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Manual punch versus automated flow-through sample desorption for dried blood spot LC-MS/MS analysis of voriconazole

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ABSTRACT

Dried blood spot (DBS) sampling is a patient-friendly alternative for plasma sampling for the purpose of therapeutic drug monitoring (TDM). To speed up the analysis time, an automated flow-through desorption method of DBS samples may be beneficial. This article describes the cross-validation of a manual punch DBS method with an automated desorption (DBS autosampler, DBSA) method for the DBS analysis of the antifungal drug voriconazole, followed by cross-validation of both DBS methods with a plasma-based method, and an assessment of agreement between DBS/DBSA and regular plasma concentration measurements (gold standard) in samples from patients on voriconazole treatment.

DBS and DBSA LC-MS/MS assays for voriconazole were validated according to the latest guidelines on bioanalytical method validation (FDA, EMA). Additional DBS-specific validation parameters included hematocrit effect and the influence of spot volume. Passing-Bablok regression and Bland-Altman plots were used to cross-validate the punch DBS, DBSA and plasma methods. The assessment of agreement between DBS/DBSA and plasma concentration measurements involved the performance of DBS/DBSA measurements to predict voriconazole plasma concentrations in patient samples.

Both DBS methods complied with all validation parameters. Sample pre-processing time was reduced from 1.5 h to 3 min when using the DBSA. Cross-validation of both DBS methods showed a proportional bias and a correction factor was needed to interchange voriconazole concentrations of both DBS methods. Similarly, the punch DBS method required a factor to correct for proportional bias compared to the plasma method, but the DBSA and plasma assays showed no bias. Limits of agreement of the DBS/DBSA and plasma assays in Bland-Altman analysis were relatively wide, i.e. 0.75–1.28 for the DBS punch method versus plasma method and 0.57–1.38 for the DBSA versus plasma assay. Interpretation of DBS, DBSA and plasma samples in terms of concentrations in or outside of the voriconazole therapeutic range agreed in 82–86% of the cases.

The variability in paired DBS/DBSA and plasma concentration measurements is considered high for TDM purposes and this limitation should be balanced against the advantages of DBS sampling of voriconazole and the speed of flow through desorption.

1. Introduction

Conventionally, drug concentrations are measured in plasma or serum obtained after venous sampling of whole blood. Dried blood spot (DBS) sampling involves sampling of whole blood by means of a finger prick and is followed by bio-analysis of the whole blood (rather than plasma or serum) sample. DBS has unique advantages over conventional plasma sampling, specifically for the purpose of therapeutic drug monitoring (TDM), which is the individualisation of drug doses based on drug concentration measurements. These advantages of DBS sampling include (I) minimally invasiveness as 1–2 drops of blood are easily obtained by means of a finger prick which can be spotted onto filter paper; (II) the possibility of home based sampling as patients can perform the finger prick themselves, possibly also increasing patient

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empowerment; (III) the possibility to obtain correctly timed drug samples (e.g. peak or trough concentrations, dependent on the drug) more easily; (IV) convenient transport of the sampling paper by regular mail; (V) after analysis of drug on the sampling paper, the results may be available prior to the next outpatient visit, allowing for direct dose adaptation, and (VI) drugs may be more stable upon storage. In addition, our recent data show that DBS sampling may decrease costs involved with TDM [1]. These advantages have been acknowledged by many groups and there has been an important increase in the development of DBS assays over the past decade [2].

Good candidate drugs for DBS sampling are those that meet the requirements for TDM *and* that require repetitive concentration measurements. Voriconazole, the pivotal azole antifungal agent used to treat invasive fungal infections, is frequently sampled for the purpose of TDM. It requires TDM to individualise drug dosing due to its high inter and intra-individual pharmacokinetic variability with nonlinear pharmacokinetic properties and pharmacokinetic interactions involving cytochrome P450 (CYP) isoenzymes [3]. Therefore, voriconazole may be an excellent candidate drug for DBS sampling.

With conventional DBS methods, the blood spots are punched out manually after which they undergo sample pre-treatment such as off-line extraction with subsequent LC-MS/MS analysis [4]. Several innovative methods have been developed in order to improve the recovery of drugs or to speed up the analysis, such as (semi-) automated punch [5] or automated flow-through desorption [6,7]. The current article describes the development of a manual punch and a flow-through desorption (DBS autosampler, DBSA) method for voriconazole. Voriconazole was measured by means of LC-MS/MS. Next, we performed a cross-validation comparing both DBS methods as well as an assessment of agreement between DBS/DBSA and plasma concentration measurements (gold standard) in samples obtained from patients on voriconazole treatment.

2. Methods

2.1. Experimental section

2.1.1. Chemicals, reagents and materials

Voriconazole was purchased from USP (Rockville, USA). The labeled internal standard (IS), $^{13}C_2{}^2H_3$-voriconazole, was purchased at Alsachim (Illkirch Graffenstaden, France). Merck acetonitrile hypergrade for LC-MS was obtained from VWR (Amsterdam, The Netherlands). Methanol absolute ULC/MS was bought from Biosolve (Valkenswaard, The Netherlands) and formic acid eluent additive for LC-MS from Sigma Aldrich (Zwijndrecht, The Netherlands). Ultrapure water was generated by a PURELAB flex 4 system purchased from Veolia Water Technologies Netherlands B.V. (Ede, The Netherlands). Due to small volumes, preparation of calibrator and QC samples was done with a Brand multistepper pipet. A Hettich centrifuge was used for analysis of the hematocrit.

DBS sampling paper and equipment was purchased from Dried Blood Spot Laboratory (DBSL, Geleen, The Netherlands), consisting of Whatman 903 filter paper and BD Microtainer® lancets (pink or blue) for finger prick sampling.

2.1.2. Preparation of DBS calibration standards, quality control (QC) solutions and IS solution

Two series of stock solutions of voriconazole were prepared (one for calibration standards and one for QC samples) in methanol at a concentration of 1.0 mg/mL, dispensed in polypropylene tubes and kept at −40 °C. Stock solutions of internal standard (IS) were also prepared at a concentration of 1.0 mg/L in methanol and kept at −40 °C.

For the preparation of calibration standards and QC samples, fresh drug-free blood was collected in lithium heparin BD Vacutainer® tubes from a healthy volunteer. Hematocrit (Hct) was determined by centrifugal force (Hettich centrifuge). A capillary tube was dipped into the blood tube, and after filling this capillary tube for about 75% with blood it was sealed at the dry end and transferred to the hematocrit centrifuge rotor. This centrifuge rotor was placed into the centrifuge (rotor radius 85 mm, relative centrifugal force [RCF] 16,000, centrifuge time 7 min). An Hct of 0.30 was created by adding an adequate volume of plasma from the same drug-free blood.

The preparation of standard 1 (15 mg/L voriconazole, at the higher limit of quantitation, HLOQ) and standard 2 (10 mg/L) was performed by directly spiking voriconazole stock solution into drug-free blood. Standard 2 was further diluted to create standards 3, 4, 5 and 6 (5, 2.5, 1 and 0.5 mg/L respectively). Standard 6 was further diluted to create standard 7 (0.10 mg/L) and standard 8 (0.05 mg/L, lower limit of quantitation, LLOQ).

QC sample QC H (12.5 mg/L) was prepared by spiking drug-free blood with voriconazole QC stock. QC H was diluted to create QC M (0.7 mg/L). QC L was created by further dilution of QC M (0.07 mg/L). Immediately after preparation, 25 µL spots were applied on Whatman 903® sampling paper with a Brand multistepper pipette yielding the calibration and QC samples. The blood spots were left to dry at least for 3 h and were stored at 4–8 °C in sealed plastic bags containing desiccant.

The extraction solvent for the manual punch method was methanol/water 70:30 (v/v) + 0.1% formic acid. The IS stock solutions were diluted with this extraction solvent to create concentrations [$^{13}C_2$,$^2H_3$]-voriconazole of 16 µg/L and were stored at 4–8 °C.

2.1.3. Sample preparation punch method

Sample preparation for the punch method was optimized by using different compositions of the extraction solvent and various extraction times. Eventually a paper disk with a diameter of 7 mm was punched out of the dried blood spots in a 2 mL safe-lock tube (Eppendorf) and 250 µL of extraction solvent with IS was added. The sample was then vortexed on a multi tube vortex for 30 min at 1200 rpm and centrifuged at 18620 RCF. Subsequently, an aliquot of 200 µL supernatant was transferred into a vial with glass insert and centrifuged again at 1910 RCF to be sure all the remaining waste was at the bottom and not injected. 10 µL was injected into the HPLC-MS/MS system.

2.1.4. On-line DBS solid phase extraction (SPE)

To perform flow-through elution of the DBS card, we used a DBS card autosampler (DBSA) coupled to an automated solid phase extraction (SPE) cartridge exchange module (ACE) for on-line SPE and a high-pressure dispenser pump (HPD) to deliver solvents (Spark Holland, Emmen, The Netherlands, see Fig. 1).

The DBS card was transferred from the card rack to the sliding cardholder by the robot arm of the DBSA. To determine the exact position of desorption, the DBSA took a picture of the card. Subsequently, the spot was eluted with a 2 mm internal clamp diameter. Labeled IS solution was added via the loop (20 µL) and flushed through the DBS card together with 1 mL desorption solvent (water + 0.1% formic acid at a flow rate of 2 mL/min). After desorption, the DBSA took a second picture. These pictures identify the card before and after extraction, which may help to identify the source of unexpected results.

A disposable Hysphere C18HD SPE cartridge was used (7 µm, 2 × 10 mm) and conditioned with 1 mL acetonitrile at a flow rate of 5 mL/min. It was subsequently equilibrated with 1 mL water + 0.1% formic acid at a flow rate of 5 mL/min before the compounds were eluted from the card onto the SPE system. After loading the SPE with the components, it was washed with 1.5 mL water/acetonitrile 95:5 (v/v) + 0.1% formic acid at a flow rate of 5 mL/min.

The HPLC pump eluted the trapped component from the SPE cartridge to the LC-MS/MS system, with a composition of mobile phase consisting of 95% solvent A and 5% solvent B (see under 2.1.5). After a gradient program of exactly 8.1 min, the DBSA-SPE system was washed with 1 mL water + 0.1% formic acid and 2 mL water/acetonitrile 30:70 (v/v) + 0.1% formic acid at flow rates of 5 mL/min. The on-line DBS-