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Poster presentation

Quantitation of raltegravir in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of LC-MS/MS

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Background

Determination of drug concentrations in plasma is the gold standard for pharmacokinetic studies. However, quantification of drug levels in dried blood spots (DBS) obtained with a simple fingerprick is a patient-friendly alternative in patient populations where intensive venous sampling is unethical or impossible. Moreover, when using DBS there is no need for plasma separation or cold sample storage. Lastly, DBS can be stored or transported without special requirements. The site of action of raltegravir is within the infected cell. Intracellular drug levels of raltegravir provide information on drug disposition in a compartment where HIV replicates and may therefore be useful in understanding its clinical pharmacology.

We here present the development, validation and application of a sensitive and fast assay for the determination of raltegravir in plasma, DBS and peripheral blood mononuclear cell (PBMC) lysate by means of liquid chromatography coupled with electrospray tandem mass spectrometry (LC-MS/MS). The assay allowed detection of the main metabolite, raltegravir-glucuronide.

Methods

Raltegravir was extracted from plasma by means of protein precipitation using only 50 μL plasma. Extraction from DBS was performed with a simple one-step extraction with a mixture of methanol, acetonitrile and 0.2 M zinc sulphate in water and extraction from cell lysate was performed in 50% methanol in water. Chromatographic separation was performed on a reversed phase C18 column (150 × 2.0 mm, particle size 5 μm) with a stepwise gradient using an acetate buffer (pH 5) and methanol, at a flow rate of 0.25 mL/min. The analytical run time was only 10 minutes. The triple quadrupole mass spectrometer was operated in the positive ion-mode and multiple reaction monitoring was used for drug quantification. The method was validated over a range of 50 to 10,000 ng/mL in plasma and DBS and a range of 1 to 500 ng/mL in PBMC lysate. Dibenzepine was used as the internal standard.

Summary of results

The method was proven to be specific, accurate, precise and robust. Method accuracy was within 85%–115% and method precision (RSD) was less than 15% across all concentration ranges tested.

Conclusion

The described method has been applied in a clinical study in patients on a raltegravir-containing salvage regimen. DBS sampling proved to be easily implemented and DBS samples were succesfully obtained from all included patients.