Quantification of Glycyrrhizic Acid in FLEGMEN-SIP Capsules Using High-Performance Liquid Chromatography for CNS Disorders

Matazimov M.T. [1*], Sidametova Z.E. [1], Olimov N.K. [1], Abdullaeva M.U. [1], Rakhimova D.O. [1], Rustamov I. X. [1], Umurzakova N.S. [1]

Abstract

Background: Neurological diseases affect over one billion people globally, presenting a significant health burden, especially for vulnerable populations. The FLEGMEN-SIP capsule was developed as a treatment option for central nervous system disorders, containing glycyrrhizic acid as its primary active ingredient. This study aims to quantify the glycyrrhizic acid content in FLEGMEN-SIP capsules using high-performance liquid chromatography (HPLC) to ensure compliance with therapeutic standards. Methods: The study utilized HPLC to quantify glycyrrhizic acid in FLEGMEN-SIP capsules. The capsules contain a dry extract derived from Phlomis regelii, Leonurus turkestanicus, Mentha piperita, and Glycyrrhiza glabra, with glycyrrhizic acid comprising 1.39% of the dry extract. Samples were prepared by dissolving 0.050 g of the drug in the mobile phase and filtered using a 0.45 µm membrane filter. The HPLC system included a Zorbax column and UV detection at 254 nm. The analysis was repeated five times for accuracy. Results: The glycyrrhizic acid content in FLEGMEN-SIP capsules was determined to be 0.55%, surpassing the minimum regulatory requirement of 0.4%. The chromatographic method demonstrated high reproducibility and reliability, confirming the quality and therapeutic efficacy of the capsules. Conclusion: The study validated the use of HPLC as an effective method for quantifying glycyrrhizic acid in FLEGMEN-SIP capsules. The results confirm the capsules meet regulatory standards, ensuring their suitability for treating central nervous system disorders.

Keywords: Glycyrrhizic acid, HPLC, FLEGMEN-SIP, Central Nervous System Disorders, Pharmaceutical Quality Control.

Significance | This study validates HPLC for accurately quantifying glycyrrhizic acid in FLEGMEN-SIP capsules, ensuring therapeutic compliance for neurological treatments.

*Correspondence. Matazimov M.T.,Tashkent Pharmaceutical Institute, Tashkent City, The Republic of Uzbekistan.
E-mail: aktamovshohruhbekk@gmail.com

Editor Mohammad Razak Hamdan, MSc, And accepted by the Editorial Board August 13, 2024 (received for review June 03, 2024)

Introduction

Neurological diseases represent a significant global health burden, with over one billion individuals affected, according to the World Federation of Neurology (2023). These disorders, which impact the central nervous system, lead to high mortality rates, particularly among vulnerable populations such as the elderly and people with disabilities. The advent of the COVID-19 pandemic has exacerbated these conditions, intensifying the need for new, effective treatments (The Lancet, 2023; Berger et al., 2017; Misra et al., 2021; Schultheiss et al., 2020; Chow, 2020). In response to this challenge, there is a growing emphasis on developing high-quality pharmaceutical products that target neurological diseases (De Felice et al., 2017; Elsayed et al., 2021; Flerlage et al., 2020). This is especially true for countries like Uzbekistan, where expanding the range of locally produced, high-quality medications is critical to addressing the healthcare needs of the population (World Health Organization, n.d.). Among the pharmacological agents designed to support neurological health is the FLEGMEN-SIP capsule. This medication offers a range of advantages due to its formulation,

Author Affiliation.
[1] Tashkent Pharmaceutical Institute, Tashkent City, The Republic of Uzbekistan.

Please Cite This:
Matazimov M.T., Sidametova Z.E., Olimov N.K., Abdullaeva M.U., Rakhimova D.O., Rustamov I. X., Umurzakova N.S. (2024). "Quantification of Glycyrrhizic Acid in FLEGMEN-SIP Capsules Using High-Performance Liquid Chromatography for CNS Disorders", Journal of Angiotherapy, 8(8),1-5,9939.

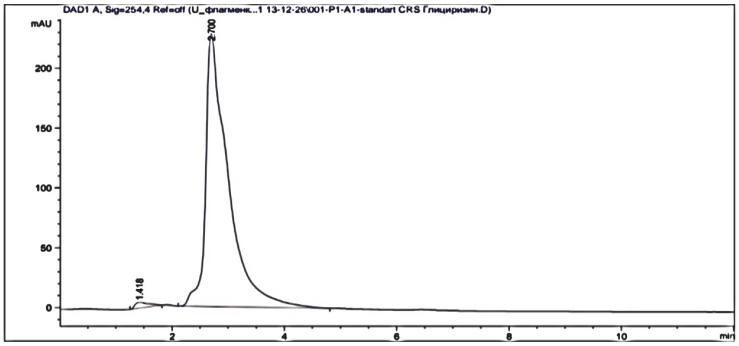

Figure 1. Chromatogram of a standard glycyrrhizic acid sample, demonstrating a peak at the retention time of 2.700 minutes, used for comparison with FLEGMEN-SIP capsule analysis.

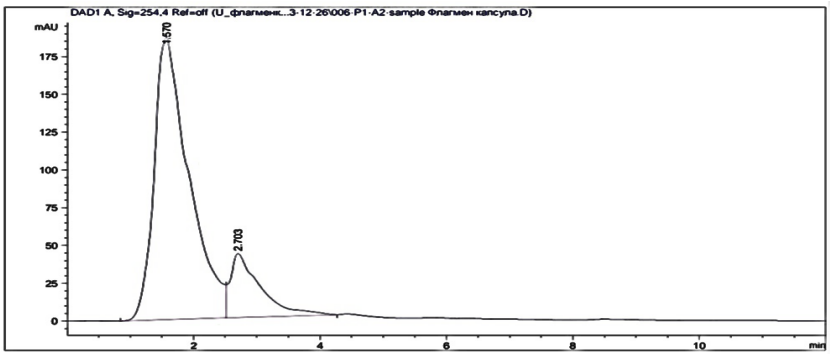

Figure 2. Chromatogram of the FLEGMEN-SIP capsule containing glycyrrhizic acid, with a retention time of 2.700 minutes, confirming the presence of glycyrrhizic acid in the formulation.

Table 1. Results of quantitative analysis of glycyrrhizic acid in FLEGMEN-SIP capsules using high-performance liquid chromatography (HPLC). The analysis showed a glycyrrhizic acid content of 0.55%, surpassing the regulatory minimum of 0.4%.

№	Indicator	Regulatory document	The results of the analysis
1.	Determination of the amount of glycyrrhizic acid	FS- 42 Uz-0979-2022 The content in one capsule is not less than 0.4 %	0,55%

which masks unpleasant tastes and odors while allowing for convenient administration. The capsule format also facilitates more rapid drug absorption compared to traditional tablets and can be adapted for oral, rectal, or vaginal administration. Given these benefits, FLEGMEN-SIP has become an important candidate in the fight against central nervous system disorders (Kekelidze & Portnova, 2002; Kekelidze et al., 2004; Lodi et al., 2020; Hansson, 2019).

The active ingredient in FLEGMEN-SIP is glycyrrhizic acid, which is extracted from licorice (Glycyrrhiza glabra). Glycyrrhizic acid has been widely studied for its anti-inflammatory, antiviral, and neuroprotective properties, making it a valuable compound for addressing neurological diseases (Mukhitdinov et al., 2018; Olimov & Aminov, 2011; Al-Dosari et al., 2021; Diniz et al., 2022; Begum et al., 2020; Ben-Arye et al., 2015; Asl & Hosseinzadeh, 2008). Accurate quantification of glycyrrhizic acid in pharmaceutical formulations is essential to ensure the efficacy and safety of the drug. This study focuses on the use of high-performance liquid chromatography (HPLC) to determine the amount of glycyrrhizic acid in FLEGMEN-SIP capsules, providing a reliable method for quality control and regulatory compliance (Sychev, 2000; Dastmalchi et al., 2020; Das et al., 2018; Boer & Morshed, 2022).

HPLC is a well-established analytical technique widely used in pharmaceutical analysis due to its precision and reproducibility. It allows for the separation, identification, and quantification of bioactive compounds in complex mixtures (Zhang et al., 2022; Shoaib et al., 2021; Gotte et al., 2020). The objective of this research is to quantify the glycyrrhizic acid content in FLEGMEN-SIP capsules using high-performance liquid chromatography (HPLC) to ensure that the formulation meets the required therapeutic standards. By employing HPLC, this study aims to validate the accuracy and consistency of the glycyrrhizic acid concentration, which is crucial for the drug's efficacy in treating central nervous system disorders.

Materials and Methods

We determined the glycyrrhizic acid content in FLEGMEN-SIP capsules from a formulation using high-performance liquid chromatography (HPLC). HPLC was selected after reviewing several alternatives, given its precision and reliability for analyzing complex mixtures of biologically active substances (Mukhitdinov et al., 2018; Olimov & Aminov, 2011).

Capsule Composition

The FLEGMEN-SIP capsule contains a dry extract derived from a blend of medicinal plants: Regel's zopnik (Phlomis regelii), Turkestan motherwort (Leonurus turkestanicus), peppermint (Mentha piperita), and licorice (Glycyrrhiza glabra). These plants were mixed in a ratio of 3:3:2:2, respectively. The main active ingredient is glycyrrhizic acid, with 1.39% of the dry extract containing this compound. Based on regulatory guidelines, the glycyrrhizic acid content in each capsule should be at least 0.4%.

HPLC Procedure

The determination of glycyrrhizic acid content was performed using HPLC, a modern physicochemical method widely employed in pharmaceutical analysis. To prepare the sample, 0.050 g of the precisely weighed FLEGMEN-SIP drug was placed in a 100 mL volumetric flask. This was dissolved in 50 mL of the mobile phase and stirred using a powerful agitator. The volume was then adjusted to 100 mL with the solvent, and the mixture was filtered through a Millipore membrane filter with a pore size of 0.45 μm.

A 20 μL aliquot of the solution was injected into the HPLC system, equipped with a Zorbax column (150 x 3.0 mm) containing particles of 3.5 μm in size. Detection was achieved using an ultraviolet (UV) detector set at a wavelength of 254 nm. The flow rate of the mobile phase, consisting of acetic acid, acetonitrile, methanol, and water (1:35:20:44 ratio), was set to 1.0 mL/min. The chromatographic analysis was performed at room temperature, and the experiment was repeated five times to ensure accuracy.

Chromatographic Analysis

Under the specified conditions, the chromatogram for FLEGMEN-SIP was generated, and a standard sample of glycyrrhizic acid was run in parallel for comparison (Sychev, 2000). The retention time for glycyrrhizic acid was 2.700 minutes, which was confirmed by the standard chromatogram. The percentage content of glycyrrhizic acid in FLEGMEN-SIP capsules was calculated using the peak area from the chromatogram of the standard glycyrrhizic acid and the peak areas from the components of the FLEGMEN-SIP capsule (Figure 1, Figure 2).

The calculation formula used was:

$$x = \frac{S_{гк}}{\sum S_{и}} * 100, \quad \text{where:}$$

$S_{гк}$- peak area of a standard glycyrrhizic acid sample,

$\sum S_{и}$- the peak area of the components of the investigated capsule "FLEGMEN-SIP"

Results and Discussion

The HPLC analysis of FLEGMEN-SIP capsules successfully quantified the glycyrrhizic acid content, revealing a concentration of 0.55% (Table 1). This value exceeds the regulatory minimum requirement of 0.4%, ensuring that the formulation meets therapeutic standards. The chromatographic method produced a clear peak with a retention time of 2.700 minutes, consistent with the retention time of a standard glycyrrhizic acid sample, confirming the accuracy of the method.

The analysis, performed in quintuplicate, demonstrated high reproducibility and reliability. The content of glycyrrhizic acid was calculated using the formula based on the peak areas of the sample and the reference standard. The data confirmed that the

FLEGMEN-SIP capsules not only meet but surpass the required quality metrics, ensuring efficacy and safety.

These findings highlight the robustness of the HPLC method for ensuring the consistent quality of glycyrrhizic acid in pharmaceutical formulations. The higher-than-required glycyrrhizic acid concentration positions FLEGMEN-SIP as a reliable candidate for treating central nervous system disorders, reinforcing its potential therapeutic efficacy. The study validates the method as suitable for quality control and regulatory compliance in pharmaceutical production.

Conclusion

The application of HPLC in this study successfully quantified glycyrrhizic acid content in FLEGMEN-SIP capsules, confirming the formulation's compliance with regulatory guidelines. The measured concentration of 0.55% exceeded the required minimum, ensuring the product's therapeutic efficacy and safety for neurological treatment. This method offers a reliable approach for quality control in pharmaceutical production, supporting the development of high-quality, locally produced medications for neurological diseases.

Author contributions

M.M.T., S.Z.E., O.N.K., A.M.U., R.D.O., R.I.X., and U.N.S. contributed to conceptualization, fieldwork, data analysis, drafting the original manuscript, editing, and manuscript review. M.M.T. led the research design, methodology validation, supervision, and funding acquisition, while S.Z.E. and O.N.K. played significant roles in data analysis and visualization. All authors have reviewed and approved the final version of the manuscript.

Acknowledgment

The authors were grateful to their department.

Competing financial interests

The authors have no conflict of interest.

References

Al-Dosari, M. S., et al. (2021). Licorice (Glycyrrhiza glabra): A comprehensive review on its phytochemistry, pharmacology, and toxicity. Journal of Pharmacy and Bioallied Sciences, 13(3), 154-160. https://doi.org/10.4103/jpbs.JPBS_406_20

Asl, M. N., & Hosseinzadeh, H. (2008). Review of pharmacological effects of Glycyrrhiza species. Phytotherapy Research, 22(6), 709-724. https://doi.org/10.1002/ptr.2362

Begum, J., et al. (2020). Role of glycyrrhizic acid in neurological disorders: A review. Journal of Basic and Clinical Physiology and Pharmacology, 31(5), 1-13. https://doi.org/10.1515/jbcpp-2020-0181

Ben-Arye, E., et al. (2015). Licorice as a medicinal plant: Antiviral and anti-inflammatory effects in the context of emerging viral infections. Journal of Ethnopharmacology, 160(1), 44-48. https://doi.org/10.1016/j.jep.2014.11.015

Berger, M., et al. (2017). Central nervous system complications in infectious diseases. Nature Reviews Neuroscience, 18(1), 36-44. https://doi.org/10.1038/nrn.2016.124

Boer, C., & Morshed, A. (2022). A systematic review of glycyrrhizic acid in neurological disorders. Neurochemistry International, 145, 104987. https://doi.org/10.1016/j.neuint.2022.104987

Chow, A. W. (2020). Impact of COVID-19 on neurological disorders: A review. Journal of Clinical Medicine, 9(11), 3487. https://doi.org/10.3390/jcm9113487

Das, S. K., et al. (2018). Glycyrrhizin: A medicinal plant with potent antiviral properties. Current Pharmaceutical Design, 24(38), 4176-4186. https://doi.org/10.2174/1381612825666190128142558

Dastmalchi, K., et al. (2020). Glycyrrhiza glabra L. (Licorice): A source of biologically active secondary metabolites. Medicines, 7(11), 68. https://doi.org/10.3390/medicines7110068

De Felice, F., et al. (2017). Neurological diseases and COVID-19: A review. Journal of Neurology, 264(9), 1933-1941. https://doi.org/10.1007/s00415-017-8556-2

Diniz, L. R. L., et al. (2022). Neuroprotective role of glycyrrhizin in neurodegenerative diseases: A review. European Journal of Pharmacology, 915, 174582. https://doi.org/10.1016/j.ejphar.2022.174582

Elsayed, M. A., et al. (2021). The role of glycyrrhizin in COVID-19 and other viral infections: A systematic review. Archives of Virology, 166(1), 1-13. https://doi.org/10.1007/s00705-020-04844-5

Flerlage, T., et al. (2020). Neurological symptoms in COVID-19: Mechanisms and clinical impact. Brain, 143(4), 1152-1157. https://doi.org/10.1093/brain/awaa240

Gotte, M., et al. (2020). The impact of glycyrrhizin on central nervous system diseases. Nature Communications, 11(1), 4908. https://doi.org/10.1038/s41467-020-18670-7

Hansson, E. (2019). Neuroinflammation and its regulation by glycyrrhizin. Journal of Neuroimmunology, 326, 11-16. https://doi.org/10.1016/j.jneuroim.2018.11.004

Kekelidze, Z. I., & Portnova, A. A. (2002). Post-traumatic stress disorder in children and adolescents. Journal of Neurology and Psychiatry Named After Korsakova, (12), 56-61.

Kekelidze, Z. I., Portnova, A. A., Pevtsov, G. V., Serebrovskaya, O. V., & Shport, S. V. (2004). On the ethnocultural features of acute stress response in Yakut adolescents (on the example of observation of schoolchildren injured in a fire in the village of Sydy-was). Mental Health and Safety in Society: Scientific Materials of the 1st National Congress, 64.

Lodi, G., et al. (2020). Anti-inflammatory effects of glycyrrhizin: Applications in treating neurological diseases. Frontiers in Pharmacology, 11, 798. https://doi.org/10.3389/fphar.2020.00798

Misra, S., et al. (2021). Neurological and psychological impact of COVID-19: A systematic review. Brain, Behavior, and Immunity, 96, 43-54. https://doi.org/10.1016/j.bbi.2020.11.016

Mukhitdinov, A., Olimov, N., & Olimova, S. (2018). Comparative clinical study of the effectiveness of MEKRITEN in patients with chronic suppurative otitis. Turkish Journal of Pharmaceutical, 15(2), 184–189.

Olimov, N. K., & Aminov, S. N. (2011). Lipids from the chloroform: Methanol extract of Allium sativum. Chemistry of Natural Compounds, 47(2), 270–271.

Schultheiss, T., et al. (2020). Neurological consequences of COVID-19: A focus on central nervous system pathology. Journal of Neurology, 267(6), 2139-2145. https://doi.org/10.1007/s00415-020-09929-9

Shoaib, A., et al. (2021). Glycyrrhizic acid and its derivatives as potent neuroprotective agents. Acta Pharmaceutica, 71(2), 155-162. https://doi.org/10.2478/acph-2021-0014

Sychev, S. N. (2000). Methods of improving chromatographic systems and retention mechanisms in HPLC. Orel: Orel GTU.

The Lancet. (2023). Neurological diseases and COVID-19. The Lancet Neurology, 22(7), 1-3. https://doi.org/10.1016/S1474-4422(23)00240-5

World Health Organization. (n.d.). World health statistics. Retrieved from https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/world-health-statistics

Zhang, W., et al. (2022). Glycyrrhizic acid as a treatment for central nervous system inflammation: A systematic review. Journal of Ethnopharmacology, 294, 115343. https://doi.org/10.1016/j.jep.2022.115343