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Fit-for-Purpose Validation of a PK Assay applying Blood Collection by Volumetric Absorptive Microsampling

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OBJECTIVES: Validation and application of a fit-for-purpose PK assay used for quantitative analysis of acetaminophen in whole blood applying volumetric absorptive microsampling using MITRA® devices.

METHODS: MITRA® devices were evaluated using whole blood samples prepared at different hematocrit values. PK assay parameters precision, accuracy, linearity, LOD, LLOQ, ULOQ, carry-over, and stability of analyte in dried whole blood on MITRA® device were validated following EMA guidelines. Acetaminophen was quantified by LC-HRMS. MITRA® devices were used on selected timepoints post-dose to collect capillary blood after finger prick. Acetaminophen was extracted using methanol containing acetaminophen-d4.

RESULTS: Adsorbed whole blood volume ranged from 9.79 ± 0.18, 9.95 ± 0.34, and 10.10 ± 0.48 µL for 20, 45 and 65 % hematocrit. Highest intra-day precision and accuracy were 11.4 % CV and -19.7 %bias at 1 µg/ml. Highest inter-day precision and accuracy were 12.3% CV and -18.8 %bias at 1 µg/mL. Measuring range was 1-25 µg/mL and LOD of 0.30 µg/mL. ACP was stable on Mitra® device tip for up to 4 weeks. Obtained results from the pharmacokinetic study were not significantly different to parameters reported in the literature using plasma samples.

CONCLUSIONS: MITRA® devices have a great potential use as an alternative whole blood collection device in comparison with other blood sampling techniques or devices.

KEYWORDS: volumetric absorptive microsampling, hematocrit, acetaminophen, pharmacokinetics, accurate mass-high resolution mass spectrometry.

INTRODUCTION

Over the past decade there has been an increased interest in as well as academic research in the pharmaceutical industry in the use of microsampling techniques of whole blood for the quantitative analysis of a plethora of analytes ranging from small molecules to large molecules. Dried blood spot (DBS) sampling using a filter-paper for the collection of small

amounts of blood (10-25 µL) has led to a rapid growth of this microsampling technique but also in general for micro sampling using other devices or technologies.

DBS were firstly described by Guthrie in the screening of neonates on phenylketonuria [1]. The popularity of DBS originates from some major benefits that DBS has, namely; small amounts of blood sample needed, fast blood collection technique that can be even used for collection of blood in hospital facilities in remote areas or directly at patient's homes [2].

But with advantages also disadvantages exist. One of the major disadvantages of DBS is the significant impact of the blood sample's hematocrit value on the quantitative analysis, but also the frequently observed inhomogeneity of DBS samples. Natural variations in hematocrit values between samples causes a significant impact on the reliability of the data obtained from using DBS. A more extensive review of advantages and disadvantages as well as challenges DBS microsampling has been discussed previously by Meesters and Hooff [3].

To overcome the challenge DBS presents with hematocrit of whole blood samples Dennif and Spooner [4] introduced in 2014, a whole blood device to circumvent the hematocrit effect and inhomogeneity of DBS samples. Authors introduced a microsampling device containing a tip (made of porous material) for accurate and reproducible collection of biofluids. Because the blood collection tip is made from a porous material, the pores of the material are designed to adsorb a fixed volume of blood via capillary action independent from the hematocrit. The microsampling technique is known as Volumetric Absorptive Microsampling (VAMS) and the device is commercially available under the name MITRA®. The device can be used for biofluid collection by not medically trained individuals including patients in a home-based setting [5]. A patient blood sampling home-based application example is the monitoring of HbA1c in blood of diabatic children [6].

MITRA® devices are not only used in diagnostic application but also in several research areas such as pharmaceutical analysis [7], therapeutic protein analysis [8], metabolomics [9, 10], lipidomics [11], monoclonal antibodies [12], environmental research [13] and recently in COVID-19 research and testing [14] as well as in other applications [15]. Previously, a pharmacokinetic study using Acetaminophen as model drug by Rincon and Meesters [16] evaluated and compared using DBS and plasma samples in pharmacokinetic applications. In present paper, MITRA® devices were applied in a fit-for-purpose validation of MITRA® devices for blood microsampling in whole blood pharmacokinetics. For that purpose, a assay was developed using MITRA® devices for whole blood microsampling post-dose. The assay was prior to use in the quantitative analysis of whole blood drug concentrations validated in adherence to recent FDA [17] and EMA guidelines on validation of bioanalytical assays [18]. The assay performance characteristics evaluated were precision, accuracy, linearity, carry-over and stability of the drug in dried blood matrix on MITRA® tips. A fit-for-purpose validation strategy was selected with the rational that was based on the validation of assay key performance characteristics such as precision, accuracy, response curve linearity and stability of the analyte ACP in a dried whole blood matrix adsorbed on a Mitra® device. Other assay performance characteristics such as specificity, interference by cross-reacting molecules and/or concomitant medications, biotransformed species and robustness have to be validated prior to using this type of assay in clinical studies. After finalization of the assay fit-for-purpose validation, the assay was used in the assessment of PK of Acetaminophen. Additionally, an assessment on the density of whole blood with different hematocrit and the volume of whole blood adsorbed at different hematocrit were performed. The PK data was obtained from a single dose of 500 mg of Acetaminophen by one healthy voluntary donor.

MATERIALS AND METHODS

Chemicals and materials

Acetaminophen (ACP), HPLC-grade methanol and acetonitrile were purchased from Sigma-Aldrich (St. Louis, USA). Acetaminophen-d4 (ACP-d4) used as an internal standard was from Cerilliant (Round Rock, TX, USA). Ultrapure water (18.2 MΩ.cm) for liquid chromatography (LC) was produced in-house by a Direct-Q® 3UV Milli-Q apparatus (EMD Millipore, Billerica, MA, USA). Capillary whole blood samples from one voluntary

healthy subject were collected after a finger prick during the pharmacokinetic study (PK) by application of MITRA® sampling devices (Neoteryx, USA). The MITRA® devices were kindly provided by Neoteryx (Torrance, CA, USA).

Liquid chromatography-high resolution mass spectrometry (LC-HRMS)

The mass spectrometric analysis of calibrators, QC samples and PK samples by LC-HRMS was performed according to the method described previously [16]. In short, LC separation was performed using an Agilent 1260 Infinity LC system. Samples were separated on a Waters Atlantis T3 column (2.1 x 100 mm x 5 µm) kept isothermally at a temperature of 35°C. A separation gradient using mobile phase A (0.1% formic acid in H_2O) and mobile phase B (100% acetonitrile) was applied to elute the analyte: 2% B from 0 to 0.5 min, 2 to 30% B from 0.5 to 2.5 min, 30 to 95% B from 2.5 to 2.6min, held at 95% B for 1.4 min followed by 2% B until 6.0 minutes. An equilibration time of 5 minutes prior to next sample injection was used. The applied flow was 0.3 mL/min and sample injection volume was 20 µL. HRMS analysis was done using an Agilent accurate mass Q-TOF 6520 mass spectrometer (MS) working in positive electrospray ionization (ESI). Optimized settings were as follows: gas temperature: 300°C (N_2); nebulizer pressure: 48 psi; capillary voltage (Vcap): 3500 Volts; drying gas: 8 L/min; fragmentor voltage: 135 Volts; skimmer voltage: 60 Volts. For quantitative analysis following LC-HRMS accurate mass-to-charge ratios for ACP (m/z 152.07110) and ACP-d4 (m/z 156.09619) were applied. The mass accuracy obtained was < 3ppm (mean mass accuracy for both analytes was -1.1 ppm).

Preparation of whole blood samples with different hematocrit

The whole blood amount that could reproducible be adsorbed onto the sampling tip of a MITRA® device was assessed by an adsorption of artificially prepared whole blood samples at three different hematocrit values (20, 45, and 65%). Whole blood samples with different hematocrit were prepared by gently mixing of different volumes of plasma and erythrocyte pellet prepared from centrifuged whole blood sample from one single healthy voluntary subject as described previously [19].

Assessment of adsorptive volumetric property of MITRA® tip

The assessment of the amount of adsorbed whole blood on a 10 µL MITRA® tip was performed by two experiments:

1. Determination of the density of whole blood at different hematocrit

One milliliter of whole blood samples with different hematocrit was pipetted into a microtube (1.5 mL). The weight of the microtube including the whole blood was determined using an analytical balance. Then five aliquots of 10 µL each, were removed from the microtube by pipetting and the weight loss of the microtube after every pipetting step was. The weight of the blood was then used for the calculation of the density of the artificially prepared whole blood with three different hematocrit values.

2. Determination of adsorbed whole blood volume

From five MITRA® devices, the weight was determined and 10 µL of whole blood at the three different hematocrit values was collected by absorption according to the protocol provided by the manufacturer. Thereafter, the weight of the wet MITRA® device was immediately determined, later, the MITRA® devices were let too dry to completely dryness prior to further sample preparation steps (120 minutes). The determination of the collected amount of adsorbed whole blood at three different hematocrit values was done according to the methodology previously reported by Dennif and Spooner [4].

MITRA® based assay validation procedures

The assay was validated according to recent validation guidelines on bioanalytical methods adapted from the 2011 EMA guidelines [18]. Whole blood standards and quality

control samples (QC) were prepared using MITRA® devices through adsorption of ACP fortified whole blood at 45% hematocrit. MITRA® QC samples were prepared at whole blood nominal concentration levels of 1.00, 3.00, 12.0 and 18.0 µg/mL ACP and were used in the validation of following assay performance parameters: carry-over, linearity, lower limit of quantification (LLOQ), limit of detection (LOD), within-day and between-day accuracy and precision as well ACP whole blood stability (1, 2 and 4 weeks) [19].

ACP Response curve

The response curve for the quantitative analysis of ACP in whole blood samples using MITRA® devices was assessed by analysis of seven whole blood matrix matched standard solutions spiked with ACP. The response curve covered an ACP concentration range of 1 – 25.0 µg/mL, plus a blank whole blood sample. Each matrix matched calibrator was measured in triplicate (n=3). ACP from the air-dried MITRA® devices (tip unmounted from holder) was extracted by using 100 uL of methanol containing IS (1 ng/mL) in a 1.5 mL microtube. Linearity between the area ratio of ACP and ACP-d4 was considered to pass acceptance criteria if the coefficient of determination (r^2) was >0.990. MITRA® calibrators for assessment of the linearity were prepared according to following protocol:
From a primary aqueous ACP stock solution of 500 µg/mL, whole blood matrix matched standards (45% hematocrit) were prepared by dilution of this primary stock solution with drug-free whole blood. Standards were prepared at concentrations of 0.00, 1.00, 2.50, 5.00, 10.0, 17.5, and 25.0 µg/mL. The selected calibration concentration range that has been validated was selected on basis of the pharmaceutical therapeutic concentration range of ACP in plasma (10–20 µg/ml) [20].

Whole blood matrix-matched response curve using MITRA® devices was prepared according to following procedure:
The MITRA® device was held at an angle of approx. 45 degrees touching the surface of a prepared whole blood matrix-based standard into a 1.5 mL microtube. The MITRA® device tip was let to adsorb the whole blood by capillary forces until the complete MITRA® tip changed color from white to red. The tip was held in this position for additional two more seconds and then the MITRA® device was removed. This adsorption procedure was done for each standard in triplicate and MITRA® devices were let too dry for 2 hours before extraction. Thereafter, the MITRA® absorbance tip was removed from the holder using tweezers and extracted by sonication using 100 µL of methanol containing ACP-d4 (1 ng/µL) for 15 minutes. Samples were centrifuged 5 minutes at 13300 rpm at 4°C and supernatant was analyzed.

Lower limits of quantification and detection (LLOQ and LOD)

LOD was determined mathematically by application of obtained response curve. The LLOQ was defined as the lowest whole blood standard that could be quantitatively analyzed with acceptable accuracy of <20% bias and precision of <20% CV, this according to the EMA validation guidelines. The limit of detection (LOD) was defined as three times the signal-to-noise ratio (S/N) of drug-free whole blood when analyzed in triplicate.

Carry-over

Carry-over effects of the MITRA® supported assay was assessed by the measurement (n=3) of MITRA® QC samples at high concentration (18.0 µg/mL ACP), followed by the measurement of a drug-free whole blood sample (n=3, extracted without the addition of IS). Carry-over was considered passing acceptance criteria if the concentration of ACP observed during the analysis of the blank sample was less than 20% of the LLOQ for ACP and 5% of the LLOQ for ACP-d4.

Within- and between day accuracy and precision

Within-run and between-run accuracy and precision were determined by injection of MITRA® QC samples at four selected concentration levels (1.00, 3.00, 12.0 and 18.0 µg/mL ACP) covering the majority of the measuring range of the assay. Each QC sample was analyzed and prepared in triplicate. Accuracy and precision were considered passing the acceptance criteria when mean observed concentrations from the triplicate analysis was within 15 %bias or 15 %CV of the nominal value, while the QC sample at the LLOQ (1.00 µg/mL) was considered passing the acceptance criteria when %CV and %bias was < 20%. Within-run accuracy and precision were determined by three consecutive runs of the QC samples within a one-single day by analysis of the QC samples. The three consecutive runs of QC samples were performed in the morning, midday and during the night (within one day). Between-run accuracy and precision were determined by analysis of the QC samples on 3 consecutive different days.

ACP stability in MITRA®-whole blood matrix

The stability of ACP in the dried MITRA® whole blood matrix was evaluated by analysis of three QC samples (3.00, 12.0 and 18.0 µg/mL ACP) after storage for 1 week, 2 weeks and 4 weeks in a desiccator at room temperature. The stability of ACP was considered passing acceptance criteria if the observed nominal concentration of all QC samples after storage was within 15% CV and 15% bias of the nominal concentration value of day 0.

Pharmacokinetic study design

The PK study design used in the feasibility study was published previously by Rincon and Meesters [16]. In short, the PK study involved a single oral fixed-dose of ACP (Tecnoquimicas, Colombia, 500 mg) purchased at a local drugstore. For the feasibility study a one single voluntary participant was used because the emphasis of the feasibility study was on the analytical feasibility of the assay and not on the pharmacokinetic feasibility of ACP. The healthy voluntary participant fasted for at least 8 hours prior to the start of the PK study and blood samples were collected using a finger prick by an automatic safety lancet (SurgiLance, St. Ingbert, Germany). For PK profiling a volume of approximately 300 µL of peripheral blood from the finger was collected at post-dose time points: 0, 30, 60, 60, 90, 120, 150 and 170 minutes. Once whole blood samples were collected, MITRA® devices were employed to quantitively collect the whole blood samples. MITRA® devices were placed into a desiccator for at least 2 hours to ensure complete drying at room temperature until extraction and analysis.

Pharmacokinetic data analysis

LC-HRMS data was processed using Mass Hunter software (Agilent, V04.00) obtaining ACP concentrations at selected time points post-dose. PK profile and PK parameters calculated were: area under the curve (AUC), ACP clearance (Cl), apparent volume of distribution (V_d), time to occurrence of peak concentration (t_{max}), peak concentration (C_{max}) and half-life time ($t_{1/2}$). PK parameters were calculated by PkSolver® software [21] using a one-compartment open model with first-order ACP absorption and first-order elimination.

RESULTS AND DISCUSSION

Assessment of the whole blood density at different hematocrit

An important part of the validation strategy of the new developed assay was the evaluation and determination of the amount of whole blood absorbed by the MITRA® devices at the three different hematocrit values to ensure it was reproducible. Before the volumetric amount of whole blood that had been absorbed for the three different hematocrit values could be determined, the density of the whole blood sample prepared at selected hematocrit values had been assessed by gravimetric analysis. The density was assessed in fivefold and resulted in a mean density of 1.05 g/mL (SD 0.03 g/mL) at 20% hematocrit, 1.07 g/mL (SD 0.02 g/mL) at 45% hematocrit and 1.09 g/mL (SD 0.03 g/mL) at 65% hematocrit.

Assessment of adsorbed amount of whole blood on VAMS device

Following the gravimetric analysis of the absorbed whole blood amount at the three hematocrit values by the MITRA® device, the absorbed amount of whole blood was calculated from the weight increase of the MITRA® device. The obtained density of the whole blood samples resulted in a mean absorbed whole blood amount of 9.79 µL (SD 0.18µµL) at 20 %, 9.95 µL (SD 0.34 µL) at 45 % and 10.10 µL (SD 0.48 µL) whole blood at 65% hematocrit. Normalizing the mean amount absorbed whole blood at 20%, and 65% hematocrit with regards to the mean amount absorbed at 45% hematocrit resulted in relative mean amounts of whole blood absorbed at this hematocrit of -1.44% less at 20% and +1.48 % more at 65% hematocrit. The observed relative differences of mean absorbed amount of whole blood at 20 and 65% of hematocrit were comparable with the relative mean differences of blood adsorbed previously reported by Dennif and Spooner [4], the authors reported less than 5% adsorbed blood volume variation across the hematocrit range of 20–70% hematocrit. In total 68 VAMS devices were used for the determination of the absorbed whole blood amount at 45% hematocrit. Two absorbed whole blood amounts calculated from gravimetric analysis were outliers (see Fig. 1a). The mean absorbed volume of whole blood using n=66 observations was 10.16 µL (SD 0.4003 µL). Figure 1b represents the frequential distribution of obtained adsorbed whole blood amounts (45% hematocrit) using the n=66 VAMS devices.

MITRA® supported assay fit-for-purpose validation

The new developed MITRA® based assay for the quantitative analysis of ACP in whole blood did undergo a fit-for-purpose validation procedure according to recent FDA and EMA guidelines on development and validation of bioanalytical assays. Selected validated assay performance parameters were linearity, within-and between day accuracy and precision, stability of ACP in dried MITRA® device tips and carry-over. The validation results are presented in following sections. All presented validation results and PK data are mean results of a triplicate analysis unless stated differently.

ACP response curve applying MITRA® devices

The response curve results obtained demonstrated a good linearity between the ratio of the ACP/area ACP-d4 and ACP concentration (µg/ml) in the selected concentration range (0-25 µg/mL ACP). The linear regression equation between the ratio of the area ACP/area ACP-d4 (y) and ACP concentration (x) and coefficient of determination (r^2) were as follows; y=0.4303x, r^2=0.9920. Linear regression includes the calculation of the intercept but the result of the linear regression which was performed using αα=0.05 (95% Confidence level) demonstrated that the intercept of the response was not significant at the selected confidence level (p=0.0709) and was therefore

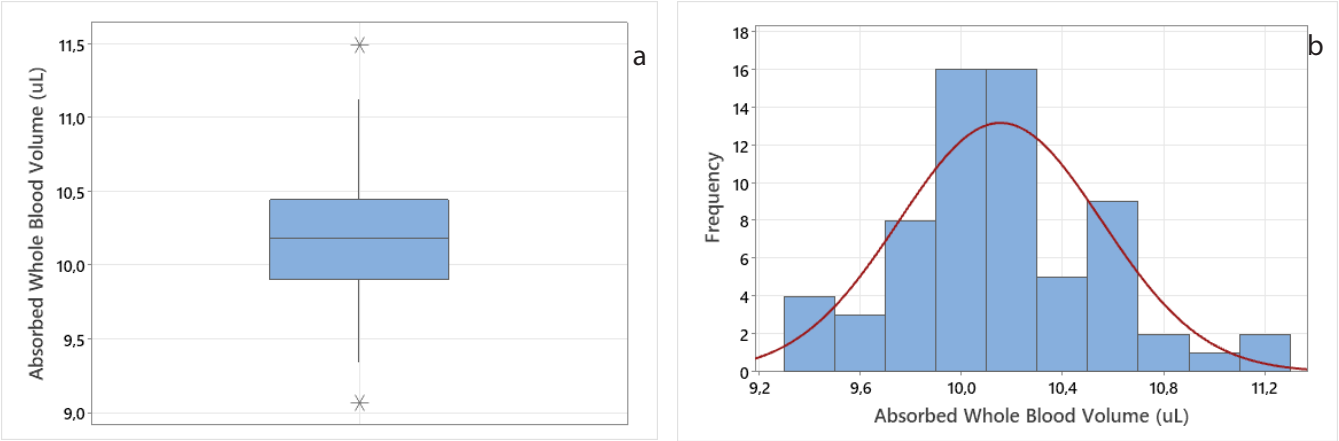

Figure 1. (a) Box-whisker's plot of adsorbed volume (µL) of whole blood (45% hematocrit) on MITRA® device. Shown are Q1 quartile, median, Q3 quartile and lowest and highest observed value and two outliers (represented by *). Whiskers from 9.35 to 11.12 µL; Q3: 10.44 µL; Median (Q2): 10.19 µL; and Q1: 9.90 µL; (b) Histogram showing the frequency of observed adsorbed volumes of blood (µL) using a MITRA® device. The hematocrit of whole blood presented was 45%

eliminated from the response curve regression line equation. The 95% confidence interval for the slope ranged from 0.4036 (lower 95%) to 0.4323 (upper 95%). The calibration curve including the 95% confidence intervals is presented in Figure 2.

Limit of Detection and Validation limits

The lower limit of quantification (LOD) was mathematically determined by using the regression equation from the obtained calibration curve. The LLOQ was defined as the lowest calibrator that could be quantitatively analyzed with acceptable accuracy and precision of <20% bias and <20% CV, this according to the EMA validation guidelines. The LLOQ of the MITRA® device bases assay was 1 µg/mL, which was the lowest calibrator used for the determination of the linear range of the calibration curve. The accuracy at the LLOQ was 15.4% bias while the obtained precision was 2.4 %CV. The limit of detection (LOD) was calculated from the obtained LLOQ by dividing the LLOQ limit by 3.3 (LOD=LLOQ/3.3) and was determined in threefold. Using the mathematical relationship presented above resulted in a calculated LOD of 0.30 µg/mL.

Within-day and between-day accuracy and precision

Observed within and between day accuracy and precision were for the lowest QC (LLOQ) less than 20 %bias and 20 %CV. At the three higher QC levels (3, 12 and 18 µg/mL ACP) observed assay accuracies and precisions were below 15 %bias and 15 %CV for all assays. All results from within and between- day accuracy and precision experiments are presented in Table 1.

Stability of ACP in the whole blood-MITRA® matrix

The stability of ACP in the dried whole blood-MITRA® matrix was assessed over a period of four weeks. This period was selected because the analysis of the whole blood samples will generally occur within a period of four weeks after sample collection. The decline of the ACP concentration in the whole blood-MITRA® matrix was observed to be less than 15% at all three QC sample concentration levels (3.00, 12.0 and 18.0 µg/mL). It was concluded that the stability of ACP over a time period of up to 4 weeks stored at room temperature was high enough for being able to store the whole blood impregnated MITRA® devices for at least four weeks prior to analysis. The observed stability of ACP is presented in Table 2.

Carry-over

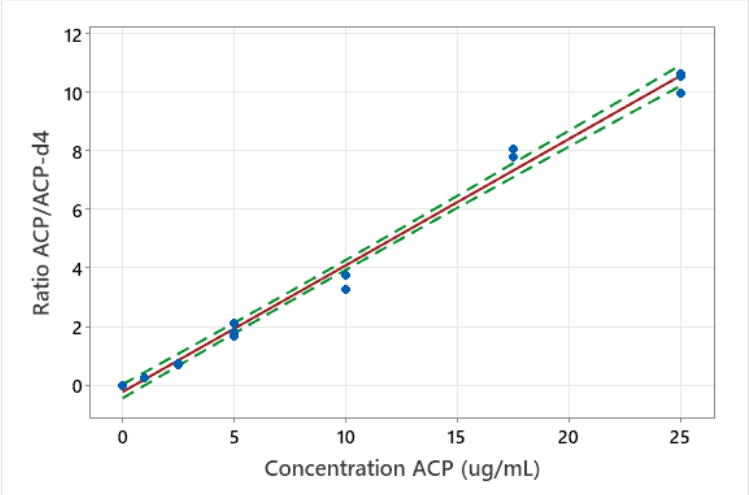

Figure 2. Response curve of Acetaminophen (ACP). The equation of the response was y=0.4303x with a Coefficient of determination (R^2) of 0.992. In the equation x represents the ACP concentration (µg/mL) and y the ratio between ACP and internal standard ACP-d4. The dotted line represents the 95 % confidence interval of the response curve.

Table 1. With-in day and between day accuracy and precision.

Within-days												
Nominal ACP concentration (µg/mL)		1.00			3.00			12.0			18.0	
	M	MD	E	M	MD	E	M	MD	E	M	MD	E
Mean observed ACP concentration (µg/mL)	0.8	0.9	0.9	2.6	2.7	2.7	11.2	11.1	11.6	19.2	19.3	19.9
Precision (%CV)	4.0	11.4	7.8	2.4	1.5	1.8	3.0	6.5	5.9	4.9	6.2	2.5
Accuracy (%bias)	-19.7	-15.1	-13.7	-13.6	-11.2	-10.2	-6.9	-7.5	-3.3	6.4	7.2	10.0
Between-days												
Nominal ACP concentration (µg/mL)		1.0			3.0			12.0			18.0	
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Mean observed ACP concentration (µg/mL)	0.8	0.8	0.8	2.5	2.7	2.7	10.9	11.4	11.5	17.0	16.9	17.3
Precision (%CV)	4.4	12.3	5.0	1.2	2.8	9.5	3.3	3.0	3.6	2.2	4.4	2.2
Accuracy (%bias)	-18.7	-18.4	-18.8	-7.10	-11.1	-10.7	-9.5	-5.3	-4.3	-5.5	-6.0	-4.1

Table 2. Stability of ACP in dried whole blood matrix on tip of MITRA® device.

	0 weeks								
Nominal ACP concentration (µg/mL)	3.00	12.0	18.0	3.00	12.0	18.0	3.00	12.0	18.0
Storage time	1 week			2 weeks			4 weeks		
Mean observed ACP concentration (µg/mL)	2.60	12.2	18.1	2.60	11.1	18.3	2.90	12.0	17.4
Precision (%CV)	1.70	3.60	8.90	3.40	5.60	4.70	6.00	2.20	5.20
Accuracy (%bias)	14.8	1.60	0.600	-12.8	-7.80	1.50	-3.30	0.00	-3.30

The validation of carry-over effects for the internal standard ACP-d4 was observed to be less than 5% and for a QC sample containing 18 µg/mL ACP and less than 20% for a blank whole blood samples spiked with the internal standard. All results were in compliance with FDA and EMA guidelines on method development and validation. The observed level of carry-over effects showed that cross-contamination of samples in a sample cue within the auto sampler could be assumed to be negligible.

Acetaminophen pharmacokinetic study

After assay validation was finished the validated assay was tested for its feasibility in a PK analysis applying whole blood samples collected post-dose at selected time points (0, 30, 60, 90, 120, 150 and 170 min). The PK curve of the post-dose collected samples applying VAMS devices is presented in Figure 3. The figures 3a-c demonstrated observed ACP concentrations (red circles) as well as predicted concentrations calculated by PKSolver® software (blue solid line) applying the one-compartment open model with first order absorption and first order elimination. Figure 3c shows the residuals calculated from applying the first order model. Comparison of the most important PK parameters (Cl, V_d, C_{max}, $t_{1/2}$ and AUC_{0-t}) showed that the mean values determined for these PK parameters were within the range of previously reported PK parameter values of previously obtained PK values measured using liquid plasma by Rincon and Meesters[16]. Reported values for Cl (clearance) were 0.68 ± 0.40 L/min, V_d (volume of distribution) 51.3 ± 24.1

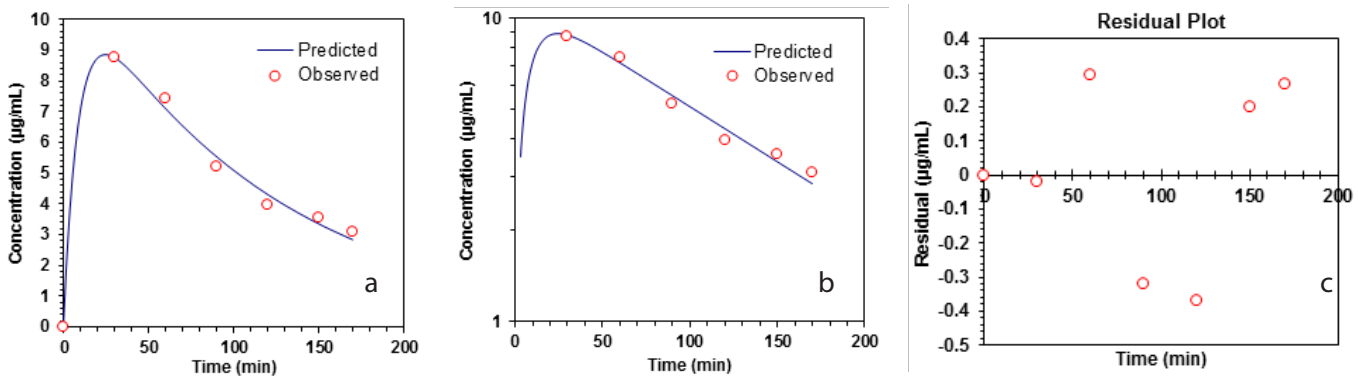

Figure 3. (a) ACP pharmacokinetic profile applying MITRA® blood collection devices presenting observed whole blood concentrations (solid blue line) and predicted whole blood concentrations (red circles) versus post-dose collection time (min); (b) PK profile presenting the natural logarithm of observed and predicted MITRA® device whole blood concentrations versus post-dose collection time; (c) presenting calculated residual concentrations (observed-predicted MITRA® device whole blood concentrations) versus post-dose collection time. Predicted MITRA® device whole blood ACP concentrations were calculated using PKSolver® software by application of a one-compartment open model with first order absorption and first order elimination.

L, AUC (area under curve) 965.7 ± 514.3 µg/mL min, $t_{1/2}$ (half-life time) 95.0 ± 48.9 min and C_{max} (maximum concentration) 6.0 ± 2.5 µg/mL. The obtained PK parameter values demonstrate the feasibility of applying MITRA® devices as a whole blood micro-collection tool in assays.

CONCLUSION

It was concluded that the assay applying MITRA® devices for the collection of post-dose whole blood collection from a voluntary healthy person has great potential to be applied in PK studies of small molecules-based pharmaceuticals in whole blood samples.

The fit-for-purpose validation strategy used to validate the assay had been proven successful. All assay performance parameters passed acceptance criteria according to FDA and EMA guideline acceptance criteria. The use of MITRA® devices for whole blood collection is a reproducible collection method of small volumes of whole blood samples even at different hematocrit values overcoming drawbacks from other whole blood microsampling techniques (such as Dried Blood Spot sampling). In larger PK studies or pharmaceutical analysis with many whole blood samples collected, stability up to 4 weeks of ACP in dried whole blood matrix stored in a desiccator at room temperature is a great advantage.

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