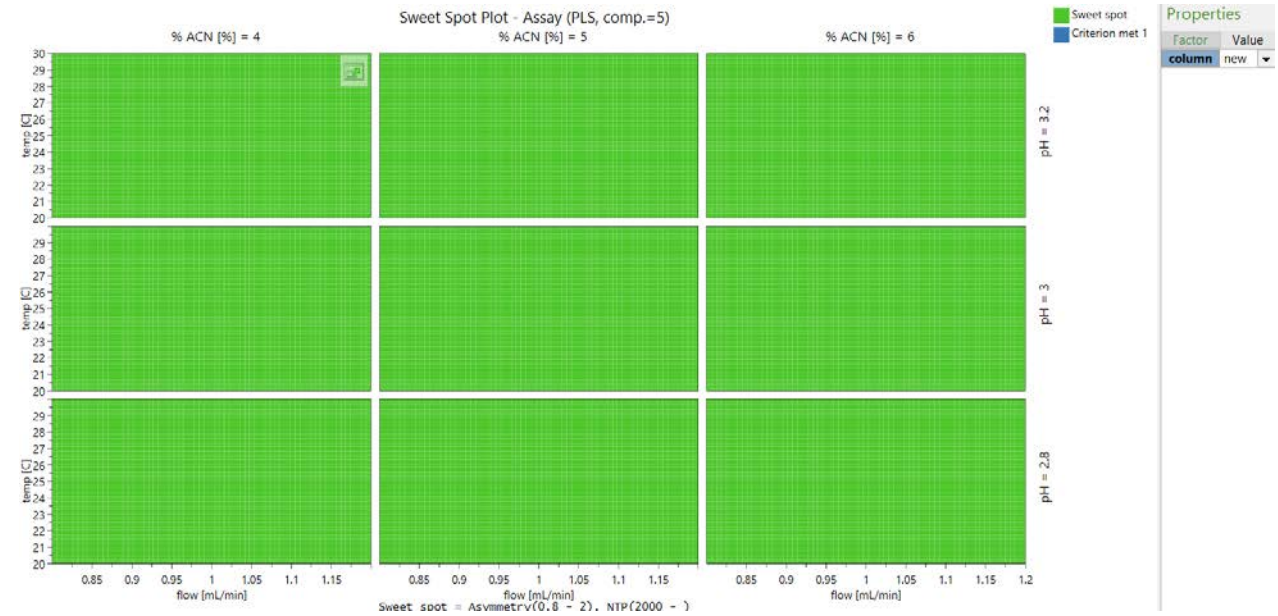

Figure 4. Sweet spot diagram (with fixed factor new column)
Slika 4. Dijagram optimalne zone (sa fiksiranim faktorom – nova kolona)

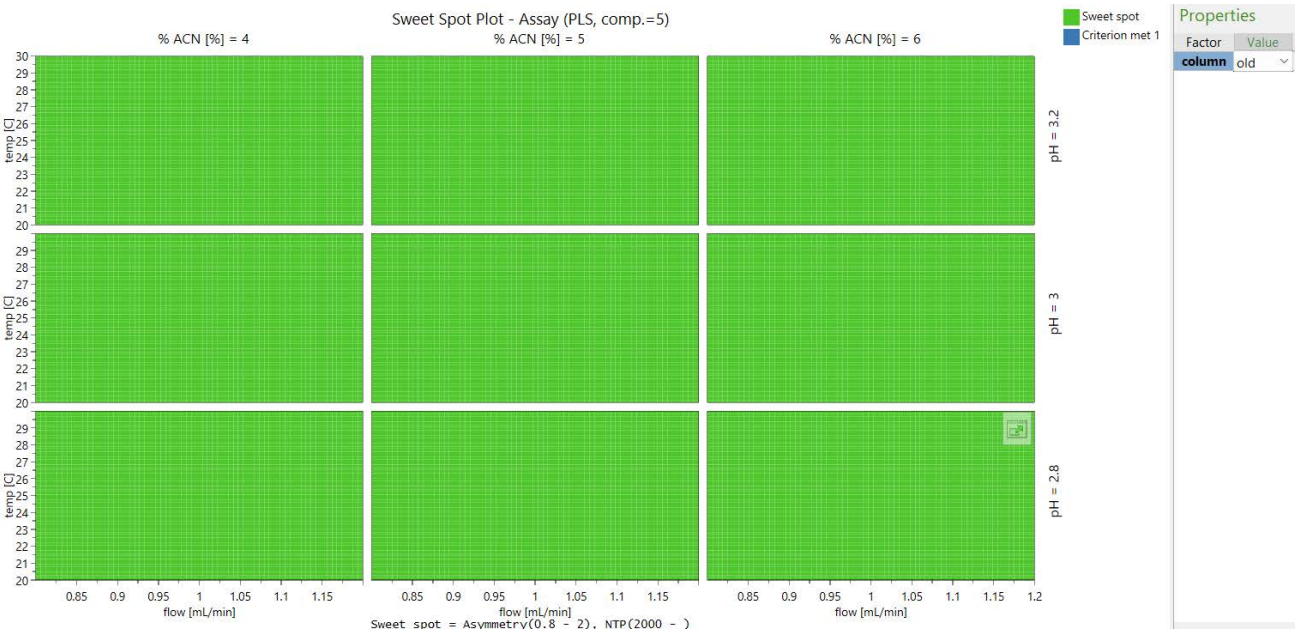

Figure 5. Sweet spot diagram (with fixed factor old column)
Slika 5. Dijagram optimalne zone (sa fiksiranim faktorom – stara kolona)

The resulting sweet spot diagrams indicate a relatively wide design space, confirming that the method remains stable across a broad range of operating conditions.

This wide operational window demonstrates that normal variations in chromatographic parameters do not adversely affect method performance. The absence of statistically significant factor effects observed in the Pareto analysis is consistent with these findings and further confirms the robustness and reliability of the developed analytical method.

Stability of Standard Solution. The stability of the standard solution was evaluated by analysing the solution immediately after preparation and at predefined time intervals while stored at room temperature. The results are presented in Table VI.

Table VI Stability of standard solution and sample solution
Tabela VI Stabilnost standardnog rastvora i pripremljenog rastvora uzoraka

	Standard solution		**Sample solution**	
Time interval	**Area**	**Recovery (%)**	**Area**	**Recovery (%)**
Initial	32.4582	/	32.5561	/
24 hours	32.5766	100.4	32.4570	99.7
48 hours	32.0167	98.6	32.3479	99.4
72 hours	32.6157	100.5	32.5728	100.1
96 hours	32.6082	100.5	32.5628	100.0
7 days	32.7118	100.8	32.5476	100.0

The obtained recoveries remained within the range of 98.6 – 100.8% over a period of 7 days, indicating no significant degradation or loss of analyte. The minimal variation in peak area over time confirms that the standard solution is chemically stable under the tested conditions. Therefore, the standard solution can be considered stable for at least 7 days at room temperature.

Stability of Sample Solution. The stability of the sample solution was assessed under the same conditions as the standard solution. The results, summarized in Table VI, demonstrate that the recoveries remained within a narrow range of 99.4 – 100.1% throughout the 7-day period.

These findings indicate that the analyte remains stable in the presence of the formulation matrix and that no significant degradation or adsorption occurs over time. The sample solution is therefore considered stable for at least 7 days at room temperature.

Filtration Study – Standard Solution. The effect of filtration on analyte recovery was evaluated by comparing the peak areas obtained from six independently filtered aliquots of the standard solution (0.45 µm regenerated cellulose filter) with those of an unfiltered solution. The results are presented in Table VII.

Table VII Filter study on standard solution and sample solution
Tabela VII Ispitivanje uticaja filtriranja standardnog rastvora i pripremljenog rastvora uzorka

	Standard solution		Sample solution	
	Area	**Recovery (%)**	**Area**	**Recovery (%)**
Unfiltered solution	32.1374	/	31.9668	/
Filtered solution	32.3917	100.8	32.0107	100.1
	32.4156	100.9	31.9672	100.0
	32.2911	100.5	31.9634	100.0
	32.4333	100.9	31.9171	99.8
	32.3928	100.8	31.9328	99.9
	32.4303	100.9	31.9686	100.0
	Mean	100.8	Mean	100.0
	RSD	0.2	RSD	0.1

The mean recovery after filtration was 100.8% with an RSD of 0.2%, indicating negligible loss of analyte during the filtration process. These results confirm that the selected filter material is suitable and does not introduce adsorption or retention of the analyte.

Filtration Study – Sample Solution. A similar filtration study was performed for the sample solution, where filtered aliquots were compared with an unfiltered (centrifuged) solution. The results are summarized in Table VII.

The mean recovery was 100.0% with an RSD of 0.1%, demonstrating excellent agreement between filtered and unfiltered samples. This confirms that filtration does not affect analyte recovery and that the selected filtration procedure is appropriate for routine analysis.

Overall, the optimized chromatographic system combined with the two-step acetonitrile–water extraction provides a selective, precise, and robust analytical platform for the quantitative determination of polar compounds in HPMC-based controlled-release formulations and is readily applicable to similar analytical challenges.

Conclusion

This work presents a comprehensive approach to overcoming one of the major analytical challenges associated with controlled-release formulations based on hydroxypropyl methylcellulose (HPMC): the quantitative recovery of polar compounds from a hydrated polymer matrix. The study systematically explores the influence of solvent composition, polarity, and agitation technique on the efficiency and reproducibility of extraction, leading to the development of a robust, mechanistically justified sample preparation procedure.

The optimized two-step protocol – comprising an initial 15-minute ultrasonic extraction with pure acetonitrile followed by 45 minutes of magnetic stirring after

addition of water – was found to effectively disrupt the swollen HPMC network, while simultaneously providing conditions favourable for complete dissolution of the polar analyte. This sequence exploits the contrasting physicochemical properties of acetonitrile and water: the former induces polymer dehydration and collapse of the gel structure, while the latter rehydrates the system in a controlled manner to promote solubilization without reforming the gel. The result is a homogeneous, particle-free solution suitable for direct chromatographic analysis. This innovative approach successfully eliminated the variability and incomplete recovery typically encountered with single-solvent or hydro-alcoholic systems.

Coupled with ion-pair reversed-phase HPLC, the developed sample-preparation strategy yielded a method characterized by high analytical performance in line with the criteria of ICH Q2 (R2). Overall, the optimized chromatographic system combined with the two-step acetonitrile–water extraction provides a selective, precise, and robust analytical platform for the quantitative determination of polar compounds in HPMC-based controlled-release formulations and is readily applicable to similar analytical challenges. By addressing the often overlooked issue of polymer–solvent interactions during analytical sample preparation, this study contributes to improving the reliability of assay methods used for controlled-release dosage forms.

Beyond its immediate analytical application, the methodology provides a transferable framework for similar systems containing polar, ionic, or weakly basic drugs. Future studies may expand its application to different HPMC viscosity grades, polymer blends, and alternative hydrophilic matrices such as hydroxyethyl cellulose or polyethylene oxide. The underlying mechanistic insight into solvent–polymer–drug interactions may also guide the design of standardized sample preparation protocols, facilitating harmonization of analytical practices across laboratories and regulatory environments. The proposed approach enhances both the scientific understanding and the practical reliability of quality control testing for modern controlled-release pharmaceutical products.

Acknowledgments

We would like to express sincere gratitude to Alkaloid AD Skopje and its leadership for providing the essential resources and the opportunity to carry out this research. Our deepest appreciation goes to our colleagues, for their invaluable guidance, constructive feedback, and continuous encouragement throughout the research process. We are also grateful to our professors and colleagues from the Faculty of Pharmacy for their support, insightful discussions, and commitment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

M.B.: conceptualization, data curation, formal analysis, validation, writing – original draft; **T.A.:** conceptualization, formal analysis; **A.A.** and **P.A.**: resources, supervision; **O.G.** and **A.P.P.**: writing – review and editing.

References

1. Borandeh S, van Bochove B, Teotia A, Seppälä J. Polymeric drug delivery systems by additive manufacturing. Adv Drug Deliv Rev. 2021;173:349–73. doi: 10.1016/j.addr.2021.03.022.
2. Chandra S, Sharma V, Kumawat S. Extended-release tablets: An overview. Int J Health Adv Clin Res. 2023;1(4):85–9.
3. Ghogare AS, Gholap PD, Gadhe KB, Ghule RA, Kolpe NM. A review on an extended-release drug delivery system. Int J Novel Res Dev. 2024;9(5):367–76.
4. Arévalo-Pérez R, Maderuelo C, Lanao JM. Recent advances in colon drug delivery systems. J Control Release. 2020;327:703–24. doi: 10.1016/j.jconrel.2020.09.026.
5. Park C, Lee JH, Jin G, Ngo HV, Park JB, Tran TT, et al. Release kinetics of hydroxypropyl methylcellulose governing drug release and hydrodynamic changes of matrix tablet. Curr Drug Deliv. 2022;19(5):520–33. doi: 10.2174/1567201818666210820101549.
6. Colombo P, Bettini R, Santi P, De Ascentiis A, Peppas NA. Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport. J Control Release. 2000;39(2–3):231–7. doi: 10.1016/S0168-3659(96)01488-8.
7. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev. 2012;64:163–74. doi: 10.1016/j.addr.2012.09.028.
8. Maboos M, Yousuf RI, Shoaib MH, Nasiri I, Hussain T, Ahmed HF, Iffat W. Effect of lipid and cellulose based matrix former on the release of highly soluble drug from extruded/spheronized, sintered and compacted pellets. Lipids Health Dis. 2018;17:136. doi: 10.1186/s12944-018-0783-8.
9. Dressman JB, Reppas C. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. Eur J Pharm Sci. 2000;11:S73–S80. doi: 10.1016/S0928-0987(00)00181-0.
10. Phaechamud T. Variables influencing drug release from layered matrix system comprising hydroxypropyl methylcellulose. AAPS Pharm SciTech. 2008;9(2):668–74. doi: 10.1208/s12249-008-9085-1.
11. Qiu Y, Zhang GGZ. Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice. Burlington: Academic Press; 2016. doi: 10.1016/C2012-0-03385-1.
12. Shoaib MH, Siddiqi SA, Yousuf RI, Zaheer K, Hanif M, Rehana S, et al. Development and evaluation of hydrophilic colloid matrix of famotidine tablets. AAPS Pharm SciTech. 2010;11(2):708–18. doi: 10.1208/s12249-010-9427-7.
13. Roy H, Brahma CK, Nandi S, Parida KR. Formulation and design of sustained release matrix tablets of metformin hydrochloride: Influence of Hypromellose and polyacrylate polymers. Int J Appl Basic Med Res. 2013;3(1):55–63. doi: 10.4103/2229-516X.112242.

14. Venkatesh DN, Meyyanathan SN, Kovacevic A, Zielińska A, Fonseca J, Eder P, et al. Effect of hydrophilic polymers on the release rate and pharmacokinetics of acyclovir tablets obtained by wet granulation: In vitro and in vivo assays. Molecules. 2022;27(19):6490. doi: 10.3390/molecules27196490.
15. Ford JL. Design and evaluation of hydroxypropyl methylcellulose matrix tablets for oral controlled release. Drug Dev Ind Pharm. 1999;25(6):647–58. doi: 10.1081/DDC-100102246.
16. Siepmann F, Siepmann J. Mathematical modeling of drug delivery. Int J Pharm. 2008;364(2):328–43. doi: 10.1016/j.ijpharm.2008.09.004.
17. ICH Q2(R2): Validation of Analytical Procedures [Internet]. International Council for Harmonization (ICH); 2023 [cited 2026 May 29]. Available from: https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf.
18. Navarro-Lupión FJ, Bustamante P, Escalera B. Relationship between swelling of hydroxypropyl methylcellulose and the Hansen and Karger partial solubility parameters. J Pharm Sci. 2005;94(7):1608-16. doi: 10.1002/jps.20370.
19. Siepmann J, Podual K, Sriwongjanya M, Peppas NA, Bodmeier R. A new model describing the swelling and drug release kinetics from hydroxypropyl methylcellulose tablets. J Pharm Sci. 1999;88(1):65–72. doi: 10.1021/js9802291.
20. Sartorius Stedim Data Analytics AB.
21. MODDE® User Guide. Version 12. Umeå, Sweden, 2017.

Optimizacija pripreme uzoraka i evaluacija analitičkih performansi model polarnog jedinjenja u tabletama sa kontrolisanim oslobađanjem na bazi hidroksipropil metilceluloze

Marijana Bozhinovska[1,2*], Tina Achkoska[1], Ana Atanasova[1], Packa Antovska[1], Olga Gigopulu[2], Ana Poceva Panovska[2]

[1]Istraživanje i razvoj, Alkaloid AD Skopje, Bul. Aleksandar Makedonski 12, 1000 Skopje, Severna Makedonija
[2]Farmaceutski Fakultet, Univerzitet Sv. Kiril i Metodij, Ul. Majka Tereza 47, 1000 Skopje, Severna Makedonija

*Autor za korespondenciju: Marijana Bozhinovska, e-mail: mtrendafilovik@alkaloid.com.mk

Kratak sadržaj

Tablete sa kontrolisanim oslobađanjem na bazi hidroksipropil-metilceluloze (HPMC) pružaju brojne terapijske prednosti, ali istovremeno predstavljaju značajan analitički izazov pri određivanju sadržaja polarnih aktivnih supstanci, usled izražene sposobnosti polimera da bubri i formira gel. Cilj ove studije je razvoj i evaluacija robustnog dvostepenog postupka pripreme uzorka koji omogućava potpunu ekstrakciju i tačno određivanje sadržaja polarne model supstance baznih osobina koja je inkorporirana u HPMC matriks. Različiti rastvarači i tehnike mešanja sistematski su upoređeni radi identifikacije uslova koji obezbeđuju kompletnu ekstrakciju analita. Optimizovani postupak obuhvata inicijalnu ekstrakciju acetonitrilom u trajanju od 15 minuta u ultrazvučnom kupatilu, nakon čega sledi hidratacija vodom u trajanju od 45 minuta uz mešanje na magnetnoj mešalici, pre analize u uslovima jon-par reverzno-fazne HPLC metode. Acetonitril efikasno narušava strukturu HPMC mreže, sprečava zarobljavanje analita u gelu i omogućava kvantitativni prinos (100 ± 0,5%) uz izuzetnu ponovljivost (RSD < 1%). Razvijena analitička metoda pokazuje linearnost (R^2 = 0,9997), tačnost (99,6%), preciznost (RSD ≤ 0,5%), kao i zadovoljavajuću robustnost i stabilnost rastvora, u skladu sa zahtevima smernice ICH Q2 (R2). Predloženi dvostepeni postupak ekstrakcije pripremom smeše acetonitril-voda predstavlja pouzdanu strategiju za određivanje sadržaja polarnih aktivnih supstanci u HPMC tabletama sa kontrolisanim oslobađanjem i može poslužiti kao metodološki okvir validacija specifičnih za određeno jedinjenje.

Ključne reči: hidroksipropil metilceluloza, tablete sa kontrolisanim oslobađanjem, polarna supstanca, priprema uzorka, ekstrakcija acetonitrilom, procena performansi metode