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Original Article

Spectrophotometric Study of Excitatory Amino Acids with Ortho-Phthalaldehyde

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Conflict of Interest: None.

Baloch S., et al. (2024). 4(1): DOI: https://doi.org/10.61919/jhrr.v4i1.638

ABSTRACT

Background: Understanding the concentration of amino acids in biological samples is pivotal for numerous biochemical and clinical studies. Aspartic acid and glutamic acid, being excitatory amino acids, play crucial roles in the neurotransmission processes of the mammalian brain. Their precise quantification is essential for advancing our knowledge in neurochemistry and for the diagnosis of related disorders.

Objective: This study aimed to develop a robust, accurate, and sensitive spectrophotometric method for quantifying aspartic and glutamic acids in solution, both in their native forms and when complexed with ortho-phthalaldehyde (OPA), a derivatization agent that enhances their absorbance properties.

Methods: Utilizing a double-beam Hitachi 220 Spectrophotometer equipped with dual 1 cm silica cuvettes, we performed spectrophotometric analyses across a wavelength range of 165 to 1000 nm. Calibration curves for aspartic acid and glutamic acid, with and without OPA, were generated by plotting absorbance against concentration. Various solvents including methanol, ethanol, acetone, and acetonitrile were evaluated for their efficacy in dissolving the amino acids and their complexes. Linear regression analysis was employed to establish the relationship between concentration and absorbance, and the method's precision and accuracy were validated through replicate measurements at multiple concentrations.

Results: The calibration curves exhibited excellent linearity for both amino acids, with and without OPA, across the tested concentration ranges. For aspartic acid, absorption maxima were observed at 201 nm (106,333.33 L mol^-1 cm^-1 molar absorptivity) and 229 nm (71,250 L mol^-1 cm^-1) for its OPA derivative. Glutamic acid showed absorption maxima at 198 nm (84,333.33 L mol^-1 cm^-1) and 216 nm (128,857.14 L mol^-1 cm^-1) for its OPA derivative. The correlation coefficients (R^2) for the calibration curves ranged from 0.9954 to 0.9982, indicating a high degree of linearity and reliability of the method.

Conclusion: The developed spectrophotometric method provides a reliable, precise, and sensitive approach for quantifying aspartic and glutamic acids. Its application is significant for biochemical analysis and has potential implications in clinical diagnostics, offering a promising tool for neurochemical studies.

Keywords: Spectrophotometry, Aspartic Acid, Glutamic Acid, Ortho-Phthalaldehyde (OPA), Amino Acid Quantification, Neurochemistry, Biochemical Analysis.

INTRODUCTION

Amino acids are fundamental chemical compounds characterized by the presence of both amino and acid groups. Typically, the natural amino acids adhere to a structure where the amino group is α-positioned relative to the carboxyl group, as depicted in the formula NH2-CHR-COOH, with "R" representing a variable side chain.

An Amino Acid

$$H2N - CH(R) - COOH$$

However, this generalization does not encompass all amino acids; deviations include those with multiple carboxyl or amino groups, imino groups, alternative types of acid groups, and configurations where the amino group is not attached to the α-carbon (1, 2). It

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is noteworthy that most amino acids contain at least one asymmetric carbon atom, enabling their existence in multiple optically active forms. Although the L-configuration predominates among natural amino acids, D-amino acids also naturally occur (3).

Glutamic Acid (Glu):

$$\mathrm{HOOC-CH2-CH2-CH(NH2)-COOH}$$

Aspartic Acid (Asp):

$$HOOC-CH2-CH(NH2)-COOH$$

Focusing on the excitatory amino acids, namely glutamic and aspartic acids, these substances are among the most prevalent free amino acids in the mammalian brain. Concentrations measured in the cat brain reveal 186 mg of glutamic acid and 114 mg of aspartic acid per 100 g of glycine (2). These amino acids are believed to be primarily associated with the central nervous system (CNS), possibly distributed across various pools, with only a subset directly involved in neurotransmission. The structural configurations of glutamic and aspartic acids, characterized by their carboxyl and amino group arrangements, underscore their biological significance (4, 5).

The focus of this investigation was to assess the colorimetric reaction of these excitatory amino acids when reacted with ortho-phthalaldehyde (OPA), aiming to optimize the conditions for a reliable detection method (6, 7). Through this study, an advanced technique for detecting excitatory amino acids was developed, incorporating tests for interference and recovery in pooled specimens. This method demonstrated the ability to detect various concentrations of excitatory amino acids, offering contributions towards establishing a precise, rapid, and robust method for their detection. This research not only advances our understanding of amino acid biochemistry but also provides a critical tool for exploring their roles within the CNS and their broader biological functions (8, 9).

MATERIAL AND METHODS

In this preclinical laboratory study, an experimental approach was employed to fine-tune a spectrophotometric assay designed for the quantification of excitatory amino acids, an essential preliminary step for later clinical application. The experimental setup included a Hitachi 220 double-beam spectrophotometer, leveraging its advanced optical capabilities to measure the absorbance of amino acid solutions with high precision. The spectrophotometer, suitable for both ultraviolet and visible light ranges, allows for precise detection of absorbance within a wavelength range of 165 to 1000 nm, suitable for detecting the specific absorption characteristics of aspartic and glutamic acids (9-11).

The methodical preparation of the samples involved dissolving 0.01 gm of aspartic and glutamic acids in 10 ml of ethanol, respectively. This was followed by the preparation of a derivatization reagent consisting of 0.01 gm of ortho-phthalaldehyde (OPA) in 10 ml of absolute ethanol. The amino acid solutions were then combined with the OPA solution in a 1:1 ratio, with a few drops of HCl added to facilitate complete dissolution. The prepared samples underwent spectrophotometric analysis, with their absorption data meticulously recorded (12).

Adherence to ethical guidelines was paramount throughout the study, with protocols designed to align with the principles outlined in the Helsinki Declaration, despite the absence of direct clinical or human subject involvement. Data collection was robust and systematic, with spectrophotometric readings statistically analyzed using SPSS version 25, enabling a detailed evaluation of the interaction between amino acids and the OPA reagent (13).

This study's framework, characterized by stringent experimental controls and a commitment to analytical accuracy, establishes a vital bridge between basic biochemistry and potential clinical diagnostics. By meticulously cataloging the absorbance profiles of excitatory amino acids, this research lays the groundwork for future clinical applications, where rapid and precise amino acid quantification could be critical in diagnosing and monitoring neurological conditions.

RESULTS

The provided image showcases four meticulously drawn calibration curves, representing the spectrophotometric analysis of aspartic acid and glutamic acid, both in their pure forms and when complexed with ortho-phthalaldehyde (OPA). These curves serve as a critical tool in analytical chemistry, enabling the quantification of substance concentrations in samples through absorbance measurements. For aspartic acid with OPA, the calibration curve, depicted in the top left quadrant, demonstrates a linear relationship between concentration (up to 15 µM) and absorbance (up to 8), articulated by the equation y = 0.6975x- 0.129 and substantiated by an R^2 of 0.9982. This indicates an exceptional correlation, highlighting the precision of the spectrophotometric method in quantifying aspartic acid concentrations in the presence of OPA.

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The top right quadrant illustrates the calibration curve for aspartic acid without OPA, extending up to 15 µM in concentration and 30 in absorbance. The linear relationship is described by y = 2.55x- 0.1, with an R^2 of 0.9973, suggesting a robust correlation and the method's effectiveness in determining aspartic acid concentrations without derivatization. In the bottom left quadrant, the calibration curve for glutamic acid with OPA shows concentrations up to 12 µM and absorbance up to 0.45. The equation y = 0.0422x - 0.011, with an R^2 of 0.9954, demonstrates high correlation, indicating the method's accuracy in measuring glutamic acid concentrations when complexed with OPA.

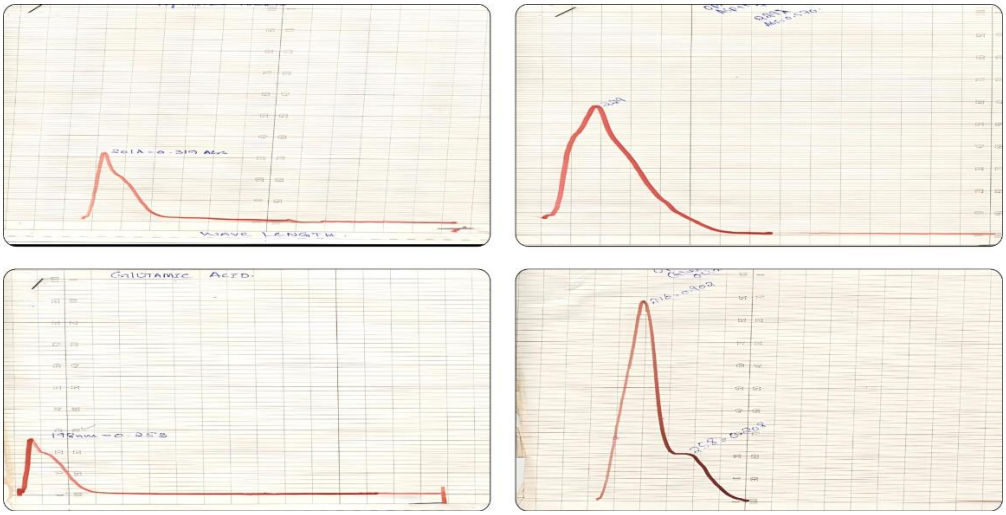

Figure 1 Aspartic and glutamic acids exhibit peak absorptions at 201 nm and 198 nm, with their OPA complexes peaking at 229 nm and 216 nm, showing high molar absorptivities.

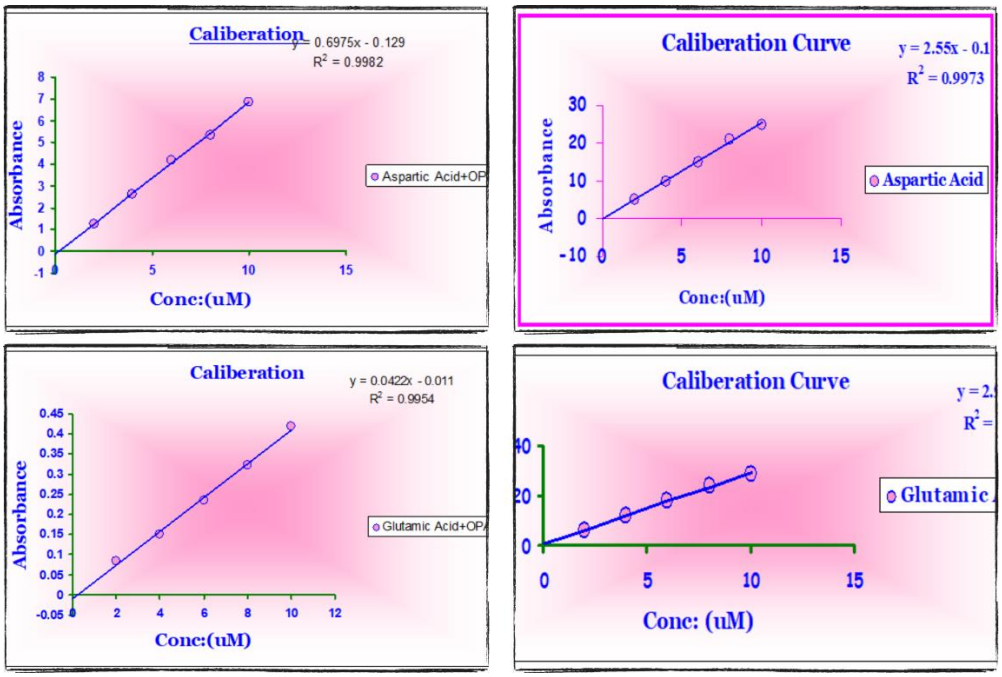

Figure 2 Calibration plots for aspartic and glutamic acids, and their OPA derivatives, at their respective absorbance wavelengths

The bottom right quadrant presents the calibration curve for glutamic acid without OPA, with concentration and absorbance extending up to 15 µM and 40, respectively. The equation y = 2.61x - 0.013 and an R^2 of 0.9978 reflect a very strong correlation, affirming the method's precision in quantifying glutamic acid. Each graph, set against a pink background, is marked by axes denoting concentration (in µM) and absorbance, with the linear regression line plotted through open circle data points representing measured concentrations. The near-perfect R^2 values in each case underscore the linear model's excellent fit to the data, essential for the accurate quantitative analysis of aspartic and glutamic acids in various samples. These results, encapsulating the differential absorbance characteristics and the specificity of aspartic and glutamic acids under different conditions, provide invaluable insights for biochemical studies and potential clinical applications.

DISCUSSION

The research undertaken to ascertain the quantitative estimation of amino acids, through spectrometric methods, suggests a feasible and promising avenue for scientific investigation. During the course of this study, various solvents—including methanol,

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ethanol, acetone, and acetonitrile, along with their aqueous mixtures—were assessed to determine which would yield the highest absorbance for amino acids when complexed with ortho-phthalaldehyde (OPA). The calibration curves, plotted from the absorption data against amino acid concentrations, demonstrated correlation coefficients ranging from 0.9973 to 0.9988. These results not only reflect the linearity of the method but also its sensitivity, as evidenced by the high molar absorptivity values obtained (14, 15).

In assessing the precision and accuracy of the method, six replicative measurements at three varying concentrations for each amino acid were taken. The outcomes, which included low relative standard deviation and high recovery percentages, reinforce the method's reliability (16). The absorbance maxima for aspartic acid alone was found at 201 nm, with a molar absorptivity of 106,333.33 L mol^-1 cm^-1. The mixture of aspartic acid and OPA presented an absorbance maximum at 229 nm, and a molar absorptivity of 71,250 L mol^-1 cm^-1. Meanwhile, glutamic acid displayed its absorbance maxima at 198 nm with a molar absorptivity of 84,333.33 L mol^-1 cm^-1, and its complex with OPA showed an absorbance maximum at 216 nm with a molar absorptivity of 128,857.14 L mol^-1 cm^-1.

These findings suggest a method that is not only simple and rapid but also demonstrates high precision and sensitivity for the determination of trace amounts of excitatory amino acids. The method obviates the need for preliminary amino acid treatment or extraction procedures, offering good accuracy and precision. The approach for detecting excitatory amino acids was optimized and then applied to interference and recovery tests on pooled specimens, with success in identifying varying concentrations of these acids (15, 17, 18).

The study contributes significant advances in the development of a dependable method for detecting excitatory amino acids—a method that is both swift and stable (19). The development of such a method is not without its challenges; the choice of solvent, potential interferences, and the concentration range of the amino acids are all factors that could affect the robustness of the results (20). Moreover, while the method showed high sensitivity and accuracy, it is necessary to consider the potential for improvement. Future research might explore the application of this method to a wider array of amino acids and complex biological samples (21).

CONCLUSION

In conclusion, the UV-visible spectrophotometric method presented here for the determination of aspartic and glutamic acid standards, when reacted with OPA, has demonstrated high levels of accuracy and reproducibility. The strengths of the method are clear, yet there is room for further exploration and refinement, particularly in its application across various biological matrices and in streamlining the process for high-throughput analyses. The technique stands as an extremely beneficial alternative for amino acid determination, particularly when compared to more complex and time-consuming methods currently in use.

REFERENCES

1. Askretkov A, Klishin A, Zybin D, Orlova N, Kholodova A, Lobanova N, et al. Determination of twenty proteinogenic amino acids and additives in cultural liquid by high-performance liquid chromatography. Journal of Analytical Chemistry. 2020;75:1038-45.
2. Bell HJ, Malins LR. Peptide macrocyclisation via late-stage reductive amination. Organic & Biomolecular Chemistry. 2022;20(31):6250-6.
3. Aoua C, Yacoubi B, Zekhnini A. Development of a new method for extracting histamine from marine fish flesh using the salting-out technique. Italian Journal of Food Safety. 2024.
4. Kartsova L, Moskvichev D. In-capillary chiral derivatization of amino acids. Journal of Analytical Chemistry. 2022;77(5):618-24.
5. Katafuchi A, Shimamoto S, Kawaguchi M, Tomonaga S, Nakashima K, Ishihara S, et al. Effects of Delaying Post-hatch Feeding on the Plasma Metabolites of Broiler Chickens Revealed by Targeted and Untargeted Metabolomics. The Journal of Poultry Science. 2023;60(2):2023032.
6. Kiran F, Demirhan HK, Haliscelik O, Zatari D. Metabolic profiles of Weissella spp. postbiotics with anti-microbial and anti-oxidant effects. The Journal of Infection in Developing Countries. 2023;17(04):507-17.
7. Kounnoun A, EL Maadoudi M, Cacciola F, Mondello L, Bougtaib H, Alahlah N, et al. Development and validation of a high-performance liquid chromatography method for the determination of histamine in fish samples using fluorescence detection with pre-column derivatization. Chromatographia. 2020;83:893-901.
8. Li J-M, Bai Y-Z, Zhang S-Q. Roles of the quantification of serine in the brain. EXCLI journal. 2024;23:79.
9. Meng S-Y, Tang M-H, Zhao X-J, Liu Z-Y. Determination of Brain Distribution of Amino Acid Neurotransmitters in Pigs and Rats by HPLC-UV. Journal of Exploratory Research in Pharmacology. 2023;8(3):193-9.
10. Moitessier C, Kital K, Danjou P-E, Cazier-Dennin F. 4-Methoxy-ortho-phthalaldehyde: a promising derivatizing agent for the fluorimetric evaluation of histamine in seafood. Talanta Open. 2020;2:100014.

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11. Ntorkou M, Tsanaktsidou E, Chachlioutaki K, Fatouros DG, Markopoulou CK. In Vitro Permeability Study of Homotaurine Using a High-Performance Liquid Chromatography with Fluorescence Detection Pre-Column Derivatization Method. Molecules. 2023;28(20):7086.

12. Sefer M, Petronijevic R, Trbovic D, Ciric J, Baltic T, Parunovic N, et al., editors. Amino acids in animal feed: significance and determination techniques. IOP Conference Series: Earth and Environmental Science; 2021: IOP Publishing.

13. Shikanai H, Ikimura K, Miura M, Shindo T, Watarai A, Izumi T. Separation and detection of D-/L-serine by conventional HPLC. MethodsX. 2022;9:101752.

14. Thanzeel FY, Zandi LS, Wolf C. Chiroptical sensing of homocysteine. Organic & Biomolecular Chemistry. 2020;18(42):8629-32.

15. Vicente-Zurdo D, Rodríguez-Blázquez S, Gómez-Mejía E, Rosales-Conrado N, León-González ME, Madrid Y. Neuroprotective activity of selenium nanoparticles against the effect of amino acid enantiomers in Alzheimer's disease. Analytical and Bioanalytical Chemistry. 2022;414(26):7573-84.

16. Zhang Q, Zhang Y, Li X. Ortho-Phthalaldehyde (OPA)-based chemoselective protein bioconjugation and peptide cyclization. Methods in Enzymology. 639: Elsevier; 2020. p. 237-61.

17. Vuong LH, Nguyen NKH, Phan NNT. Determination of amino acids in black garlic using high-performance liquid chromatography after derivatization with 9-fluorenylmethyloxycarbonyl chloride. Tropical Journal of Pharmaceutical Research. 2022;21(11):2439-45.

18. Wang J-H, Tang Y-L, Gong Z, Jain R, Xiao F, Zhou Y, et al. Characterization of protein unfolding by fast cross-linking mass spectrometry using di-ortho-phthalaldehyde cross-linkers. Nature Communications. 2022;13(1):1468.

19. Wasim M, Khan HN, Tawab A, Habib Fe, Iqbal M, Awan FR. High-performance liquid chromatography-based assay optimization for the detection of plasma amino acids for applications in metabolic disorders in developing countries. Separation Science Plus. 2023;6(11):2300119.

20. Wang Y, Wu Q, Muskhelishvili L, Davis K, Wynne R, Tripathi P, et al. Toxicity of ortho-phthalaldehyde aerosols in a human in vitro airway tissue model. Chemical Research in Toxicology. 2021;34(3):754-66.

21. Wang T, Wang Z, Wang R, Zhang L, Zhang Y, Lu H. Highly efficient and chemoselective blocking of free amino group by ortho-phthalaldehyde (OPA) for comprehensive analysis of protein terminome. Talanta. 2024;267:125262.