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Stability Indicating 1st Derivative Synchronous Spectrofluorimetric Method for the Determination of the Newly Approved Antiviral Drug Daclatasvir in Presence of Its Oxidative and Photolytic Degradation Products: Application to Tablet Dosage Form

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Abstract

A highly sensitive, simple and rapid first derivative synchronous spectrofluorimetric method was utilized for the determination of daclatasvir dihydrochloride (DCV) in presence of its oxidative and photolytic degradation products. Where synchronous 1st derivative spectrofluorimetric approach was utilized to quantitatively determine DCV at 373 nm in presence of its oxidative degradation product and at 388 nm in presence of its photolytic degradation product that is obtained by exposing DCV to UV light at 312 nm, these were the zero-crossing wavelengths of degradation products without interference. The synchronous fluorescence was scanned at Δ λ of 80 nm. The method was found to be linear across the concentration range of 0.5-5.0 ng/mL with lower detection limit of 0.090 and lower quantification limit of 0.275 ng/mL (at 373 nm) and 0.268 ng/mL (at 388 nm). The adopted approach was successfully applied to commercial tablet and the results exhibited that the derivative synchronous fluorescence spectroscopy is a stability-indicating method, suitable for routine use within a short analysis time. The proposed method was carefully validated for linearity, accuracy, precision, specificity and robustness.

Keywords: *Angelica sinensis*; *Glycyrrhiza uralensis*; *Rhodiola rosea*; Chemometrics; UPLC; NO scavenging; PCA

Introduction

Chemically, Daclatasvir (DCV) is methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl] pyrrolidin-2-yl]-1H-imidazol-5-yl] phenyl] phenyl]-1H-imidazol-2-yl] pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl] carbamate [1] (Figure 1).

DCV is a recently approved direct acting hepatitis C virus NS5A inhibitor. It acts as a P-glycoprotein inhibitor, organic anion transporting polypeptide 1B1 and 1B3 inhibitor, and breast cancer resistance protein inhibitor [2].

Daclatasvir is available as an oral antiviral agent that inhibits the NS5A region of the hepatitis C virus (HCV). It is concomitantly administered with other oral antiviral agents for treatment of chronic hepatitis C [3].

DCV was approved by the British Pharmacopoeia (BAN 2017) [4]. Since the drug is newly approved, the review of its literature did not reveal plenty of analytical methods that were reported up to date. These reported analytical methods involved LC [5-11], liquid chromatography coupled to mass spectrometry [12-16], HPLC / DAD [17], a chiral HPLC method [18], a single electrochemical method [19] and spectrophotometric methods [20-22]. So far there is only a single recently reported spectrofluorimetric analysis method for determination of DCV [23].

Some of the previously cited methods were concerned with the study of the degradation behavior of DCV under different stress conditions [5,11,17,22].

Of these studies, some reported that DCV was only liable to alkaline degradation [5]. Kekan et al. [22] and Baker et al. [17] declared that DCV was liable to photolytic, alkaline and oxidative degradation, meanwhile, it was stable under thermal, acidic and neutral conditions. The recently published stability method [11] highlighted that the forced degradation results showed in different previous studies were discrepant with each other, and confirmed that DCV was susceptible to alkaline, oxidative and photolytic degradation. Those finding were in good agreement with those of the European Medicines Agency (EMA) report of DCV. The EMA reported that DCV is susceptible to degradation at basic conditions and at high intensity UV and visible light. DCV shows minor degradation under oxidative conditions [24].

Spectrofluorimetric strategies have been discovered more selective than typical UV-spectroscopy because of measurement of substance at excitation and emission wavelengths [25,26]. Derivative spectrofluorimetry gives a more prominent selectivity than normal spectrofluorimetry [27-29]. It is the great approach for determination of one analyte whose peak is extensively overlapped by another analyte in multi-component analysis. Synchronous fluorescence spectroscopy (SFS) has been found to have a few points of interest, for example, sharp spectra, high selectivity and low interference [30,31]. The blend of synchronous spectrofluorimetry with derivative technique is favorable regarding sensitivity, spectral discrimination and more dependable distinguishing proof of certain species in multi component analysis [32,33].

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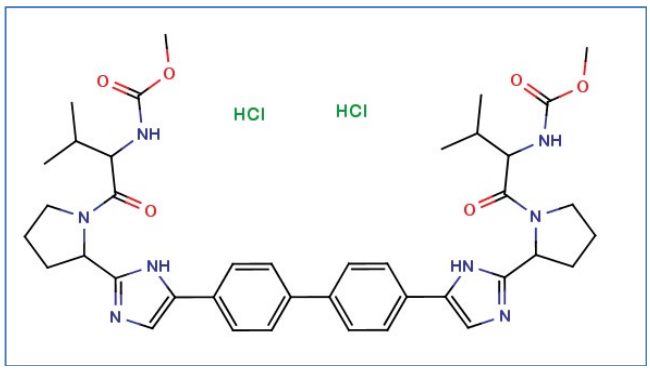

Figure 1: The structural formulae of DCV.

Therefore, the aim of the present work was to develop a highly sensitive, economic 1st derivative synchronous spectrofluorimetric methods for the determination of DCV in presence of its oxidative and photolytic degradation products. Moreover, the method was further applied for the analysis of DCV in its tablet dosage form. The degradation behavior of DCV was carefully studied under different ICH induced stress condition, in an attempt to resolve the drug from its degradation product.

Experimental

Apparatus

A FP-8200 JASCO Spectrofluorophotometer (Japan) with a Xenon arc lamp was used with the excitation and emission slits widths was set at 10 mm. A 1-cm quartz cell was used for all measurements. Fluorescence data manager software was used to obtain the derivative spectra. JASCO Spectra Manager ™ CFR, for FP-800 series, Copyright 2011, JASCO, Tokyo, Japan. UV-1800 Shimadzu, UV spectrophotometer, Shimadzu corporation, Kyoto, Japan, was utilized in applying comparison method.

Seven2Go S2. -Basic; pH/mV portable meter, Mettler Toledo (USA), was used for pH measurements. VILBER LOURMAT UV-lamp, CN-15. MC, dual wavelength 312/254 nm, Size LxWxH [mm]: 505 x 415 x 280(France) was used in the UV-degradation study. An ultrasonic bath, model Branson 2800 was utilized as well. A&D GR300 analytical balance.

Materials and reagents

All chemicals were of analytical grade, solvents were of HPLC grade and deionized water was used throughout the work.

Daclatasvir dihydrochloride was kindly provided by BDR Life Sciences. Pvt. Ltd, Vadodara, Gujarat, India. Batch No. DCZN160028. The purity percentage of DCV was certified to be 99.3 (% W/W) . The purity was established by applying HPLC method on anhydrous basis. Javidacla film coated tablets , labeled to contain 60 mg DCV /tablet, Product of Future Pharmaceutical Industries for Multicare Egypt for Pharmaceutical Industries. It was purchased from the local pharmacy. Sodium dodecyl sulphate and sodium acetate trihydrate, were obtained from Lobachemie, Mumbai, India. Methanol, acetonitrile, acetone and n-propanol were obtained from Sigma- Aldrich (Germany). Glacial acetic acid was obtained from BDH laboratory supplies. England. Hydrochloric acid (product of Sigma-Aldrich, Austria). NaOH pellets and boric acid were obtained from central drug house (CDH), New Delhi, India. Tween- 80, methyl cellulose, borax were all obtained from Assagaf pharma, Saudi Arabia. Acetate buffer (0.2 mol/L, pH 3.5–5.5) and borate buffer (0.2 mol/L, pH (6.0–8.7) solutions were freshly prepared. SDS, methylcellulose and Tween-80 were prepared as 1.0 % w/v aqueous solutions.

Pharmaceutical formulations

Pharmaceutical preparation containing the studied drug were purchased from commercial sources in the local pharmacy.

Javidacla® Film coated tablets (Batch no# 160404A) labeled to contain 60 mg daclatasvir (DCV) per tablet. It was manufactured by Future Pharmaceutical industries for Multicare Egypt for Pharmaceutical industries, Egypt.

Standard solutions

Stock solution of concentration 100.0 µg/mL of DCV was obtained by dissolving 10 mg of pure drug powder in 100 mL of methanol utilizing an ultrasonic bath. Working standard solution of 100 ng/mL was obtained through appropriate dilution of the stock solution with deionized water. All solutions were stored in the refrigerator and were found to be stable for at least 7 days without alteration.

Construction of calibration graph: Accurately measured aliquots of the working standard solutions of the drug were transferred into a series of 10 mL volumetric flasks to obtain a final concentration in the range of 0.5- 5.0 ng/mL of DCV. The volumes were completed by adding deionized water. The synchronous fluorescence spectra of the solutions were derived by scanning at Δ λ=80 nm for oxidative and photolytic degradation, and first derivative synchronous fluorescence spectra were recorded. The peak amplitudes of the first (1D) derivative spectra were recorded at 373 nm and 388 nm, respectively. A blank experiment was performed simultaneously. The peak amplitude of the first (1D) derivative technique was then plotted against the final concentration of the drug in ng/mL to get the calibration graphs.

Procedures for tablets: An accurately weighed amount of the mixed contents of 10 powdered tablets equivalent to 10.0 mg of DCV was transferred into a 100 mL volumetric flask and about 30.0 mL of methanol were added. The contents of the flask were sonicated for 30 min, completed to the mark with the same solvent and filtered through cellulose acetate syringe filter. Flasks were then diluted to volume with deionized water to get working standard solution for assay by applying the general procedure as described under "construction of calibration graph". The nominal content was calculated utilizing a previously plotted calibration graph or the corresponding regression equation.

Preparation of the degradation products: To conduct the degradation studies, working solution of concentration 1 µg/mL was prepared by suitable dilution of the stock solution with deionized water. Volumes of 2.5 mL of this solution (equivalent to 2.5 µg) were then transferred into series of small tubes for alkaline, oxidative and photolytic degradation, where the following procedures were adapted:

- **For alkaline degradation**: Aliquots of 5 mL of 1 M NaOH were added to the tubes. The solutions were heated under reflux in a boiling water bath for 10 min. At the specified time, the contents of each tube were removed from the water bath, cooled and neutralized to pH 7.0. The solutions were then quantitatively transferred into a series of 25 mL volumetric flasks. The volumes were adjusted with deionized water. Aliquots of these solutions (0.4 mL) were transferred into a series of 10 mL volumetric flasks and made to volume with deionized water. The procedure under "construction of calibration graphs" for the first method was then conducted.

- **For oxidative degradation**: Five milliliters of 30 % H_2O_2 were added to each tube. The solutions were then heated in a thermostatically controlled water bath at 80°C for 10 min. At the specified time intervals, the contents of each tube were cooled and quantitatively transferred to

25 mL volumetric flask. The volumes were completed to the mark with deionized water. Aliquots of these solutions (0.4 mL) were transferred into a series of 10 mL volumetric flasks and made to volume with deionized water. The procedure under "construction of calibration graphs" for the first method was then conducted.

- **For UV light degradation**: Suitable aliquots of the working solution (equivalent to 2.5 µg) were transferred into 25 mL volumetric flasks and completed to volume with deionized water. The flasks were exposed to UV-light at 254 and 312 nm for 4 h in a closed cabinet, where the distance between the source and the sample solution was kept at 15 cm. Aliquots of these solutions (0.4 mL) were transferred into a series of 10 mL volumetric flasks and the procedure under "construction of calibration graphs" was then applied.

Results and Discussion

Prescan of the fluorescence spectra of DCV, revealed that DCV exhibits an intense native fluorescence in water at 400 nm after excitation at 310 nm (Figure 2).

Furthermore, the stability of DCV was studied by subjecting the drug to different stress conditions as per ICH guidelines.

The oxidative alkaline and photolytic degradation products of DCV could not be resolved from its original spectrum either by conventional or synchronous fluorimetry (Figure 3).

Due to the great overlap shown among the fluorescence spectra of DCV and its different degradation products, derivative synchronous Fluorimetry (DSF) technique was adapted for their resolution. Unfortunately, DCV could not be resolved from its alkaline degradation product by either 1st, 2nd, 3rd or 4th derivative synchronous Fluorimetry. DCV was separated from its oxidative degradation products by first derivative synchronous Fluorimetry (FDSF) at 373 nm (Figure 4). Furthermore, DCV was resolved from its photolytic degradation products that is produced by exposure to UV radiation of 312 nm by FDSF at 388 nm (Figure 5). Thus approving the stability-indicating power of the proposed method.

Derivative synchronous Fluorimetry as a technique is far more simple in comparison to the complicated modern HPLC techniques [5-17], less time consuming, and of higher sensitivity than conventional spectrophotometric ones [19-21]. In addition, water was the diluting solvent throughout the method this adds to the advantages that the method is less expensive and eco-friendly.

Optimization of experimental conditions

Effect of different organized media: The synchronous fluorescence properties of DCV in various organized media were studied, using anionic surfactant (SDS), nonionic surfactant (Tween-80) and a macromolecule (methyl cellulose, MC) where 1 mL of each surfactant (1.0 % w/v) was added to an aqueous solution of the drug (final concentration 2 ng/mL DCV). SDS and MC use resulted in pronounced decrease of DCV synchronous fluorescence intensity (SFI), where tween addition caused no pronounced effect on the SFI, in addition

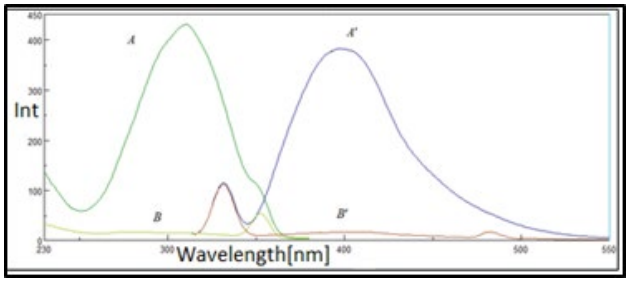

Figure 2: Luorescence spectra of: **(A)** Excitation and **(A')** Emission spectra of (2 ng/mL) of DCV in deionized water. **(B)** Excitation and **(B')** Emission spectra of deionized water.

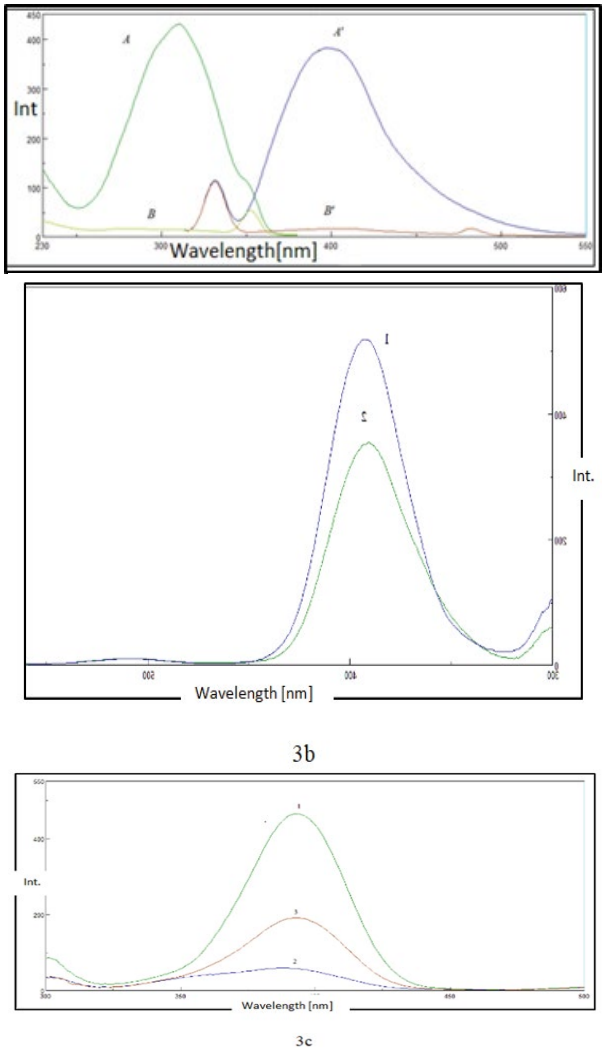

Figure 3: Synchronous fluorescence spectra of:
(3a) (1) DCV (4.0 ng/mL) (2) alkaline degradation product; at Δ λ=80 nm.
(3b) (1) DCV (4.0 ng/ mL) (2) oxidative degradation product; at Δ λ=80 nm.
(3c) (1) DCV (4.0 ng/mL) (2) photolytic degradation product at 313 nm, (3) photolytic degradation product at 254 nm; at Δ λ=80 nm.

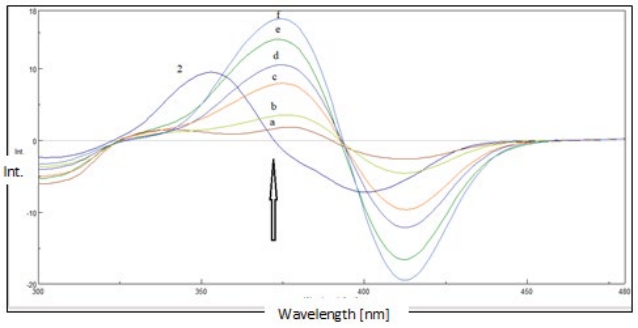

Figure 4: First derivative synchronous fluorescence spectra of: (a-f) of DCV (0.5, 1.0, 2.0, 3.0, 4.0 & 5.0 ng/mL) at 373 nm (2) oxidative degradation product.

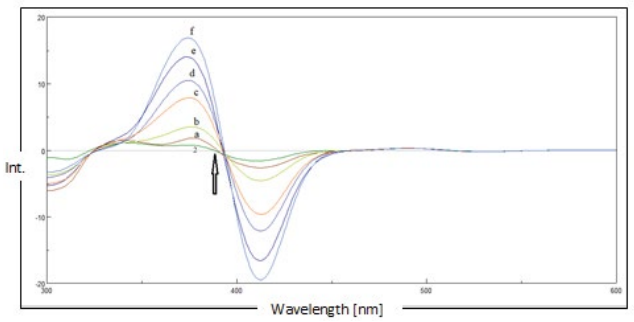

Figure 5: First derivative synchronous fluorescence spectra of: (a-f) of DCV (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ng/mL) at 388 nm (2) photolytic degradation product at 313 nm.

to broadening of the synchronous fluorescent peak of DCV. Thus, SFI is optimum when none of the pre-mentioned surfactant was added (Figure 6).

Effect of different pH: The influence of pH on the SFI of DCV was studied using different types of buffers covering the whole pH range, such as 0.2 mol/L acetate buffer over the pH range 3.5–5.6 and 0.2 mol/L borate buffer over the pH range 6.5-8.5. In addition to 0.1 N HCl and 01.N NaOH. The use of buffer did not enhance the SFI over the entire pH range studied. It was found that maximum SFI was obtained in 0.1 N NaOH however that was not exceeding the SFI in aqueous solution without any pH adjustment (Figure 7).

Effect of diluting solvent: The effect of different diluting solvents on the SFI of DCV was studied using water, methanol, ethanol, acetonitrile, n-propanol, acetone, 0.1 N NaOH and 0.1 N HCl. Complete quenching of the synchronous fluorescence intensity was observed upon using acetone. Propanol and 0.1 N HCl caused marked decrease in SFI of DCV. Acetonitrile, methanol, and ethanol all resulted in reduction of SFI of DCV, where ethanol showed a high blank reading with a broad peak as well. 0.1 N NaOH and H_2O showed the highest SFI, where H_2O showed low blank reading value compared to 0.1 N NaOH. Hence H_2O was the diluting solvent of choice producing a symmetric peak that increased quantitatively with change in DCV concentration (Figure 8).

Effect of time: The effect of time on the SFI of DCV was studied. It was found that the synchronous fluorescence intensity remained constant for at least 1 h.

Effect of temperature: Another factor that affects the synchronous fluorescence intensity is temperature. The effect of temperature was studied in the range 40°C-100°C in a thermostatically controlled water bath. It was found that increasing the temperature resulted in a decrease in the SFI. This effect is mainly attributed to the high internal conversion as the temperature increases, facilitating non radiative deactivation of the excited singlet state [34]. Therefore, all the experiments were carried out at room temperature.

Choice of optimum Δ λ: Choice of optimum Δ λ is a very important factor that must be considered when scanning the synchronous Fluorimetry as it could significantly influence the sensitivity, resolution or bands symmetry. Therefore, varying range of Δ λ (20-140 nm) was carefully investigated.

Δ λ of 80 nm demonstrated best band shapes with highest sensitivity for both oxidative and photolytic degradation. Lower and higher values of Δ λ, resulted in low synchronous fluorescence intensity of DCV and its degradation products.

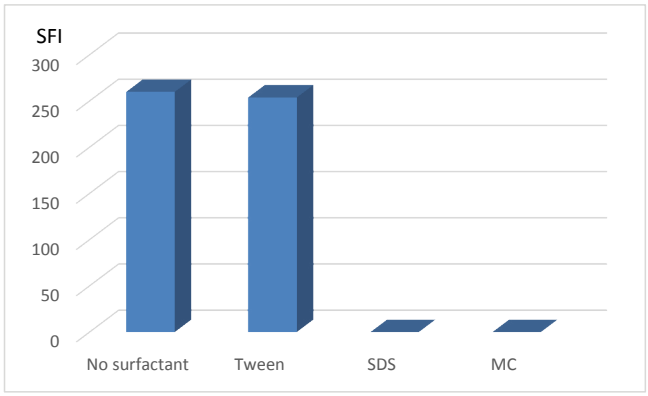

Figure 6: Effect of different organized media on the synchronous fluorescent intensity (SFI) of DCV (2 ng/mL).

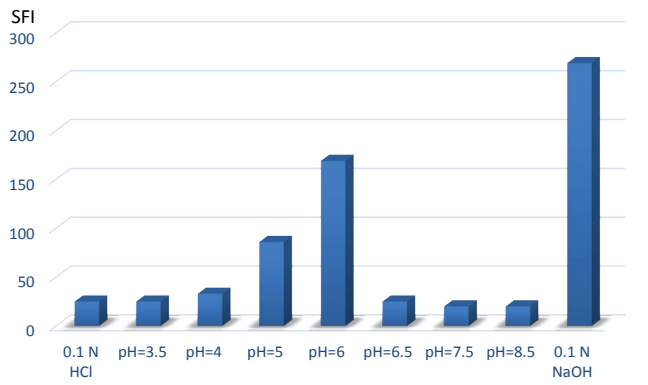

Figure 7: Effect of Different pH on the synchronous fluorescence intensity (SFI) of DCV (2 ng/mL).

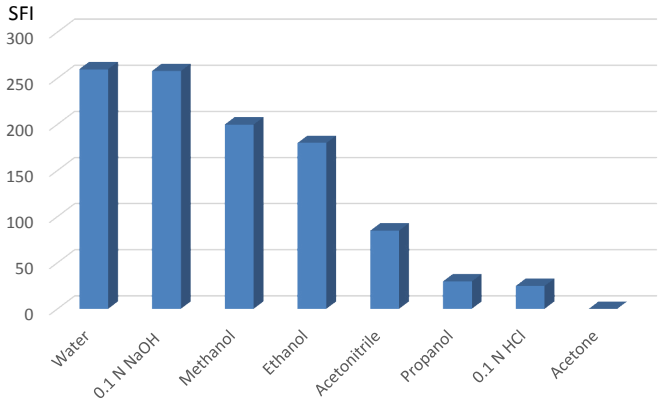

Figure 8: Effect of different diluting solvents on the synchronous fluorescent intensity (SFI) of DCV (2 ng/mL).

However, very low or very high Δ λ values caused irregularities in the spectral shape.

Validation of the Method

The validity of the method was determined by testing its linearity, specificity, accuracy, repeatability and precision in accordance to ICH recommendations [35].

Linearity

It was conducted through replicate analysis of six standard

concentrations of DCV. Calibration graphs of DCV were constructed by plotting the peak amplitude of (1D) against the drug concentration in ng/mL. The data for regression equations and correlation coefficients were summarized in Table 1. In the proposed methods, good linearity was achieved across the range of 0.5-5.0 ng/mL as demonstrated by the high values of correlation coefficients (>0.999).

Linear regression analysis of the data gave the following equations:

^1D=0.411+3.343 C (r=0.9998) at 373 nm

^1D=0.009+0.060 C (r=0.9998) at 388 nm

Where: ^1D is the 1st derivative synchronous fluorescence intensity, C is the concentration of VPS in ng/mL and r is the correlation coefficient.

Statistical analysis [36] of the data demonstrates high value for the correlation coefficient (r) of the regression equation, small values for the standard deviation of residuals (Sy/x), of intercept (Sa), and of slope (Sb), and small value of the percentage relative standard deviation and the percentage relative error (Table 1). These data proved the linearity of the calibration graph.

Limits of quantification (LOQ) and limits of detection (LOD)

The limits of quantitation (LOQ) were determined by assessing the lowest concentrations that can be measured according to the ICH Q2 (R1) recommendation [35]. The limits of detection (LOD) were determined by evaluating the lowest concentrations of the drug that can be readily detected. The results are also abridged in Table 1. The values of LOQ and LOD were calculated according to the following equation [35]:

LOQ=10Sa/b

LOD=3.3Sa/b

Where Sa is the standard deviation of the intercept of the regression line and b is the slope of the calibration graph.

Accuracy and Precision

Statistical analysis [36] of the results obtained by the proposed and reference methods [20] using Student's t-test and variance ratio F-test showed no significant differences between the two methods regarding accuracy and precision (Table 2). The reference method was based on measuring the UV absorption spectrum of an aqueous solution of the drug at 214 nm.

The intraday precision was evaluated by determination of three concentrations of DCV in pure forms on three successive occasions. The interday precision was also evaluated through replicate analysis of three concentrations over 3 successive days. The results of intraday and interday precision are summarized in Table 3. The relative standard deviations were found to be very small indicating appreciable repeatability and intermediate precision of the proposed methods.

Parameter	Value	
	FDSF at 373	FDSF at 388
Linearity and range (ng/mL)	0.5-5.0	0.5-5.0
Correlation coefficient (r)	0.9998	0.9998
Slope	3.343	1.588
Intercept	0.411	0.009
$S_{y/x}$, S.D. of the residuals	0.12	0.06
S_a, S.D. of the intercept	0.09	0.04
S_b, S.D. of the slope	0.03	0.01
S.D.	1.13	1.26
%RSD [a]	1.13	1.26
%Error [b]	0.46	0.51
LOD [c]	0.09	0.09
LOQ [d]	0.275	0.268

[a] Percentage relative standard deviation
[b] Percentage relative error
[c] Limit of detection
[d] Limit of quantitation

Table 1: Analytical performance data for the determination of DCV by the proposed methods.

Selectivity

The proposed method was found to be selective for DCV in its tablet, where, satisfactory results were obtained and no interference was encountered from common excipients (Table 4). Furthermore, derivative synchronous fluorimetry method was found to be selective for DCV determination in presence of its oxidative and photolytic degradation products.

Pharmaceutical applications

The proposed fluorimetric strategy was effectively used for the determination of DCV in its commercially available tablets (Table 4). The outcomes shown in Table 4 are in great concurrence with those got utilizing the comparison method [20].

Factual investigation of the outcomes acquired by the proposed approach and the comparison method [20] utilizing Student's t test and variance ratio F test at 95 % confidence level [36] uncovered no huge distinction between the execution of the methodologies in regards to the precision and accuracy, separately.

Degradation study

Several methods were reported regarding the study of the stability of DCV. Apparently, they discrepant with each other's, and even with the report of the European Medicines Agency (EMA) for DCV [24]. The results of stability study by the proposed method was found to be in agreement with those reported by Zaman and Hassan [11]. In alkaline medium, the amide linkages in the side chains of both sides of the molecule are cleaved ending with pyrazole ring structure. The rigidity of the resulting compound decreases with decrease of synchronous fluorescence intensity. The oxidative degradation- on the other hand- is proposed to proceed *via* oxidation of the pyrazole ring giving the N-oxide derivative with subsequent decrease of fluorescence (Figure 9).

Conclusion

The proposed strategy represents an extremely sensitive, simple time saving 1st derivative synchronous spectrofluorimetric technique for the quantitative assay of small deliberate concentrations of DCV in

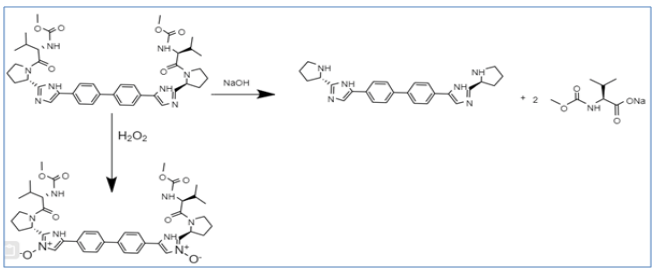

Figure 9: Proposed degradation scheme for DCV under alkaline and oxidative stress conditions.

Parameter	Concentration taken (ng/mL)	Proposed Method				Comparison Method [20]	
		FDSF at 373 nm		FDSF at 388 nm			
		Concentration Found (ng/mL)	% Found	Concentration Found (ng/mL)	% Found	Concentration taken (µg/mL)	% Found
DCV	0.5 1.0 2.0 3.0 4.0 5.0	0.505 0.984 2.001 2.990 4.057 4.963	101.06 98.40 100.06 99.67 101.42 99.26	0.493 1.008 2.033 2.971 3.961 5.034	98.50 100.76 101.66 99.04 99.03 100.69	6.0 8.0 10.0	100.72 98.92 100.43
X ± SD		99.98 ± 1.13		99.95 ± 1.26		100.02 ± 0.97	
t-test		-0.06 (2.36)		-0.09 (2.36)			
F-test		1.36 (19.0)		1.69 (19.29)			

*Figures between parentheses are the tabulated t and F values at p=0.05 [36].
Each result is the average of three separate determinations.

Table 2: Application of the proposed method for the analysis of DCV in its pure form.

Parameters		At 373 nm			At 388 nm		
		concentration (ng/mL)			concentration (ng/mL)		
		2.0	3.0	4.0	2.0	3.0	4.0
Intraday	%Found	100.06	99.76	101.42	101.66	99.04	99.03
		99.58	98.57	101.54	99.63	97.82	97.97
		100.17	100.11	99.79	98.45	98.34	99.55
	$(\bar{x})$ ± S.D.	99.94 ± 0.32	99.48 ± 0.81	100.92 ± 0.98	99.91 ± 1.62	98.40 ± 0.61	98.85 ± 0.81
	%RSD	0.32	0.81	0.97	1.63	0.62	0.81
	%Error	0.18	047	0.56	0.94	0.36	0.47
Inter-day	%Found	100.06	99.76	101.42	101.66	99.04	99.03
		98.93	100.33	99.14	98.75	100.11	100.34
		99.76	98.47	98.88	100.14	98.65	100.57
	$(\bar{x})$ ± S.D.	99.58 ± 0.59	99.52 ± 0.95	99.81 ± 1.40	100.18 ± 1.46	99.27 ± 0.76	99.98 ± 0.83
	%RSD	0.59	0.96	1.40	1.45	0.76	0.83
	%Error	0.34	0.55	0.81	0.84	0.44	0.48

N. B. Each result is the average of three separate determinations.

Table 3: Precision data for the determination of DCV by the proposed method.

Parameter	Concentration taken (ng/mL)	Proposed Method				Comparison Method [20]	
		FDSF at 373 nm		FDSF at 388 nm			
		Concentration Found (ng/mL)	% Found	Concentration Found (ng/mL)	% Found	Concentration taken (µg/mL)	% Found
DCV	2.0 3.0 4.0	1.9752 2.9742 4.0104	98.76 99.14 100.26	2.0264 2.9619 4.0196	101.32 98.73 100.49	6.0 8.0 10.0	100.76 98.68 100.43
$\bar{X}$ ± SD		99.39 ± 0.78		100.18 ± 1.32		99.96 ± 1.12	
t-test		0.72 (2.78)		0.22 (2.78)			
F-test		2.05 (19.0)		1.39 (19.0)			

*The figures between parentheses are the tabulated t and F values at P=0.05 [36].

Table 4: Assay results for the determination of DCV by the proposed method in its tablet.

its pharmaceutical tablets, on the contrary of the complicated modern HPLC techniques [5-17] or the low sensitivity spectrophotometric ones [19-21]. Stability studies were also conducted utilizing distinctive stress induced conditions as indicated by ICH recommendations. Luckily, first derivative synchronous Fluorimetry was ideally suited to separate the synchronous fluorescent spectra of DCV from its oxidative and photolytic degradation products at 373 and 388 nm, respectively, after recording the synchronous Fluorimetry utilizing Δ λ of 80. Thus, the

stability indicating capability of this approach can be evaluated.

Conflict of Interest

The authors declare no conflict of interest.

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