



## ORIGINAL ARTICLE

# Direct injection HILIC–MS/MS analysis of darunavir in rat plasma applying supported liquid extraction



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## Abstract

A novel bioanalytical method was developed and validated for the quantitative determination of darunavir (DRV) in rat plasma by employing hydrophilic interaction chromatography and tandem mass spectrometry (HILIC–MS/MS) with supported liquid extraction (SLE). Irbesartan (IRB) was used as an internal standard (IS). The analyte in rat plasma (200  $\mu$ L) was isolated through SLE using ethyl acetate as the eluting solvent. The chromatographic separation was achieved on Luna-HILIC (250 mm  $\times$  4.6 mm, 5  $\mu$ m) column with a mobile phase of 0.1% of formic acid in water:acetonitrile (5: 95, v/v), at a constant flow rate of 1.0 mL/min. The MS/MS ion transitions for DRV (548.1  $\rightarrow$  392.0) and IS (429.2  $\rightarrow$  207.1) were monitored on an ion trap mass spectrometer, operating in the multiple reaction monitoring (MRM) mode. The lower limit of quantitation (LLOQ) was 0.2 ng/mL and quantitation range was 0.2–5000 ng/mL. The method was validated for its selectivity, sensitivity, carryover, linearity, precision, accuracy, recovery, matrix effect and stability. The method was successfully applied to pharmacokinetic study in rats.

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## 1. Introduction

Hydrophilic interaction chromatography (HILIC) has been gaining importance and utilization since this concept was first introduced by Alpert in 1990 [1]. HILIC is complimentary to

reverse phase liquid chromatography and provides good retention and unique selectivity for polar compounds. It has been proven to be a valuable technique for analyzing polar drugs and metabolites in biological matrices. The highly volatile organic mobile phase used in HILIC can provide increased sensitivity with ESI-MS. In addition, the organic extracts from protein precipitation (PPT), liquid–liquid extraction (LLE) or solid phase extraction (SPE) are compatible with the higher organic content in HILIC mobile phases and can often

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be directly injected onto a HILIC column [2–9]. This results in elimination of the time-consuming evaporation and reconstitution steps during preparation of biological samples. In bioanalysis, mostly, liquid samples including whole blood, plasma, serum, urine or saliva are handled. Thus an appropriate sample preparation technique should be properly chosen according to the needs of the developed method. Such methods should facilitate a sample preparation step keeping the main focus on decrease of sample amount and solvent consumption, shortening sample preparation time, reducing the number of steps, minimizing analysis cost and enabling automation. PPT, LLE and SPE are among the most commonly used sample cleanup techniques for biological sample analysis. However, supported liquid extraction (SLE) is a newly developed sample cleanup technology which is particularly suitable for the 96-well format operation. Similar to the traditional LLE, SLE provides very clean extracts with a high recovery and such bioanalytical LC–MS/MS or GC–MS assays have been developed and validated using SLE packed with diatomaceous earth material [10–18]. Also several methods have been published to quantify darunavir (DRV) in different biological matrices alone [19–22], and in combination [23–33] with many other protease inhibitors, nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI), integrase inhibitor, raltegravir and an entry inhibitor, maraviroc. The above methods require solvent evaporation and reconstitution steps prior to sample injection, while the present method facilitates direct injection. To the best of our knowledge, there has been no published HILIC–MS/MS method for the analysis of DRV alone in rat plasma using SLE. The aim of the present work was to demonstrate the application of a combination of SLE with direct injection of the organic extract into a HILIC–MS/MS system, for the simple and rapid analysis of DRV in rat plasma adopting SLE for sample preparation. The SLE extract can be injected directly onto the HILIC column, eliminating the need for lengthy evaporation and reconstitution steps, and increasing higher throughput. The retention times of DRV and the internal standard (IS) were low enough (3.3 and 4.5 min), allowing a short run time of 5.0 min. These improvements helped to develop a rapid, simple and sensitive SLE–HILIC–MS/MS method for the determination of DRV in rat plasma and thus the method was successfully applied to a pharmacokinetic study.

## 2. Experimental

### 2.1. Chemicals and materials

Reference standard of DRV (purity, 99.4%) and irbesartan (IRB) (purity, 99.8%) were procured from Hetero Drugs Limited (Hyderabad, India). HPLC grade methanol, acetonitrile, methyl *tert*-butyl ether, diethyl ether, ethyl acetate, dichloro methane, *iso*-propyl alcohol and formic acid were obtained from Merck Specialties Pvt. Ltd. (Mumbai, India). Water used in the entire analysis was prepared from Milli-Q water purification system procured from Millipore (Bangalore, India). Freshly obtained drug free rat plasma was collected from male Wistar rats in our laboratory and stored at  $-80^{\circ}\text{C}$  prior to use.

### 2.2. LC–MS/MS analysis

Chromatographic separation was accomplished by HILIC using the isocratic mode of elution. Chromatography was performed on Luna–HILIC (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) column. LC–MS/MS studies were performed using an Agilent 1100 MSD ion-trap–SL mass spectrometer with ESI source in positive-ion mode equipped with a degasser (G1379A), binary pump (G1312A), autosampler (G1329A),

autosampler thermostat (G1329B) and diode array detector (G1315B). All the systems were procured from Agilent Technologies (Waldbronn, Germany). The data were acquired and processed using Chemstation 5.3 (Bruker, Waldbronn, Germany). An isocratic elution with 0.1% formic acid in water:acetonitrile (5:95, v/v) as mobile phase was pumped at a flow rate of 1.0 mL/min; the sample injection volume was 20  $\mu\text{L}$  and the column temperature was maintained at ambient conditions. The sensitivity of the multiple reaction monitoring (MRM) was optimized by testing with an infusion of 0.1  $\mu\text{g}/\text{mL}$  analyte and IS in mobile phase. Detection of the ions was carried out in the MRM, by monitoring the transition pairs of  $m/z$  548.1 precursor ions to the  $m/z$  392.2 for DRV and  $m/z$  429.2 precursor ion to the  $m/z$  207.0 product ion for the IS. Nitrogen was the nebulizer gas. Collision-induced dissociation was achieved using helium gas as collision gas. The ion source conditions were set as follows: temperature,  $300^{\circ}\text{C}$ ; nebulizer gas, 35 psi; dry gas, 10.0 L/min; skimmer, 40.0 V; capillary exit, 128.0 V; trap drive, 44.5; maximum accumulation time, 200 ms; Icc target, 30,000.

### 2.3. Preparation of standard solution, calibration standards and quality control (QC) samples

The standard stock solution of DRV (1000  $\mu\text{g}/\text{mL}$ ) was prepared by dissolving requisite amount in methanol. Calibration standards and QC samples were prepared by spiking blank plasma with stock solution. Calibration curve standards were made at 0.2, 0.5, 1.0, 4.0, 10.0, 50.0, 100, 350, 800, 1500, 3500 and 5000 ng/mL concentrations while QC samples were prepared at four concentration levels, 4000 ng/mL (HQC, high quality control), 2500 ng/mL (MQC, medium quality control), 3.0 ng/mL (LQC, low quality control) and 0.2 ng/mL (LLOQ QC, lower limit of quantification quality control). Stock solution (500  $\mu\text{g}/\text{mL}$ ) of the IS was prepared by dissolving 2.5 mg of IS in 5.0 mL of methanol. An aliquot of 20  $\mu\text{L}$  of this solution was further diluted to 10.0 mL in the same diluent to obtain a solution of 1.0 ng/ $\mu\text{L}$ . All the solutions (standard stock, calibration standards and QC samples) were stored at  $2-8^{\circ}\text{C}$  until use.

### 2.4. Sample preparation

SLE extraction was carried out with five different solvent systems for the effective extraction of analyte and their compatibility of being directly injected into HILIC–MS/MS system. Diethyl ether, ethyl acetate, dichloro methane, *iso*-propyl alcohol and methyl *tert*-butyl ether were found to be compatible for direct injection. A 200  $\mu\text{L}$  sample aliquot was diluted with 200  $\mu\text{L}$  of water, fortified with 50  $\mu\text{L}$  of 1 ng/ $\mu\text{L}$  IS working solution and loaded onto an isolute SLE+ cartridge. After the analyte was allowed to equilibrate with the sorbent for a minimum of 5 min, it was eluted with 1 mL extraction solvent but the process was hastened by applying a little pressure from the top with a rubber bulb initiating a reasonably faster elution [7,17], and approximately 20  $\mu\text{L}$  of the solution was injected directly into the HILIC–MS/MS system for analysis.

### 2.5. Method validation

The validation process was carried out according to Guidance for Industry–Bioanalytical Method Validation, recommended by U.S. FDA [34]. The assay was validated for selectivity, sensitivity (LLOQ), linearity, precision, accuracy, recovery, matrix effect (ME) and stability.

## 2.6. Pharmacokinetic studies

The applicability of the developed bioanalytical method (SLE-HILIC/ESI-MS) for DRV in rat plasma was demonstrated by the results obtained from pharmacokinetic studies conducted in six male Wistar rats weighing  $180 \pm 10$  g approximately which were fasted overnight before and 4 h after DRV dosing (The study was approved of by the Animal Ethical Committee of Indian Institute of Chemical Technology, Hyderabad). Each rat received an oral dose of 50 mg/kg of DRV in gum acacia suspension. Blood samples (0.5 mL) were collected from orbital sinus into EDTA coated tubes at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h time intervals after drug administration. The blood samples were centrifuged at 5000 rpm for 10 min and the plasma samples were stored at  $-80^\circ\text{C}$  until analysis. The plasma samples were then analyzed by using the developed SLE-HILIC/ESI-MS method. Plasma drug concentration–time data were subjected to noncompartmental pharmacokinetic analysis using linear trapezoidal rule.

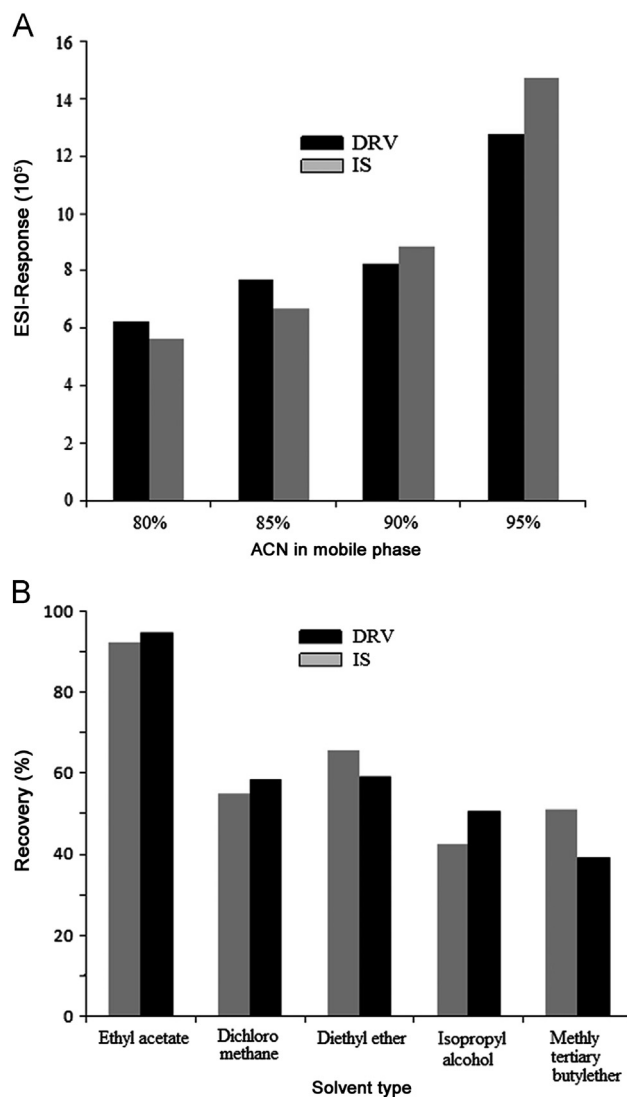
## 3. Results and discussion

### 3.1. Mass spectrometry

Mass spectrometric parameters were tuned in both positive and negative ionization modes for DRV and IS. However, comparatively a good response was found in positive ionization mode. The MS parameters were optimized to maximize the response. Acetonitrile offered a higher response than methanol and was therefore chosen as the organic modifier in the eluent. Prior to analysis a thorough study was undertaken to arrive at a suitable solvent system. It was observed that a decrease in polarity enhanced the chromatographic selectivity and facilitated a greater sensitivity with respect to mass spectrometric signal intensities (Fig. 1A). These subtle but distinguishable features were monitored with various combinations of water:acetonitrile but only water:acetonitrile (5:95 v/v) with 0.1% formic acid offered the best of selective separation and made a huge chromatographic distinction with no noticeable deterioration of peak shape with a typical run time of 5 min. The amount of formic acid was optimized to obtain a maximal MS response. Addition of 0.1% formic acid to the mobile phase was thus found to be an important factor for acquiring the higher sensitivity. The mass spectra for DRV and IS showed peaks as protonated molecular ions  $[\text{M}+\text{H}]^+$  at 548.1 and 429.2  $m/z$ , respectively. The major fragment ions observed in each product spectrum were at 392.2  $m/z$  and 207.0  $m/z$  for DRV and IS, respectively (Fig. 2).

### 3.2. Liquid chromatography

The liquid chromatography was optimized on a HILIC column under isocratic conditions. Under HILIC conditions, the analyte interacts with a hydrophilic stationary phase and is eluted with a high concentration of organic solvent (typically acetonitrile with a small percentage of water/buffer). The highly organic mobile phase can result in increased sensitivity with ESI-MS detection. At the same time, HILIC columns often allows direct injection of organic extract into the LC–MS/MS system so that the dry-down and reconstitution steps can be eliminated without compromising the chromatographic peak shape and quality. Choosing the appropriate IS is an important aspect to achieve acceptable method

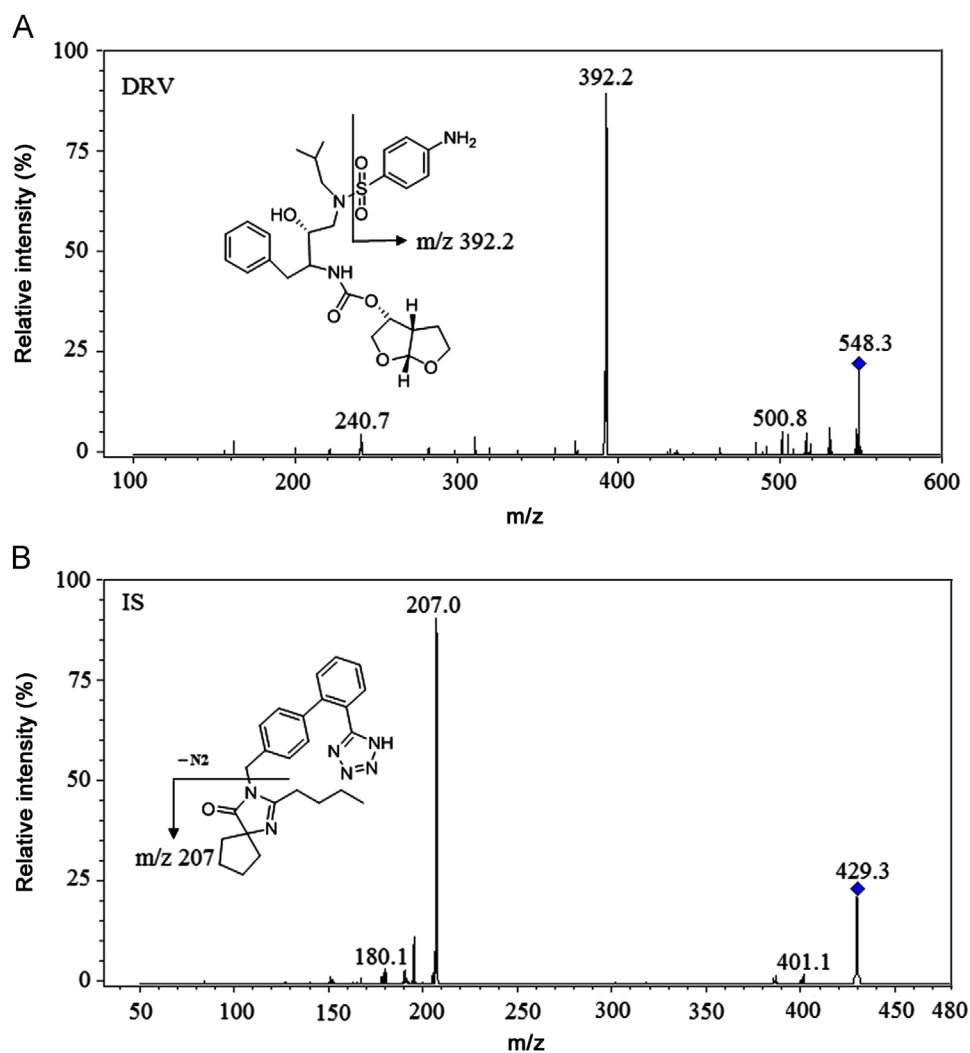


**Fig. 1** (A) ESI-MS signal at different portions of ACN in mobile phase and (B) effect of different solvent types on the extraction efficiency.

performance, so different compounds were analyzed to arrive at a suitable IS but IRB offered high recovery, less analytical run time and well separated from the analyte under study with better chromatographic selectivity. Chromatographic separation was accomplished using isocratic mobile phase system consisting of 0.1% formic acid and acetonitrile (5:95, v/v), and HILIC column facilitated good peak shape and response even at the lowest concentration level for both the analyte and IS (Fig. 3). The mobile phase was operated at a flow rate of 1.0 mL/min. The retention time of DRV and the IS was low enough (3.3 and 4.5 min), allowing a short run time of 5.0 min.

### 3.3. Optimization of sample preparation

Traditional LLE is the most commonly used effective extraction technique across a wide range of applications, but its requirement for off-line preparation renders the method unsuitable for high-throughput sample preparation. SLE is a newly developed sample preparation technique that is similar to the traditional LLE. The



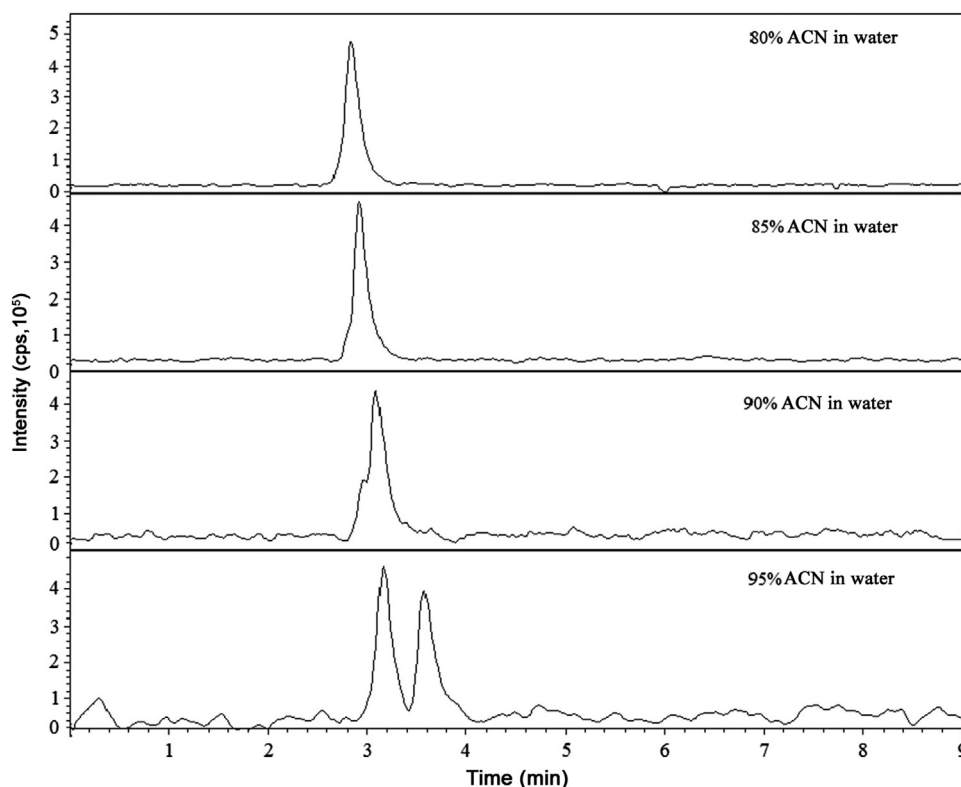
**Fig. 2** Product ion mass spectra of (A) DRV ( $m/z$  548.1→392.0, scan range 100–700 amu) and (B) IS ( $m/z$  429.3→207.0, scan range 50–500 amu) in positive ion mode.

SLE cartridge is packed with a modified form of diatomaceous earth. As the analyte under study is in plasma as 1:1 sample/ aqueous solution phase, a selective elution of the analyte alone is now required. Therefore, a solvent which is immiscible with water was used for preferential and quantitative elution of analyte alone, leaving the aqueous portion having plasma behind which is like a simple partition. In addition to sample preparation, adequate chromatographic selectivity is an important requirement for valid, accurate and rugged analytical methods. Herein, a new approach to analyze DRV by HILIC–ESI–MS/MS is reported. This method features the direct injection of sample into an HPLC system, without solvent evaporation and reconstitution steps. In the SLE experiments, a number of organic solvents as diethyl ether, ethyl acetate, dichloride methane, *iso*-propyl alcohol and methyl *tert*-butyl ether were used for the elution. The percentage recoveries of analyte are given in Fig. 1(B). It is evident from Fig. 1(B) that with dichloro methane, *iso*-propyl alcohol and methyl *tert*-butyl ether the recoveries of DRV and IS were in the range of 45–55% and 40–60%, respectively. Elution with diethyl ether gave reasonable recoveries of DRV and IS in the range of 68% and 65% respectively. Among all the solvents studied, ethyl acetate was found to give cleaner extract and higher recovery (>90%) and

selected as an optimum solvent for extraction. Here, a combination of 500, 1000 and 1500  $\mu$ L eluent and 50, 100 and 200  $\mu$ L of plasma was also investigated. We found that when extracting 200  $\mu$ L plasma and 1000  $\mu$ L eluent improved the extraction recovery.

### 3.4. Method validation

Selectivity of the assay method was assessed by evaluating potential interference or background noise at the LC retention times for the analyte and IS from endogenous compounds. Six randomly selected blank plasma samples were tested. However, no significant interfering peak was observed in any of these 6 lots of blank plasma samples of analyte and IS, indicating that comparatively SLE was a cleaner procedure than other extraction methods [35] (Fig. 4). The linearity of the method was tested by adding appropriate amount DRV to blank plasma to yield the following concentrations: 0.2, 0.5, 1.0, 4.0, 10.0, 50.0, 100, 350, 800, 1500, 3500 and 5000 ng/mL, and analyzed in triplicate. The calibration curve was constructed by plotting the peak area ratios of analyte to IS vs the nominal concentration in plasma. The data were



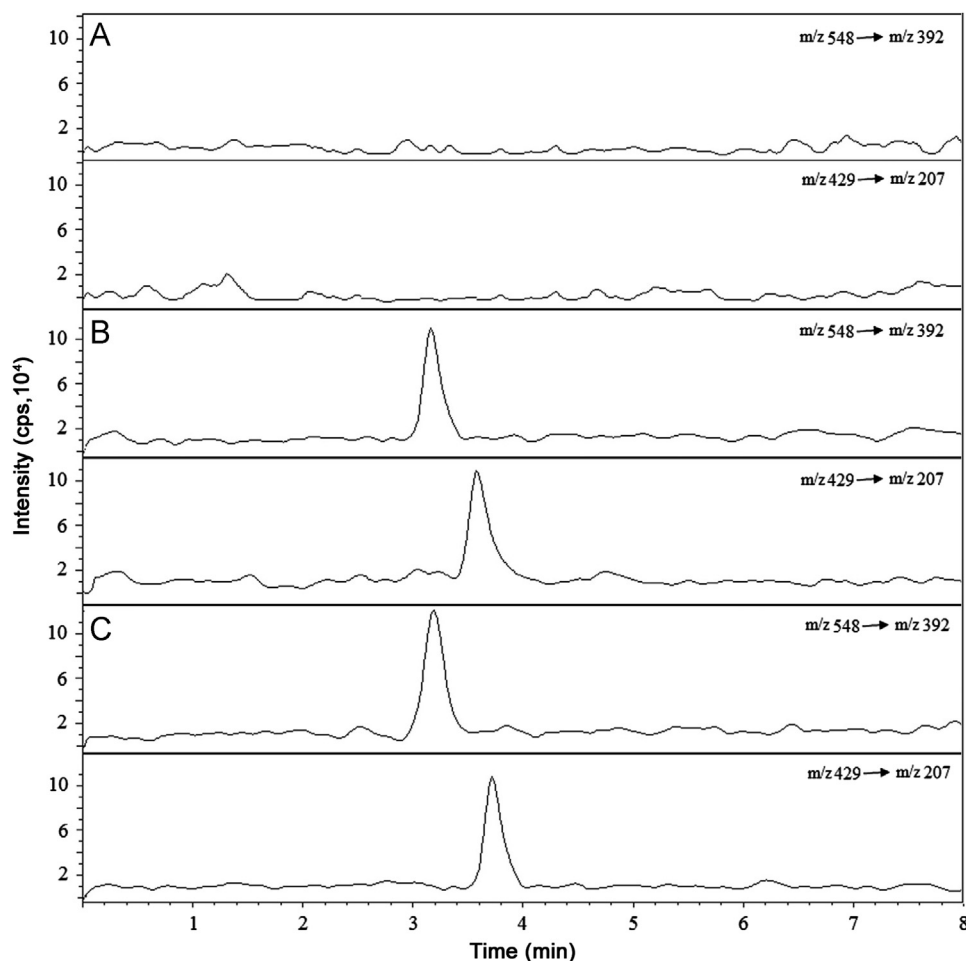
**Fig. 3** Effects of acetonitrile content in the mobile phase on DRV (100 ng/mL) retention time on the HILIC column.

subjected to statistical analysis using a linear regression model, showing that the regression equation was  $y=0.00012x-0.043$ , correlation coefficient ( $r^2$ ) was greater than 0.998 for DRV where ‘y’ represents the peak area ratio of analyte to that of IS and ‘x’ represents the concentration of analyte in ng/mL. The intra-day assay precision and accuracy were obtained by analyzing six replicates of LLOQ and QC samples on a single day. The inter-day assay precision and accuracy were obtained by analyzing six replicates of LLOQ and QC samples on 3 different days. **Table 1** shows a summary of intra- and inter-day precision and accuracy data for LLOQ and QC samples containing DRV. Both intra- and inter-precision CV (%) values ranged from 0.38% to 8.66%. The accuracy values were within 95.0–107.6%. These results indicated that the present method had the acceptable accuracy and precision. The extraction recovery of each analyte for three levels of QC samples was assessed by comparing the peak areas of extracted spiked plasma samples with the peak areas of pure compounds of the same concentrations in solvent. The recovery of the IS was evaluated at the concentration used in sample analysis. Matrix effect was investigated to ensure precision, selectivity and sensitivity that were not compromised by the matrix screened. Blank biological samples were extracted and then spiked with the analyte at three QC levels and IS in six replicates. The corresponding peak areas were compared to those of standard solutions, and the peak area ratio was defined as the matrix effect. **Table 2** shows the results of the recovery and the matrix effect for DRV and IS. The results suggested that ion suppression or enhancement from the plasma matrix was negligible in this study. The carryover was evaluated by analyzing a blank sample immediately after the ULOQ (5000 ng/mL) sample of the standard curve. The carryover level should be <20% of the response observed for the analyte with respect to LLOQ and <5% of the response observed for the IS at

the working concentration. No significant carryover was observed in the carryover analysis. Stability experiments should reflect the conditions likely to be encountered during sample transfer, handling and analysis. The stability studies were evaluated by measuring the area response (DRV/IS) of stability samples against freshly prepared comparison standards at LQC and HQC levels. The stabilities were examined under different study conditions, i.e., by keeping the samples at ambient temperature for 5 h (bench-top stability) and storing at  $-30\text{ }^\circ\text{C}$  for 50 days (long-term stability) and the stabilities of DRV in plasma extracts were also tested by placing samples at ambient temperature for 24 h (autosampler stability). Freeze/thaw stability was determined after freezing ( $-30\text{ }^\circ\text{C}$ ) and thawing for three cycles. All the stability studies were conducted at LQC and HQC levels using six replicates at each level. Samples are considered to be stable if assay values are within the acceptable limits of accuracy (<15% SD) and precision. The results are summarized in **Table 3**. The results indicate that DRV was stable for the entire period of the experiment.

### 3.5. Application

We have succeeded in applying this established SLE-HILIC/ESI-MS method to study pharmacokinetics of DRV in rats. The mean plasma concentration versus time profile of DRV is shown in **Fig. 5**. The non-compartmental pharmacokinetic parameters of DRV are summarized in **Table 4**. The peak plasma concentration ( $C_{\max}$   $130\pm 4$  ng/mL) was achieved at 3 h ( $T_{\max}$ ). The elimination half-life ( $t_{1/2}$ ) of DRV was  $5.31\pm 0.71$  h, while the  $AUC_{(0-t)}$  and  $AUC_{(0-\infty)}$  were  $1014\pm 48$  ng mL/h and  $1064\pm 49$  ng mL/h respectively. The ratio of mean value of  $AUC_{(0-t)}$  to that of  $AUC_{(0-\infty)}$  was 95.3%.



**Fig. 4** Representative MRM chromatograms of (A) blank rat plasma, (B) rat plasma spiked with 0.2 ng/mL (LLOQ) DRV, 50 ng/mL IS and (C) a rat plasma sample obtained 1.5 h after an intravenous administration of DRV.

**Table 1** Intra- and inter-day precision and accuracy for the detection of DRV in rat plasma.

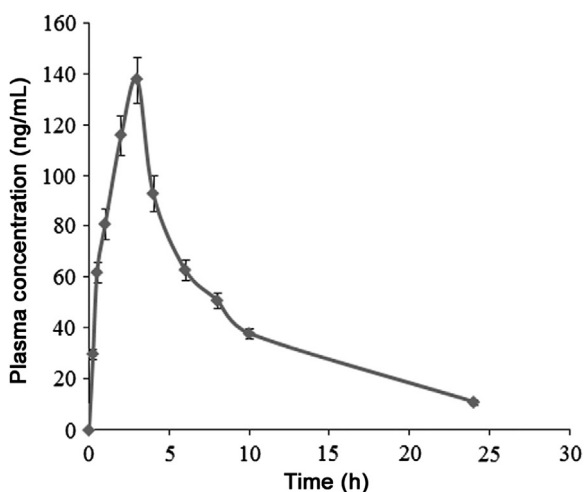
Analyte	Spiked concentration (ng/mL)	Intra-day ( $n=6$ )			Inter-day ( $n=6$ )		
		Mean (ng/mL)	Accuracy (%)	CV (%)	Mean (ng/mL)	Accuracy (%)	CV (%)
DRV	0.2	0.19	95.0	5.27	0.21	105.0	4.71
	3.0	3.12	104.0	6.84	3.23	107.6	8.66
	2500.0	2543.13	101.7	0.43	2552.05	102.0	0.53
	4000.0	4063.08	101.5	0.38	4074.14	101.8	0.59

**Table 2** The recovery and matrix effect of DRV and IS ( $n=6$ ).

Analyte	Concentration (ng/mL)	Recovery (%)		Matrix effect (%)	
		Mean $\pm$ SD	RSD (%)	Mean $\pm$ SD	RSD (%)
DRV	3	95.27 $\pm$ 2.52	2.65	94.13 $\pm$ 2.54	2.68
	2500	96.42 $\pm$ 2.19	2.28	95.56 $\pm$ 4.01	4.17
	4000	94.16 $\pm$ 1.34	1.42	96.81 $\pm$ 3.51	3.64
IS	50	92.13 $\pm$ 1.84	1.99	93.16 $\pm$ 3.87	4.16

**Table 3** Stability of DRV in rat plasma ( $n=6$ ).

Stability tested	Concentration added (ng/mL)	Concentration found (ng/mL)	Precision (RSD, %)	Accuracy (RE, %)
Autosampler stability (at 10 °C for 24 h)	3.0	2.95 ± 0.11	3.72	−3.33
	3000.0	3025.13 ± 3.21	1.28	1.25
Bench-top stability (5 h at room temperature)	3.0	3.35 ± 0.05	8.47	−1.66
	3000.0	3013.24 ± 3.58	0.88	0.75
Freeze–thaw stability (three cycles)	3.0	3.18 ± 0.04	6.34	5.00
	3000.0	3020.17 ± 2.89	0.71	1.00
Long-term stability (at −70 °C for 50 days)	3.0	2.98 ± 0.03	4.68	6.60
	3000.0	2994.3 ± 4.24	1.05	0.75

**Fig. 5** Mean plasma concentration–time profile of DRV after an oral 50 mg/kg dose of DRV to male Wistar rats.**Table 4** Pharmacokinetic parameters of DRV after oral administration at a dose of 50 mg/kg to rats ( $n=6$ ).

Pharmacokinetic parameters	Mean ± SD
$C_{max}$ (ng/mL)	130 ± 4
$T_{max}$ (h)	3.00 ± 0.28
$t_{1/2}$ (h)	5.31 ± 0.71
$AUC_{0-t}$ (ng h/mL)	1014 ± 48
$AUMC_{0-t}$ (ng h <sup>2</sup> /mL)	6518 ± 386
$AUC_{0-\infty}$ (ng h/mL)	1064 ± 49
$AUMC_{0-\infty}$ (ng h <sup>2</sup> /mL)	8408 ± 914
$MRT_{0-\infty}$ (h)	7.89 ± 0.83

#### 4. Conclusions

A high-throughput SLE-HILIC–MS/MS method for the determination of DRV in rat plasma has been successfully developed and validated. The SLE is a clean sample preparation technique with better extraction efficiency. The HILIC column allows the direct injection of the SLE extracts. Thus, the tedious manual steps of evaporation and reconstitution are eliminated but facilitated an increased and high throughput recovery. The advantages of the present approach include avoiding loss of compound during evaporation and reconstitution due to instability, evaporation, or adsorption. Furthermore a very low column back pressure

produced under HILIC conditions facilitates increased mobile phase flow rate to enhance the throughput. The method employed herein is a simple but novel, rapid and selective analytical option. SLE extraction with direct injection is an unique value addition to the present day requirement of higher throughput through cleaner technologies where an analyte is detected at LLOQ levels of 0.2 ng/mL using 0.2 mL plasma with a calibration range of 0.2–5000 ng/mL. Thus, the present method finds a potential utility for the bioassay of chemical compounds.

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#### References

- [1] A.J. Alpert, Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds, *J. Chromatogr. A* 499 (1990) 177–196.
- [2] Q. Song, W. Naidong, Analysis of omeprazole and 5-OH omeprazole in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry (HILIC–MS/MS) – eliminating evaporation and reconstitution steps in 96-well liquid/liquid extraction, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 830 (2006) 135–142.
- [3] Y. Hsieh, Potential of HILIC–MS in quantitative bioanalysis of drugs and drug metabolites, *J. Sep. Sci.* 31 (2008) 1481–1491.
- [4] H.P. Nguyen, K.A. Schug, The advantages of ESI-MS detection in conjunction with HILIC mode separations: fundamentals and applications, *J. Sep. Sci.* 31 (2008) 1465–1480.
- [5] T.M. Baughman, W.L. Wright, K.A. Hutton, et al., Determination of zanamivir in rat and monkey plasma by positive ion hydrophilic interaction chromatography (HILIC)–tandem mass spectrometry, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 852 (2007) 505–511.
- [6] B. Dejaegher, D. Mangelings, Y. Vander Heyden, et al., Method development for HILIC assays, *J. Sep. Sci.* 31 (2008) 1438–1448.
- [7] P. Jiongwei, J. Xiangyu, C. Yu-Luan, et al., Automatic Supported Liquid Extraction (SLE) coupled with HILIC–MS/MS: an application to method development and validation of erlotinib in human plasma, *Pharmaceutics* 2 (2010) 105–118.
- [8] J. De Smet, K. Boussery, P. De Cock, et al., A bio-analytical hydrophilic interaction LC–MS/MS method for the simultaneous quantification of omeprazole and lansoprazole in human plasma in support of a pharmacokinetic omeprazole study in children, *J. Sep. Sci.* 33 (2010) 939–947.
- [9] H. Nordberg, G. Jerndal, R.A. Thompson, et al., Direct injection of lipophilic compounds in the organic phase from liquid–liquid

- extracted plasma samples onto a reversed-phase column, *Bioanalysis* 3 (2011) 1963–1973.
- [10] R. Nageswara Rao, K. Guru Prasad, K.V. Sravan Kumar, et al., Diatomaceous earth supported liquid extraction and LC–MS/MS determination of elvitegravir and ritonavir in rat plasma: application to a pharmacokinetic study, *Anal. Methods* 5 (2013) 6693–6699.
- [11] A.L. Edel, M. Aliani, G.N. Pierce, et al., Supported liquid extraction in the quantitation of plasma enterolignans using isotope dilution GC/MS with application to flaxseed consumption in healthy adults, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 912 (2013) 24–32.
- [12] A.Q. Wang, W. Zeng, D.G. Musson, et al., A rapid and sensitive liquid chromatography/negative ion tandem mass spectrometry method for the determination of an indolocarbazole in human plasma using 96-well diatomaceous earth plates for solid–liquid extraction, *Rapid Commun. Mass Spectrom.* 16 (2002) 975–981.
- [13] H. Jiang, C. Randlett, H. Junga, et al., Using supported liquid extraction together with cellobiohydrolase chiral stationary phases-based liquid chromatography with tandem mass spectrometry for enantioselective determination of acebutolol and its active metabolite diacetolol in spiked human plasma, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 877 (2009) 173–180.
- [14] G. O'Maille, S.M. Pai, X. Tao, et al., An improved LC–ESI-MS–MS method for simultaneous quantitation of rosiglitazone and N-desmethyl rosiglitazone in human plasma, *J. Pharm. Biomed. Anal.* 48 (2008) 934–939.
- [15] G. Liu, J. Liu, K. Hara, et al., Rapid determination of cyanide in human plasma and urine by gas chromatography–mass spectrometry with two-step derivatization, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 877 (2009) 3054–3058.
- [16] S. Wu, W. Li, T. Mujamdar, et al., Supported liquid extraction in combination with LC–MS/MS for high-throughput quantitative analysis of hydrocortisone in mouse serum, *Biomed. Chromatogr.* 24 (2010) 632–638.
- [17] G. Zhilong, C. Kiresha, W. Stephen, et al., Simple and rapid determination of norethindrone in human plasma by supported liquid extraction and ultra performance liquid chromatography with tandem mass spectrometry, *Talanta* 91 (2012) 77–82.
- [18] S. Camilla, P.H. Gunnar, L.D. Svensson, et al., Development and validation of a method using supported liquid extraction for the simultaneous determination of midazolam and 1'-hydroxy-midazolam in human plasma by liquid chromatography with tandem mass spectrometry detection, *J. Pharm. Biomed. Anal.* 58 (2012) 71–77.
- [19] A. Yilmaz, A. Izadkhashti, R.W. Price, et al., Darunavir concentrations in cerebrospinal fluid and blood in HIV-1-infected individuals, *AIDS Res. Hum. Retroviruses* 25 (2009) 457–461.
- [20] C. Delaugerre, G. Peytavin, S. Dominguez, et al., Virological and pharmacological factors associated with virological response to salvage therapy after an 8-week of treatment interruption in a context of very advanced HIV disease (GigHAART ANRS 097), *J. Med. Virol.* 77 (2005) 345–350.
- [21] M. Takahashi, Y. Kudaka, N. Okumura, et al., The validation of plasma darunavir concentrations determined by the HPLC method for protease inhibitors, *Biol. Pharm. Bull.* 30 (2007) 1947–1949.
- [22] L. Goldwirt, S. Chhuna, E. Rey, et al., Quantification of darunavir (TMC114) in human plasma by high-performance liquid chromatography with ultra-violet detection, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 857 (2007) 327–331.
- [23] V.J. Sekar, E. Lefebvre, E. De Paepe, et al., The effect of different meal types on the pharmacokinetics of darunavir (TMC114)/ritonavir in HIV-negative healthy volunteers, *Antimicrob. Agents Chemother.* 51 (2007) 958–961.
- [24] N.L. Rezk, N.R. White, S.H. Jennings, et al., A novel LC–ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma, *Talanta* 79 (2009) 1372–1378.
- [25] A. Fayet, A. Béguin, B. Zanolari, et al., A LC–tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, maraviroc, darunavir, and etravirine, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 877 (2009) 1057–1069.
- [26] R. ter Heine, H. Rosing, E.C.M. van Gorp, et al., Quantification of protease inhibitors and non-nucleoside reverse transcriptase inhibitors in dried blood spots by liquid chromatography–triple quadrupole mass spectrometry, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 867 (2008) 205–212.
- [27] R. ter Heine, C.G. Alderden, H. Rosing, et al., Fast and simultaneous determination of darunavir and eleven other antiretroviral drugs for therapeutic drug monitoring: method development and validation for the determination of all currently approved HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 21 (2007) 2505–2514.
- [28] A. D'Avolio, M. Siccardi, M. Sciacandra, et al., HPLC–MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 859 (2007) 234–240.
- [29] J. Martin, G. Deslandes, E. Dailly, et al., A liquid chromatography–tandem mass spectrometry assay for quantification of nevirapine, indinavir, atazanavir, amprenavir, saquinavir, ritonavir, lopinavir, efavirenz, tipranavir, darunavir and maraviroc in the plasma of patients infected with HIV, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 877 (2009) 3072–3082.
- [30] A. D'Avolio, M. Simiele, M. Siccardi, et al., HPLC–MS method for the quantification of nine anti-HIV drugs from dry plasma spot on glass filter and their long term stability in different conditions, *J. Pharm. Biomed. Anal.* 52 (2010) 774–780.
- [31] L. Else, V. Watson, J. Tjia, et al., Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 878 (2010) 1455–1465.
- [32] R. ter Heine, M. Davids, H. Rosing, et al., Quantification of HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in peripheral blood mononuclear cell lysate using liquid chromatography coupled with tandem mass spectrometry, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 877 (2009) 575–580.
- [33] A. D'Avolio, M. Simiele, M. Siccardi, et al., A HPLC–MS method for the simultaneous quantification of fourteen antiretroviral agents in peripheral blood mononuclear cell of HIV infected patients optimized using medium corpuscular volume evaluation, *J. Pharm. Biomed. Anal.* 54 (2011) 779–788.
- [34] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry, Bioanalytical Method Validation, May, 2001, available at: (<http://www.fda.gov/cder/guidance>).
- [35] O.A. Ismaiel, T. Zhang, R.G. Jenkins, et al., Investigation of endogenous blood plasma phospholipids, cholesterol and glycerides that contribute to matrix effects in bioanalysis by liquid chromatography/mass spectrometry, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 878 (2010) 3303–3316.