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QUANTITATIVE ANALYSIS OF SOME AROMATIC AMINO ACIDS BY SPECTROPHOTOMETRIC MATHEMATICAL DERIVATIZATION

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ABSTRACT : A simple UV spectrophotometric differential derivatization method was performed for the simultaneous quantification of three aromatic amino acids of tryptophan, the polar tyrosine and phenylalanine TRP, TYR and PHE respectively. The avoidance of the time and reagents consuming steps of sample preparation or analyze separation from its bulk of interferences made the approach environmentally benign, sustainable and green. The linear calibration curves of differential second derivative were built at the optimum wavelength for each analyze (218.9, 236.1 and 222.5 nm) for PHE, TRP and TYR respectively. Quantification for each analyze was in the concentration range of (1.0– 45, 0.1–20.0 and 1.0– 50.0 µg/ml) at replicates of (n=3) with a reasonable linearity R^2 value of (0.9983, 0.9970 and 0.9990) for PHE, TRP and TYR, respectively. The good repeatability of the approach was expressed by the low values of relative standard deviations which were less than 1.03%. Recovery study was implemented to confirm the accuracy of the method which was (97.35-99.65, 99.90-96.10 and 98.30-99.03) for PHE, TRP and TYR, respectively. The proposed approach can be applied satisfactorily to the analysis of the quoted amino acids in pharmaceutical formulations.

Key words : Tyrosine, tryptophan, phenylalanine, zero-crossing point, differential derivative spectrometry, simultaneous estimation.

INTRODUCTION

Peptides and hence the proteins are made of amino acids. They are organic compounds with a tetrahedral carbon atom attached to an amino and carboxyl functional group. Amino acids mainly differ from each other by their side chains alkyl group. However, twenty of them appears in the genetic code and are known as the standard or the essential amino acids (Hammer and McPhee, 2018).

Three out of the twenty are characterized for their aromatic hydrophobic sidechain; Phenylalanine, Tryptophan and Tyrosine as shown in Fig. 1. PHE is a precursor of thyroxine, dopamine, melanin and norepinephrine (National Center for Biotechnology Information, 2019a). The brain employs it to produce the Catecholamine namely Norepinephrine molecule that sendoff signals between nerve cells and the cerebrum to preserve you awake and attentive, functions as an antidepressant, reduces starvation pains, and helps develop memory (Dale, 2012). Tryptophan (TRP) is vital for the construction of the proteins, enzymes, including muscle tissue. It also affects the synthesis of niacin. TRP supplements can help relieve insomnia, reduce anxiety,

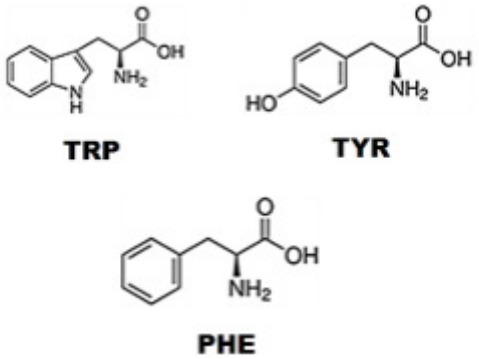

Fig. 1 : Structures of the amino acids with hydrophobic aromatic sidechain; Phenylalanine (PHE), Tryptophan (TRP) and Tyrosine (TYR).

depression and migraine headaches. It also helps the immune system by reducing the risk of cardiac spasms (National Center for Biotechnology Information, 2019b). Tyrosine (TYR) is naturally occurring and can be synthesized in vivo from phenylalanine. It is a crucial nutrient for patients diagnosed with phenylketonuria as they do not have the ability to convert phenylalanine into tyrosine (Hay *et al*, 2018). TYR has an important role in the production of protein, enzymes, catecholamines, thyroxine (thyroid hormones) and melanin. It acts as an

anti-depressant, memory improver and mental alertness booster. Deficiency in TYR may lead to low blood pressure and low body temperature (National Center for Biotechnology Information, 2019c).

Different procedures were reported in the literature for the analysis and *in vivo* and *in vitro* determination of aromatic amino acids; as spectrometrically (Kumar, Sharma and Kalonia, 2005; Luo *et al*, 2019; Al-Janabi, Mahmood and Luaibi, 2020), chromatographic techniques (Maneglier *et al*, 2004; Sanchez-Machado, Chavira-Willys and López-Cervantes, 2008; Al-Janabi *et al*, 2012; Kämpfer *et al*, 2019), LC-MS(Le *et al*, 2019), voltammetry (Majidi *et al*, 2006) and electrochemically (Idili *et al*, 2019). Differential derivative spectrophotometric methods in which a correlation of the absorbance intensity as a function to the change in the wavelength is measured were preferred over other traditional analytical techniques for their simplicity, affordability, high sensitivity, and good specificity (Nozaki, 1990; Erk, 2000; Shah and Patel, 2019). The mathematical derivatization of a spectrum makes insignificant spectral changes considerably measurable. Where the first derivative of a graph is a drawing for the spectral slope as a function for the wavelength. While the second derivative spectrum represents a derivative of the first derivative spectrum. Generally, the measurement of both peak height and peak area directly expresses the concentration of the analyte. While the second derivative function has reduced bandwidth as an additional privilege over them which is dependent on the shape of the peak. This fact offers a better resolution of overlapped peaks and an improved sensitivity (Perkampus and Threlfall, 2013).

Another common spectrometric method is the Vierordt s method (Giriraj and Sivakkumar, 2014) and so-called simultaneous equation method (SE), which is an approach usually applied to quantify pharmaceutical mixtures that contain more than one active material. It involves the selection of the λ_{max} for each component in the mixture as well as the isobestic point among them to form the simultaneous equations (Vichare *et al*, 2010).

Consequently, the application of mathematical derivative spectrometry is preferred for real-time determination of PHE, TRP and TYR as it proposes a great qualitative and quantitative mean of analyses of pharmaceutical mixtures (Dave and Thakkar, 2007). This research aims to prove the ability resolving and outdo the problem of overlapping spectral bands using the second derivative mathematical methodology and permits the simultaneous determination of phenylalanine, tryptophan, and tyrosine without the need for prior sample preparation and separation.

Experimental

Instrumentation and reagents

- Spectrophotometric measurements were made using a UV-Visible Spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan).
- The program used for the purpose of mathematical derivatization for the absorbance spectra: UV-Probe Software Version 2.42, Shimadzu Corporation, Kyoto, Japan.
- The acidity of the prepared solutions and buffers were adjusted using a digital pH meter (pH/mV Bench Meter, Hanna Instruments) with a combined glass pH electrode.
- Analytical Electronic balance (Sartorius, with four decimals).
- All chemicals and solvents were of analytical grade.
- The preparation of PHE, TRP and TYR stock solutions in the concentrations of (1000 µg/ml) were accomplished by the dissolution of 50 mg of each amino acid in 50 ml of distilled water. Five ml(s) of the above solutions were diluted separately into 50 ml of distilled water to produce 100 µg/ml each of PHE, TRP and TYR in distilled water.
- The stock solutions in the concentration of 100 µg/ml were diluted with distilled water to make the required standard solutions of each analyze and their mixtures as well.

RESULTS AND DISCUSSION

The overlaid graph in Fig. 2 shows the zero-order absorption spectra of TRP (10 µg/ml), TYR (40 µg/ml) and PHE (40 µg/ml) in distilled water. The spectra noticeably exhibit a significant overlap, therefore the traditional Vierodet s method and its modulation for analyzing mixtures seem to be inapplicable.

Simultaneous determination of PHE, TYR and TRP in their mixture can be accomplished from the second-order derivative differential spectra (2D) presented in Fig. 3. The derivative simultaneous spectra of the three amino acids mixture have a good characteristic that it does have zero-crossing points that can make use of it to determine the concentration of each analyte at a certain wavelength. The concerned analyze should have an absorbance value at a certain wavelength where the other components do not.

The second-order differential derivative spectra of PHE, TYR and TRP exhibited in Fig. 3 shows the capability of the simultaneous determination of PHE at

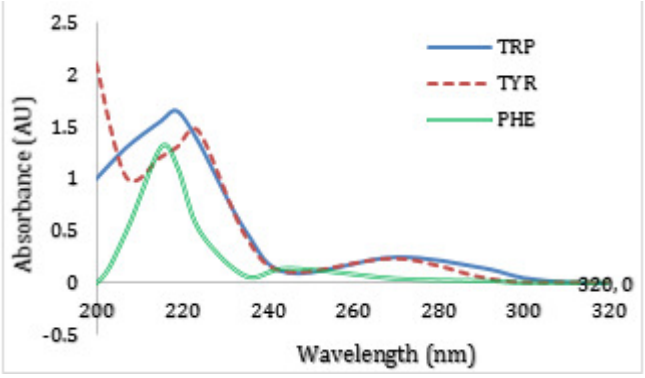

Fig. 2 : Overlaid graph of the Zero-order UV absorbance Spectra for aqueous solutions of (40 µg/ml) of PHE, (40 µg/ml) of TYR and (10 µg/ml) of TRP.

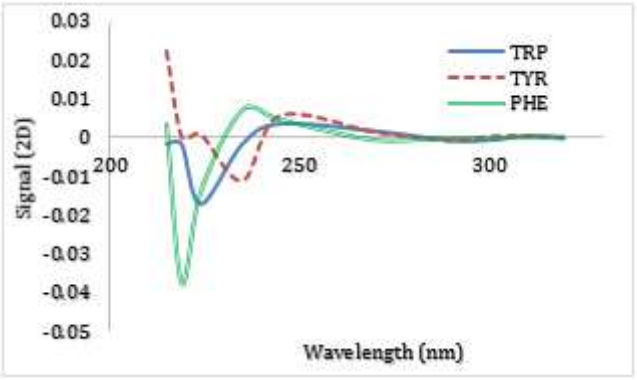

Fig. 3 : Overlaid graph of the 2nd order derivative UV absorbance Spectra for aqueous solutions of (40 µg/ml) of PHE, (40 µg/ml) of TYR and (10 µg/ml) of TRP.

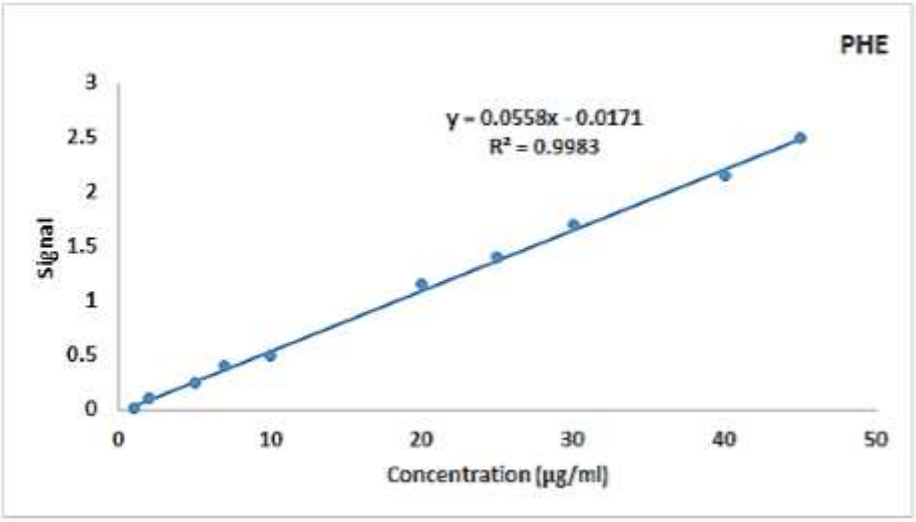

Fig. 4 : The calibration curve of PHE second derivative absorption spectrum at the wavelength of 218.9 nm.

Table 1 : Common parameters of the differential derivatization spectrometry of PHE, TRP and TYR analyzes.

Factor	PHE	TRP	TYR
Measurement wavelength (nm)	218.9	236.1	222.5
Quantification range (µg/ml) (x)	1.0-45.0	0.1-20.0	1.0-50.0
Curve linearity (R^2)	0.9983	0.9970	0.9990
Regression equation (y)	0.0558x - 0.0171	0.0743x + 0.0129	0.0338x + 0.0103
Slope (sensitivity) (b)	0.0558	0.0743	0.0338
Intercept (a)	-0.0171	0.0129	0.0103
Y= a+bX			

the optimum working wavelength of 218.9 nm where TRP and TYR are at the zero-crossing point which means they do not contribute to the value of 2D signal at that wavelength. However, TYR can be determined simultaneously in the presence of TRP at the wavelength of 236.1 nm but in the absence of PHE as it will interfere with the analysis. The same issue can be examined with the simultaneous determination of TRP at the wavelength of 222.5 nm in the presence of TYR as PHE will interfere with the analysis. The observations of the obtained results revealed the ability PHE can be determined in the presence of TYR and TRP, while the contrary is not applicable as PHE contribute in 2D signal value at the optimum wavelength value of the second derivative of TYR and TRP. Common parameters worked out from the application of the least-squares regression analysis of the differential derivatization spectrometry on the calibration curves of PHE, TRP and TYR analyzes (Figs. 4, 5, 6) and as were listed in Table 1.

The method validity and applicability were verified by laboratory-prepared mixtures in the concentration

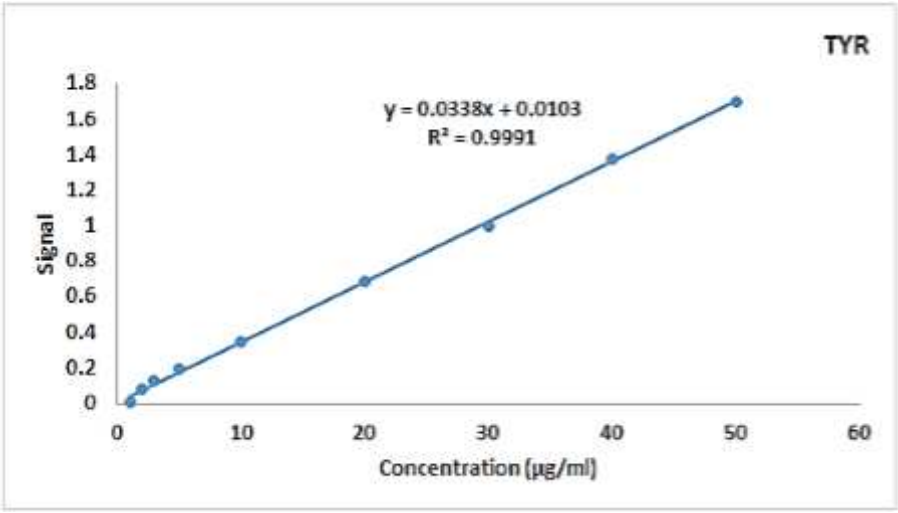

Fig. 5 : The calibration curve of TYR second derivative absorption spectrum at the wavelength of 236.1 nm.

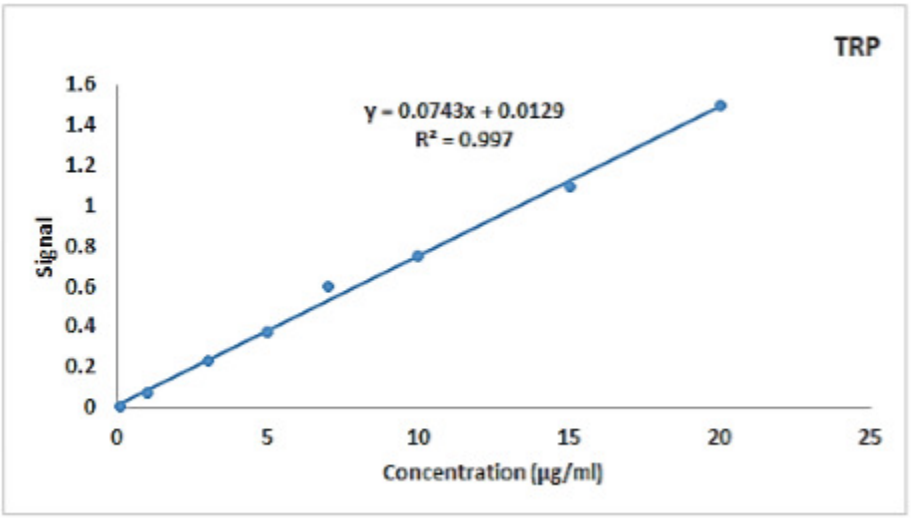

Fig. 6 : The calibration curve of TRP second derivative absorption spectrum at the wavelength of 222.5 nm.

Table 2 : Method validation and statistical values for the differential derivatization spectrometric determination method of PHE, TRP and TYR analyzes.

Parameters value	PHE	TRP	TYR
Actual concentration (µg/ml)	20.0	10.0	30.0
Measured concentration (µg/ml)	19.7	9.8	29.6
Standard Deviation (SD)	±0.23	±0.19	±0.11
Recovery	97.35-99.65	99.90-96.10	98.30-99.03
Relative SD (RSD%)	0.73	0.78	1.03
Relative Error (Er%)	0.69	0.61	1.07

ranges displayed in Table 1. The relative standard deviation (RSD%) was calculated for each amino acid separately and found within the range of 0.73–1.03% while the relative errors were in the range of 0.61-1.07% demonstrating satisfactory precision, reliability and reproducibility (Table 2).

CONCLUSION

Spectrometric differential derivatization with zero-crossing approach has been applied for the analysis of the aromatic amino acids of PHE, TYR and TRP in their mixture. The proposed approach proved to be simple, reproducible, sensitive, rapid, affordable, as well as sustainable and environmentally benign since it does not require solvents for the extraction or other sample preparation steps. The analytical outcomes that the method of the zero-crossing technique for the derivatized spectrophotometric spectrum can offer accurate, precise results and expected to be applicable for the examination of other aromatic amino acids.

REFERENCES

Al-Janabi K W S (2012) Direct Acetylation and Determination of Chlorophenols in Aqueous Samples by Gas Chromatography Coupled with an Electron-Capture Detector. *J. Chromatographic Sci.* **50**(7), 564–568. doi: 10.1093/chromsci/bms040.

Al-Janabi K W S, Mahmood A K and Luaibi H M (2020) Determination of the Dissociation Constants of Metformin from a Second Derivative UV Spectrum. *Int. J. Res. Pharmaceut. Sci.* **11**(1), 790–796.

Dale T (2012) *AARP Revitalize Your Hormones: Dr. Dale s 7 Steps to a Happier, Healthier and Sexier You*. Wiley. Available at: https://books.google.iq/books?id=5kRr25CbD5AC.

Dave H N, Mashru R C and Thakkar A R (2007) Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. *Analytica Chimica Acta* **597**(1), 113–120.

Erk N (2000) Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods. *J. Pharmaceutical and Biomedical Analysis* **23**(6), 1023–1031. doi: 10.1016/S0731-7085(00)00371-X.

Giriraj P and Sivakkumar T (2014) New Simple Spectrophotometric Method for the Simultaneous Estimation of Paracetamol and Flupirtine Maleate in Pure and Pharmaceutical Dosage Form. *Int. J. Spectroscopy* pp. 1–6. doi: 10.1155/2014/968420.

Hammer G D and McPhee S J (2018) *Pathophysiology of Disease: An Introduction to Clinical Medicine 8E*. McGraw-Hill Education. https://books.google.iq/books?id= 6Cp9Dw AAQBAJ.

Hay W W *et al* (2018) *Current Diagnosis and Treatment Pediatrics.* Twenty-Fourth Edition. McGraw-Hill Education. Available at: https://books.google.iq/books?id=KudXDwAAQBAJ.

Idili A *et al* (2019) An electrochemical aptamer-based sensor for the rapid and convenient measurement of l-tryptophan. *Anal. Bioanal. Chem*. **411**(19), 4629–4635. doi: 10.1007/s00216-019-01645-0.

Kämpfer P *et al* (2019) Quantitative determination of 58 aromatic amines and positional isomers in textiles by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *J. Chromatography A* **1592**, 71–81.

Kumar V, Sharma V K and Kalonia D S (2005) Second derivative tryptophan fluorescence spectroscopy as a tool to characterize partially unfolded intermediates of proteins. *Int. J. Pharmaceutics* **294**(1–2), 193–199.

Le T T *et al* (2019) Development and validation of a simple LC-MS/MS method for the simultaneous quantitative determination of trimethylamine-N-oxide and branched chain amino acids in human serum. *Anal. Bioanal. Chem*. **411**(5), 1019–1028.

Luo Y R, Armaghan Shafaei, Angela Genoni, Claus Christophersen, Amanda Devine, Johnny Lo, Philippa Lyons Wall and Mary C. Boyce Analy(2019) Azo coupling-based derivatization method for high-sensitivity liquid chromatography–tandem mass spectrometry analysis of tetrahydrocannabinol and other aromatic compounds. *J. Chromatography A* **1597**, 109–118.

Majidi M R, Jouyban A, Abdollahi H and Asadpour-Zeynali K (2006) Simultaneous voltammetric determination of cysteine, tyrosine and tryptophan by using principal component-artificial neural networks. *Asian J. Chem.* **18**(4), 2445–2457.

Maneglier B,Christine Rogez-Kreuz, Paulette Cordonnier, Patrice Therond, Charles Advenier, Dominique Dormont, Pascal Clayette and Odile Spreux-Varoquaux (2004) Simultaneous measurement of kynurenine and tryptophan in human plasma and supernatants of cultured human cells by HPLC with coulometric detection. *Clin. Chem.* **50**(11), 2166–2168.

National Center for Biotechnology Information (2019a) *Phenylalanine , CID=6140*, *PubChem Database*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Phenylalanine (Accessed: 8 July 2019).

National Center for Biotechnology Information (2019b) *Tryptophan, CID=6305*, *PubChem Database*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Tryptophan (Accessed: 8 July 2019).

National Center for Biotechnology Information (2019c) *Tyrosine, CID=6057*, *PubChem Database*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Tyrosine (Accessed: 8 July 2019).

Nozaki Y (1990) Determination of tryptophan, tyrosine, and phenylalanine by second derivative spectrophotometry. *Arch. Biochem. Biophy.* **277**(2), 324–333. doi: 10.1016/0003-9861(90)90587-O.

Perkampus H H, Grinter H C and Threlfall T L (2013) *UV-VIS Spectroscopy and Its Applications*. Springer Berlin Heidelberg (Springer Lab Manuals). Available at: https://books.google.iq/books?id=6ejwCAAAQBAJ.

Sanchez-Machado D I, Chavira-Willys B and López-Cervantes J (2008) High-performance liquid chromatography with fluorescence detection for quantitation of tryptophan and tyrosine in a shrimp waste protein concentrate. *J. Chromatography B* **863**(1), 88–93.

Vichare V *et al* (2010) Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation. *Int. J. PharmTech Res.* **2**(4), 2512–2516.

Shah M and Patel H (2019) Development And Validation Of Absorbance Correction Method And First Order Derivative Spectrophotometric Method For Simultaneous Estimation Of Gallic Acid, Ellagic Acid And Curcumin In Polyherbal Antidiabetic Dosage Forms. *Int. J. Pharmaceutical Res.* **11**, 1.