Volume 3(1),2025, Page 32-40

Analytical comparison between two spectroscopic methods used for the quantitative determination of olanzapine in its pure form

Ahmad Khalid Hussein [1], Mahmood Z. Lafta [2], Mumin F. Hamad Al-Samarrai [1], Ahmad Lateef [1]*, Saba Thamer Mahmood [1], Mahasen Raad Mahmood [1], Sama Ali Hussein [1]

[1] Samarra University - College of Education - Department of Chemistry. Samarra, Salah Al-Din, Iraq
[2] Salah al-Din Education Directorate - Samarra Education Department. Samarra, Salah Al-Din, Iraq
* Correspondence: ahmad.hasan.14418@gmail.com

Abstract: Statistical analysis compared two spectroscopic methods for determining olanzapine in its pure form, revealing similar results. The first method utilized the area under the peak within the wavelength range of 272-308 nm, demonstrating linearity within the range of 0.1-3 μg/ml. Results showed a recovery percentage of 96.05-102.93% and coefficient of variation percentage of 0.007-0.063%. Conversely, the second method employed a multi-wavelength technique at specific wavelengths (276, 284, 292, and 300 nm), with linearity within the range of 0.3-2 μg/ml. Results showed a recovery percentage of 98.6-100.97% and coefficient of variation percentage of 0.009-0.119%. Both methods exhibited sensitivity and accuracy, suggesting their applicability for the precise estimation of olanzapine in laboratory settings focused on quantitative drug analysis.

Keywords: multi-wavelengths; Quantitative determination; olanzapine; analytical comparison; UV-Visible spectrophotometer

1. Introduction

Olanzapine, a medication known for its ability to elevate serotonin levels within cells, holds therapeutic significance in the treatment of depression, schizophrenia, and psychotic syndromes. Chemically classified as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b][1,5]benzodiazepine (see Figure 1), this compound's multifaceted pharmacological properties make it a cornerstone in the management of various mental health disorders [1-3].

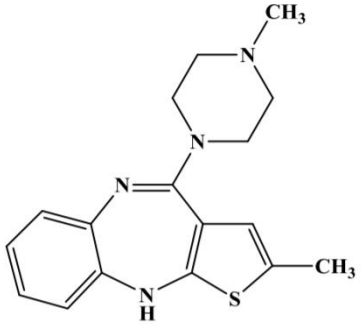

Figure 1. Chemical Composition of Olanzapine drug.

In pharmaceutical or pure formulations, various techniques are utilized for the determination of olanzapine. These include color methods such as ion pair [4], charge transfer complexes [5] and derivative of spectral ratio [6,7], capillary zone electrophoresis [8-10], gas chromatography-mass spectrometry [11,12], cyclic voltammetry [13], high-performance thin-layer chromatography (HPTLC) [14], and high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) [15,16] or ultraviolet detection (HPLC-UV) [17-23].

2. Materials and Methods

2.1. Devices

- UV-Visible Spectrophotometer: Spectra for Olanzapine were recorded using a UV-Visible spectrophotometer manufactured by Shimadzu Company, model 1650. Cuvettes with a width of 1 cm were utilized for sample containment.
- Sensitive Balance: A Sartorius BL 210 S sensitive balance, manufactured in Germany, was employed for precise weighing.

The spectra were recorded within the wavelength range of 190-380 nm, utilizing a fast-scanning speed with a rate of change of 0.1 nm and a bandwidth of 2 nm.

2.2. Solutions

The raw materials were sourced from the State Company for the Pharmaceutical Industry and Medical Devices (SDI) - Samarra, Iraq.

- Olanzapine Standard Solution: A 1000 μg/ml Olanzapine standard solution was prepared by dissolving 0.05 g of Olanzapine in ethanol:water (1:9) within a 50 ml volumetric flask.

The wavelength ranges of 272-308 nm and 276-300 nm were selected based on the absorption characteristics of olanzapine, as it exhibits strong absorption within these ranges, allowing for accurate spectral signals to determine concentration. These ranges correspond to the wavelengths where the absorption of the compound is most prominent, minimizing interference from other compounds and ensuring more precise results. The 272-308 nm range shows a strong response for olanzapine absorption, making it suitable for constructing an accurate calibration curve. The 276-300 nm range was chosen to enhance measurement accuracy and reduce interference from other substances in the sample.

2.3. The procedure

1- multi-wavelengths

- The solvent chosen for the experiment was ethanol:water (1:9) ratio.
- Different concentrations of Olanzapine were prepared ranging from 0.1 to 30 μg/ml.
- Absorption spectra were recorded for each concentration.
- Four wavelengths close to each other were selected for analysis: 276 nm, 284 nm, 292 nm, and 300 nm.
- Calibration curves were constructed for each wavelength.
- It was observed that the linearity of the calibration curves complied with the Beer-Lambert law within the concentration range of 0.3-2 μg/ml.
- The equations of the straight lines were algebraically summed to derive a single straight-line equation for subsequent analysis.

2- area under-curve

A series of concentrations ranging from 1.0 to 300 μg/ml of Olanzapine standard solution was prepared in 10 ml volumetric flasks and adjusted to the mark with a solvent composed of ethanol and water.

Absorption spectra were recorded across wavelengths ranging from 190 to 380 nm. After conducting several experiments on peak areas within the wavelength range of 250-325 nm, it was determined that the optimal area for constructing the calibration curve was between wavelengths 272-308 nm.

Linearity within this wavelength range was found to be between 0.1 and 3 μg/ml, making it suitable for conducting the study on the drug using the proposed method.

Linearity Range Differences:

The difference in linearity ranges (0.1-3 μg/ml vs. 0.3-2 μg/ml) reflects the impact of practical conditions on choosing the appropriate method.

0.1-3 μg/ml range (Area Under the Curve method):

Suitable for measurements requiring accurate determination of low to medium concentrations. This method provides better sensitivity for ensuring precise results at low concentrations, making it ideal for samples with small amounts of the active compound.

0.3-2 μg/ml range (Multi-wavelength method):

Better suited for samples containing higher concentrations of the active substance. This method is more effective when the quantities are higher, as it reduces spectral interference and achieves greater accuracy.

Practical Application:

The differences in linearity range influence the choice of method based on sample type and analytical needs. For situations requiring precise measurement of very low concentrations, the Area Under the Curve method is ideal for accurate determination within a broader measurement range. In contrast, the Multi-wavelength method is more suitable when dealing with higher concentrations or complex spectral interference.

3. Results

3.1. Absorption Spectraz

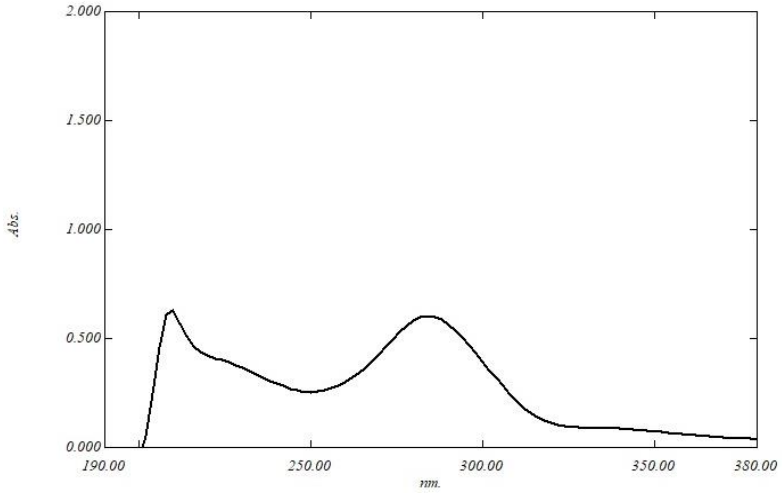

Figure 2: Olanzapine absorption spectrum (1µg/ml^{-1})

The absorption spectrum of olanzapine was recorded using a spectrophotometer within the wavelength range of 190-380 nm. Figure 2 illustrates the absorption spectrum of olanzapine at a concentration of 1 µg/ml.

3.2. Multi-Wavelengths method

When observing the spectrum of olanzapine, its wide peak makes accurate distinction challenging. Therefore, we employed the multi-wavelength method to mitigate this issue [24-28].

To proceed with this approach, four specific wavelengths were selected as previously described. Calibration curves were then constructed for each wavelength, yielding individual linear equations. These equations were algebraically combined to derive a single straight-line equation for analysis. Figure 3 depicts the selected wavelengths of the drug along with a series of concentrations.

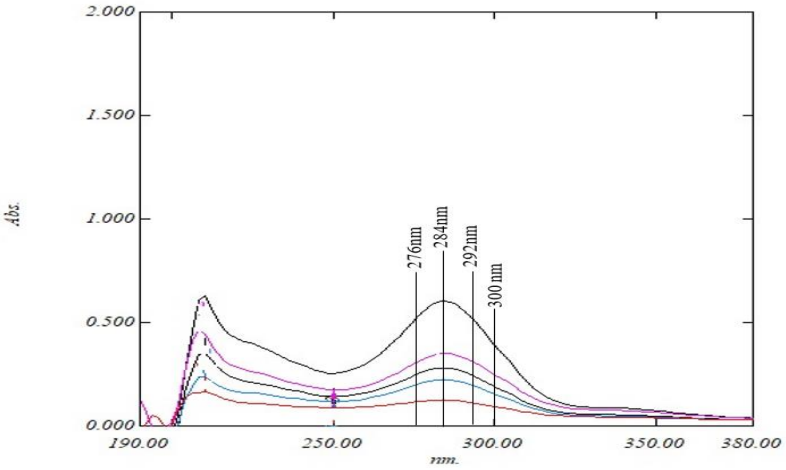

Figure 3: The chosen wavelengths for a series of conc.

3.3. area under-curve

The area under the curve was determined at wavelengths ranging from 272 to 308 nm using the UVProbe program for each concentration. It was observed that the area was directly proportional to the increase in drug concentrations within the method's range of (0.1-3 µg/ml).

After plotting the calibration curve between the concentrations and the corresponding area under the curve, Figure 4 illustrates the selected area for different concentrations of the drug.

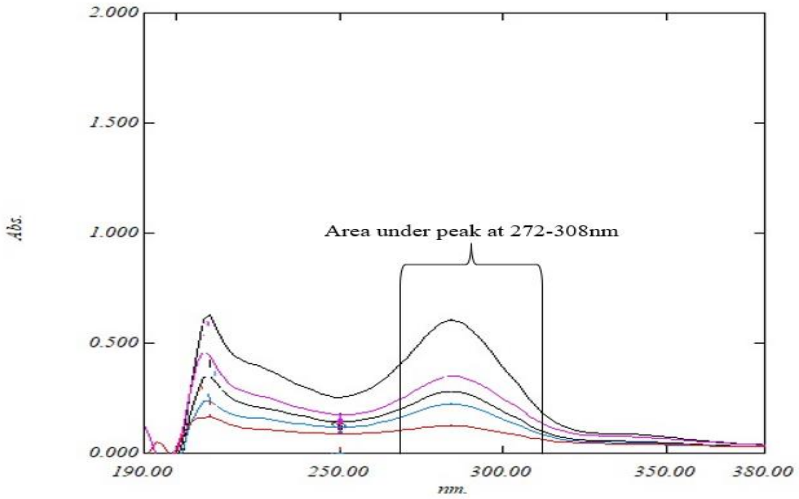

Figure 4: the selected area at 272-308nm.

3.3. Calculations & Calibration Curves

1.multi-wavelenths method

After determining the optimal solvent, calibration curves were constructed, revealing concentrations that adhered to Beer-Lambert's law. Concentrations within the range of 0.3-2 µg/ml demonstrated linearity for all wavelengths, with correlation coefficient values ranging from 0.9985 to 0.9989 for olanzapine. Figures 5-8 display the calibration curves of olanzapine at each wavelength.

By summing the equations of the individual straight lines, a single equation was derived (y = 3.3513x - 0.3512). This combined equation was utilized for further statistical analysis to demonstrate the sensitivity and accuracy of the method. The limit of detection (LOD) was calculated to be 0.0906, while the recovery percentage (Rec%) ranged from 98.6 to 100.97, with coefficients of variation (CV%) between 0.009 and 0.119.

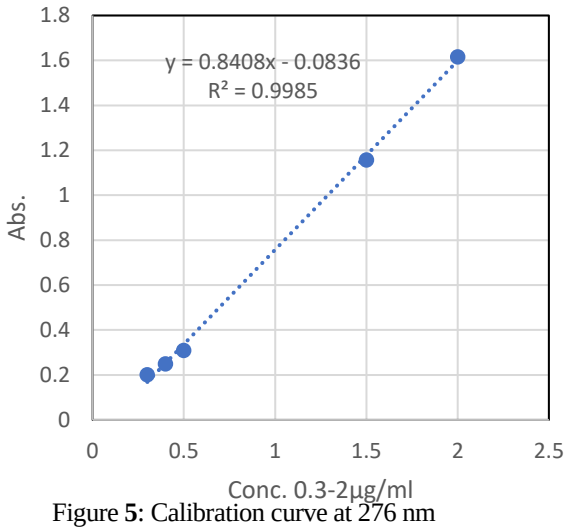

Figure **5**: Calibration curve at 276 nm

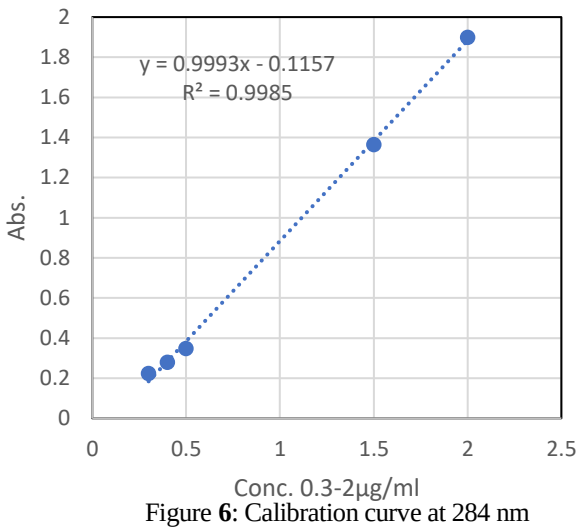

Figure **6**: Calibration curve at 284 nm

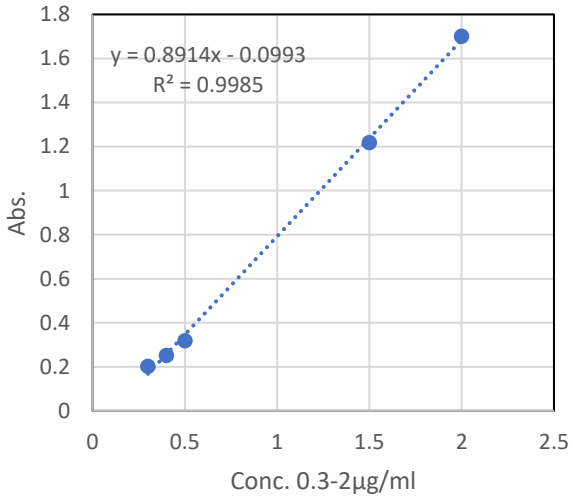

Figure **7**: Calibration curve at 292 nm

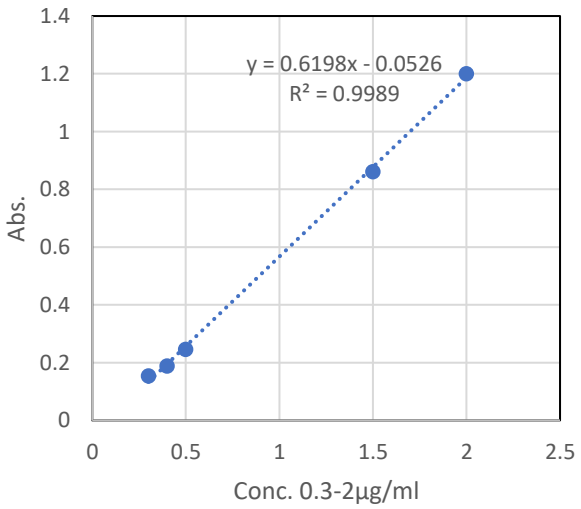

Figure **8**: Calibration curve at 300 nm

2.area under peak method

A series of concentrations ranging from 0.1 to 30 µg/ml was prepared, as previously described. Upon plotting the curve between the area under the peak and the concentrations, it was determined that the method exhibited linearity within the range of 0.1-3 µg/ml. Figure 9 illustrates the calibration curve obtained.

The limit of detection (LOD) was calculated to be 0.0693. The recovery percentage (Rec%) fell within the range of 96.05-102.93, with coefficients of variation (CV%) ranging from 0.007 to 0.063.

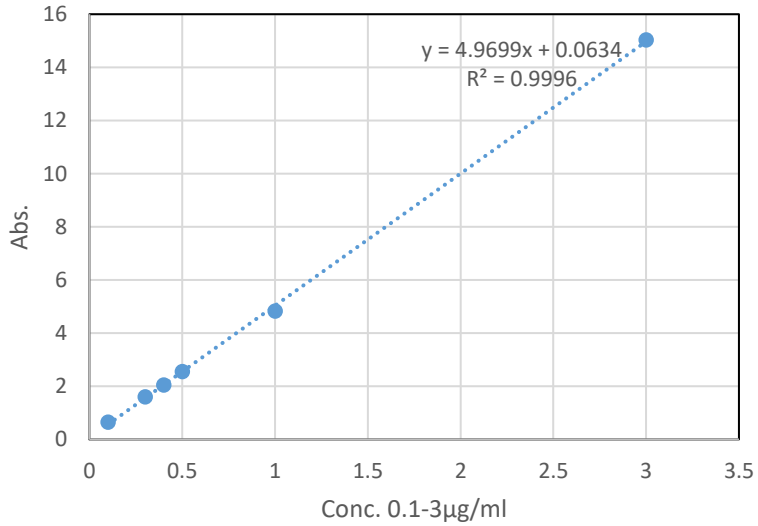

Figure 9: Calibration curve by area under peak.

3.4. The precision & accuracy of the proposed methods

From the straight-line equation resulting from the combination in the first method, three concentrations (0.4, 1.5, and 2 µg/ml) were selected to assess the accuracy and precision of the method. The recovery percentages (Rec%) ranged from 98.06 to 100.97, with coefficients of variation (CV%) ranging from 0.009 to 0.119.

For the second method, concentrations ranging from 0.3 to 3 µg/ml were chosen. The recovery percentages (Rec%) fell within the range of 96.05 to 102.93, with coefficients of variation (CV%) ranging from 0.007 to 0.063, as summarized in Table 1.

Table 1: precision & accuracy of the work methods

Method	**Taken Conc. µg/ml**	**Found Conc. µg/ml**	**Recovery%**	**CV%**
Multi-wavelengths	0.4	0.395	98.68	0.119
	1.5	1.479	98.60	0.088
	2	2.019	100.97	0.009
Area under peak	0.3	0.309	102.93	0.063
	1	0.961	96.05	0.021
	3	3.012	100.39	0.007

Table 2 shows the t-test results indicate that the t-value of -0.185, along with a P-value greater than 0.05, suggests that there is no statistically significant difference between the two methods in terms of recovery percentages. This implies that both methods yield similar results with respect to accuracy in estimating the drug concentration.

Furthermore, the F-value of 1.02 indicates that the variances between the two methods are close, supporting the idea that either method can be used with equal confidence in practical applications, as both demonstrate comparable precision and reliability.

Table 2: T-test calculation

Variable	Multi-Wavelength Method	Area Under Peak Method	Mean Difference	Standard Deviation	t-value	P-value	F-value
Recovery%	98.68, 98.60, 100.97	102.93, 96.05, 100.39	-0.37	3.50	-0.185	>0.05	1.02

Accuracy was assessed through recovery experiments conducted at three different concentrations (0.4, 1.5, 2 µg/ml). The results showed recovery percentages ranging from 96.05% to 102.93%, which aligns with the ICH Q2(R1) guidelines, confirming the reliability of the method. While samples were analyzed three times per concentration on the same day (Intra-day). The analysis was repeated on a different day (Inter-day). The coefficient of variation (CV%) was calculated, and it was found to be below 2% for each method, indicating excellent repeatability according to USP and ICH standards. The linearity of the data was assessed using the correlation coefficient ($R^2 > 0.998$). The limits of detection (LOD) and quantitation (LOQ) were measured to ensure the methods' sensitivity.

4. Comparison of the results of the proposed methods

The statistical results obtained under the working conditions, along with the comparison of the proposed methods, indicate that the methods are statistically similar and excel in various aspects, including cost-effectiveness, accuracy, and precision. Table 2 provides a comparison demonstrating the close similarity between the methods.

These findings suggest that these methods hold potential for utilization in laboratories focused on the estimation of medicinal drugs.

Table 2: Comparison of the results of the proposed methods

Methods / **Statistical operations**	**Multi-wavelengths***	**Area under peak***
Linearity	0.3-2	0.1-3
correlation coefficient	0.9989	0.9996
Rec%	100.97	100.39
LOD µg.ml^{-1}	0.0906	0.0693

LOQ µg.ml^{-1}	0.2719	0.2081
CV%	0.009	0.007

*Proposed method

The multi-wavelength method offers higher accuracy and reduced spectral interference by utilizing data from multiple wavelengths, which improves concentration determination and enhances quantitative analysis, especially for substances with strong spectral overlaps. In comparison, the Area Under the Curve (AUC) method, while useful, may be less accurate when interferences occur within the same spectral range. The multi-wavelength approach provides more detailed spectral information, allowing for more precise compound identification and reducing errors associated with peak-area-based methods [29, 30].

The choice of solvent significantly impacts the absorption response of the studied compound. Using solvents that are incompatible with the active substance can lead to skewed results due to solvent absorption or interactions with the compound. Therefore, selecting an appropriate solvent, such as ethanol or water, ensures accurate results. Similarly, sample concentration plays a crucial role; both extremely high and low concentrations can negatively affect measurement accuracy. At low concentrations, weak spectral signals can reduce precision, while at high concentrations, spectral interference may occur. It is essential to maintain concentration within the linearity range for accurate determination. The sensitivity of the spectrophotometric instrument also directly influences the system's ability to accurately measure low concentrations, with highly sensitive instruments providing more precise recovery measurements, particularly for small concentrations. Finally, experimental errors, whether due to environmental conditions or variability in laboratory equipment, can lead to variations in results. Reducing such errors requires conducting multiple trials and utilizing tools that minimize discrepancies between measurements.

Reference

1. Moffat,A.C.; Osselton, M.D. ; Widdop,B. (2011), Clarke's analysis of drugs and poisons, 4, Pharmaceutical Press, London.
2. . Shen, W. W. 1999. The metabolism of atypical antipsychotic drugs: an update. Ann. Clin. Psychiatry 11:145-158
3. Mahmoud, A. T.; Nagiba, Y. H.; Nariman, A. E. and Mohamed, A, (2013), Simultaneous determination of olanzapine and fluoxetine hydrochloride in capsules by spectrophotometry, TLC-spectrodensitometry and HPLC, Journal of Advanced Research, 2(4), 173-80.
4. Kanakapura, B. SA, MEER A. A. and Kanakapura, B. V, (2009), Simple and Sensitive Spectrophotometric Determination of Olanzapine in Pharmaceutical Formulations Using, Journal of Food and Drug Analysis, 17(6), 434-42.
5. Khalaf, F. A.; Sarmad, B. D. and Mumin, F. A, (2015), Spectrophotometric determination of ephedrine-hydrochloride in pure and pharmaceutical preparations based on charge transfer complexation method, International Conference on Recent Trends in Engineering Science and Management, 5758-66.
6. Hanoon, I.T., Al-Samarrai, M.F., & Lafta, M.Z. (2022). Immediate Real-time Estimation of a Combination of Drugs Containing Amino Groups with Ultraviolet Spectroscopy.
7. M. Hamad Al-Samarrai, E. Hanon, A. Khaleel. Development of derivative of subtracting spectra method for the simultaneous determination of some decongestant drugs, Samarra Journal of Pure and Applied Science 2021, 3(3): 18-30.
8. Chunmei, Z.; Huaizhong, G. and Li, G. (2011), Determination of Olanzapine by injection capillary electrophoresis International Journal of Chemistry, 1(3), 202-06.
9. Deng, D.; Deng, H.; Zhang, L. and Su, Y. (2014), Determination of ephedrine and pseudoephedrine by field-amplified sample injection capillary electrophoresis, Chromatogr Sci, 52(4), 357-62.
10. Raggi, M. A., Casamenti, G., Mandrioli, R., Izzo, G. and Kenndler, E. 2000. Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry. J. Pharm. Biomed. Anal. 23: 973-981.
11. Ranieri, T. and Ciolino, L., (2008), Rapid selective screening and determination of ephedrine alkaloids using GC-MS footnote mark, Phytochem Anal, 19(2), 127-35.
12. A. A. Elian, Forensic Sci. Inter., 1998, 91, 231.

13. Biryol, I. and Erk, N. 2003. Voltammetric, spectrophotometric, and high-performance liquid chromatographic analysis of olanzapine. Anal. Lett. 36: 2497-2513.
14. Shah, C. R., Shah, N. J., Suhagia, B. N. and Patel, N. M. 2007. Simultaneous assay of olanzapine and fluoxetine in tablets by column high-performance liquid chromatography and high-performance thin-layer chromatography. J. AOAC Int. 90: 1573-1578.
15. Z. Zhou, X. Li, K. Lin, Z. Xie, Z. Cheng, W. Peng, F. Wang, R. Zhu, and H. Li, J. Chromatogr., B, 2004, 802, 257.
16. M. J. Bogusz, K. D. Kruger, R. D. Maier, R. Erkwoh, and F. Tuchtenhagen, J. Chromatogr., B, 1999, 732, 257.
17. L. J. Dusci, L. P. Hackett, L. M. Fellows, and K. F. Ilett, J. Chromatogr., B, 2002, 773, 191.
18. O. V. Olsen and K. Linnet, J. Chromatogr., B, 1998, 714, 309.
19. K. Titier, S. Bouchet, F. Pehourcq, N. Moore, and M. Molimard, J. Chromatogr., B, 2003, 788, 179.
20. . M. A. Raggi, G. Casamenti, R. Mandrioli, G. Izzo, and E. Kenndler, J. Pharm. Biomed. Anal., 2000, 23, 973.
21. Al-Samarrai, M.F., Lafta, M.Z., Al-Abbasee, M.J., & Muhammad, O.J. (2024). Development and Validation of a RP-HPLC Method for Simultaneous Quantification of Paracetamol and Phenylephrine Hydrochloride. International Journal of Design & Nature and Ecodynamics.
22. D. W. Boulton, J. S. Markowitz, and C. L. De Vane, J. Chromatogr., B, 2001, 759, 319.
23. O. V. Olsen, B. Poulsen, and K. Linnet, Therap. Drug Monit., 2001, 23, 51.
24. H. Weigmann, S. Hartter, S. Maehrlein, W. Kiefer, G. Krammer, G. Dannhardt, and C. Hiemke, J. Chromatogr., B, 2001, 759, 63.
25. Samadi Naser, Mina Salamati, and Abdolhossein Naseri. "Model based multi-wavelength spectrophotometric method for calculation of formation constants of phenanthrenequinone thiosemicarbazone complexes with some metallic cations." Current Chemistry Letters 2.2 (2013): 69-76.
26. M. Lafta, N. Hassan and M. Al-Samarrai. Spectrophotometric Estimation of Paracetamol in its Pharmaceutical form (tablets) by multi-Wavelength method. Education for Chemical Engineers 2022, 40-(7): 1–8.
27. M. Hamad Al-Samarrai. "Spectrophotometric Determination for Metronidazole drug in pharmaceutical form (tablet) by Multi-wavelengths method." Tikrit Journal of Pure Science 2023.
28. Mouhamed, A.A., Nadim, A.H., Mostafa, N.M. et al. Application of smart chemometric models for spectra resolution and determination of challenging multi-action quaternary mixture: statistical comparison with greenness assessment. BMC Chemistry 18, 44 (2024). https://doi.org/10.1186/s13065-024-01148-9.
29. Khayata, W., & Zakri, D. A. L. (2017). Two simple spectrophotometric methods for the simultaneous determination of amoxicillin trihydrates and flucloxacillin sodium. Research Journal of Pharmacy and Technology, 10(5), 1327–1332.
30. Hegazy, M. A., Lotfy, H. M., & Mowaka, S. (2016). Evaluation of the efficiency of continuous wavelet transform as processing and preprocessing algorithm for resolution of overlapped signals in univariate and multivariate regression analyses. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 164, 15–23.