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A clinical study for the evaluation of pharmacokinetic interaction between daclatasvir and fluoxetine

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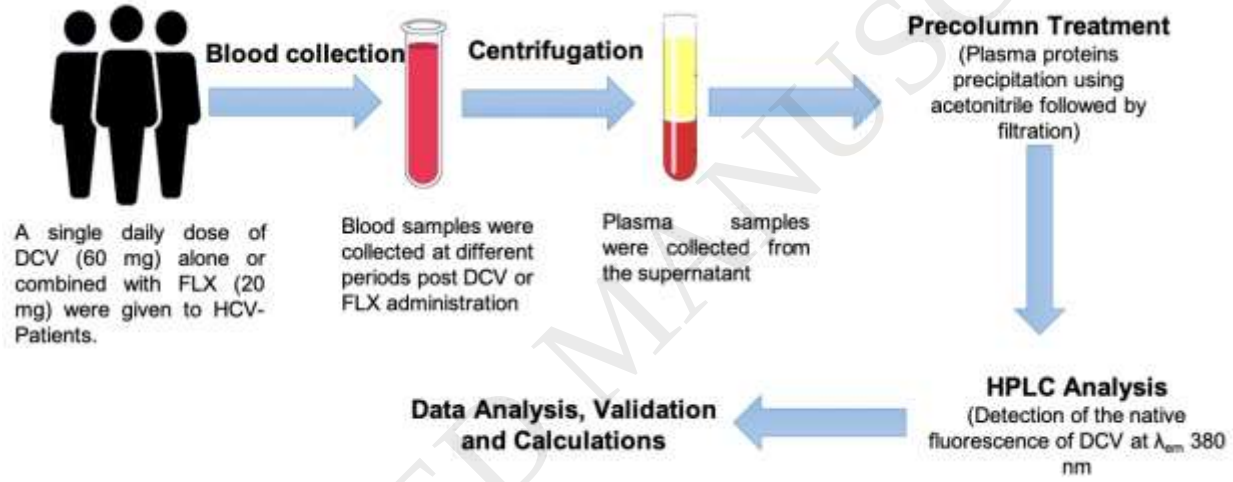
Graphical abstract



Highlights

- **Hepatitis C virus is an epidemic problem with high global rate was recorded in Egypt**
- **Treatment of HCV is based on using direct acting antiviral agents that has been recommended by the WHO**
- **Patients diagnosed with HCV have symptoms of depression**
- **Pharmacokinetic study between daclatasvir and fluoxetine is reported**
- **HPLC coupled with fluorometric detector provides ultrasensitive analysis**

Abstract

A simple and sensitive chromatographic method has been developed for the quantitative analysis of an antiviral agent, daclatasvir (DCV), that commonly prescribed for the treatment of hepatitis C viral (HCV) infection. The method was applied to detect DCV in human plasma and real blood samples collected from patients diagnosed with HCV and treated with DCV. The analysis strategy was based on recording the native fluorescence of DCV in plasma, after pre-column treatment to precipitate the plasma proteins using a readily applicable protocol. Chromatographic conductions, factors influencing the fluorescence and stability studies were also investigated. Furthermore, the method was validated according to the International Conference on Harmonization (ICH) guidelines and could be used to detect DCV in plasma over a linear range of 1.0 to 4000 ng/mL, with an acceptable sensitivity as the limit of detection (LOD) was 0.025 ng/mL. In addition, the study was extended to evaluate pharmacokinetic interaction between DCV and a co-prescribed antidepressant drug, fluoxetine (FLX) in real blood samples, collected from volunteering patients who were diagnosed with HCV and treated with DCV alone or combined with FLX. The results showed a significant influence of FLX on the pharmacokinetic profile of DCV. The findings observed in this study could be used by clinical pharmacists adjust the DCV dose, when combined with FLX, during the HCV treatment.

Keywords: HCV treatment; Daclatasvir; HPLC analysis; Pharmacokinetic interaction; Fluoxetine.

1. Introduction

Hepatitis-C virus (HCV) is a chronic viral infection that is responsible for the annual death of approximately 700.000 humans while the highest global infection rate in a population was recorded in Egypt [1, 2]. During the early stages of HCV infection, patients may experience no or mild symptoms that become more severe if not treated. The major complication of HCV infection is the chronic liver cirrhosis that may lead to further fetal problems including liver cancer, liver failure, and death [2]. During the last decades, the trend for the treatment of HCV was based on using pegylated interferons, however, the major concern was that their adverse effect on mental condition of patients, while depression was the most frequently reported disorder [3]. Recently, direct-acting antiviral (DAA) agents were discovered and recommended by the World Health Organization (WHO) for the treatment of HCV instead of pegylated interferon. Such drugs have been listed in the WHO Essential Medicines for 2015 and were reported to achieve a recovery rate up to 95% [4]. However, psychiatric disorders in patients diagnosed with HCV are still a challenge when treated with DAA. For example, studies have shown that ~50% of the HCV patients were suffered from depression whereas anxiety was reported for almost 41% of HCV patients and other psychiatric disorders, such as bipolar and stress, were also seen [5]. While the exact reason for these psychiatric changes, associated with HCV, is still unknown, nevertheless, the U.S. National Institutes of Health recommended the management and treatment of depression before and during the HCV treatment [6]. This necessitates the use of a combination between DAA and an antidepressant to control the HCV and manage any related depression.

Daclatasvir (DCV) is a highly effective DAA agent that has been reported for the treatment of HCV via the blocking of viral RNA replication. While DCV is a highly selective against HCV the major

advantage of this drug is the high potency, which permits a once daily dose [7]. To manage the psychiatric disorders and depression associated with HCV infection, selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (FLX) have been recommended [8]. This combined therapy could be helpful for the treatment of HCV and management of depression, it could increase the drug-drug interactions that could negatively influence the treatment efficiency [9]. Due to the limited available data for studying the potential pharmacokinetic interaction between DCV and antidepressants [10], further research is needed to study any possible interaction between DCV and FLX; as a commonly co-prescribed antidepressant.

Many methods have been reported for the detection of DCV in bulk or biological fluids including chromatographic [11-15], electrochemical [16], spectrofluorimetric and spectrophotometric methods [17-21]. Recently, a high-performance liquid chromatography (HPLC) coupled with fluorescence detection was utilized for the analysis of DCV over the range from 20 to 200 ng/mL, based on its native fluorescence, [22]. In addition, the native fluorescence of DCV has been employed to investigate the pharmacokinetic interaction between DCV and another antiviral agent [23]. To the best of our knowledge, none of the previously reported methods have been applied to investigate any potential interaction between DCV with other antidepressant drugs that are usually combined during the HCV treatment. In addition, most of these methods are time-consuming, require many chemicals for analysis and well-trained personnel. Further, such methods utilize a sophisticated procedure for analyte extraction from the plasma, prior to the analysis, while the reported sensitivity is relatively low [16, 22]. This reflects the requirement for a simple and sensitive method for the analysis of DCV in the presence of the co-administrated antidepressant drug (FLX) to investigate any potential pharmacokinetic interactions. Such study could be useful for physicians and clinical pharmacists to evaluate the treatment process, monitor any potential interaction and adjust the required DCV dose.

The main aim of this study is to develop and optimize a simple analytical method for the simultaneous and sensitive detection of DCV in the plasma samples collected from HCV patients. This method harnesses the detection of the native fluorescence of DCV, combined with HPLC for efficient separation, after pre-column extraction of DCV from plasma samples using acetonitrile to precipitate plasma proteins followed by simple filtration. This enabled the detection of DCV without any interferences from the plasma components and with relatively high sensitivity. In addition, many factors that could influence the procedure have been studied and optimized to improve the sensitivity, selectivity and detection range. Furthermore, the method was analytically validated according to the International Conference on Harmonization (ICH) guidelines to permit the application in quality control laboratories. In addition, the proposed method was also applied for studying the pharmacokinetic interaction between DCV and FLX in HCV patients. Overall, the proposed method exhibits many advantages in comparison to the previously reported methods for the determination of DCV in terms of simplicity, few chemicals needed, and considerable sensitivity.

2. Materials and chemicals

2.1. Chemicals

Pure DCV powder was obtained from Optimus Drugs Ltd., India. Acetonitrile and methanol, both were of for HPLC grade, were purchased from Merck (Darmstadt, Germany). Double distilled water, produced by water purification system from Aquatron A4000 (Cole-Parmer Ltd, Staffordshire, UK), was used throughout this work. Sartorius handy balance H51 (Hannover, Germany), laboratory centrifuge (Megafuge 11 from Thermo Electron Industries, France) and Whatman syringe filter (0.45 µm; Sigma-Aldrich, MO, USA) were also used.

2.2. Preparation of solutions

A standard stock solution of DCV with a concentration of 1.0 mg/mL was prepared by dissolving in methanol. Further dilutions were made up with ethanol to produce the working standard solutions with concentration ranged from 1.0 up to 4000 ng/mL. All the solutions were prepared in amber mini-vials, wrapped with aluminum foil and stored at -8 °C until further use.

2.3. Chromatographic conditions and DCV detection

The HPLC system was composed of Sykam S 1130 HPLC quaternary pump with optional integrate online vacuum degasser (Sykam GmbH, Gewerbering, Germany). A Wakopack Handy-ODS column (150 × 4.6 mm; diameter of 5 µm from Wako Pure Chemical industries, Osaka, Japan) was used in this study. The fluorometric measurements were performed using a detector (RF-20A, Shimadzu, Kyoto, Japan) coupled with the HPLC system at 380 nm (λ_{em}) after excitation at 320 nm (λ_{ex}). The mobile phase used for separation of DCV was a mixture of acetonitrile: water (65:35 v/v) and flowed at 1.0 mL/min.

2.4. Plasma collection

Volunteers participated in this study were HCV-patients at the outpatient clinics at the Hospital of Sohag University, Sohag, Egypt. All these volunteers were informed about the aim and application of the study and gave a written permission to use samples collected from them for research. Blood samples were collected from the forearm vein into K_2-EDTA tubes and centrifuged at 2000g for 10 min at room temperature. The resultant plasma samples (supernatant) were placed in mini-amber vials, wrapped with aluminum foil and kept at -20°C prior to use.

2.5. Construction of the calibration curve for DCV using spiked human plasma

Into separated amber mini- vials, 25 µL of drug-free plasma sample were mixed with 20 µL of the working solutions of DCV, prepared at different concentrations (1-4000 ng/mL). The volume was completed to 250 µL with acetonitrile to precipitate the plasma protein content as reported [24]. The mixture was mixed well for 1.0 min and then centrifuged at 2000g for 10 min at room temperature. The supernatant was quantitatively transferred into a clean amber mini-vials after filtration with Whatman syringe filter. Finally, 20 µL of the filtrated solution was injected into the HPLC system against blank treated similarly without DCV. The calibration curve was constructed using six replicates of each concentration versus the mean of the corresponding peak area for each concentration.

2.6. Stability studies

Stability of DCV, spiked in human plasma, was tested after 12 h and 24 h at room temperature (25 ± 2 ◦C) or after storing in the fridge at 4 ◦C. Additionally, long-term stability was determined after

storage at -20◦C for 1 month before analysis and freeze thaw stability (three cycles at -20◦C and room temperature) was also investigated. All the results (n = 5) were compared with those obtained from freshly prepared samples.

2.7. Procedures for analysis of DCV in HCV patients

Volunteers in this study, that were diagnosed with HCV and depression, gave a written consent to collect and use their blood samples for the research conducted. All volunteers were administered a single daily dose of either DCV (60 mg/tablet) alone or combined with FLX (20 mg/tablet). Blood samples were collected into tubes containing K_2-EDTA prior to drug/s administration or after 0.5, 1.0, 2.0, 4.0, 8.0, 12 and 24 h. Samples collected were then centrifuged at 2000 g for 10 min at room temperature and the resultant plasma (in the supernatant) were collected and stored as previously mentioned. Following this, 25 µL of these plasma were transferred to amber vials and the volumes were completed to 250 µL with acetonitrile. The mixture was treated similarly as previously described for plasma that was spiked with DCV.

2.8. Pharmacokinetic study between DCV and FLX

Pharmacokinetic interaction between DCV and FLX was investigated using plasma samples collected from HCV volunteers who were given a single oral daily dose of either DCV (60 mg/tablet) or DCV (60 mg/tablet) and FLX (20 mg/tablet). Samples were collected at the time periods as abovementioned in the previous section. The concertation of DCV was analyzed using the calibration curve and the maximum serum concentration (C_{max}) and maximum concentration peak time (T_{max}) were calculated. The equation of 0.693/k (k= rate constant) was used to calculate the elimination half-life ($T_{1/2}$). Area under the curve for concentration vs. time ($AUC_{0\text{-}24h}$) was also calculated using moment analysis model. All the data are presented as the mean ± standard deviation (SD, n=5). Statistical analysis was carried out using the Microsoft Excel software with the built-in functions to calculate the student's *t*-test (t-value, confidence limit > 95%) and the probability (*p*-value); the results were compared with the critical values.

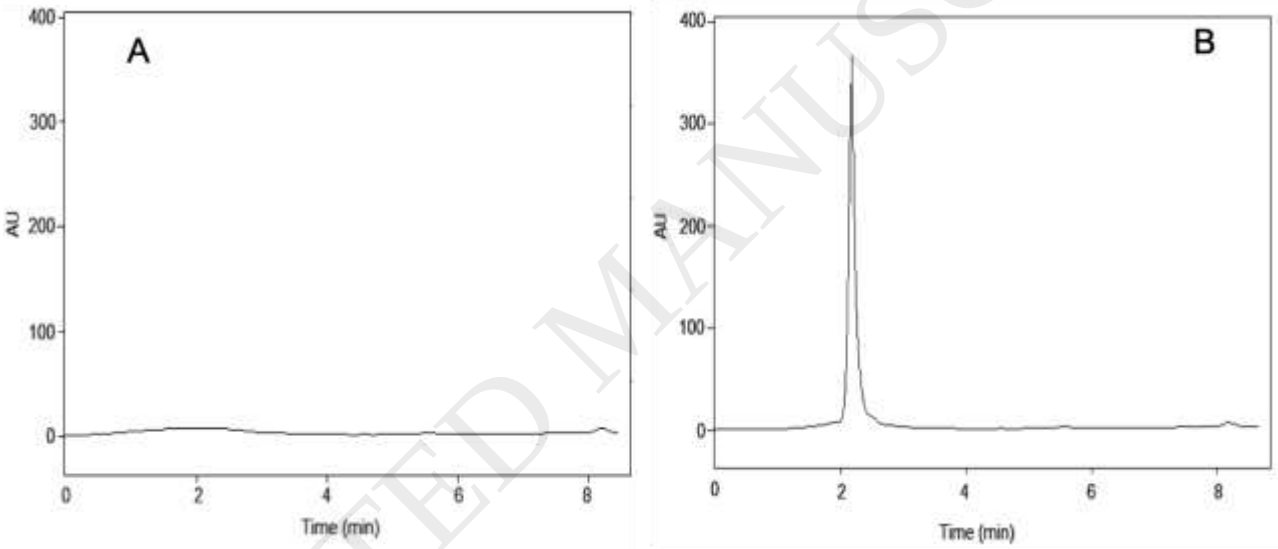
3. Results and discussion

3.1. Sample preparation and chromatographic conditions

Preliminary experiments were performed to optimize a method for extraction of DCV from plasma samples to achieve the maximum drug recovery that could improve the analysis sensitivity. Liquid-liquid extraction technique, that has been previously reported for extraction of drugs from plasma, was attempted [25]. Different solvents (methanol, chloroform, ethyl acetate, diethyl ether, and dichloromethane) were used for extraction of DCV after spiking in plasma. However, this technique exhibited a relatively low recovery of DCV after extraction from plasma. In addition, noticeable interference peak was observed in the chromatogram background due to plasma components (data not shown), which indicates inefficient extraction. Therefore, liquid-liquid extraction method was excluded from further experiments. Further, solid phase extraction was also tried to extract DCV from human plasma [26]. This procedure was able to extract more than 90% of spiked DCV, however, this technique requires tedious experimentation and is

expensive. In comparison, plasma protein precipitation has been reported for drug extraction from plasma with a good recovery [24]. This method is based on using of acetonitrile for precipitation of plasma proteins followed by a simple filtration to isolate the precipitated proteins and collect the spiked drug. Therefore, this simple procedure of extraction was employed in this study to efficiently extract the DCV from plasma samples and was used for all further experiments.

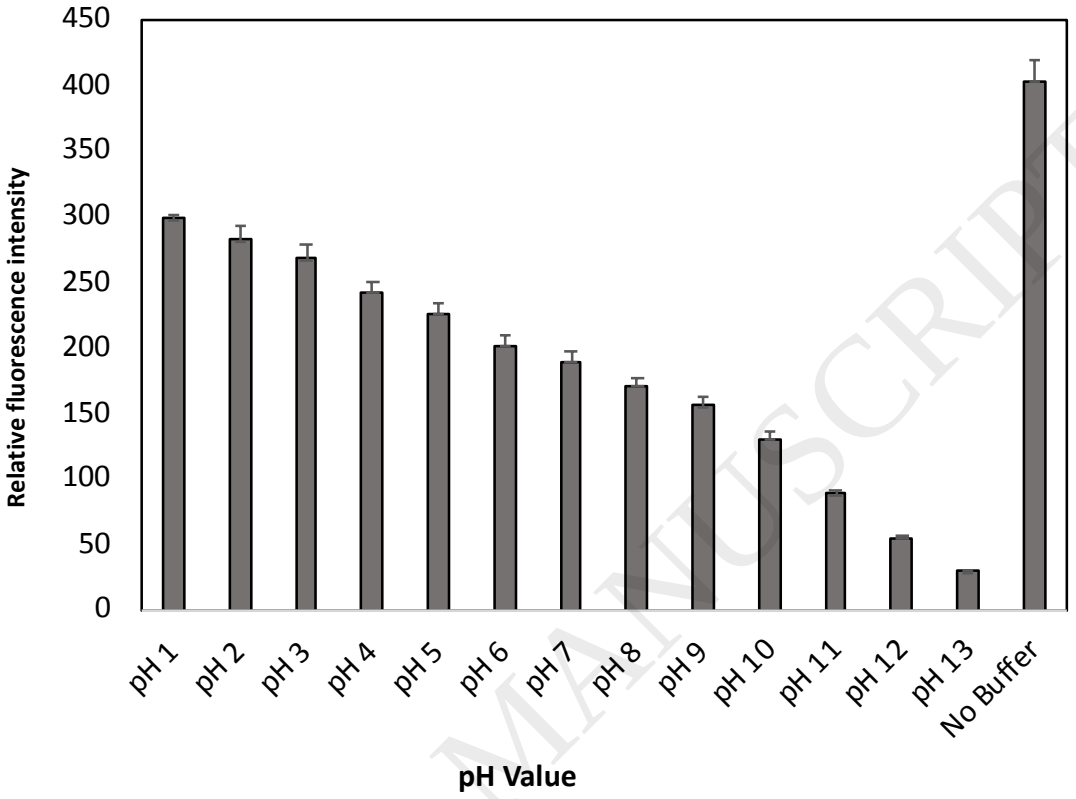
Meanwhile, different chromatographic conditions (mobile phase compositions, ratios, and HPLC columns) were optimized to achieve an intense (sensitive) peak for DCV without any interferences. The results showed that the optimum chromatographic conditions were obtained using an isocratic system of acetonitrile - water (65: 35 v/v) that was flowed at 1.0 mL/min through a Wakopack Handy-ODS® column (150 × 4.6 mm, diameter = 5 µm). After ~2.5 min of elution, an intense chromatogram peak for DCV was obtained as showed in **Figure 1.**



3.2. Optimization of experimental conditions

The analytical strategy reported in this study was based on using the native fluorescence of DCV for the detection, after extraction from plasma, precipitation of plasma proteins and separation on HPLC, using the optimized mobile phase. Therefore, many factors (pH, acid or base, temperature, time) that could influence the fluorescence intensity of DCV were investigated.

The effect of acid or base on the fluorescence intensity of DCV solution was studied using 0.1 M of either HCl (pH 1.0) or 0.1 M NaOH (pH 13.0) while the effect of pH value was studied using a wide ranged pH buffer system (0.2 M Teorell and Stenhagen buffer [27]) from pH 2 up to 12. The results showed that using acid, base or buffer (with different pH values) did not improve the fluorescence intensity when compared to the native fluorescence of DCV, recorded in ethanol. Increasing the pH value significantly quenched the native florescence of DCV. Therefore, acids, bases and buffer solutions were excluded from the present study (**Figure 2**).



Further, the effect of time on the fluorescence intensity of DCV was also studied at different time intervals ranged from 10 min up to 4 hours from sample preparation. This was performed to study the stability of the signal over time. The results showed that the fluorescence intensity did not significantly change over time which indicates the suitability of the method for analysis of DCV within 4 hours.

The effect of temperature on the native fluorescence intensity of DCV was investigated by heating the solution in different temperature to 70 ºC for 5 minutes. Increasing the temperature significantly decreased the fluorescence intensity of DCV solution. Therefore, measurements were performed at room temperature (25 ± 5 ºC).

3.3. Method validation

The proposed method for the analysis of DCV was validated in accordance with the official guidelines for analytical method validation as published by the International Conference on Harmonization (ICH) to permit application in quality control laboratories. Many statistical parameters including method linearity, range, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision and selectivity were detected and calculated [28].

3.3.1. Calibration curve parameters

Calibration curve of standard DCV spiked in plasma, with different concentrations, was established using the method described for extraction and precipitation for plasma proteins followed by HPLC analysis. The peak height of the resulting chromatograph for each concentration was used for construction of the calibration curve and the results showed a linear relationship was obtained over the concentration ranged from 1.0 to 4000 ng/mL with a good correlation coefficient (**Table 1**).

To examine the method sensitivity, the limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equation of LOD = 3 σ / S, LOQ = 10 σ / S, where σ is the standard deviation of intercept and S is the slope of the calibration curve [29]. As in **Table 1**, the method is considered as highly sensitive as LOD and LOQ were 0.025 and 0.083 ng/mL respectively. In comparison, previously reported methods for the fluorometric detection of DCV lack of good sensitivity and only can be used for the determination over a narrow linear range [22, 23], as illustrated previously in **Table 1**. The results obtained in this study render our method applicable over an extended linear range with relatively high sensitivity.

Table 1: Statistical parameters of the calibration curve of DCV analysis using the proposed method. (n =6). The least square equation was applied to calculate the linearity of the calibration

Parameter	Value
Linear range (ng/mL)	1.0-4000
Correlation coefficient (r)	0.9993
Limit of detection (LOD)	0.025 ng/mL
Limit of Quantification (LOQ)	0.083 ng/mL

3.3.2. Selectivity

Selectivity study was performed to detect DCV in the presence of other drugs such as ledipasvir, sofosbuvir, tenofovir, vildagliptin and linagliptin. None of these drugs exhibited any fluorescence peak at the selected wavelengths of DCV. Furthermore, FLX (10 ng/mL) was also added to the plasma sample spiked with DCV (n=6) and no chromatogram peak was found for FLX in the presence of DCV. This is due to that FLX does not have emission at 380 nm. These results indicate the selectivity of the proposed method for determination of DCV in human plasma.

3.3.3. Precision and accuracy

Precision and accuracy of the proposed method to detect DCV were evaluated using six replicates at four concentration levels within the linear range (1.0, 50, 1000 and 3000 ng/mL). The results were represented as the percentage of recovery ± relative standard deviation (RSD). The RSD for intraday precision (n =6) was less than 3.5 % of the recovery percentage for each corresponding concentration. While for interday precision, the RSD value was below 7.50. This determines the acceptable precision of the method to detect DCV in plasma (**Table 2**). In addition, the accuracy of the method was determined and expressed as recovery percentage of the spiked DCV. The results showed that the percentage of recovery of DCV, of all four concentrations spiked in plasma, was ranged from 95 to 100 %. As represented in **Table 2** the results indicate the acceptable precision and accuracy of this method to detect DCV in plasma.

Table 2: The precision and accuracy of the proposed method to detect DCV in human plasma.

Concentration added (ng/mL)	Intra-day assay (n=6)			Inter-day assay (n=6)		
	Conc. Found (ng/mL)	RSD (%)	Recovery (%)	Conc. Found (ng/mL)	RSD (%)	Recovery (%)
1.0	0.981	3.50	98.10	0.952	7.50	95.20
50.0	50.0	3.0	100.0	48.20	7.22	96.40
1000	990	3.20	99.0	973	7.0	97.30
3000	2925	2.60	97.50	2850	6.60	95.0

3.3.4. Stability studies

Stability experiments of DCV spiked in human plasma were examined using low, medium and high concentrations of DCV at room temperature (at 12 h and 24 h), after overnight storage in the fridge (-4 ^{o}C) and freezing at -20 ^{o}C for 1 month. The percentage of recovery of each concentration of DCV under each specified condition was calculated (n=5) from the obtained chromatogram and compared with a freshly prepared sample containing the same concentration. As shown in **Table 3**, the obtained percentage of recovery under different conditions were ranged from 94.22 to 98.54 which indicates the sufficient stability of DCV in plasma either at room temperature, after store in the fridge or freezing, provided that the experiments should be performed within the time period specified for each storage condition.

Table 3: The stability study of DCV in plasma detected under different storage conditions and different time periods.

Condition	Spiked concentration		
	(1.0 ng/mL)	(1000 ng/mL)	(3000 ng/mL)
Room temperature (12 h)	97.45 ± 1.26	98.54 ± 1.62	96.45 ± 1.36
Room temperature (24 h)	96.80 ± 1.92	97.68 ± 1.29	97.85 ± 1.92
Overnight at -4^{o}C	97.32 ± 1.53	96.92 ± 1.35	98.23 ± 1.51
Freezing at -20^{O}c for month	95.62 ± 2.02	96.52 ± 2.02	97.62 ± 1.22

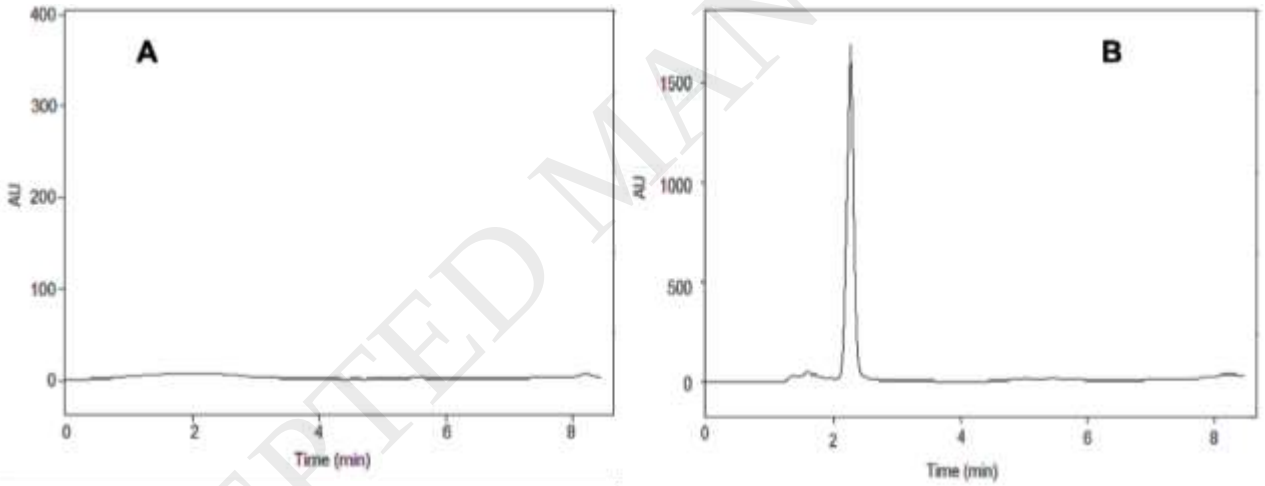
3.3.5. Dilution integrity

Dilution integrity experiments were performed to determine the optimum dilution range that can be used without influencing the analysis results. Six replicates of DCV prepared at three different concentrations (5, 250 and 500 ng/mL) were spiked in plasma and then diluted with 5-fold of

human plasma. DCV chromatogram was tested for three successive days. The mean percentage of recovery was calculated. The results showed minimal effect of plasma dilution on the resultant peak as the percentage of recovery was comparable to the results obtained previously. Moreover, the RSD value was below 3% of the mean which indicates the neglected effect of dilutions on the precision and accuracy of the method.

3.4. Pharmacokinetic study between DCV and FLX

After optimizing the method for the analysis of DCV in human plasma, this analytical method was applied to study any potential pharmacokinetic interaction between DCV and the most commonly prescribed antidepressant drug (FLX). Patients volunteered in this study were classified into two groups; one who administered DCV alone (60 mg/single daily dose) and others who were given a combination of DCV (60 mg/day) and FLX (20 mg/day). Blood samples were collected at different times post administration of drugs (either DCV or DCV combined with FLX) and the samples were treated as described in the experimental part. Analysis of DCV was performed as described in this study. As shown in **Figure 3**, the chromatogram obtained was similar to that obtained for DCV, spiked in plasma without any interreference from FLX. Using the calibration curve, the amount of DCV found in plasma equals to 940.8 ng/mL.



Further, blood samples were collected from both two groups after different time periods of DCV or DCV and FLX administration. Pharmacokinetics parameters (T_{max}, C_{max}, $T_{1/2}$ and AUC_{0-24}) were calculated for each group. As illustrated in **Table 4**, patients who were given FLX with DCV showed a significant change in the pharmacokinetic profile for the administered DCV, compared to those who administered DCV only. Such concentration-time profile for DCV administered alone or combine with FLX was determined and the results are shown in **Figure 4**. These results reflected that the level of DCV when co-administered with FLX was significantly elevated. This may be explained by the delayed metabolism of DCV, when administered with FLX, as both drugs are metabolized by cytochrome P450 3A4 isoenzyme [30]. Generally, administration of FLX with DCV significantly changed the pharmacokinetic parameters of DCV. Interestingly, the calculated C_{max} was changed from 930.70 ± 0.340.50 ng/mL to 1150.50 ± 275.80 and the AUC_{0-24} was significantly

changed from 11932.25±2345.05 to 16058.90±2284.40 ng.h/mL for those treated with DCV alone or combined with FLX, respectively.

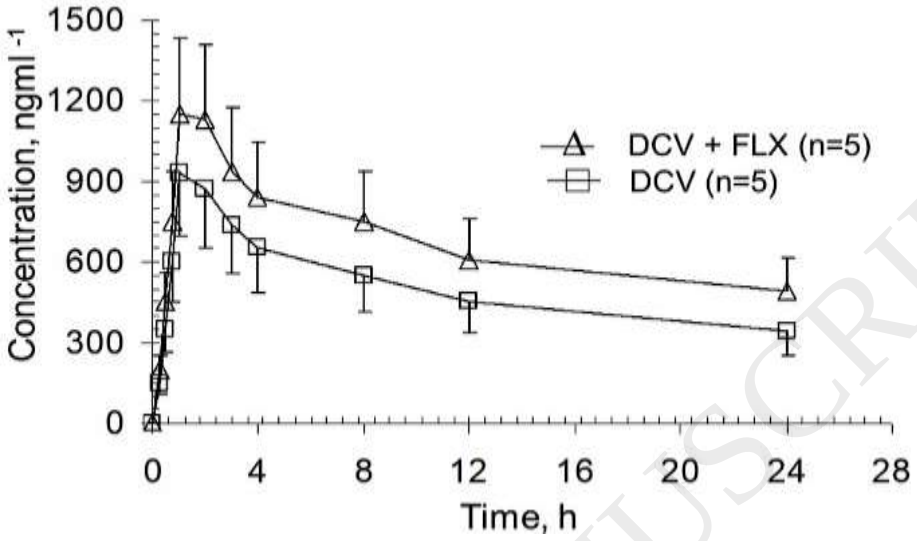


Table 4: The pharmacokinetics parameters for DCV in patients plasma when administered alone or combined with FLX

Parameter	**DCV**	**DCV + FLX**	***p* value***
T_{max} (h)	1.10 ± 0.30	1.20 ± 0.30	0.621
C_{max} (ng/mL)	930.70 ± 340.50	1150.50 ± 275.80	0.031*
$T_{1/2}$ (h)	11.50 ± 1.50	12.20 ± 2.10	0.215
AUC_{0-24}(ng·h/mL)	11932.25 ± 2345.05	16058.90 ± 2284.40	0.018*

* When *p*> 0.05 means the result is not significantly different.

To the best of our knowledge, no previous study has shown such pharmacokinetic interaction between DCV and FLX before. We postulated such interactions due to the influence of both drugs on cytochrome P 450 activity. However, further research is needed to study the underlying mechanism of such interaction. Overall, this study reflects the need for dose readjustment of DCV when co-administered with antidepressant drugs such as FLX as an exemplar.

In comparison to other previously reported methods for the determination of DCV, the proposed method exhibits many advantages in terms of simplicity, few chemicals needed, and considerable sensitivity as shown in **Table 5**. While the reported sensitivity of some previous methods was extremely high, as those based on using the ultra-performance liquid chromatography with a tandem mass detector (UPLC/MS-MS). Nevertheless, these methods require a lot of chemical and steps for analyte extraction and the analysis process, which renders their application in clinical laboratories difficult. In comparison, the proposed method exhibits high sensitivity, short

time of analysis and utilize few chemicals for extraction and analysis that permits application in quality control and clinical laboratories.

Table 5: A comparison between the previously reported methods and the proposed method for the detection of DCV

Method/Technique	Range	LOD	Sensitivity	Reference(s)
Spectrophotometry	2 - 50 µg/mL	0.15 µg/mL	Very low	[19-21]
Spectrofluorometry	0.2 - 2000 ng/mL	0.8 - 0.1 ng/mL	Relatively high	[17, 18, 22, 23]
Electrochemical sensor	738 ng/mL - 8.8 µg/mL	650 ng/mL	Moderate	[16]
UPLC/MS-MS detector	0.05 - 5000 ng/mL	0.05 - 5 ng/mL	Extremely high	[11-13, 15]
HPLC/Fluorescence detector	20 - 200 ng/mL	4.46 ng/mL	Relatively low	[22]
HPLC/UV detector	0.05 -10 µg/mL	0.02 µg/mL	Low	[14]
Our method	1 - 4000 ng/mL	0.025 ng/mL	Relatively high	

4. Conclusions

A simple and sensitive method has been developed for the quantitative analysis of an antiviral agent, daclatasvir, in real plasma samples collected form volunteering patients. This method was based on coupling a fluorometric detector with a high-performance liquid chromatography (HPLC) instrument to detect the studied drug over a linear range between 1.0 to 4000 ng/mL. Pre-column treatment procedure for the precipitation and filtration of plasma proteins, using acetonitrile, was performed prior to the analysis to get rid of any interfering species. Furthermore, the method was also validated according to the official guidelines. Additionally, the study was extended to study the pharmacokinetic interaction between DCV and a co-prescribed antidepressant drug, fluoxetine, in plasma samples collected from volunteering HCV patients. Plasma was collected from patients who treated with either daclatasvir alone or combined with fluoxetine were involved in this study to investigate such effect. In comparison to previous methods, the proposed method exhibits many advantages for the determination of DCV in terms of simplicity, few chemicals needed, and high sensitivity. Our findings reflect the need for further research to study the exact mechanism, nevertheless, this study could be useful for the physicians and clinical pharmacists to re-adjust doses of daclatasvir when co-prescribed with an antidepressant drug to achieve the maximum treatment efficiency.

5. Acknowledgements

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6. Conflict of Interest

The authors confirm that there is no conflict of interests.

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Figure 1: (A) Chromatogram of DCV free human plasma samples. (B) Plasma spiked with DCV (1.0 ng/mL) and the chromatogram was established detected using the proposed procedure after plasma protein precipitation.

Figure 2: The influence of different pH values on the fluorescence intensity of DCV (1.0 nm/mL) spiked in human plasma. Fluorescence was recorded at 380 nm using an excitation at 320 nm.

Figure 3: (A) Chromatogram obtained from human plasma before starting the administration of DCV or FLX. (B) After 3 hours of giving the patients a combination of DCV (60mg/tablet) and FLX (20mg/tablet).

Figure 4: Concentration-time profile of DCV in human plasma after a single administration of DCV (60mg/tablet) alone or combined with FLX (20mg/tablet). Data are expressed as mean ± SD (n= 5).