Simultaneous Determination of Four HIV Protease Inhibitors by HPLC-MS/MS for use in a Therapeutic Drug Monitoring Service

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INTRODUCTION

- Current therapy for HIV consists of a combination of drugs. This results in a potential for drug interactions leading to elevated or reduced plasma drug concentrations.
- Plasma drug concentrations of the HIV protease inhibitors (PIs) correlate with antiviral efficacy [1].
- As antiviral activity of the PIs requires maintenance of therapeutically effective plasma levels, therapeutic drug monitoring (TDM) can ensure adequate drug exposure.
- A viable TDM service needs simultaneous quantification of the PIs with precision and accuracy. A method is required that analyses a large number of samples in a short time using a sensitive assay that requires a small sample volume.
- The aim was to develop and validate a high performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) method to determine the concentrations of the HIV PIs, Indinavir (IDV), Ritonavir (RTV), Saquinavir (SQV) and Nelfinavir (NFV) from plasma samples obtained from HIV positive patients.

METHODS

- Standard curves were prepared using IDV (0.075–15µg.ml⁻¹), RTV (0.1–20µg.ml⁻¹), SQV (0.05–10µg.ml⁻¹) and NFV (0.075–15µg.ml⁻¹) added to blank plasma. Quality control samples (QCs) were prepared by spiking plasma with all four drugs.
- PIs and internal standard (IS: Ro 31-9564) were extracted with acetonitrile by protein precipitation prior to injection onto the HPLC-MS/MS system.
- IDV, RTV, SQV, NFV and IS were eluted on a Hypurity Elite SC18 Column (5µm: 250 x 4.6mm, Hypersil) with a mobile phase of 20mmol.L⁻¹ ammonium formate buffer-acetonitrile (30:70; v/v) maintained at 1.2ml.min⁻¹. Run time was 10 min.
- IDV, RTV, SQV, NFV and IS were analysed by fragmentation of the parent compounds and quantification of the resulting two fragment ions (Table 1), using a mass spectrometer (LCQ Duo), operating at atmospheric pressure.

RESULTS

- The inter-assay and intra-assay variability was determined from six replicates with different QCs of IDV, RTV, SQV and NFV. External QCs (from the International Quality Control Program for Therapeutic Drug Monitoring in HIV infection) were also analysed.

Table 1. Parent and Fragment ions of the PIs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parent ion (m/z)</th>
<th>Fragment ions (m/z)</th>
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<tbody>
<tr>
<td>IDV</td>
<td>614.4</td>
<td>465.3, 596.3</td>
</tr>
<tr>
<td>RTV</td>
<td>721.4</td>
<td>426.1, 296.0</td>
</tr>
<tr>
<td>SQV</td>
<td>671.4</td>
<td>570.3, 433.2</td>
</tr>
<tr>
<td>NFV</td>
<td>568.3</td>
<td>467.2, 330.1</td>
</tr>
<tr>
<td>IS</td>
<td>674.4</td>
<td>573.3, 388.2</td>
</tr>
</tbody>
</table>

- The lower limits of detection were between 100 – 200pg.ml⁻¹.
- The inter-assay and intra-assay coefficients of variation (CV) with accuracy (% bias) are shown in Tables 2 and 3.
- All samples analysed were within 7% of the actual concentration in the external QC programme.

COSTCLUSIONS

- A rapid, sensitive, specific and validated method for the quantitative determination of IDV, RTV, SQV and NFV in human plasma has been developed.
- This fully validated HPLC-MS/MS method can be used to analyse, with accuracy and precision, routine samples in a TDM service.
- In addition the method is currently being used in a number of pharmacokinetic trials for analysis of plasma, intra-lymphocytic, semen and CSF concentrations of PIs.

REFERENCES


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