

Therapeutic drug monitoring of antiretroviral drugs in rural Tanzania using a fully automated dried blood spot extraction method

Urs Duthaler¹, Benjamin Berger¹, Stefan Erb², Manuel Battegay², Emili Letang³, Stefan Gaugler⁴, Stephan Krähenbühl¹, Manuel Haschke^{1*}

⁽¹⁾ Division of Clinical Pharmacology & Toxicology, University Hospital Basel and Department of Biomedicine, University Hospital Basel, Switzerland

⁽²⁾ Division of Infectious Diseases and Hospital Epidemiology, Departments of Medicine and Clinical Research, University Hospital Basel, Switzerland

⁽³⁾ Swiss Tropical and Public Health Institute, Switzerland and ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Spain

⁽⁴⁾ CAMAG, Muttenz, Switzerland

BACKGROUND

The dried blood spots (DBS) technique is a micro sampling tool, whereby capillary blood is spotted onto a filter paper. After complete drying, a fixed blood spot area can be punched out for drug analysis.

Adherence to antiretroviral therapy is paramount as patients with suboptimal adherence are at risk of HIV progression and the development of drug resistance. Particularly in developing countries, adherence assessment by healthcare providers is performed infrequently. Adherence assessment by DBS is an attractive option in such resource-constrained settings, for the following reasons:

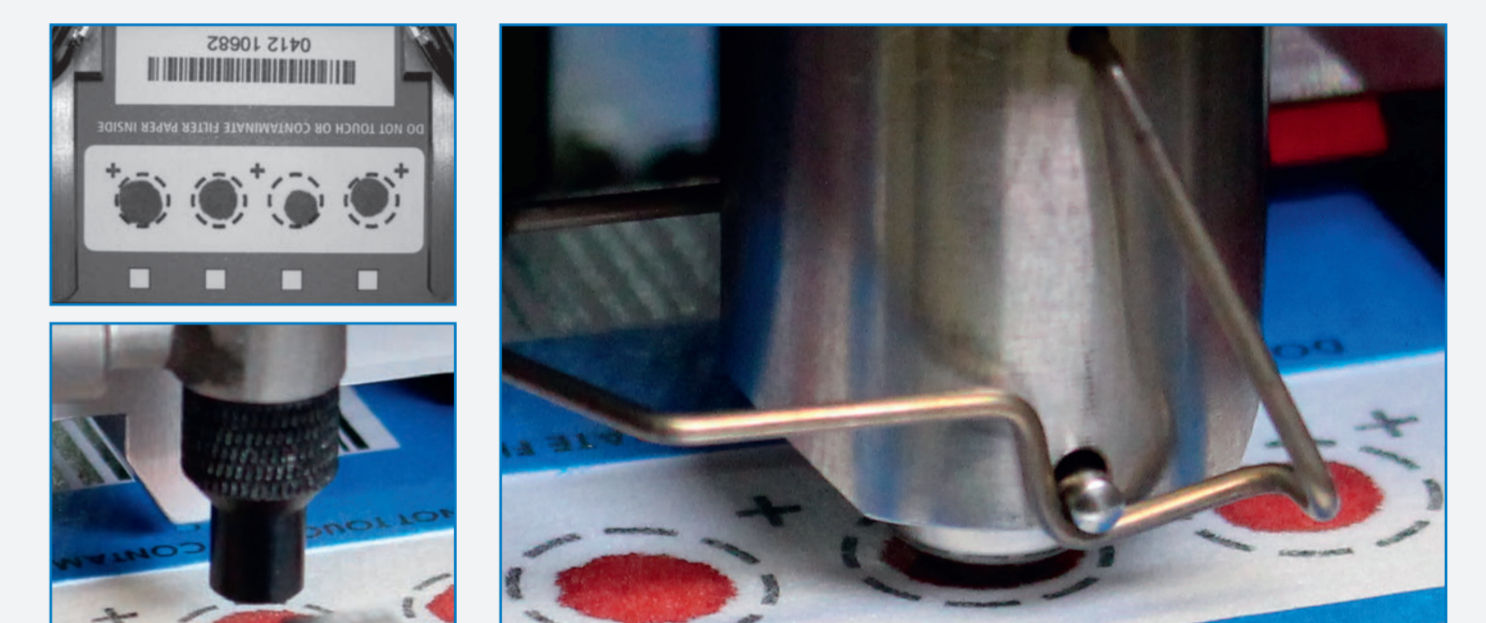
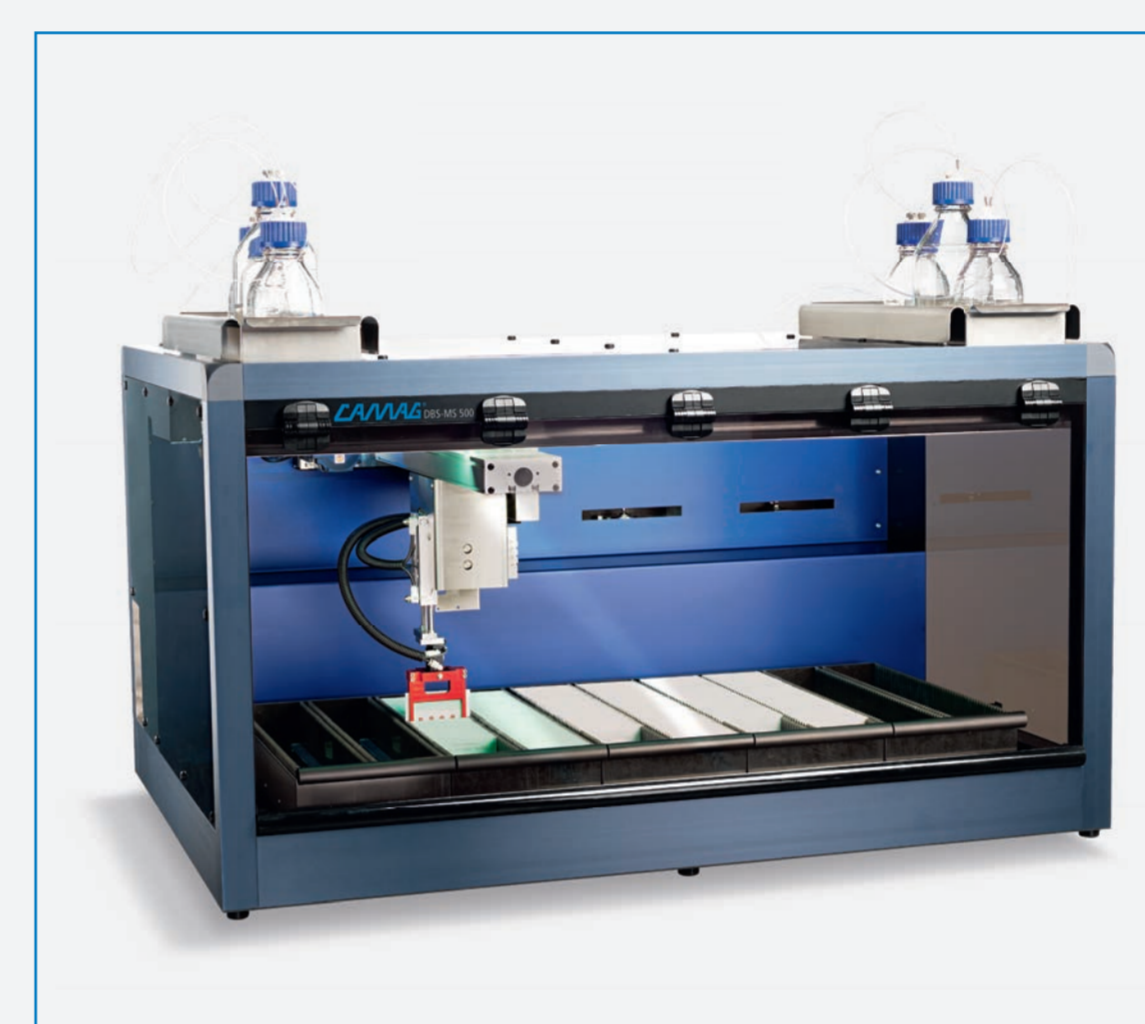


- Minimally invasive finger prick
- No trained phlebotomist is required
- Only a few drops of blood are withdrawn
- Minimised biohazardous risk
- Generally stable at room temperature

MATERIALS & METHODS

DBS and plasma samples (nevirapine n=192, efavirenz n=482, or lopinavir n=66) were collected in the framework of an adherence assessment study in Tanzania including 299 patients. A fully automated DBS-MS 500 autosampler (CAMAG AG, Switzerland) was used for the extraction of DBS samples. Following extraction, samples were analysed by liquid chromatography tandem mass spectrometry:

CAMAG DBS-MS 500 AUTOSAMPLER

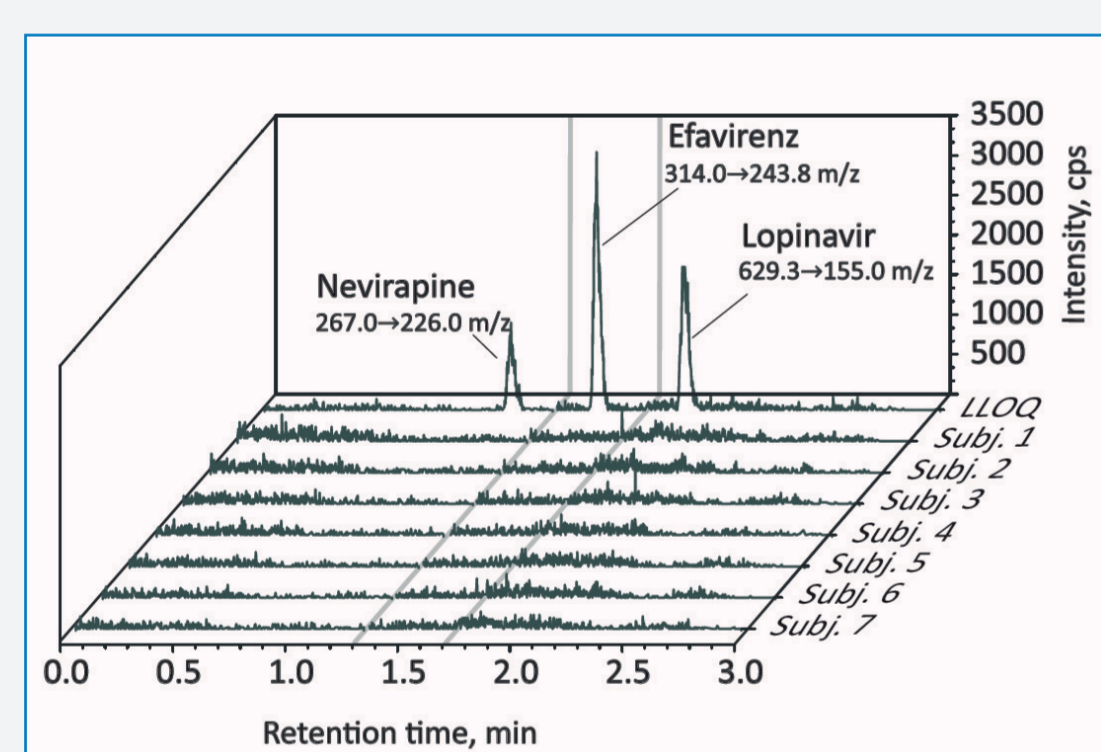


- Imaging of the DBS cards
- Spraying internal standard
- Automated extraction

RESULTS

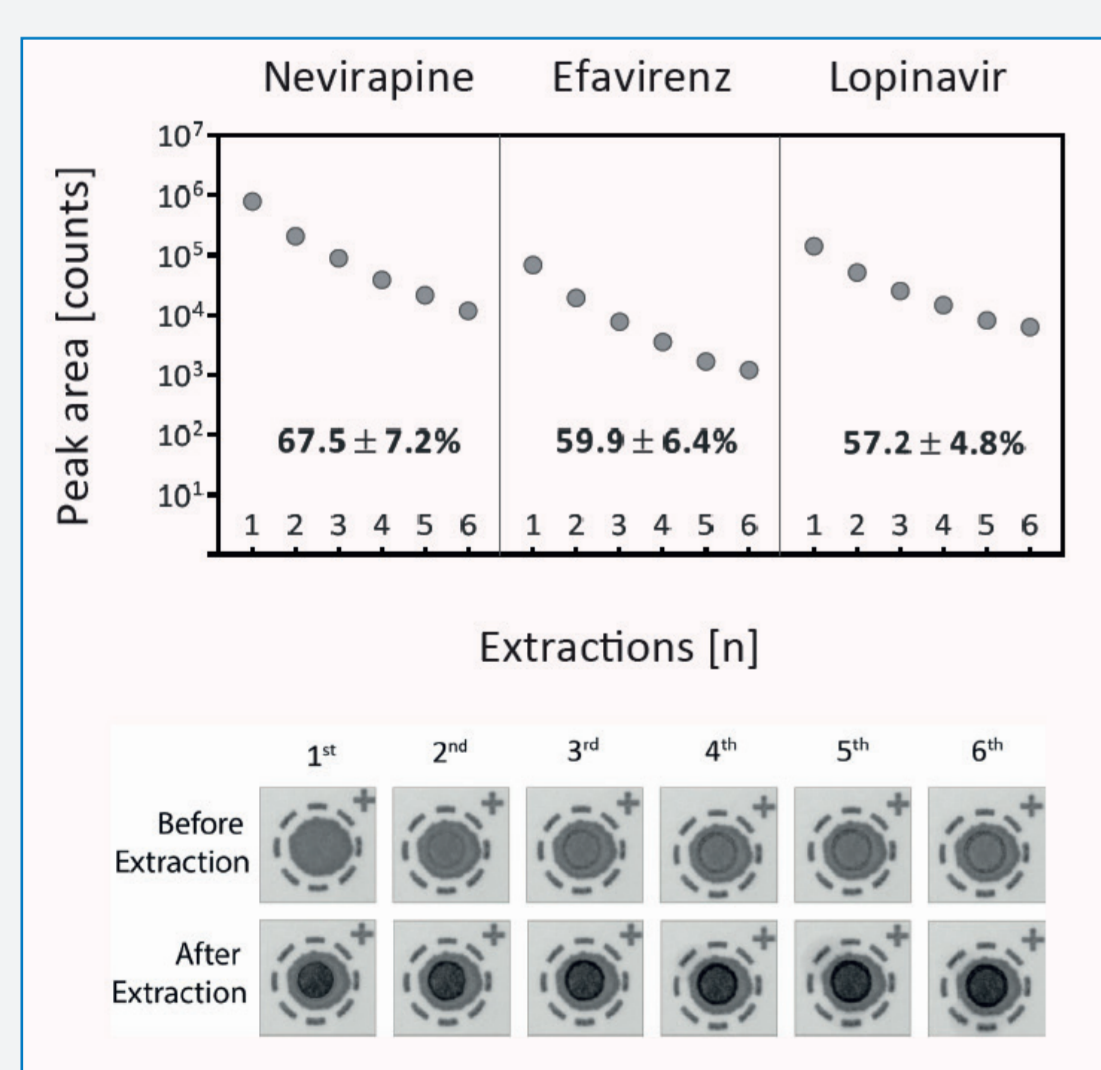
A) METHODOLOGY

Method Selectivity



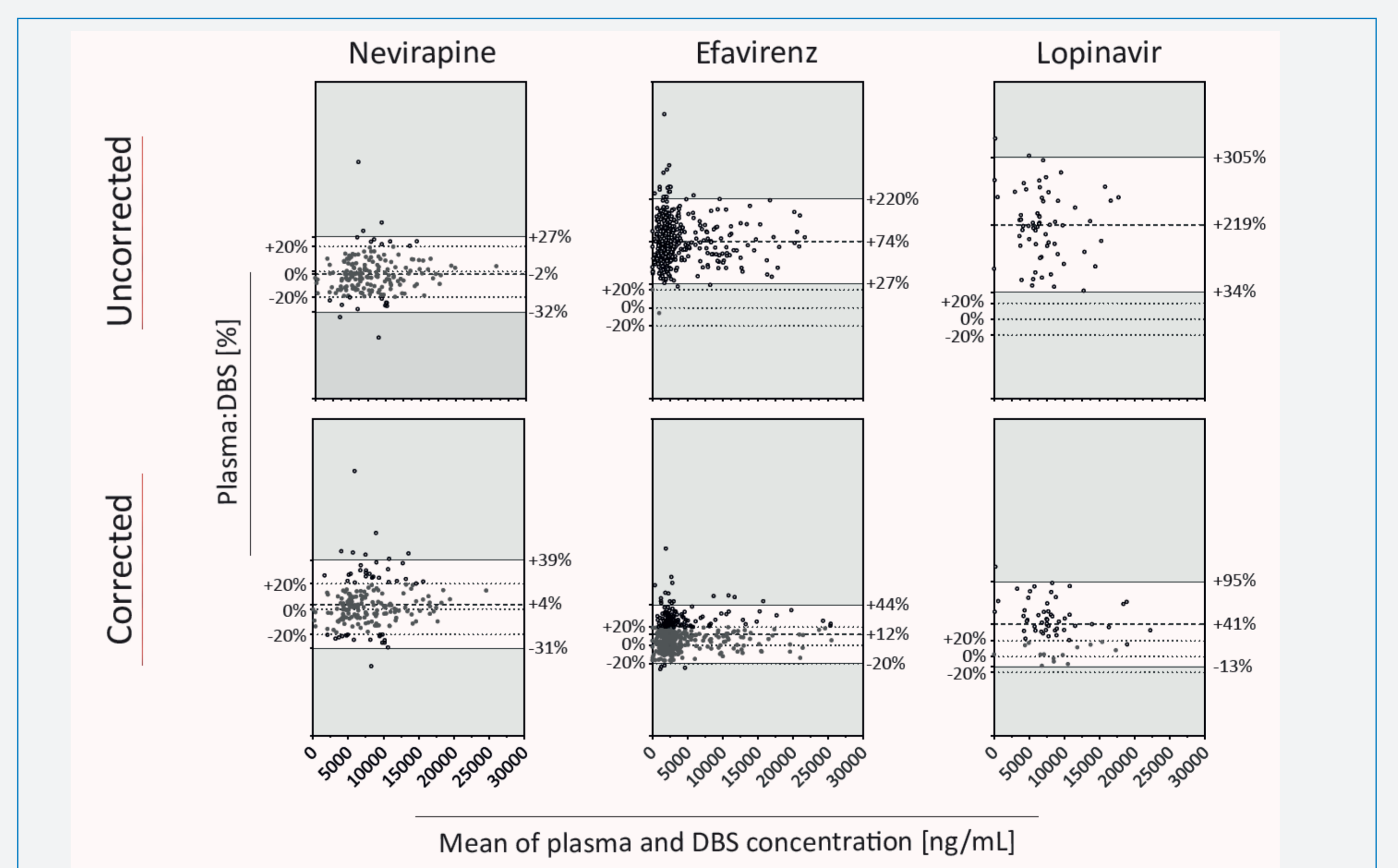
- Analytes were baseline separated
- No interference (n=7 blank DBS)
- Quantification limit of 10 ng/mL

Recovery



- Extraction recovery was determined by multiple extraction of the same DBS spot
- A recovery of 57-67% was calculated
- A bias in recovery of less than 7.2% was determined between subjects (n=6), indicating high consistency in the sample extraction process

B) APPLICATION



- No bias between plasma and DBS was detected for nevirapine
- Plasma concentrations were 75% and 200% higher than in DBS for efavirenz and lopinavir, respectively
- Correcting the DBS concentrations with the hematocrit and with protein binding removed the bias for efavirenz and improved the agreement for lopinavir

CONCLUSIONS

Our study confirms that TDM of nevirapine and efavirenz in DBS is a suitable alternative to conventional plasma analysis, especially when samples have to be collected in remote settings. For efavirenz a correction for hematocrit and protein binding is required to obtain comparable results to plasma. Automated DBS extraction was reliable and facilitated the analysis of large number of samples.