Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 91 (6) 589–602 (2026)
JSCS–5512
Original scientific paper
Published 2 July, 2026

Embracing green chromatography principles in perindopril, amlodipine and indapamide drug mixture analysis using *β*-cyclodextrin modified mobile phase

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(Received 4 December 2025, revised 6 February, accepted 11 March 2026)

Abstract: Raising the level of environmental awareness in the field of liquid chromatography is considered indispensable while the use of *β*-cyclodextrin, as additive, in a mobile phase is promising strategy in this regard. This study presents a method development in line with ICH Q14 regulatory requirements for introducing sustainability and method life-cycle management to separate components of a cardiovascular multi-drug tablet formulation. At the beginning, the analytical method target profile was defined, separation of perindopril, amlodipine, and indapamide in a shortest possible analytical run time. Following risk analysis pointed out that the mobile phase constituents represent the critical method parameters affecting the chromatographic analyses. Design of experiments methodology and desirability function calculation was employed to simultaneously optimize the levels of concentration of *β*-cyclodextrin solution, pH value and acetonitrile content in the mobile phase investigated in the ranges 5–15 mM, 4.0–6.0 and 20–30 vol. %, respectively. The optimal chromatographic conditions consisted of 10 mM *β*-cyclodextrin (pH 5.4) and acetonitrile in the volume ratio 70:30, 2 mL min^{-1} flow rate, RP-18e column kept at 25 °C, 215 nm detection wavelength, and 10 µL injection volume. The eco-friendliness of the method was assessed using the AGREE tool indicating a green and sustainable method was successfully developed.

Keywords: HPLC method development; design of experiments; desirability function; cardiovascular multi-drug tablet formulation; AGREE assessment tool.

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https://doi.org/10.2298/JSC251204013O

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INTRODUCTION

Achieving sustainability in the domain of pharmaceutical sciences involves integrating environmental and social considerations from the earliest stages of development through to end-of-life management. This includes using green chemistry principles, developing eco-friendly analytical methods, designing sustainable manufacturing processes and optimizing drug product formulations, packaging and distribution to reduce waste and energy consumption.[1]

Multi-drug formulations have increasingly become a significant challenge in the field of drug analysis due to the complexity and resource demanding requirements associated with resolving these compounds. This is attributed to the growing interest in rationally designed multi-target drugs, also known as multimodal drugs, network therapeutics or designed multiple ligands. These drugs have emerged as an appealing drug discovery paradigm over the past decade to address diseases with complex etiologies and those exhibiting substantial drug resistance.[2,3] Multi--drugs are also means to sustainable environment as less resources are used in their manufacture and consecutive quality control analysis.

Multi-component drug formulation used in this research comprised of a mixture of perindopril *tert*-butylamine (also referred as perindopril erbumine), amlodipine besylate and indapamide active pharmaceutical substances which structures are presented in Fig. 1. Their respective drug formulation has been successfully used for the treatment of cardiovascular diseases, considering that perindopril is an angiotensin-converting enzyme inhibitor that lowers blood pressure by reducing sodium and water retention,[4] amlodipine besylate is a calcium channel blocker that lowers blood pressure by relaxing blood vessels[5] and indapamide is a diuretic that causes water elimination hence reducing the pressure inside blood vessels.[6]

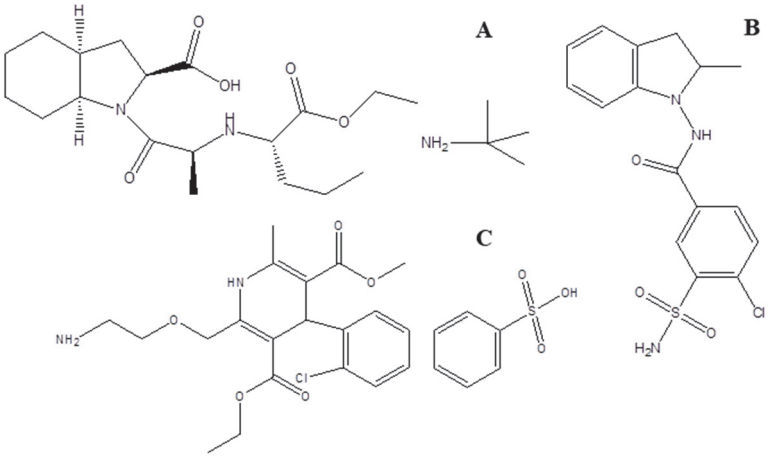


Fig. 1. Chemical structures of: A) perindopril erbumine, B) indapamide and C) amlodipine besylate.

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In the realm of drug analysis, high pressure liquid chromatography (HPLC) stands as the de facto gold standard. Among the various HPLC techniques, reversed phase high performance liquid chromatography (RP-HPLC) predominates, comprising approximately 75 % of the reported methodologies. Historically, the technique has predominantly utilized acetonitrile as preferred organic solvent in the mobile phase. At the same time, contemporary scientific advancements, particularly the green analytical chemistry (GAC) concept, emphasize the primary objective of reducing or eliminating the utilization and production of substances that pose risks to human health or the environment, as delineated by Anastas.[1] It is evident now that HPLC inherently lacks environmental friendliness due to the substantial quantities of solvents employed, which ultimately become waste at the end of the analysis. These wastes become more hazardous to the environment as well as the health and wellbeing of analysts involved if the solvents used are toxic. In the light of the growing need to achieve the global Sustainable Development Goals, SDG 9.4, which aims to enhance research and upgrade industrial technologies, particularly in developing countries, by 2030. SDG 12.4–6 also focuses on the environmentally sound management of chemicals and waste, aiming to reduce their release and minimize adverse impacts on human health and the environment.[7] However, new and promising strategies highlighted as green chromatography principles that rely on the adjustment of the mobile phase composition emerge, thereby enhancing sustainability of the HPLC method without compromising its performance.[8–11] There have been several assessment tools for measuring such achievement of a method, such as the GAPI, ESA and NEMI index. However, recently released AGREE tool incorporated in an open access software outstands out for his notable and relevant advantages. In addition to the ecological criteria evaluated by other tools, AGREE considers the number of analytes determined in a single run, sample throughput, automation, and the use of chemicals from renewable sources.[12,13]

The inclination towards introducing GAC concept in the field of drug analysis and the literature review of the reported HPLC methods used for the quality control of ternary drug combination containing perindopril erbumine, amlodipine besylate and indapamide revealing that these methods have predominantly utilized extensive amounts of toxic organic solvents, indicated that it is necessary to offer analytical procedure improvement by means of development of an alternative sustainable and eco-friendly HPLC method.[14,15]

Cyclodextrins (CDs) are semi-natural compounds derived from starch (a renewable resource) through enzymatic conversion. Their diverse applications in chemistry, pharmaceuticals, food and cosmetics stem from their non-toxicity and cost-effectiveness.[16] When CDs are used as additives into the mobile phases in HPLC analysis they could reduce the organic solvent–water ratio without compromising selectivity or resolution. The potential usefulness of CDs in HPLC separations comes out of the ability of CDs to form inclusion complexes with the guest

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molecules thus affecting their retention. The efficiency of complexation depends on the structural compatibility between the CD and the guest molecule. The height and internal diameter of the CD cavity are determined by the number of glucose units. α-CD has a lower internal diameter compared to β-CD and γ-CD, enabling them to incorporate low molecular weight compounds with aliphatic chains, β-CD can accommodate heterocyclic and aromatic compounds, spanning a wide range of active pharmaceutical substances (APIs), while γ-CD can accommodate complex macrocycles and steroids. Among others, β-CDs are the most utilized CDs in pharmaceutical formulations. Furthermore, β-CD's weak adsorption onto C18 columns ensures preserving high column performance, facilitates easy washing, and minimizes damage compared to other CDs. At the same time, all CDs possess a unique advantage over other commonly used mobile phase additives by being transparent within the ultraviolet-visible range predominantly used in detectors in HPLC instruments.[13,17,18]

Sustainability in the field of development of analytical methods may be achieved by using various computer technology related products such as advanced data processing software and strategies that enable reduction of experimental work while maintaining high quality of gathered data. In that respect, the application of Design of Experiments (DoE) methodology has been recognized in the field of drug analysis over past decades. DoE is a robust structural approach based on the multifactorial planning of order and number of experiments to be executed thus reducing the use of resources as well the generation of waste, but as the most important fact, enabling better insight into factor interactions and factor response relations. DoE serves as a technique for optimizing multivariate systems, such as liquid chromatography, where various interdependent mechanisms, including column efficiency, retention factor, ionization efficiency and ion suppression, analytes' solubility, significantly impact the analysis.[19–21] At the same time, according to the International council for harmonization of technical requirements for pharmaceuticals for human use (ICH) Q14 guideline, adopting smarter method development approach supported by principles of quality risk management, enhances the reliability of analytical methods.[22] Developed methods also undergo the systematic process of method validation in accordance with ICH Q2(R1/R2)[23] guideline which is considered as necessary to ensure that the developed method is fit for its intended use.

Having all this in mind, the aim of this study was focused on the DoE supported development and validation of a HPLC method with β-CD-modified mobile phase as an eco-friendly HPLC alternative for the separation of the three APIs, perindopril erbumine, amlodipine besylate and indapamide from a comercially available tablet formulation.

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EXPERIMENTAL

Chemicals and reagents

β-CD of 98 % purity was purchased from Acros Organics, USA. An HPLC grade acetonitrile, water and ethanol were purchased from J.T. Baker Inc., USA. Perindopril erbumine, amlodipine besylate and indapamide reference substances were of Ph. Eur. quality. Co-Amlessa tablets containing 4 mg of perindopril erbumine, 10 mg of amlodipine besylate and 1.25 mg of indapamide per one tablet (Krka-Farma, Serbia) were purchased from a local drug store.

Chromatographic conditions and equipment

The experiments were performed on a Vanquish Core 3000 HPLC system (Thermo Fisher Scientific) equipped with quaternary pumps, autosampler, thermostated column department, and PDA detector. Chromatographic data was collected using Chromeleon® 7.0 and Chrom Quest 4.2 chromatography data system. Merck Chromolith RP-18e column (100 mm×4.6 mm, macropore size 2 μm, mesopore size 13 nm) was used for separations. *β*-CD aqueous solutions were prepared in the concentration range 5–15 mM. The organic part of mobile phase consisted of acetonitrile in range 20–30 vol. %. Column temperature was 25 °C, flow rate 2 mL min^{-1}, injection volume 20 μL and detection wavelength 215 nm.

Stock and working solutions preparation

Stock solutions were prepared by dissolving the powder reference substances in 50 vol. % ethanol to attain concentrations of 1 mg mL^{-1} for each API. Stock solutions were further diluted using the mixture of 10 mM *β*-CD (pH 5.4) and acetonitrile (70:30 volume ratio) as solvent to prepare one working solution containing 20 μg mL^{-1} of perindopril erbumine, 50 μg mL^{-1} of amlodipine besylate and 6.25 μg mL^{-1} of indapamide to be used within method optimization. A series of five working solutions containing raising concentrations of all APIs were prepared from stock solutions by dilution with the aforementioned solvent to be used within method linearity evaluation. Raising concentrations of working solutions were as follows: 10, 15, 20, 25 and 30 μg mL^{-1} for perindopril erbumine, 25.0, 37.5, 50.0, 62.5 and 75 μg mL^{-1} for amlodipine besylate and 3.125, 4.688, 6.250, 7.813 and 9.325 μg mL^{-1} for indapamide.

Sample preparation

The stock sample solution was prepared with the appropriate amount of powdered tablet mass containing 4 mg of perindopril erbumine, 10 mg of amlodipine besylate and 1.25 mg of indapamide and transferring it to a 50 mL volumetric flask. The volumetric flask was filled with 50 vol. % ethanol and the solution was filtered. Working sample solution was prepared by transferring of 2.5 mL of stock solution in 10 mL volumetric flask and dilution with the aforementioned solvent to attain concentrations 20, 50 and 6.25 μg mL^{-1} for perindopril erbumine, amlodipine besylate and indapamide, respectively, denoted as 100 % concentration level with respect to the declared value for each API. This sample solution was used both for method optimization and method repeatability test (6 sample solutions repetitive analysis) and intermediate method precision test (6 working solutions repetitive analysis performed on three separate days by different analysts) within method validation procedure. The similar procedure was applied for preparation of test sample solutions for accuracy test within method validation procedure with the difference that respective volumes of stock solution were transferred in a 50 mL volumetric flask, mixed with placebo and filled to volume with the aforementioned solvent to attain concentrations 80, 200 and 25 μg mL^{-1} for perindopril erbumine, amlodipine besylate and indapamide, respectively. This solution was filtered and diluted with the same solvent to attain test solution

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containing 16, 40 and 5.00 μg mL^{-1} for perindopril erbumine, amlodipine besylate and indapamide, respectively, representing 80 % concentration level for each API. Similarly, test solutions containing 20, 50 and 6.25 μg mL^{-1} representing 100 % concentrations level and 24, 60 and 7.50 μg mL^{-1} representing 120 % concentration level for perindopril erbumine, amlodipine besylate and indapamide, respectively, were prepared. The test sample solutions were prepared in triplicate for each of these concentration levels.

Method optimization

The influence of experimental factors on the retention behavior of the analytes was assessed with experimental design whose plan was constructed as well as obtained results processing using multiple regression analysis was performed using Design Expert 11.0.0 software (Stat-Ease Inc., USA). The Box–Behnken response surface design was used comprising of a total of 15 runs within which 3-level factors are varied in a predefined manner involving factor levels at the midpoints of the edges of the experimental space and level positioned at the center of the experimental space. The center point experiment was repeated 3 times in order to evaluate the experimental error.[15,16] Simultaneous optimizations of multiple responses or multi-objective method optimization, was also performed using the same software upon setting the specific numerical target values for each of the observed responses. Firstly, individual desirability functions are calculated with a value of 1 for the most ideal outcome for each of the observed responses reaching its target value and 0 for the least desirable outcome. Afterwards, the global desirability function was calculated combining the results of individual desirability functions assigning an overall score also expressed in a range of values 0–1. The overall desirability function represents a compromise and points out to the optimal experimental setup for which all observed responses are the closest possible to their individual target value.

Greenness assessment

Using the AGREE assessment scale, the method was graded for its greenness according to a twelve-point metric system adopted from the 12 GAC principles. Each of the 12 input variables is transformed into a scale in the 0–1 range, and the final result is the product of the assessment results for each GAC principle. The output is a clock-like graph, with the overall score and color representation generated using AGREE calculator (University of Vigo, Spain).

RESULTS AND DISCUSSION

According to the analytical method development strategy described within ICH Q14 recommendations, the first step is to define the target analytical profile of the new method. The optimization goals were accordingly aligned for the developed method to demonstrate satisfactory separation of adjacent peaks achieved within the least possible total analysis time. The observed chromatographic responses recognized as critical method attributes for achieving these optimization goals (CMAs) were retention factor (k) of the last eluting compound from the mixture, indapamide, as well as measure of separation, selectivity factors between all peak pairs (according to the observed elution order, the selectivity factors were: α_{1-2} between amlodipine and perindopril peaks and α_{2-3} between perindopril and indapamide peaks).

Multiple factors may affect the retention properties of the APIs under review. They may be classified as analysts, samples, mobile phase, column, instrument and

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detection related factors, as presented in Ishikawa or fishbone diagram (Fig. 2). The factor classification followed with constant, noise and experimental (CNX) evaluation as a risk analysis approach, enables insight into the critical method parameters (CMPs) of the analytical method.[21] The CNX risk-based approach defines the method parameters that should be kept under control, at constant level (marked with yellow color), which ones may be disregarded as noise factors (colored in green) and which parameters require detail experimental evaluation (colored in red). From the perspective of the GAC concept, special attention must be paid to materials, energy and waste. Factors that influence solvent consumption and directly affect the amount of generated wasted include the composition of the mobile phase. Therefore, the selection of suitable solvents is considered the most important step in the intended method development process.

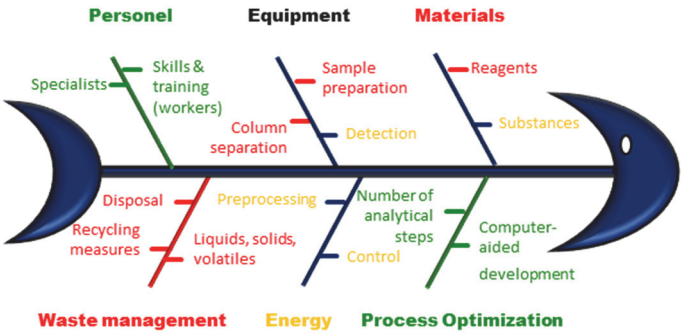


Fig. 2. Classification of important analytical method aspects with risk-assessment.

C18 reversed-phase stationary phase and the type of chromatographic column was selected before the method is optimized, based on previous experience and recommendations from the literature.[18] The stationary phase of monolithic columns is composed of highly porous continuous silica network, with macro- and mesopores. Macropores are 2 μm in size, and they are responsible for low resistance towards mobile phase flow. Therefore, monolithic columns are compatible with a high mobile phase flow rate, even up to 10 mL min^{-1}, accompanied by low pressure in the system. On the other hand, mesopores are smaller in comparison to macropores and they account for a huge active surface, approximately 300 m^2 g^{-1}, which enables efficient chromatographic separation. Allowing faster mobile phase flow rates and thus shortening the duration of chromatographic analyses, together with its compatibility with highly viscous mobile phases, such as CD-modified mobile phases, made the monolithic column the ideal choice for the separation of the intended drug mixture. With reference to other studies dealing with the use of β-CD in HPLC analysis, a study performed by Đajić *et al.*[18] recommended the use of β-CD aqueous solutions in a concentration range of 5–15 mM. The pH range for intended β-CD solutions was considered to be aligned with the pK_a values of the APIs. It

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was considered reasonable to for perindopril being the least lipophilic compound, to select a pH range where he is present in an undissociated form (pK_a values of perindopril, indapamide and amlodipine are 3.79, 8.85 and 9.60, respectively), while for other two compounds being very lipophilic to select pH value where they will have smaller affinity towards reversed-phase stationary phase (log *P* values of perindopril, indapamide and amlodipine are 0.63, 2.52 and 2.20, respectively). Therefore, the pH range of pH 4.0–6.0 was selected. To provide an appropriate total analytical run time, a relatively small amount of acetonitrile (20–30 vol. %) was used to complete the mobile phase. The relationship between selected CMPs whose levels were investigated inside experimental space bordered as described and CMAs was investigated using DoE supported regression analysis. The plan of experiments according to Box–Behnken response surface design together with results obtained for observed responses is presented in Table I.

TABLE I. DoE plan of experiments and results obtained for observed responses

β-CD concentration, mM	Acetonitrile content, vol. %	pH	$\alpha_{1\text{-}2}$	$\alpha_{2\text{-}3}$	*k*
15	20	5	11.388	7.834	13.024
15	25	6	3.301	8.378	6.992
5	20	5	7.097	2.589	5.030
15	25	4	1.945	5.474	4.392
10	25	5	5.087	8.060	7.171
10	25	5	3.884	6.668	6.234
5	30	5	7.920	3.174	4.158
15	30	5	2.507	6.610	4.222
5	25	4	2.812	6.783	7.325
10	25	5	3.693	7.557	7.270
10	30	6	3.191	7.574	4.089
10	20	6	4.960	3.478	4.952
10	30	4	2.469	5.953	4.051
10	20	4	2.863	10.806	14.114
5	25	6	4.885	6.100	3.522

Processing the data in Design Expert 11.0.0 software, regression mathematical models were obtained which enabled interpreting the dependence of the selected responses on the examined factors in their corresponding ranges. All models were in the form of a quadratic polynomial equation, while the need for response transformation was indicated by the software in some cases: the response $\alpha_{1–2}$ was used as is, while the response $\alpha_{2–3}$ was used as inverse square root and the response *k* was transformed with the power function, as shown in Table II. To facilitate the presentation of the polynomial equation, investigated factors are coded as follows: *A* stands for *β*-CD concentration (mM), *B* for acetonitrile content, vol. % and *C* for pH value of the aqueous part of the mobile phase. Only coefficients of regression

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model whose *p*-values were lower than 0.05 threshold limit indicating their statistical significance to the observed response were presented. The negative value of a coefficient in a polynomial equation indicates that the observed response decreases with the increase of the investigated factor levels, while the positive value of a coefficient indicated the same direction of a change of levels of investigated factors and values of observed responses. According to the size of the absolute values of coefficients in polynomial equations, it was evident that acetonitrile content, vol. %, appeared as the most influential factor for all observed responses, usually resulting in the observed response decrease, while the influence of other factors as well as the intensity of two-factor interactions changed from response to response. Regression models were also evaluated according to obtained values of coefficients of determination (R^2, adjusted R^2 and R^2 predicted), and the statistical significance of lack of fit value (Table III). The closeness of coefficients of determination to 1 and *p*-values of lack of fit being greater than 0.5 threshold limit indicated satisfactory ability of mathematical models to describe the observed chromatography systems and thus may be used for predicting retention properties of APIs according to predefined optimization goals.[19,20]

TABLE II. Coefficients of the regression models

Coefficient	$\alpha_{1\text{-}2}$	$1/(\sqrt{\alpha_{2\text{-}3}})$	$k^{2.36}$
Intercept	4.220	0.370	0.011
A	–0.450	–0.097	–0.005
B	–1.280	–0.021	0.011
C	0.780	0.065	0.005
AB	–2.430	0.023	0.004
AC	–0.180	–0.140	–0.016
BC	–0.340	–0.051	–0.005
A^2	1.440	0.120	0.007
B^2	1.570	–0.004	0.006
C^2	–2.420	0.021	0.008

TABLE III. Statistical profile of the regression models

Model	R^2	Adjusted R^2	Predicted R^2	Lack of fit *p*-value
$\alpha_{1\text{-}2}$	0.9367	0.8228	0.7602	0.2825
$1/(\sqrt{\alpha_{2\text{-}3}})$	0.9872	0.9641	0.8281	0.2701
$k^{2.36}$	0.9958	0.9882	0.9775	0.8938

3D-response surfaces were constructed and used as visualization tools for easier understanding of the APIs' retention behavior (Fig. 3). They show the dependence of each of the observed CMAs from the two selected CMPs while the remaining CMP is kept on a constant level. The analysis of the 3D response surfaces indicated that the increase in the acetonitrile content led to a decrease in

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retention of all APIs. When observing the influence of acetonitrile on the selectivity factors, the wavy appearance of response surfaces indicated the significant presence of factor interactions or mutual influence of acetonitrile in combination with other CMPs. In addition, the increase of β-CD concentration demonstrated a similar influence on retention pointing out that significant complexation between drug guest molecules and β-CD occurred enabling the mobile phase to retain appropriate elution strength.

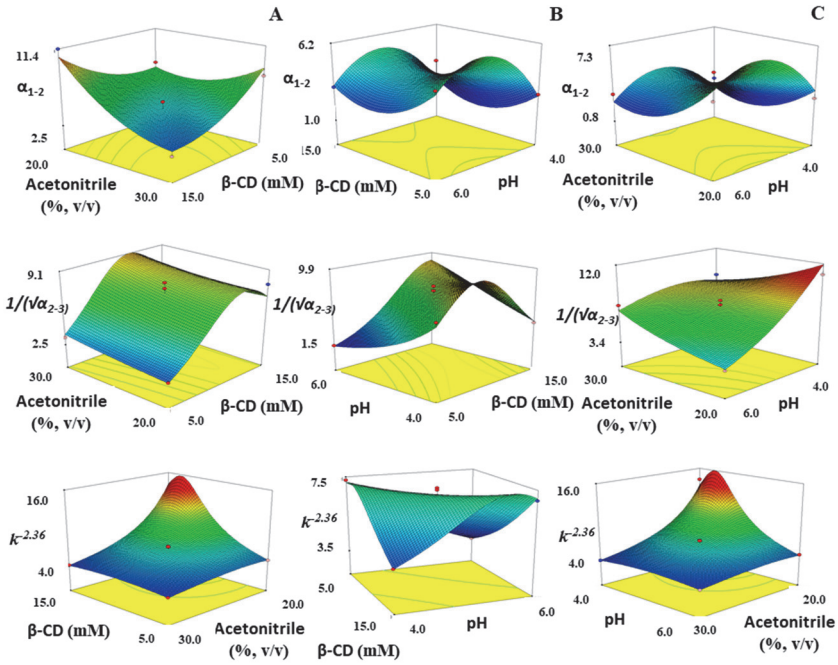


Fig. 3. Response surfaces describing dependencies of observed responses $\alpha_{1\text{-}2}$, $1/(\sqrt{\alpha_{2\text{-}3}})$ and $k^{2.36}$, respectively from factors: acetonitrile content (vol. %) and β-CD concentration (mM), *A*, pH value and β-CD concentration (mM), *B*, and pH value and acetonitrile content (vol. %), *C*.

The steep slope of the response surfaces describing the variation of β-CD concentration indicated the most significant influence of this factor on all observed responses, while the pH of the aqueous part of the mobile phase was always of lower importance. Interestingly, although the appearance of the response surfaces for the response $1/(\sqrt{\alpha_{2-3}})$ pointed out to very dramatic shifts within investigated ranges of CMPs, its values revealed relatively good separation of amlodipine and indapamide peaks, while other CMPs needed more detail considerations prior to selection of the optimal conditions. Having in mind that the responses α_{1-2} and $k^{2.36}$ demonstrated different trends upon the influence of investigated factors, it was difficult to define from the response surfaces what would the optimal solution be.

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Therefore, numerical multi-objective optimization was performed using the desirability function (*D*) calculation.[11] It is considered that the desired fulfillment of predefined CMAs is reached if the maximal value of *D* is equal to 1, indicating that the compromise solution is met for which all the optimization goals are as close as possible to the predefined goals. For the proposed method, analytical target profile of the method was achieved using the following chromatographic conditions: 10 mM *β*-CD (pH 5.4) and acetonitrile (70:30 volume ratio), 25 °C column temperature, 215 nm detection wavelength, 2 mL min^{-1} flow rate and 10 μL injection volume. In accordance with ICH Q14 concept,[22] measures of quality assurance were further considered in order to prove that CMAs will be reached under these conditions with appropriate probability and therefore model coefficients uncertainty were discussed. Low values of standard errors of model coefficients (varying from model to model in range –0.026–0.090) and low correlation of residuals for all regression models (0.182, 0.093 and 0.091, respectively) were noted thus proving appropriate regression model quality.

The verification chromatogram was recorded both with mixture of reference substances and sample solution under selected optimal chromatographic conditions with a total analytical run time within three min, as shown in Fig. 4.

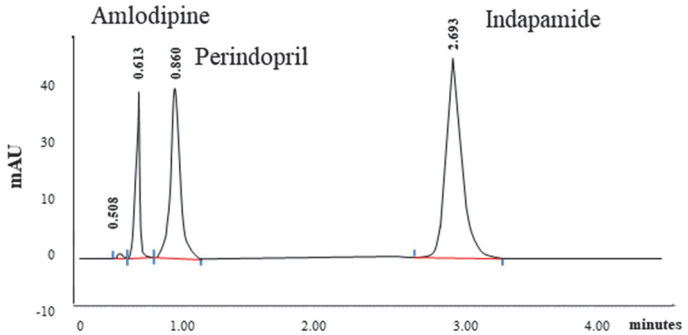


Fig. 4. Representative HPLC chromatogram recorded using mobile phase composed of 10 mM *β*-CD (pH 5.4) and acetonitrile (70:30 volume ratio).

System suitability test parameters were further evaluated: the asymmetry factor (*As*) meeting the acceptance criteria $0.8 < As < 1.5$, the number of theoretical plates (*N*) greater than 2000 and appropriate system precision expressed as percent relative standard deviation value (*RSD*) being lower than 1 % (Table IV). The HPLC method was validated to prove its suitability for intended use, the quality control of commercially available tablets. Method linearity and range, precision, repeatability and accuracy were tested following the procedure required by ICH guideline.[23] From the data shown in Table IV, it may be seen that all validation parameters met appropriate acceptance criteria. Method linearity was demonstrated by the value of calculated correlation coefficient being greater than 0.998.

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The *RSD* values indicated good method repeatability and intermediate precision since they were lower than 2 and 3 % in average, respectively. Average percent Recovery values calculated for 3 concentration levels were in range 98–102 % indicating good method accuracy.

Table IV. Results of method validation and system suitability tests

Parameter	Amlodipine besylate	Perindopril erbumine	Indapamide
Asymmetry factor	1.33	1.45	1.36
Number of theoretical plates	4150	5907	4856
System precision, *RSD* / %	0.39	0.72	0.12
Linearity range, μg mL^{-1}	25–75	10–30	3.125–9.325
Correlation coefficient	0.9997	0.9991	0.9993
Repeatability, *RSD* / %	1.35	1.25	1.77
Intermediate precision, *RSD* / %	1.41	1.30	1.56
Accuracy (Recovery, %)	98.53	99.32	100.01

In order to evaluate HPLC method greenness and sustainability profile, the assignment of penalty points to every step of the analytical procedure was performed in the accordance with the procedure of calculating AGREE score.[12,13] In that respect, acetonitrile present in the mobile phase was labeled as danger and so appropriate penalty points were taken. In contrast, *β*-CD was considered safe. Then, it was noted that none of the chemicals used exceeded the amount of 20 mL per analysis. Afterwards, energy consumption was considered. HPLC instruments commonly use more than 1.5 kWh or less than 0.5 kWh of electrical currency per sample, and therefore penalty points are assigned to this technique. The PDA detector was used, which takes less than 0.5 kWh of electrical energy per sample. The generated waste was collected and it is possible to be recycled. HPLC system used is hermetically closed system. Therefore, penalty points accounting for produced waste are assigned only on the basis of its amount. The positioning of the analytical instrument was also taken into consideration, which is an inline analysis. This adds to greenness points as there's is elimination of manual sampling and reduction of waste. Considering all mentioned, the final AGREE score obtained was 0.7 (Fig. 5) which is higher compared to the values obtained from similar studies analyzing

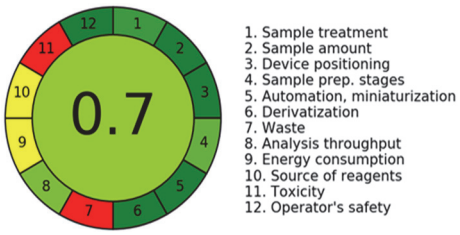


Fig. 5. AGREE greenness assessment results.

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the same three APIs (0.45 and 0.5).[14,15] This method is therefore declared as far superior in its eco-friendliness.

CONCLUSION

Understanding that in recent times there is a need to develop analytical procedures for the drug analysis which are sustainable and eco-friendly is of outmost importance. The contribution of every stakeholder in the industry is much needed towards the achievement of the sustainable development goals, in this regard the present work is the kind contribution of the authors to develop a new greener and sustainable HPLC method for the analysis of perindopril, amlodipine and indapamide in a ternary mixture. The method has AGREE score 0.7 indicating the most compliant profile to sustainability and GAC principles compared to previous reports.

Acknowledgement. The research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through Grant Agreements with University of Belgrade Faculty of Pharmacy, No. 451-03-33/2026-03/200161 and 451-03-34/2026-03/ /200161.

И З В О Д

ПРИМЕНА ПРИНЦИПА ЗЕЛЕНЕ ХРОМАТОГРАФИЈЕ У АНАЛИЗИ СМЕШЕ ПЕРИНДОПРИЛА, АМЛОДИПИНА И ИНДАПАМИДА КОРИШЋЕЊЕМ *β*-ЦИКЛОДЕКСТРИНА КАО МОДИФИКАТОРА МОБИЛНЕ ФАЗЕ

ХУСЕИНАТУ ОСМАН, ЈЕВРЕМ СТОЈАНОВИЋ, АНА ПРОТИЋ, МИРА ЗЕЧЕВИЋ и БИЉАНА ОТАШЕВИЋ

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Подизање нивоа еколошке свести у домену развоја метода течне хроматографије је неопходно, при чему употреба *β*-циклодекстрина као адитива мобиле фазе представља релативно новију стратегију која обећава. Овај рад приказује развој методе усаглашен са захтевима ICH Q14 смернице за сагледавањем одрживости и управљањем животним циклусом методе, са циљем да се обезбеди хроматографска анализа вишекомпоненте таблетне формулације која се користи у терапији кардиоваскуларних болести. Најпре је дефинисан жељени профил методе, постизање добре раздвојености пикова периндоприла, амлодипина и индапамида у што краћем времену, а затим је урађена анализа ризика које је указала да компоненте мобиле фазе представљају критичне параметре методе који утичу на ток хроматографске анализе. Методологија дизајна експеримената и израчунавање функције пожељних одговора, искоришћени су за истовремену оптимизацију нивоа концентрације раствора *β*-циклодекстрина, pH вредности и удела ацетонитрила у мобилној фази који су испитивани у опсезима редом 5–15 mM, 4,0–6,0 и 20–30 запр. %. Оптимални хроматографски услови укључивали су: 10 mM раствор *β*-циклодекстрина (pH 5,4) и ацетонитрил у запреминском односу 70:30 при протоку од 2 mL min^{-1}, RP-18e колону загрејану на 25 °C, таласну дужину детекције од 215 nm и запремину узорка од 10 μL. Процена еколошке прихватљивости методе помоћу AGREE алата је потврдила да је успешно развијена зелена и одржива хроматографска метода.

(Примљено 4. децембра 2025, ревидирано 6. фебруара, прихваћено 11. марта 2026)

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REFERENCES

1. P. Anastas, *Crit. Rew. Anal. Chem.* **29** (2010) 167 (https://doi.org/10.1080/10408349891199356)
2. W. Löscher, *Front. Pharmacol.* **12** (2021) 730257 (https://doi.org/10.3389/fphar.2021.730257)
3. J. J. Lu, W. Pan, Y. J. Hu, Y. T. Wang, *PLOS One* **7** (2012) 40262 (https://doi.org/10.1371/journal.pone.0040262)
4. S. Julius, J. N. Cohn, J. Neutel, M. Weber, P. Turlapaty, Y. Shen, V. Dong, A. Batchelor, H. Lagast. *J. Clin. Hypertens.* **25** (2007) 10 (https://doi.org/10.1111/j.1524-6175.2004.02440.x)
5. T. Godfraind, *J. Cardiovasc. Pharmacol. Ther.* **19** (2014) 501 (https://doi.org/10.1177/1074248414530508)
6. D. B. Campbell, *Curr. Med. Res. Opin.* **8** (1983) 9 (https://doi.org/10.1185/03007998309109832)
7. United Nations General Assembly, *Transforming our world: the 2030 Agenda for Sustainable Development*, A/RES/70/1 (2015) (https://docs.un.org/en/A/res/70/1)
8. M. Yabré, L. Ferey, I. T. Somé, K. Gaudin, *Molecules* **23** (2018) 1065 (https://doi.org/10.3390/molecules23051065)
9. S. El Deeb, *Molecules* **29** (2024) 3205 (https://doi.org/10.3390/molecules29133205)
10. J. Stojanović, J. Krmar, B. Otašević, A. Protić, *Arh. Farm.* **73** (2023) 146 (https://doi.org/10.5937/arhfarm73-43479)
11. N. Đajić, J. Krmar, J. Stojanović, B. Svrkota, B. Otašević, A. Malenović, A. Protić, *Arh. Farm.* **74** (2024) 235 (https://doi.org/10.5937/arhfarm74-49572)
12. F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, *Anal. Chem.* **92** (2020) 10076 (https://doi.org/10.1021/acs.analchem.0c01887)
13. L. A. Yahya, C. Vakh, O. Dushna, O. Kalisz, S. Bocian, M. Tobiszewski, *Anal. Chim. Acta* **1357** (2025) 344052 (https://doi.org/10.1016/j.aca.2025.344052)
14. K. Patel, *Sep. Sci. Plus* **3** (2020) 175 (https://doi.org/10.1002/sscp.202000010)
15. K. N. Prashant, Y. Pragathi, S. Yenduri, *Green Anal. Chem.* **12** (2024) 100182 (https://doi.org/10.1016/j.greeac.2024.100182)
16. S. Agatonović-Kustrin, M. Lebete, M. E. Brown, D. W. Morton, B. Glass, *J. Serb. Chem. Soc.* **81** (2016) 1037 (https://doi.org/10.2298/JSC150915018A)
17. V. González-Ruiz, A. G. León, A. I. Olives, M. A. Martín, J. C. Menéndez, *Green Chemistry* **13** (2011) 115 (https://doi.org/10.1039/C0GC00456A)
18. N. Maljurić, J. Golubović, B. Otašević, M. Zečević, A. Protić, *Anal. Bioanal. Chem.* **410** (2018) 2533 (https://doi.org/10.1007/s00216-018-0911-3)
19. U. A. Thorsteinsdóttir, M. Thorsteinsdóttir, *J. Mass Spectrom.* **56** (2021) 4727 (https://doi.org/10.1002/jms.4727)
20. S. N. Hidayah, M. Mazidatu lakmam, S. M. F. Bukhari, R. N. Yani, *J. Serb. Chem. Soc.* **90** (2025) 1203 (https://doi.org/10.2298/JSC250124062S)
21. B. Svrkota, J. Krmar, F. Petronijević, A. Protić, B. Otašević, *J. Sep. Sci.* **48** (2025) 70136 (https://doi.org/10.1002/jssc.70136)
22. European medicinal agency, *ICH Q14 Guideline on analytical procedure development* (2023) (https://www.ema.europa.eu/en/ich-q14-analytical-procedure-development-scientific-guideline)
23. European medicinal agency, *ICH Q2(R2) Guideline on validation of analytical procedures* (2024) (https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures-scientific-guideline).

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