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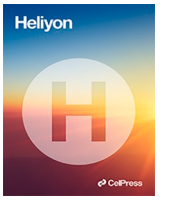

Innovative and sustainable deconvoluted amplitude factor spectrophotometric method for the resolution of various severely overlapping pharmaceutical mixtures: Applying the complex-GAPI-tool

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ABSTRACT

UV spectroscopy is considered the simplest, the most money and time investor technique in analytical research. Besides its lowered solvent and energy consumption leading to greener outcomes, its practicality is wide and suitable for a wide range of applications. Multicomponent mixtures are always representing themselves as a problematic challenge for any analytical technique fortunately UV spectroscopic methods found many ways to tackle these mixtures. Fourier self-deconvolution (FSD) was recently applied in UV spectroscopy as an effective tool for the resolution of binary mixtures unfortunately like any other method may fail to completely resolve severely overlapping mixtures. In this paper, we epitomize the newly developed deconvoluted amplitude factor (DAF) spectrophotometric approach which couples the concepts of both the FSD and the amplitude factor methods for the resolution of tadalafil (TAD) in its binary mixtures with dapoxetine hydrochloride (DAP) or tamsulosin hydrochloride (TAM). The embraced approach was assessed regarding its greenness utilizing different assessing protocols to give evident proof for its sustainability. The innovative approach showed an enhancement in the resolution of binary mixtures and showed high sensitivity as noticed from limits of detection and quantitation which were (0.374, 1.136 μg/mL), (0.269, 0.817 μg/mL), and (0.518, 1.569 μg/mL) for TAD, DAP, and TAM, respectively. The method was validated as per ICH guidelines recommendations and also was statistically compared with recently reported methods which revealed no statistically significant difference. A very handy and reader-friendly data presentation approach was followed for the ease of statistical data interpretation and evaluation.

1. Introduction

Erectile dysfunction is the disability to achieve and maintain a hard enough erection for sex caused by physical issues, including

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obesity, heart diseases, high cholesterol, and diabetes, or physiological matters, including stress, anxiety, and depression [1]. This can be treated using erectile dysfunction medication such as phosphodiesterase inhibitors (tadalafil). However, tadalafil may not always be enough to cure patients with this condition, as they may also suffer from premature ejaculation or lower urinary tract symptoms related to benign prostatic obstruction. Thus treating such severe conditions necessitated the administration of at least a single dose of each tadalafil and other premature ejaculation drugs as serotonin reuptake inhibitors (dapoxetine) before sexual intercourse, which made the person feel unsatisfied as some medications required administration 1 h before sexual intercourse and others required administration for hours. For this reason, new pharmaceutical fixed-dose combined products are being emerged on a daily basis to aid in the treatment of various disease conditions [2].

Tadalafil (TAD) combined pharmaceutical products represent an excellent example of a continually developed section of the pharmaceutical research and industry. TAD was recently found in the combination of many other drugs to serve various complex morbidities. TAD and tamsulosin hydrochloride (TAM) combination was found clinically effective in the management of erect dysfunction accompanied by lower urinary tract symptoms in men [3]. Also, TAD was found in combination with dapoxetine hydrochloride (DAP) for the management of erectly dysfunction conditions accompanied by premature ejaculation [4]. The development of high throughput UV spectrophotometric techniques for the resolution of TAD in its binary mixtures with DAP or TAM is important for the quality control activities regarding the manufacturing of these products. Only one traditional UV spectrophotometric method was developed for the determination of TAD in presence of TAM [5]. Also, TAD in the presence of DAP was resolved by two UV spectrophotometric methods [6,7].

The previously reported spectrophotometric methods based on ratio spectra (RSM) were characterized by their accuracy and sensitivity but suffer from the required multi-step processing [8–10]. Recently, several simple, and sensitive coupled UV spectrophotometric methods that can resolve binary mixtures with minimal steps are preferred over other traditional spectrophotometric methods as it reduces the time required for signal processing [11,12]. One of these methods is the Fourier self-deconvolution (FSD) which is a mathematical approach that eliminates broadening in UV spectral signals resulting in narrower peaks with the evolution of zero crossing points enabling binary mixtures resolutions [13]. In some severely overlapped spectra, FSD may resolve one component and fails to resolve the second, to treat this drawback coupling with another capable method to resolve the second component is the best reasonable option [12]. The amplitude factor method (AMP-F) [14] is a mathematical manipulation method that could resolve binary mixtures if one were determined at zero-crossing of the first component. The proposed DAF methodology was superior to the conventional ratio spectra based methodologies (RSM) in terms of simplicity, cost-effectiveness, and ease of implementation. It did not require any sophisticated software to perform the analysis. In contrast, the RSM methods involved multiple iterations (such as devisor selection) to resolve the investigated mixtures and some of them required time-consuming processes such as mean centering. Furthermore, in comparison to the reported chromatographic methods; the proposed UV spectroscopic methodologies had some advantages over chromatographic methods, especially in cases of less complicated mixtures such as binary and ternary pharmaceutical mixtures as being faster, having lower ecological foot print and more cost effective.

In this work, we presented, for the first time ever, the DAF methodology which proved successful in resolving different completely overlapping binary mixtures in their bulk and pharmaceutical dosage forms. Also, we applied other various UV spectrophotometric methods as dual and induced dual-wavelength methods for resolving TAD in its binary mixture with TAM proving their efficacy as simple mathematically manipulated UV spectrophotometric approaches compared with the reported methodologies.

Several greenness assessment protocols were utilized to assess the sustainability of the embraced UV spectroscopic methodologies. Besides, reader-friendly data presentation tools were exclusively employed for the ease of presentation, evaluation, and interpretation of the obtained statistical data.

2. Experimental

2.1. Materials and reagents

Mash premiere pharmaceutical industries produced and graciously supplied tadalafil pure standard with confirmed purity of 99.98% (Cairo, Egypt). Tamsulosin certified to be 98.54% was generously provided by the Adwia Company (Elobour, Egypt). Hikma Pharma (Cairo, Egypt) gratefully provided dapoxetine hydrochloride with certified purity of 99.42%.

A local pharmacy sold us Contiflo T® Capsules, a product of Sun Pharmaceutical Industries Ltd (Mumbai, India), which included tamsulosin (0.4 mg) and tadalafil (5 mg). DEJAC T® tablet a product of Intas Pharmaceuticals Ltd (Ahmedabad, India), which included dapoxetine hydrochloride (30 mg) + tadalafil (10 mg) was also purchased from the local market. Methanol HPLC grade was purchased from Thermo Fisher (USA) and was employed as a solvent.

2.2. Instrumentation and software

A double-beam spectrophotometer (Jasco, Japan) was used for all spectrophotometric mensuration. Pharmaceutical samples were prepared using a sonicator (DAIHAN WUC-A01H, USA) and centrifuge (Centurion Scientific, UK). Software called Spectra Manager was utilized to conduct spectral scanning and treatments on the investigated absorption spectra. The obtained and reported data were investigated through a comparative statistical analysis using Minitab 2019 and Microsoft Excel 2010.

2.3. Procedures

2.3.1. Standard stock and working solutions

Individual standard stock solutions of TAD, DAP, and TAM were prepared by carefully weighing 10 mg of each component. Each 10 mg was separately and quantitatively transferred into three separate 100 mL volumetric flasks then 70 mL of methanol was added and the flasks were vigorously shaken for 10 min and exposed to an ultrasonic bath for another 20 min till the complete dissolution of all powders. Eventually, the volumes were made up to the mark by methanol to obtain TAD, DAP, and TAM stock solutions of 100 μg/mL. Standard solutions were stored under refrigeration (8 °C) when not in use.

2.3.2. Construction of calibration curves

Using methanol as a blank, TAD, TAM, and DAP individual solutions with varying concentrations were scanned across the wavelength range of 200–400 nm. Calibration curves for TAM in its binary mixture with TAD were plotted using prepared working solutions in the ranges of 2–25, and 2–30 μg/mL, respectively. While, DAP in its binary mixture with TAD working solutions in the ranges of 2–40, and 2–50 μg/mL were used for plotting their respective calibration curves. Each calibration curve for TAD, DAP, and TAM generated a regression equation which was then utilized to calculate each corresponding concentration in each binary mixture as shown below in detail.

2.3.3. Induced dual wavelength (IDW)

Two different wavelengths, namely 270 and 285 nm, were chosen from the zero-order absorption spectra of TAD to determine TAD in the presence of TAM by applying IDW. Then an equality factor (F) obtained from the TAM spectrum was used to rectify the absorbance. The adjusted data absorbance difference was shown as a function of concentration.

2.3.4. Dual wavelength (DW)

TAM was determined in the presence of TAD utilizing the DW method that relied on graphing the difference in zero-order absorption spectra at 277 and 290 nm against each corresponding concentration.

2.3.5. Deconvoluted amplitude factor (DAF)

The DAF method was stratified for the quantitative analysis of TAM and DAP in their binary combinations with TAD. In this method, zero-order absorption spectra of TAD (2–25 μg/mL) and TAM (2–30 μg/mL) were computed. The obtained spectra were then adjusted by deconvolving them with a full width at half maximum (FWHM) value of 90 using the self-deconvolution tool. To remove TAD's interfering effect at the 282 nm amplitude of the TAM, an amplitude factor (F_{amp}) for TAD was derived using its amplitude values at 282 nm and 295 nm. A calibration curve for TAM was created by plotting its amplitude values at 282 nm versus TAM corresponding concentrations. The resulting regression equation was then utilized to quantify TAM in laboratory mixtures and pharmaceutical formulations after modifying its deconvoluted amplitude values by the calculated amplitude factor.

Also, DAP was determined by the same concept in the presence of TAD. First, both TAD (2–25 μg/mL) and DAP (2–40 μg/mL) zero-order spectra were deconvoluted as described above but at an FWHM of 60 utilizing the FSD filter. A F_{amp} for TAD was computed at 277 nm and 291 nm to alleviate its interference with DAP at its major amplitude at 291 nm. A calibration curve for DAP deconvoluted amplitudes at 291 nm was plotted and the resulting regression equation was utilized to determine the DAP pure concentration at 291 nm.

TAD deconvoluted amplitudes (FWHM of 90 & 60) were directly resolved from TAM at 295 nm (FWHM of 90) and DAP at 277 nm (FWHM of 60) in each corresponding mixture. For the determination of TAD concentration in each mixture, two calibration curves were plotted for TAD in each mixture. The resulting regression equations were applied for the determination of TAD concentration in each mixture.

2.3.6. Laboratory-prepared mixtures

Several synthetic lab mixtures simulating DAP and TAM binary mixtures with TAD were prepared in varying ratios by carefully transferring suitable aliquots of each paired drug into a series of 10 mL volumetric flasks and volumes were made up with methanol as diluent.

2.3.7. Analysis of pharmaceutical dosage form

Content of ten capsules from Contiflo T® capsules (claimed to contain 0.4 mg TAM and 5 mg TAD) were milled to produce a uniform powder, weighed, and the average mass was calculated. An equivalent weight to one capsule was dissolved in a volumetric flask of 200 mL containing 100 mL of methanol with the help of an ultrasonic bath for 15 min. After that, the concentration of the solution was enriched by 6 mL of the TAM stock solution where the volume was completed to the mark with the same solvent to get a final concentration of 25 μg/mL for TAD and 5 μg/mL for TAM. The resulting solution was centrifuged for 20 min at 8 °C and 10,000 rpm. The supernatant was then withdrawn for applying the procedures under IDW and DW. While in the case of FSD coupled with the P-factor method for the same mixtures, an equivalent weight to one tablet was dissolved in a volumetric flask of 250 mL containing 100 mL of methanol with the help of shaking for 15 min and ultrasonic bath for another 15 min. After that, the concentration of the solution was enriched by 1 mL of the TAM stock solution, where the volume was completed to the mark with the same solvent to get a final concentration of 20 μg/mL for TAD and 2 μg/mL for TAM. The resulting solution was centrifuged for 15 min at 4 °C and 10,000 rpm and ready for applying the discussed procedures under these methods.

Another ten tablets of DEJAC T® tablets (claimed to contain 10 mg TAD and 30 mg DAP) were weighed, pulverized and the average tablet weight was carefully transferred to a 100 mL volumetric flask. The powder content was dissolved into 70 mL of methanol with vigorous shaking, and sonication for 30 min till complete dissolution, and the final volume was made up of methanol. A suitable volume was then cool centrifuged at 4 °C and 10.000 rpm for 15 min and 1 mL aliquot from the supernatant was quantitatively transferred to a 10 mL volumetric flask and volume was completed with methanol to obtain a final concentration of 10 μg/mL TAD and 30 μg/mL DAP.

3. Results and discussion

The current work involves an innovative coupled UV spectrophotometric methodology for the resolution and quantitation of TAD in its binary pharmaceutical mixtures with DAP or TAM. The presented methodologies had the advantage of simplicity as being based totally on simple mathematical manipulations without the need for external software or frustrating multiple steps.

The innovative DAF method represents a newly developed technique capable of resolving severely overlapping binary mixtures with minimal effort whose resolution was challenging if performed by a traditional single method. FSD is considered a powerful tool for UV spectroscopic signals resolution; it functions by narrowing the UV bands of the supplied spectra through the elimination of the physical convolutions in the transmitted signal which is the main reason for signals broadening and the consequence overlapping of UV multicomponent spectra. FSD results in more focused and sharp amplitudes with zero-crossing regions at which interference is a minimum (approaching zero nm) and one component can be resolved in the presence of the interfering component. Unfortunately, some multicomponent spectra are severely overlapped so that only one component can be resolved after applying the FSD method. To solve this; we came up with an innovative coupled approach between FSD and AMP-F methods called the DAF method. The developed DAF approach states that the FSD method is applied first and one component (Ex: A) is determined, then calculated the F_{amp} for the determining component is considered as interfering with the second component (Ex: B). The following mathematical representation summarizes the theory behind the DAF approach:

$$D_{\lambda x}\ [A+B] = A, \text{when D } [B] \approx \text{zero}$$

Where $D_{\lambda x}$ [A + B] is the deconvoluted spectrum of mixture [A + B], λ_x is the wavelength point at which D [B] is approximately zero.

$$F_{amp\ [A]} = D\ (A_{\lambda 1}) \,/\, D\ (A_{\lambda 2})$$

Where, λ_1 and λ_2 represent the maximum absorbance point for B and its zero-crossing point, respectively.

$$D_{(pure)}\ [B] = D_{\lambda 1}\ [A+B] - \left(D_{\lambda 2}\ [A+B] * F_{amp\ [A]}\right)$$

Moreover, a greenness assessment of the embraced methodologies was accomplished by multiple assessing protocols which revealed green outcomes of the applied methods. Also, a thorough statistical analysis of the obtained results was achieved for better data interpretation. Statistical data presentation utilizing the Tukey test, interval, Box, and normal probability plot of residuals represented an eye-friendly package for ease of data visualization and interpretation [15–18].

3.1. Proposed spectrophotometric methods

3.1.1. Induced dual wavelength (IDW)

An adaptation of the previously published DW was used here since the zero-order absorbance spectra of TAM revealed no spots of equal absorbance [19]. In our research, TAD was calculated in the presence of TAM by choosing two variant wavelengths on the TAD zero-order spectrum where the variance in absorbance for TAM was not equal to zero. To alleviate the interference produced by TAM, we multiplied one of the computed absorbances by the equality factor (F) to release a zero difference in the data of absorbance of TAM, whereas this difference was still substantial for TAD Fig. 1. The equality factor (F_{eq}) was computed from zero-order absorption spectra of TAM by dividing the absorbance at 285 by the absorbance at 270, which was observed to be 1.213.

Calibration graphs were created by charting the TAD absorbance variance as a function of concentration. The regression equation, which was calculated and reported in Table 1, helped assess TAD in laboratory-prepared mixes and pharmaceutical dosage forms in Table 2 and 3 (a-c).

3.1.2. Dual wavelength (DW)

DW is a spectrophotometric approach that has shown its advantage over other spectrophotometric techniques due to its ability to identify severe overlapping spectra without needing a divisor or extensive manipulation of zero-order absorption spectra [12].

The overlay zero-order absorption spectra of TAD and TAM showed substantially overlapping spectra, making it successfully simple to determine TAM in the presence of TAD by applying DW. Based on zero-order absorption spectra for TAM, two variant wavelengths, namely λ_{277} and λ_{290}, were chosen where TAM was quantified. On the other hand, TAD could not be determined utilizing the same wavelengths as the difference in absorbance was equal to zero Fig. 2. The calibration plots were detailed in the preceding section, and linear regression was used to fit them.

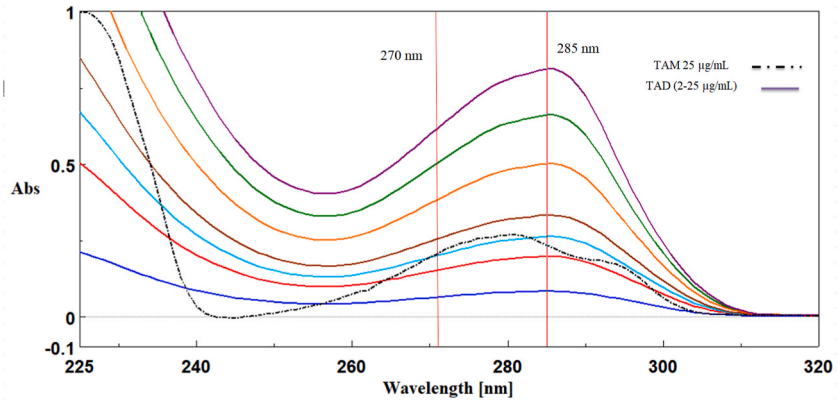

Fig. 1. Overlain absorption spectra of TAD (2–25 µg/mL) and TAM (25 µg/mL) utilized for applying IDW method for TAD at 270 and 285 nm.

Table 1
Summary of Calibration data for the analysis of TAD, DAP, and TAM.

Validation parameters	TAD and TAM mixture				TAD and DAP mixture	
	TAD		TAM		TAD	DAP
	IDW	FSD	DW	DAF	FSD	DAF
Wavelength (nm)	270–285	295	277–290	282–295	277	277–291
Linear range (µg/mL)	2–25	2–20	5–25	2–30	2–25	2–40
Slope	0.0036	0.0354	0.0030	0.0362	0.0480	0.0300
Intercept	- 0.0010	0.0320	- 0.0079	- 0.0086	- 0.0239	- 0.0077
Correlation Coefficient (r)	0.9996	0.9999	0.9999	0.9998	0.9998	0.9999
LOD (µg/mL)	0.572	0.223	0.393	0.518	0.374	0.269
LOQ (µg/mL)	1.732	0.747	1.189	1.569	1.136	0.817
Accuracy (Recovery % ± SD)[a]	101.20	99.86	99.75	100.19	99.92	100.88
	±0.649	±0.393	±1.160	±1.095	±1.712	±0.418
Precision (RSD) Intra-day[b]	±0.318	±0.239	±0.146	±0.229	±0.668	±0.467
Inter-day[c]	±0.566	±0.510	±0.179	±0.705	±1.124	±0.9313

[a] Mean of five determinations.
[b] Mean of three various concentrations (10, 15, 20 µg/mL) for TAD and DAP and (10, 15, 20 µg/mL) for TAD and TAM recurred three times within the day.
[c] Mean of three various concentrations (10, 15, 20 µg/mL) for TAD and DAP and (10, 15, 20 µg/mL) for TAD and TAM recurred three times in three different days.

Table 2
Analysis of laboratory prepared mixtures.

TAD and DAP mixture				TAD and TAM mixture					
TAD	DAP	% Recovery[a] of TAD	% Recovery[a] of DAP	TAD	TAM	% Recovery[a] of TAD		% Recovery[a] of TAM	
		FSD	DAF			FSD	IDW	DW	DAF
10	30	99.44	100.11	10	5	99.72	99.25	101.67	99.09
20	20	101.31	99.92	5	5	99.53	99.32	100.58	100.66
20	30	100.38	99.41	15	5	101.15	100.60	101.79	99.16
10	40	99.76	100.14	10	10	101.60	98.52	102.04	98.43
25	25	100.76	100.53	2	20	99.73	99.50	100.26	98.81
5	25	100.74	99.17	20	10	99.80	101.09	100.98	100.67
				25	5		101.79	101.41	
Mean% ± SD		100.40 ± 0.690	99.88 ± 0.500	Mean% ± SD		100.26	99.71	101.25	99.47
						±0.882	±0.953	±0.66	±0.962

[a] Mean of three determinations.

3.1.3. Deconvoluted amplitude factor method (DAF)

This method is based on coupling the concepts of both FSD and AMP-F methods revealing the deconvoluted amplitude factor (DAF) method which had the advantage of simplicity as all signals are first deconvoluted, following the parameters of the FSD methodology, from which one component is directly determined using the zero-crossing points at the deconvoluted spectra.

After that, the second component can be determined after calculating a factor that eliminates the effect of the interfering signal(s),

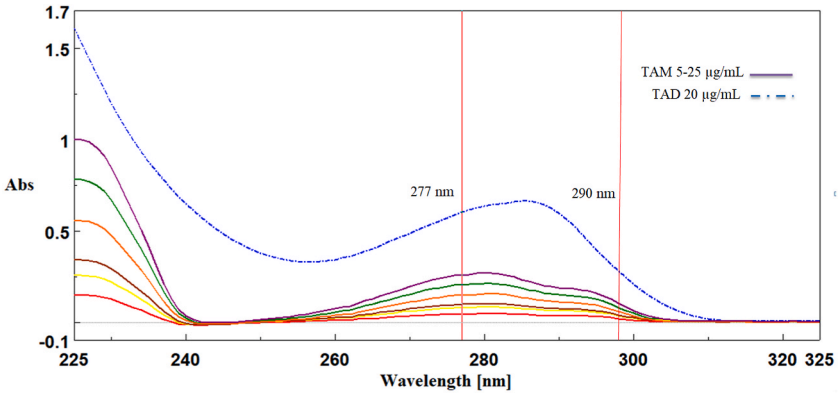

Fig. 2. Overlain absorption spectra of TAM (5–25 µg/mL) and TAD (20 µg/mL) utilized for applying DW method for TAM at 277 and 290 nm.

and then by applying the AMP-F method the second component is determined. The entire process is comprised of two simple steps; first, all UV spectra are deconvoluted, and then an amplitude factor (F_{amp}) is calculated for the interfering component to be knocked out.

AMP-F is regarded as a modification of the absorption correction method (ACM). As one of ACM's requirements is to have an extended spectrum of one pure drug, TAM has a significant absorbance at the somewhat overextended λ_{max} of TAD in this case. Also, DAP has an insufficient overextension beyond 310 nm which has insignificant absorbance values making it impossible for ACM to be applied in this study Fig. 3 (a and b).

FSD has subsequently been employed in various signal-processing applications, particularly spectroscopy. It is considered a computational approach for fixing highly overlapping spectral peaks by compressing their bandwidth in such a manner that each one can be distinguished from the others [13]. Also, it reverses the distorting effects of the recorded spectrum, such as instrumental errors and random noise, which occur during spectrophotometric measurements.

Additionally, using the FSD technique alone was insufficient to identify either DAP or TAM in presence of TAD directly because TAD crossing amplitudes were not approximating zero or because of the nonlinear incidental parts of TAM and DAP deconvoluted amplitudes as shown in Fig. 4 (a and b). This defect was addressed by the innovative DAF approach, which was based on calculating the F_{amp} for the TAD deconvoluted amplitude in each mixture to eradicate its interference with TAM at 282 nm or DAP at 291 nm after deconvoluting the zero-order spectra of each according to its specific FWHM as mentioned earlier.

In mixtures of TAD and TAM; the F_{amp} was computed for TAD by dividing TAD's deconvoluted amplitude values at 282 nm by its values at 295 nm as shown below, and a mean value of 2.753 was obtained.

$$F_{amp\ (TAD)} = TAD_{(D282nm)} \ / \ TAD_{(D295nm)}$$

Furthermore, the pure TAM at 282 nm was determined using the F_{amp} after eliminating the TAD peak Fig. 5. A calibration curve for TAM deconvoluted amplitudes at 282 nm was built up where the regression equation Table 1 assisted in the analysis of TAM in pharmaceutical formulation and laboratory mixtures after correcting its deconvoluted amplitudes with the calculated F_{amp} as following:

$$TAM_{(pureD282nm)} = TAM_{(D282nm)} - \left[TAM_{(D295nm)} * F_{amp\ (TAD)}\right]$$

Where; D represented the deconvoluted amplitudes of TAD at 282 nm and 295 nm and TAM at 295 nm and 282 nm.

While in mixtures of TAD and DAP the F_{amp} for TAD was calculated and meant to give an average value of 1.346 as follows:

$$F_{amp\ (TAD)} = TAD_{(D291nm)} \ / \ TAD_{(D277nm)}$$

The pure values of DAP deconvoluted amplitudes at 291 nm were determined after canceling TAD interference at this point Fig. 6 through $F_{amp\ (TAD)}$ and are given as follows:

$$DAP_{(pure\ D\ 291\ nm)} = DAP_{(D291nm)} - \left[DAP_{(D277nm)} * F_{amp\ (TAD)}\right]$$

Where; D represented the deconvoluted amplitudes of DAP at 291 nm and 277 nm in its mixture with TAD.

Then a calibration curve for DAP deconvoluted amplitudes at 291 nm was plotted and the resulting regression equation was utilized to calculate the pure DAP concentration in its laboratory mixtures and dosage form with TAD.

TAD was determined in the presence of DAP at its zero-crossing point 277 nm (FWHM of 60) as in Fig. 7. While TAD in the presence of TAM was determined at 295 nm (FWHM of 90) which is the TAM zero-crossing point as shown in Fig. 8. The following equations illustrate further the resolution of TAD in both mixtures using two different FSD approaches.

$$D_{(277nm)}\ [TAD + DAP] = D_{pure}\ [TAD], \text{ Where the FWHM} = 60 \text{ and } D_{(277nm)}\ [DAP] \text{is approximately zero.}$$

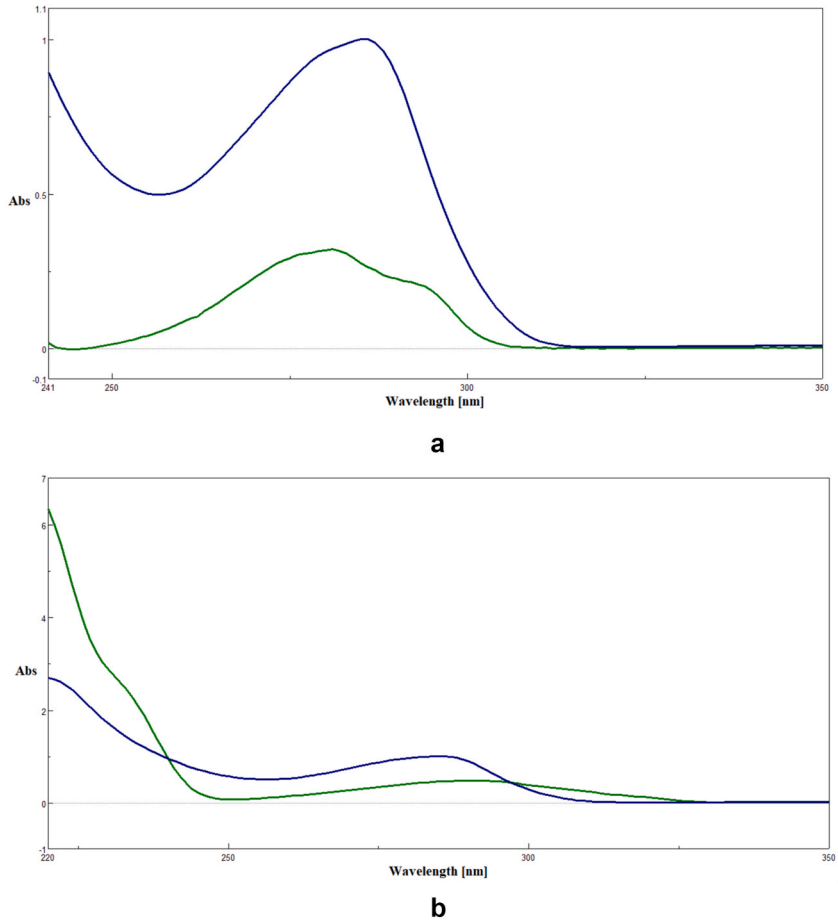

Fig. 3. (a): Zero order absorption spectra of TAD (blue line) and TAM (green line), showing sever overlapping. Fig. 3 (b): Zero order absorption spectra of TAD (blue line) and DAP (green line), showing sever overlapping.

$$D_{(295\ nm)}\ [TAD + TAM] = D_{pure}\ [TAD], \text{Where FWHM} = 90 \text{ and } D_{(295nm)}\ [TAM] \text{is approximately zero}$$

3.2. Greenness assessment of the proposed UV spectrophotometric methods

The idea of green chemistry is becoming more prevalent within the realm of chemical research. Specialized evaluation tools are needed to assess a chemical process's environmental impact accurately [20,21]. For the assessment of greenness, many methods can be used. Four fundamental criteria were used to determine whether analytical procedures were environmentally friendly: high chemical use levels and associated risks, consumption of high energy, workplace risks, and waste production. In addition to economic effectiveness, the suggested method's greenness was evaluated using five methodological approaches.

The National Environmental Methods Index (NEMI), which labels greenness with a pictogram split into four quadrants, is the oldest assessment still in use [22]. The quadrant will be colored green if all of the following conditions are met: 1) None of the reagents are classified as persistent, bio-accumulative, and toxic reagents by the Environmental Protection Agency's Toxic Release Inventory (EPA's list) [23], 2) The chemicals used in the procedure are not registered on the EPA's list of hazardous wastes, 3) The pH is between 2 and 12, 4) The waste generated during the work is lower than 50 g. Despite being simple to read, the NEMI pictograms are nonetheless regarded as a qualitative tool since each quadrant is designated as being below or over a certain threshold. Analysis of the suggested approach revealed that it displayed three green-shaded quadrants with a green profile, as illustrated in Fig. 9 (a).

Raynie et Driver; developed an assessment of the green profile tool, a semi-quantitative approach, which depicts the evaluation as a pentagram split into five jeopardy potentials, namely energy, environment, waste, safety, and health [24]. Each possibility has three shading options: green, yellow, or red. Based on National Fire Protection Association (NFPA) standards, the safety and health category are highlighted: in red and blue, respectively [25]. The quantity consumed/produced throughout the operation calculates environmental dangers and waste. Furthermore, energy is determined using the technique chosen. Fig. 9 (b) revealed that the solvent employed in the procedure had a minor health hazard with an NFPA health hazard score of 1 (green shading), which was associated with the green coloring of the safety hazard pictogram that revealed an instability score of 0. The suggested approach also spent less

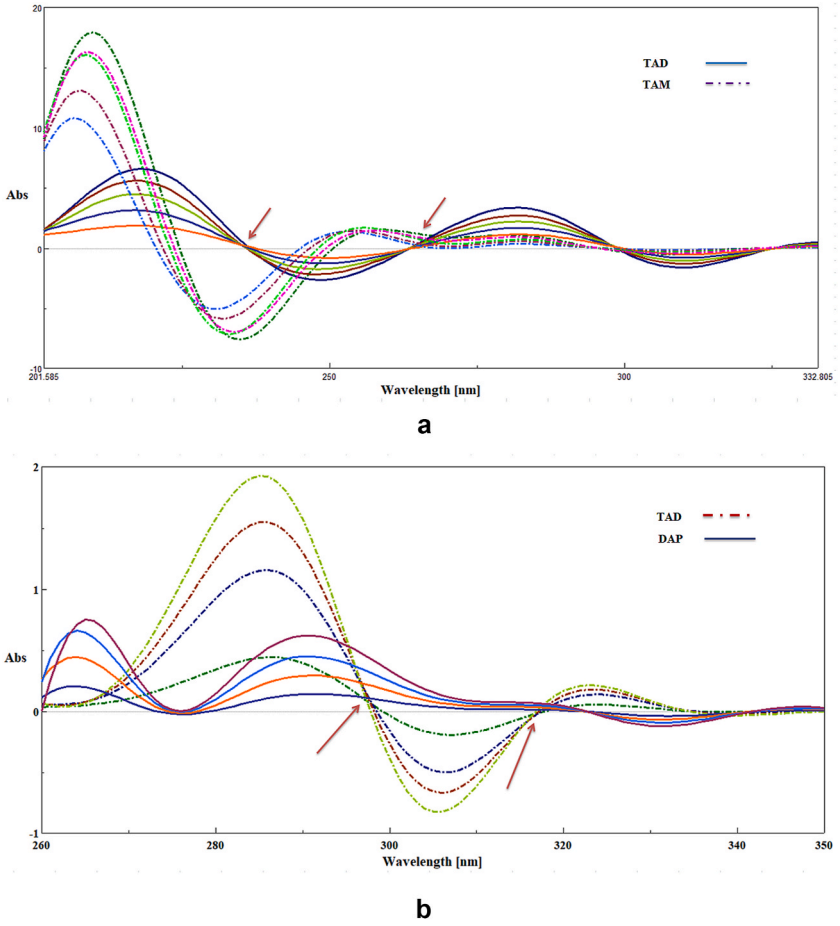

Fig. 4. (a): Fourier self deconvoluted spectra (FWHM = 90) of both TAD & TAM, showing the difficulty of resolving TAM from TAD, Fig. 4 **(b):** Fourier self deconvoluted spectra (FWHM = 60) of both TAD & DAP, showing the difficulty of resolving DAP from TAD.

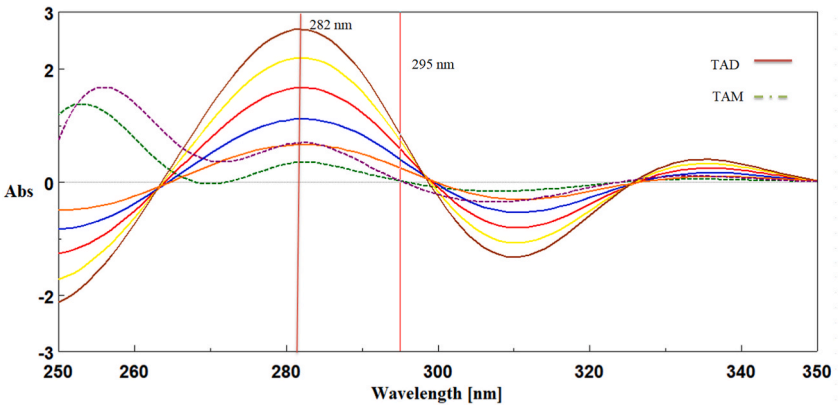

Fig. 5. Deconvoluted amplitude factor spectra of TAM determined at 282 nm in the presence of TAD.

energy since it was based on the use of a UV spectrometer, which was the reason behind the shading energy pictogram green. Furthermore, the environmental risks and waste quantity pictograms were highlighted in green since the ecological hazards and the overall waste amount was less than 50 gm.

The result of an analytical Eco-Scale analysis is a score, which is found by taking the total number of penalty points for the following factors and subtracting that number from the overall score of 100 [26]. These characteristics consist of data relating to solvents, including quantities and risks of solvents employed, that align with the data regarding instruments, including the quantity of waste

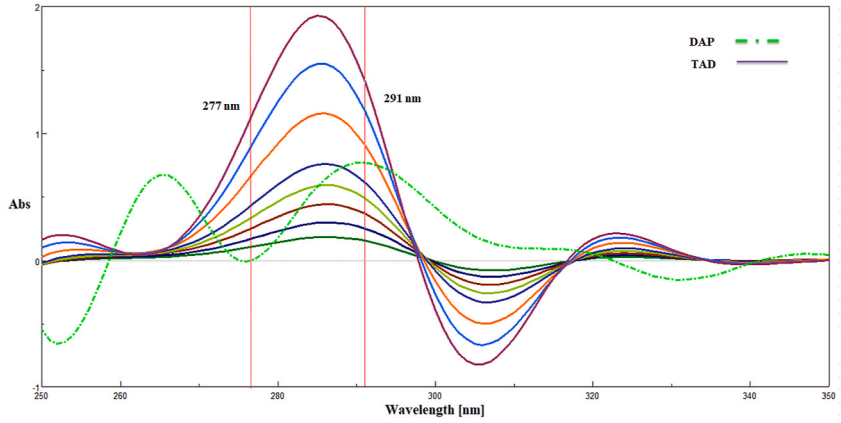

Fig. 6. Deconvoluted amplitude spectra factor of DAP determined at 291 nm in the presence of TAD.

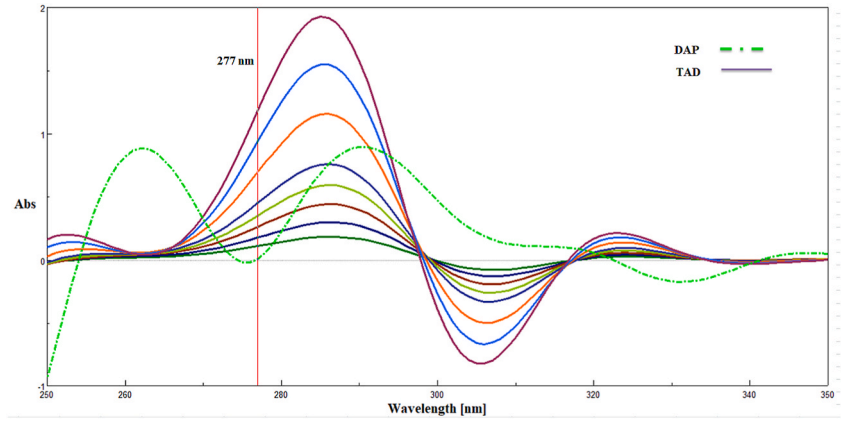

Fig. 7. Fourier self deconvoluted spectra of TAD (2–25 μg/mL) determined at 277 nm zero-crossing point of DAP deconvoluted spectrum.

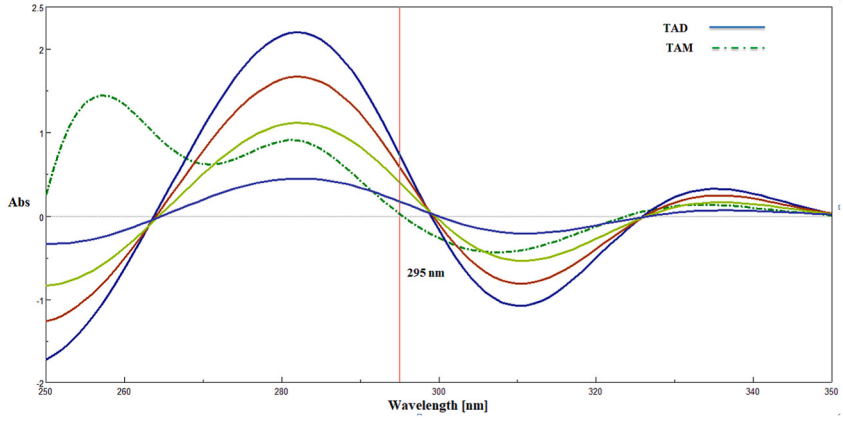

Fig. 8. Fourier self deconvoluted spectra of TAD (2–20 μg/mL) determined at 295 nm zero-crossing point of TAM deconvoluted spectrum.

created, the amount of energy required by the device, and occupational hazards. Table 4 demonstrated the advantage that comes with using the strategy that was proposed.

The green analytical procedure index (GAPI) is regarded as a qualitative and quantitative technique for assessing the level of environmental friendliness of the complete analytical process, from the first stages, such as sample collection and preparation, through the last step of analysis [27]. The GAPI assessment is based on a pictogram made up of five pentagons, each of which stands for a

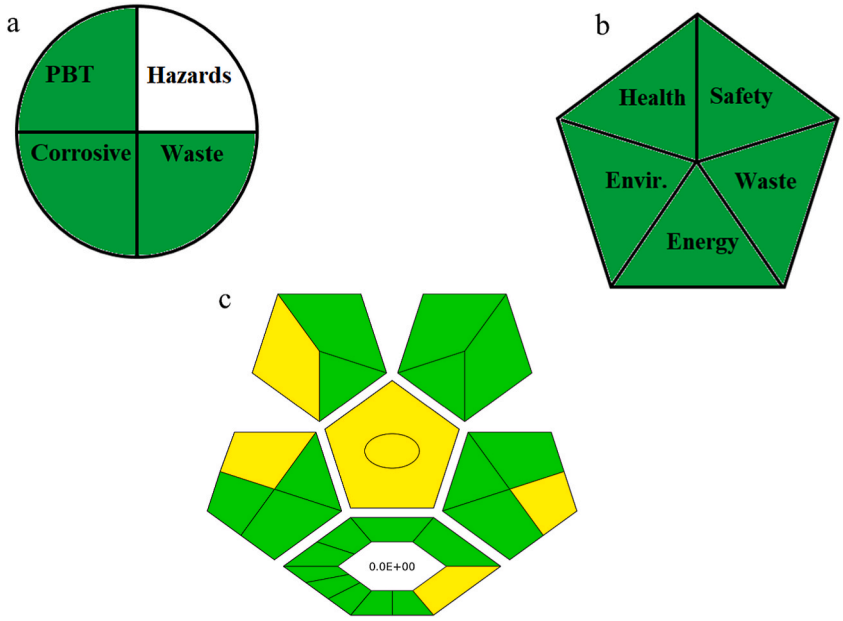

Fig. 9. (a): National Environmental Method Index for the developed UV spectrophotometric method. Fig. 9 **(b):** Raynie and Driver tool for assessing the greenness of the suggested UV spectrophotometric method. Fig. 9 **(c):** Green analytical procedure index and Complex green analytical procedure index for assessing the greenness of the suggested UV spectrophotometric method.

different stage of the procedure. Three color scale levels are linked to this tool, indicating the impact of the analytical steps on the ecology. The green level indicates minor environmental impact, the yellow level indicates considerable ecological implications, and the red level indicates severe ecological danger. Fig. 9 (c) displayed the suggested method's green GAPI evaluation.

For the assessment of analytical techniques based on the GAC characteristics, another metric tool called ComplexGAPI was applied, which comprises the use of components and chemicals produced before the analytical step. The ComplexGAPI metric enlarges the pictogram for GAPI by appending a second hexagonal domain at the bottom. This domain reflects the green nature of pre-analysis procedures [28]. It addresses six aspects: reagents and solvents, instrumentation, yield and conditions, relation to green economy workup, and final product purification. The modified technique uses three assessment levels for each step, using color standards similar to GAPI. The produced pictogram can be utilized to assess and estimate the minimal, moderate, and significant ecological consequences connected to each step of the pre-analysis procedure and the analytical approach, from green to yellow to red. Each area represents a distinct aspect of the given operations and analytical technique, which will fill with green text when all the conditions are satisfied. Fig. 9 (c) displayed the suggested method's green ComplexGAPI evaluation.

3.3. Validation

Validation of the proposed methods was achieved following the ICH guidelines recommendations including linearity and range, limits of detection and quantitation, accuracy, and precision [29].

3.3.1. Linearity and range

The linearity of each proposed method was investigated through calibration curves for each of TAD, DAP, and TAM with the calculation of the corresponding correlation coefficients (r). The r values were in the range of (0.9996–0.9999) which indicated the linearity of the investigated concentration ranges. The concentration ranges for TAD, DAP and TAM through the proposed methods were (2–55 μg/mL), (2–40 μg/mL) and (2–30 μg/mL), respectively.

3.3.2. Limits of detection and quantitation

Limits of detection and quantitation in each proposed method were investigated according to the ICH guidelines as follows:

LOD = 3.3* SD of intercept/slope.

LOQ = 10* SD of intercept/slope.

The LOD and LOQ values for TAD, DAP, and TAM were acceptable values indicating the sensitivity of the proposed methods as summarized in Table 1.

3.3.3. Accuracy

Investigating the accuracy of the proposed methods aimed to compare the method capability to concentration determination with the actual values [30]. This was investigated through successive determinations of three different concentrations each repeated three

Table 3a
Application of standard addition technique for the analysis of DEJAC T ® tablets using deconvoluted amplitude factor.

Drug	Pharmaceutical taken (μg/mL)	Pure added (μg/mL)	Pharmaceutical found[a] (μg/mL)	Recovery[b] (%R)
TAD	10	5	10.03	101.17
		10		98.34
		15		99.68
	Mean ± %RSD			99.73
				±1.423
DAP	**Pharmaceutical taken (μg/mL)**	**Pure added (μg/mL)**	**Pharmaceutical found [a] (μg/mL)**	**Recovery [b] (%R)**
	30	2	29.56	98.69
		5		101.86
		10		99.83
	Mean ± %RSD			100.13
				±1.599

[a] Mean of three determinations.
[b] Mean of three determinations.

Table 3b
Application of standard addition technique for the analysis of Contiflo T ® capsules using DW coupled with IDW method.

Drug	Pharmaceutical taken (μg/mL)	Pure added (μg/mL)	Pharmaceutical found[a] (μg/mL)	Recovery[b] (%R)
				IDW
TAD	20	2	19.88	99.49
		4		99.73
		5		98.93
	Mean ± %RSD			99.38
				±0.413
TAM	Pharmaceutical taken (μg/mL)	Pure added (μg/mL)	Pharmaceutical found[a] (μg/mL)	Recovery[b] (%R)
				DW
	1.6	3.4	1.59	101.04
		6.4		99.18
		8.4		99.57
	Mean ± %RSD			99.93
				±0.984

[a] Mean of three determinations.
[b] Mean of three determinations.

Table 3c
Application of standard addition technique for the analysis of Contiflo T ® capsules using deconvoluted amplitude factor.

Drug	Pharmaceutical taken (μg/mL)	Pure added (μg/mL)	Pharmaceutical found[a] (μg/mL)	Recovery[b] (%R)
				FSD
TAD	5	3	4.96	99.87
		5		99.42
		10		98.15
	Mean ± %RSD			99.15
				±0.901
TAM	Pharmaceutical taken (μg/mL)	Pure added (μg/mL)	Pharmaceutical found[a] (μg/mL)	Recovery[b] (%R)
				DAF
	0.4	5	0.399	101.01
		10		99.05
		15		99.23
	Mean ± %RSD			99.77
				±1.087

[a] Mean of three determinations.
[b] Mean of three determinations.

times. Values of the average recovery percentage (R %) and standard deviation (SD) came to indicate the accuracy of the investigated methods as summarized in Table 1. Also, the accuracy of each proposed method was further indicated by applying the standard addition technique and the values of average R% and SD came in line with the predetermined level of accuracy and prove the capability of the proposed methods to resolute each drug in its dosage form from the accompanying excipients as shown in Table 3 (a-c).

3.3.4. Precision

The precision of the proposed methods is intended to investigate the accordance among the determined concentrations when

Table 4
Assessment of the analytical method greenness utilizing the eco-scale tool.

Reagents	Penalty points of proposed method
Methanol	6
Instrument	
Energy	0
Occupational hazards	0
Waste	3
Total Penalty points	Σ 9
Analytical eco-scale total score[a,b]	91

If the score is > 50, it represents acceptable green analysis.
If the score is < 50, it represents inadequate green analysis.
[a] Analytical Eco-Scale total score = 100- total penalty points.
[b] If the score is > 75, it represents excellent green analysis.

Table 5
Statistical analysis of the adopted spectrophotometric methods and the reported method for simultaneous determination of TAD, and TAM mixtures, and TAD and DAP mixtures.

Parameters	TAD and TAM						TAD and DAP			
	TAD			TAM			TAD		DAP	
	Reported method	IDW	FSD	Reported method	DW	DAF	Reported method	FSD	Reported method	DAF
Mean	100.06	100.69	99.15	100.19	100.56	99.39	100.10	100.30	98.73	98.54
S.D.	0.723	0.941	0.893	0.629	0.934	0.634	0.530	0.589	0.390	0.512
n	5	5	5	5	5	5	6	3	6	3
Variance	0.523	0.885	0.797	0.396	0.872	0.402	0.281	0.347	0.152	0.262
t-test	-(2.31)	1.19	1.77	-(2.31)	0.73	2.00	-(2.36)	0.50	-(2.36)	0.57
F-test	-(6.39)	1.69	1.53	-(6.39)	2.20	1.02	-(5.79)	1.24	-(5.79)	1.72

applying each method. This was achieved by investigating the intra-day precision by measuring three different concentrations on the same day but at differing consecutive times repeatedly. While the investigation of the inter-day precision was achieved by measuring three different concentrations on variable consecutive days repeatedly. The results were assessed by observing the RSD% values of each proposed method and were summarized in Table 1.

3.3.5. Selectivity

The selectivity of the proposed methods was investigated to guarantee the capability of these methods to fully resolute both DAP and TAM in their various ratios with TAD from their binary laboratory-prepared mixtures. The results of the laboratory-prepared mixtures analyses (average R% and SD) supported the investigated selectivity for each proposed method as shown in Table 2.

3.4. Analysis of pharmaceutical dosage form

TAD, TAM, and DAP determination in their pharmaceutical formulation were successfully examined using the recommended approaches. The mean recoveries and RSD% were determined and were shown in Table 5. Furthermore, the efficacy of the methods was assessed by implementing the standard addition strategy Table 3 (a-c).

3.5. Statistical analysis

Statistical analysis of the proposed spectrophotometric methods was achieved by comparing the obtained percentage recoveries from the proposed methods with each corresponding reported method [31,32] by applying the student t-test and f-test whose results were summarized in Table 5. The t and F-test results came less than their critical values which indicated that there was no statistically significant difference between the reported and proposed methods.

Also, for the best visualization and evaluation of the data, a set of eye-friendly graphing tools were utilized to present our data as; interval plots for the proposed and the reported methods Figs. S1–S4 (a). The interval plots showed the results of R% in the form of intervals which if showed intersecting patterns they reveal no significant difference between the presented methods. Box plots were also plotted to further illustrate the distribution of the R% results within each of the proposed and the reported methods Figs. S1–S4 (b). Normal probability plots Figs. S1–S4 (c) showed the distributions of residuals of each set of methods which if showed a normal distribution pattern of the residuals they reveal that there were no statistically significant differences between the compared sets of methods. Furthermore, Tukey's honestly significant difference test was applied and the results were displayed as a matrix providing a confidence interval for each pair of comparisons. According to Figs. S1–S4 (d), there was no appreciable variation in the mean between methods based on the overlap between the intervals. All these graphical representations saved the time and effort for the analysts to assess the performance of their proposed versus the reported methods.

4. Conclusion

For the first time, a newly innovated spectrophotometric approach named deconvoluted amplitude factor was used to assess TAD in the presence of DAP, and TAM in their pure standard forms, laboratory-prepared mixes, and pharmaceutical formulations. Besides, different manipulated spectrophotometric methods including dual and induced dual wavelengths were applied to determine these two binary mixtures. Also for more data interpretation, a comparative statistical investigation relied on the *t*-test and the F-test revealed that there was no statistically significant variance between the designed spectrophotometric methods and the reported HPLC approaches. Moreover for ensuring such a comparative study, different confirmatory plots namely; interval plots, boxplots, normal probability plots, and Tukey's simultaneous significant difference test were applied to represent an eye-friendly package for ease of data visualization and interpretation. Furthermore, a newly ComplexGAPI assessment was applied for the first time in spectrophotometric methods, for checking the pre-analytical step, proving the safety during the application of this method, besides this assessment was associated with other greenness assessments of the embraced methodologies revealing green outcomes of the applied methods.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Authors' contributions

Maya S. Eissa: review & editing, Supervision. Ahmed Elsonbaty: Conceptualization, Investigation, Writing original draft. Khaled Attala: Conceptualization, Investigation, Writing original draft. Randa A. Abdel Salam: review & editing, Supervision. Ghada M. Hadad: review & editing, Supervision. Mohamed A. Abdelshakour: review & editing, Supervision. Aziza E. Mostafa: review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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