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VALIDATION OF THE METHOD FOR QUANTIFYING NARINGIN IN GRAPEFRUIT (*CITRUS PARADISI*) EXTRACT USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract. The study validates the HPLC method for quantifying naringin in a grapefruit extract. The demonstrated high accuracy, precision, and reproducibility were achieved with recovery ranging from 99.73% to 100.65%. The method linearity was confirmed by a correlation coefficient of 0.999. These findings have significant implications for grapefruit-based pharmaceutical development.

Keywords: naringin; naringenin; validation; linearity; HPLC-DAD.

1. Introduction

The global market for herbal medicines has been growing steadily in recent years. Using herbal remedies for the prevention and treatment of diseases is particularly important in chronic processes where long-term treatment is necessary, and the lack of obvious side effects is important due to the complex effect of medicinal plants and preparations on the human body.

The choice of analytical procedure was based on a comprehensive review of existing methodologies and optimization for the specific matrix of interest. Quintão *et al.*[1] clarified a procedure for detecting naringenin in skin layers using a reversed-phase C18 column and a methanol/phosphoric acid mobile phase. While this methodology demonstrated high sensitivity, accuracy, and precision, its specific focus on skin permeation may not be directly applicable to grapefruit extract analysis. Jha *et al.*[2] delineated a correlation between HPLC and UV spectrophotometric methods for quantifying naringenin in solid dispersions. Although UV spectroscopy appeared to be a cost-effective alternative, the precision and specificity offered by HPLC for grapefruit extract quantification rendered it the preferred method. Ribeiro and Ribeiro[3] presented an HPLC technique for the simultaneous determination of naringin and naringenin concentrations in citrus juices. While the focus on citrus matrices was promising, the method required further optimization for grapefruit extract. Caccamese and Chillemi[4] explored chiral HPLC for studying naringin racemization in pummelo, offering insights into citrus fruit bitterness variations. Although insightful for citrus fruit maturation studies, the method's specific focus on chirality might not be directly applicable to the current study's objective. Asghari *et al.*[5] proposed a sample preparation technique aimed at reducing time and solvent consumption compared to standard solid-phase extraction. The focus on multiple flavonoids in citrus fruit juice suggested potential applicability for grapefruit extract. Liu *et al.*[6] showcased a rapid-resolution liquid chromatography technique for the simultaneous determination of multiple bioactive flavonoids in Citri Reticulatae Pericarpium. While the method was time-efficient and sensitive, it required validation for grapefruit extract analysis. Baranowska *et al.*[7] introduced an HPLC method, combined with mass spectrometry, for chiral separation and quantification of flavonoid enantiomers. Despite its potential for analyzing the chiral nature of flavonoids in natural products, its complexity may not be necessary for the quantification of naringin in grapefruit extract. Considering the reviewed methodologies, an HPLC method was deemed the most suitable due to its precision, specificity, and versatility. The method was then tailored and validated for the quantification of naringin in grapefruit extract, yielding promising results with high accuracy, precision, and reproducibility. The validated method promises significant utility for grapefruit-based pharmaceutical development.[8]

Validation of the method for the quantification of naringin in grapefruit extract by high-performance liquid chromatography is the process of assessing the parameters of the method to establish its suitability for use in practice.[9] Method validation is necessary to ensure accurate and reliable analysis results. Therefore, the main purpose of the study is to assess the validation of the method for

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determining the amount of naringin in grapefruit extract obtained from grapefruit residues using high-performance liquid chromatography (HPLC). To achieve the above purpose, the following tasks were established and implemented: determine the naringin content in grapefruit extract by high-performance liquid chromatography; the method should be validated to ensure the validity and accuracy of its results. It will provide reliable data on the naringin content of grapefruit extract and guarantee its quality.

2. Experimental

The following reagents were used to validate the method for the determination of naringin in grapefruit extract obtained from grapefruit residues grown in Azerbaijan: standard naringin (CAS 10236-47-2, reference standard, Sigma-Aldrich, Germany), acetonitrile (CAS 75-05-8, HPLC gradient, Sigma-Aldrich, Germany), acetic acid (CAS 64-19-7, HPLC gradient, Sigma-Aldrich, Germany), ammonium oxalate (CAS 1113-38-8, HPLC gradient, Sigma-Aldrich, Germany), dimethylformamide (CAS 68-12-2, HPLC gradient, Sigma-Aldrich, Germany), matrix (mixture of pectin, CAS 9000-69-5; methylcellulose, CAS 9004-67-5; chlorhexidine, CAS 55-56-1; dimethyl sulfoxide, CAS 67-68-5; and propylene glycol, CAS 57-55-6), model mix, empty solution (mobile phase used). The substances used in the research were used directly and were not subjected to any further purification steps. In this study, the reagents were weighed using a precision analytical balance from Mettler-Toledo, specifically chosen for its suitability for high-accuracy tasks such as this one. For this particular study, we utilized a model that offers a precision of up to 0.0001 g, ensuring the utmost accuracy in our weighing processes integral to the validation of the HPLC method for naringin quantification. For the solution preparation part, the label indicated: "1129 S" Solution preparations:

1. Preparation of the mobile phase. In a 1000-mL volumetric flask with 800 mL of deionized water, 200 mL of acetonitrile and 0.5 mL of acetic acid were added and stirred, followed by degassing in an ultrasonic water bath.

2. Preparation of the solvent for the sample solution: 3.55 g of ammonium oxalate was placed in a 1000-mL volumetric flask, 200 mL of deionized water was added, shaken, and then the volume of the solution was supplemented with deionized water. The resulting solution was incubated in an ultrasonic bath for 15 minutes.

3. Preparation of the solvent for the standard: 0.06 g of ammonium oxalate was placed in a 1000-mL volumetric flask, 200 mL of deionized water was added, shaken, and the volume of the solution was supplemented with deionized water. The resulting solution was then incubated in an ultrasonic bath for 15 minutes.

4. Empty solution. The mobile phase was used without the addition of a sample or standard.

Preparation of the model mixture (grapefruit extract and the active ingredient in the required proportions were used to determine the validation values):

1. To prepare the matrix, 2 g of pectin was soaked in 25 mL of chlorhexidine until fully swollen. In parallel, 1 g of methylcellulose was first dissolved in 25 mL of deionized water to form a clear solution. This methylcellulose solution was then added to the swollen pectin-chlorhexidine mixture. Once combined, the mixture was homogenized in a water bath at 50-60 °C. After cooling, 2 mL of dimethyl sulfoxide and 2 mL of propylene glycol were introduced, and the mixture was stirred until completely homogenized. Lastly, additional deionized water was added to bring the final volume to the mark on the volumetric flask.

2. To prepare the test solution, 50 mL of the obtained grapefruit extract was placed in a 200 mL volumetric flask, and 10 mL of 0.025 mol/mL ammonium oxalate and 10 mL of dimethylformamide were added and shaken. The solution was then stored in an ultrasonic bath for 5 minutes. The volume of the solution was supplemented with deionized water and stirred. This solution was then filtered through a polytetrafluoroethylene filter with a pore size of 0.45 μm.

3. To prepare a standard solution, 10 mg of standard naringin was placed in a 25-mL volumetric flask, and 10 mL of dimethylformamide was added and dissolved by shaking. The volume of the solution was then supplemented with 0.01 mol/mL ammonium oxalate and stirred. After obtaining the solution, it was filtered through a polytetrafluoroethylene filter with a 0.45 μm pore size.

The experiments were performed on an Agilent 1100 HPLC system with a UV detector (Agilent Technologies, USA). A Zorbax SB-C18 column (Agilent Technologies, USA) with a particle size of 5 μm was used as the stationary phase. Chromatographic conditions: column temperature 30°C, mobile phase flow rate 1 mL/min, injection volume 10 μL. The analysis duration was 20 minutes. The amount of naringin in the grapefruit extract was calculated by high-performance liquid chromatography using the formula:

$$X = \frac{S \cdot M_s \cdot V_1}{S_0 \cdot M_n \cdot V_2} \cdot 100\,\%, \qquad (1)$$

where: X is the percentage content of naringin in the grapefruit extract; S is the average peak area of the sample (relative units); S_0 is the average peak area of the reference (relative units); M_s: mass of the standard (mg); M_n – mass of the sample (mg); V_1 – volume of the standard (mL); V_2 – volume of the sample (mL).

The analytical method validation values are determined according to the requirements of the relevant ana-

lytical regulations, namely: the Chemical Method Validation Manual for the Food FDA Programme and the ICH Harmonised Tripartite Manual.[10-13] The accuracy assessment was determined by analyzing six repetitions of the quality control samples. The chromatographic response of the LOQ (quantification of the smallest amount of the analyzed substance) must be five times higher than the blank response, with an accuracy of 80–120%. Together with the calibration curve, five repetitions of the standard were prepared. Linearity was assessed by a linear regression (correlation coefficient, R^2). The accuracy of the method was assessed using three concentrations of grapefruit extract: low, medium, and high. In addition, accuracy was determined by comparing each concentration with its coefficient of variation (CV%), standard error (SE), and relative standard deviation (RSD).

3. Results and Discussion

The research team determined optimal conditions by conducting a comprehensive review of existing methodologies and then optimizing the chosen method for the specific grapefruit extract matrix. They adjusted parameters such as the mobile phase composition, flow rate, and detection conditions to ensure the method's accuracy, precision, and specificity for grapefruit extract analysis. Optimal conditions were determined: solution volume 10 µL, flow rate 1 mL/min, temperature 30°C, mobile phase ratio 200/800, Zorbax column SB-C18 (4.6x100 mm). A naringin standard with a known level of purity was used to assess the accuracy of the method. Several standard solutions of naringin with different concentrations were prepared for this purpose. First, 14.4 mg of standard naringin was transferred into a 25-mL volumetric flask. Then 10 mL of dimethylformamide was added and dissolved by shaking. The volume of the solution was brought to the label with 0.01 mol/mL ammonium oxalate and mixed thoroughly. Thus, a solution with a concentration of 0.576 mg/mL was obtained. Similar steps were taken for the preparation of standard solutions of naringin with concentrations of 0.72 mg/mL (18 mg naringin) and 0.864 mg/mL (21.6 mg naringin). Three chromatograms were taken for each solution using high-performance liquid chromatography, and particular values were calculated (Figs. 1 and 2, Table 1).

To assess the accuracy of the method for the quantification of naringin in grapefruit extract by HPLC, six repeat quality control samples were analyzed. This analysis was repeated five times on two different days to assess the accuracy and validity of the method and intra-day variability (Tables 2 and 3). Thus, method validation involved an accuracy assessment based on a comparison of the results of six samples from a homogeneous series. These procedures were performed according to the protocol for sample preparation, obtaining HPLC chromatograms, and calculating the corresponding indices.[7] Following a protocol will help to ensure the repeatability and reliability of results and ensure that the method meets quality requirements and regulatory standards.

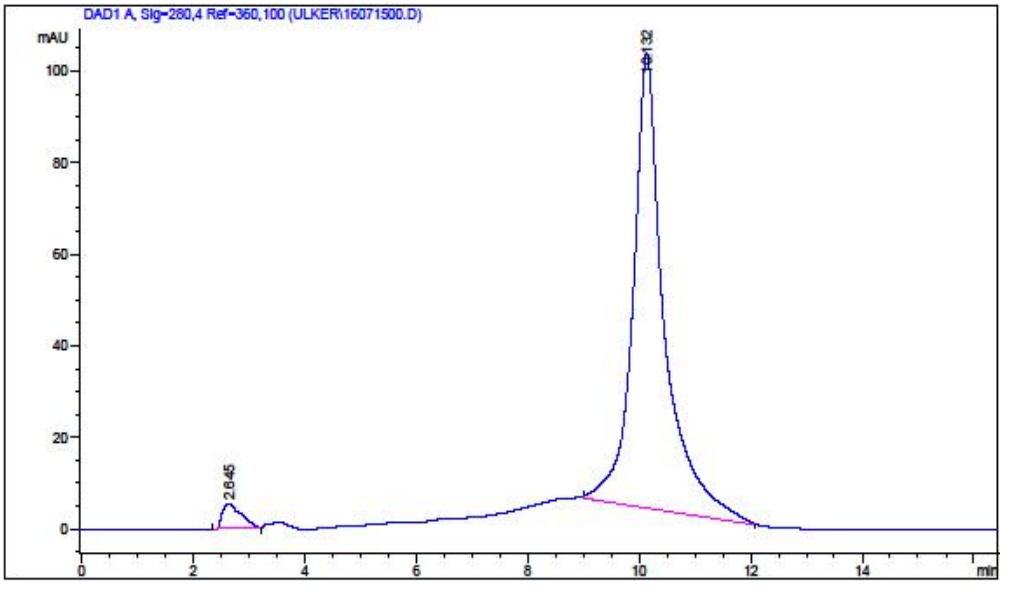

Fig. 1. Chromatogram of a standard sample of naringin obtained by HPLC
Source: compiled by the authors.

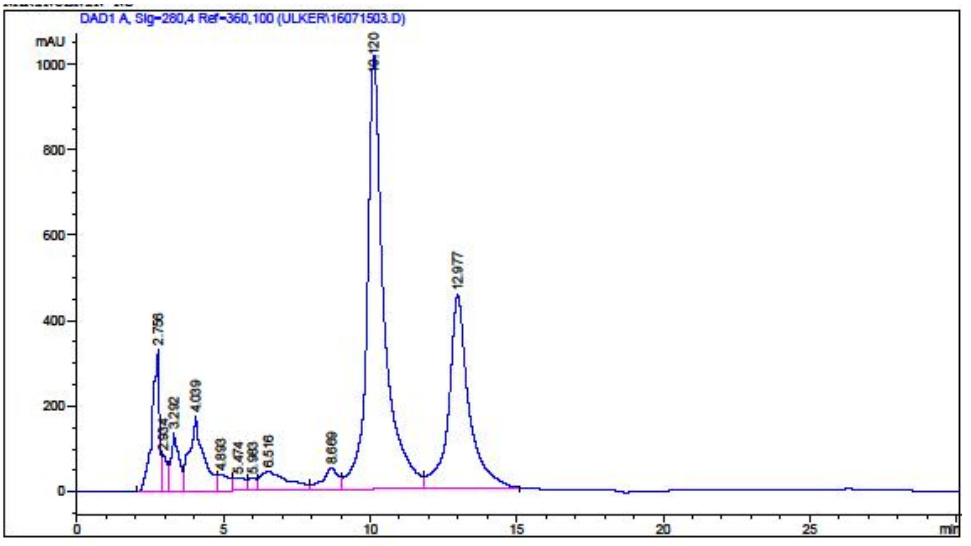

Fig. 2. Chromatogram of naringin in a test sample obtained by HPLC
Source: compiled by the authors.

Table 1. Method accuracy in the determination of naringin by HPLC

Amount taken, mg	Peak area, AU	Quantity found, mg	Restoration*, %
14.4	2584.1	14.3	99.5
	2580.3	14.2	98.8
	2584.0	14.4	100.1
18	3230.2	18	99.8
	3229.1	18.2	100.8
	3229.1	18.1	100.6
21.6	3876.2	21.7	100.3
	3876.5	21.6	100.2
	3872.4	21.6	100.0
Root-mean-square deviation (%)			0.6
Standard error (SE)			0.2
Variation coefficient (%)			0.6
Lower limit of the confidence interval (%, p=95%)			99.6
Upper limit of the confidence interval (%, p=95%)			100.5

Note: *the ratio between the amount of narginin determined experimentally and taken by weight.
Source: compiled by the authors.

Table 2. Determination of the interdaily accuracy of the method for the quantification of naringin using HPLC

Amount taken, mg	Time, min	Peak area, a.u.	Quantity found, mg	Restoration, %
18.6	10.12	3225.4	18.7	100.7
18.6	10.12	3230.2	18.7	100.4
18.6	10.13	3219.3	18.7	100.6
18.6	10.12	3301.1	18.6	99.9
18.6	10.11	3221.9	18.7	100.6
18.6	10.11	3228.3	18.6	99.7
Root-mean-square deviation (%)				0.4
Standard error (SE)				0.2
Variation coefficient (%)				0.4
Confidence interval (p=95%)				0.4

Source: compiled by the authors.

Table 3. Determination of the intra-day precision of the method for the quantification of naringin by HPLC

Amount taken, mg	Time, min	Peak area, a.u.	Quantity found, mg	Restoration, %
18.6	10.11	3268.3	18.7	100.6
18.6	10.11	3229.1	18.7	100.7
18.6	10.11	3239.4	18.8	100.9
18.6	10.12	3300.1	18.5	99.4
18.6	10.12	3231.9	18.7	100.7
18.6	10.12	3238.6	18.5	99.4
Root-mean-square deviation (%)				0.7
Standard error (SE)				0.3
Variation coefficient (%)				0.7
Confidence interval (p=95%)				0.7

Source: compiled by the authors.

A narrower confidence interval indicates more accurate and reliable analysis results.

By analyzing the data obtained, it can be observed that there is a linear relationship between the concentration of the sample tested and the average area of the peak. It indicates that the method has a linear range, *i.e.*, the ability to quantitatively measure different concentrations of naringin in samples. Consequently, as the concentration of naringin in the sample increases, the average peak area will increase accordingly. The linear correlation is a desirable property of the method, as it allows accurate quantitative results for different concentrations of naringin. These results confirm that the method for quantifying naringin in grapefruit extract by HPLC is linear and suitable for use in practical applications where the concentration of naringin in samples needs to be measured with high accuracy and confidence.

Table 4. Calibration curve parameters

No.	Concentration (mg/ml)	Peak area, a.u.
1	0.3	2512.3
2	0.4	2822.9
3	0.4	3125.4
4	0.4	3457.9
5	0.5	3791.5
Correlation coefficient		0.9
Slope		7983.5
Y-intercept		-51.4
Correlation coefficient (R^2)		0.9
Standard error (SE)		12.7
Linear regression		y=7983.475x-51.39

Source: compiled by the authors.

Based on this data, it can be concluded that the provided calibration curve demonstrates good quality and accuracy. It allows a quite accurate prediction of naringin concentration based on the measured peak area, and this is confirmed by the high correlation coefficient, coefficient of determination, and low standard error.

Limits of detection (LOD): the smallest amount of an analyte that can be reliably detected by an analytical method but is not necessarily quantified with high accuracy. Limits of quantification (LOQ): the smallest amount of an analyte that can be quantitatively measured with a given accuracy and precision. Detection and quantification limits have been determined according to the guidelines of the ICH Harmonized Triangular Manual. These parameters are used to assess the sensitivity of the analytical method. Using the LOD and LOQ values allows for lower limits within which the method can be applied for the detection and quantification of naringin in grapefruit extract. If the concentration of naringin in the sample is below the LOD value, its presence may be questionable or uncertain. If the concentration of naringin is between LOD and LOQ, the method can detect its presence, but with limited accuracy. When the concentration of naringin exceeds the LOQ, the method can quantify its concentration with a given accuracy and precision. In the conducted study, the LOD was 0.005, which means that the method can detect the presence of naringin in grapefruit extract at a concentration as high as 0.005, but possibly without the ability to quantify it accurately. In addition, the LOQ was 0.016, which means that the method can quantify the presence of naringin in grapefruit extract at a concentration as low as 0.016 with a specified accuracy (Table 5).

Thus, after all these validation steps, the method for the quantitative determination of naringin in grapefruit extract using HPLC can be concluded to be reliable, accurate, and applicable to this analysis.

Our findings align with several previous studies, reinforcing the reliability of our method. Quintão *et al.*[1] validated a chromatographic method for naringenin quantification, demonstrating the importance of robust quantification techniques in related research areas. Suleria *et al.*[14] conducted screening and characterization of phenolic compounds in fruit peels, emphasizing the significance of

understanding the composition of fruit extracts for various applications. Martín and Liras[15] explored the biosynthesis of naringenin and related chalcones in actinobacteria and plants, shedding light on the production of bioactive metabolites.

Table 5. Parameters for LOD and LOQ values

Parameter	Value
Limits of detection (LOD)	0.005
Limits of quantification (LOQ)	0.016
Linear range	0.32-0.48
Correlation coefficient (R^2)	0.999
Accuracy (by restoration)	98.5%
Reproductivity (RSD)	1.9%

Source: compiled by the authors.

It is worth noting that our results are consistent with those of Guttman *et al*.,[16] who found that new grapefruit cultivars exhibited low cytochrome P4503A4-inhibition activity, supporting the accurate quantification of naringin in grapefruit extracts. Additionally, Ferreira-Nunes *et al*.[17] developed a versatile chromatographic method for catechin determination in natural extracts, showcasing the importance of analytical methods in studying complex matrices.

The correlation between our findings and those of Jha *et al*.[2] further supports the robustness of our method. They correlated two validated methods for the quantification of naringenin, highlighting the relevance of different analytical techniques for naringin analysis. Moreover, Agrawal *et al*.[18] emphasized the pharmacological significance of hesperidin and hesperetin, two citrus flavonoids, in various applications, including potential antiviral compounds.

The potential implications of our validated method extend to research related to grapefruit-based medicines, as suggested by Qurtam *et al*.,[19] who explored the mechanistic antidiabetic potential of citrus flavanone narirutin. Additionally, our results could inform the optimization of analysis parameters, such as mobile phase composition, flow rate, and thermostatic conditions, as proposed by Priyadarsani et al.[20] in the context of supercritical carbon dioxide extraction.

4. Conclusions

In conclusion, it has become clear that evaluating the HPLC method's applicability for real-world applications requires validating its capacity to quantify naringin in grapefruit extract. The comprehensive evaluation of method parameters has yielded highly promising outcomes. The established HPLC method for quantifying naringin has demonstrated remarkable precision and accuracy, with recovery values spanning from 99.7% to 100.7% and an average recovery of 100.3%. The data further substantiates the intraday accuracy, with all naringin recovery values consistently falling within the range of 99.4% to 100.9%. The low standard deviation of 0.7% signifies minimal variability throughout the experiment, affirming the method's repeatability and stability.

Moreover, the narrow coefficient of variation (0.67%) underscores the method's reliability, indicating a consistently low level of variability across samples and time points. The 0.7% confidence interval instills confidence in the precision of the results, strengthening the accuracy of the estimates. The calibration curve, which shows a correlation coefficient of 0.9, demonstrates the method's strong linearity. This high linear relationship between naringin concentration and peak area enhances the method's ability to predict naringin concentrations accurately. The coefficient of determination, at 0.9, explains a substantial 99.9% of the variability in peak area, further emphasizing the method's reliability.

In summation, the outcomes of this study firmly establish the HPLC method's accuracy, reproducibility, and linearity in quantifying naringin. These validated results hold significant practical implications for the pharmaceutical industry and research endeavors focused on the development of grapefruit-based medicinal products. Future research avenues may involve optimizing analytical parameters, including mobile phase composition, flow rate, and thermostatic conditions. These refinements could potentially unlock even greater potential for harnessing grapefruit's bioactive compounds for therapeutic applications.

References

[1] Quintão, W.S.C.; Ferreira-Nunes, R.; Gratieri, T.; Cunha-Filho, M.; Gelfuso, G.M. Validation of a Simple Chromatographic Method for Naringenin Quantification in Skin Permeation Experiments. *J. Chromatogr. B.* **2022**, *1201-1202*, 123291. https://doi.org/10.1016/j.jchromb.2022.123291

[2] Jha, D.K.; Shah, D.S.; Talele, S.R.; Amin, D. Correlation of Two Validated Methods for the Quantification of Naringenin in its Solid Dispersion: HPLC and UV Spectrophotometric Methods. *SN Appl. Sci.* **2020**, *2*, 698. https://doi.org/10.1007/s42452-020-2536-3

[3] Ribeiro, I.A.; Ribeiro, M.H.L. Naringin and Naringenin Determination and Control in Grapefruit Juice by a Validated HPLC Method. *Food Control* **2008**, *19*, 432–438. https://doi.org/10.1016/j.foodcont.2007.05.007

[4] Caccamese, S.; Chillemi, R. Racemization at C-2 of Naringin in Pummelo (Citrus Grandis) with Increasing Maturity Determined by Chiral High-Performance Liquid Chromatography. *J. Chromatogr. A* **2010**, *1217*, 1089–1093. https://doi.org/10.1016/j.chroma.2009.10.073

[5] Asghari, A.; Barfi, B.; Barfi, A.; Saeidi, I.; Ghollasi Moud, F.; Peyrovi, M.; Beig Babaei, A. Comparison between Conventional

Solid Phase Extraction and Its Simplified Method for HPLC Determination of Five Flavonoids in Orange, Tangerine, and Lime Juice Samples. *Acta Chromatogr.* **2014**, *26*, 157–175. https://doi.org/10.1016/j.chroma.2013.08.078
[6] Liu, E.-H.; Zhao, P.; Duan, L.; Zheng, G.-D.; Guo, L.; Yang, H.; Li, P. Simultaneous Determination of Six Bioactive Flavonoids in Citri Reticulatae Pericarpium by Rapid Resolution Liquid Chromatography Coupled with Triple Quadrupole Electrospray Tandem Mass Spectrometry. *Food Chem.* **2013**, *141*, 3977–3983. https://doi.org/10.1016/j.foodchem.2013.06.077
[7] Baranowska, I.; Hejniak, J.; Magiera, S. Development and Validation of a RP-UHPLC-ESI-MS/MS Method for the Chiral Separation and Determination of Flavanone, Naringenin and Hesperetin Enantiomers. *Talanta* **2016**, *159*, 181–188. https://doi.org/10.1016/j.talanta.2016.06.020
[8] Csuti, A.; Sik, B.; Ajtony, Z. Measurement of Naringin from Citrus Fruits by High-Performance Liquid Chromatography – A Review. *Crit Rev Anal Chem*. **2022**, 1–14. https://doi.org/10.1080/10408347.2022.2082241
[9] Suleymanov, T.A.; Balayeva, E.Z.; Akhmedov, E.Yu. Development and Determination of Validation Parameters for the HPLC Method of Thymol Quantification in "Kalinol Plus" Syrup. *News of Pharmacy* **2016**, 3. https://doi.org/10.24959/nphj.16.2117
[10] Foods Program Methods Validation Processes and Guidelines. 2021. https://www.fda.gov/food/laboratory-methods-food/foods-program-methods-validation-processes-and-guidelines
[11] Foods Program Compendium of Analytical Laboratory Methods. 2023. https://www.fda.gov/food/laboratory-methods-food/foods-program-compendium-analytical-laboratory-methods
[12] Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products 3rd Edition U.S. Food and Drug Administration Foods Program. 2019. https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Validation_Methods/b-Chemical-Methods-Validation-Guidelines_3rd-ed_RSSC-508_final_12_06_19.pdf
[13] ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). ICH: Geneva, 1995.
[14] Suleria, H.A.R.; Barrow, C.J.; Dunshea, F.R. Screening and Characterization of Phenolic Compounds and their Antioxidant Capacity in Different Fruit Peels. *Foods* **2020**, *9*, 1206. https://doi.org/10.3390/foods9091206
[15] Martín, J.F.; Liras, P. Comparative Molecular Mechanisms of Biosynthesis of Naringenin and Related Chalcones in Actinobacteria and Plants: Relevance for the Obtention of Potent Bioactive Metabolites. *Antibiotics* **2022**, *11*, 82. https://doi.org/10.3390/ANTIBIOTICS11010082
[16] Guttman, Y.; Yedidia, I.; Nudel, A.; Zhmykhova, Y.; Kerem, Z.; Carmi, N. New Grapefruit Cultivars Exhibit Low Cytochrome P4503A4-Inhibition Activity. *Food Chem. Toxicol.* **2020**, *137*, 111135. https://doi.org/10.1016/J.FCT.2020.111135
[17] Ferreira-Nunes, R.; Angelo, T.; da Silva, S.M.M.; Magalhães, O.; Gratieri, T.; da Cunha-Filho, M.S.S.; Gelfuso, G.M. Versatile Chromatographic Method for Catechin Determination in Development of Topical Formulations Containing Natural Extracts. *Biomed. Chromatogr.* **2018**, *32*, e4062. https://doi.org/10.1002/bmc.4062
[18] Agrawal, K.; Agrawal, C.; Blunden, G. Pharmacological Significance of Hesperidin and Hesperetin, Two Citrus Flavonoids, as Promising Antiviral Compounds for Prophylaxis Against and Combating COVID-19. *Nat. Prod. Commun.* **2021**, 16. https://doi.org/10.1177/1934578X211042540
[19] Qurtam, A.A.; Mechchate, H.; Es-safi, I.; Al-zharani; M., Nasr, F.A.; Noman, O.M.; Aleissa, M.; Imtara, H.; Aleissa, A.M.; Bouhrim, M. Citrus Flavanone Narirutin, *in vitro* and *in silico* Mechanistic Antidiabetic Potential. *Pharmaceutics* **2021**, *13*, 1818. https://doi.org/10.3390/pharmaceutics13111818
[20] Priyadarsani, S.; Patel, A.S.; Kar, A.; Dash, S. Process Optimization for the Supercritical Carbondioxide Extraction of Lycopene from Ripe Grapefruit (*Citrus paradisi*) Endocarp. *Sci. Rep.* **2021**, *11*, 10273. https://doi.org/10.1038/s41598-021-89772-6

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ВАЛІДАЦІЯ МЕТОДУ КІЛЬКІСНОГО ВИЗНАЧЕННЯ НАРИНГІНУ В ЕКСТРАКТІ ГРЕЙПФРУТА (*CITRUS PARADISI*) ЗА ДОПОМОГОЮ ВИСОКОЕФЕКТИВНОЇ РІДИННОЇ ХРОМАТОГРАФІЇ

Анотація. *У дослідженні валідовано метод ВЕРХ для кількісного визначення нарингіну в екстракті грейпфрута. Продемонстровано високу точність, прецизійність і відтворюваність методу з вилученням від 99,73 % до 100,65 %. Лінійність методу була підтверджена коефіцієнтом кореляції 0,999. Ці результати мають важливе значення для фармацевтичної розробки препаратів на основі грейпфрутів.*

Ключові слова: *нарингін, нарингенін, валідація, лінійність, ВЕРХ-ДМД.*