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Development of validated methods for the simultaneous quantification of Finasteride and Tadalafil in newly launched FDA-approved therapeutic combination: greenness assessment using AGP, analytical eco-scale, and GAPI tools†

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The primary objectives of green chemistry are the reduction of generation and use of hazardous substances. In healthcare, the most active areas of research in green chemistry are medication manufacturing and analysis. Analysts take serious steps for converting traditional analytical methods to eco-friendly ones that minimize the negative effects of solvents and chemicals on the environment and improve the healthcare. In the proposed work, two analytical methods are presented for the quantification of Finasteride (FIN) and Tadalafil (TAD) simultaneously in newly launched FDA-approved dosage form without prior separation. The first method is derivative spectrophotometry, which is based on measuring the amplitudes of first derivative spectrophotometric peaks of FIN and TAD in ethanolic solution at 221 nm and 293 nm, respectively. On the other hand, measuring the peak-to-peak amplitudes of second derivative spectrum of TAD solution at 291–299 nm was also performed. Regression equations show good linearity for FIN and TAD in the ranges of 10–60 μg mL^{-1} and 5–50 μg mL^{-1}, respectively. The second method is the RP-HPLC method, where the chromatographic separation was achieved using the XBridgeTM C18 (150 × 4.6 mm, 5 μm) column. The eluent was the mixture of acetonitrile:phosphate buffer with triethylamine, 1% (v/v) adjusted to pH = 7 in the ratio of 50 : 50 (by volume). The flow rate was 1.0 mL min^{-1} with DAD-detection at 225 nm. This analytical procedure was linear over the ranges of 10–60 μg mL^{-1} and 2.5–40 μg mL^{-1} for FIN and TAD, respectively. The presented methods were validated (regarding ICH guidelines) and statistically compared by applying the *t*-test and *F*-test with the reported method. The greenness appraisal was performed using three different tools. The proposed validated methods were found to be green, sensitive, selective, and can be successfully used for quality control test.

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1. Introduction

The most common diseases among males around the world are benign prostatic hyperplasia (BPH) and its associated lower urinary tract symptoms.[1,2] Finasteride (FIN) (Fig. 1) is one of the 5α-reductase inhibitors available for the treatment of BPH. FIN inhibits the type II isoenzyme, which has prostate higher activity level than the type I isoform.[3,4] In October 2011, one of the most famous phosphodiesterase-5 inhibitors drugs, Tadalafil (TAD) (Fig. 1) was used daily for treating BPH. This drug represented a new action for the treatment of BPH symptoms.

The treatment of men who were at risk of BPH progression by FIN combination therapy represented an innovation in therapeutics. In December 2021, a combination of FIN and TAD was approved by FDA for the treatment of urinary tract symptoms caused by BPH in men after oral administration under the trade name Entadfi™.

Many analytical methods were reported for the analysis of FIN and TAD in their pure forms, dosage forms, and in different biological fluid including spectroscopic techniques and different chromatographic techniques.[5–15]

As a latterly approved combination, there is an urgent need for the development of selective analytical methods for their quantitative determination in bulk or dosage forms. In the literature, two analytical techniques were developed for the simultaneous determination of FIN and TAD, one of these methods is the chromatographic method using the LC-MS/MS

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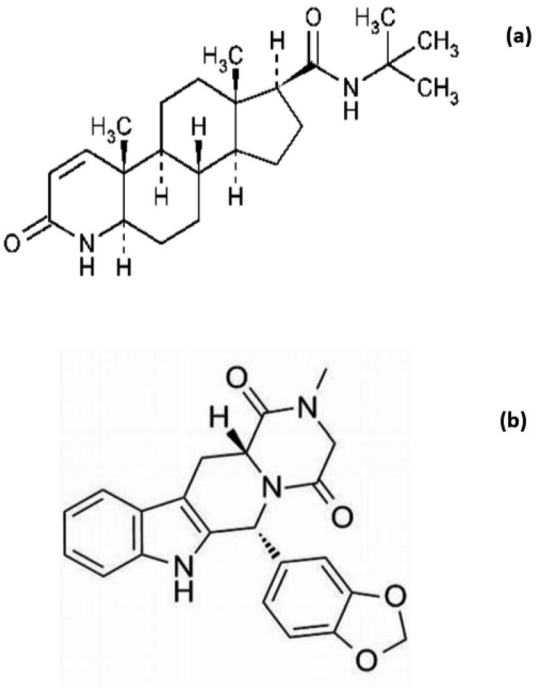


Fig. 1 Chemical structures of (a) FIN and (b) TAD.

technique for the determination of the studied drugs in human plasma.[16] Although LC-MS/MS is a specific and highly sensitive technique, it is considered a sophisticated technique and affects the speed and cost of the analysis of the compounds of interest. In July 2022, a spectroscopic technique was developed for the determination of FIN and TAD in a capsule.[17] Literature data lack any chromatographic method for the assaying of the proposed combination in its dosage form.

Since 1999, the term "green analytical chemistry" was suggested for consideration. Most actions in making chemical procedures greener emphasize the need for using more benign solvents, reagents reduction, and lowering the energy consumption. Anastas, the father of green chemistry, shed light on the need to develop green analytical methods. Since that time, the concern in applying the principles of green analytical chemistry has grown.[18–22]

One of the challenges in green analytical chemistry is the assessment of the greenness of analytical processes. It is renowned that the process that cannot be measured cannot be managed. Thus, the goal of the proposed work is to design, develop, and optimize green analytical methods for the simultaneous quantitation of FIN and TAD in their recent FDA-approved capsules. In addition, the greenness evaluation of the proposed techniques was performed by applying analytical greenness profile (AGP), eco-scale penalty points approach, and green analytical procedure index (GAPI). All developed methods were fully validated.

2. Experimental

2.1. Materials and reagents

Pharmaceutical grade of Finasteride and Tadalafil were supplied from Sigma-Aldrich (GmbH, Steinheim, Germany). Ethanol, TEA, and buffer components are from Sigma-Aldrich®, St. Louis, MO, United States. HPLC grade acetonitrile is from Tedia, Ohio, USA.

2.2. Pharmaceutical formulations

A commercial product (Entadfi™ labeled to contain 5 mg FIN and 5 mg TAD per capsule) was studied.

2.3. Instrumentation

2.3.1. For the derivative spectrometric method. A double beam PerkinElmer-25 Lambda – UV-Visible spectrophotometer and 1 cm quartz cells were used for all measurements.

2.3.2. For the HPLC method. The HPLC system (Agilent, Germany) containing Agilent 1260 Series Quaternary pump G1311C was used. This model contains an integrated vacuum degasser in a solvent cabinet and a four-channel gradient pump. The detector is Agilent 1260 Series Diode Array G1315D, multiple wavelength. LC separations were performed on an XBridgeTM C18 (150 × 4.6 mm, 5 μm) column, part no. 186003116.

The eluent consisted of acetonitrile and phosphate buffer adjusted to pH 7 with 0.1% TEA (50 : 50). Then, it was degassed and filtered using a 0.45 μm pore size membrane filter. The samples were filtered using 0.45 μm disposable filters. The flow rate was adjusted to be 1 mL min^{-1}.

2.4. Standard solutions

1 mg mL^{-1} stock standard solutions of FIN and TAD were prepared separately in ethanol. Then, dilution was performed by ethanol to 100 μg mL^{-1} (working standard solutions).

2.5. Construction of calibration graphs

2.5.1. For the derivative spectrophotometric method. Aliquots from the working standard solutions of both drugs, as stated in Table 1, were accurately transferred into separate sets of 10 mL volumetric flasks. Dilution was done with ethanol, and the UV spectrum for each solution was recorded against ethanol. The first derivative (1D) of FIN spectra (obtained using $\Delta\lambda = 3$ nm) were recorded. On the other hand, in TAD, the first derivative (1D) spectra were recorded using $\Delta\lambda =$ 9 nm. The values of the 1D were measured at 221 and 293 nm for FIN and TAD, respectively, and plotted *versus* the corresponding concentrations to obtain a linear relationship. On the other hand, the second derivative of (2D) were recorded using $\Delta\lambda = 9$ nm for the TAD spectra. The values of 2D amplitudes were measured from peak to peak at 291–299 nm. The calibration curve for TAD was designed by plotting the 2D values *versus* concentration and the regression equation was computed.

2.5.2. For HPLC method. Working stock solutions were diluted with the eluent to obtain standard solutions of required concentrations, as shown in Table 1. For each concentration, triplicate 10 μL injections were made and studied by chromatography under the mentioned conditions. The peak areas were drawn against the corresponding concentrations of both the drugs to obtain the calibration curves.

Table 1 Validation parameters of the proposed methods for the simultaneous determination of FIN and TAD[a]

	FIN		TAD		
	Derivative spectrophotometric method		Derivative spectrophotometric method		
Parameters	^{1}D at 221 nm	HPLC method	^{1}D at 293 nm	^{2}D from peak to peak (291–299 nm)	HPLC method
Accuracy* (mean ± RSD%)	101.87 ± 1.5	98.9 ± 1.1	100.31 ± 1.5	100.14 ± 0.8	98.2 ± 0.7
Precision** (RSD%)					
Intraday	1.80	0.99	0.50	0.91	0.55
Interday	1.14	1.25	1.89	1.54	0.89
LOD***	2.03	2.00	1.32	1.29	0.88
LOQ***	6.16	6.5	4.01	3.91	2.9
Linearity range***	10–60	10–60	5–60	5–60	2.5–40
Intercept (a)	1.788×10^{-3}	−7.81	4.45×10^{-3}	1.65×10^{-2}	7.58
Slope (b)	3.48×10^{-3}	8.31	1.14×10^{-2}	2.61×10^{-2}	44.95
r	0.9995	0.9995	0.9998	0.9998	0.9998
S_a	1.71×10^{-3}	5.01	2.99×10^{-3}	6.66×10^{-3}	8.85
S_b	4.69×10^{-5}	0.13	8.76×10^{-5}	1.95×10^{-4}	0.39
$S_{y/x}$	2.15×10^{-3}	5.38	4.58×10^{-3}	1.02×10^{-2}	13.10
S_b%	1.35	1.56	0.77	0.75	0.87
F	5514.54	4168.66	17 040.37	17 898.21	13 021.60
Significance F	8.39×10^{-9}	3.40×10^{-7}	1.36×10^{-11}	1.18×10^{-11}	3.54×10^{-8}

[a] *Calculated as mean% recovery from the analysis of synthetic mixtures of different ratios (FIN/TAD: 30/10, 10/10, 10/20 μg mL^{-1}); $n = 9$. ** Calculated as mean RSD% from the analysis of the synthetic mixtures; $n = 9$. *** in μg mL^{-1} r is correlation coefficient, S_a is standard deviation of intercept, S_b is standard deviation of slope, and $S_{y/x}$ is standard deviation of residuals.

2.6. Analysis of synthetic mixture

Adjusted volumes of each of FIN and TAD working stock solutions were transferred into a set of 10 mL volumetric flasks. The content of each flask was diluted to volume with ethanol (for derivative spectrophotometric method) and mobile phase (for HPLC method) such that the drugs' concentrations were within the ranges mentioned in Table 1.

2.7. Procedure for capsule analysis

The developed methods were applied for the quantification of FIN and TAD in capsules. Ten capsules were emptied and their content weighed. A measured quantity, corresponding to 25 mg FIN and 25 mg TAD, was transferred to a 25 mL volumetric flask. 15 mL ethanol was added. Sonication was done for 20 min. After completing the volume with ethanol, the solution was filtered. 1 mL filtrate was diluted to 10 mL with ethanol to obtain the final capsules extract containing FIN/TAD of 100/100 μg mL^{-1} for FIN and TAD analysis. Steps were followed as under Section 2.5.

3. Results and discussion

3.1. The derivative spectrophotometric method

Spectrophotometry is a recognized technique for routine drug testing in quality control laboratories around the world. In addition, spectrophotometry has also gained an essential role toward green analytical chemistry. FIN and TAD are combined in capsule formulation in a ratio of 1 : 1. The UV absorption spectra for FIN and TAD solutions in ethanol were scanned (Fig. 2). The spectra showed complete overlap between the investigated drugs in the region of 200–300 nm. It appeared from Fig. 2 that FIN is a weakly absorbing compound in comparison with TAD, which shows high UV absorbance. The challenge is to simultaneously determine the two drugs that are combined in a 1 : 1 ratio in the dosage form. Thus, it subsequently necessitates an effective mathematical separation procedure. The first derivative of the absorption spectra (1D) was applied successfully for the determination of both FIN and TAD while the second derivative of absorption spectra (2D) was applied only for the quantification of TAD.

3.1.1. Optimization of the derivative spectrophotometric method. The main parameter that affects the derivative spectra shape is the wavelength difference ($\Delta\lambda$) by which the derivative

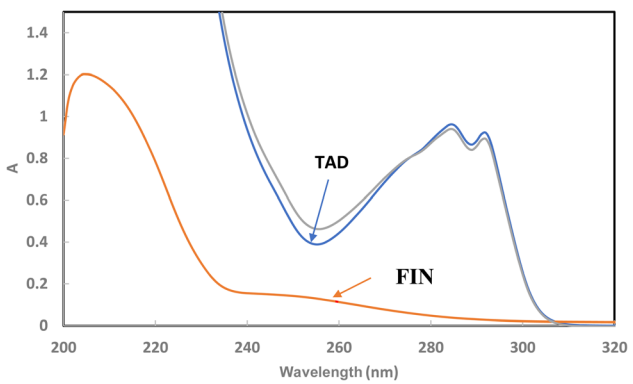


Fig. 2 Absorption spectra of 30 μg mL^{-1} FIN, 30 μg mL^{-1} TAD and their synthetic mixture (30/30 μg mL^{-1}).

is calculated.[23] This parameter requires to be optimized to give a well-resolved peaks and good selectivity for the simultaneous determination of drugs in the mixture. Actually, if the value of $\Delta\lambda$ is too high, the resolution of the spectra is very poor, and if it is small, the noise will appear. Thus, the optimum value of $\Delta\lambda$ must be detected by taking into consideration the level of noise and the resolution of the derivative spectrum.

Fig. 3 shows the 1D and 2D spectra of both drugs. FIN can be successfully estimated by measuring its 1D value at 221 nm, which corresponds to a zero-crossing for TAD, whereas TAD can be determined by measuring its 1D value at 293 nm and its 2D values from peak-to-peak at 291–199 nm (corresponding to no contribution from FIN). The influence of several values of $\Delta\lambda$ on the 1D and 2D spectra was tested. $\Delta\lambda = 3$ nm was selected for the 1D determination of FIN, and $\Delta\lambda = 9$ nm was chosen for the 1D and 2D determination of TAD as the optimal condition to obtain a good signal-to-noise ratio.

Generally, the characteristics of the derivative spectra can constitute a specialized fingerprint for drug identification as the amplitude ratios at the chosen wavelength can be considered to be suitable parameters for the confirmation of drug purity.[24] The ratio of absolute 2D values in the range of 291–299 nm for TAD standard solutions and capsules was calculated to detect the presence of any interferences. The obtained results show that the ratio was 2.085 with RSD% of 0.34 ($n = 8$). These RSD% value are less than 2%. Therefore, the 2D method is specific for TAD and can be used to examine its identity and purity.

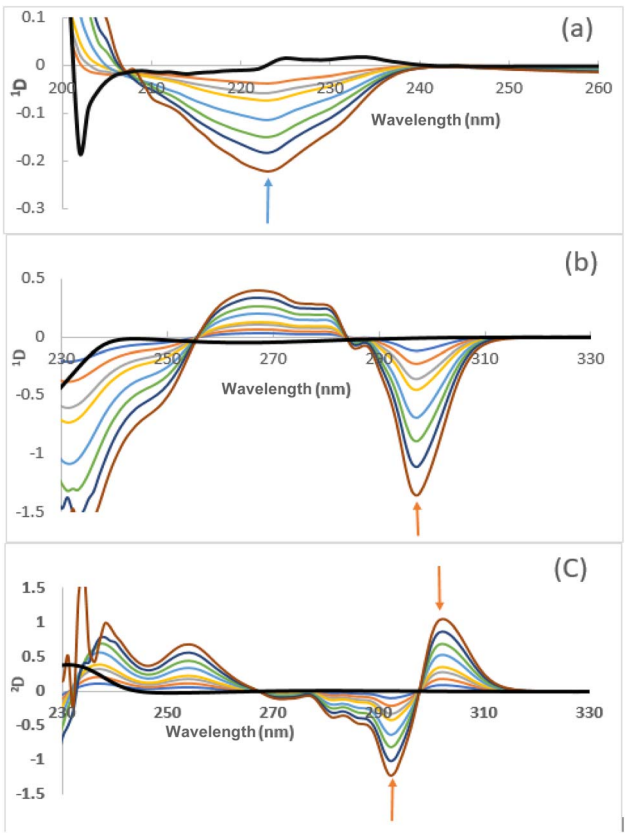


Fig. 3 (a) First derivative spectra of 10, 15, 20, 30, 40, 50, and 60 μg mL^{-1} FIN and 10 μg mL^{-1} TAD, (b) first derivative spectra, (c) second derivative spectra of 5, 10, 15, 20, 30, 40, 50, and 60 μg mL^{-1} TAD and 30 μg mL^{-1} FIN in ethanol.

3.2. The HPLC method

The proposed HPLC method aimed to develop a chromatographic system capable of eluting and separating FIN and TAD from one another. In addition, its target is to fulfill the system's general requirements.

3.2.1. Optimization of chromatographic conditions. To maximize the selectivity and sensitivity of the analytical procedure, the following experimental conditions were optimized.

3.2.1.1. Stationary phase. Different stationary phases were tried. Using both C8 and C18 (250 × 4.6 mm) columns, the two peaks of FIN and TAD were eluted at very high retention times. The C18 (150 × 4.6 mm) column succeeded efficiently in separating the two drugs within acceptable retention times.

3.2.1.2. Organic modifier. The type of organic modifier greatly affects the peak shape of both investigated drugs. Different organic modifiers have been tried including ethanol, methanol, and acetonitrile. Using ethanol and methanol in mobile phase led to peak broadening, decreased efficiency, and caused distortion of the shape of FIN and TAD peaks. On the other hand, the use of acetonitrile allowed the elution of FIN and TAD with acceptable peak shape.

Binary mixtures of drugs were injected with the mobile phase containing different percentages of acetonitrile. Fig. S1† shows the retention times obtained for the two compounds as a function of acetonitrile percentage in the eluent. 50% acetonitrile was selected to give optimum separation. At lower acetonitrile proportions, separation occurred but with the excess tailing of the TAD peak and increasing retention time for the FIN peak. Using a higher ratio of acetonitrile succeeded in separating the two drugs with reasonable retention times but with decreasing greenness of the method.

3.2.1.3. pH of the aqueous part of the mobile phase. The effect of pH of the aqueous component of the eluent was studied. Various pH values (from 2.0 to 7.0) using phosphate buffer (adjusted by orthophosphoric acid or sodium hydroxide) together with acetonitrile in a ratio of 50 : 50 v/v were tried. The best pH value for the optimum resolution of the components under analysis was 7. Below this value, the asymmetry of the FIN peak appeared and the forked tailed TAD peak was obtained. Triethylamine (TEA) was also added to the aqueous part not only to provide the required pH but also to prevent peak tailing of FIN and TAD.

3.2.1.4. Detection wavelength. To increase the selectivity and sensitivity of the method, different wavelengths were tried for the quantification of proposed drugs. It was found that the detection at 225 nm for both FIN and TAD gave the best selectivity in addition to sensitivity.

3.2.1.5. Flow rate. The impact of flow rate was tested in the range of 0.5–1.5 mL min^{-1}. A flow rate of 1 mL min^{-1} was selected as it was associated with reasonable retention times and the highest theoretical plates. A flow rate of more than 1 mL min^{-1} resulted in low resolution between FIN and TAD,

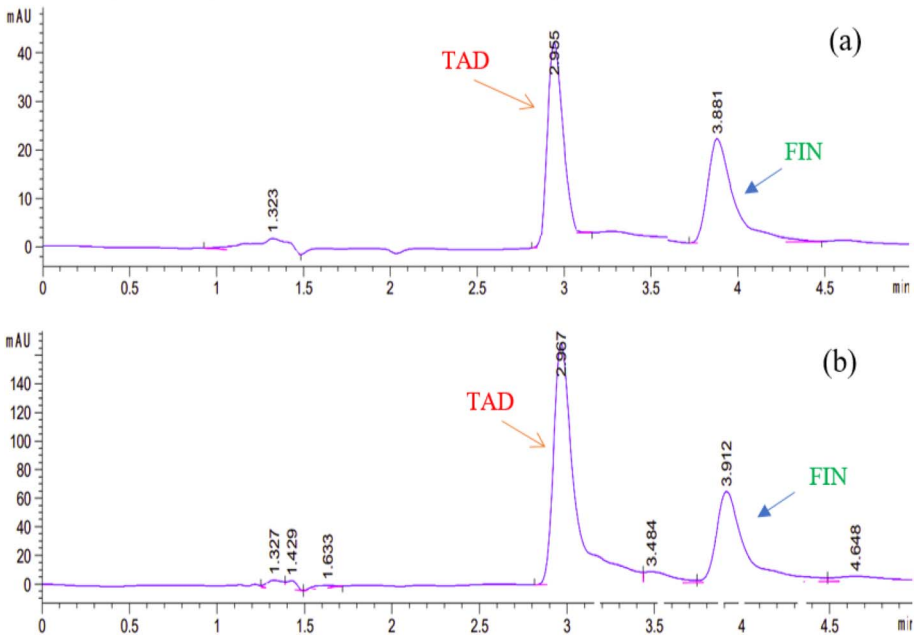


Fig. 4 Typical HPLC chromatograms of (a) a standard mixture of 10 μg mL^{-1} FIN and 10 μg mL^{-1} TAD, (b) prepared capsule solution containing 40 μg mL^{-1} of both FIN and TAD using the optimized chromatographic conditions.

whilst a flow rate less than 1 mL min^{-1} resulted in high retention times of both the drugs. Thus, the optimum chromatographic conditions stated previously were adopted for all measurements. Fig. 4 shows the significant separation of FIN and TAD with optimum run time, peak sharpness, and resolution.

3.2.2. System suitability of the chromatographic method. Many system suitability parameters were calculated. All these parameters are listed in Table S1.† The results in Table S1† demonstrate satisfactory selectivity, acceptable degree of peak asymmetry, and good efficiency of the proposed HPLC method for the separation of FIN and TAD binary mixture.

3.3. Validation of the proposed methods

To guarantee the analytical validity of the proposed methods, different parameters of ICH guidelines were evaluated.

3.3.1. Linearity, limit of detection, and limit of quantitation. Under the above-described experimental conditions, linear relationships were observed by plotting 1D, 2D values (for derivative spectrophotometric method) and peak areas (for HPLC method) against corresponding concentrations of both the drugs. The concentration ranges, calibration equations, limit of detection (LOD), limit of quantitation (LOQ), and other statistical parameters are listed in Table 1. Acceptable linearity was revealed by nearness of the correlation coefficients (r) to unity and low values of variance ration (F-value).

3.3.2. Accuracy and precision. The accuracy of the proposed methods was appraised (Table 1). The results in Table 1 show satisfactory recoveries and small relative standard deviations (RSD%), indicating acceptable accuracy of the proposed methods. The precision was assessed by repeating the assay three times on the same day (intraday precision) and by the assay of the sets of samples on three different days (interday precision). The RSD% values depicted in Table 1 shows that both methods provide acceptable intraday and interday variation of FIN and TAD concentrations.

3.3.3. Robustness. The robustness of the proposed methods were assessed by analyzing FIN and TAD at the same concentration levels mentioned in Section 3.3.2. Various parameters were slightly changed as indicated in Table 2.

It was found that slight intended changes in the parameters had no significant influence on the determination of FIN and TAD using the proposed methods. Acceptable and satisfied robustness was indicated by the low RSD% of 1D and 2D in the derivative spectrophotometric method and the nearly unchanged capacity factor (k/) values of both drugs in the HPLC method (Table 2).

3.3.4. Selectivity. The selectivity was checked by applying the proposed methods for the determination of the two drugs in capsules. Accepted percentage recoveries without interference from the excipients such as carrageenan, lactose monohydrate, potassium chloride, magnesium stearate, sodium lauryl sulfate, sodium starch glycolate, and silicified microcrystalline cellulose were obtained, indicating the selectivity of the methods (Table 3).

Moreover, the derivative spectrophotometric method, the ratios of 2D values between (292–299 nm), as shown in Fig. 3, were measured to test the presence of any interferences. The results for several concentrations of TAD standard solutions and capsules are indicated in Table S2.† The obtained results show RSD% values less than 2%, indicating that the derivative spectrophotometric method is specific for TAD and can be used to test its purity.

The peak purity of FIN and TAD was tested using a G1315D PDA detector for the HPLC method. The purity angle was within

Table 2 Robustness of the proposed methods for the simultaneous determination of FIN and TAD[a]

Parameters	FIN*		TAD*			
Derivative spectrophotometric method	SD of ^{1}D at 221 nm	RSD%	SD of ^{1}D at 293 nm	RSD%	SD of ^{2}D from peak to peak (291–299 nm)	RSD%
(1) Spectrophotometer of different models**	0.006	1.14	0.019	1.57	0.012	1.67
(2) Ethanol of different lots	0.021	1.73	0.014	1.95	0.023	1.82

Parameters	FIN*		TAD*	
HPLC method	RSD% of peak areas	$k/ \pm$ SD	RSD% of peak areas	$k/ \pm$ SD
(1) Acetonitrile percentage in mobile phase (45, 50, and 55%)	1.80	1.21 ± 0.015	0.98	1.93 ± 0.009
(2) pH of the aqueous phase (6.2, 6.6, 7, 7.2, and 7.5)	1.56	1.20 ± 0.010	1.55	1.93 ± 0.008
(3) Flow rate of the mobile phase (0.8, 1, and 1.2 mL min^{-1})	1.10	1.19 ± 0.006	1.64	1.90 ± 0.006
(4) Wavelength of detection (220, 222, 225, 227, and 230)	1.95	1.21 ± 0.005	1.75	1.93 ± 0.003

[a] * Average of three concentrations 10, 30, 50 μg mL^{-1} and 5, 30, 60.0 μg mL^{-1} for FIN and TAD, respectively. ** PerkinElmer Lambda EZ201 UV-visible spectrophotometer and Thermo-Spectronic UV-vis spectrophotometer connected to a Harvest computer system.

Table 3 Assay of FIN and TAD in Entafi® capsules using the proposed derivative spectrophotometric and HPLC methods

	FIN			TAD			
	Derivative spectrophotometric method			Derivative spectrophotometric method			
Entafi® capsules[a]	^{1}D at 221 nm	HPLC method	Reported method[17]	^{1}D at 293 nm	^{2}D from peak to peak (291–299 nm)	HPLC method	Reported method[17]
Mean% recovery ± SD[b]	98.80 ± 0.751	100.36 ± 1.181	99.90 ± 1.560	99.62 ± 0.804	99.91 ± 0.515	100.05 ± 0.915	99.32 ± 0.484
Er (%)	−1.20	0.36	−0.10	−0.38	−0.09	0.15	−0.68
t^c	0.67	0.53	—	0.71	1.87	1.68	—
F^c	4.31	1.71	—	2.76	1.13	3.57	—

[a] Labeled to contain 5 mg FIN and 5 mg TAD/capsule. [b] Mean ± standard deviation of five determinations. [c] Theoretical values of t and F are 2.31 and 6.39, respectively, at 95% confidence limit.

the purity threshold limit for both the drugs in the capsule sample solution, indicating the ability of the HPLC method to determine FIN and TAD without interferences from co-formulation adjuvants.

3.3.5. Stability in solutions. The study of stability of FIN and TAD in their solutions during the analysis was performed. At room temperature, standard solutions of both drugs were kept for 3 h. Then, they were analyzed using the developed methods. It was found that there is no significant changes in 1D, 2D and the peak areas values throughout the analysis time. Thus, the two drugs are considered stable in solutions for at least 3 h.

3.4. Application on dosage form (Entafi® capsules)

The proposed methods were performed to assay FIN and TAD in Entafi® capsules. Five determinations of capsule sample solutions were measured. The results in Table 3 provided reasonable RSD% and recovery% values for both the investigated drugs. Thus, FIN and TAD were found to match the label claims. As shown in Table 3, the results of the proposed methods were statistically compared with that of the reported one.[17] The values of t and F tests do not surpass the theoretical values, thus advocating that there is no significant variation between the developed methods in the proposed study and the reported one.

3.5. Greenness appraisal

3.5.1. Greenness appraisal of the proposed methods. The sustainability of developed methods was evaluated using thee different greenness appraisal tools including AGP, Eco-scale penalty points, and GAPI. The first assessment tool is the AGP method,[25] where it is demonstrated as a pentagram shape divided into five parts. Each part can be green or yellow or red. All parts indicate the impact of five roles: safety, environmental, health, energy, and waste. The AGP pentagrams of the proposed methods are represented in Fig. S2.† As shown in Fig. S2,† the pictograms rich with green and yellow color indicate that the methods are highly eco-friendly. However, the derivative spectrophotometric method provides a greener alternative to HPLC.

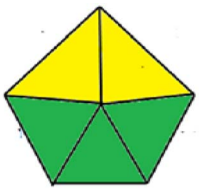
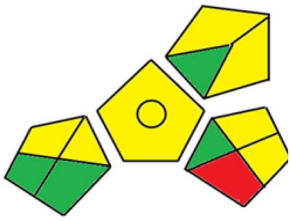
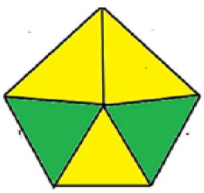
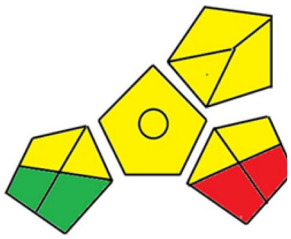
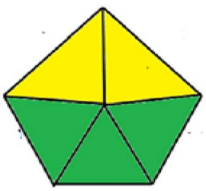
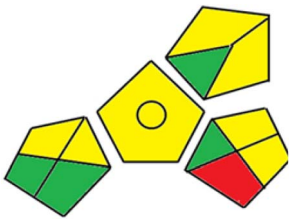
The second assessment tool is the eco-scale penalty points.[18,26] The eco-scale penalty points were calculated for the proposed methods. All calculations are cited in Table 4. It was observed that the derivative spectrophotometric and HPLC methods are highly eco-friendly. This is confirmed by the calculated values of analytical eco-scale score, which is more than 75 (Table 4). Thus, the proposed methods are excellent in greenness assessment.

Finally, the third assessment tool is the GAPI method,[21] which is also illustrated by a pictogram. This colored pictogram is divided into 15 segments. Each segment can take green or yellow or red color regarding the level of greenness. The proposed methods' GAPI pictograms are shown in Fig. S3.† The

Table 4 Greenness assessment of the proposed methods using three different tools, analytical greenness profile (AGP) (a), analytical eco-scale (b), and green analytical procedure index (GAPI) (c)

Reagents/instruments	Spectrophotometric method	HPLC method
(a) Analytical Greenness Profile (AGP)		
Health hazard	Moderately toxic, NFPA = 2	Moderately toxic, NFPA = 2 and 3
Safety hazard	Highest NFPA flammability and instability score = 3 and 0	Highest NFPA flammability and instability score = 3 and 0
Environmental hazard	<50 mL	<50 mL
Energy	Very little solvent evaporation	Energy of HPLC
Waste amount	≤50 mL	≤50 mL
Health hazard	Moderately toxic, NFPA = 2	Moderately toxic, NFPA = 2 and 3
(b) Analytical eco-scale penalty points		
Reagents		
Ethanol	12	—
Acetonitrile	—	8
Phosphate buffer pH 7	—	Not hazardous
TEA	—	8
Instruments		
Spectrophotometry	0	—
HPLC	—	1
Occupational hazard	0	0
Waste	3	5
Total penalty points	15	22
Analytical Eco-scale total score	**85**	**78**
(c) Green analytical procedure index (GAPI)		
Sample preparation		
Collection (1)	At-line	At-line
Preservation (2)	None	None
Transport (3)	None	None
Storage (4)	Under normal conditions	Under normal conditions
Type of method: direct or indirect (5)	Filtration	Filtration
Reagent and solvents		
Amount (9)	<10 mL	10–100 mL
Health hazard (10)	NFPA health hazard score = 2	NFPA health hazard score = 1, 2, and 3
Safety hazard (11)	Instability score = 0 Flammability score = 3	Instability score = 0 Flammability score = 3
Instrumentation		
Energy (12)	≤ 0.1 kW h per sample	≤ 1.5 kW h per sample
Occupational hazard (13)	—	—
Waste (14)	1–10 mL	> 10 mL
Waste treatment (15)	No treatment	No treatment
Quantification	Yes	Yes

Table 5 Greenness comparison between the proposed methods and the reported method for the assay of the binary mixture of FIN and TAD

Methods	AGP	Analytical eco-scale total score	GAPI
Proposed derivative spectrophotometric method		85	
Proposed HPLC method		78	
Reported method[17]		85	

GAPI pictogram of the derivative spectrophotometric method demonstrates four green segments, seven yellow segments, and only one red segment, while the GAPI pictogram of the HPLC method shows two green segments, eight yellow segments, and two red segments. These results provide that the proposed methods are green and eco-friendly. As shown in Fig. S3,† few number of red batches are observed in case of the derivative spectrophotometric method, which is due to the use of only one solvent, using direct method for analysis and production of few amounts of waste. This approach indicates that the developed derivative spectrophotometric analysis is relatively greener than the chromatographic one.

3.5.2. Greenness appraisal of the proposed methods in comparison with the reported one. Table 5 shows a comprehensive greenness behavior difference between the proposed methods and that of the reported one[17] for the quantification of FIN and TAD. Regarding the eco-scale applied tool, the eco-scale scores of the three methods were above 75, revealing the excellent green analytical method of analysis. Meanwhile, for the AGP tool, it was found that the proposed derivative spectrophotometric method and reported method had the same figure and greenness result. On the other hand, the proposed HPLC has one yellow segment more than them. For the GAPI tool, both the proposed derivative spectrophotometric and reported method provided the same number of green, yellow, and red segments. On the other hand, the proposed HPLC has fewer green segments, higher yellow segments, and one red segment more than them because of its higher amount of waste.

4. Conclusion

The present study proposes two fully validated analytical techniques for the simultaneous determination of FIN and TAD in bulk and capsules. Both proposed methods are sensitive, selective, and can be used for routine quality control investigation. Also, they were ecologically assessed using three greenness assessment tools. The findings indicate that they are environmentally benign. The present derivative spectrophotometric method has advantages of low cost of operation and greenness when compared to HPLC. The suggested HPLC method has advantages over the reported method as it is straight forward, does not need further mathematical treatment of measured data, having higher selectivity and taking a short time as the analysis time is only 5 min. The main advantage of the proposed derivative spectrophotometric method in comparison with the reported method is the greenness as it used ethanol instead of methanol.

Conflicts of interest

There are no conflicts to declare.

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