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Multiplexed therapeutic drug monitoring (TDM) of antiviral drugs by LC–MS/MS

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ABSTRACT

Background: Therapeutic drug monitoring (TDM) can be a useful tool in the clinical management of anti-hepatitis C virus (anti-HCV) drugs. Methods for the determination of various types of anti-HCV drugs in biological samples are, therefore, needed for clinical laboratories.
Objective: In this work, employing the LC–MS/MS approach, we aimed to develop a multiplexed method for identification of the following anti-HCV drugs: Ribavirin (RBV), Boceprevir (BOC), Telaprevir (TVR), Simeprevir (SIM), Daclatasvir (DAC), Sofosbuvir (SOF) and its metabolite GS 331007 (SOFM) in liquid plasma and in dried plasma spots (DPSs).
Method: A single-step extractive-deproteinization was employed for both liquid plasma and DPSs. Reverse-phase liquid chromatography coupled with MRM detection was developed for multiplexed drug detection and quantification.
Results: Sensitivities (expressed as LOQ) were 10 (± 1.2), 10 (± 4.9), 10 (± 4.4), 10 (± 4.4), 10 (± 6.4), 10 (± 3.4), 10 (± 6.4) ng/ml for RBV, SOFM, SOF, DAC, BOC, TVR, and SIM, respectively; accuracy (expressed as BIAS%) was < 10% for all drugs; reproducibility (intra- and inter-day CV%) was < 10% for all drugs; dynamic range was 10–10,000 ng/ml for all drugs.
Conclusions: A novel, simple, rapid and robust LC–MS/MS multiplex assay for the TDM of various anti-HCV drugs that are currently in the clinic was successfully developed. Application to DPS samples enabled TDM to be used for outpatients as well.

1. Introduction

Chronic hepatitis C is a well-established cause of liver cirrhosis and is strongly associated with hepatocellular carcinoma. Hepatitis C is a widespread health issue that currently affects about 200 million individuals worldwide [1]. The standard of care for treatment of hepatitis C, thus far, has been a combination of pegylated alpha-interferon and ribavirin (PEG-IFN/RBV). This so called *"double therapy"* has shown limited therapeutic efficacy and numerous side effects [2]. More recently, the introduction of direct-acting antiviral agents (DAAs) has revolutionized the field of hepatitis C therapy and enabled low toxicity IFN-free regimens characterized by improved efficacy [3,4]. DAAs are being increasingly employed in the clinic.

In this work, we describe the development of an analytical method for the measurement of currently used anti-HCV drugs: the classical nucleoside analog RBV; the first-generation viral non-structural protease NS3/4A inhibitors telaprevir (TVR) and boceprevir (BOC); the more recent NS3/4A inhibitor simeprevir (SIM); the NS5A inhibitor daclatasvir (DAC); and the NS5B inhibitor sofosbuvir (SOF) and its GS331007 metabolite (SOFM). Their chemical structures are shown in Fig. 1.

Knowledge of the blood concentration of these drugs would be useful both in clinical research and routine management of HCV pharmacotherapy. This has been demonstrated for RBV, whose circulating levels in the blood have been associated with dose-dependent side effects, in particular hemolytic anemia [7]. Non-randomized studies have shown that RBV dose adjustment, via therapeutic drug monitoring (TDM), could increase its therapeutic efficacy and reduce its toxicity [8]. Trough levels above 2 µg/ml at treatment week 4 are considered necessary to ensure virological response, although trough levels above 3.5 µg/ml have been correlated with an increased risk of anemia [7,8]. Less is known about circulating levels of DAAs, especially

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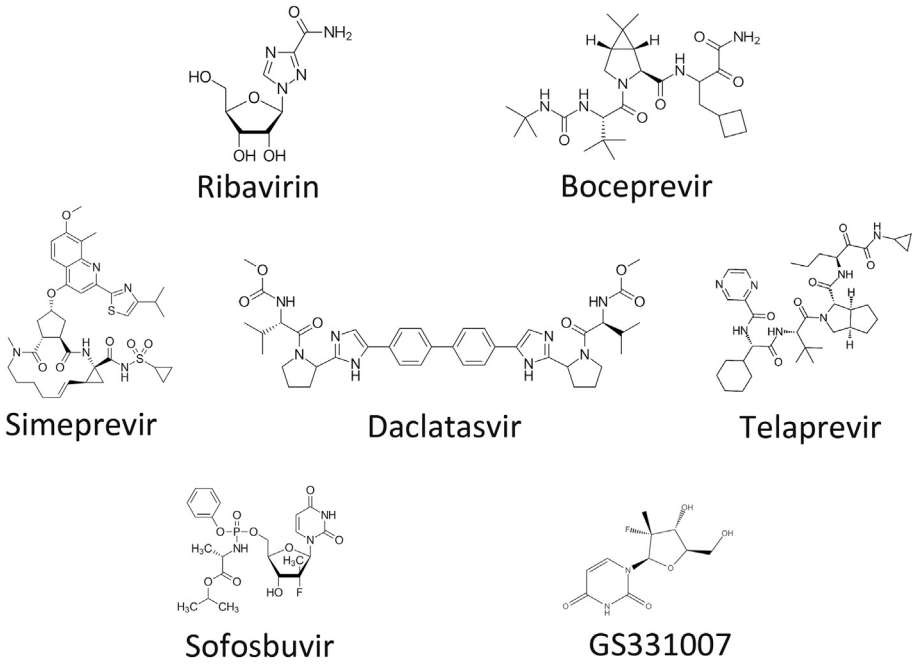

Fig. 1. Chemical structures. Different chemical structures of the seven analytes determined with the proposed method.

in larger and more heterogeneous patient populations than those investigated during its clinical trials. DAA levels are expected to exhibit marked inter- and intra-individual variability due to (i) absorption variations as a function of their oral delivery in combination with food [5], (ii) drug interaction on the basis of their common P450-cytochrome metabolism, and/or (iii) P-glycoprotein mediated transport with antiretrovirals, immunosuppressants and antidepressants, commonly used in HCV infected patients [6]. Investigation of compliance, and drug adsorption and bioavailability, based on individual factors, is expected to be helpful for clinicians in providing treatment. DAAs are enzyme inhibitors and maintaining blood levels over a certain threshold would ensure effective antiviral action and prevent low-level replication and development of viral resistance [5].

Ideally, for TDM data to have an operational impact in the clinic, results need to be available to clinicians within 24–48 h after blood draw; and since HCV patients are usually on multiple drug regimens, a multiplexed approach would assist the laboratory achieve these turnaround times.

Although various methods for the determination of anti-HCV drugs have been reported [9–19], it appears that a method for the multiplexed determination of RBV, BOC, TVR, SIM, DAC, SOF and its main circulating metabolite SOFM, has yet to be published. Due to the large differences in the chemical structures (Fig. 1) and solubility of these drugs (ranging from the hydrophilic RBV to the highly lipophilic TVR, BOC and SIM) a chromatographic assay for their simultaneous analysis represents an analytical challenge. However, by employing LC–MS/MS, we have developed a rapid, robust and efficient assay for these analytes. Plasma sample treatment, based on a single-step extractive-deproteinization, enabled simultaneous extraction of all drugs with acceptable yields. Rapid-gradient chromatography enabled discrimination of analytes from their known isobaric interferences; in particular, RBV from its analog nucleoside uridine (U). MRM detection with optimized transition-specific parameters enabled specific and sensitive multiplexed detection.

After development of the method for liquid plasma samples, we developed a slightly modified procedure for dried micro-sample analysis, for which there is growing interest in the clinical laboratory: dried microsamples are convenient compared to conventional liquid samples since they can be obtained by a simple, even self-performed, finger prick; they provide patients with better comfort and safety; and they can be easily shipped to remote hub laboratories without the need for refrigeration or complex transportation procedures.

When choosing a micro-sample type, we preferred to employ dried plasma spots (DPSs) instead of whole blood spots (DBSs), because DPS are not affected by hematocrit-related spot size and distribution variability, which can generate biases in quantitative analysis [20]. Although devices for obtaining DPS directly from a finger prick are commercially available, we did not employ them in our study. We spotted plasma on classical paper cards after blood centrifugation, instead. These laboratory-produced DPSs, even though they do not simplify sampling, can be useful for simplifying shipping from peripheral centers to hub laboratories where the LC–MS technology is available. In this study, we demonstrate that concentration levels in those DPSs are comparable to those obtained from liquid plasma samples.

The developed procedures were designed to meet laboratory and clinical requirements of routine implementation in an ISO15189 environment [21,22], and were applied to the measurement of anti-HCV drugs in a number of real patients samples.

2. Materials and methods

2.1. Chemicals

RBV, TVR, BOC, DAC, SIM, SOF, SOFM and [$^{13}C_5$]RBV, as an internal standard (IS) were purchased from Alsachim (Strasbourg, France). Acetic acid (HAc) was of glacial grade and obtained from J.T. Baker (Center Valley, PA, USA). Ammonium acetate (AmAc) and zinc sulphate heptahydrate ($ZnSO_4{\cdot}7H_2O$) were from Sigma-Aldrich (Saint Louis, MO, USA). Formic acid (FOA), trichloroacetic acid (TCA), water (H_2O), acetonitrile (ACN), methanol (MeOH) and dimethyl sulfoxide (DMSO), all LC–MS grade, were obtained from VWR (Radnor, PA, USA).

2.2. Instrumentation

The HPLC consisted of two Shimadzu (Kyoto, Japan) LC-20A pumps, a DGU-20A5 degasser and a SIL-20AC HT autosampler. This was coupled to an API 4000 QTRAP mass spectrometer from Sciex (Toronto, Canada), equipped with an electrospray ionization (ESI) TurboIonSpray source. The software to control the systems and to process the data was Analyst software, version 1.5.2 from Sciex.