Green spectrophotometric platforms for resolving overlapped spectral signals of recently approved antiparkinsonian drug (Safinamide) in presence of its synthetic precursor (4-Hydroxybenzaldehyde): Applying ecological appraisal and Comparative statistical studies

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ABSTRACT

Background: Safinamide, a highly specific inhibitor of monoamine oxidase B, is a new approved prodigious therapy used to cure Parkinson's disease.

Objective: Before marketing and selling a medicine, manufacturers must guarantee that the manufacturing process is consistent by monitoring levels of process-related chemicals and drug contaminants. Therefore, five precise, fast, and accurate spectrophotometric techniques were employed and evaluated for the simultaneous measurement of safinamide and its synthetic precursor 4-Hydroxybenzaldehyde.

Method: The first derivative, derivative ratio, ratio difference, dual wavelength, and Fourier self-deconvolution methods worked well to resolve spectral overlap of safinamide and its synthetic precursor 4-Hydroxybenzaldehyde.

Results: Safinamide detection limits ranged from 0.598 to 1.315 µg/mL, whereas 4-Hydroxybenzaldehyde detection limit was found to be as low as 0.327 µg/mL.

Conclusion: According to ICH criteria, all procedures were verified and confirmed to be accurate, robust, repeatable, and precise within reasonable range. No considerable variation was found when comparing the outcomes of the suggested approaches to the findings of previously published method. The ecological value of established methods was measured: The national environmental methods index (NEMI), the analytical Eco-scale, the Analytical Greenness Metric (AGREE), and the green analytical process index (GAPI) were used.

Highlights: First spectrophotometric determination of safinamide drug in presence of its synthetic precursor. Five simple and efficient spectrophotometric approaches were employed to determine newly approved antiparkinsonian drug in presence of synthetic precursor simultaneously. Ecological appraisal was performed for the developed methods using four assessment tools.

Keywords: Safinamide, First derivative, Derivative ratio, Fourier self-deconvolution and COVID-19

Introduction

One of the most prevalent causes of mortality and illness in the world today is neurodegenerative disease, which affects the elderly(1). When it comes to neurological illnesses, Parkinson's Disease (PD) is in second place behind only Alzheimer's disease (AD) in terms of prevalence(2). One million Americans and tens of millions of others throughout the world are estimated to be affected by Parkinson's disease. Regrettably, by 2030, the number of persons living with PD will have increased from 8.7 million to 9.3 million, according to studies. Healthcare costs range from $2,000 to $20,000 for each patient, and prescription costs range from $1,000 to $6,000 (3, 4). The stiffness of the respiratory muscles in elderly people with more severe PD makes them more susceptible to COVID-19 (5). The severity of infection with COVID-19 might be exacerbated if the cough reflex is impaired due to possible brain stem involvement and coexisting dyspnea(6). Monoamine oxidase-B (MAO-B) inhibitors, which suppress dopamine breakdown, have been recommended to treat people with PD (7). A drug called safinamide mesylate (SAF) Figure.(S1.a) is amongst the most crucial MAO-B inhibitors(8). It was approved by the European Union and the FDA in February 2015 and March 2017, respectively, to be used either alone or in conjunction with current Parkinson drugs(9). Its chemical composition is: (S)-2- [[4-[(3-fluorophenyl) methoxy] phenyl] methyl] aminopropanamide methanesulfonate(10). Many publications on the assessment of SAF alone or in blend with other medications have been authored in peer-reviewed journals which include the following: HPLC (11–18) ,HPTLC (11, 19), UPLC (11),thermal analysis (20). There is no published spectrophotometric technique for determining SAF in the presence of its synthetic precursor impurity 4-hydroxybenzaldehyde (4-HB) Figure.(S1.b), which has been described as its synthetic precursor (15, 21–23).

Spectrum overlap and lack of specificity in a drug combination make spectrophotometric component measurement more challenging. This has resulted in the

development of novel spectrophotometric approaches that employ basic software and arithmetic to quickly, precisely, and inexpensively separate overlapping spectra. As a result, the fundamental purpose of this research was to create innovative, selective, sensitive, and accurate spectrophotometric methods for the simultaneous determination of the recently FDA approved antiparkinson's drug in tablets and bulk powder in the presence of its synthetic precursor without prior separation. Four approaches were used to assess the ecological value of the suggested methods: the national environmental methods index (NEMI)(24) , the analytical Eco-scale(25, 26), the Analytical Greenness Metric (AGREE)(27), and the green analytical process index (GAPI)(25).

Experimental

Devices and software

A dual beam spectrophotometer (Jasco, Japan) was used for all spectrophotometric measurements. Spectra management software was installed on the Spectrophotometer to conduct spectral treatments on the obtained absorption spectra. A comparative statistical analysis of the analyzed and reported data was conducted using Minitab 2019. In the processing of pharmaceutical samples, a sonicator (DAIHAN WUC-A01H, USA) was employed.

Material and reagents

LOBA Chemie Pvt. Ltd (Mumbai, India) supplied 4-HB with a purity certification of 98 percent. SAF was acquired from October Pharm, Cairo, Egypt, with a purity certification of 99.7 percent. SAF tablets (Safinozol®) supposed to contain 100 mg of SAF per tablet were generously donated by October Pharm, Cairo, Egypt. Merck (Germany) provided the HPLC-grade methanol.

Standard solutions

Standard stock solutions of SAF and 4-HB (100 µg/ mL) were made by weighing and accurately transferring 10 mg of each standard powder into a 100-mL volumetric flask. They were then dissolved and agitated in 70 mL methanol, and the volume was adjusted to 100 mL with methanol. The solution remained stable for 14 days when refrigerated at 4°C.

Creation of calibration curves

In the wavelength range of 200–400 nm, 4-HB was scanned against methanol as a blank, and the absorbance at 290 nm was determined directly without interference from SAF. The regression equation was produced by establishing a calibration curve between the absorbance at 290 nm and the required 4-HB concentrations (1-10 µg/mL), while SAF approaches comprise the following procedures:

(a) ***Method of first derivative spectrophotometry (D^1)***

The first derivative corresponding to every absorption spectra was recorded to determine SAF in the presence of 4-HB. Over the concentration range of 5-30 µg/mL, the amplitude values were recorded at 241nm.

(b) ***Method of first derivative ratio (DD^1)***

SAF was calculated in the presence of 4-HB by dividing the recorded zero-order spectra for SAF working solutions by the spectrum of 4-HB (7 µg/mL) as a devisor. The first derivative of every ratio spectrum was recorded, and the produced amplitudes of SAF at 238 nm were calculated against their respective concentration ranges of 5–35 µg/ mL.

(c) ***Method of ratio difference (RD)***

SAF was determined in the presence of 4-HB by dividing the recorded spectra of SAF across the concentration range 5–35 µg/mL by the spectra of 7 µg/mL of 4-HB as a divisor. The calibration curves for SAF were created by plotting the amplitude difference of ratio spectra at 217 nm and 233 nm versus their respective concentrations in µg/mL, then computing the regression equations.

(d) ***Method of dual wavelength (DWL)***

The difference in absorbance of the saved spectra was evaluated at 226 and 259 nm throughout the concentration region 5–30 µg/mL to determine SAF in the presence of 4-HB.

(e) ***Method of Fourier self-deconvolution (FSD)***

The saved zero-order spectra were deconvoluted using the Fourier deconvoluted function incorporated into spectrophotometer software with a full width at half maximum value (FWHM) of 65 to determine SAF in the presence of 4-HB. The amplitudes of SAF generated at 234 nm were then plotted versus their concentrations (5–30 µg/mL).

Analysis of laboratory-prepared mixtures

A series of 10-mLvolumetric flasks were used to correctly transfer aliquots of SAF and 4-HB from their respective standard stock solutions (100 µg/mL). After that, the flasks were filled with methanol to create laboratory-prepared solutions with different concentrations of SAF and 4-HB (10:10, 20:8, 25:5, 30:9, and 30:10 µg/mL, respectively). The zero-order spectra of every laboratory created combination was recorded against methanol, and then saved in the computer and the operation followed as described before.

Pharmaceutical dosage form analysis

The contents of 10 Safinozol® tablets were precisely weighed and combined. A properly weighed quantity of 100 mg SAF was transferred to a 100-mL volumetric flask, and then 50 mL methanol was added. The prepared solution was sonicated for 25 minutes before being cooled and finally completed to volume with methanol. The solution was filtered and diluted to accomplish a final concentration of 1000 µg/mL. Different SAF and 4-HB concentrations were obtained by diluting the stock solution with methanol. The appliance of regression equations assessed the medicine concentration.

Results and discussion

In the presence of 4-HB, SAF UV spectra are severely overlapped. Five new UV-spectrophotometric platforms have been developed for selective analysis of SAF by removing 4-HB interference.

Method of first derivative (D^1)

SAF and 4-HB first derivatives(28, 29) are obtained and the drug of concern is measured while the 4-HB crosses zero, as illustrated in Figure. 1. Measurements of amplitude at 241nm were used to determine SAF in this study.

Method of first derivative ratio (DD^1)

The ratio spectra technique first derivative (30, 31) was calculated by dividing each drug absorption spectrum by the sum of the spectra of the synthetic precursor. However, selecting an appropriate divisor is a crucial step that must be fine-tuned in order to get correct results. Therefore, different concentrations of the divisors were examined, and it was determined that divisor 7 µg/mL was the most suited divisor without interference from 4-HB. After obtaining the absorption spectrum of the saved ratio spectra (SAF/4-HB). The first ratio derivative of each SAF concentration was observed at 238 Figure. 2.

Method of ratio difference (RD)

The difference between any two spots on the preceding resultant ratio spectrum (SAF/4-HB) will be directly proportional to the concentration of the targeted medication(32, 33). It was found that the best results came from the difference in the peak amplitudes (ΔP) of the ratio spectra at 217 and 233 nm for SAF Figure. 3.

Method of dual wavelength (DWL)

The inclusion of two wavelengths in which the interfering element exhibits identical absorbance and the desired element varies greatly in absorbance with concentration is a critical consideration in using the dual-wavelength approach (34, 35) . Because selecting suitable wavelengths for sensitivity and selectivity is critical, different wavelengths were investigated, and it was observed that the best results for sensitivity and selectivity were obtained through the absorbance difference at 226 nm and 259 nm for determining SAF, where 4-HB shows zero absorbance difference Figure. 4.

Method of Fourier self-deconvolution (FSD)

The Fourier-self deconvolution technique (FSD) is a unique spectrophotometric approach for analyzing binary mixtures(36, 37). It is a basic and uncomplicated mathematical strategy for resolving substantially overlapped zero-order spectra by reducing their bandwidth by utilizing the Fourier or deconvolution function of spectrophotometer software. By overlaying the medicinal combinations spectra, zero-crossing or no-contribution sites were produced, allowing the identification of one component without impact from the other. Deconvolution of SAF spectrum at 234 nm was performed to calculate the SAF concentration, and a regression equation was applied to get the SAF concentration. Figure. 5.

Method validation

The ICH Q2 (R1) criteria were used to examine linearity, the limit of detection (LOD), the limit of quantitation (LOQ), selectivity, accuracy, and precision(38).

(a) *Linearity*

Linearity of the suggested spectrophotometric techniques for SAF and 4-HB quantification was evaluated in triplicate by measuring varied concentration absorbance's in the ranges shown in Table 1. The adopted approaches presented respectable linearity (correlation coefficient, $R \geq 0.9992$). Table 1 displays the regression parameters of the suggested approaches.

(b) *The limit of detection (LOD) and limit of quantification (LOQ)*

The limits of detection (LOD) and quantification (LOQ) were evaluated using ICH Q2 (R1) guidelines by calculating the lowest concentrations that could be recognized and quantitatively assessed, as shown in Table 1.

LOD =3.3 S/b and LOQ = 10 S/b

Where S is the standard deviation of the intercept of the calibration curve, and b is the slope of the calibration curve.

(c) ***Accuracy***

The suggested methods were tested by comparing the five laboratory prepared concentrations of each medicine that were measured to their genuine values. Table 1 displays the average percentage of recoveries calculated.

(d) ***Precision***

The Precision of each suggested approach was checked intraday by repeating each analyte analysis three times on the same day. Repeating the technique three times in a row allowed us to calculate the RSD percent of the inter-day precision. The findings are displayed in Table 1.

(e) ***Selectivity***

The selectivity of the suggested approaches was evaluated via the use of laboratory-prepared mixes with different SAF: 4-HB ratios. Average recovery percentages were found to be within acceptable ranges, as shown in Table 2.

Analysis of dosage form

These spectrophotometric methods were used to determine the amounts of SAF in its dosage form (Safinozol tablet®). Using the standard addition approach, the validity of the suggested processes was further examined, and no interference from excipients was observed. As shown in Table 3, the described approaches yielded recoveries with high percentages.

Assessment of the proposed approaches environmental impact

It is essential to replace toxic solvents and reagents with less hazardous alternatives if the analytical procedure is to be ecologically friendly. Analytical tools such as the NEMI, analytical Eco-scale, AGREE, and GAPI are well-known in this field. Ecological evaluation using these tools has been reported in many publications (39–49) . The four aforementioned instruments for evaluating environmental friendliness analyzed the proposed procedures (Table 4). NEMI is a graph with four quarters. The four green quarts indicate that the solvents used are non-hazardous, non-bioaccumulative, non-corrosive, and create negligible quantities of waste. Eco-scale is yet another point-based assessment system. The method begins with a score of 100. If the base value deviates from the standard, penalty points are removed from it. The proposed techniques were

given an eco-scale score of 88. GAPI is a novel aspect comprised of five pentagrams that reflect ecological impact. The items are colored green, yellow, and red to represent mild, moderate, and severe environmental impacts, respectively. AGREE was recently reported as well as built on GAC twelve principles. It presents a graph in the form of a clock with twelve segments around its circumference, each of which represents a distinct GAC principle based on its intuitive color and weight expressed by segment breadth. The AGREE color-codes range in hue from red to yellow to green. The final score and color in the center of the suggested techniques pictogram proved the greenness of the suggested approach.

Statistical analysis

Methods were compared with each other and with published results using a variety of statistical techniques. When comparing the reported and recommended techniques, a student t-test and an F-test were used, and neither of them showed a significant difference, Table 5. One-way ANOVA findings showed that the estimated F-values were below the critical one, which suggested that there was no variation between groups, Table 6. However, ANOVA was not the only statistical method used to verify the results.

The interval plot test was the second method(50). Confidence intervals are shown in the form of vertical lines, with the center point matching to the interval average. Each approach data group intervals overlap each other in the figure. Figure (S2) illustrates that there is no weighty variance among the techniques that have been proposed and those that have been reported.

Boxplots are an additional essential data visualization technique(51), which denotes the diffusion of data across groups, Figure (S3) displays boxplots of the proposed and published methods. The middle quartile is depicted by the center box, which includes a line indicating the data median, upper lines indicating higher values, and whiskers indicating lower values. The boxplot illustrates the data distribution within each data category.

The normal probability plot (52) is a further approach for verifying whether data is normally distributed Figure (S4). The data satisfy the normal distribution if the straight line passes across the greater part of the data sets.

The ultimate statistical instrument is Tukey's simultaneous significant difference test (50). It is a potent instrument for identifying any disparities between the mean values of the various groups. The data interval for each group is shown in Figure. (S5) as a horizontal line with a dot passing through the mean value of each data group. The overlap among the intervals indicated that the average values of the planned and reported methods were not substantially different.

Conclusion

This study included five spectrophotometric methods for the first time to evaluate SAF in the presence of 4-HB in their standard powdered form, laboratory-prepared mixes, and drug product. A statistical analysis employing the t-test and the F-test noted no statically significant difference between the intended and stated spectrophotometric methods. To aid in data visualization, interval plots, boxplots, normal probability plots, and Tukey's simultaneous significant difference test were employed to determine that there were no significant differences between the suggested and documented methods. NEMI, GAPI, AGREE, and analytical Eco-scale, are fulfilled by the offered approaches, which proved to have the minimal environmental effect.

References

1. Silva, S., Almeida, A.J., & Vale, N. (2021) *Pharmaceutics* **13**. doi:10.3390/pharmaceutics13040508

2. Müller, T. (2017) *Expert Opin. Drug Metab. Toxicol.* **13**, 693–699. doi:10.1080/17425255.2017.1329418

3. Li, W., Yang, X., Song, Q., Cao, Z., Shi, Y., Deng, Y., & Zhang, L. (2020) *Bioorg. Chem.* **97**, 103707. doi:10.1016/j.bioorg.2020.103707

4. Suarez-Cedeno, G., Suescun, J., & Schiess, M.C. (2017) *Parkinsons. Dis.* **2017**, 1–10. doi:10.1155/2017/9358153

5. Elbeddini, A., To, A., Tayefehchamani, Y., & Wen, C. (2020) *J. Clin. Mov. Disord.* **7**, 1–7

6. Hand, A. & Chaudhuri, K.R. (2020) *Br. J. Neurosci. Nurs.* **16**, 121–124

7. Finberg, J.P.M. & Rabey, J.M. (2016) *Front. Pharmacol.* **7**, 1–15. doi:10.3389/fphar.2016.00340

8. Blair, H.A. & Dhillon, S. (2017) *CNS Drugs* **31**, 169–176. doi:10.1007/S40263-017-0408-1

9. Bette, S., Shpiner, D.S., Singer, C., & Moore, H. (2018) *Ther. Clin. Risk Manag.* **14**, 1737

10. Teixeira, F.G., Gago, M.F., Marques, P., Moreira, P.S., Magalhães, R., Sousa, N., & Salgado, A.J. (2018) *Drug Discov. Today* **23**, 736–744. doi:10.1016/j.drudis.2018.01.033

11. Bachhav, P.C.B. and R.S. (2021) *World J. Pharm. Sci.* **9**, 114–119

12. Adhao, V.S., Thenge, R.R., Sharma, J., & Thakare, M. (2020) *Jordan J. Pharm. Sci.* **13**, 149–159

13. El-Kosasy, A.M., Hussein, L.A., Mohamed, N.M., & Salama, N.N. (2020) *J. Chromatogr. Sci.* **58**, 789–795. doi:10.1093/chromsci/bmaa043

14. R. Tammisetty, M., R. Challa, B., & B. Puttagunta, S. (2020) *Int. J. Pharma Bio Sci.* **10**, 55–61. doi:10.22376/ijpbs/lpr.2020.10.2.p55-61

15. Zou, L., Sun, L., Zhang, H., Hui, W., Zou, Q., & Zhu, Z. (2017) *J. AOAC Int.* **100**, 1029–1037. doi:10.5740/jaoacint.16-0218

16. Zhang, Q., Zhang, Q., Liu, J., & Guo, W. (2016) *China Pharm.* **19**, 582–584

17. Redasani, V.K., Mali, B.J., Patil, A.S., & Shirkhedkar, A.A. (2013) *Anal. Chem. An Indian J.* **13**, 127–130

18. Zhang, K., Xue, N., Shi, X., Liu, W., Meng, J., & Du, Y. (2011) *J. Pharm. Biomed. Anal.* **55**, 220–224. doi:10.1016/j.jpba.2010.12.030

19. Redasani, V.K., Mali, B.J., & Surana, S.J. (2012) *ISRN Anal. Chem.* **2012**, 1–4. doi:10.5402/2012/135208

20. Nandedkar, M., Sexena, G., & Oswal, R. (2021) *Int. J. Progress. Res. Sci. Eng.* **2**, 11–12

21. Higa, V.M. & Omori, A.T. (2021) *Synlett* **32**, 1433–1436

22. Reddi, A., Mujahid, M., Sasikumar, M., & Muthukrishnan, M. (2014) *Synthesis (Stuttg).* **46**, 1751–1756. doi:10.1055/s-0033-1341104

23. Leonetti, F., Capaldi, C., Pisani, L., Nicolotti, O., Muncipinto, G., Stefanachi, A., Cellamare, S., Caccia, C., & Carotti, A. (2007) *J. Med. Chem.* **50**, 4909–4916. doi:10.1021/jm070725e

24. Keith, L.H., Gron, L.U., & Young, J.L. (2007) *Chem. Rev.* **107**, 2695–2708

25. Tobiszewski, M. & MarE, M. (2015) *Molecules* **20**, 10928

26. Gałuszka, A., Migaszewski, Z.M., Konieczka, P., & Namieśnik, J. (2012) *TrAC - Trends Anal. Chem.* **37**, 61–72. doi:10.1016/j.trac.2012.03.013

27. Pena-Pereira, F., Wojnowski, W., & Tobiszewski, M. (2020) *Anal. Chem.* **92**, 10076–10082. doi:10.1021/acs.analchem.0c01887

28. Elmasry, M.S., Hassan, W.S., El-Mammli, M.Y., & Badrawy, M. (2022) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **266**, 120436. doi:10.1016/j.saa.2021.120436

29. Abdelrahman, M.M., Abdelwahab, N.S., Abdel-Rahman, H.M., & Fares, M.Y. (2017) *Anal. Chem. Lett.* **7**, 356–368. doi:10.1080/22297928.2017.1322533

30. A. Sayed, R., Mohamed, A.R., Hassan, W.S., & Elmasry, M.S. (2021) *Drug Dev. Ind. Pharm.* **47**, 867–877. doi:10.1080/03639045.2021.1908341

31. Sebaiy, M.M., El-Adl, S.M., & Mattar, A.A. (2020) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **224**, 117429. doi:10.1016/j.saa.2019.117429

32. Sayed, R.A., Mohamed, A.R., Hassan, W.S., & Elmasry, M.S. (2021) *Drug Dev. Ind. Pharm.* **47**, 1469–1480

33. Meselhy, E.M., Aboul Kheir, A.A., El Henawee, M.M., & Elmasry, M.S. (2020) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **230**, 118083. doi:10.1016/j.saa.2020.118083

34. Ghoniem, N.S., Hussien, E.M., Atta, M.Y., & Hegazy, M.A. (2022) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **270**, 120745. doi:10.1016/j.saa.2021.120745

35. Lotfy, H.M., El-Hanboushy, S., Fayez, Y.M., & Abdelkawy, M. (2019) *Futur. J. Pharm. Sci.* **5**, 1–14. doi:10.1186/s43094-019-0003-z

36. Attala, K. & Elsonbaty, A. (2021) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **258**, 119855. doi:10.1016/j.saa.2021.119855

37. Elmasry, M.S., Hassan, W.S., Merey, H.A., & Nour, I.M. (2021) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **254**, 119609. doi:10.1016/j.saa.2021.119609

38. ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: Text and methodology. Q2(R1), Current Step 4 Version, Parent. Guidelines on Methodology Dated November 6; 2005 Geneva, Switzerland

39. Elbordiny, H.S., Elonsy, S.M., Daabees, H.G., & Belal, T.S. (2022) *Sustain. Chem. Pharm.* **25**, 100580

40. Yosrey, E., Elmansi, H., Sheribah, Z.A., & Metwally, M.E. (2022) *Luminescence* **37**, 569–576

41. Ayad, M.M., Hosny, M.M., Ibrahim, A.E., El-Abassy, O.M., & Belal, F.F. (2020) *Acta Chromatogr.* **33**, 51–56

42. El-Kafrawy, D.S., Abo-Gharam, A.H., Abdel-Khalek, M.M., & Belal, T.S. (2022) *Microchem. J.* **176**, 107236

43. Ibrahim, A.E., El Deeb, S., Abdellatef, H.E., Hendawy, H.A.M., El-Abassy, O.M., & Ibrahim, H. (2022) *Separations* **9**, 49

44. El-Kafrawy, D.S., Abo-Gharam, A.H., Abdel-Khalek, M.M., & Belal, T.S. (2022) *Sustain. Chem. Pharm.* **28**, 100709

45. El Sharkasy, M.E., Tolba, M.M., Belal, F., Walash, M., & Aboshabana, R. (2022) *Luminescence*

46. Sayed, R.A., Mohamed, A.R., Hassan, W.S., & Elmasry, M.S. (2022) *Sustain. Chem. Pharm.* **27**, 100694

47. Elbordiny, H.S., Elonsy, S.M., Daabees, H.G., & Belal, T.S. (2022) *Sustain. Chem. Pharm.* **27**, 100684

48. Tolba, M. & Elmansi, H. (2021) *R. Soc. open Sci.* **8**, 210110

49. Ibrahim, A.E., Elmansi, H., & Belal, F. (2020) *J. Sep. Sci.* **43**, 3224–3232

50. Elmasry, M.S., Hassan, W.S., El-Mammli, M.Y., & Badrawy, M. (2022) *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **266**, 120436

51. Elsonbaty, A., Hasan, M.A., Eissa, M.S., Hassan, W.S., & Abdulwahab, S. (2021) *J. Fluoresc.* **31**, 97–106

52. Attala, K., Eissa, M.S., Hasan, M.A., El-Henawee, M.M., & Abd El-Hay, S.S. (2020) *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **240**, 118533. doi:10.1016/j.saa.2020.118533

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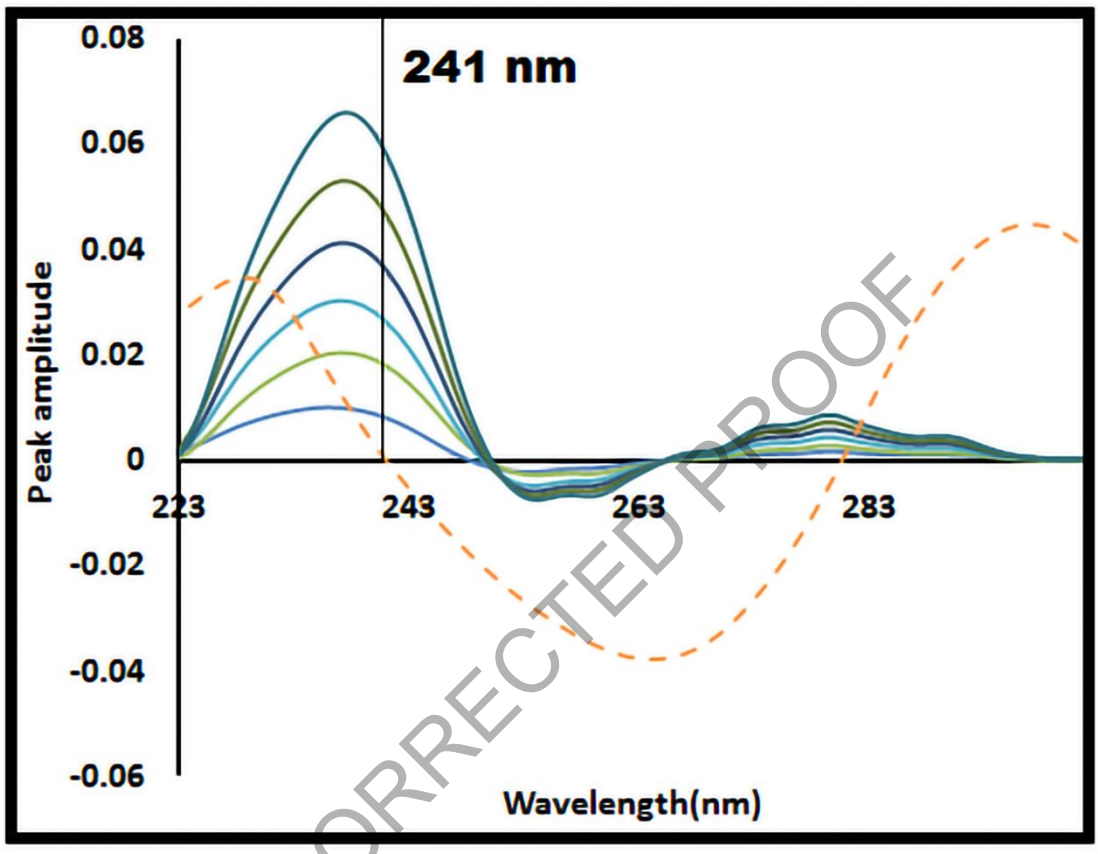


Fig 1. First derivative of Safinamide at 5–30 µg/mL, and 4-Hydroxybenzaldehyde (----) in methanol

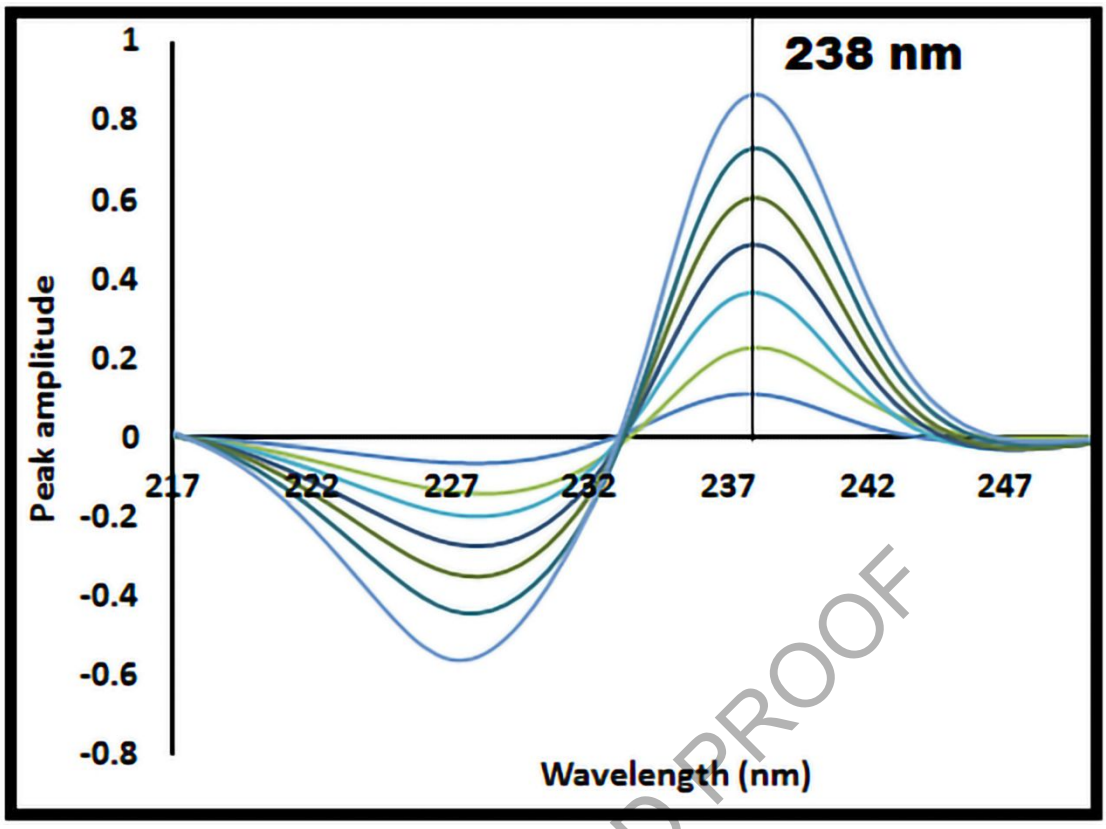


Fig 2. First derivative of ratio spectra of safinamide (5–35 µg/mL) in methanol at 7 µg/mL 4-Hydroxybenzaldehyde as a divisor.

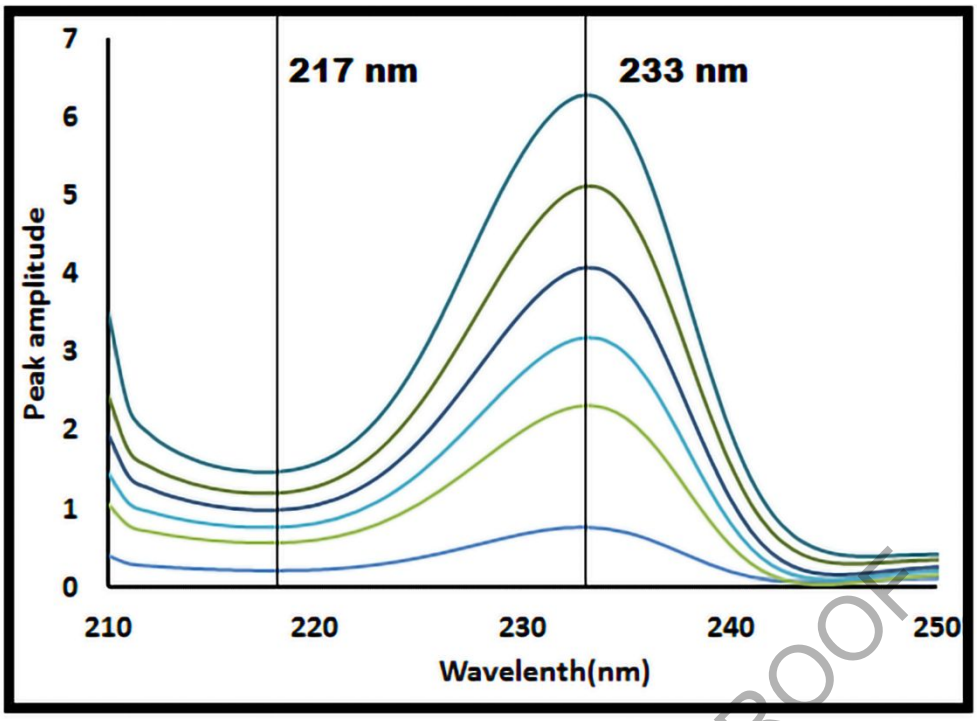


Fig 3. Ratio difference of safinamide (5–35 μg/ml) using 4-Hydroxybenzaldehyde (7 μg/ml) as divisor in methanol.

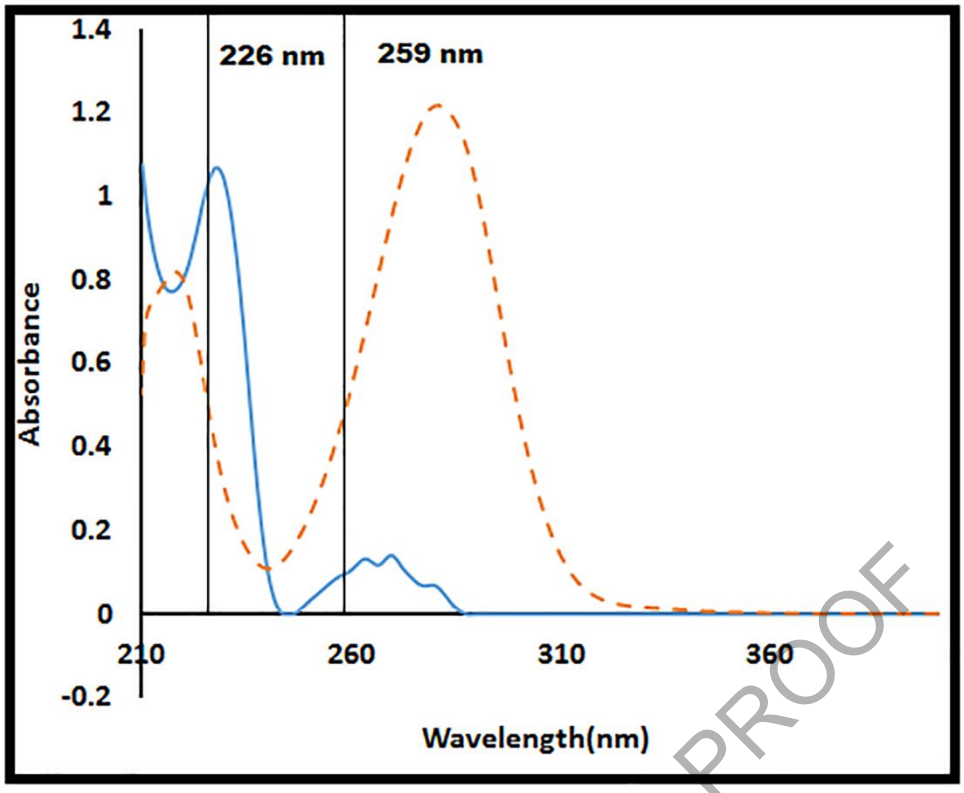


Fig 4. Zero-order absorption spectra of 25 µg/mL of Safinamide (——), 25 µg/mL of 4-Hydroxybenzaldehyde (- - -); illustrating dual wavelength determination of safinamide at 226–259 nm in methanol.

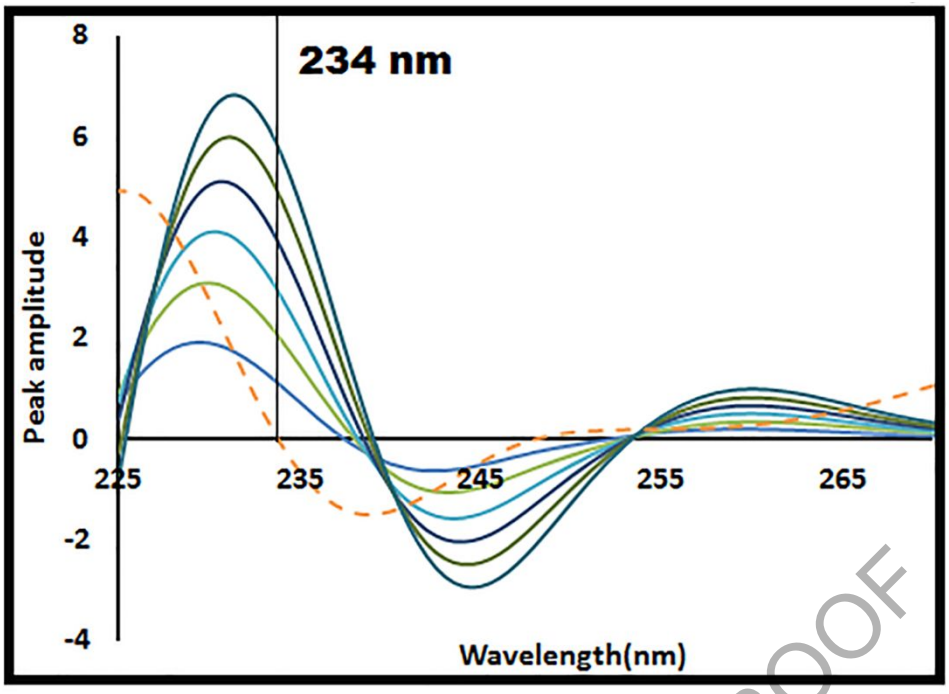


Fig 5. Deconvoluted spectra of safinamide (5–30 µg/mL) in methanol.

Table 1. Validation data for determination of safinamide and 4-Hydroxybenzaldehyde by the proposed methods

Validation parameters	SAF					4-HB
	D[1]	DD[1]	RD	DWL	FSD	Direct
Wavelength (nm)	241	238	217 and 233	226 and 259	234	290
Linearity (µg/mL)	5-30	5-35	5-35	5-30	5-30	1-10
Slope	0.001	0.025	0.132	0.039	0.183	0.140
Intercept	-0.002	-0.016	-0.192	-0.038	0.101	-0.006
Correlation coefficient (R)	0.9992	0.9998	0.9994	0.9994	0.9998	0.9992
LOD (µg/mL)	1.222	0.644	1.315	1.038	0.598	0.327
LOQ (µg/mL)	3.704	1.954	3.985	3.145	1.813	0.992
Accuracy (recovery % ± SD)[a]	99.82±1.319	100.20±1.058	99.12±1.009	99.70±1.114	99.65±1.211	100.33±1.226
Precision (% RSD)						
- Intra-day	1.278	1.110	0.756	1.037	0.918	1.231
- Inter-day	1.152	1.193	0.982	1.345	0.848	0.536

[a] Mean of five determinations

Table 2. Analysis of laboratory prepared mixtures by the proposed methods

Methods	D[1]	DD[1]	RD	DWL	FSD	Zero order
Concentration (µg/mL)			**Found %**			**Found %**
			SAF			**4-HB**
SAF:4-HB						
10:10	101.99	98.69	98.91	100.46	101.66	99.54
20:8	98.84	101.13	98.11	98.08	100.33	100.43
25:5	100.76	100.51	99.56	98.21	98.02	99.00
30:9	101.54	99.16	99.11	100.82	98.07	101.36
30:10	101.54	98.50	101.57	100.32	98.62	98.44
Mean±SD[a]	100.93±1.25	100.60±1.16	99.45±1.29	99.58±1.31	99.34±1.60	99.75±1.15

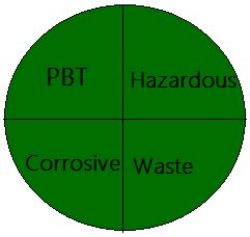
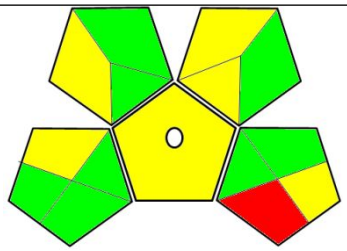
[a]Mean of five determinations.

Table 3. Determination of safinozol ® tablets by the proposed method using standard addition technique

Drugs	**SAF**				
Methods	**D[1]**	**DD[1]**	**RD**	**DWL**	**FSD**
Pharmaceutical dosage form[a] (found% ± SD)	100.32±0.79	100.55±0.80	100.11±1.37	100.58±0.94	99.78±1.16
Standard Addition (recovery% ±SD)[b]	99.51±1.49	100.20±1.07	99.48±0.79	99.60±1.04	100.21±1.24

[a]Safinozol claimed to contain 100 mg/mL.
[b]Average of five determinations.

Table 4. The outcomes of the evaluation of the proposed approaches greenness

1. NEMI pictogram	**2. Green analytical procedure index (GAPI)**	**3. Analytical Greenness Metric (AGREE)**
		

4. Analytical Eco-scale score

Item	Number of pictograms	Signal of words	Word sign	Penalty points
Reagents: volume				
Methanol 10 mL	2	3	(1) Warning	6
Instrument				
Spectrophotometer				0
Energy [<0.1 kWh per sample]				0
Waste (1–10 mL, no treatment)				6
Occupational hazards(analytical process hermetization)				0
Total penalty points				12
Analytical Eco-Scale score[a]				88 Excellent green method

[a] Analytical Eco-Scale total score = 100- total penalty points, where score >75 represents excellent green analysis, score >50 represents acceptable green analysis, and score < 50 represents inadequate green analysis

Table 5. Statistical analysis of proposed and reported methods for safinamide in Safinozol® tablet.

Parameters	SAF					
	Proposed Methods					Reported method [12]
	D[1]	DD[1]	RD	DWL	FSD	
Mean	100.32	100.55	100.11	100.58	99.78	99.72
SD	0.796	0.807	1.370	0.940	1.169	0.626
n	5	5	5	5	5	5
Variance	0.634	0.652	1.881	0.883	1.366	0.392
Student's t-test (2.306)[a]	1.33	1.83	0.59	1.71	0.10	
F-value (6.39)[a]	1.62	1.66	4.80	2.25	3.48	

[a] Theoretical of t and F values at p = 0.05

Table 6. One-way ANOVA results for determination of proposed and reported methods of safinamide in Safinozol® tablet

Sum of variation	Sum of squares	Degree of freedom	Mean of squares	F-value	P-value	Critical F
Between group	3.44	5	0.68	0.71	0.62	2.62
Within group	23.25	24	0.96			
Total	26.69	29				