Clinical Validation of a Dried Blood Spot Assay for 8 Antihypertensive Drugs and 4 Active Metabolites

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Background: Drug nonadherence is one of the major challenges faced by resistant hypertension patients, and identification of this problem is needed for optimizing pharmacotherapy. Dried blood spot (DBS) sampling is a minimally invasive method designed to detect and determine the degree of nonadherence. In this study, a DBS method for qualifying 8 antihypertensive drugs (AHDs) and 4 active metabolites was developed and validated using ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS).

Method: The DBS assay was validated analytically and clinically, in accordance with FDA requirements. Analytical validation was accomplished using UHPLC-MS/MS. For clinical validation, paired peak and trough levels of DBS and plasma samples were simultaneously collected and comparatively analyzed using Deming regression and Bland–Altman analyses. All concentrations below the set lower limit were excluded. Deming regression analysis was used to predict comparison bias between the collected plasma and DBS samples, with DBS concentrations corrected accordingly.

Results: The UHPLC-MS/MS method for simultaneously measuring 8 AHDs and their metabolites in DBS, was successfully validated. With Deming regression no bias was observed in N = 1; constant bias was seen in N = 6 and proportional bias in N = 11 of the AHDs and metabolites. After correction for bias, only one metabolite (canrenone) met the 20% acceptance limit for quantification, after Bland–Altman analyses. In addition, amlodipine, valsartan, and [enalaprilate] met the 25% acceptance limit.

Conclusions: A novel DBS assay for simultaneously qualifying and quantifying 8 AHDs and their metabolites, has been successfully developed and validated. The DBS assay is therefore a suitable method to detect drug nonadherence. However, with the exception of canrenone, the interchangeable use of plasma and DBS sampling to interpret drug quantities should be avoided.

Key Words: hypertension, clinical validation, drug monitoring, chromatography, dried blood spot

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BACKGROUND

Hypertension is associated with increased risks of cardiovascular events and end-organ damage.[1,2] The use of antihypertensive therapy to decrease blood pressure (BP) reduces stroke rates in as high as 30% of the respective patient population.[3] Antihypertensive drugs (AHDs) are an effective hypertension therapy. However, nonadherence to antihypertensive medication is one of the most common causes of uncontrolled BP, hence the incorrect label "resistant hypertension" in as high as 50% of the respective patient population.[4,5] Ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) is a reliable method for measuring plasma AHD concentrations.[6–8] However, its use is hampered by logistic challenges and its vulnerability to bias, attributable to white-coat adherence. The dried blood spot (DBS) method is a more convenient and patient-friendly technique for drug concentration measurements. DBS can be performed by a simple finger prick, enabling immediate sampling (in the general practitioner's office) when nonadherence is suspected, minimizing the risk of white-coat adherence.[9–11] However, minute differences exist between DBS and plasma samples, and should be taken into account when using this method. First, whole blood as opposed to plasma, is used for the DBS assay, and is beneficial to drugs that adhere to red blood cells, which normally have lower plasma concentrations. Furthermore, because only a drop of blood is sampled, the blood viscosity, and thereby hematocrit, influences the quantity used for measurements. More so, the shape of the drop is important for obtaining reliable results during drug concentration measurements. It was therefore necessary to validate this method extensively, to observe for the similarities/equality in drug concentrations, compared with plasma-only measurements. Although the DBS method has been used to accurately measure immunosuppressive and antipsychotic drug concentrations,[12–14] it has

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not been validated for AHD measurements.[15–17] In this study, we developed (following analytical validation) and clinically validated a DBS method for 8 commonly used AHDs from the 4 most frequently prescribed drug classes, by qualifying and quantifying the AHD and active metabolite (displayed within [brackets]) concentrations, using UHPLC-MS/MS. Validation of the DBS-assay will give clinicians a reliable and valuable new tool to address nonadherence in patients with hypertension, which will benefit the patients by preventing cardiovascular events.

MATERIALS AND METHODS

Analytical Validation of the DBS Method to Determine Reliability

Method Development

A sensitive UHPLC-MS/MS (Thermo Scientific, Waltham, MA) assay was developed for the quantification of 8 AHDs and [their metabolites] in DBS. These include angiotensin converting enzyme inhibitors; enalapril [enalaprilate] and perindopril [perindoprilate], angiotensin II receptor blockers (ARB); losartan [losartan carboxylic acid (ca)] and valsartan, diuretics; hydrochlorothiazide and spironolactone [canrenone], and calcium channel blockers; amlodipine and nifedipine. The UHPLC setup consisted of a Dionex Ultimate system connected to a TSQ Vantage MS with a triple quadrupole and heated electrospray ionization probe. Plasma and DBS samples were prepared (plasma only) and analyzed (plasma and DBS) as previously described.[7] For DBS sampling, filter papers (Whatman protein saver 903 card, Cardiff, United Kingdom) were used, and one blood spot contained one drop of whole blood. Using a manual disk puncher, a 6-mm diameter sample was punched out of the blood spot. Enalaprile-d5-maleate dissolved in a mixture of acetonitrile and methanol (1:1), was used as internal standard, and added to 7 AHD samples. For hydrochlorothiazide, an internal standard of hydrochlorothiazide-13CD2 was used. Thereafter, samples were sonicated, centrifuged, and diluted with eluent.[7]

DBS assay validation was performed following the FDA/EMA guidelines on bioanalytical method validation,[18,19] and the concept guidelines of the alternative sampling committee of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDCMT), and as previously described.[12,20,21]

Hematocrit

To investigate the influence of blood viscosity on different AHD concentrations, blood with different hematocrit levels (0.3, 0.35, 0.4, and 0.45 L/L) was administered to increasing AHD concentrations on DBS.[22,23] Samples were measured using the UHPLC-MS/MS method, as previously described.[7]

Stability

The DBS samples were analyzed in batches. Although studies indicate the relative stability of these drugs in DBS,[20,24–26] we studied AHD stability in our samples. Hence, quality control (QC) samples (QC low, medium, and high) were used, and then stored in a desiccator for 11 and 26 days after sampling, at room temperature. The 11-day storage period was considered clinically acceptable. Sample concentrations were comparable to those mentioned on **Supplemental Digital Content 1** (see **Table 1**, http://links.lww.com/TDM/A375). Measurements were done in 2-fold. Based on the guidelines of the alternative sampling committee of IATDCMT, a ±15% degradation in the nominal value was considered acceptable.[27]

The Clinical Validation Method: DBS Concentrations versus Plasma Concentrations

Patients

Samples were collected between October 2016 and July 2018. A total of 135 patients were included, aiming for 40 paired samples/drug to validate the DBS method and ensure statistical test validity, when comparing DBS and plasma AHD/[metabolite] concentrations.[24] Patients were selected from the outpatient clinic (internal medicine, cardiology, and nephrology) at the Erasmus Medical Center, Rotterdam, The Netherlands.

As inclusion criteria, the automated BP measurement (ABPM) (Datascope Accutor Plus, Paramus, NJ) or office BP had to be <135/85 mm Hg, to optimize the chances of sampling an adherent patient, and the use of ≥2 AHDs for which the DBS method had been developed. All available drug dosages were exploited, in a bid to test and increase their usability in clinical practice. Exclusion criteria included the inability to provide written informed consent and proven nonadherence, after sampling and inclusion; based on nonmeasurable drug concentrations derived from plasma and DBS samples when a peak drug level was expected. This study was approved by the medical ethical committee of the Erasmus Medical Centre, Rotterdam, The Netherlands (MEC-2016-162).

DBS and Plasma Sampling

DBS samples were obtained using the finger prick method, following predetermined instructions; washing hands, finger massage before and after puncture, and sampling without card contact. The spots were allowed to dry for at least 20 minutes, and the cards folded to keep the blood spots away from light, to prevent the degradation of the photo labile compounds amlodipine and nifedipine.[28] The DBS cards were then stored at room temperature in plastic zipper bags containing silica desiccant, transported to the laboratory, and placed in a desiccator until further analyses. Venous whole blood samples were collected in ethylenediaminetetraacetic acid tubes during a regular venipuncture, preferably obtained within the hour before or after DBS sampling. For study purposes, an extra tube of blood was drawn (asides that requested by the physician), sent to the pharmacy laboratory, centrifuged, and the resulting plasma stored at −80°C until analyses.

For each AHD, 2 hospital visits were required, because the trough and peak levels of the same patient were measured to validate the entire potential drug concentration range.[24,29] The first visit, where BP was measured using ABPM, was

planned in the morning to ensure drug levels were measured approximately 1–7 hours after AHD intake (peak levels). During the second visit (approximately 2 weeks later), venipuncture and DBS were performed to measure trough levels. Here, patients were asked to delay their AHD intake until after the appointment, to maximize trough levels at an interval of ≥20 hours after intake. After attaining 20 samples (peak and trough levels from 10 patients) per drug, the peak levels for the remaining samples were then measured, as required for validation. This adaptation improved the convenience of study participation, as only 1 visit to the hospital was required. Furthermore, it limited the number of negative values (trough levels >24 hours after the last intake were not observed). Additional trough levels were obtained solely from patients who accidently failed to self-medicate on the day of measurement. Patients were allowed to participate more than once.

Data Analysis

Analytical Validation

To determine DBS method reliability, the following quantification limits were calculated, following the FDA/EMA guidelines on bioanalytical method validation[18,19]: linearity (by means of a calibration curve), limits of quantification, intraday and interday accuracy, precision, and stability. Matrix effects were measured as a whole, as opposed to individually, because different matrices were used for subsequent days. Reproducibility data were then used to determine any matrix effects.

Clinical Validation

To compare quantitative data from DBS and plasma samples, all samples below the lower limit of quantification (LLOQ) were discarded. Outliers were determined using boxplot analyses, because samples with extreme values were excluded in further analyses. Deming regression analyses were then performed to assess any constant and/or proportional bias between plasma and DBS AHD/[metabolite] measurements.[12] A proportional bias was obtained if the 95% confidence interval (95%-CI) of the regression line slope did not contain 1. When the 95% CI of the regression line intercept did not contain 0, the data were considered to have a constant bias. These results were used to calculate the "estimated plasma concentration."[30] The following formula was used, and adjusted with respect to constant and/or proportional bias: Estimated plasma concentration = (DBS concentration − intercept of Deming regression line)/slope of Deming regression line.

Using a Bland–Altman plot, the plasma AHD/[metabolite] concentration measured from venipuncture samples was compared with the "estimated plasma concentration" derived from DBS samples.[24,31] According to the European Guidelines on Bioanalytical Method Validation, ≥67% of the measurements per drug should be within 20% of the mean of the differences between the both methods.[18]

This difference was adopted as the acceptance limit, although alterations could be made, based on clinical relevance.[12,32] Previous work showed that a within 25% difference is also acceptable in AHDs, owing to their wide therapeutic range.[16] More so, to determine the suitability of the DBS method in clinical practice, the number of DBS false negatives, compared with those of plasma, were calculated. Here, all samples were included independent of their LLOQ.

Analyses were performed using the IBM SPSS statistics v24.0 software for Windows (IBM, Armonk, NY) and GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

RESULTS

Analytical Validation of the Method

A summary of the quantification limits is presented on **Supplemental Digital Content 1** (see **Tables 1–3**, http://links.lww.com/TDM/A375). The lower limit of detection (LLOD) and LLOQ for all drugs measured with DBS were determined during analytical validation. For most analytes, the lowest QC was higher, compared with the lowest measured DBS concentration. Hence, almost all low QCs failed to meet the requirements. Therefore, the LLOQ was raised (in comparison with the plasma method) to an acceptable value, in accordance with the QCs.

Hematocrit

No differences were observed in AHD concentrations, despite increasing hematocrit quantities. Therefore, no correction was required for measured DBS AHD/[metabolite] concentrations, regarding hematocrit.

Stability

DBS cards with low, middle, and high concentrations of the 8 AHDs and their metabolites were prepared on different dates, and assessed for stability (see **Table 4**, **Supplemental Digital Content 1**, http://links.lww.com/TDM/A375). Amlodipine, losartan, [losartan-ca], [perindoprilate], and enalapril, at all concentrations, showed no relevant differences in almost all QCs, after 11 and 26 days (<15% from the nominal value). [Canrenone] displayed a difference in QC low and medium after 11 and 26 days, which exceeded the acceptance limit. Although valsartan and spironolactone showed no relevant differences at low and median concentrations, their QC high values were above the acceptance limit after 11 and 26 days. Nifedipine stability data showed >20% degradation at medium and high concentrations. Hydrochlorothiazide and [enalaprilate] proved unstable at different concentrations at both time points.

Clinical Validation of the DBS Method

Sampling

A total of 195 samples were obtained from 135 unique patients. Patients (N = 3) were excluded when negative plasma and DBS analyses were found in peak drug levels, an indication of total or partial nonadherence.

Deming Regression Analyses

Before these analyses, samples below the LLOQ for DBS and/or plasma were initially excluded (Table 1),

followed by the exclusion of 2 [enalaprilate] sample outliers. These outliers were seen in the venipuncture, and corresponding DBS samples. The results of the Deming regression analyses are presented in Table 1. Constant and proportional biases were observed for the following 6 drugs of which [1 metabolite]: hydrochlorothiazide, losartan, nifedipine, perindopril, [perindoprilate], and spironolactone. Proportional bias was observed for the following 5 drugs of which [3 metabolites]: amlodipine, [canrenone], [enalaprilate], valsartan, and [losartan-ca] (see **Fig. 1A1–3**, **Supplemental Digital Content 1**, http://links.lww.com/TDM/A375).

Bland–Altman Analyses

Results of the proportion within the 20% and 25% range calculated using Bland–Altman analyses are presented in Table 2. Only [canrenone] met the 20% acceptance limit (Fig. 1A). For the 25% acceptance limit, additional AHDs; amlodipine, valsartan, and one metabolite; [enalaprilate], met the criteria. Moreover, the metabolites showed a better agreement, compared with their respective parent drugs (appendix; enalapril versus [enalaprilate], Fig. 1B versus C).

DBS False Negatives

False negatives for DBS compared with plasma are displayed in **Supplemental Digital Content 1** (see **Table 5**, http://links.lww.com/TDM/A375). Overall, most negative values were observed for the parent drugs: enalapril (5.2%), losartan (4.8%), perindopril (4.9%), and spironolactone (20.9%). Amlodipine, nifedipine, and [enalaprilate] only had a single false negative, whereas valsartan, [canrenone], and [losartan-ca] had no false negatives. Nevertheless, hydrochlorothiazide and [perindoprilate] had more false negatives for DBS (9.4% and 9.8%, respectively), compared with other measured drugs. These false negatives were found in random samples, independent of drug dosage.

DISCUSSION

In this study, a clinically convenient DBS method for qualifying and quantifying 8 AHDs and 4 metabolites from the most frequently prescribed drug classes, was validated using UHPLC-MS/MS. To the best of our knowledge, this is the first study that compared AHDs in DBS and plasma in an extensive number of samples from actual patients, instead of spiked plasma samples. A good agreement was observed with Deming regression analyses, after correction for outliers and bias. Based on Bland–Altman analyses, only [canrenone] met the 20% acceptance limits, implying that DBS and plasma sampling could be used interchangeably when quantifying [canrenone]. Also, the 25% acceptance limit was met by valsartan, amlodipine, and [enalaprilate].

Analytical Validation

DBS is a reliable method for qualifying and quantifying 8 AHDs and 4 of their metabolites. However, the LLOD and LLOQ were higher in DBS, compared to plasma (see **Table 3**, **Supplemental Digital Content 1**, http://links.lww.com/TDM/A375). This was mainly due to the deviation in the lowest QC of most drugs, during analytical validation. If

TABLE 1. Results of Deming Regression Analyses After Correction With the Conversion Formula for the Comparison of Plasma and Estimated Plasma Concentrations (Derived From DBS)

Analyte	N*	N ≤ LLOQ DBS	N ≤ LLOQ Veni	Conversion Formula	Slope	95% Confidence Interval	Y-Intercept	95% Confidence Interval
Amlodipine	57	7	4	EPC = DBS × (1/slope)	1.443	1.258 to 1.628	2.537	−0.990 to 6.606
Enalapril	25	12	7	No bias	0.960	0.737 to 1.182	6.272	−3.901 to 16.450
Enalaprilate	27†	9	0	EPC = DBS × (1/slope)	0.416	0.349 to 0.484	4.348	−2.822 to 11.520
Hydrochlorothiazide	42	26	42	EPC = ((DBS-intercept) × (1/slope))	3.540	2.225 to 4.856	−105.900	−276.600 to 64.680
Losartan	24	18	10	EPC = ((DBS-intercept) × (1/slope))	0.359	0.289 to 0.428	29.660	3.226 to 56.100
Losartan-ca	24	18	0	EPC = DBS × (1/slope)	0.506	0.330 to 0.682	5.337	−86.460 to 97.140
Nifedipine	15	25	4	EPC = ((DBS- intercept) × (1/slope))	0.622	0.330 to 0.915	29.250	10.580 to 47.910
Perindopril	14	26	23	EPC = ((DBS- intercept) × (1/slope))	0.745	0.623 to 0.866	5.782	2.360 to 9.204
Perindoprilate	19	22	1	EPC = ((DBS- intercept) × (1/slope))	0.485	0.328 to 0.642	3.688	0.480 to 6.896
Spironolactone	9	30	18	EPC = ((DBS- intercept) × (1/slope))	0.527	0.242 to 0.812	18.650	10.530 to 26.780
Canrenone	22	20	2	EPC = DBS × (1/slope)	0.876	0.764 to 0.988	6.748	−7.330 to 20.840
Valsartan	42	0	0	EPC = DBS × (1/slope)	0.553	0.479 to 0.627	−6.017	−211.200 to 199.100

*After exclusion of samples below the LLOQ of DBS and/or plasma.
†Enalaprilate numbers were decreased as a result of 2 outliers.
ca, carboxylic acid; EPC, estimated plasma concentrations; N, number of samples; Veni, sample collected by venipuncture.

TABLE 2. Results of Bland–Altman Analyses of Plasma Versus Estimated Plasma Concentrations (Derived From DBS)

Analyte	N*	Mean Bias	95% Limits of Agreement	Δ Within 20% of Average (%)	Δ Within 25% of Average (%)
Amlodipine	57	1.76	−6.36 to 9.87	57.9	**71.9**‡
Enalapril	25	5.17	−30.13 to 40.48	36.0	44.0
Enalaprilate	27†	10.45	−32.41 to 53.32	66.7	**77.8**‡
Hydrochlorothiazide	42	−0.03	−113.58 to 113.51	45.2	52.4
Losartan	24	−0.01	−291.88 to 291.86	12.5	16.7
Losartan-ca	24	10.61	−435.21 to 456.43	41.7	45.8
Nifedipine	15	0.00	−48.53 to 48.54	26.7	33.3
Perindopril	14	0.00	−11.99 to 11.99	28.6	28.6
Perindoprilate	19	0.01	−12.77 to 12.78	31.6	31.6
Spironolactone	9	35.39	14.82 to 55.96	33.3	33.3
Canrenone	22	7.69	−24.44 to 39.82	**77.3**‡	**86.4**‡
Valsartan	42	−0.09	−1457.49 to 1457.31	61.9	**73.8**‡

*After exclusion of samples below the LLOQ of DBS and/or plasma.
†Enalaprilate numbers were decreased as a result of 2 outliers.
‡Values in agreement of the acceptance limit of >67% of the samples within 20% or 25% difference of the mean.
ca, carboxylic acid.

the DBS method is used for AHD quantification, accurate measurement of trough levels could be a challenge.

Our stability data showed that spironolactone and [canrenone] were close to the acceptance limit of a <15% degradation. This reduced stability was expected for spironolactone, as shown by van der Nagel et al[7] for plasma. [Canrenone] exceeded the limits for stability in QC low and medium after 11 and 26 days, contrasting an earlier study by Suyagh et al[33] showing its DBS stability for as long as 1 month postsampling. Also, [enalaprilate] and hydrochlorothiazide stability data were hard to interpret, because they were stable at different concentrations and time points. Technical issues in sample collection, capable of influencing the quality of the spot, might have influenced stability outcome. Nifedipine was the only unstable AHD after 11 days. Generally, these inconsistencies can be attributed to the fact that DBS sampling is more susceptible to interindividual variability. Therefore, it is believed that most drugs are more stable than observed, with the exception of nifedipine.

Clinical Validation

Clinical validation was necessary to determine whether DBS and plasma can be interchangeably used to quantify drugs. Here, the LLOQ is one of the most important parameters. Quantitative measurement is only accurate above the LLOQ. Therefore, only measurements above the LLOQ should be used in clinical validation studies. Given the raise in DBS LLOQ, more samples than expected had to be excluded. Before sample measurements, approximately 40 samples per drug were collected to meet the guideline-based clinical validation criteria. However, only 3 of the 8 drugs measured in this study had the appropriate amount of samples after excluding samples below the LLOQ, and outliers. For nifedipine, perindopril, [perindoprilate], and spironolactone in particular, the number of excluded samples was so high, denting the results from the Bland–Altman plot. Spironolactone is rapidly converted to [canrenone] after intake, a probable reason why only few samples of spironolactone were above the LLOQ. Therefore, for better quantitative value interpretation, it is necessary to include [canrenone] when measuring spironolactone. Fortunately, [canrenone] had more samples above LLOQ and could be used to determine plasma concentration after spironolactone intake.

However, for enalapril, losartan, and [losartan-ca], which failed to meet the Bland–Altman criteria and had <40 working measurements, it is unlikely that adding samples will enable the attainment of the required acceptance limits.

The number of measurable concentrations for hydrochlorothiazide was low in our study population, for DBS and plasma, compared with the quantity of samples collected. Approximately half of all samples were excluded, owing to a relatively high LLOQ, established via analytical validation. This is probably due to a rapid decrease in plasma concentrations, whereby hydrochlorothiazide levels are already below the LLOQ at 12 hours after intake.[34,35] UHPLC-MS/MS optimization is therefore necessary for improving hydrochlorothiazide LLOQ. However, in clinical practice, hydrochlorothiazide is often administered as a combination tablet; hence, the presence of other drugs can, in this case, be used as indication of hydrochlorothiazide intake.

When comparing blood drug concentrations without adjustment for bias, higher DBS concentrations, compared with plasma, were found for amlodipine, hydrochlorothiazide, nifedipine, and perindopril. Conversely, for [perindoprilate], [enalaprilate], losartan, [losartan-ca], and valsartan, higher plasma concentrations were observed. This was independent of the drugs' protein binding or lipophilicity properties. However, the higher drug concentrations in whole blood have been attributed to strong adhesion to red blood cells.[36,37] It is unclear whether these findings are related to the outcomes of the Bland–Altman analyses.

The Deming regression analyses of losartan and perindopril showed a great variation between plasma and

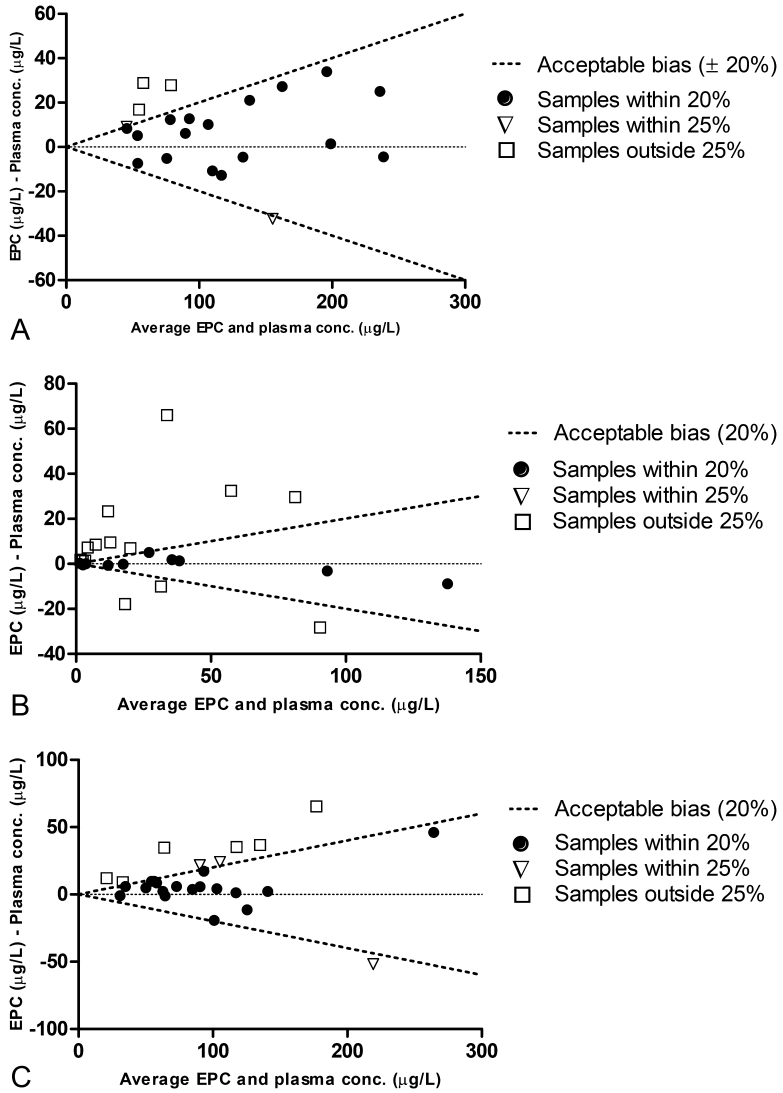

FIGURE 1. Bland–Altman analyses of [canrenone] (A), enalapril (B), and [enalaprilate] (C). The dotted line in each graph depicts 20% acceptable bias when comparing plasma and DBS measurements. Enalaprilate and canrenone are the active metabolites of respectively enalapril and spironolactone and are shown within brackets. EPC, Estimated plasma concentrations.

DBS at peak levels. The latter could be attributed to the fact that not all plasma and DBS samples were taken simultaneously, but often 30–60 minutes apart. Losartan and perindopril peak concentrations have reportedly been attained an hour after intake.[38,39] [Losartan-ca] and [perindoprilate], the active metabolites of their parent drugs, attain peak concentrations at 3–4 hours after drug intake.[40,41] In this study, sampling was often done between 1 and 5 hours after drug intake, corresponding to the metabolite and parent drug peak concentration times. At peak concentrations, steep slopes exist, emphasizing the importance of time between sampling. This is one possible explanation for not meeting the Bland–Altman acceptance criteria.

Also, perindopril and [perindoprilate] DBS and plasma values were much lower, compared with valsartan for instance (highest concentration for [perindoprilate] vs. valsartan measured with DBS: 23.51 versus 3883.06 mcg/L). This difference is mainly attributable to the settings of the UHPLC-MS/MS method. As a result, deviations in [perindoprilate] plasma and DBS concentrations have a much greater impact on the Bland–Altman outcomes.

False negatives can result in wrong assumptions toward patient nonadherence. [Perindoprilate] had the most false negatives, compared with other metabolites. However, these results can be attributed to the sampling time after drug intake. For instance, one sample was taken <1 hour after drug intake, and another, 36 hours after intake (trough sample). This explains why both samples showed no [perindoprilate] for the DBS method. Wrong patient information on the time of drug intake may have also skewed results. Here, [perindopril] was totally absent in the plasma and DBS sample, and low [perindoprilate] concentrations were observed in the plasma samples. These findings were in accordance with samples used for trough level measurements, indicating that the information given by the patients on time of drug intake was

inaccurate. Other false negatives were mainly observed for parent drugs, and were nullified by results from their corresponding metabolites. It is therefore very important for any nonadherence detection method to include the parent drug, and their metabolites.

Considerations when Using DBS

The main advantage DBS has above plasma measurements is the ability of sampling to occur at the same time as BP measurements, and at any given location, without the need for an additional visit to a blood sampling facility. DBS sampling is less invasive and less time-consuming for the patient, compared with venipuncture. For the 8 AHDs measured, the metabolites were more often detected, compared with the parent drugs. Furthermore, for the AHDs with the best correlation, such as [canrenone] and [enalaprilate], DBS could also be useful for PK/pharmacodynamic (PD) modeling, with more intense sampling. This could be convenient for patients who have great variation in drug concentrations when using the same dose, including the elderly and female patients.[42]

Differences between DBS and plasma can be explained from several angles; stability, DBS sampling method, and blood spot quality,[43] with the sampling method and blood spot quality being of great importance. According to previous studies, measuring a different spot on the same card, which theoretically contains the same drug concentration, showed a difference in mean, from −25% to almost 40%.[43] This could be attributed to different spot sizes, irregularities, spot overlap, or multiple spots in one area. Some suboptimal spots were also seen in our samples, particularly in the drug concentrations that deviated from the Bland–Altman acceptance limits. It is therefore recommended to evaluate spot quality before analyses, because not all blood spots are similar and/or of good quality. This is also the most likely explanation for the stability results observed in this study, during analytical validation. Stability challenges were observed for nifedipine when the drug was sampled with DBS, which may explain why it failed to meet the acceptance limits for Bland–Altman analyses.

Despite the possible stability and technical sampling problems, all AHDs in DBS were measured, with some agreement in the Bland–Altman analyses, when DBS and plasma measurements were compared. This implies the efficiency of DBS in determining nonadherence for all measured AHDs over all available dosages, because it could measure the presence of a drug with a high accuracy for as long as 24 hours after intake; the most important factor. This can preserve a good patient–physician relationship, which is key to properly discussing the outcome of the absence of drugs in the blood. The use of DBS, and discussions on the outcome, to improve nonadherence, is currently being studied in a large multicenter trial called RHYME-RCT (NL6736). However, to quantify drug concentrations, it is important to choose one of the methods (DBS or plasma) and avoid interchanges. Also, a more detailed analyses with respect to the quantitative values, may be considered in the future, to determine long-term adherence.

CONCLUSIONS

DBS is a reliable and accurate method for measuring AHDs, and is therefore applicable for nonadherence assessment/detection. DBS sampling enables AHD quantification. However, when measuring trough levels, venipuncture is more accurate. In addition, DBS and plasma should not be used interchangeably when quantifying AHDs, with the exception for valsartan.

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