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Berm, Elizabeth J. J.; Odigie, Blessing; Bijlsma, Maarten J.; Wilffert, Bob; Touw, Daan J.; Maring, Jan G.

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A clinical validation study for application of DBS in therapeutic drug monitoring of antidepressants

Background: A bridging study of plasma and DBS concentrations for therapeutic drug monitoring of antidepressants was performed. **Results & methodology:** Potassium-based hematocrit analysis was included. In addition, we defined acceptance criteria based on the differences between individual data points of plasma and DBS concentrations. These criteria were applied to test acceptability of error found in predicted nortriptyline plasma concentrations. Potassium-based hematocrit predicted a negative bias for DBS concentrations of amitriptyline, but not for the other compounds. To predict plasma concentrations of antidepressants based on DBS concentrations, a factor of 0.8, 0.65, 0.84 and 0.78 was found for nortriptyline, desmethylclomipramine, venlafaxine and desmethylvenlafaxine, respectively. **Discussion & conclusion:** Application of the factor and newly formulated acceptance criteria demonstrated prediction of nortriptyline plasma concentrations based on DBS concentrations.

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Keywords: antidepressants • clinical validation • DBS • plasma • therapeutic drug monitoring

DBS analysis is increasingly described as an alternative sampling method for therapeutic drug monitoring (TDM) [1]. This is due to its various advantages, such as patient comfort, more flexibility, simplicity and the lack of biohazard risk of sending samples by post [2]. Although there are many reports of validated DBS assays, there are few of clinical validations which hamper the application of DBS in clinical practice. For a clinical validation and implementation of a DBS assay, there are issues that need to be addressed.

First, DBS concentrations mirror whole blood concentrations. Therefore, reporting of DBS concentrations is of limited value for clinicians, because most, if not all, therapeutic reference concentrations are determined in plasma or in serum. To overcome this problem, paired analysis of DBS and plasma samples in patients is needed. With such an approach, the possibility of reporting calculated plasma values based on DBS values is

possible and reference values for plasma can be used.

Ideally, the relationship between DBS and plasma concentrations is constant. However, there are different aspects in DBS analysis which can influence this relationship. An important aspect is the variation in hematocrit (hct) between patients. The amount of hct influences the viscosity of blood and thereby the spreading of a blood drop on the DBS card. Higher hct values will lead to smaller and more concentrated spots [3,4]. Variation in hct values also affects sample homogeneity, matrix effect, the blood-to-plasma concentration ratio of an analyte, and recovery [5–7]. In addition, the influence of hct can be dependent on the type of cards on which the blood spots are collected [8]. Thus, deviating hct values may greatly influence analytical results, leading to an unknown factor of uncertainty [9]. Various studies have stated that despite the many advantages of

Elizabeth JJ Berm¹, Blessing Odigie¹, Maarten J Bijlsma², Bob Wilffert^{1,3}, Daan J Touw³ & Jan G Maring^{*,4}

¹Department of Pharmacy, Section Pharmacotherapy & Pharmaceutical Care, University of Groningen, 9712 CP Groningen, The Netherlands

²Unit PharmacoEpidemiology & PharmacoEconomics (PE2), Department of Pharmacy, University of Groningen, 9712 CP Groningen, The Netherlands

³Department of Clinical Pharmacy & Pharmacology, University Medical Center Groningen, University of Groningen, 9712 CP Groningen, The Netherlands

⁴Laboratory for Drug Analysis & Toxicology, Diaconessen Hospital Meppel & Bethesda Hospital Hoozevee, Meppel, The Netherlands

*Author for correspondence:
Tel.: +31 522 234 900
maring@isala-diac.nl

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DBS, its implementation in TDM is mainly limited by hct effects [10,11]. Recently, Capiou *et al.* demonstrated that potassium measurement in DBS could serve as a predictor of the hct in venous DBS [11]. As such, a possibility to determine hct in patient capillary DBS samples became available to study hct effects in patient samples.

Another issue is the evaluation of the outcomes from clinical validation studies. Pearson's correlation coefficient could be used to describe the relationship between two methods, but there is no clear interpretation available for a 'good,' 'low' or 'insufficient' correlation coefficient. Moreover, regression methods which either take error in both the (x) and (y) values into account, or make less error distributional assumptions on the (x) and (y) values, are more suitable for analysis of method comparisons [12]. Deming (weighted) regression and Passing–Bablok (PB) regressions are such regression methods, and they could be supplemented with Bland–Altman plots [12–16]. Although these techniques give a good indication of the average comparability of the two methods, they provide no information about deviations on an individual level which might still lead to unwanted errors. This problem is illustrated by Vu *et al.*, who found DBS and plasma analysis of desacetyl rifampicin were comparable according to Deming regression, however, according to the authors, correlation between the assays was low ($r^2 = 0.69$) [17]. Therefore, criteria based on single data points would be useful in addition to the previous mentioned techniques. In another clinical validation study, de Wit *et al.* applied a limit of $\leq 25\%$ bias for single data points. This limit was based on available dosage forms for dose adaptations [18]. Although this is a clear limit, generalizability to other compounds seems a problem. Another criterion that can be considered is the proportion of different clinical interpretations based on one method versus the other [18,19]. This comparison gives valuable information, but it can be biased by a lack of heterogeneity among patient samples. For example, when more patients with concentrations around the lower or upper limit of the reference range are selected, more differences will be observed. Based on the mentioned limitations, there is a need for application of simple and uniform acceptance criteria for validation of bridging studies of DBS and plasma concentrations based on individual data points.

Previously we developed two DBS assays for the analysis of antidepressants. One for determination of amitriptyline (ATP), nortriptyline (NTP), clomipramine (CMP) and desmethylclomipramine (DCMP) and another for the determination of venlafaxine (VEN) and *O*-desmethyl-venlafaxine (ODV). Both assays were analytically validated according to US

FDA guidelines [20,21]. In addition, we found indications for a proportional difference between DBS and plasma concentrations. As such, a translation factor is needed to calculate plasma concentrations based on DBS concentrations. In this current study we aimed to establish this factor. We first assessed if the differences found in patient samples were related to variance in hct values that were calculated based on potassium concentrations in capillary DBS. Next, we established the relationship between plasma and DBS concentrations in patient samples and explored the possibility to report calculated plasma concentrations. In addition, the comparability of clinical interpretations based on plasma or DBS-based analysis was assessed. Finally, we propose and apply acceptance criteria for individual data points of calculated plasma concentrations and demonstrate the application of these criteria on an additional set of NTP samples.

Methods

Materials potassium analysis

Water (HPLC quality) was purchased from Macron Fine Chemicals (Gliwice, Poland). Potassium chloride was purchased from Merck (Damstadt, Germany). Whatman FTK DMPK-C cards were used for DBS collection and purchased from GE Healthcare (Hoevelaken, The Netherlands). Potassium ion (K^+) concentrations were measured by indirect potentiometry using a Roche Cobas 6000 analyzer (Roche Diagnostics, Almere, The Netherlands) with technical limits of 1.5 and 10 mM. Hct was measured with a Sysmex XE-5000 hematology analyzer (Sysmex, Etten-leur, The Netherlands). Blank Li-heparin blood for preparation of the calibration and QC samples for potassium analysis was obtained from healthy volunteers.

Method potassium analysis

The method for potassium extraction was based on the reported extraction method by Capiou *et al.* [11]. Among some of the collected patient samples not all four spots were of sufficient quality to be analyzed. Therefore, we did not analyze potassium concentrations in duplicate as was described by Capiou *et al.* As a result of prior internal validation studies, parts of the procedure were adapted. A 6 mm DBS punch was extracted with 200 μ l KCl (2.5 mM) for two subsequent extractions. During extraction, samples were vortexed for 10 s and placed in an ultrasonic bath for 5 min. After centrifugation, 200 μ l of the extract was placed into a vial. A second extraction step with 200 μ l extraction fluid was performed following the same procedure, however before the samples were placed in the ultrasonic bath, they were left for 2 h at room temperature. The extract of the second extraction step was

pipetted into the same vial (total 400 μ l) for potassium ion analysis. During each run of potassium analysis, four calibration and QC samples were analyzed. Two thirds of the QC samples had to be within the $\pm 15\%$ limits of the nominal hct concentrations for acceptance of the analytical results [22]. We analyzed DBS potassium concentrations of 20 volunteers (single and duplo data points) and in 26 patients (single data points only) of whom the hct concentration was measured in venous blood by the Sysmex hematology analyzer as well. We analyzed the relationship between potassium concentration and measured hct by least square regression. In addition, we analyzed the calculated and measured hct of the patient samples with PB regression and a Bland–Altman comparison. Finally, we analyzed the potassium concentrations in the remaining patient samples to assess the relationship between calculated hct and bias between DBS and plasma concentrations of antidepressants (ADs).

Methods antidepressant analysis

ATP, NTP, CMP, DCMP, VEN and ODV concentrations in DBS and plasma were determined by validated methods which met acceptance criteria of international guidelines. A 6 mm DBS punch was extracted with a acetonitrile:methanol; 1:3 solution. The DBS methods are described more in depth elsewhere [20,21]. Plasma concentrations were analyzed by a validated routine method which proved to be robust and reliable in external quality control programs. Both the DBS and plasma methods used LC–MS/MS for quantification of compounds and all used promazine as an internal standard.

Sample collection

Samples were collected between 1 January 2013 and 1 August 2015. After they gave written informed consent, paired samples of capillary DBS (i.e., obtained by fingerprick) and venous plasma were collected from patients who visited the clinic for routine TDM. Approval for obtaining the additional DBS samples was obtained from the Medical Ethics Committee of the Diaconessen Hospital Meppel. Spots were collected by qualified personnel who received written instructions, but no specific training. Instructions included disinfection of the finger, discharge of the first blood drop and only gentle pressure on the finger to form blood droplets. To fill the preprinted circle on the DBS card, spots composed by one or two blood droplets were allowed. The resulting variation in spotting volume was allowed, since a spotting volume ranging from 20 to 100 μ l was found not to introduce any systematic bias at low or high concentrations of the antidepressants [20,21]. After at least 2 h of drying at room temper-

ature, samples were placed in a sealed plastic bag and stored with a desiccant at 4°C until analysis. Under these conditions, validation samples were found stable for at least 8 months and all patient DBS samples were analyzed within 5 months after the collection date.

In addition to the patient samples, we used paired spiked venous DBS and plasma samples to assess the relationship between DBS and plasma concentrations. Blood from a healthy volunteer was used for the preparation of the samples. Samples were spiked at concentrations in the validated range (tricyclic ADs: 30–450 μ g/l [n = 9]; VEN/ODV: 100–1100 μ g/l [n = 12]). Details about the preparation of the spiked samples are described in prior work [21].

Data analysis

Bridging DBS & plasma concentrations

The deviation (bias%) between the DBS and plasma concentrations of the AD were plotted against calculated hct values and visually inspected for a linear relationship caused by hct effects. PB regression was used to test for significant differences between the DBS and plasma method [14]. If found significant, the intercept or slope of the regression analysis were considered as translation factors [23]. In addition to the relation found in patient samples, we also calculated the ratio of the plasma and venous DBS concentrations based on spiked (nonpatient) samples.

The difference in clinical interpretation was assessed by comparison of the clinical interpretation based on the measured plasma concentrations and DBS-based plasma concentrations. The clinical interpretation was based on Dutch reference ranges [24,25]. The sensitivity of DBS-based plasma concentrations was calculated as the amount of identical advices/total advices [19].

Calculation of limits for acceptance of DBS-based calculated plasma concentrations

In addition to the PB analysis and clinical interpretation, we wanted to assess the error for individual data points and formulate uniform acceptance criteria for this error. To find such acceptance criteria, we used the allowed 15% variance (or 20% between LLOQ and QC low level) in accuracy and precision as is allowed according to FDA and EMA guidelines. In R version 3.0.2 we simulated method validations of two methods which were both on the borderline of FDA and EMA guidelines. A Monte Carlo simulation was performed by random draws from a dataset with a normal distribution with accuracy and precision on the borderline of FDA and EMA guidelines. Next, we calculated the relative differences of single data points between the two methods (A and B) and calculated the 95% prediction interval (PI) for this difference. To compare our

results with the criteria for incurred sample analysis of the EMA (67% of samples should be within 20% of original value), we also calculated the differences at the 67% PI [26]. A total of 10,000 method validations, of each 15 data points, were simulated to calculate the PIs. To demonstrate the application of these additional acceptance criteria, we applied them to an additional dataset of NTP patient samples.

Results

A total of 162 paired patient samples were included in this validation study. Since samples were collected alongside routine care, different sets of samples were available for the different compounds and calculated hct analysis (Table 1). Due to the collection of blood by fingerprick and direct spotting, spots differed in spotting volume and quality (Figure 1). Prior validation indicated little bias was observed when spotting volume was varied [20,21]. Therefore, as long as a punch of 6 mm was possible with both the front and backside of the paper saturated, we included the sample in our analysis.

Bias due to variation in hematocrit

Based on least square linear regression, the squared correlation coefficients between potassium concentration and hct were 0.53, 0.68 and 0.50 for the single data points of volunteers, duplo data points of volunteers and single data points of patient samples, respectively. Concentrations of calculated hct in the capillary patient DBS samples were higher than the concentrations found in venous blood ($n = 26$; mean difference Bland–Altman comparison: 0.02; PB analysis: slope: 0.73 [95% CI: 0.50–1.11]; intercept: 0.10 [95% CI: -0.07–0.20]).

In Figure 2, the relation between calculated hct and the bias between concentrations found in DBS and plasma is shown for ATP and NTP, respectively. For

the other compounds see Supplementary File 1. Visual inspection of the plots suggested that there was no quantifiable linear relationship between the calculated hct and the bias, except a weak relationship for ATP. The relationship between the bias and calculated hct concentration had a squared correlation coefficient of 0.27, for calculated hct values between 0.35 and 0.55 l/l.

Relationship between DBS & plasma concentrations

Results of the analysis of the paired spiked and patient (*in vivo*) samples are shown in Table 2. The regression plots are shown in Figure 3. The regression analyses indicated no constant bias between plasma and DBS analysis (all intercepts included zero). For ATP, NTP, DCMP, VEN and ODV proportional differences were found between the methods. The differences found in the patient samples were comparable to the results found in the spiked venous samples. The confidence interval around the slope estimate of CMP was much broader compared with confidence intervals of other compounds.

Results for the comparison of clinical interpretations based on capillary DBS-derived plasma concentration and measured plasma concentrations are shown in Table 3. For TDM of ATP, NTP and VEN, interpretation based on DBS-based plasma concentrations were highly comparable (sensitivity >96%) to plasma-based advises. For CMP, TDM based on DBS did result in a different clinical interpretation in 25% of the comparisons.

Acceptance criteria for differences between DBS derived plasma concentrations & measured plasma concentrations

After simulating method validations at an accuracy and precision of 15 or 20% in both methods, the following criteria were found:

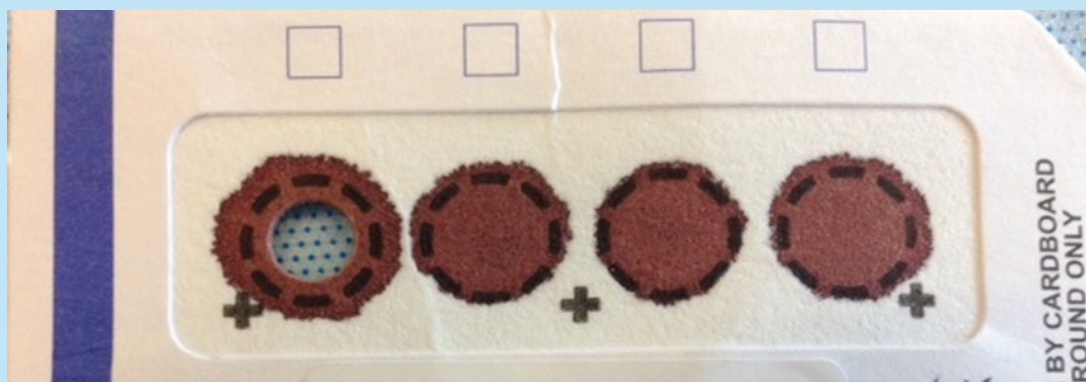
Table 1. Samples included in analyses.

AD used	K ⁺ analyzed	AD analyzed	Paired AD samples		
			Above LLOQ	Below LLOQ	Total
ATP	18	ATP	27	2	29
		NTP	21	8	90 [†]
NTP	44	NTP	59	2	
CMP	42	CMP	38	6	44
		DCMP	42	2	44
VEN	23	VEN	23	5	28
		ODV	27	1	28

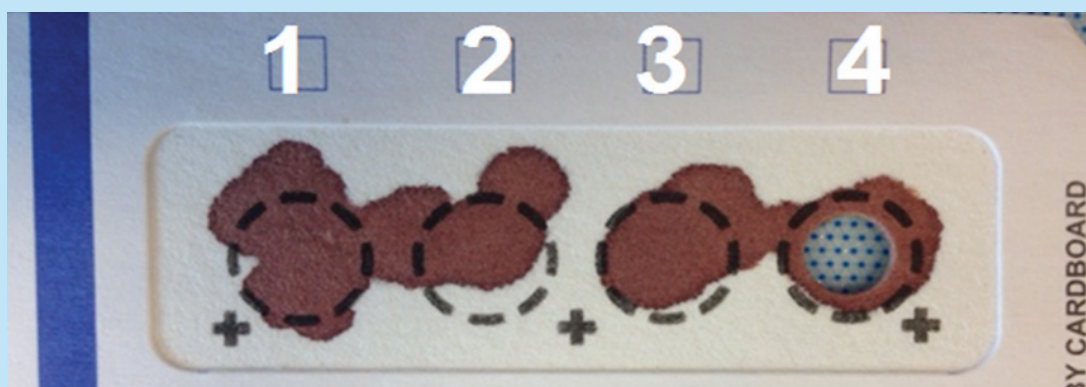
[†]Out of the 80 samples above LLOQ, 40 random samples were used for regression analyses and the remaining samples were used for testing of additional acceptance criteria.

AD: Antidepressant; ATP: Amitriptyline; CMP: Clomipramine; DCMP: Desmethylclomipramine; NTP: Nortriptyline; ODV: O-desmethyl-venlafaxine; VEN: Venlafaxine.

(A) All spots of good quality



(B) Spot 2 of insufficient quality, spot 1 and 3 of sufficient quality, spot 4 best quality and used for analysis



(C) Backside of the DBS card. Spot 1 and 4 of insufficient quality, spot 3 of sufficient quality, spot 2 of best quality and used for analysis

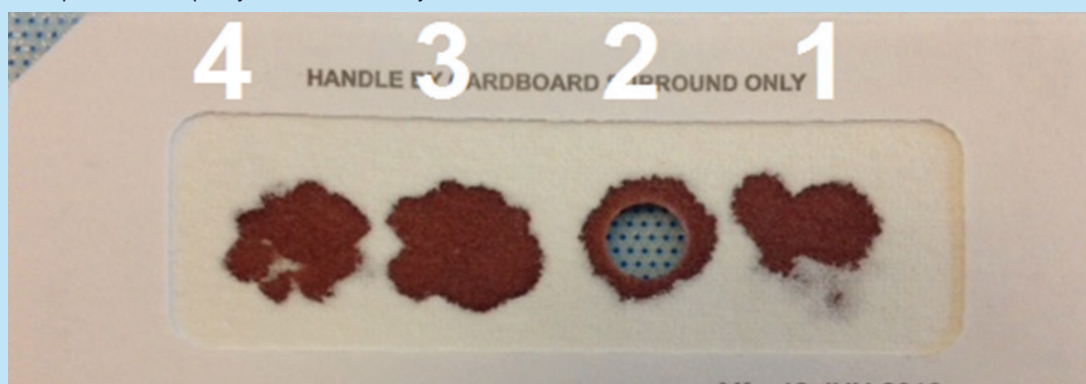


Figure 1. Example of quality of the samples used for analysis. (A) All spots of good quality. **(B)** Spot 2 of insufficient quality, spot 1 and 3 of sufficient quality, spot 4 best quality and used for analysis. **(C)** Backside of the DBS card. Spot 1 and 4 of insufficient quality, spot 3 of sufficient quality, spot 2 of best quality and used for analysis.

- At an accuracy and precision of 20% in both methods, no more than 5% of the data had a difference between method A and B of >(48%). This limit should be applied at DBS concentrations ranging from LLOQ to QC low concentrations.
- At an accuracy and precision of 15% in both methods, no more than 5% of the data had a difference between method A and B of >(36%). This limit should be applied at DBS concentrations ranging from QC low concentrations and higher.

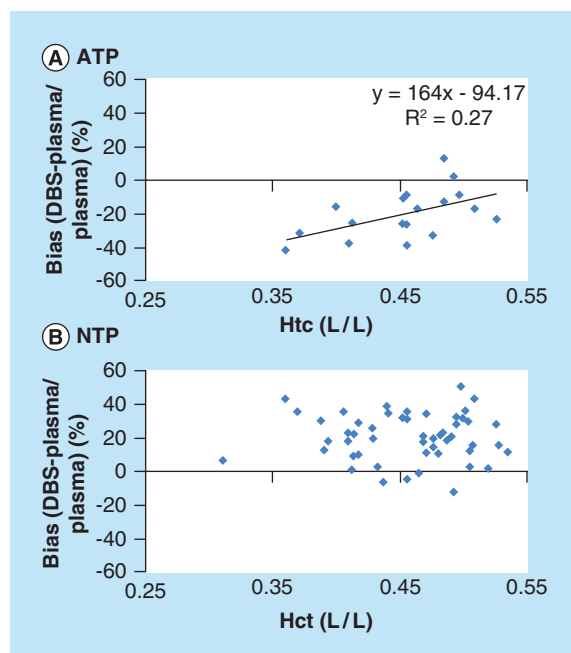


Figure 2. Relationship between calculated (i.e., potassium based) hct and bias (bias = between DBS and plasma concentrations) of (A) ATP and (B) NTP. For CMP, DCMP, VEN and ODV see Supplementary File 1.

Histograms of the differences between the single data points of the simulation can be found in Supplementary File 2. In our simulation, 67% of the samples had a bias <25% at an accuracy and precision of 20% (i.e., LLOQ level) and <18% at an accuracy and precision of 15%.

Application of acceptance criteria

We applied the found translation factor for NTP ($[DBS] \cdot 0.80 = [plasma]$) to another dataset of NTP samples and plotted the remaining differences (Figure 4). We found one sample (2.5%) was outside the range for maximum bias, which was within our acceptance criterion of 5%. As such, we established a translation factor,

which proved to give reliable results for calculation of plasma concentrations based on DBS concentrations.

Discussion

To compare plasma and DBS analyses of antidepressants, a large set of paired patient samples was analyzed. We found variance in calculated hct influenced bias between the DBS and plasma assay of ATP, although based on the sample size ($n = 27$) no firm conclusion can be drawn from these results. Nevertheless, calculation of ATP plasma concentrations based on DBS concentrations becomes more complex. The possible translation factor found with the PB regression might be dependent on the hct range which is covered by the samples. As such, our estimate contains uncertainty. To gain better insight into the correct translation factor, more samples should be collected with a better hct measurement (in duplicate) and better controlled hct of reference samples used for the AD analysis. For the other compounds, calculated hct had no quantifiable influence on our results. We found significant proportional differences between DBS and plasma concentrations of NTP, DCMP, VEN and ODV. The results of the spiked samples were in line with the results from the patient samples which strengthen our findings. The slopes of the PB analysis which were significant were used for calculation of plasma concentrations, based on DBS analysis. When this correction was applied on the DBS concentrations, it was found that the clinical interpretation based on DBS analysis of ATP, NTP, VEN and ODV was highly comparable to clinical interpretation based on plasma analysis. For these compounds, sensitivity was >96% which was slightly higher than was reported sensitivity of TDM by DBS of antipsychotics (i.e., sensitivity of 92%) [19]. However, for TDM of CMP, a substantial difference and lower sensitivity was found. To study what is inducing this error, further research is needed.

Table 2. Relation between antidepressant concentrations in plasma and dried blood samples.

AD	Ratio spiked plasma/ DBS Mean \pm SD	Ratio <i>in vivo</i> plasma/ DBS Mean \pm SD	Slope PB (95% CI) <i>in vivo</i> plasma-DBS	Literature Plasma/whole blood \pm SD	Ref.
ATP	1.13 \pm 0.12	1.27 \pm 0.22 [†]	1.22 (1.13–1.34) [†]	1.45 \pm 0.14	[31]
NTP	0.73 \pm 0.07	0.84 \pm 0.15	0.80 (0.71–0.89)	0.86 \pm 0.06	[31]
CMP	0.94 \pm 0.09	1.15 \pm 0.25	1.15 (0.94–1.38)	0.72–1.07 [‡]	[34]
DCMP	0.70 \pm 0.11	0.66 \pm 0.11	0.65 (0.57–0.75)	Not found	
VEN	0.87 \pm 0.06	0.89 \pm 0.14	0.84 (0.76–0.92)	Not found	
ODV	0.80 \pm 0.05	0.83 \pm 0.09	0.78 (0.71–0.86)	Not found	

[†]Note that this relationship was influenced by hematocrit of the bloodspots.

[‡]Study performed in rats, ratio was dependent on concentration, 0.72 at 5 μ g/ml and 1.07 at 0.50 μ g/ml.

AD: Antidepressant; ATP: Amitriptyline; CMP: Clomipramine; DCMP; Desmethylclomipramine; NTP: Nortriptyline; ODV: O-desmethyl-venlafaxine; PB: Passing and Bablok regression analysis; SD: Standard deviation; VEN: Venlafaxine.

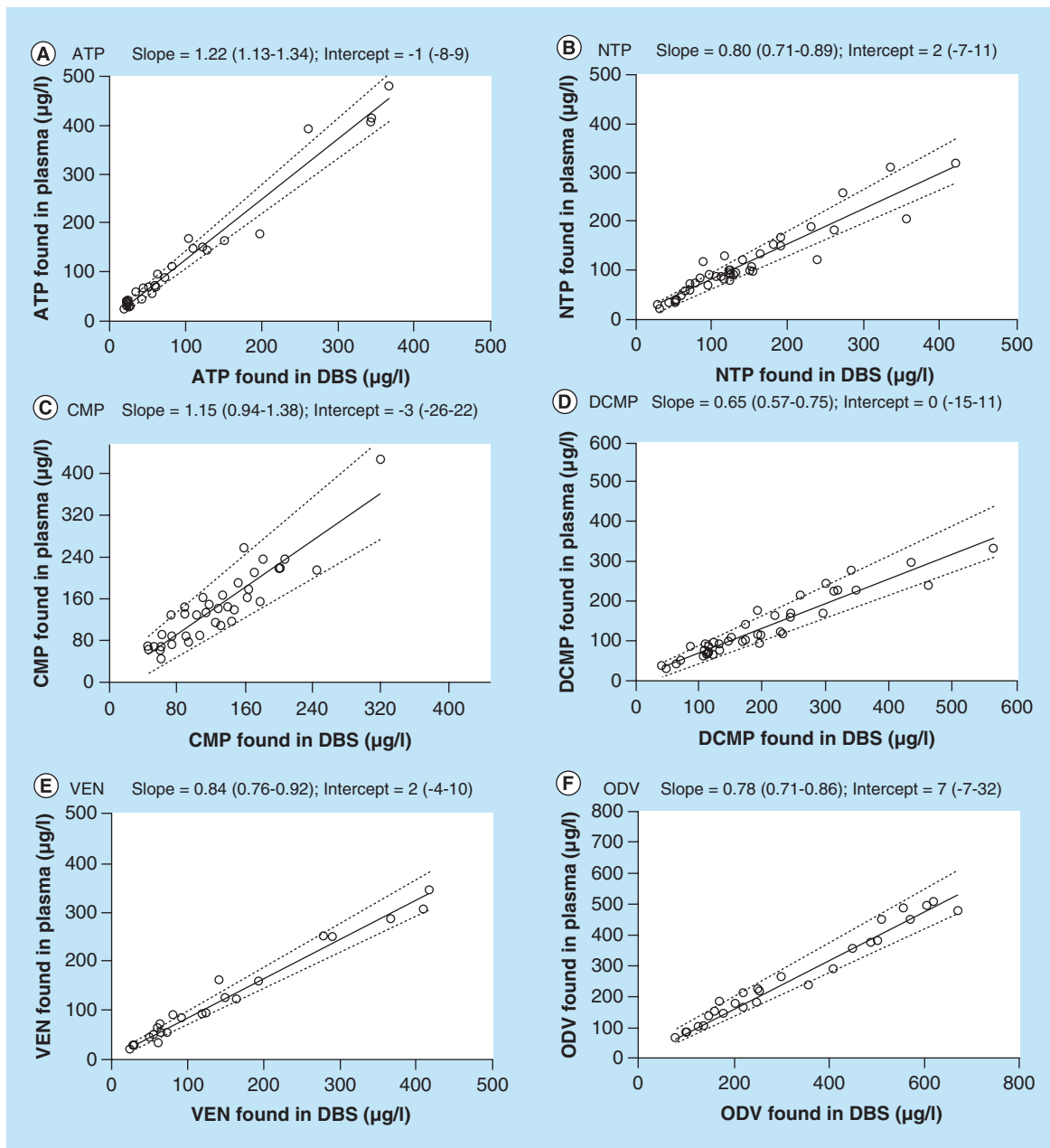


Figure 3. Passing–Bablok regression of patient DBS and plasma concentrations for (A) ATP, (B) NTP, (C) CMP, (D) DCMP, (E) VEN and (F) ODV.

ATP: Amitriptyline; CMP: Clomipramine; DCMP: Desmethylclomipramine; NTP: Nortriptyline; ODV: *O*-desmethylvenlafaxine; VEN: Venlafaxine.

To take clinical validation one step further, we established acceptance criteria for 95% of single data points and applied these criteria on an additional dataset of NTP. We found our results met these criteria, demonstrating the validity of the DBS assay and the translation factor *in vivo*. Notably, criteria which were derived from our simulation were in line with the criteria for incurred sample analysis of the EMA [26]. However, they were more specific since they include two different criteria, depending on the different level of

accuracy and precision at LLOQ or higher concentrations. In addition, they take 95% of the samples into account instead of 67%.

In this study, potassium-based hct analysis was conducted on patient samples using capillary blood obtained by fingerprick. A higher (mean difference Bland–Altman comparison: 0.02) calculated hct was found in capillary DBS (without anticoagulation) when compared with measured hct in venous EDTA blood. This is in contrast to the findings of Capiou *et al.*,

Table 3. Clinical interpretation based on measured plasma concentration and compared with DBS-derived plasma concentrations.

	ATP and NTP	NTP	CMP and DCMP	VEN and ODV
Therapeutic range ($\mu\text{g/l}$) [†]	100–300	50–150	200–400	100–400
<LLOQ (n)	2	2	2	1
<Therapeutic range (n)	14	9	20	4
Within therapeutic range (n)	9	44	15	12
>Therapeutic range (n)	6	8	9	12
Identical interpretation (n)	28	59	33	28
Total (n)	29	61	44	28
Sensitivity (%)	96.5	96.7	75.0	100.0

If significant, the slope of Passing–Bablok regression as reported in **Table 2** was used to calculate DBS derived plasma concentrations.
[†]Reference values which were used followed the Dutch guidelines [24,25]. AGPN guideline advices: ATP and NTP: 80–200 $\mu\text{g/l}$; NTP: 70–170 $\mu\text{g/l}$; CMP and DCMP: 230–450 $\mu\text{g/l}$; VEN and ODV: 100–400 $\mu\text{g/l}$ [37].
 ATP: Amitriptyline; CMP: Clomipramine; DCMP: Desmethylclomipramine; NTP: Nortriptyline; ODV: O-desmethyl-venlafaxine;
 VEN: Venlafaxine.

who found a lower (mean difference Bland–Altman comparison: -0.02) concentration of calculated hct in venous lithium heparin DBS compared with measured hct in venous EDTA blood [11]. However, our results were in line with results from Burger *et al.* who also found higher (mean difference Blant–Altman comparison: 0.04) concentrations in capillary DBS samples [27]. Although these differences could be related to the difference in sample collection, our sample size was too small to draw any firm conclusions [28,29]. Nevertheless, we considered the correlation between the measured and calculated hct sufficient to continue our study and assess the relationship between calculated hct and bias between the DBS and plasma assay of the ADs. With respect to this we found no hct effects based on calculated hct, except for ATP. This did not correspond with our prior findings in which we found a minor hct effect for all compounds in spiked venous DBS samples [20,21]. Others who applied potassium-based analysis of hct effect in venous DBS samples did find a negative bias of up to -30% at a hct level of 0.20 l/l [10]. There are different explanations possible for our findings. First, our results could be explained by the small hct range which was studied. All patient samples had a calculated hct of >0.30 l/l. Nevertheless, we do believe this range is a good representation of our populations hct (i.e., patients treated with antidepressants), since hct values of <0.30 l/l are usually found in severely ill patients [23]. Second, the lack of hct effects could be due to compensation of the negative bias by an increased recovery at lower hct levels. This effect referred to as ‘recovery bias’ was recently demonstrated by Abu-Rabie *et al.* [7]. Such compensation could disappear when whole spot punches are used instead of the sub-punches we used in our assay. However, compounds with a high absolute recovery ($>60\%$) are less

sensitive to recovery bias at varying hct [7]. Therefore, recovery bias did probably not have a major influence on our assay since all compounds except ODV had a high absolute recovery ($>80\%$, ODV = 67%) [20,21]. Moreover, any recovery bias was likely already present in our prior validation and is therefore unlikely to explain our current findings. A more reasonable explanation for our findings would be the preparation procedure of the samples. As reported in previous work, the red blood cell fraction was pipetted into the plasma fraction and the hct was calculated based on the pipetted fractions [20,21]. As such, a lower hct could have been obtained than anticipated. This was very recently demonstrated by Koster *et al.* [30]. Therefore, we could have overestimated the hct effect in our prior work. Last, our findings could be explained by the moderate correlation found between calculated hct and measured hct. The calculated hct might simply not be precise enough to differentiate between a hct bias of approximately $\pm 20\%$ and other influences of bias in capillary patient samples.

The results of analysis of both spiked and patient samples showed that with the current sample size and standard deviation there was no significant difference in CMP concentrations between plasma and DBS. For NTP, DCMP, VEN and ODV, higher concentrations were found in DBS. For ATP, lower concentrations were found in DBS. For ATP, NTP and CMP, these results were in line with previous reported estimates [31–35]. For DCMP, VEN and ODV, no estimates were found in literature. Nevertheless, our estimates were supported by the consistent results between our spiked and patient samples. It has been reported that tertiary amines like ATP and CMP have more affinity toward plasma than to the red blood cell fraction when compared with their secondary amines metabolites NTP

and DCMP, respectively. [31]. Our findings were in line with these characteristics.

Our study has its limitations. Although we collected samples for more than 2 years, the sample size was still relatively small. Due to practical constraints, we could not further increase the inclusion period. According to Passing and Bablok, a sample size of $n = 30$ would have enough power to detect a slope <0.87 or >1.15 under the assumption that the coefficient of variation of both methods was approximately 7% [36]. This assumption was made based on our previous validation work. Indeed, for VEN and ODV, we were able to detect significant proportional differences. However, for CMP we did not reach significance for a slope estimate of 1.15. This could be due to a higher coefficient of variation than found during prior validation. Further validation seems recommended due to the observed biases and relative low clinical comparability. The results based on clinical interpretation of ATP, NTP and VEN were promising. Nevertheless, we only validated the translation factor of NTP on an additional independent dataset which fully guarantees the robustness of this factor. Taking into account the high clinical comparability of the clinical interpretation of ATP, VEN and ODV, it is questionable if the efforts to undertake collection of an additional dataset would be necessary for implementation of the DBS assay in clinical practice. Regulatory or international consensus guidelines should be issued to give clear recommendations about the comprehensiveness of clinical validation studies.

We calculated additional acceptance criteria for bridging studies of DBS by a simulation of comparison of methods. These comparisons assumed two methods which were both on the borderline of FDA limits. It

is important to realize that similar acceptance criteria could be found when simulating two methods of which one is well (e.g., 5% bias) within and one is outside these limits (e.g., 30% bias). This implies a very precise reference method would be able to compensate for substandard performance of a comparative method. However, this problem would arise in any current technique for method validation and is not limited to our acceptance criteria. Nevertheless, this underlines the importance of a full analytical validation to ensure bias of both methods meets the FDA and EMA limits.

Conclusion

This study assessed DBS-based TDM of antidepressant in clinical practice. For ATP, bias induced by calculated variations in hct influenced the relationship between plasma and DBS samples. For all other compounds, these variations had no influence. For NTP, DCMP, VEN and ODV, a translation factor to calculate plasma concentrations based on DBS concentrations was established. For ATP and CMP, further validation of this translation factor is needed. The agreement between the clinical interpretation based on DBS or plasma concentrations of ATP, NTP and VEN was high. Acceptance criteria for single data points were calculated. We demonstrated application of these acceptance criteria toward an additional dataset of NTP samples and found our results met these criteria.

Future perspective

Based on the many analytical validation reports of DBS analysis, many clinical validation studies will likely follow in the near future. Guidelines for requirements of a clinical validation of DBS analysis are needed.

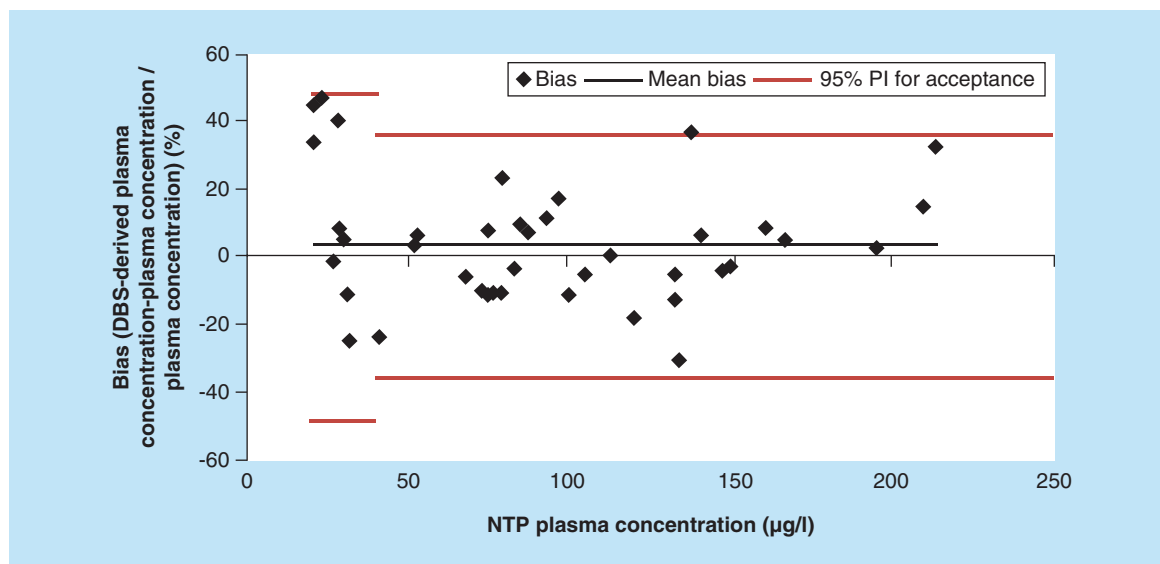


Figure 4. Method comparison plots showing the difference between calculated plasma concentrations and measured plasma concentrations of nortriptyline in patient samples.

Such guidelines should preferably include acceptance criteria for maximum differences between individual data points which are uniform and can be applied in a similar way to all compounds as demonstrated in this study. In addition, guidelines should include acceptance criteria for agreement between the clinical interpretation. This agreement is dependent on the therapeutic range and concentrations of compounds found in the samples. Therefore, samples included in this analysis should, for example, include at least five observations above and five below the therapeutic range to demonstrate the DBS-based assay is able to differentiate between clinical interpretations.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/full/10.4155/bio.15.255

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- The relationship between plasma and capillary DBS concentrations of amitriptyline, nortriptyline, clomipramine, desmethylclomipramine, venlafaxine and desmethylvenlafaxine was studied.
- To estimate the influence of hematocrit on this relationship, potassium analysis of DBS was included.
- Criteria for acceptance were calculated based on a Monte Carlo simulation and applied to test acceptability of error found in DBS-derived nortriptyline plasma concentrations.

Bias due to variation in hematocrit

- Potassium-based hematocrit predicted a negative bias for DBS concentrations of amitriptyline.

Relationship between DBS & plasma concentrations

- To predict plasma concentrations of antidepressants based on DBS concentrations, a factor of 0.8, 0.65, 0.84 and 0.78 was found for nortriptyline, desmethylclomipramine, venlafaxine and desmethylvenlafaxine, respectively.
- The clinical interpretation for DBS-based TDM of NTP, ATP, VEN and ODV was highly comparable (>96%) to plasma-based TDM.

Acceptance criteria

- Application of newly formulated acceptance criteria demonstrated reliable prediction of nortriptyline plasma concentrations based on DBS concentrations.

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