TDM of Antiseizure Medications

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Therapeutic drug monitoring (TDM) of antiseizure medications (ASMs) represents a valuable tool to establish an appropriate patient therapy, to collect important information about drugs' interactions and to evaluate patient's metabolic capabilities. In recent years, a new volumetric absorptive microsampling technique using VAMS® technology and Mitra® devices, consisting of a sampling technique for the collection of fixed-volume capillary blood, was developed. These new devices provide a new home-sampling technique for whole blood that has been spread out to simplify sample collection from finger-pricks.

Keywords: volumetric absorptive microsampling ; therapeutic drug monitoring ; bioanalysis ; whole blood analysis ; antiseizure medications ; blood-to-plasma ratio

1. Introduction

Epilepsy is a chronic brain disease that affects approximately 50 million people worldwide. Etiology is very variable encompassing genetic forms, metabolic diseases, brain injuries etc. Epilepsy is characterized either by recurrent seizures or absences that arise from uncontrolled electrical discharges in different brain districts. Seizures involving the whole body are known as generalized seizures while partial seizure refers to episodes that affect only a single part of the patient's body.

Seizures can be controlled, and the main treatments are antiseizure medications (ASMs). Moreover, for some ASMs (carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT), and valproic acid (VPA)) Cytochrome P450 enzymes family metabolizes most of the ASMs in the liver and this may result in metabolic interactions among different ASMs and/or other concomitant drugs [1]. In order to assess these interactions and monitor ASM efficacy and toxicity, TDM is usually performed in patients with epilepsy.

Currently, TDM is a necessary tool for individualizing drug treatment and optimizing patient outcomes. –response relationship to a certain ASM as well as possible pharmacological interactions between different ASMs and/or other concomitant drugs. Only 70% of patients with epilepsy respond to treatment with one or two ASMs [2]. Therefore, and since dose–response relationships may change over time, TDM is the necessary tool to make ASM treatment highly patient-specific, even in the case of multi-drug treatments or polypharmacy.

These matrices have been obtained from venous blood (sample volume ranging from 500 µL to 5 mL); thus, blood sampling requires a collection by trained personnel for venipuncture procedure. All of these factors have led to the development of alternative sampling techniques that overcome the above-listed drawbacks and require less sample volume (<50 µL). Regarding microsampling techniques, dried blood spot (DBS) is the most common. They used dried blood samples to detect phenylketonuria in a population of newborn infants by measuring phenylalanine concentrations in whole blood.

The first is the quality of the sample where it has been reported that insufficient spot quality led to poor results. A new and simple approach to blood collection that includes all the advantages of DBS sampling but overcomes the aforementioned issues is represented by volumetric absorptive microsampling (brand name Mitra®devices and VAMS®technology; Neoteryx, Torrance, CA) [3][4]. Since the withdrawal can be made in the absence of qualified personnel, it allows for sample collection at home. Today, VAMS®may represent a very promising microsampling technique (reviewed in [5][6][7]) (a complete list of published papers involving VAMS®use can be found in [8]).

2. Volumetric Absorptive Microsampling Analysis Using VAMS® Technology

VAMS®sampling in feasibility studies, such as those illustrated in this review, in most cases is performed by contacting the blood surface (avoiding full immersion), for a few seconds with the VAMS®tip, using a standard venous blood tube. To date, studies involving actual sampling from the finger are still rare. Among those analyzed in this review, only two studies make use of finger pricking or capillary blood for ASMs [9][10]. [11] published a pharmacokinetic study of radiprodil oral suspension in healthy adults comparing conventional venous blood sampling with two microsampling techniques, comprising VAMS®technology.

Sample preparation usually takes place after a drying period of the Mitra®device (often at room temperature) ranging from 1 h to 3 h, sometimes in the presence of a bag with desiccant. Velghe and co-workers [10] have described VAMS®and DBS microsampling for therapeutic drug monitoring of antiseizure medications in children with Nodding syndrome and epilepsy. and then the first drop of mixed blood and interstitial fluid was removed. This strategy avoids diluting whole blood with interstitial fluids and must be considered very carefully when planning TDM studies based on VAMS®sampling.

[12] have reported a clinical verification of volumetric absorptive microsampling for therapeutic drug monitoring of anti-epileptic drugs. In this study, different ASMs (10-OHOXC, CBZ-E, CBZ, OXC, PRM, PHT, ETS, PB, GBP, TPM, VPA, LTG, PGB, LCM, LEV, and BRV) were determined in whole blood collected by VAMS®. Samples were prepared by dipping the Mitra®microsampler devices into the ASM-spiked whole blood. After 45 min of shaker and centrifuge, 25 µL of supernatant was diluted into 350 µL of water (for samples containing VPA, ETS, and PB

D'Urso and colleagues [13] also have reported a new method to quantify 14 different antiseizure medications (LEV, LCM, ETS, RFN, ZNS, FBM, LTG, OXC, CBZ, PB, PRM, PHT, TPM, and PMP) and 2 active metabolites (10-OHOXC and CBZ-E) in samples collected by volumetric absorptive microsampling. Samples were collected by dipping only the lower part of the VAMS®tip of Mitra®device, in order to avoid oversampling, into whole blood in K3-EDTA containing tubes. Few seconds after the tip's surface became entirely red, time necessary to ensure complete blood absorption; tips were removed and desiccated at least one hour at room temperature. Subsequently, dried tips were rehydrated in a 96-well plate with 200 µL of LC-MS/MS grade water and 250 µL of acetonitrile containing deuterated internal standards were added.

Velghe et al. [14] and D'Urso et al. [13] both highlight the need to avoid overfilling of the Mitra®devices not completely immersing the VAMS®tip into the blood.

The sample preparation strategy described by Moorthy et al. [15] in their study involves drying the sample for 60 h at room temperature, in a special dryer with desiccant gel, covered with aluminum foil. Then samples are extracted with 250 µL of a mixture consisting of 0.1 M zinc sulfate, 0.1 M ammonium acetate and 0.1% formic acid in acetonitrile. The entire diluted supernatants (500 µL) were then transferred to a solid phase extraction (SPE) plate for sample cleanup before injection Supernatants diluted (1:1 (v/v)) with water or mobile phase were injected in LC–MS/MS system.

In the study from Pigliasco et al. [9] VAMS®samples were extracted with 200 µL methanol, sonicated and centrifuged. Extracted samples were then purified with an SPE online purification system.

The common features among the reported studies are the intensive laboratory trial to set different screening assays to optimize the extraction conditions from VAMS®and the subsequent trial to set an appropriate analytical method. Often two or more organic solvents (i.e., methanol and acetonitrile) were tested in different combinations combined with different steps of re-hydration and/or sonication.

All the validated and reported procedures involve the use of water and an organic solution as precipitating agent, and a shaking step that varies from 10 min to 45 min or a sonication treatment. In addition, very different extraction temperatures were investigated.

Common steps in sample preparation are: sample drying for an appropriate length of time (at least 2 h), extraction of drugs from tips polymer by the mean of rehydration combined with protein precipitation. Of particular significance, at least in our hands, was the rehydration step to ensure the minimum possible variability in extraction recovery. No further purification steps were needed, apart from a dilution in water or mobile phase in order to achieve a good and reproducible signal. Only studies on CBD monitoring, reported further purification steps, namely SPE plates [15] or SPE online [9].

All methods showed that the extraction recovery percentage values met European Medicines Agency (EMA) guidelines [16] for method development.

Summary of investigated ASMs by mean of VAMS® technology, extraction methods and blood/plasma ratio.

PP: protein precipitation; SPE: solid phase extraction; RT: room temperature; Nd: not determined.

all rely on ASMs detection by mean of triple quadrupole LC-MS/MS analysis with similar mobile phases and reversed phase C18 columns. Drugs were detected with electrospray ionization (ESI) or heated-electrospray ionization (H-ESI) ionization probe, either in positive or negative mode with comparable accuracy, precision, and recovery. Clinical method validation was carried out comparing data obtained from Mitra®devices equipped with VAMS®tips with routine methods on plasma/serum routinely used in respective laboratories. compared their results with serum concentrations obtained using chemiluminescent magnetic microparticle immunoassay technology (CMIA, Abbott Diagnostics).

The two studies differ in the use of ionization probes. Results are comparable and methods were both validated although only Pigliasco et al. referred to international guidelines on bioanalytical method validation [16][17]. Data comparison was conducted against venipuncture-derived plasma values, obtained with a method developed previously by Pigliasco et al. [9] with addition of an on-line SPE purification.

[15] developed a different strategy. Whole blood samples were spiked with different concentrations of CBD. The same sample was loaded on VAMS®tips and the remaining was centrifuged in order to obtain plasma with same drug concentration. One limitation of the study was that only one clinical pharmacokinetic curve (obtained from one volunteer), was reported.

It is of interest to note that the analytical methods presented in the herein reported studies show a number of similarities to one another, for example all studies used LC–MS/MS and all with basic mobile phases. This demonstrates easy and feasible use of TDM for ASMs from extracts of VAMS®tips. Indeed LC-MS/MS instrumentation may be necessary for measuring extracts from VAMS®tips because of the improved sensitivity and specificity of this technique compared to other platforms such as immunoassay. To adapt the use of this particular micro method to these techniques it would be necessary to develop other extraction methodologies.

The effect of the stability of ASMs on VAMS®samples have been investigated by a number of groups across a range of temperatures (−60 °C, 37 °C, room temperature, 4 °C, –20 °C and −78 °C), and durations (1–60 days). The results indicated that time and temperature have different effects on specific ASMs and are summarized inSupplemental Material Table S1.

[14] reported that VPA, PB, PHT, CBZ, and CBZ-E, stored at temperatures ranging from 4 °C to 60 °C for four days or one week, showed a percentage difference from fresh sample did not exceed ±15% except for the CBZ metabolite (CBZ-E). In another study, Velghe and colleagues [10] went one-step further, testing clinical samples collected in Uganda that were mailed to laboratories in Belgium and finally stored −20 °C. To assess stability, QC samples, stored at −20 °C, in zip-closure plastic bags containing desiccant gel, were examined after storage times ranging from 4 days to 31 days. QC samples with nine leftover hospital patient samples were also examined after 93 and 186 further days of storage.

[12] concluded that their stability study enabled an accurate detection of a wide variety of ASMs from VAMS®extracts within 2 days post sampling. However, they also reported that ETS, LTG, OXC, PB, PHT, PGB, and PRM appeared to suffer negative biases as a result of a lack of temperature control, showing a loss of more than 15% in concentration when exposed at room temperature for 1 days, 2 days, 3 days, and 7 days. There was instead an improvement of stability when the VAMS®samples were stored at –20 °C for 1days and 7 days: a decrease of degradation was observed for LTG and PHT but still less than –15% of percentage difference from day 1. This demonstrates a clear improvement of stability on dried VAMS®compared to liquid blood, observed when samples were stored at 37 °C for 2 h and 48 h where all differences from day 1 are within ±15%.

Stability studies have thus suggested that ASMs on VAMS®were stable until one week at controlled temperature, allowing the potential implementation of VAMS®assays from remotely collected samples. However, it is necessary to carry out a further stability investigation for some specific compounds, such as OXC focusing on the effect of the physico-chemical properties of both the drug and microsampler surface.

Moorthy et al. [15] tested VAMS®tips under very different time/temperature combinations (summarized inTable S1). The authors found an acceptable stability for CBD at temperatures below 0 °C, in the autosampler at 10 °C for 24 h and for QC samples stored for 1 week sealed in a dedicated environment. An acceptable stability was also demonstrated also for post-extracted samples when stored at −80 °C.

Pigliasco et al. [9] also studied the stability of CBD on Mitra®devices at 1 weeks and 4 weeks at two different temperatures (−20 °C and 25 °C) with satisfactory results at a range of concentrations. In fact, the reported variation of accuracy and CV varied between 90–99% and between 3–8% respectively.

The stability studies have shown that Mitra®devices with VAMS®tips allow the exchange and storage of ASMs samples between the patient and the laboratory, even over long distances and/or over longer periods, within acceptable stability limits. For this reason, the aforementioned stability studies were of the utmost importance, to demonstrate future implementation of robust assays from remotely collected samples.

Data collected in the studies reviewed in this paper, clearly demonstrate that Mitra®devices with VAMS®tips meet this need in both experimental and real-life settings. The stability of OXC remains a matter of debate and further studies will certainly be necessary, possibly on large numbers of real finger pricking samples. However, OXC, although pharmacologically active, is the pro-drug of 10-OHOXC, as it is rapidly metabolized to the active metabolite of OXC. Therefore, most laboratories do not measure OXC routinely.

One of the main advantages of VAMS®is that accurate volumes of blood can be collected independent of HCT values. To measure the HCT effect, a comparison of peak areas of compound(s) spiked into blood samples with a range of HCTs values (typically 25–65%) has been performed. The percentage recovery of these samples is compared to corresponding blank blood samples extracted under the same conditions with standards spiked in post extraction.

[13] observed absence of a significant HCT bias across all drugs and HCT levels (35%, 45%, and 55%) tested. Similar results were collected by Velghe and co-workers [14], that investigated a wider range of HCT values (21%, 42%, 52%, and 65%). It was observed that even when high HCT values seem to correspond to a lower recovery, there are not statistically significant differences between the different HCTs, except for the recovery of VPA at 62% HCT compared to 42% in samples prepared by pipetting 10 µL onto the samplers. (data shown in Supplemental Digital Content 1,http://links.lww.com/TDM/A381(accessed on 10 February 2021).

Canisius et al. [12] evaluated HCT effect by calculating the percentage of deviation recovery against HCT values: HCT value varied from 30% to 55% and more than 90% of measurements were within 15% of deviation (OXC was excluded). Finally, the HCT effect was not investigated by Pigliasco, F et al. [9] assuming from previous studies that VAMS

Moorthy et al. [15] evaluated the impact of three HCT levels (20.5%, 39.9%, and 67.2%) on the quantitation of CBD. CBD had a matrix effect of 100–108%, across two concentration levels thus demonstrating that CBD had optimal recovery and minimal matrix effect following extraction from Mitra®devices with VAMS®tips employing the described method.

As discussed earlier, Mitra®devices with VAMS®tips have been developed to solve spot area mediated HCT biases seen from DBS post punch extractions. Therefore, the exclusion of a HCT effect represents a crucial point of these method validations because if the contribution of the HCT is considered null or limited, a validation of robust assays across a range of HCTs is possible. Indeed, all studies reviewed within, clearly show HCT independent assays on Mitra®devices with VAMS®tips for measurement of ASM concentrations in whole blood over a wide HCT range is possible.

Velghe and Stove [14] revealed that blood-to-plasma ratio (R) of VPA, PB, PHT, and CBZ were in line with published data, despite the small number of measures obtained; also measured blood-to-plasma ratio for several antiseizure medications separating those with ratio around 1 from those with ratio different than 1 as evidence of the different capability of binding to red blood cells (RBC). As an example, in this study authors found R = 2.7 ± 0.8 for ZNS reflecting the fact that ZNS is well known to bind substantially to RBC [18][19]. It is already known from a study published from Patsalos P.N. [20] on PMP PK using radiolabeled drug that PMP has a blood/plasma ratio covering a range from 0.55 to 0.59, as expected for drugs with a very high plasma protein binding (>95%).

[10] had published an article as application of previously validated method, measuring the levels of VPA, PB, and CBZ in patients with Nodding syndrome, from Uganda and Democratic Republic of the Congo, comparing also VAMS®with dried blood spots (DBS). This study confirmed the blood-to-plasma ratios already published but with some differences between the method for VAMS®measure and DBS and serum concentration. Velghe and colleagues, comparing DBS and VAMS®samples, reported a lower variability in incurred sample reanalysis test for DBS. The authors found an overestimation of VAMS®concentrations compared with DBS concentrations that most relevant especially for samples containing PB.

Canisius et al. [12] did not investigate blood-to-plasma ratio.

Regarding CBD, only Moorthy and colleagues [15] reported data on this issue. These authors studied CBD partitioning in both extracted plasma and Mitra®devices with VAMS®. R-values measured in plasma ranged from 0.714 to 0.775, showing a good correlation with that found measuring R on Mitra®devices with VAMS®as the reported R for VAMS®ranged from 0.696 to 0.827.

Data on blood/plasma ratio are discussed in more detail inSection 3, Discussion.

3. Conclusions

Volumetric Absorptive Microsampling using Mitra®devices based on VAMS®technology is bringing a revolution in the sampling, pre-treatment, and analysis of biological fluids, especially regarding pharmacokinetic and toxicological studies.

In addition, VAMS®has proved less invasive and therefore is helping in therapeutic drugs monitoring by increasing subject recruitment and retention.

Despite all the advantages already demonstrated, other points (analyte stability, repeated analysis, correlation between plasma and blood concentrations) need to be clarified before the technique will be accepted as a routine bioanalytical procedure. This approach could be adopted as the sampling method of choice in several areas, not only for drug monitoring, but also for toxicokinetic and clinical studies, as well as other fields such as forensics purpose. Further studies are needed to validate this sampling method on real patient finger/heel pricking and to assess the clinical usefulness of the new sampling method.

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