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Specific, highly sensitive and simple spectrofluorimetric method for quantification of daclatasvir in HCV human plasma patients and in tablets dosage form

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Abstract: A simple, selective and highly sensitive spectrofluorimetric method for the quantitative detection of an antiviral drug, daclatasvir (DCV) has been developed and analytically validated in its pure form, in its commercially available pharmaceutical preparations, and in hepatitis-C (HCV) patient's plasma. The method was based on recording the native fluorescence of DCV that exhibit an emission wavelength maximum at 380 nm upon excitation at 320 nm. Many factors affecting the fluorescence intensity and the method sensitivity including pH, type of surfactant and solvent have been studied and optimized. In addition, a stability-indicating study was performed in accordance with the guidelines of the International Conference on Harmonization (ICH), to detect the drug in the presence of its degradation products which validates the method for application in quality control laboratories. In addition, extraction of DCV from the plasma proteins was performed using a simple technique that was based on using methanol and borate buffer (pH 9) that gave a recovery of ~95%. The results showed that DCV could be detected using this method in the pure form (with a linear range of 2-1000 ng/mL), commercially available tablets and in plasma samples (with a linear range of 5-1000 ng/mL), without any interferences. Furthermore, the method was also analytically and clinically validated according to the Food and Drug Administration (FDA) guidelines, with limit of detection (LOD) of 0.3 and 0.5 ng/mL for DCV in the pure form and plasma samples, respectively.

Keywords: Daclatasvir; sofosbuvir; spectrofluorimetric; stability-indicating; half-lives; plasma; and dosage forms; HCV; hepatitis.

1 Introduction

Hepatitis-C virus (HCV) is a global health problem responsible for chronic infections for approximately 170 million people leading to 700 000 deaths [1]. According to the World Health Organization (WHO), the highest rate of HCV infection in the world has been recorded in Egypt where approximately 15% of its population have been diagnosed with HCV [2]; 150 000 Egyptians are carrying the latent virus and almost 40 000 people die every year [3,4]. The treatment of HCV is based on the application of direct-acting antiviral agents (DAAs) in conjunction with other medicines such as ribavirin that can achieve a highly successful recovery rate of more than 95% [5-7]. Daclatasvir (DCV, Figure 1) is a DAA that was listed in the WHO Essential Medicines for the treatment of HCV and introduced into the global market in 2015 [8].

Many methods have been reported for the detection of DCV, in tablets or biological fluids, including UPLC/MS/MS [9-11], LC/MS/MS [12, 13], HPLC [14-16], and electrochemical method[17]. However, the application of such methods for the analysis of DCV in low socioeconomic countries, where HCV is an epidemic, is limited due to the high cost of operations and the rare availability of equipment. Further, most of these methods require well-trained staff, extensive extraction protocols and are time-consuming. Alternatively, spectrofluorimetry offers a simple, sensitive, and selective technique for the quantification of drugs in their pure state, dosage forms or in biological fluids, without the need for an extensive extraction process. This study describes a validated spectrofluorimetric method for the quantitative detection of DCV in its pure form, and which is commercially

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Figure 1: Structure formula of DCV.

available in tablet form and in human plasma samples that were collected from HCV patients treated with DCV. This method was almost a thousand times more sensitive than the previously reported HPLC methods [14-16] and with a comparable sensitivity to the reported LC/MS/MS methods [12-13]. The study was analytically validated according to the FDA guidelines and was extended to investigate the stability/degradation process of DCV under various stress conditions. This validated the described protocol for the analysis of DCV in the presence of its degradation products and in the quality control laboratories.

2 Experimental

2.1 Apparatus and measurement conditions

Fluorescence measurements were recorded, using 1 cm-quartz cells, on a fluorescence spectrometer FS-2 (Scinco, Korea) with grating excitation and emission monochromators, slit width was adjusted at 10. The excitation and emission wavelength were set at 320 nm and 380 nm respectively. Jenway PH meter model 350, a centrifuge (Bremsen ECCO, Germany), at the speed of 10000 rpm, and a Shimadzu UV-1601 PC UV-visible spectrophotometer (Tokyo, Japan) with 1 cm quartz cell were used. CAMAG UV-lamp, dual wavelength (254/366), 2×8W (Muttenez Switzerland) was used to initiate the photo-degradation process.

2.2 Material and reagents

Throughout this research, all chemicals were of analytical grade and double distilled water was used. Daclatasvir dihydrochloride (DCV), a pure standard, with a purity of ~99.99% as measured using a reported method¹⁴, was purchased from Bristol-Myers Squibb, USA and was used as received. Sofosbuvir (SFB) was purchased from Virdev Intermediates Pvt Ltd., India while ribavirin (RBV) was obtained from Modern Times Helpline Pharma; New Delhi;

India. Methanol; ethanol, isopropanol, acetonitrile, tween 80, PEG 6000, sodium lauryl sulfate, polyoxyethylene 50 stearate, carboxymethyl cellulose, hydrogen peroxide (30%), sodium hydroxide and hydrochloric acid (33%) were purchased from El Nasr Chemical Company, Cairo, Egypt. Acetate buffer solutions (0.2 M), in the range of pH 3.6 to 5.6, and borate buffer (0.2 M) in the range of pH 6.5 to 9.5, were prepared. All tablets that contained the dosage of 60 mg of DCV (Daklinza® from Bristol-Myers Squibb company, Daclavirocyl® manufactured by Mercyril Pharmaceutical Industries, Dakasvir® produced by Pharma-5 and Daclanork® by Mash Premiere Company) were purchased from the Egyptian local market.

2.3 Preparation of standard and working solutions for pure drug and plasma analysis

For pure drug solution: a stock standard solution of 1 mg/mL was prepared in methanol. Further dilutions were made using methanol to prepare the working standard solution (10 µg/mL) and concentrations in the range of 2-80 ng/mL.

For plasma: 10 mg of DCV were weighed, transferred into a 10 mL-volumetric flask, dissolved in methanol and completed to 10 mL with the same solvent (*solution S*). From this solution, 32.0 µL was transferred into a 10 mL-volumetric flask and then completed to 10 mL with methanol to produce a solution with a concentration of 1 µg/mL (*Calib. G*). Different volumes from the *Calib. G* solution were further diluted to 4 mL with methanol to obtain the working standard concentrated solutions suitable for the analysis (Table 1). The QC stock solution was prepared as described for the calibration stock solution by weighing 10 mg separately.

2.4 Procedure for calibration curve construction

For pure drug solution: Aliquots of 1 mL of the working solution were transferred into a series of 10 mL volumetric

Table 1: Procedure for preparation of plasma working standard solutions.

Calib. name	Volume (μL) taken from Calib. G	Amount of methanol added (μL)*	Resulted concentration(ng/mL)
Calib. A	20	3980	5
Calib. B	60	3940	15
Calib. C	180	3820	45
Calib. D	480	3520	120
Calib. E	1300	2700	325
Calib. F	2700	1300	675

*The solution required is 4000 μL

flasks, wrapped with aluminum foil. The volumes were made up with methanol to prepare solutions with concentrations of 2-1000 ng/mL (2, 5, 50, 100, 200, 400, 500, 600, 700, and 1000 ng/mL). The relative fluorescence intensity was recorded against the corresponding concentration to construct the calibration curve.

For plasma: A 200 μL of human plasma was transferred into a series of test tubes, wrapped with aluminum foil, spiked with 25 μL of the working DCV solutions that were used to construct the calibration solutions (Calib A, B, C, D, E, F, and G), to prepare samples with concentrations of 5, 15, 45, 120, 325, 675, and 1000 ng/mL, respectively.

For protein precipitation: 200 μL borate buffer (pH 9) was added into the plasma samples followed by addition of 2 mL of methanol for each solution; the content of each test tube was mixed by vortex for 5 min and centrifuged at 10 000 rpm for 10 min. The clear supernatant was carefully transferred into a quartz cell and measured directly by the fluorimeter. The relationship between the relative fluorescence intensity was established versus the corresponding drug concentration and the regression equation was also calculated. A blank experiment was carried out on drug-free plasma samples treated similarly without the addition of DCV. The samples were prepared as described for calibration samples with concentrations of 6, 400, and 800 ng/mL and 15, 400, and 800 ng/mL for pure and plasma samples respectively.

2.5 Procedure for tablets

An accurately weighed quantity equivalent to 10 mg of DCV of ten grounded tablets was transferred into a 100 mL volumetric flask, dissolved in 50 mL of methanol and sonicated for 30 min. The flask content was completed to the level with the same solvent to obtain a concentration of 10 μg/mL. Then the procedure was processed as described

under the calibration curve construction. The nominal content of tablets was calculated using the calibration graph or the corresponding regression equation.

2.6 Clinical study

After approval by the Ethics Committee of the Faculty of Medicine, Al-Azhar University, Assuit, Egypt, all plasma samples were collected from the outpatient at the Division of Internal Medicine with written informed consent obtained from all patients.

2.7 Plasma sample preparation

Appropriate preventive measures were taken during the process of collection, processing and the storage of the plasma samples as DCV is light-sensitive. Samples were handled under yellow light and not UV or sunlight, and then stored in the dark. Additionally, all glassware and equipment were wrapped with aluminum foil. All analysis steps were conducted under light-protected conditions. Stock and standard solutions were stored in brown glass vials at -5°C. Plasma samples were taken from eight hepatitis C patients during their treatment with SFB and DCV regimen. Four plasma samples (subject 1-4) were collected after 2h of oral administration while the rest samples (subject 5-8) were taken 10h post oral dose. Following this, 5.0 mL of human blood samples were collected into K₂-EDTA tube after 2h and 10h following the last oral administration. The tubes content was centrifuged at 10 000 rpm for 10 min followed by transferring 200 μL of plasma into centrifuge tubes, wrapped with aluminum, and then 200 μL of borate buffer (pH 9) were added for each tube. For protein precipitation 2 mL of methanol was added, the solution was mixed by vortex for 5 min

Figure 2: Excitation and emission spectra of RBV (A, A*), SFB (B, B*), and DCV (C, C*) in spiked human plasma.

and centrifuged at 10 000 rpm for 10 min. The clear supernatant was carefully transferred into a quartz cell, and the relative fluorescence intensity was measured. The procedure was repeated three times on three different days to obtain the intraday and inter-day assay.

2.8 Procedure for stress degradation studies

2.8.1 Acidic, alkaline and oxidative degradation studies

An aliquot of DCV equivalent to 400 ng/mL was transferred into a series of test tubes, to which 5 mL of 1M HCl, 1M NaOH or 10% H₂O₂ were added. The content of each tube was boiled at 100 °C for different time intervals of 10, 20, 30, 40, 50, and 60 minutes and then cooled and neutralized to pH 7. The solutions were then transferred into 25-mL volumetric flasks and completed to the water level. 1.5 mL of each solution was transferred into a 10-mL volumetric flask and the volume was completed to the appropriate level with methanol to end up with a final concentration of 60 ng/mL. The general procedure that was described under the “construction of calibration curve” was followed.

2.8.2 photolytic degradation study

An aliquot of DCV equivalent to 600 ng/mL was transferred into a 10-mL volumetric flask, sealed well and completed with methanol (final concentration 60 ng/mL) up to 10 mL.

The flask was exposed to the UV-light at a wavelength of 254 nm for 24 h using an UV-lamp followed by measuring the fluorescence intensity.

2.8.3 Stability in direct sunlight

Human plasma samples were infused with the drug (200 ng/mL) and exposed to direct daylight. In parallel, methanolic solutions of drug (60 ng/mL) were exposed to direct daylight at room temperature using glass containers which were directly positioned close to the laboratory window for 0, 1, 2, 4, 6, 8, 10, and 12 hours. All samples were processed according to the described extraction and fluorimetric method.

3 Results and discussion

A DCV solution in methanol exhibits an intense native fluorescence maximum at ~380 nm (Figure 2). However, quantitative determination of DCV in biological fluids and pharmaceutical preparation based on using its native fluorescence signals has not been reported yet. This encouraged us to develop a simple analytical method for the quantitative detection of DCV using its native fluorescence signal, permitting its application in many laboratories, especially in low-income countries where sophisticated alternatives such as LC/MS/MS are not available.

Figure 3: Effect of different pH on the RFI of DCV (60 ng/mL).

DCV is combined with other drugs including SFB and RBV; therefore, any method developed for the assay must be able to determine DCV in the presence of such co-administrated drugs. Further, factors that may influence fluorescence measurements such as pH, surfactant, diluting solvent must be optimized to maximize the signal recorded. Therefore, we started this study by studying and optimizing the effect of such factors on the native fluorescence signal of DCV.

3.1 Effect of pH

The effect of pH on the fluorescence intensity of DCV was studied using 2.0 mL of acetate buffer (pH from 3.5 ± 0.1 to 5.5 ± 0.1); borate buffer (pH from 6.5 ± 0.1 to 9.5 ± 0.1), acidic (0.1M HCl) or basic conditions (0.1M NaOH). The results showed that the fluorescence intensity of DCV was not significantly increased upon pH variations (Figure 3). As a result, buffer solutions, acids and bases were excluded during the analysis of DCV.

3.2 Effect of surfactant

It has been reported that surfactants could improve the fluorescence intensity of many molecules *via* micelle formation. Therefore, the interplay between different surfactants and the fluorescence signal of DCV was investigated using 1 mL of aqueous solution of each surfactant (tween 80, PEG 6000, sodium lauryl sulfate, polyoxyethylene 50 stearate, and carboxymethylcellulose).

Figure 4: Effect of various surfactant on fluorescence intensity of DCV (60 ng/mL).

The results showed that polyoxyethylene 50 stearate and carboxymethylcellulose did not influence the fluorescence intensity while other surfactants such as PEG 60000, sodium lauryl sulfate or tween 80 slightly reduced the recorded fluorescence (Figure 4). As a result, no surfactant was used throughout this work.

3.3 Effect of diluting solvent

Different diluting solvents including methanol, ethanol, isopropanol, acetonitrile or water were attempted to study their effects on the fluorescence intensity of DCV. As in Figure 5, the results showed that the highest fluorescence signal of DCV was obtained when methanol was used for dilution. Therefore, methanol was used as the optimal diluting solvent for further work.

3.4 Recovery study

An optimal extraction protocol should be identified before the proposed method to detect DCV in pharmaceutical preparations and plasma samples can be applied; it has been reported that more than 99% of DCV were attached to the plasma proteins which indicates that the extraction

Figure 5: Effect of diluting solvent on the fluorescence intensity of DCV.

of DCV from the protein prior to analysis is required [20]. Accordingly, a protein precipitation technique was utilized to extract the drug from the attached proteins using a precipitating solvent. In this work, three precipitating solvents namely methanol, acetonitrile, and isopropanol were tested; results showed that methanol and borate buffer (pH 9), in the ratio of 2:1, gave the highest percentage of drug recovery at ~95% (Figure 6). This high extraction recovery using methanol and borate buffer (pH 9), indicates the efficiency of methanol to precipitate the plasma protein and to extract the free drug into the organic layer [21]. The mean recoveries of DCV from plasma were calculated at three concentration levels; low, middle and high, using six replicates for each concentration. The obtained recoveries ranged from 90 ± 0.4 to $105 \pm 1.6\%$ which could validate our method for the quantification of DCV in biological fluids, such as plasma (Table 3) (Figure 6).

Figure 6: Percentage recovery for different solvents used for extracting DCV from plasma

3.5 Validation of the proposed method

The method was validated in accordance with the FDA recommendations in the terms of linearity, range, LOD, LOQ, accuracy, precision, and specificity according to FDA [19].

3.5.1 Linearity and range

The linear range of the proposed method to detect DCV was assessed by analyzing sets of DCV ($n=10$ or 7 for calibration curve) in its pure form and in plasma samples. The relationship between the concentration and fluorescence intensity was established. The results showed that a linear relationship could be observed over

the range of 2-1000 ng/mL and 5-1000 ng/mL for pure drug solution and in plasma samples, respectively. Statistical analysis of the data including intercept (a), slope (b), percentage of relative standard deviation (%RSD), and percentage of standard error (% Err) are listed in Table 2.

3.5.2 Limit of detection and limit of quantification

The author's method was used to calculate the limit of detection (LOD) and the limit of quantification (LOQ) for the detection of DCV in its pure form and plasma. This was performed by calculating the lowest and highest concentrations that could be detected using the proposed method. The results revealed that the LOD values were 0.3 and 0.5 ng/mL for pure and plasma samples, respectively

Table 2: Analytical performance data for the spectrofluorimetric quantification of DCV in pure form and spiked plasma.

Parameter	Pure form	Plasma
Wavelength ($\lambda_{\text{ex}} / \lambda_{\text{em.}}$) nm	320/380	320/380
Linearity and range (ng/mL)	2-1000	5-1000
Lower limit of detection LLOD (ng/mL)	0.300	0.501
Lower limit of quantification LLOQ (ng/mL)	1.21	2.66
Intercept (a)	112.33 + 0.87	145.53 + 1.32
Slope (b)	30.33 + 0.031	21.55 + 0.67
r	0.9996	0.9998
r ²	0.9993	0.9997
%RSD	1.02	1.87
% Error	0.87	1.34

while LOQ was either 1.21 or 2.66 ng/mL for pure or plasma, respectively (Table 2).

$$\text{LOD} = 3.3 \text{ Sa/bLOQ} = 10 \text{ Sa/b}$$

Where: Sa is the standard deviation of intercept of calibration graph; b is slope of the calibration graph.

3.5.3 Accuracy and precision

The proposed method was analytically validated according to the recommendations published by the Food and Drug Administration (FDA) [19]. Accuracy was calculated as the ratio between the mean of recovery for different drug concentrations and the nominal value. Precision was defined as the standard deviation (SD) of the mean of at least three readings, expressed in percent. These values were calculated within each batch and for different batches. For this purpose, batches (n=3) containing DCV within eight calibration points and 18 QC samples at three different concentrations (QC A, B, and C) were analyzed. From these values the accuracy and the precision of the method were calculated and expressed as mean values $\pm$ SD. Overall the precision measured as coefficient of variance (CV) and was ranged from 4.4-8.3% for pure form and 7.2-8.4% for plasma while accuracy value ranged from 95.9 to 99.5% for pure form of DCV and 97.7-102.1% for plasma samples (Table3).

3.5.4 Selectivity and specificity

The selectivity of the proposed method was assessed to determine DCV in the presence of the two co-administered

drugs, SFB and RBV. The results showed that the fluorescence signal of DCV was detected without any interference from the added two drugs that did not exhibit any fluorescence signal at the specified excitation and emission wavelengths of DCV (Figure 2). Therefore, we could consider our method as selective to determine DCV in the presence of the co-administered drugs (SFB and RBV).

3.6 Application in pharmaceutical preparation

The application of the proposed method for analysis of DCV in pharmaceutical formulations were performed. The results gave a considerable recovery percentage ranging from 97.4 $\pm$ 1.7 to 102.3 $\pm$ 0.6% (Table 4). These results were comparable with a previously reported method [14]. Student's t-test and F-test were calculated to compare our method with the previously reported methods for the detection of DCV. The values of these tests revealed that there were no significant differences between the reported method and the proposed method at a 95% confidence level.

3.7 Application to HCV plasma patients

DCV was well-absorbed by the intestines after oral administration, with median peak concentrations observed within 1 to 2 hour's post-dose. The reported C_{max} of DCV following multiple oral administrations of 60 mg, once daily, was approximately 973 ng/mL [18]. This concentration lies within the linear range of our method which indicates the suitability of the proposed method to determine DCV

Table 3: Validation results for DCV in pure form and plasma matrix with the extraction recovery percentage.

		Pure samples			Plasma		
		QC A	QC B	QC C	QC A	QC B	QC C
Concentration ^a		10	400	800	15	400	800
Within-batch							
1	Mean [ng/mL]	9.86	390.20	800.01	14.17	394.17	827.83
	Accuracy [%]	98.58	98.00	100.01	94.51	98.10	103.76
	Precision [%CV]	7.23	2.46	2.42	7.88	7.64	11.12
2	Mean [ng/mL]	9.60	400.05	799.81	14.64	390.83	798.83
	Accuracy [%]	95.98	100.13	99.72	97.61	97.52	99.75
	Precision [%CV]	7.18	3.87	2.72	7.44	5.57	6.40
3	Mean [ng/mL]	9.59	390.70	790.70	15.20	404.50	822.00
	Accuracy [%]	95.93	99.19	99.56	101.03	101.25	102.70
	Precision [%CV]	8.34	5.81	4.43	5.80	11.28	6.41
Batch-to-batch							
Mean [ng/mL]		9.68	393.64	796.84	14.66	395.83	816.22
Accuracy [%]		95.93	99.19	99.56	97.71	98.92	102.06
Precision [%CV]		7.58	4.07	3.19	7.04	8.16	7.97
% Recovery $\pm$ SD					99.3 $\pm$ 1.3	105 $\pm$ 1.6	90 $\pm$ 0.4

^a Nominal analyte concentrations [ng/mL]

in human plasma of hepatitis-C patients, treated with such a drug. This experiment was performed against blank plasma samples, free of DCV, to ensure that there was no interference from the plasma content. The results showed that the recovery percentage of DCV in the patient's plasma ranged from 92.3 to 112.2%, 2 hours post oral dose (subject 1-4) followed by a sharp decline to 45.1 - 69.2% (Figure 7, Table 6), 10 hours after oral administration of DCV.

3.8 Stability indicating study

A stability-indicating study was conducted to quantitatively determine DCV in the presence of its degradation products [22]. This study was performed to validate our method application in the quality control laboratories.

3.8.1 Effect of direct daylight on DCV stability in human plasma and methanol matrix.

As DCV is light-sensitive, direct exposure of DCV to the daylight could influence the practical handling. To study

Table 4: Results of the degradation study of DCV under different stress conditions.

Parameter	Sunlight		1M HCl	1M NaOH	10% H ₂ O ₂
	pure	plasma			
$t_{1/2}$ (min) ^a	2.73	6.42	56.75	52.47	33.53
r^2	0.85	0.99	0.95	0.91	0.94

^a $t_{1/2}$ for sunlight is calculated as hours

the effect of direct daylight on DCV stability, human plasma samples were spiked with DCV; drug solutions in methanol were separately prepared as well and exposed to direct daylight for different time intervals. The results showed that approximately 73% of DCV in plasma and 93% of DCV solutions in methanol were degraded after exposure to direct daylight after 12 h. Further, the half-life value of DCV in pure solution or plasma was calculated using the equation of $t_{1/2} = \ln 2 / k \approx 0.693 / k$ [24]. The half-life of DCV in human plasma (6.78 h) was longer than that obtained from the pure solution in methanol (2.6 h), which could be postulated to the slightly yellow color of human plasma and its consistency, which could offer

Figure 7: Effect of 1M HCl, 1M NaOH, 10% H₂O₂ (A), and direct sunlight (B) on DCV stability

some sort of protection in prolonging the half-life (Figure 8 and Table 4). Additionally, the effect of UV light on the stability of DCV was studied by continuous exposure of DCV to the UV lamp at 254 nm for 24h which resulted in the degradation of ~ 15% of DCV, in methanol, after the first 24 hours of UV exposure.

3.8.2 Effect of Acid, Alkaline, oxidation, and UV degradation

Stress conditions that could accelerate the degradation of DCV such as extreme acid, alkaline or oxidative conditions were investigated using 1M HCl, 1M NaOH or 10% H₂O₂ in combination with heating for a different time interval. The results showed that the fluorescence intensity gradually decreased upon increasing the degradation time Figure 8, Table 6). Both acid and alkaline conditions showed the same degradation impact on DCV as ~66% of the original

drug was degraded after boiling for 1 hour in either 1 M HCl or 1 M NaOH. In contrast, DCV underwent faster degradation under the specified oxidation conditions (10% H₂O) with a shorter half-life on DCV compared to the acid or alkaline degradation.

4 Conclusion

The present work describes a simple and efficient spectrofluorometric method for the detection of an antiviral drug, daclatasvir (DCV), which is commonly used for the treatment of hepatitis C (HCV). The method was successfully applied to determine this drug in its pure form, in plasma samples collected from hepatitis-C patients who were treated with DCV and in pharmaceutical preparations. In addition, a direct, rapid and cost-effective protein precipitation extraction method was employed

Table 5: Results of quantification of DCV in tablets dosage form.

Tablet dosage form 60 mg/tablet	Proposed method ^a (Recovery %±SD)	Reported method ^a (Recovery %±SD)	t test ^b	F test ^b
Daclanork	98.7±1.2	99.6±0.7	0.89	2.9
Daklinza	102.3±0.6	100.7±1.5	0.43	6.8
Daclavirocyl	100.1±0.8	99.8±1.1	0.65	5.9
Dakasvir	97.4±1.7	100.4±0.4	0.97	8.6

^a Mean of five determinations. ^bThe tabulated values of t- and F-values at 95% confidence limit are 2.78 and 6.39, respectively [23].

Table 6: Percentage recovery of DCV in real human plasma samples.

Subject number ^a	Found mean ^b ±SD	(Recovery %)
1	892.24 ±0.7	92.4
2	1091.35 ±1.2	112.4
3	931.85 ±1.1	99.5
4	1016.47 ±1.9	105.7
5	577.42 ±1.1	59.3
6	600.24 ±1.8	61.13
7	673.66 ±0.6	69.23
8	438.33±1.4	45.05

^a (subjects 1-4)samples collected 2hrs post oral administration while (subjects 5-8) were collected 10hrs post-dose

^b Mean of five determinations

to extract DCV from the patient plasma using methanol: borate buffer (pH 9), in the ratio of 2:1. The method was validated according to FDA recommendations which revealed that the potential method was sensitive enough to detect DCV in pharmaceutical formulations and in human plasma even in the presence of the co-administer drugs (sofosbuvir and ribavirin), which are usually combined with DCV for the treatment of HCV. The simplicity of the proposed method together with the high sensitivity and considerable selectivity facilitate the application of such a method in low socioeconomic countries where HCV is an epidemic and other (sophisticated extraction techniques or chromatographic methods) methods may not be available. In addition, a stability-indicating study validated this method for application in the quality control laboratories.

Conflict of interest: Authors declare no conflict of interest.

Figure 8: Results of quantification of DCV in HCV treating patients.

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