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Validated Stability-Indicating Derivative Spectrophotometry and Synchronous Fluorescence Spectroscopy Methods for the Determination of Dapoxetine Hydrochloride in the Presence of its Degradation Product and Co-Formulated Drugs

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Abstract

The stability of dapoxetine hydrochloride (DP) was studied in the presence of its acidic degradation product namely, (+)-N, N-dimethyl-1-phenyl-3-propanolamine (Deg 1) and the co-formulated drugs Vardenafil (VR) and Tadalafil (TD) using derivative spectrophotometry and synchronous fluorescence spectroscopy methods.

Method I, stability indicating derivative spectrophotometry 1D was developed for the determination of DP in the presence of its hydrolytic degradation product and the co-formulated drug VR. The amplitude of the first derivative spectra 1D at λmax of 240 nm and 227 nm was measured for DP and VR, respectively.

Method IIA, stability indicating synchronous fluorescence spectroscopy (SFS) was described for the determination of DP in the presence of its hydrolytic degradation product and the co-formulated drug TD. In this method (SFS) was performed at Δλ of 70 nm in acetonitrile medium and the synchronous fluorescence intensities of TD were measured at 212 nm.

Method IIB, The first derivative synchronous fluorescence spectra FDSFS were applied for measuring the amplitudes of FDSFS at 295 nm and 242 nm for analysis of DP and TD, respectively.

The degradation products was obtained in acidic stress condition of 5 M hydrochloric acid, separated, and identified by IR and mass spectrometry to confirm its structure, and elucidate degradation pathway. The methods were applied for stability-indicating assay of DP (method I and II), VR (method I) and TD (method II) in bulk powders, laboratory prepared mixtures and co-formulated pharmaceutical preparations containing degradation product of DP. The results obtained were satisfactory compared with those obtained from the comparison methods and no significant differences were found. The two stability indicating methods were validated as per ICH guidelines.

Keywords: Stability-indicating spectroscopy methods; Degradation pathway; Dapoxetine; Vardenafil; Tadalafil

Introduction

Dapoxetine hydrochloride (DP); (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1-phenyl propan-1-amine hydrochloride, (Figure 1); is a short-acting selective serotonin reuptake inhibitor that is rapidly absorbed and eliminated in the body. It is one of the most potent drugs that are recently used for the treatment of premature ejaculation (PE) in men. It can be taken in combination with other PDE-5 inhibitors, since it doesn't show any pharmacokinetic interactions [1,2].

Vardenafil (VR); 4-[2-Ethoxy-5-(4-ethylpiperazin-1-yl) sulfonyl-phenyl]-9-methyl-7-propyl-3, 5, 6, 8-tetrazabicyclo[4.3.0]nona-3,7,9-trien-2-one, (Figure 1), and Tadalafil (TD); (6R-trans)-6-(1,3-benzodioxol-5-yl)-2, 3, 6, 7, 12, 12a-hexahydro-2-methyl-pyrazino [1', 2':1,6] pyrido[3,4-b]indole-1,4-dione, (Figure 1), are selective long-acting phosphodiesterase-5 inhibitors that are as well very effective in the treatment of erectile dysfunction. The maximum recommended dose is 20 mg per day for VR and TD [3-5].

Snovitra Super Power tablets and T-ject® tablets are novel prescription drugs that combine Dapoxetine hydrochloride DP with Vardenafil VRand DP with Tadalafil TD, respectively, to improve male sexual health. These combinations were introduced recently into the market as novel co-formulated drugs due to the synergistic effect of these drugs in the treatment of PE and ED [6,7]. The assay of the proposed drugs in their drug substances and co-formulated preparations are not officially recorded in any pharmacopoeia; therefore much more investigations are required for their analysis.

A literature survey revealed that the simultaneous determination of DP and VR (method I) was addressed in few methods. These involve the use of spectrophotometry, derivative synchronous fluorescence spectroscopy; thin layer chromatography and high performance liquid chromatography for analysis of the selected drugs in combined dosage form [8-13].

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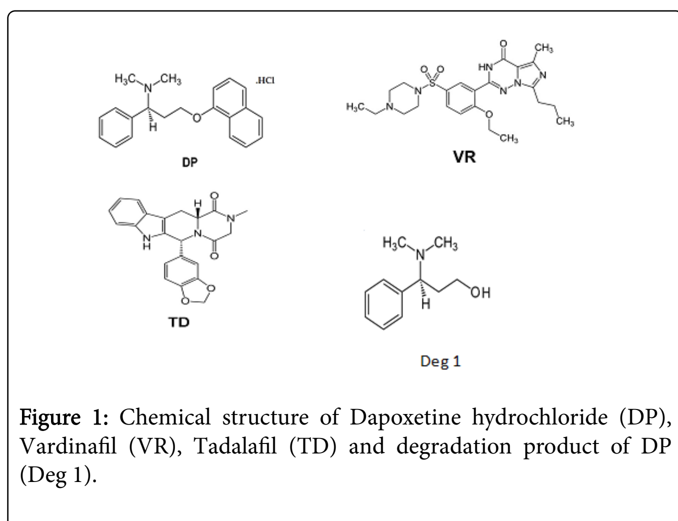

Figure 1: Chemical structure of Dapoxetine hydrochloride (DP), Vardinafil (VR), Tadalafil (TD) and degradation product of DP (Deg 1).

Few analytical procedures have been reported for the simultaneous determination of DP and TD (method II) in bulk powder and co-formulation. These procedures include spectrophotometry, synchronous fluorescence spectroscopy and high performance liquid chromatography [14-17].

For commercial samples containing DP alone, spectrophotometric methods are reported in the recent literature [18-20]. HPLC methods have also been reported for determination of DP enantiomer, process related impurities and metabolites in pharmaceutical preparations and/or biological samples [21-23].

Although there are reported stability-indicating HPLC methods for analysis of DP in bulk drug and formulation to the best of our knowledge, the two stability indicating 1D spectrophotometry and first derivative synchronous fluorescence spectroscopy methods FDSFS for determination of DP in the presence of its hydrolytic degradation product and its co-formulated drugs VR and TD have yet been developed before [24,25].

Up till now neither stability-indicating 1D and FDSF methods nor possible degradation mechanism has been reported for determination of DP in the presence of its hydrolytic degradation product and its co-formulated drugs VR and TD. The proposed methods able to prevent cross-interferences arising from absorption and/or emission by DP hydrolytic degradate and its co-formulated drugs VR and TD. Therefore; the aim of the present study is to develop simple, inexpensive and selective two stability-indicating 1D and FDSFs methods for analysis of the cited drugs in drug substances, laboratory prepared mixtures and co-formulated pharmaceutical preparations containing acidic degradation product of DP namely, (+)-N, N-dimethyl-1-phenyl-3-propanolamine (Deg 1) (Figure 1).

Experimental

Instrumentation

Spectrophotometer: A double beam PerkinElmer UV-visible spectrophotometer connected to a Perkin Elmer UV Win lab Explorer software. The spectral slit width was 2 nm, Δλ was 4 nm, scaling factor (CF) was 10 and scanning speed was 480 nm/min.

Spectrofluorometer: The fluorescence spectra and measurements were recorded using Hitachi F 7000 fluorescent; model 5J1-0004 connected with FL Solutions 2.1 Software program. It is equipped with a 150 watt Xenon arc lamp. Ex and Em slit was 10 nm. Scan speed was 12000 nm/min, Δλ was 70 nm and PMT Voltage was 400 V.

Additional instruments were used including: A thermostatic water bath (Memmert, Germany), a Rotavapor (Buchi, Switzerland), a sonicator (crest, New York), a digital pH meter (Hanna pH211, Romania), TLC aluminum sheets (20 cm × 20 cm, 0.2 mm), Silica gel F254 (Merck KGaA Darmstadt, Germany) and a UV lamp with a short wavelength of 254 nm (Desaga Germany).

Materials and methods

Authentic samples

Dapoxetine and Vardenafil standard materials were kindly supplied by Inspire Pharma, Lilly-Egypt. Their purity was found to be 99.31% ± 0.61% and 99.38% ± 1.54% for DP and VR, respectively, according to the reference HPLC method. The quantification of the two drugs was carried out on C18 column and a mobile phase consisting of buffer (KH_2PO_4): acetonitrile: triethylamine (55: 45: 0.1 v/v/v) at 266 nm [13].

Dapoxetine and Tadalafil standard materials were kindly supplied by Bayer medical companies-Egypt; the purity was found to be 99.22% ± 1.35 and 99.02 ± 1.02% for DP and TD, respectively, according to the reference HPLC methods. Determination of the two drugs was achieved on C18 column using a mobile phase consisting of acetonitrile: 0.1% triethyl amine in water pH 4.0 (80: 20) at 229 nm [16].

Market samples

Snovitra Super Power® tablets (batch No. 108002) labeled to contain 60 mg of DP base equivalent to 67.162 mg of Dapoxetine HCl and 20 mg of VR per tablet in a ratio of 3:1 was purchased from online pharmacy.

T-ject® tablets (batch No. 176030) labeled to contain 60 mg of DP and 20 mg of TD per tablet in a ratio of 3:1 was manufactured by Spier and purchased from online pharmacy.

Chemicals and solvents

HPLC grade acetonitrile was Lab-Scan (Poland), while methanol and ethanol were Fischer Chemical (UK).

Degradation of the sample

100 mg of DP refluxed with 100 ml of 5M HCL solution in a 250 ml round bottom flask for 30 h at 90°C. The sample tested for complete degradation by TLC using toluene-methanol (8.5:1.5 v/v) as a mobile phase and the developed plates were visualized at 254 nm under a UV lamp. Only one spot was observed not corresponding to DP. The degraded solution was then cooled and neutralized with 5 M NaOH until pH 7. The solution was evaporated on a water bath nearly to dryness, cooled and transferred into a 100 ml volumetric flask with methanol. The volume was completed to the mark with methanol and filtered. The degraded solution was used for the IR and mass spectral scan and for testing the two stability-indicating methods.

Standard and degradation product solutions

Stock standard solutions of DP and VR (Method I): Stock standard solutions of 1 mg/mL of each of DP and VR were prepared in methanol. Further dilution was prepared in methanol to obtain working standard solutions of (0.1 mg/mL) of each drug.

Stock standard solutions of DP and TD (Method IIA and IIB): Stock standard solutions of 0.1 mg/mL of each of DP and TD were prepared in acetonitrile. Further dilution was prepared in acetonitrile to obtain working standard solutions of (0.01 mg/mL) of each drug.

Stock degradation product solution (1 mg/ml) in methanol: The solution was prepared by refluxing 100 mg of DP with 100 ml of 5M HCl solution in 250 ml round bottom flask for 30 h at 90°C. The degraded solution was then cooled and neutralized with 5M Na OH until pH 7. The solution was evaporated on a water bath nearly to dryness, cooled and transferred into a 100 ml volumetric flask with methanol. The volume was completed to the mark with methanol and filtered.

Working degradation product solution (DP Deg I) 0.1 mg/ml in methanol for method I: It is prepared by transferring 10 ml degradation product stock solution (1 mg/ml) into a 100 ml volumetric flask and the volume was completed with methanol.

Working degradation product solution (DP Deg I) 0.01 mg/ml in methanol for method IIA and IIB: It is prepared by transferring 1 ml degradation product stock solution (1 mg/ml) into a 100 ml volumetric flask and the volume was completed with methanol.

General procedures

1D spectrophotometry (Method I): Linearity and construction of calibration curves: Aliquots equivalent to 2-120 µg/mL of DP and 5-120 µg/mL of VR standard solution (100 µg/ml) were transferred into two separate sets of 10 ml volumetric flasks. The volume was completed with methanol so that the final concentration is in the range of 0.2 µg/mL to 12 µg/mL and 0.5 µg/mL to 12 µg/mL for DP and VR, respectively.

Then 1D spectra of each sample solution was computed with scaling factor of 10 and Δλ of 4 nm. The peak amplitudes of 1D for both DP and VR at 240 nm and 227 nm were measured, respectively.

Linear calibration curves were constructed by plotting the peak amplitudes at 240 nm and 227 nm to the corresponding concentrations of DP and VR, respectively, and the regression equations were computed.

Laboratory-prepared mixtures contain different ratios of DP and VR in presence of hydrolytic degradation product of DP.

Aliquots (1.08 ml to 0.12 ml) of DP and co-formulated drug VR were accurately transferred from their working solutions (0. 1 mg/mL) equivalent to (108 µg/mL to 12 µg/mL) into a series of 10 mL volumetric flasks.

Aliquots (0.12 ml to 10.08 ml) of DP degradation product working solutions (0.1 mg/mL) equivalent to (12 µg/mL to 108 µg/mL) were added and then volumes were made up with methanol to prepare mixtures containing (10% to 90% w/w) of the degradation product. The procedure described under linearity was then followed and the percentage recoveries of the each drug were calculated from the corresponding regression equation.

Synchronous fluorescence spectroscopy (Method II): Method IIA- Linearity and construction of calibration curves: Aliquots equivalent to 0.1 µg/mL to 10 µg/mL of DP and 0.1 µg/mL to 7 µg/mL TD were transferred into two separate series of 10 ml volumetric flasks. The volume was then completed with acetonitrile so that the final concentration is in the range of (0.01 µg/mL to1 µg/mL) and (0.01 µg/mL to 0.7 µg/mL) for DP and TD, respectively.

The instrument was adjusted to synchronous mode by scanning both monochromators at a constant wavelength difference at Δλ of 70 nm, the normal synchronous spectra were recorded within the range of 200 nm-350 nm against acetonitrile as a blank. The synchronous fluorescence intensities of TD were measured at λem 212 nm (no interference from DP).

Linear calibration curves were constructed by plotting the normal synchronous fluorescence intensities of TD at 212 nm to the corresponding concentrations of TD and the regression equation was computed.

Linearity and construction of calibration curves: Method IIB: After following the procedures mentioned in method IIA, the first derivative synchronous fluorescence scan FDSFS of DP and its degradation and co-formulated drug TD were derived from the normal synchronous spectra using FI Solution software at Δλ 70 nm, and the fluorescence intensities of FDSFS were estimated at 295 nm and 242 nm both DP and TD, respectively. A blank experiment was performed simultaneously.

A Linear calibration graph was constructed relating the peak amplitudes of FDSFS at 295 nm and 242 nm to the corresponding concentrations of DP and TD, respectively, and the regression equations were computed.

Laboratory-prepared mixtures contain different ratios of DP and TD in presence of hydrolytic degradation product of DP.

Aliquots (0.63 ml to 0.07 ml) of DP and co-formulated drug TD were accurately transferred from their working solutions (0.01 mg/mL) equivalent to 6.3 µg/mL to 0.7 µg/mL into a series of 10 mL volumetric flasks.

Aliquots (0.07 ml to 0.63 ml) of DP degradation product working solutions (0. 01 mg/mL) equivalent to (0.7 µg/mL to 6.3 µg/mL) were added, and then volumes were made up with acetonitrile to prepare mixtures containing (10% to 90%) of the degradation product. The procedures described under linearity method (II A and IIB) were then followed the percentage recoveries of the each drug were calculated from the corresponding regression equation.

Application to pharmaceutical formulation

Ten tablets of Snovitra Super Power® tablets or T-ject® tablets were separately weighed and finely powdered. An accurately weighed amount of the powdered tablets equivalent to 60 mg of DP and 20 mg of VR (Method I) and equivalent to 60 mg of DP and 20 mg of TD (Method IIA and IIB) were transferred into two separate 100 ml volumetric flask. 50 ml methanol was added and the two solutions were sonicated by shaking in ultrasonic bath for about 30 min. The volumes was then completed to the mark with methanol and filtered. Suitable dilutions were made using methanol to prepare a tablet solutions containing 0.1 mg/ml (Method I) or 0.01 mg/ml (Method IIA and IIB). Aliquots of each tablet solution were diluted with the appropriate solvent to obtain final concentration within the working concentration ranges. The procedure previously described under each

method was followed and standard addition techniques were applied. The concentrations of standard added were calculated from the corresponding regression equation.

Results and Discussion

The stability of DP was studied according to ICH guidelines [26] for acid stress using 0.1M, 1M HCl for 10 h, 2M, 3M HCl for 10 h, 4 M HCl for 20 h and 5 M HCl for 20 h and 30 h.

The degradation processes were monitored by TLC using toluene-methanol (8.5:1.5 v/v) as a mobile phase. DP was found to be liable to acid degradation and appearance of only one spot after complete degradation indicates formation of a single component. The structure of the isolated acid degradation product of DP was confirmed by TLC and MS.

The IR spectrum of intact DP (Figure 2A) is characterized by C-H stretching aromatic at 3050 cm^{-1}, C=C aromatic at 1600 cm-1 and O-R ether function group at 1100 cm^{-1}. On the other hand, the IR spectrum of DP degradation product (Figure 2B) indicates a broad band of a hydrogen bond (appearance of -OH stretching) at 3426 cm^{-1}, C-H stretching aliphatic at 2924 cm^{-1} and the spectrum showed absence of the characteristic O-R ether function group present in the IR spectrum of the intact DP at 1100 cm^{-1}.

A mass spectrum of degraded DP (Figure 2C) shows the mass ion peak of $C_{11}H_{17}NO$ at (m/z 180.34). The other fragment was suggested to be naphthalene which is known to be volatile. These studies suggested the degradation pathway and confirm the structure of the hydrolytic degradation products of DP. The proposal of the reaction pathway of DAP with 5M HCl was presented in (Scheme 1).

1D spectrophotometry (Method I)

Derivative spectrophotometry [27,28] has proved to be usefulness and simple technique for quantitative analysis, characterization, and quality control of drugs in mixtures. This technique offers various advantages over the conventional methods such as the enhancement of the resolution of specific spectral interference from degradation products, co-formulated drugs, and the elimination of interference from the non-specific absorption from other formulation components.

In Method A, the recorded zero order absorption spectra (0D) of DP and VR showed overlapped spectra in the range of 200 nm-350 nm, as described in (Figure 3). DP is liable to degradation under acidic condition. The hydrolytic degradation product that was formed exhibited a detectable absorbance and also shows an interfering spectrum with DP and VR. This observation prevents the use of direct analysis of DP in presence of its degradation and co-formulated drug VR, as shown in (Figure 3). Therefore, the first derivative method 1D was applied to resolve this overlapping. Upon examining the 1D spectra of DP, its degradation and the co-formulated drug VR, it was observed that DP and VR were found to exhibit zero crossing point at the maxima of the selected drug and at zero crossing point of the interfering one, where the hydrolyzed degradation has no contribution and exhibits zero reading.

DP can be a determined at λ 240 nm (Figure 4) in presence of its acidic degradation and the co-formulated VR, also VR could be analyzed at λ 227 nm (Figure 5) in presence of DP and its acidic degradation.

Linearities were obtained between the values of peak amplitudes of 1D at specified wavelengths and the corresponding concentrations of the drugs in the range of 0.2 to 12 and 0.5 µg/mL- to 12 µg/mL-1for DP and VR in methanol, respectively.

The proposed stability-indicating 1D method was successfully applied for determination of DP and VR in drug substances in presence of DP degradation product up to 90% without interference with mean recoveries of 99.90 ± 0.98% and 99.83 ± 0.94%, respectively, as shown in Table 1.

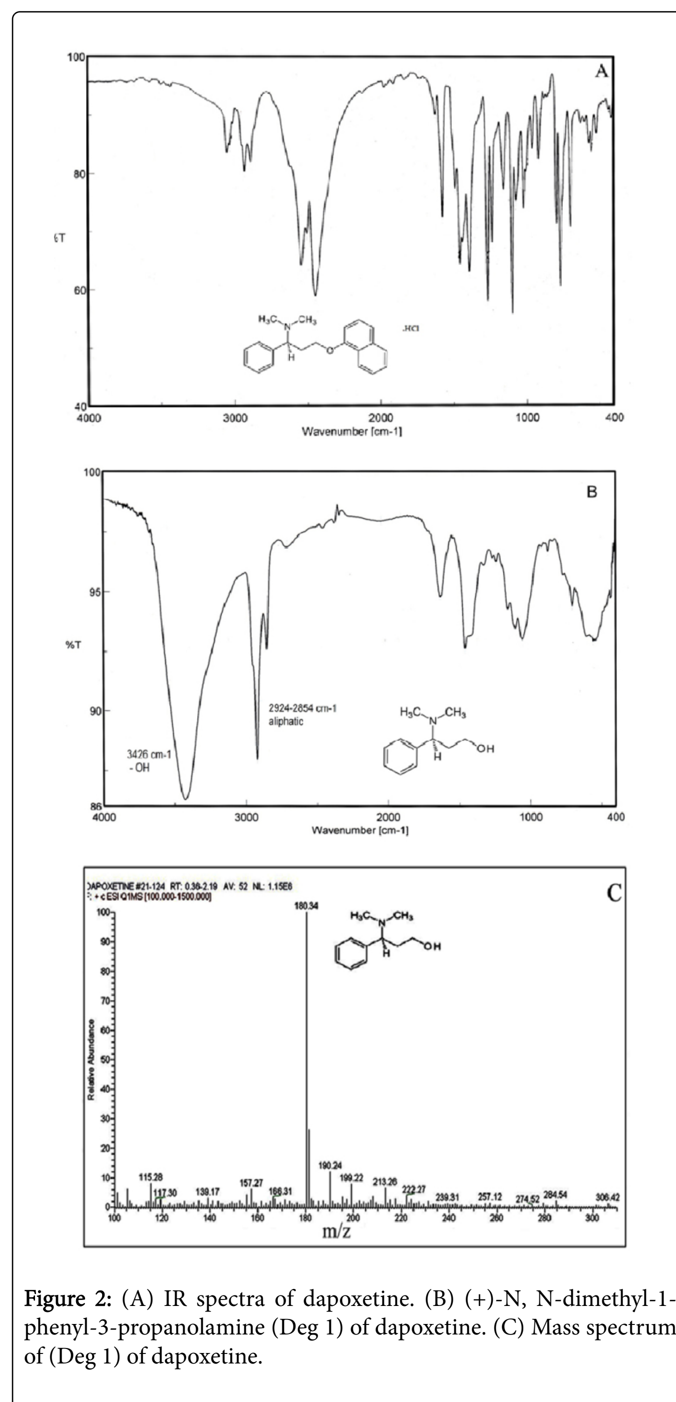

Figure 2: (A) IR spectra of dapoxetine. (B) (+)-N, N-dimethyl-1-phenyl-3-propanolamine (Deg 1) of dapoxetine. (C) Mass spectrum of (Deg 1) of dapoxetine.

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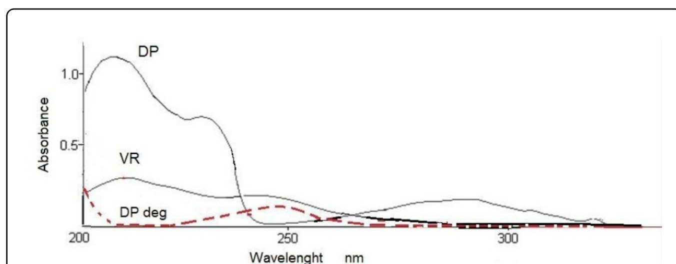


Figure 3: Zero order absorption spectra of DP and its degradation product (9 µg/ml each) and VR (3 µg/ml) in methanol.

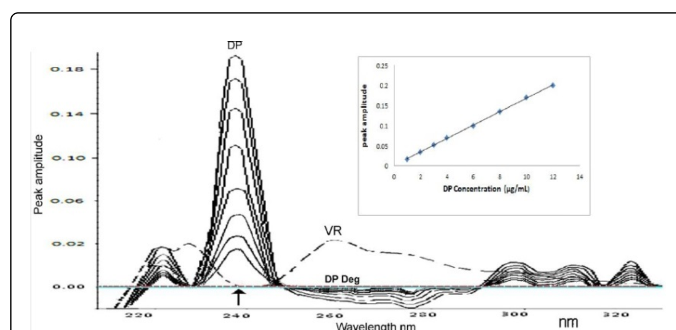


Figure 4: Linearity of the first derivative spectra of DP (0.2-12 µg/mL) at λ 240 nm, its degradation product and VR (12 µg/mL each) in methanol.

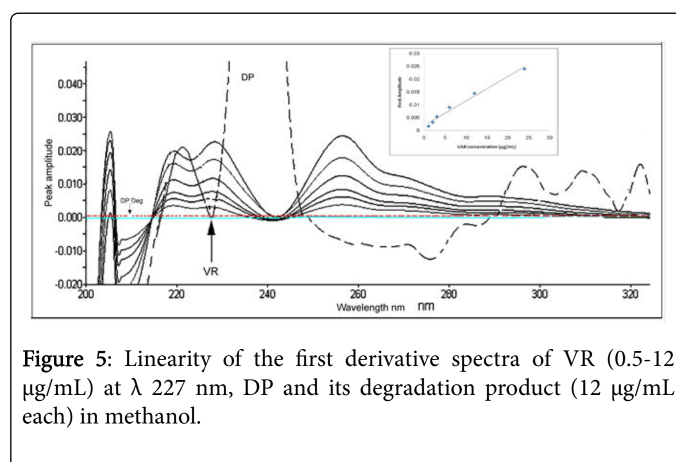


Figure 5: Linearity of the first derivative spectra of VR (0.5-12 µg/mL) at λ 227 nm, DP and its degradation product (12 µg/mL each) in methanol.

Synchronous fluorescence spectroscopy

The excitation and emission spectra of DP in acetonitrile were recorded in the range of 200 nm-500 nm as shown in (Figure 6), it exhibit maximum emission at 366 nm when excited at 235 nm and 299 nm. While, a similar concentration of DP degradate solution has no fluorescence emission at the same excitation wavelength. TD also showed native fluorescence in acetonitrile solution, it exhibit maximum emission at λ em 360 nm after an excitation maximum at 290 nm. Both the excitation and emission spectra of DP and TD are greatly overlapped (Figure 6). Therefore, the SFS technique was proposed for analysis of DP, TD in presence of degradation product of DP.

A synchronous fluorescence spectroscopy SFS has become a well-established technique for the determination of drugs in mixtures and combined dosage forms due to the considerable advantages of spectral simplification, light scattering reduction, and selectivity improvement over spectrophotometry and conventional fluorometry [29]. The selectivity and resolution are further enhanced by the combination of SFS approaches with derivative technique (DSFS) [30].

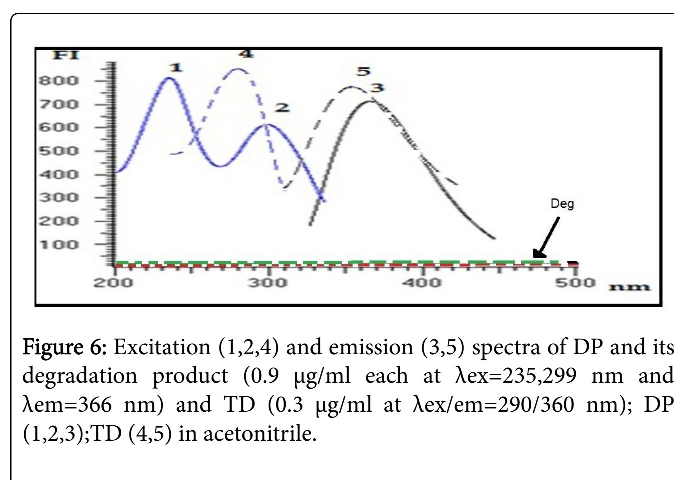
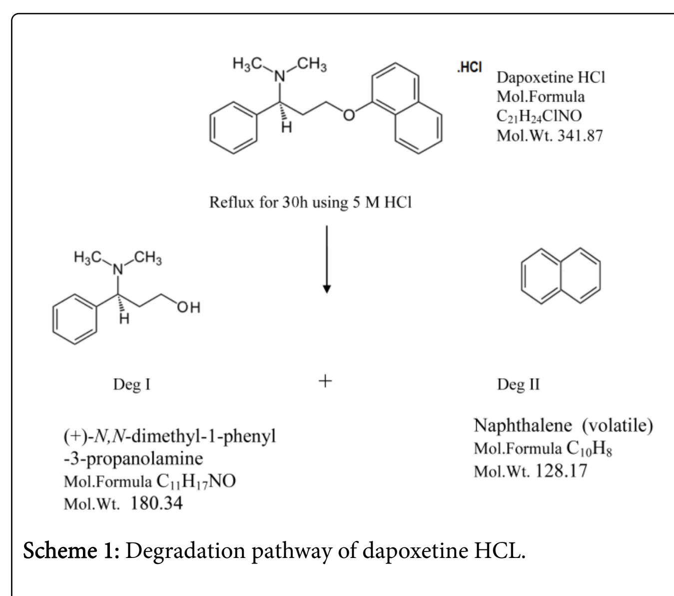


Figure 6: Excitation (1,2,4) and emission (3,5) spectra of DP and its degradation product (0.9 µg/ml each at λex=235,299 nm and λem=366 nm) and TD (0.3 µg/ml at λex/em=290/360 nm); DP (1,2,3);TD (4,5) in acetonitrile.



Scheme 1: Degradation pathway of dapoxetine HCL.

The proposed stability-indicating SFS (Method II) were successfully applied for determination of TD (Method IIA) and analysis of DP and

Parameter	1D Spectrophotometry		Spectrofluorometry methods		
	DP at 238 nm	VR at 227 nm	SFS	FDSFS	
			TD at 212 nm	DP at 295 nm	TD at 242 nm
Rang µg/mL	0.2-12	0.5 – 12	0.01-0.7	0.01-1.0	0.01-0.7
Slope	0.016	0.0001	2.4 × 10-3	3.6 × 10-3	17.6 × 10-3
SD of slope	0.002	0.00007	0.0001	0.007	0.009
Intercept	0.012	0.002	0.166 × 10-3	0.97 × 10-3	1.21 × 10-3
SD of intercept	0.003	0.00001	0.0014	0.003	0.009
(r)[a]	0.9991	0.9989	0.9989	0.9991	0.9975
LOD[b]	0.07	0.18	1.9 × 10-3	3.0 × 10-3	1.7 × 10-3
LOQ[b]	0.2	0.5	5.8 × 10-3	8.3 × 10-3	5.1×10-3
Accuracy[c]	99.90 ± 0.98	99.83 ± 0.94	98.54 ± 0.52	99.70 ± 0.98	99.00 ± 0.62
Selectivity [d]	98.93 ± 0.65	99.28 ± 0.71	99.61 ± 0.96	99.47 ± 0.34	99.22 ± 0.80
Precision RSD%					
Intra-day[e]	0.82	1.1	0.63	0.49	0.64
Inter-day[f]	1.25	1.25	0.7	0.53	0.7

Table 1: Assay validation report of the proposed methods for the simultaneous determination of DP, VR and DP, TD in their drug substances. [a]Correlation coefficient,[b]LOD=3.3 SD/slope, [b]LOQ=10 SD/slope. [c]Accuracy (mean ± SD) average of five determinations. [d]Selectivity (mean ± SD) for the laboratory prepared mixtures. [e]Repeatability (n=3), RSD of three different concentrations of the cited drugs repeated three times within the day. [f]The intermediate precision (n=3), RSD of the same three concentrations of the cited drugs repeated three times in three successive days.

Method IIA: The SFS was employed for analysis of TD using Δλ of 70 nm (no interference from DP) in the presence of DP and its hydrolytic degradation product in drug substances and co-formulated tablets. The SFS technique depends upon the native fluorescence of both DP and VR observed in acetonitrile due to the presence of conjugated systems in their fused aromatic rings and the absence of fluorescence of DP degradation product solution (Scheme 1). Different concentrations of DP (0.01 µg/ml to 1 µg/ml) at 280 nm in presence of its degradation product of DP (1 µg/ml) and TD (0.3 µg/ml) were represented, where no complete spectral resolution was observed for DP (Figure 7). On the other hand, TD in concentration range (0.01 µg/ml-0.7 µg/ml) was determined using SFS technique in presence of DP and its degradation product (1 µg/ml each) and quantified at 212 nm without any interference of DP or its degradation product as given in (Figure 8).

TD (Method IIB) in bulk powder in the presence of DP degradation product up to 90% without interference with mean recoveries of 98.54 ± 0.52%, 99.70 ± 0.98% and 99.00 ± 0.62%, respectively, as shown in Table 1.

Optimization of the proposed experimental conditions

To optimize the proposed 1D method it was necessary to study the influence of different variables; for example, the effect of different smoothing factors (Δλ at 2, 4, and 8) values and scaling factor (CF) values (10, 100 and 1000) were studied. A smoothing factor at Δλ=4 and CF=10 were selected proved good spectral resolution of DP and VR.

The effect of different solvents (Figures 11 and 12) was examined including bi-distilled water, methanol, ethanol and acetonitrile. It was observed that methanol gave the highest absorbance values of DP and VR in (Method I) (Figure 11). A significant hyperchromic effect was observed in acetonitrile which gave the highest FI and the lowest blank reading for DP and TD (Figure 12).

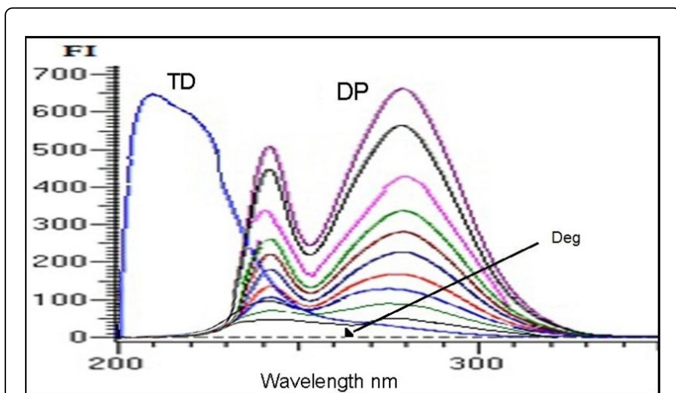


Figure 7: Linearity of synchronous fluorescence spectra of DP (0.01-1 μg/ml), its degradation product (1 μg/ml) at 280 nm and TD (0.3 μg/ml) in acetonitrile.

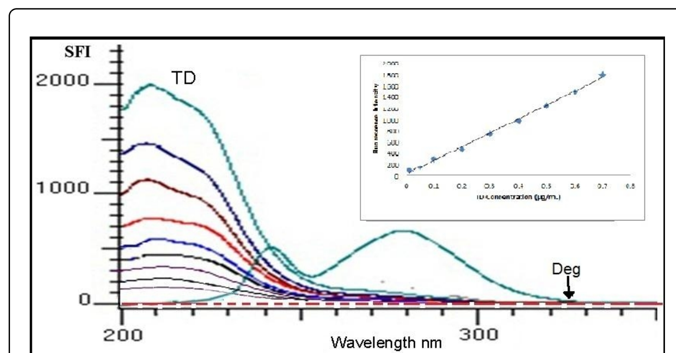


Figure 8: Linearity of synchronous fluorescence spectra of TD (0.01 μg/ml-0.7 μg/ml) at 212 nm, DP and its degradation product (1 μg/ml each) in acetonitrile.

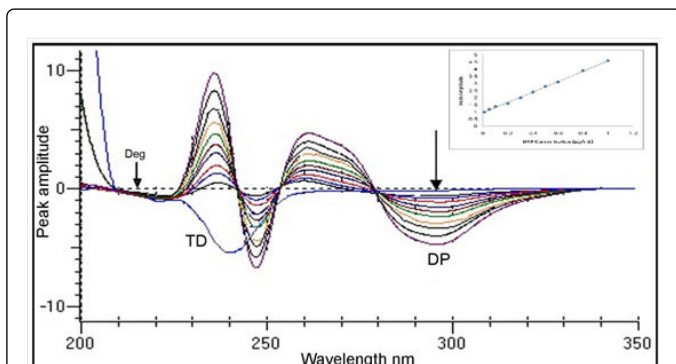


Figure 9: Linearity of the first derivative synchronous fluorescence spectra FDSFS of DP (0.01-1 μg/ml) at 295 nm, its degradation product (1 μg/ml), and TD (0.3 μg/ml) in acetonitrile.

Method IIB: FDSFS using zero-crossing technique was performed, which resulted in complete spectral resolution and enabled determination of DP and its co-formulated drug TD in the presence of DP degradation at Δλ of 70 nm in acetonitrile. Linearities were obtained between the values of amplitudes of FDSFS at 295 nm and 242 nm and the corresponding concentrations of the cited drugs in concentration ranges of (0.01 μg/ml to 1 μg/ml) and (0.01 μg/ml to 0.7 μg/ml) for determination of DP and TD, respectively, as shown in (Figures 9 and 10).

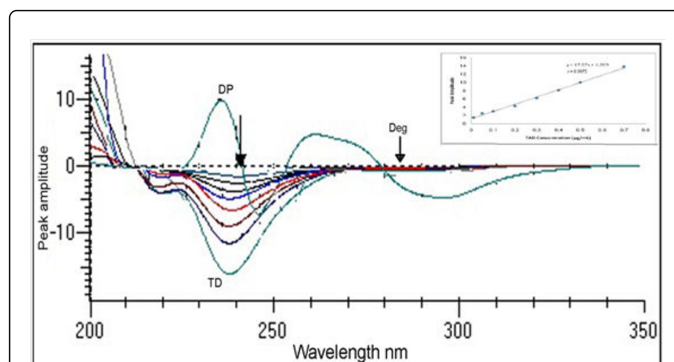


Figure 10: Linearity of the first derivative synchronous fluorescence spectra FDSFS of TD (0.01-0.7 μg/ml) at 242 nm, DP and its degradation product (1 μg/ml) in acetonitrile.

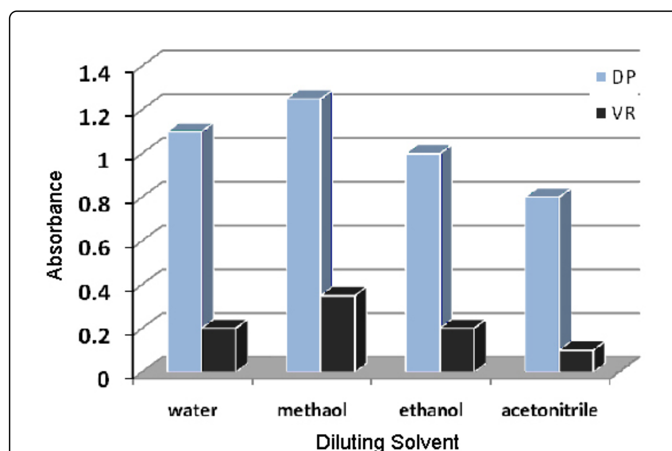


Figure 11: Effect of diluting solvent on the absorbance of DP, its degradation product (9 μg/mL each) and VR (3 μg/mL) in methanol.

The main important principle stated by the green chemistry is to use safer solvents that generate less hazardous waste, less harmful to humans and to the environment [31,32]. Hence methanol and acetonitrile were selected as diluting solvents for the proposed methods.

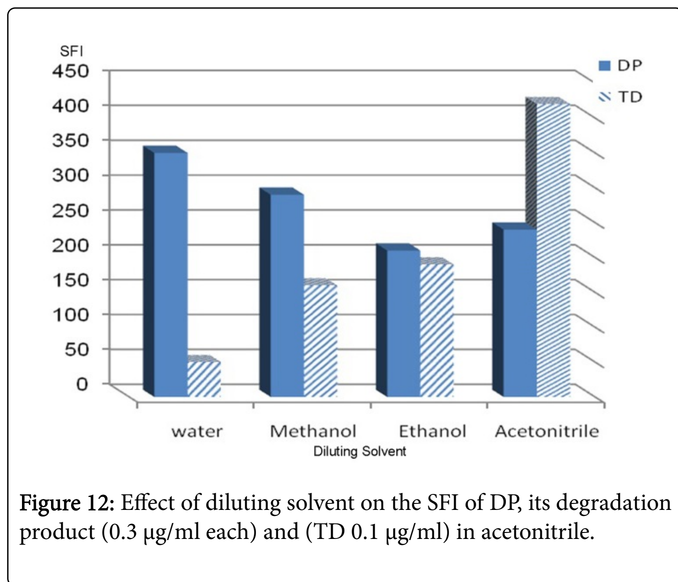


Figure 12: Effect of diluting solvent on the SFI of DP, its degradation product (0.3 µg/ml each) and (TD 0.1 µg/ml) in acetonitrile.

In method II, Different experimental and instrumental parameters affecting the performance of SFS were carefully studied and optimized. They include scanning range, the effect of optimal Δλ on SFS, excitation and emission band width and diluting solvents.

The synchronous wavelength range of 200 to 700 was scanned while changing (Δλ). The effect of different values of Δλ on the resolution of the emission spectra of both DP and TD from (Δλ of 10 to 100 nm) was examined, and the observation indicates Δλ of 70 nm gave the best result for resolution of both drugs, since it reduced the spectral interference caused by each compound in the mixture (Figures 9 and 10). Various excitation and emission band width were tested (5, 10 and 20 nm) and fixed at 5 nm, where it provides high fluorescence intensities.

In addition, the effect of time on stability of the fluorescence intensity was also studied and the result indicates that the intensity of the developed fluorescence was stable up to 2 h.

Method Validation

Validation of the proposed methods was assessed as per ICH guidelines [33].

Linearity: The linearity of the proposed methods was evaluated by triplicate analysis of the same concentration range as the corresponding calibration graph. The characteristic parameters of the regression equations are stated in (Table 1).

Repeatability and intermediate precision: Repeatability as intra-day precision was studied by analysing freshly prepared solutions of concentrations (0.5 µg/ml, 5 µg/ml and 12 µg/ml) of DP and VR (method A), (0.01 µg/ml, 0.3 µg/ml and 0.7 µg/ml) of TD (SFS method B) and (0.05, 0.1 and 0.7 µg/ml) of each of DP and TD (FDSFS method B) in triplicate within the same day. The inter-day precision was studied by analysis of the same concentrations in triplicate for three successive days to determine variation observed from the proposed methods, which was expressed as% RSD. The results are listed in (Table 1).

Detection and quantification limits: The sensitivity of the proposed method was determined with respect to LOD and LOQ. The lowest detectable amount of the selected drugs that could be detected and quantified was calculated and the results were listed in (Table 1).

Accuracy: Accuracy studies were evaluated by assaying freshly prepared solutions of the cited drugs prepared at each recovery level and analyzed versus standard solutions. The percentage mean recovery values ranged from 98.54 ± 0.52% to 99.90 ± 0.98%, indicating accurate results as summarized in (Table 1).

Also the Accuracy of the methods for assay of the cited drugs in tablets was tested by applying the standard addition technique. The obtained results indicate good recovery (Table 2).

Specificity: The specificity of the proposed methods was evaluated by analysis of laboratory-prepared mixtures containing different percentages of the selected drugs and different percentages of the DP degradation product. The results were recorded in (Table 3).

1D spectrophotometry Method I						FDSFS Method IIB					
Claimed taken (µg/mL)		Added µg/mL		Recovery of standard added% [a]		Claimed taken (µg/mL)		Added µg/mL		Recovery of standard added% a	
DP	VR	DP	VR	DP	VR	DP	TD	DP	TD	DP	TD
3	1	4	1	99.46	98.43	0.3	0.1	0.1	0.1	98.3	99.33
3	1	5	2	101.2	99.27	0.3	0.1	0.2	0.2	98.64	98.68
3	1	6	3	98.66	100.7	0.3	0.1	0.3	0.3	99.87	99.42
3	1	8	6	99.51	99.19	0.3	0.1	0.4	0.4	98.53	100.6
Mean				99.71	99.4					98.84	99.51
± RSD				1.07	0.95					0.7	0.8

[a]Average of three determinations

Table 2: Results of determination of DP, VR (Method I) and DP, TD by the proposed methods and application of standard addition technique.

Citation: Soliman SM, El-Azizy HMY, El Bayomi AEA (2017) Validated Stability-Indicating Derivative Spectrophotometry and Synchronous Fluorescence Spectroscopy Methods for the Determination of Dapoxetine Hydrochloride in the Presence of its Degradation Product and Co-Formulated Drugs. Pharm Anal Acta 8: 564. doi:10.4172/2153-2435.1000564

Recovery[a] of intact drug%					
Degradation% (w/w)of DP	**Method I**		**Method II**		
			SFS IIA	**FDSFS IIB**	
	DP at 238 nm	**VR at 227 nm**	**TD at 212 nm**	**DP at 295 nm**	**TD at 242 nm**
10	98.13	100.8	100.66	99.41	100.99
30	98.52	100.5	98.92	99.9	99.5
50	100.3	99.7	101.41	99.39	99.88
70	99.34	101.3	99.75	100.3	100.33
80	99.3	99.9	99.4	100.8	98.75
90	99.22	100.55	98.42	100.1	100.76
Meana ± RSD%	99.35 ± 0.73	100.63 ± 064	99.77 ± 1.10	99.70 ± 0.98	100.03 ± 0.84
[a]Average of three determinations.					

Table 3: Specificity results of the proposed methods for determination of DP, VR (Method I), TD (Method IIA) and DP, TD (Method IIB) in presence of degradation product of DP (10-90 w/w) in laboratory prepared mixtures.

In method I, a stability indicating 1D was specific for analysis DP and VR in presence of up to 90% of DP degradation product at 240 nm and 227 nm, respectively.

In method IIA, SFS was specific for assay of DP in presence of up to 90% of its hydrolytic degradation product and up to 10% of co-formulated drug TD at 280 nm. SFS was specific for assay of TD in presence of DP its degradation product up to 90% at 212 nm.

In method IIB, FDSFS was specific for determination of DP and TD in presence of up to 90% of degradation product of DP at 295 nm and 242 nm, respectively (Table 3).

Stability: In the present study, methanol and acetonitrile were appropriate solvents for preparing standard stock solutions and laboratory-prepared mixtures solutions. The stability of DP or VR standard solutions in methanol and DP or TD standard solutions in acetonitrile were determined. The RSD% values of analysis were below 2%which indicate stability of the selected drugs in the stock solutions for 3 days without significant changes.

Application of the proposed methods to pharmaceutical preparations: The usefulness of proposed methods to the analysis of DP and VR as well as DP and TD in their co-formulated preparations was studied. No interfering spectra were observed from any of the inactive ingredients. The direct assay of the cited drugs in their co-formulated tablets without interference from the excipients proves the simplicity and selectivity of the proposed methods as shown in (Table 4).

The proposed stability-indicating methods have also been employed to assay DP and VR in Snovitra Super Power® expired tablets and also to quantify DP and TD in T-ject® expired tablets in which minor degradations were occurred. The results were recorded in Table 4.

Statistical analysis: Recovery data obtained by the suggested methods for determination of drug substances were statistically compared to those obtained by the reference ones [13,16] the calculated t-and F-tests values did not exceed the theoretical ones, indicating no significant differences with respect to accuracy and precision as given in (Table 5).

Conclusion

The developed 1D spectrophotometry and FDSFS methods are simple, less expensive, time saving methods and environmentally friendly in comparison to the other published ones. They allow analysis of DP, VR and TD in drug substances, laboratory prepared mixtures and their co-formulated tablets without interference from excipients or DP hydrolytic degradation product up to 90%, so they could be applied in QC laboratories for routine analysis. The acidic degradation products were defined as (+)-N, N-dimethyl-1-pheny l-3-propanolamine (Deg 1) and Naphthalene (Deg 2), confirmed by IR and Mass analysis.

The proposed 1D spectrophotometry (Method I)						
Product	**Concentration taken µg/mL**		**Concentration found µg/mL**		**Recovery[a]%**	
Snovitra Super Power® tablet Batch No# 108002 60 mg DP/20 mg VR/ tablet	DP	VR	DP	VR	DP	VR
	12	4	11.917	3.942	99.31	98.56
	9	3	8.971	2.962	99.68	98.72
	6	2	6.036	1.989	100.6	99.46
Mean ± RSD Snovitra Super Power® tablet expired batch Batch No# 080177					99.86 ± 0.66	98.91 ± 0.48
					98.45 ± 0.26	98.70 ± 0.18
The proposed FDSFS (Method IIB)						
T-ject ® tablet Batch No# 176030 60 mgDP/20 mg TD/tablet	**Concentration taken µg/mL**		**Concentration found µg/mL**		**Recovery[a]%**	
	DP	TD	DP	TD	DP	TD
	0.3	0.1	0.2976	0.1001	99.2	100.1
	0.6	0.2	0.5917	0.1986	98.61	99.31
	0.9	0.3	0.8929	0.2977	99.21	99.24
Mean ± RSD T-ject ® tablet expired batch Batch No#07081					99.01 ± 0.34	99.55 ± 0.48
					98.52 ± 0.20	97.11 ± 0.14
[a]Average of three determinations						

Table 4: Results obtained for the determination of DP, VR (Method I) and DP, TD (Method IIB) in their co-formulated tablets by the proposed methods.

Items	**[1]D spectrophotometry**		**Reported method[a]**		**SFS**	**DSFS**		**Reported method[b]**	
	Method I				**Method IIA**	**Method IIB**			
	DP	VR	DP	VR	TD	DP	TD	DP	TD
Mean	99.9	99.83	99.31	99.38	98.54	99.7	99	99.22	99.02
SD	0.98	0.94	0.61	1.54	0.52	0.98	0.62	1.35	1.02
Variance	0.96	0.88	0.37	2.37	0.27	0.96	0.38	1.82	1.04
n	5	5	5	5	5	5	5	5	5
t-test[c]	1.14	0.56			0.94	0.64	0.04		
	2.306	2.306			2.306	2.306	2.306		
F-test[c]	2.58	2.68			3.85	1.9	2.71		
	6.388	6.388			6.388	6.388	6.388		

[a]The quantification was carried out on C18 column and a mobile phase consisting of buffer (KH_2PO_4): acetonitrile: triethylamine (55: 45: 0.1 v/v/v) at 266 nm (13).

[b]Determination s was achieved on C18 column with mobile phase consisting of acetonitrile: 0.1 % triethyl amine in water pH 4.0 (80: 20) at 229 nm(16). .

[c]The values in the parenthesis are the corresponding theoretical values of t and F at (P = 0.05).

Table 5: Statistical analysis of the results obtained by the proposed methods and the reported ones for the determination of DP, VR (Method I), TD (Method IIA) and DP, TD (Method IIB) in their pure forms.

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