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CONFERENCE

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TITLE

LC-MS/MS Determination of Rifabutin and 25-*O*-deacetyl Rifabutin in Human Plasma

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ABSTRACT

Novel Aspect: A high-throughput method was developed by using solid support liquid-liquid extraction.

Introduction

Rifabutin is used for the treatment of pulmonary tuberculosis in patients and also used as a prophylaxis against mycobacterium avium complex (MAC) in patients with AIDS. One of the two major metabolites is 25-O-desacetyl rifabutin. Many drug interaction studies have required the quantitation of rifabutin and its metabolite, 25-O-desacetyl rifabutin in human plasma. In this study, we have developed a semi-automatic method using solid support liquid-liquid extraction on Tomtec. This method has the advantage over traditional manual liquid-liquid extraction, in that it uses an environmental friendly solvent, n-butylchloride, and is less labor intensive. We report here development and validation of an LC/MS/MS assay for Rifabutin and 25-*O*-Deacetyl Rifabutin in human plasma.

Methods

Human plasma samples (50 mL) were spiked with internal standard and immediately processed by solid-supported liquid-liquid extraction cartridge on Tomtec and extracted with 1.4 mL of Methyl tert-Butyl Ether (MtBE):Ethyl Acetate / 50:50 (v:v). The supernatant was transferred and evaporated to dryness under nitrogen. The extracts were then reconstituted with 400 mL of methanol:water:acetic acid/ 45:55:0.1 (v:v:v) for LC/MS/MS analysis. Chromatographic separation was achieved with gradient elution on a reverse phase 30x2.1 mm HPLC column with a total run time of six minutes. Detection was by positive turboionspray tandem mass spectrometry on Sciex API4000.

Preliminary results

The LC/MS/MS assay was validated over a range of 1 to 1000 ng/mL for Rifabutin and 25-*O*-Deacetyl Rifabutin in human plasma using 50 µL of the human plasma. The precursor → product ion transitions under multiple reaction monitoring (MRM) were: m/z 847.5 $(M+H)^+$ → 815.7 (for Rifabutin), and m/z 805.7 $(M+H)^+$ → 773.7 (for 25-*O*-Deacetyl Rifabutin). Quality control samples at 4 concentrations were used to determine precision and accuracy. Intra-assay precision ranged from 2.0 to 11.8% for Rifabutin, 2.1 to 9.9% for 25-*O*-Deacetyl Rifabutin, while Inter-assay precision ranged from 4.2 to 9.4% for Rifabutin, 3.8 to 9.3% for 25-*O*-Deacetyl Rifabutin. The Intra-assay accuracy (defined as % error from nominal) ranged from -7.6 to 6.8% for Rifabutin, -11.0 to 6.4% for 25-*O*-Deacetyl Rifabutin and the Inter-assay accuracy ranged from -4.3 to 3.9% for Rifabutin, -6.7 to 5.0% for 25-*O*-Deacetyl Rifabutin. The analytes have been shown to be stable in plasma for 19 hours at room temperature and after 8 freeze/thaw cycles. The analytes are also stable in processed sample for 131 hours at 4°C and are found to be stable for at least 76 days of storage at -20°C. In addition, the selectivity, dilution reproducibility, matrix effect and recovery were also demonstrated. The assay was shown to be sensitive, selective, accurate, reproducible and reliable. The method was successfully applied to support drug interaction studies.