A UHPLC-MS/MS method for the quantification of direct antiviral agents simeprevir, daclatasvir, ledipasvir, sofosbuvir/GS-331007, dasabuvir, ombitasvir and paritaprevir, together with ritonavir, in human plasma

This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/1620348 since 2016-12-13T15:30:36Z

Published version:
DOI:10.1016/j.jpba.2016.04.031

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(Article begins on next page)
Title: A UHPLC-MS/MS method for the quantification of direct antiviral agents simprevir, daclatasvir, ledipasvir, sofosbuvir/GS-331007, dasabuvir, ombitasvir and paritaprevir, toghether with ritonavir, in human plasma.

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Keywords: liquid chromatography; tandem mass spectrometry; HCV; DAAs; TDM; multiplexed.

Conflicts of interest: The authors disclose no conflicts.

Funding: This study was supported by internal founding.
Abstract

To date, the new standard for treatment of chronic hepatitis C is based on the administration of novel direct acting antivirals. Among these, sofosbuvir, simeprevir, daclatasvir, ledipasvir, dasabuvir, ombitasvir and paritaprevir already entered the clinical use. Anyway, since few pharmacokinetic studies have been conducted on these drugs in a “real life” context poor knowledge is available about their optimal therapeutic range. Without this background, therapeutic drug monitoring is not applicable for treatment optimization. Up to now, a few methods are reported to quantify these drugs in human plasma, and none of them in a simultaneous way. The aim of this work was to develop and validate a simple, fast and cheap, but still reliable UHPLC-MS/MS method for the quantification of these drugs, feasible for a clinical routine use.

Solid phase extraction was performed using HLB C18 96-well plates. Chromatographic separation was performed on a BEH C18 1.7 μm, 2.1 mm×50 mm column, settled at 50°C, with a gradient run of two mobile phases: ammonium acetate 5mM (pH 9,5) and acetonitrile, with a flow rate of 0.4 mL/min for 5 minutes. Tandem-mass detection was carried out in positive electrospray ionization mode.

Both inter and intraday imprecision and inaccuracy were below 15%, as required by FDA guidelines, while both recoveries and matrix effects resulted within the acceptance criteria. The method was tested on 80 patients samples with good performance.

Being robust, simple and fast and requiring a low plasma volume, this method resulted eligible for a clinical routine use.
1. INTRODUCTION

To date, HCV infection affects more than 130 million people worldwide, being an important cause of liver cirrhosis, hepatocellular carcinoma and liver transplantation [1].

HCV is a single strand RNA virus, belonging to the Flaviviridae family with a genome coding for 10 proteins, among which protease NS3-4A and polymerases NS5A/NS5B have been identified as druggable targets.

Up to 2011, the standard for treatment of HCV infection consisted in the combination of ribavirin (RBV) and pegylated-interferon α (Peg-IFNα). More recently, the better knowledge of viral life cycle and of its enzymes lead to the development of new direct acting antivirals (DAAs) [2].

The first generation of protease inhibitors, telaprevir (TVR) and boceprevir (BOC), has been approved in 2011 for use in combination with Peg-IFNα/RBV and, despite the enhanced response rate, these agents caused important side effects: anemia, neutropenia, and disguise for BOC and anemia, skin rash, and anorectal symptoms for TVR [2, 3].

The goal of the research of new therapies is to develop drugs with pangenotypic activity, high genetic barrier and fewer side effects for patients with HCV.

These drugs include Nucleoside Inhibitors (NIs) and Non-Nucleoside Inhibitors (NNIs) of viral polymerase NS5A/5B and Protease Inhibitors (PIs) [4].

Among NIs, sofosbuvir (SOF) is currently the most used, because of its high pangenotypic effectiveness, alone or in combination with Peg-IFNα/RBV or with PIs (such as simeprevir, SMV) or NNIs (as ledipasvir, LDV, or daclatasvir, DAC), with or without RBV. An issue emerging in the evaluation of SOF kinetics is its fast metabolism to its main plasma metabolite (>90%), GS-331007, which can be considered a good marker for SOF plasma exposure [5].

Other than SOF-based regimens, therapeutic alternatives include the co-administration of SMV or DAC with Peg-IFNα/RBV and, more recently, the single tablet formulation of ritonavir-boosted ombitasvir and paritaprevir (OMV and PAR, a NNI and a PI, respectively), with or without dasabuvir (DBV, a NI) and/or RBV [4]. However, poor knowledge about these drugs
pharmacokinetics in plasma and, going further, about the possible correlation between plasma concentrations and therapeutic response and/or toxicity is currently available. Analyses on previous anti-HCV drugs, as RBV, BOC and TVR, already revealed in the past years the relationship between plasma concentration of these drugs and some of adverse effects, such as anemia, or therapeutic failure [6, 7]. Indeed, the therapeutic drug monitoring (TDM) of anti-HCV drugs plasma concentration could represent a useful tool for the clinicians to evaluate drug efficacy and to prevent adverse events, in order to optimize the therapy. Treatment optimization through TDM is already reported to improve the quality of life and the efficacy of the therapy itself, but also it could lead to a cost saving, reducing side effects and consequent clinical cost for patient’s care, in many different contexts. At the moment only few methods have been developed for the quantification of some of the new DAAs, and not altogether [8-12]. For these reasons, a robust quantification method for all the currently used drugs is currently needed. Therefore, the aim of this work was to develop and validate a new high-throughput UHPLC-MS/MS method for the simultaneous quantification in human plasma of SOF/GS-331007, SMV, DAC, LDV, OMV, PAR and DBV, togheter with RTV, eligible for a wide routine use following FDA guidelines [13].
2. EXPERIMENTAL

2.1 Chemicals
DAC and \([^{13}\text{C}_2, ^2\text{H}_6}\text{-DAC}\) (d-DAC), SMV, SOF and its metabolite GS-331007, DBV, OMV and PAR were all purchased from Alsachim (Illkirch Graffenstaden, France); LDV was purchased from Selleckchem (Munich, Germany). Acetonitrile (ACN) HPLC grade and Methanol HPLC grade were purchased from J.T. Baker (Deventer, Holland). HPLC grade water was produced with Milli-DI system coupled with a Synergy 185 system by Millipore (Milan, Italy). 6,7-Dimethyl-2,3-di(2-pyridyl) quinoxaline (QX), RTV and formic acid were obtained from Sigma-Aldrich (Milan, Italy). Blank plasma from healthy donors was kindly supplied by the Blood Bank of Maria Vittoria Hospital (Turin, Italy). Ammonium acetate and DMSO were purchased from Sigma Aldrich.

2.2 Chromatographic conditions
Chromatographic analysis was performed on a Shimadzu Nexera X2 ® LC system coupled with a LC-8050 ® tandem mass spectrometer (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on an Acquity UPLC BEH C18 column, (2.1 mm×50 mm, 1.7 µm; Waters, Milan, Italy) maintained at 50°C through the column oven. Compounds separation was obtained through a gradient (Table 1) of mobile phases A (Ammonium acetate 5mM buffer, ph 9.5) and B (ACN) at flow rate of 0.4 mL/min and a time run of 5 minutes. Auto-sampler was settled at 4°C and the injection volume was 0.3 µL, with a sampling rate of 1 µL/sec. Data processing and system control was managed through the LabSolution ® software (Shimadzu, Kyoto, Japan) version 1.0.

2.3 Stock Solutions, Standards and Quality Controls
Stock solutions of DAC, SMV, LDV, SOF/GS-331007, DBV, OMV and PAR were prepared in DMSO at a concentration of 1 mg/mL and stored at -80 °C. QX, RTV and d-DAC stock solutions
(1 mg/mL) were prepared in pure methanol and stored at 4°C until analysis. Internal standard working solution (IS) was made with QX and d-DAC (both at [0.625 µg/mL]) in water:methanol (50:50 v:v) at the time of the analysis.

The highest standard sample (STD 9) and the three quality controls, high (H), medium (M) and low (L), were prepared by spiking blank plasma with stock solutions; Lower STDs were prepared by serial 1:1 dilution from STD 9 to STD 1 with blank plasma, in order to obtain 9 different spiked concentrations plus a blank sample (STD 0). STDs and QC were stored at -80°C.

Calibration ranges and QCs concentrations for all drugs are listed in Table 2.

2.4 Sample extraction

HLB C18 96 wells plates were chosen for the samples extraction. Each well (cartridge) was activated with 1 mL of pure methanol and equilibrated with 1 mL of water, in a positive pressure-96 manifold ® (Waters, Milan, Italy).

Two hundred microliters of plasma were diluted 1:2 with H2O 1% phosphoric acid, added with 40 µL of IS working solution and centrifuged at 21000 x g for 10 minutes: then, the supernatants were loaded into the corresponding wells. After a washing step with 200 µL of pure water, the samples were eluted in a 96 well 2 mL collection plate with 500 µL of methanol:ACN 90:10 (vol:vol): 0.5 µL of the resulting extracts have been injected in the chromatographic system.

2.5 Mass conditions

Tandem mass spectrometric detection was carried-out through electrospray ionization source set in positive ionization mode (ESI+) for all the considered analytes.

Ionization conditions were optimized by directly injecting solutions containing each single drug, prepared in a mixture of the two mobile phases (A and B) 50:50 (vol:vol), bypassing the column.
(Fast Injection Analysis, FIA): the optimization process was automatically performed using the “optimization for method” function of the chromatographic system. The optimized instrument parameters were as follows: capillary voltage 4 kV, nebulizing gas flow 3 L/min, drying gas flow 10 L/min, heating gas flow 10 L/min, interface temperature 300 °C, heating block temperature 400 °C, desolvation line temperature 250 °C.

The ion monitoring was performed by positive electrospray ionization (ESI+) in multiple reaction monitoring (MRM) mode, with the mass transitions reported in table 3. Each drug was monitored at two different transitions (except for simeprevir): the first was used to quantify (quantification trace) and the second as confirmation (secondary ion trace, not reported).

2.6 Real samples collection

To obtain a confirmation of the applicability of this method to clinical routine this was tested by use on 80 real plasma samples from 40 HCV+ patients (2 samples for each patient) in treatment with the novel DAAs, all giving informed consent as requested by the local Ethical Committee guidelines, in the context of the approved “Kineti-C” clinical study (protocol number 186/2014).

Blood samples from patients were collected in lithium heparin tube (7 mL) at the end of dosing interval (Ctrough) and plasma was obtained after centrifugation at 1400 g for 10 min at +4°C (Jouan Centrifuge, Model BR4i, Saint-Herblain, France). Plasma samples were immediately stored at -80°C until analysis.

2.7 Specificity and selectivity

Interference from endogenous compounds was investigated by analysis of 6 different blank plasma samples. A possible “interfering peak” has been considered as a peak which exhibited a retention time within 0.1 minutes from the analytes ones.
2.8 Accuracy, precision, calibration and limit of quantification

Inter-day precision and accuracy were determined by assaying QC samples in double replicate in 6 different validation sessions. Intra-day precision was evaluated in 5 intra-day replicates for each QC level. Accuracy was calculated as the percent deviation from the nominal concentration. Inter-day and intra-day precision were expressed as the relative standard deviation (RSD) at each QC concentration. Calibration curves were obtained by processing chromatograms of STDs by peak areas. “Quadratic through zero” regression models were used for all curves, in order to compensate a slight saturation phenomenon. The fitting to the calibration model was evaluated up to ten times concentration of STD 9 for each drug.

The limit of detection (LOD) in plasma was defined as the concentration that yields a signal-to-noise ratio of 3:1. Percent deviation from the nominal concentration (measure of accuracy) and RSD (measure of precision) of the concentration considered as the limit of quantification (LOQ) had to be < 20%, and it was considered as the lowest calibration standard, as requested by FDA and EMA guidelines.

2.9 Recovery

Recovery was evaluated in six different sessions by comparing the peak areas obtained from the three QC samples with peaks corresponding to dry extracts of blank plasma, reconstituted with spiked solutions at the same concentrations (post-extraction addition).

2.10 Matrix Effect

The “matrix effect” was investigated on six lots of blank plasma from individual donors, as requested by guidelines. Peak areas from blank extracts spiked with all analytes at three QC concentrations were compared with peak areas from direct injection of standard solutions (prepared in methanol:ACN 90:10 v:v) spiked with the analytes at the same concentration, as described by
Taylor (post-extraction addition method) [14]. The “matrix effect” was calculated as percentage of deviation in peak area between these two conditions at high, medium and low levels.

2.11 Carry-over

Carry-over was investigated by comparing peak areas obtained from three blank plasma samples injected after a sample containing target analytes concentration 5-fold higher than STD9. A signal lower than 20% of the lower limit of quantification (LLOQ) for each target drug and a value lower than 5% for the IS were considered as an acceptable carry-over.

2.12 Stability

All DAA drugs were evaluated for long-term stability in plasma samples at -80°C for 3 months, at high, medium and low QC levels. “Bench-top” stability was evaluated after 24h on the bench at room temperature, by comparison with the signal from samples stored at -80°C and immediately extracted. Autosampler stability was evaluated on QCs extracts maintained in the autosampler at 10°C for 24h, by comparing their concentrations with fresh extracts. “Freeze and thaw” stability has been evaluated after one to three freezing and thawing cycles, by comparison with freshly prepared QCs. Acceptable stabilities have been considered as percent differences in concentration lower than 5%.
3. RESULTS

3.1 Calibration curve

All calibration curves for all drug shown a mean regression coefficient \( r^2 \) ranged from 0.997 to 0.999. A quadratic through zero regression was chosen for all drugs, due to a slight saturation phenomenon at higher concentrations.

3.2 Specificity and selectivity

No interference with other potential concomitants drugs was found in the assay. The retention time of DAAs, QX and d-DAC are represented in Figure 1. No endogenous interferences were observed in six different plasma lots, taking in account the analytes retention time windows. Secondary ion traces were used to confirm the nature of the peaks (except for simeprevir).

3.3 Accuracy and precision

All accuracy, inter-day and intra-day precision parameters fitted the limits requested by FDA guidelines at each one of the QC levels. Data are summarized in table 2.

3.4 Lower limit of quantification (LLOQ) and limit of detection (LOD)

The evaluation of LOD was performed by diluting the lowest calibration point (STD1) several times. The LLOQ corresponded to the STD 1, as shown shown in table 2. Overlaid chromatograms of LLOQ and of a blank sample for each drug is showed in figure 2. There was not any interfering peak at the retention times of the analytes of interest.

3.5 Recovery

Mean recovery (and RSD%) for each analyte are summarized in table 4. Recovery resulted reproducible for all the considered analytes.
3.6 Matrix effect
Mean matrix effect data (and RSD%) for each drug are summarized in table 4. Matrix effect resulted reproducible between different plasma lots.

3.7 Carry-over
DAAs peaks observed in blank samples, injected immediately after mixes at high concentrations of analytes, were all lower than the corresponding LOD.
Likewise, mean carry-over of IS was lower than 1%. These data showed the absence of relevant carry-over.

3.8 Stability
Considering “freeze and thawing”, bench-top, autosampler and -80°C storage stability testing, all the analytes showed percent degradations lower than 5%, thus resulting stable in our working conditions.

3.9 “Real” samples testing
Among the 40 patients enrolled, 16 were treated with SOF+SMV, 10 with SOF+DAC, 4 with SOF+LDV and 10 with DBV+OMV+PAR and RTV. 29 out of 30 patients treated with showed undetectable concentrations of SOF, while GS-331007 was quantifiable in all samples, with a median concentration of 319.0 ng/mL (interquartile range, IQR 193.2 – 494.0 ng/mL). All samples from patients treated with SMV, DAC or LDV had quantifiable drugs concentrations, with median levels of 776.5 ng/mL (IQR 284.7 – 2390.2 ng/mL), 198.0 ng/mL (IQR 83.5 – 414.5 ng/mL) and 135.0 ng/mL (99.7 – 385.5 ng/mL), respectively. Likewise, all samples from patients treated with DBV+OMV+PAR and RTV had quantifiable concentrations, with median levels of 223.5 ng/mL (IQR 120.6 – 357.0 ng/mL) for DBV, 28.0 ng/mL (IQR 14.0 – 58.2 ng/mL) for OMV, 31.2 ng/mL (IQR 5 – 198 ng/mL) for PAR and 33.1 ng/mL (14.2 – 74.5 ng/mL) for RTV.
4. DISCUSSION and CONCLUSION

In this work the validation of a UHPLC-MS/MS method for the simultaneous quantification of the main DAAs already in use for treatment of HCV infection is described. The use of the HLB 96 wells plates allowed a fast and clean solid phase extraction in a high-throughput, but still highly sensitive and specific manner.

In literature we can find a few methods developed for the quantification of DAAs: one method by Rezk et al. [11], allows the quantification of SOF and its metabolite GS-331007 in human plasma, starting from 500 ul of human plasma and operating a cumbersome liquid-liquid extraction. The calibration range that Rezk et al. chose for the GS-331007 probably cannot cover the expected concentration range in all types of patients and, anyway, it is not adequate to make AUC analyses; moreover, this method started from a higher volume of sample and the extraction process could be too long if the number of sample is high, as in a clinical routine context.

A second method, developed by Jiang et al. [8], included the quantification of DAC, asunaprevir and beclabuvir in human plasma, using a liquid-liquid extraction followed by an evaporation step: anyway, this method did not include SOF, the currently most used DAA.

Other available methods were developed for the quantification of some of the other DAAs in different combinations, but they were validated for use on rat plasma [9, 10, 12].

During the method development, we tried different columns and chromatographic conditions: we compared an 2.1 x 150 mm HSS T3 1.8 μm column with acidic mobile phase to a 2.1 x 150 mm BEH C18 1.7 μm column, with basic mobile phase. We chose the second condition due to a better peak shape and higher sensitivity, especially for GS-331007 and SMV. Also protein precipitation was tried instead of SPE, but it did not work well for all the compounds (high matrix effect was observed, data not shown). Several SPE-plates were tested with different sorbents and finally we chose the HLB plate, which showed the highest (and most stable) recovery for all the compounds.
Although in clinical practice the trough concentration is the most used in antiviral therapy (and our method has been tested on Ctrough, accordingly), the choice of calibration ranges was based on the reported AUCs in clinical trials, with the aim of covering all the expected concentrations in patients. Considering method performance on “real” samples from patients, it was capable of successfully quantifying each drug in all sample, except for SOF concentrations, which resulted almost always undetectable. However, this behavior was already known in literature, as SOF is very rapidly metabolized in its active triphosphate intracellular metabolite and then converted in GS-331007 [15]: this compound was already described as a good marker for SOF exposure and showed plasma concentrations above our LLOQ in all the tested samples.

The choice of the ISs was based on chemical, analytical and economical criteria: QX was chosen because of its relatively high LogP, its well known stability, its high recovery in our conditions and its low cost, while d-DAC was used to normalize the signal for DAC, which exhibited a different recovery and matrix effect respect to the other drugs and QX.

Concluding, the high accuracy and precision of the assay, taken together with the good and stable recovery and the contained matrix effect, made this method eligible for use in clinical studies for the determination of optimal therapeutic ranges for each DAA (still unknown) and, then, for a future routine TDM of these drugs, becoming a useful tool for treatment management.


Figure Legends

Figure 1: Overlaid MRM chromatograms for each analyte from the injection of STD 9.

Figure 2: Overlaid peaks from analysis of LLOQ and blank plasma extracts for each analyte.
**Table 1:** Chromatographic gradient of mobile phases A (Ammonium acetate 5mM pH 9.5) and B (acetonitrile).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solvent B %</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>3</td>
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<td>3.60</td>
</tr>
<tr>
<td>5.00</td>
<td>3</td>
<td>5.00</td>
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</tbody>
</table>
Table 2: Summary of calibration ranges, quality controls concentrations and validation parameters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ULOQ ng/mL</th>
<th>LLOQ ng/mL</th>
<th>LLOD ng/mL</th>
<th>QC H (n=6) CONC. ng/mL</th>
<th>Accuracy (intraday)</th>
<th>Precision (%)</th>
<th>QC M (n=6) CONC. ng/mL</th>
<th>Accuracy (intraday)</th>
<th>Precision (%)</th>
<th>QC L (n=6) CONC. ng/mL</th>
<th>Accuracy (intraday)</th>
<th>Precision (%)</th>
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<tbody>
<tr>
<td>DAC</td>
<td>3000</td>
<td>11,7</td>
<td>1,5</td>
<td>2000</td>
<td>1,3%</td>
<td>5,8%</td>
<td>11,7</td>
<td>0,9%</td>
<td>4,1%</td>
<td>11,7</td>
<td>0,9%</td>
<td>4,1%</td>
</tr>
<tr>
<td>SOF</td>
<td>3000</td>
<td>11,7</td>
<td>0,7</td>
<td>2000</td>
<td>1,3%</td>
<td>9,9%</td>
<td>11,7</td>
<td>0,9%</td>
<td>6,9%</td>
<td>11,7</td>
<td>0,9%</td>
<td>6,9%</td>
</tr>
<tr>
<td>SMV</td>
<td>8000</td>
<td>31,2</td>
<td>4,9</td>
<td>6000</td>
<td>-2,3%</td>
<td>4,7%</td>
<td>14,1%</td>
<td>4,7%</td>
<td>14,1%</td>
<td>14,1%</td>
<td>4,7%</td>
<td>14,1%</td>
</tr>
<tr>
<td>LDV</td>
<td>3000</td>
<td>11,7</td>
<td>2,0</td>
<td>2000</td>
<td>-3,5%</td>
<td>5,4%</td>
<td>12,7%</td>
<td>5,4%</td>
<td>12,7%</td>
<td>12,7%</td>
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<tr>
<td>OMV</td>
<td>1000</td>
<td>3,9</td>
<td>2,0</td>
<td>800</td>
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<td>8,1%</td>
<td>14,1%</td>
<td>8,1%</td>
<td>14,1%</td>
<td>14,1%</td>
<td>8,1%</td>
<td>14,1%</td>
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<tr>
<td>DBV</td>
<td>3000</td>
<td>11,7</td>
<td>5,9</td>
<td>2000</td>
<td>-2,3%</td>
<td>5,4%</td>
<td>12,7%</td>
<td>5,4%</td>
<td>12,7%</td>
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<td>5,4%</td>
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<tr>
<td>FAR</td>
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<td>2000</td>
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<td>6,9%</td>
<td>11,7</td>
<td>0,9%</td>
<td>6,9%</td>
</tr>
<tr>
<td>RTV</td>
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<td>0,7</td>
<td>2000</td>
<td>1,3%</td>
<td>9,9%</td>
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<tr>
<td>GS-331007</td>
<td>10000</td>
<td>39,1</td>
<td>10,5</td>
<td>6000</td>
<td>-6,6%</td>
<td>12,6%</td>
<td>14,4%</td>
<td>12,6%</td>
<td>14,4%</td>
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<td>12,6%</td>
<td>14,4%</td>
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</table>
**Table 3**: Summary of the monitored quantification traces for the considered analytes

<table>
<thead>
<tr>
<th>ANALITE</th>
<th>Precursor (m/z)</th>
<th>Quantification trace (m/z)</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAC</td>
<td>739.40</td>
<td>565.10</td>
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<tr>
<td>SOF</td>
<td>530.05</td>
<td>243.05</td>
<td>22</td>
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<tr>
<td>SOF-G</td>
<td>258.1</td>
<td>239.1</td>
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<tr>
<td>SMV</td>
<td>751.15</td>
<td>314.90</td>
<td>33</td>
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<tr>
<td>LDV</td>
<td>890.30</td>
<td>733.00</td>
<td>41</td>
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<tr>
<td>OMV</td>
<td>895.40</td>
<td>588.3</td>
<td>50</td>
</tr>
<tr>
<td>DBV</td>
<td>492.15</td>
<td>476.85</td>
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</tr>
<tr>
<td>PAR</td>
<td>766.40</td>
<td>571.05</td>
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<tr>
<td>RTV</td>
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<td>295.95</td>
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<td>IS QX</td>
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<td>IS d-DAC</td>
<td>747.40</td>
<td>569.00</td>
<td>45</td>
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</table>
Table 4: Mean recovery and matrix effect (and Relative Standard Deviations) for each drug.

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>Mean Recovery</th>
<th>Recovery RSD</th>
<th>Mean Matrix Effect</th>
<th>Matrix effect RSD</th>
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<tbody>
<tr>
<td>DAC</td>
<td>99%</td>
<td>13.3%</td>
<td>14%</td>
<td>2.5%</td>
</tr>
<tr>
<td>SOF</td>
<td>95%</td>
<td>10.2%</td>
<td>7%</td>
<td>1.3%</td>
</tr>
<tr>
<td>GS-331007</td>
<td>100%</td>
<td>5.6%</td>
<td>2%</td>
<td>0.9%</td>
</tr>
<tr>
<td>SMV</td>
<td>50%</td>
<td>13.2%</td>
<td>1%</td>
<td>2.9%</td>
</tr>
<tr>
<td>LDV</td>
<td>88%</td>
<td>4.7%</td>
<td>6%</td>
<td>3.5%</td>
</tr>
<tr>
<td>DBV</td>
<td>80%</td>
<td>4.3%</td>
<td>4%</td>
<td>5.8%</td>
</tr>
<tr>
<td>OMV</td>
<td>62%</td>
<td>7.5%</td>
<td>24%</td>
<td>6.2%</td>
</tr>
<tr>
<td>PAR</td>
<td>52%</td>
<td>13.2%</td>
<td>6%</td>
<td>3.5%</td>
</tr>
<tr>
<td>RTV</td>
<td>91%</td>
<td>3.1%</td>
<td>7%</td>
<td>1.8%</td>
</tr>
<tr>
<td>IS QX</td>
<td>86%</td>
<td>1.5%</td>
<td>8%</td>
<td>2.5%</td>
</tr>
<tr>
<td>IS d-DAC</td>
<td>99%</td>
<td>11.2%</td>
<td>14%</td>
<td>2.1%</td>
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