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A UHPLC-MS/MS Method for the Simultaneous Quantification of Directly Acting Anti-HCV drugs in patients plasma

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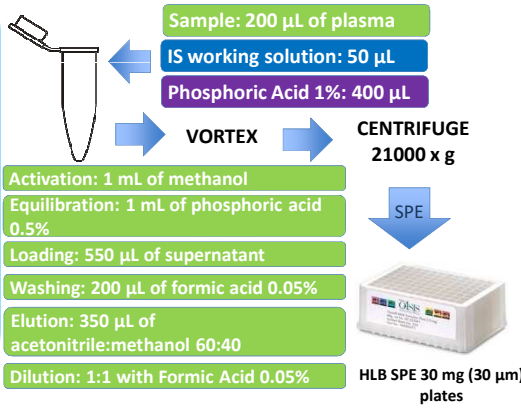
INTRODUCTION

To date, the infection by **Hepatitis C Virus (HCV)** affects more than 130 million patients worldwide and causes **hepatic cirrhosis**, **hepatocarcinoma** and **liver transplantation**. In the recent years, **Directly Acting Antivirals (DAAs)** greatly improved the effectiveness of anti-HCV treatments. Despite this, some issues concerning patients with comorbidities and polytherapies (eg. drug-drug interactions) are still present. For this reason, measurement of DAAs concentrations in patients plasma could be useful for the pharmacokinetic research or Therapeutic Drug Monitoring (TDM).
Nevertheless, no method is available in literature to quantify all the new DAAs, so the aim of this work was the validation of a **UHPLC-MS/MS multiplexed method to quantify 12 currently administered anti-HCV drugs**.

MATERIALS AND METHODS

After the addition of Internal Standard (1 µg/mL of thymidine, 100 ng/mL of 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline, D_4-Daclatasvir and D_3-Ombitasvir), the sample preparation consisted in a Solid Phase Extraction (SPE) protocol with **Oasis ® HLB SPE 30 mg plates** (Waters), graphically summarized on the right.
After samples elution and dilution, the resulting extracts were injected onto a **LX-50 ® chromatographic system (Perkin Elmer) equipped with a Acquity HSS T3 1.8 µm 2.1 x 100 mm (Waters).** Chromatographic separation was obtained with a **8 minute gradient (figure 1)** of water and acetonitrile, both acidified with 0.05% of formic acid, at **45°C** .
Tandem mass spectrometer was a QSight 220 ® (Perkin Elmer) and the quantification was performed by multiple reaction monitoring (MRM) with **ESI positive**, except for **glecaprevir, dasabuvir and ledipasvir, ionized in negative mode**.



VALIDATION PROCEDURE

Validation: A full method validation was performed, evaluating **accuracy, intra- and inter-day precision, recovery and matrix effect**. "Carry-over" was evaluated by injecting a "zero" sample after the highest standard sample. The lowest limit of quantification (LLOQ) and the limit of detection (LOD) were defined as the lowest concentration associated with accuracy and precision within the 20% of imprecision and inaccuracy, and a signal to noise of at least 3, respectively. Analytes stability evaluation is still ongoing.

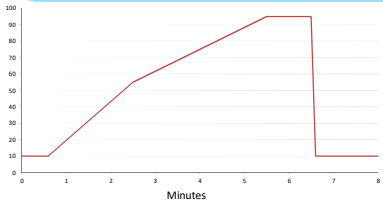


Figure 1: Graphical representation of the Phase B percentage in the chromatographic gradient.

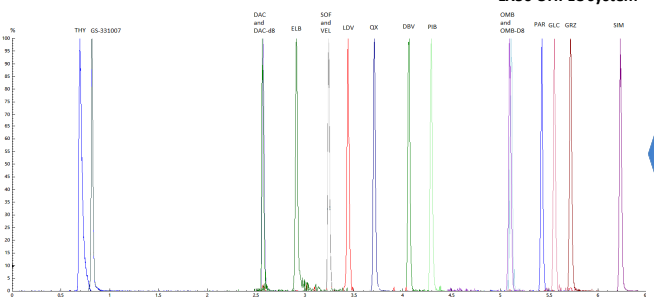


Figure 2: Overlaid chromatograms of the analytes in the highest standard sample. The peaks are represented in relative intensity.

General mass settings		
	Positive	Negative
Capillary voltage (V)	5000	-4500
Nebulizing gas Flow (L/h)	300	
Source Temperature (°C)	300	
HSID Temperature (°C)	270	
Drying gas flow (L/h)	130	
Multipole 1 RF (V)	420	

ANALYTE-SPECIFIC PARAMETERS																
MASS PARAMETERS	THY	GS-331007	DAC	DAC-d8	SOF	VEL	LDV	QX	DBV	PIB	OMB	OMB-d8	PAR	GLC	GRZ	SIM
MOTHER ION (m/z)	243.0	261.1	739.5	747.5	530.2	883.5	887.6	313.2	492.1	557.3	884.7	903.2	766.4	837.3	767.4	750.3
QUANTIFIER (m/z) DAUGHTER ION	127.0	113.1	339.2	339.2	243.1	709.2	855.5	78.1	399.2	146.1	255.2	259.2	571.2	335.1	352.3	315.1
ENTRANCE VOLTAGE	6	16	61	67	22	75	-52	44	-56	36	43	54	36	-67	42	51
COLLISION CELL LENS 2	-40	-44	-212	-284	-88	-240	152	-100	144	-104	-180	-220	-136	176	-156	-128
COLLISION ENERGY	-16	-15	-79	-78	-31	-56	35	-72	45	-33	-37	-38	-27	64	-46	-42
IONIZATION	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+	ESI-	ESI+	ESI-	ESI+	ESI+	ESI+	ESI+	ESI-	ESI+	ESI+

Table 1: General and analyte-specific mass settings for all the considered analytes. **THY=Thymidine; DAC=Daclatasvir; SOF=Sofosbuvir; VEL=Velpatasvir; LDV=Ledipasvir; QX= 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline; DBV=Dasabuvir; PIB=Pibrentasvir; OMB=Ombitasvir; PAR=Paritaprevir; GLC=Glecaprevir; GRZ=Grazoprevir; SIM=Simeprevir.**

RESULTS and APPLICATION

The drugs were successfully recovered by SPE and chromatographically separated (Figure 2). **All extraction recoveries and matrix effect data resulted consistent** (>80%) **and contained** (<20%), respectively, and **reproducible** (both CV% < 15%). Accuracy, intra- and inter-day precision values resulted within the limits indicated by EMA and FDA guidelines. Calibration curves were linear (R^2 > 0.996) for all the considered drugs, except for Simeprevir, showing a quadratic fitting. Stability studies are still ongoing. The method is now being applied to a clinical pharmacokinetic study (KINETI-C), resulting useful for the identification of clinically relevant drug-drug interactions

CONCLUSION

The described method is the first capable of simultaneously quantifying all the most used anti-HCV drugs, resulting widely applicable in both the pharmacokinetic research and clinical TDM context