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Abstract

Background: Oral fluid (OF) is an interesting alternative for conventional blood testing in therapeutic drug monitoring (TDM). OF can be used for screening but its value for quantification has to be established.

Methods: To evaluate the value of OF for quantification of 11 commonly used antipsychotics and 5 metabolites, an ultra-high performance liquid chromatography-tandem mass spectrometric (UHPLC-MS/MS) method was validated. OF was obtained from psychiatric patients using a Quantisal™ collection device. OF to serum concentration ratios were determined, taking into account the exact volume of collected OF.

Results: Linearity was evaluated at 7 or 8 calibration levels. Accuracy criteria were fulfilled, except for pipamperone at QC low. The intraday precision ranged 0.88-14.73% and interday precision ranged 1.92-16,17%. The mean recovery from the collection pad was 37.1% at QC low and 40.3% at QC high for 1 ml of collected OF; for 0.5 ml collected OF mean recovery was 35.0% at QC low and 37.3% at QC high. When 0.1 ml OF was collected, recovery data were unreliable. Mean absolute matrix effect was 101.1% (82.0-120.0%). OF patient samples (n=89) containing 269 antipsychotics and metabolites were acquired and the mean volume of collected OF was 0.562 ml (0.057-1.232 ml). The OF to serum ratios were above 1 for all antipsychotics (1.54-28.50), except for aripiprazole (0.21) and zuclopenthixol (0.66). A broad range of calculated ratios for all antipsychotics was obtained.

Conclusion: This validated UHPLC-MS/MS method can be used to reliably quantify antipsychotics in OF, even when recovery is low. Since the correlation between OF and serum concentrations was low and in addition results were highly variable, it can only be concluded that OF is a potentially interesting matrix, particularly for screening for noncompliance.

Abbreviations:

7OH-NDA-QUE: 7-hydroxy-N-desalkyl-quetiapine; **7OH-QUE**: 7-hydroxy-quetiapine; **AMI**: amisulpride; **AP**: antipsychotics ; **ARI**: aripiprazole; **BRO:** bromperidol; **CLO**: clozapine; **CI:** confidence interval; **dMRM:** dynamic multiple-reaction monitoring; **ESI:** electrospray ionisation; **HAL:** haloperidol; **IS:** internal standard; **LC:** liquid chromatography; **LC-MS/MS:** liquid chromatography-tandem mass spectrometry; **MS:** mass spectrometry; **MTBE:** methyl *tert*-butyl ether; **NORCLO:** N-desmethyl-clozapine; **NOROLA:** N-desmethyl-olanzapine; **OLA:** olanzapine; **OF**: oral fluid ; **PAL:** paliperidone; **PIP:** pipamperone; **QUE:** quetiapine; **RHAL:** reduced haloperidol; **RIS:** risperidone; **SIL-IS:** stable isotope labelled internal standards; **TDM:** therapeutic drug monitoring; **UHPLC-MS/MS:** ultra-high performance liquid chromatography-tandem mass spectrometry; **UV:** ultraviolet detector; **ZUC:** zuclopenthixol

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1. Introduction

Antipsychotics (APs) are used for treatment of psychotic symptoms in patients with schizophrenic, schizophreniform, schizoaffective, psycho-organic and bipolar disorders [1-4]. A combination of psychotherapy and pharmacotherapy can improve symptoms significantly. However, APs show interindividual variability in clinical response while having narrow therapeutic ranges with a high risk for side effects. Monitoring of APs in serum or plasma is recommended for almost all currently used APs. Therapeutic drug monitoring (TDM) can aid in finding the right therapy, explaining non-response, pharmacokinetic interactions or poor response [5, 6].

Oral fluid (OF) is a mixture of saliva (an aqueous secretion of the salivary glands), proteins, electrolytes, cell and food debris and bacteria [7]. OF sampling is an interesting alternative for conventional blood testing, especially since psychiatric patients consider blood withdrawal as unpleasant and even frightening. One of the biggest problems in psychiatry is the high frequency of adherence problems. Approximately 40% of the schizophrenic patients are poorly adherent to their AP(s) at any time [8]. OF can be of significant interest when the presence of APs has to be confirmed, like in acute situations with forced admission to a psychiatric hospital where the psychiatrist wants to know if the patient is compliant or not. OF has a lot of advantages over blood collection: e.g. it can be readily sampled by nonmedical personnel, sampling is noninvasive and sample adulteration is minimized because of direct observation [9-11]. The detection time-window of OF is more similar to blood, with the presence of a high amount of parent drug in comparison to urine. This makes OF a highly interesting matrix for screening. Consequently, the question that arises is whether or not OF is also suitable for quantification purposes. Therefore, it should be highlighted that OF collection also includes several drawbacks. Firstly, secretion of OF is influenced by numerous factors, like food, drugs, emotional state, hunger etc. Secondly, a high inter- and intra-individual variation in drug concentrations is also dependent on the technique used for OF collection [12]. Thirdly, OF drug concentrations are predominantly dependent on the pH of the OF and blood, the protein binding and the pKa of the drug. In normal healthy persons the pH of OF is usually between 6.2 and 7.4. For basic and lipophilic drugs, concentrations in OF are higher than in blood since OF is usually more acidic and lipophilic substances diffuse

more easily due to ion trapping. Because most APs are lipophilic and basic compounds, OF to plasma or serum ratios are expected to be greater than one [6, 9, 12, 13].

TDM of drugs in OF has been studied for more than 40 years, especially for anticonvulsants [12, 14, 15]. However, its use for TDM of APs is only described in a limited number of publications for a limited number of compounds [7, 16-19]. Most of the analytical methodologies for detection of drugs in OF are adaptations of their plasma or serum method [12]. Jain and colleagues compared haloperidol (HAL) concentrations in OF and serum using liquid chromatography (LC) coupled to an ultraviolet detector (UV). OF was collected by drooling, using citric acid to facilitate secretion. The influence of citric acid on the pH of OF was not determined [17]. Two other publications describe the detection of risperidone (RIS) and its metabolite 9-OH risperidone (9OH-RIS) in plasma and OF using LC-tandem mass spectrometry (LC-MS/MS) and LC with coulometric detection, respectively [18, 19]. A multi-analyte LC-MS/MS method for quantification of 8 atypical APs and 1 metabolite in plasma, serum, OF and haemolysed whole blood was published by Fisher et al. Sample preparation was identical for the four different matrices [16]. OF was obtained by drooling into a plastic tube to avoid altering salivary pH as occurs by stimulation. OF concentrations were compared with whole blood and plasma [7].

We evaluated an ultra-high performance LC-tandem mass spectrometric (UHPLC-MS/MS) method for quantification of 11 commonly prescribed APs and 5 of their metabolites in OF based on our previously published serum method [20]. 9-Hydroxyrisperidone (also called paliperidone) is a metabolite of RIS but is also used as an AP itself. In the present study, the term 9OH-RIS is used for the metabolite of RIS and paliperidone (PAL) is used to describe the prescribed drug. We aimed to derive the value of OF for TDM of APs by defining the OF to serum concentration ratios from patients under chronic AP therapy, taking into account the exact amount of OF collected with a Quantisal™ collection device (Immunalysis, Pomona, CA).

2. Materials and methods

a. Chemicals and reagents

7-Hydroxy-N-desalkyl-quetiapine dihydrochloride (7OH-NDA-QUE), 7-hydroxy-quetiapine (7OH-QUE), amisulpride (AMI), aripiprazole (ARI), bromperidol (BRO), clozapine (CLO), HAL, N-desmethyl-clozapine (NORCLO), N-demethyl-olanzapine (NOROLA), olanzapine (OLA), PAL, pipamperone dihydrochloride (PIP), quetiapine hemifumarate (QUE), reduced haloperidol

(RHAL), RIS, and zuclopenthixol succinate salt (ZUC) were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). The stable isotope labelled internal standards (SIL-IS) 7OH-NDA-QUE-d_8 dihydrochloride, 7OH-QUE-d_8, AMI-d_5, ARI-d_5, CLO-d_8, HAL-d_4, NORCLO-d_8, NOROLA-d_8, OLA-d_8, PAL-d_4, PIP-d_{10} dihydrochloride, QUE-d_8 fumarate, RHAL-d_4, RIS-d_4, and ZUC-d_4 succinate salt were also purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). OF was collected using the Quantisal™ collection device (Immunalysis), consisting of a collector pad with a blue indicator (change of color when 1 ml ± 10% is collected) and a transport tube containing 3 ml of buffer. Acetonitrile, acetic acid, formic acid, and methyl *tert*-butyl ether (ethanol stabilized) (MTBE) were purchased from Merck (Darmstadt, Germany). All chemicals were of LC quality.

b. Standards

Methanolic stock solutions of 7OH-NDA-QUE, 7OH-QUE, AMI, BRO, HAL, RHAL, PIP, QUE, and ZUC were prepared at a concentration of 1 mg/ml. ARI, CLO, NORCLO, NOROLA, OLA, PAL and RIS stock solutions were prepared in acetonitrile at a concentration of 1 mg/ml. Working solutions of each analyte (100, 10 and 1 µg/ml) were prepared by further dilution of the stock solutions with acetonitrile.

Methanolic stock solutions of 7OH-NDA-QUE-d_8, 7OH-QUE-d_8, AMI-d_5, HAL-d_4, RHAL-d_4, PIP-d_{10}, QUE-d_8, and ZUC-d_4 were prepared at a concentration of 100 µg/ml. ARI-d_8, CLO-d_8, NORCLO-d_8, NOROLA-d_8, OLA-d_8, PAL-d_4 and RIS-d_4 stock solutions were prepared in acetonitrile at a concentration of 100 µg/ml. A working solution containing a mixture of all SIL-IS was prepared in acetonitrile by dilution of the stock solutions. The final concentration of the deuterated compounds ranged between 8 and 240 ng/ml (concentration in neat OF), i.e. in the range of calibration level 3 or level 4 of the non-deuterated compounds.

The calibration standards consisted of a mixture of the working solutions containing the 16 analytes at 7 or 8 concentration levels. The internal quality control (QC) standards were also prepared as a mixture from the different working solutions at 3 concentration levels (QC low, QC mid and QC high). All solutions were stored at -20°C. Twenty µl of the calibration and QC standards were spiked to 500 µl of blank OF/buffer solution (corresponding to 125 µl of neat OF). In Table 1 the obtained concentrations of the calibration standards and quality control samples in neat OF are summarized.

c. OF collection

Blank OF, used for the validation experiments, was obtained from healthy, drug-free volunteers. Blank samples were not pooled in order to account for interpatient variability. From every

volunteer blank OF was collected by drooling and by the use of the Quantisal™ collection device. OF collection with the Quantisal™ device was performed as recommended by the manufacturer. The collector pad was placed under the tongue until the volume-adequacy indicator turned blue, indicating that 1 ml of neat OF was collected. The pad was removed and placed in the transport tube with 3 ml buffer solution. To verify whether exactly 1 ml of OF was collected, the collected volume was determined by weighing. The OF-buffer solution was decanted into a polypropylene tube and stored at 4°C.The back-calculated concentrations in neat OF of calibration and QC samples were determined by multiplying the obtained concentration with a dilution factor of 4, since 1 ml of neat OF was diluted in 3 ml of buffer solution.

Both OF and serum samples were collected from psychiatric patients at the same time point. Patients had a clinical diagnosis of schizophrenia, schizo-affective or bipolar disorder based on the criteria of DSM-TR-IV. The study was approved by the ethics committee (Reference 13/30/300) of the University Hospital of Antwerp and the 3 participating psychiatric hospitals in Belgium (Sint-Norbertus, Duffel, Belgium; Broeders Alexianen, Boechout, Belgium; Sint-Amadeus, Mortsel, Belgium). All patients signed the informed consent. Samples were collected in the morning, at least 12h after the last medication dose (trough concentration), which means that contamination of the oral cavity with APs was avoided. OF was collected using the Quantisal™ device. Patients were not allowed to drink or eat within the 30 min before OF collection. After collection, OF samples were stored during 1 week at 4°C to allow elution of the drugs from the pad, as was described by Wille et al. [21]. Subsequently, the OF-buffer solution was decanted into a polypropylene tube and stored at 4°C until analysis. Collecting 1 ml of OF from psychiatric patients is difficult. Reported collection times in the literature vary between 2 and 10 min before the indicator turns blue, which is a very long time for patients who are rapidly agitated and impatient [10, 22]. Moreover, a high number of these patients have a dry mouth caused by anticholinergic side effects of the administered APs (for example CLO, OLA or RIS) or other co-medication (especially antidepressants), which makes it almost impossible to wait until 1 ml of neat OF is collected [12]. Therefore, the volume of collected OF was determined.

d. Sample preparation

Sample preparation was almost identical to the serum method, except the use of 500 µl of OF-buffer solution from the Quantisal™ device instead of 200 µl of serum [20]. After the addition of an internal standard (IS) mixture, a simple liquid-liquid extraction was performed using 1 ml of methyl *tert*-butyl ether (MTBE) at pH 9.5. The upper organic layer was transferred and

evaporated to dryness. Finally, the extract was reconstituted in 50 µL of acetonitrile and a volume of 0.3 µL was injected into the UHPLC-MS/MS.

e. Instrumentation and analytical method

Samples were analyzed on an Agilent 1290 Infinity LC system (Agilent Technologies, Santa-Clara, California, U.S.A.) coupled with an Agilent 6460 Triple Quadrupole mass spectrometer (MS) run in Jetstream® electrospray ionization (ESI) mode.

The LC system was optimized for rapid resolution using an Agilent SB C_{18} reversed phase column (2.1 x 50 mm, 1.7 µm) (Agilent Technologies) with a column oven temperature at 40°C. The mobile phase comprised of aqueous ammonium acetate (10 mM) at pH 3.7 (A) and acetonitrile (B) at a flow rate of 0.5 mL/min. Gradient elution was programmed as follows: starting conditions 10 % B; increase to 75 % B between 0 and 2.5 min; further increase to 95 % B between 2.5 and 3 min; retain 95% B between 3 and 4.5 min; back to initial conditions with 10% B from 4.6 to 6 min. The MS conditions were: positive mode, nebulizer gas: nitrogen, sheat gas temperature: 400°C, sheat gas flow: 12 L/min, nebulizer pressure: 50 psi, capillary voltage: 3000 V, and nozzle voltage: 0 V.

The MS was operated in dynamic multiple-reaction monitoring (dMRM) mode, monitoring 3 ion transitions for each analyte around their retention time (± 0.25 min). The mass spectrometric conditions for each analyte are identical to our serum method (supplemental digital data table 1) [20].

f. Method validation

When a minor change is made to a validated analytical method, like the use of another matrix, it is acceptable to perform a partial re-validation in that other matrix [23]. Since our serum method was validated according to EMA guidelines, validation of the OF method consisted of a more limited number of parameters, namely selectivity, linearity, accuracy, precision, recovery, matrix effects, stability and incurred sample reanalysis [20, 23, 24].

Selectivity was evaluated by the use of blank OF samples from 3 different sources, 2 zero samples (blank OF + SIL-IS mix) and 2 samples spiked with analytes and no SIL-IS. Linearity was evaluated using 8-point calibration curves measured on each of 5 consecutive days. The lowest calibration point was defined as the lower limit of quantification (LLOQ). Whenever this point did not fulfill the criteria, level 2 was considered as LLOQ and evaluated according to the criteria. At each of these 5 days, duplicates of LLOQ, low, medium and high concentration levels (QC low, QC mid, QC high) were analyzed. An ANOVA-calculation as described by Wille et al. was used for

248 determination of intra- and inter-day precision and accuracy [25]. Accuracy and precision were
249 acceptable when the % bias and coefficient of variation (%CV) was lower than 15% (20% for
250 LLOQ).
251
252 Recovery and matrix effects (ME) were calculated at two concentration levels (QC low and QC
253 high), based on the post-extraction addition technique as described by Matuszewski et al. [26].
254 ME were calculated as the percent ratio of peak areas of the analytes spiked after extraction and
255 the OF-buffer free solution prepared in acetonitrile (n=5). Relative ME were calculated as the
256 percent ratio of the IS corrected peak areas of the analytes spiked after extraction and the OF-
257 buffer free solution (n=5). % CV of the relative ME should not exceed 15%. In order to determine
258 the extraction recovery from the collection pad, blank OF from 3 different sources was spiked
259 with QC low or QC high and applied on the collection pad. Samples were analyzed after 1 day of
260 interaction between the pad and buffer (n=3). The influence of the amount of collected OF was
261 tested by applying 1 ml, 0.5 ml and 0.1 ml of spiked OF on the collection pad. Recovery of the
262 APs from the pad (ER_{pad}) was calculated as the percent ratio of the peak areas of the analytes
263 spiked on the pad and the analytes spiked in buffer solution without presence of the pad. The
264 influence of the OF matrix (OF + buffer, no collection pad) was also tested by calculating the ER
265 (ER_{matrix}) as the percent ratio of the (IS corrected) peak areas of the analytes spiked in OF matrix
266 without presence of the pad and the analytes spiked after extraction (post-extraction).
267
268 Stability of the compounds in the collection tube was tested during 7 days at 4°C after spiking
269 the collection pad with QC low and QC high (n=3). Concentrations were calculated based on the
270 daily calibration curves. Incurred sample reanalysis was performed on 20 different OF patient
271 samples with a time interval of 3 months between initial analysis and reanalysis. During those 3
272 months, samples were stored at 4°C. Acceptance criterion is a % difference between both
273 measurements of ± 20% of the mean for two-thirds of the samples [23].
274
275 **g. OF to serum concentrations**
276 All APs found in the patient samples were used to describe the relationship between OF and
277 serum concentrations. The whole collection device was weighed after sample collection. The
278 mean weight of an empty collection device with buffer solution (9.9425 g, CV % 0.87, n=8) was
279 used to determine the amount of OF collected from the patient, presuming that 1 ml of neat OF
280 weighs 1 g. This weighing method was also used by Wille et al. and Langel et al. [27, 28]. A
281 dilution factor was defined for every patient sample and used for calculation of the AP
282 concentrations in neat OF. Not only the amount of OF, but also the recovery from the pad

(ERpad), determined as the mean recovery from 1 ml of spiked neat OF, was taken into account to calculate AP concentrations. Ratios were determined per AP and the OF and serum methods were compared using linear regression analysis.

3. Results

a. Validation experiments

Linearity was evaluated on 5 calibration curves during 5 consecutive days. Calibration curves were analyzed with both unweighted and weighted 1/x linear regression. Inclusion of the zero value in the 95% confidence interval (CI) of the y-intercept, indicating absence of constant error, and a correlation coefficient of 0.99 or higher was pursued. Linear regression results without weighting and with 1/x weighting were almost identical with R^2 of 0.995 or higher for all compounds and inclusion of the zero value in the 95% CI for all compounds, except for HAL. As a result, it was more convenient to work with 1/x weighting, as we did for the serum method, than to use unweighted linear regression. Accuracy was evaluated based on the criteria that the back-calculated concentration should be within 15% of the nominal value (20% for LLOQ). For some compounds, the lowest level of the calibration curve was not detected (7OH-NDA-QUE, 7OH-QUE, OLA, NOROLA) or did not fulfill the identification criteria (RHAL, ZUC) [20]. All calibration curves proved to be linear in the proposed range, except for PIP and QUE at LLOQ. For these 2 compounds, but also for the compounds for which level 1 was not detected, level 2 was considered as LLOQ. Consequently, for these 8 compounds calibration curves with 7 instead of 8 concentration levels were used (Table 1).

Accuracy and precision were determined at four concentration levels (LLOQ, QC low, QC mid and QC high) and analyzed in duplicate on 5 consecutive days. ANOVA analysis was used to calculate accuracy (% bias), intraday precision (repeatability) and inter-day precision (intermediate precision) [25, 29]. All data are summarized in Table 2. Except for PIP at QC low, all accuracy data were within the acceptance criteria (bias ≤ 15%, for LLOQ ≤ 20%). All compounds fulfilled the criteria for intraday and inter-day precision (CV ≤ 15%, for LLOQ ≤ 20%).

In Table 3, an overview of the data concerning ME is shown. Ion suppression is seen when ME are below 100 %, ion enhancement when ME are higher than 100 %. The absolute mean ME was 106.3 % (range 94.3-131.5 %) and mean IS corrected ME was 101.1 %, (range 82.0-120.0 %). As can be concluded, ME were acceptable with limited ion enhancement for most of the

compounds. CV % of the IS corrected ME was < 15 % for all compounds, except for RHAL at QC low (CV 34.4%) and QUE at QC high (CV 16.3%).

To determine the amount of compounds that stay on the collection pad, the extraction recovery was calculated after 1 day of contact between the spiked OF, the buffer solution and the pad (ER_{pad}). The influence of the amount of spiked OF (1, 0.5 and 0.1 ml) was also evaluated. For 1 ml of neat OF, the mean absolute ER_{pad} varied between 37.1% for QC low (range 13.5-94.7%) and 40.3% for QC high (range 25.3-53.7%) (Table 4). For 0.5 ml of neat OF, the absolute ER_{pad} was comparable (mean ER_{pad} 35.0% for QC low, range 11.0-84.6%; 37.3% for QC high, range 19.6-56.5%). When 0.1 ml of neat OF was spiked, ER_{pad} was even lower with a broad 95% CI for almost all compounds (mean ER_{pad} 29.0% for QC low, range 5.2-96.8%; 15.6% for QC high, range 5.0-29.6%). Recoveries were highly variable between QC low and QC high for NOROLA and a wide 95% CI was seen at QC low. Recoveries obtained with 0.1 ml of neat OF were highly variable with even negative 95% CIs. For ZUC, peaks were not found with 0.1 ml of spiked neat OF. From these data, it can be concluded that a small amount of OF (< 200 µl) will result in unreliable recoveries and thus unreliable AP concentrations.

On the other hand, the influence of the OF-buffer matrix on ER (ER_{matrix}), not taking the influence from the collection pad into consideration, was calculated on 1 ml of neat OF. The mean absolute ER_{matrix} varied between 57.8% for QC low (range 26.2-73.0%) and 66.1% for QC high (range 39.2-86.6%) (Supplemental data table 2). As can be expected, the mean IS corrected ER_{matrix} was much higher, 86.1% for QC low (range 75.2-99.6%) and 90.2% for QC high (range 82.7-107.4 %).

Stability of the compounds in the collection device was evaluated during 7 days at 4°C, which was representative for the actual storage conditions of the samples during the study. Samples were analyzed after 2, 5 and 7 days of storing at 4°C, and compared with samples analyzed on day 1, since this was the minimum time necessary to allow extraction of the compounds from the pad. All of the APs at QC low and QC high were stable at 4°C during 7 days, only NOROLA showed a decrease after day 5 and 7, but only for QC low and not for QC high (Figure 1). On the other hand, an increase of the concentration would suggest that a longer time is needed to allow extraction of the APs from the collection pad. After 7 days of extraction, a small increase in the concentrations of all APs was seen for both QC low (mean increase 11.7 %, median 14.1%) and QC high (mean increase 13.5%, median 15.2%). However, this small increase can also be attributed to deviation in OF concentrations and to measurement uncertainty. Only for NOROLA, a mean decrease in concentration of 35.6% was seen after 7 days for QC low, while the range of these measurements was wide (2.0-69.2% decrease). When looking at the NOROLA results of QC

high, this decrease was not confirmed (mean decrease of 2.5%, range +23.5% to -28.5%). It can be concluded that extraction of the APs from the pad is complete after day 1 and the extraction will not significantly change after 7 days of interaction between the pad and the buffer solution, except for NOROLA for which stability is highly variable.

Twenty OF patients' samples were reanalyzed 3 months after initial analysis. These 20 patient samples contained 56 APs and metabolites. The % difference between the results should be within 20% of their mean for two thirds of the 56 concentrations and this criterion for incurred sample reanalysis was fulfilled [23].

b. Patient samples

Eighty-nine OF samples were collected from 85 psychiatric patients (55 male, 30 female; age range 19-65 years). The mean collected volume of neat OF was 0.562 ml (median 0.514 ml; range 0.057 – 1.232 ml). Samples with a neat OF volume below 0.2 ml (n=10) were not used for calculation of the OF to serum ratios nor for regression analysis. Based on the recovery experiment, samples with a neat OF volume of 0.1 ml should be excluded since their results would be unreliable. Eleven APs and 6 of their metabolites were found. The OF to serum ratio was above 1 for all APs (mean ratios between 1.54 and 28.50) (Table 5). Only for ARI and ZUC, the ratio was below 1 (0.21 and 0.66, respectively). The ranges of these ratios were extremely wide for all compounds. Since 42 of the 89 samples had a neat OF volume between 0.1-0.5 ml, a comparison was made between the obtained OF to serum ratios if only samples with a neat OF volume above 0.5 ml were included and if samples with a neat OF volume above 0.2 ml were included (Supplementary Digital Concent Table 3 and Figure 1). Since the 25 and 75% percentiles were comparable between the two groups, it was decided to include samples with a neat OF volume between 0.2-0.5 ml. Moreover, inclusion of these samples is more representative for the actual patient sample collection.

Scatter plots and trend lines are summarized in Figure 2. There was a (low) correlation for AMI (R^2 0.68), ARI (R^2 0.53), CLO (R^2 0.13), NORCLO (R^2 0.23), RIS (R^2 0.45), 9OH-RIS (R^2 0.23), PAL (R^2 0.14), QUE (R^2 0.49), 7OH-QUE (R^2 0.64) and 7OH-NDA-QUE (R^2 0.62). However, for OLA and NOROLA (R^2 0.00 and 0.03, respectively), no correlation was seen and data points were highly variable. For BRO, HAL, RHAL, PIP and ZUC, no scatter plot was presented since the number of data were too limited to draw definitive conclusions.

4. Discussion

Different collection devices are on the market and not one is clearly superior concerning design or use [12]. The most important issues highlighted in the literature are the variable volume of

collected OF which is sometimes not enough for analysis, the recovery which is highly influenced by adsorption of compounds to the pad, and the influence of buffer solution and other materials on the device which will influence stability and can cause interferences with the analysis [27, 30]. According to the manufacturer, the Quantisal™ collection device is able to collect exactly 1 ± 0.1 ml neat OF. Langel et al. compared nine commercially available collection devices. The volume of collected OF was determined and the Quantisal™ device showed one of the lowest % CVs when 1 ml of OF was collected, both in vitro (n=6) and for volunteers (n=6). Moreover, the amount of buffer solution, according to the manufacturer 3 ± 0.15 ml, was also evaluated (3.015 ml, 0.4 %CV, n=6) and showed less variation when compared to the other collection devices containing buffer solution. Questioning of volunteers resulted in the most positive evaluation for Quantisal™. Drug recoveries (mostly drugs of abuse) were all above 80% [30]. Based on these results, Quantisal™ was chosen as the device for OF collection in psychiatric patients. This way of collecting the OF was easy and sample collection could be performed under supervision of the researchers. Requesting OF by simple drooling, as was undertaken by Fisher et al., was not considered practical for patients [16]. There are no publications regarding quantification of APs in OF using a collection device. Therefore, validation of our method had to include evaluation of the recovery of APs, taking into consideration the amount of OF collected and the influence of the collection pad.

The sample preparation was identical to the serum method. However, 500 µl of OF-buffer solution was used for quantification instead of the 200 µl used in the serum method. 500 µl of OF-buffer solution should contain 125 µl of neat OF when 1 ml of OF is collected. Since the collected volume was often less than 1 ml, the amount of neat OF in a sample can be much lower and consequently, the AP concentrations will be low. To be able to measure these small amounts of APs, we analyzed 500 µl of OF.

The obtained ME were low for this method, both for the absolute and IS corrected ME. Since absolute ME were limited, the compensating effects of the deuterated analogues of the APs used as IS mixture was less pronounced. Preservation buffers of the collection devices contain salts, non-ionic surfactants, stabilizing chemicals and anti-bacterial agents which can induce matrix effects in LC-MS analysis. Manufacturers do not disclose the exact contents of these buffer solutions. For example, high ion enhancement (318-516%, n=10) was seen with the determination of amphetamines with LC-MS/MS using Quantisal™ as collection device [11]. Apparently, the sample preparation of this method (LLE with MTBE) removed the buffer compounds which could have an influence on ME.

The ER_{pad} was rather low but still sufficiently high for this method due to the use of a highly sensitive UHPLC-MS/MS method. This incomplete recovery could be attributed to adsorption of the APs to the pad, since ER_{matrix} determined by spiking OF-buffer solution without the presence of a collection pad resulted in higher recoveries (Supplemental data, Table 2). Comparison of these results with previous publications is difficult, since most articles do not take the influence of the pad into account or it is not clearly stated if testing was undertaken in the presence of the pad [10, 11, 30]. Only one article describes the determination of both types of ER for antidepressants. The ER_{pad} ranged between 51.4 and 87.4% while the ER from the OF matrix without collection pad was higher (range 89.2-97.0%) [31]. Stabilizing buffers in OF devices have different capabilities: guaranteeing stability, reducing viscosity of OF and diminishing adsorption of drugs onto the collection pad. In general, lipophilic drugs will be highly adsorbed to the collection pad, resulting in lower recoveries [27].

As was seen for our patient samples, the amount of collected OF was highly variable (range 0.057 – 1.232 ml), since it was impossible to wait until the indicator turned blue or to use a fixed collection time. Moreover, when a fixed collection time of 5 min is used, as described by Wille et al., a range of 0.04 to 1.55 g of OF was obtained with the Quantisal™ device (n=10) [21]. According to this publication, drug concentrations tend to decrease with an increasing salivary flow, meaning that the influence of the volume is maybe not that important as was believed. Of course, the pH will also vary when salivary flow is stimulated and this will also have an influence on drug concentration. In another study, OF was collected with the Quantisal™ devices using the volume adequacy indicator or a collection time of 5 min, whichever occurred first. Twenty percent of the specimens had a volume below 1 ml of neat OF but no weight correction was applied [22]. Knowing that the difference in collected volume can be quite high, it is advisable to determine the mean weight of the empty device and use this weight for calculation of the amount of neat OF. However, the exact weight of an empty collection device appeared to be different from batch to batch (own data: range 9.9425-10.1626 g, determined on 3 different batches; literature: 10.0715 g) [27]. Since the difference between batches can be as large as 0.220 g, this will have an enormous influence on the calculation of the final drug concentrations. The mean weight of an empty collection device should thus be determined per batch. As was already highlighted, for the determination of the AP concentrations in our patient samples both the amount of neat OF and the ER_{pad} was taken into consideration, particularly since this ER_{pad} was low.

APs are mostly basic and lipophilic compounds for which high OF to serum ratios are expected due to higher concentrations in OF as compared to serum [6, 9, 13]. As can be seen in Table 5, this was indeed the case for most APs, except for ARI and ZUC. Due to the limited literature, comparison of our results is difficult. Jain et al. found that OF concentrations were 2.3 fold higher for HAL than plasma concentrations and the relation was linear with an R^2 of 0.93 [17]. The ratio was lower than our ratio (mean 6.28), but this can be caused by the stimulation of the OF flow with citric acid. In the present study, a distinction was made between the concentrations of patients taking PAL as AP drug and patients taking RIS with 9OH-RIS as metabolite, although these compounds have an identical chemical structure. Consequently, OF to serum ratios were comparable (mean PAL ratio 1.75, mean 9OH-RIS ratio 1.69). For RIS and 9OH-RIS, Flarakos et al. demonstrated OF to plasma ratios which were lower as compared to our results (range 0.06-0.84 for RIS, 0.50-1.18 for 9OH-RIS, n=7). The number of tested patients was low and the range was also very wide. In another publication, the RIS and 9OH-RIS ratios were between 0.78-1.64 and 0.88-1.50, respectively (n=6). These results were more comparable to our results. Both methods collected OF by simple drooling. For ARI and ZUC, the high protein binding in blood (> 99% for ARI, 98% for ZUC) and thus the low unbound fraction of these compounds could explain their low concentration in OF and the low OF to serum ratio [7, 13]. The OF to plasma ratios which were defined by Fisher et al. were lower, but for most of the APs a wide range, with ratios being from below 1 to way above 1, was seen. OF was collected by drooling and this will stimulate salivary flow rate in a different way than the Quantisal™ device. Overall, as can be seen both in literature and in our results, the OF to plasma/serum ratios are highly variable due to all the different factors that alter OF concentrations like pH, salivary flow rate, protein binding, binding to the collection pad etc. Since our patients were under chronic AP therapy and a trough concentration was measured, it was expected to see less variation between OF and serum concentrations. However, as can be seen from the scatter plots, concentrations in OF are highly variable and mostly much higher than the concentrations in serum.

It should be noted that the current study has some limitations, since only the influence of the collection device was studied. It was not possible to determine the pH of the neat OF of the patients after sample collection, since the sampling device contains a buffer solution. Other factors which could have contributed to the high variability in results are the small sample size per AP, the determination of the exact amount of neat OF by weighing and the use of a calculated dilution factor to derive the AP concentrations in neat OF. Since there was a correlation for some of the APs (R^2 = 0.00-0.78), we can conclude that OF can be an interesting matrix for AP testing, at least for qualitative interpretation of the results. The high variance in OF to serum ratios needs to be confirmed by other investigators, using a larger number of patient

samples. Nevertheless, a standardized procedure for OF collection is not yet established, which implies that interpretation and comparison of results remains difficult, especially when different collection devices are used [28].

5. Conclusion

Based on the validation results, it was demonstrated that our UHPLC-MS/MS method can be used for reliable quantification of APs in OF, despite the fact that the ER_{pad} was rather low. Some small changes of our serum method were necessary to measure the low concentrations in the OF-buffer mixture, like a higher sample volume (500 µl) and the use of 8 calibration levels instead of 7 for some compounds. However, when OF results of the APs found in our patient samples were compared with serum concentrations, high variations were seen. As already concluded for many other compounds, OF concentrations of APs are highly variable and should not be used to calculate serum concentrations due the wide range of OF to serum ratios [7, 9, 18, 28]. In this study, only the influence of the collection device was evaluated, while OF concentrations probably fluctuate due to a number of different causes; for example OF flow rate, pH of OF, the pKa of the compound, the protein binding of the compound and the type of collection device used. Since there was a correlation between OF and serum concentrations while results were highly variable, we can only conclude from these preliminary results that OF is a potentially interesting matrix, particularly for screening for noncompliance.

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643 **Figure 1:** Seven-day stability of the APs in 1 ml of OF spiked on Quantisal collection devices at QC low (A) and QC high (B) at 4°C.

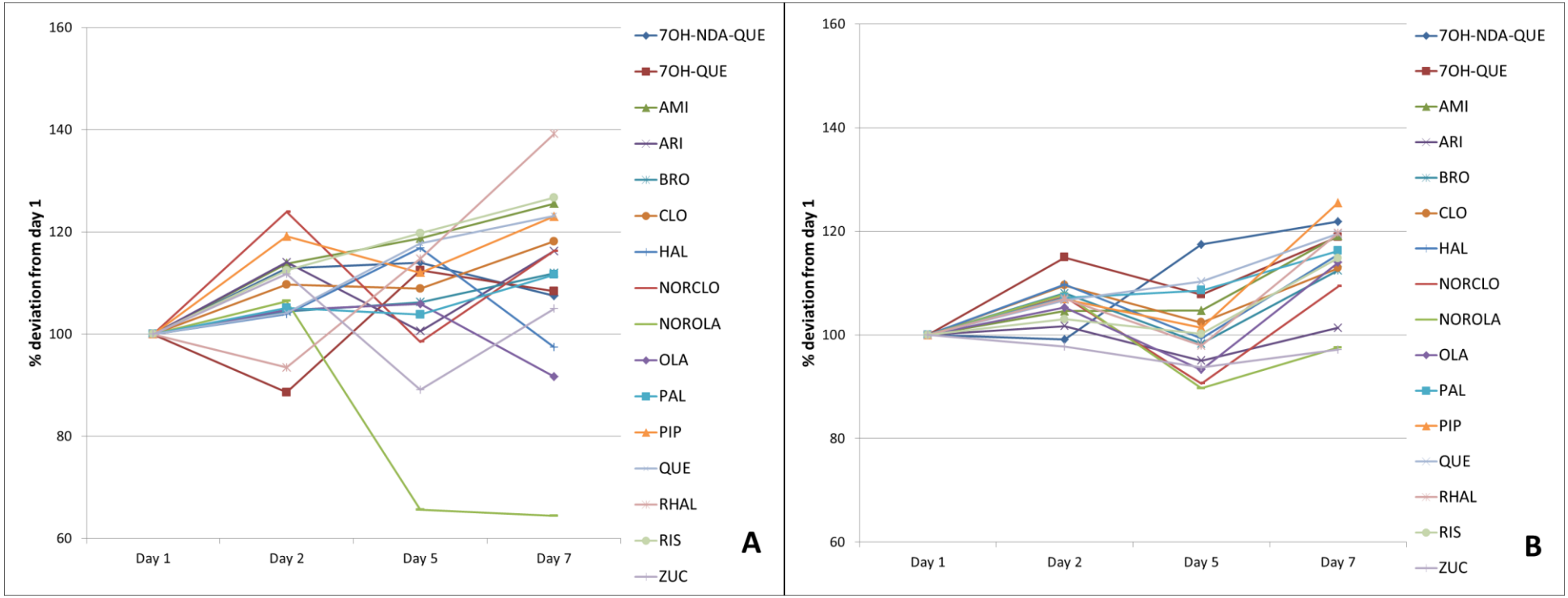

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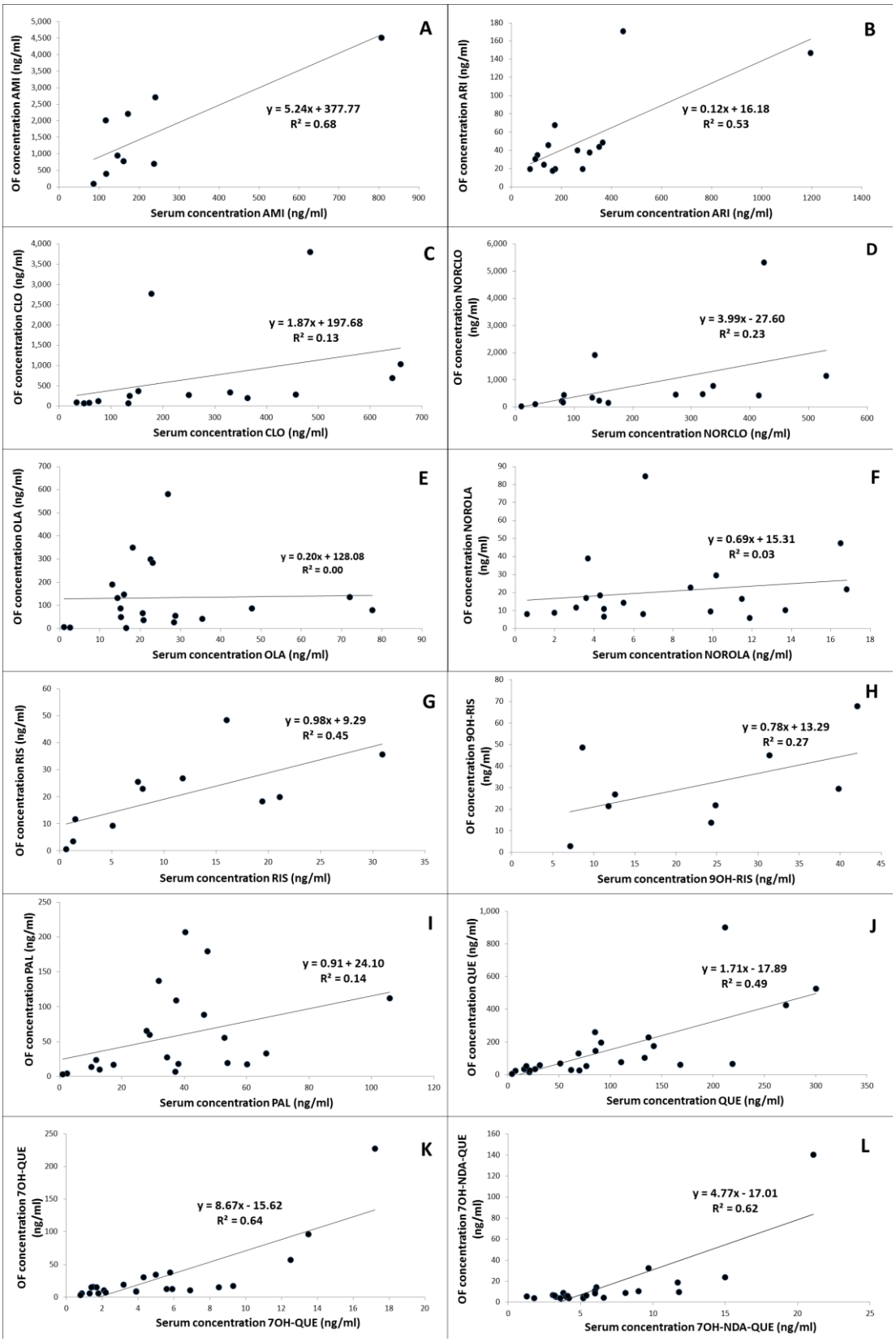
Figure 2: Scatter plots and trend lines of serum and oral fluid (OF) concentrations in ng/ml of patients taking A amisulpride (AMI); B aripiprazole (ARI); C clozapine (CLO); D N-desmethylclozapine (NORCLO), metabolite of CLO; E olanzapine (OLA); F N-desmethylolanzapine (NOROLA), metabolite of OLA; G risperidone (RIS); H 9-hydroxyrisperidone (9OH-RIS), metabolite of risperidone; I paliperidone (PAL); J quetiapine (QUE); K 7OH-quetiapine (7OH-QUE), metabolite of QUE; L 7OH-N-desalkylquetiapine (7OH-NDA-QUE), metabolite of QUE.



653 **Table 1:** Neat oral fluid concentrations of calibration standards and quality control samples of all antipsychotics

Analyte	Abbreviation	Calibration standards (ng/ml)								Internal quality control samples (ng/ml)		
		L1	L2	L3	L4	L5	L6	L7	L8	QC low	QC med	QC high
Amisulpride	AMI	3.20	16.0	80	160	240	480	960	1920	48	400	1360
Aripiprazole	ARI	6.40	32.0	80	400	800	1200	1600	2400	96	1040	2000
Bromperidol	BRO	0.32	1.6	8	16	24	48	96	160	4.8	40	128
Clozapine	CLO	16.00	60.0	160	400	800	1200	1600	2400	240	1040	2000
N-desmethylclozapine	NORCLO	-*	16.0	80	160	320	800	1200	2400	48	560	1840
Haloperidol	HAL	0.16	0.8	1.6	4	8	16	40	80	2.4	12	56
Reduced haloperidol	RHAL	-*	0.8	1.6	4	8	16	40	80	2.4	12	56
Olanzapine	OLA	-*	1.6	16	40	80	160	240	480	4.8	120	360
N-desmethylolanzapine	NOROLA	-*	1.6	8	16	24	48	96	160	4.8	40	128
Paliperidone	PAL	0.32	1.6	16	40	80	160	240	480	4.8	120	360
Pipamperone	PIP	-*	16.0	80	240	480	800	1200	1600	48	640	1360
Quetiapine	QUE	-*	16.0	80	400	800	1200	1600	2400	48	1040	2000
7OH-N-desalkyl-quetiapine	7OH-NDA-QUE	-*	1.6	8	16	24	48	96	160	4.8	40	128
7OH-quetiapine	7OH-QUE	-*	1.6	8	16	24	48	96	160	4.8	40	128
Risperidone	RIS	0.32	1.6	8	16	40	80	120	240	4.8	64	160
Zuclopenthixol	ZUC	-*	1.6	8	16	80	160	240	480	4.8	120	360

* Criteria for identification, accuracy and/or precision were not fullfilled. L2 was evaluated as LLOQ.

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Table 2: Accuracy and precision data for all antipsychotic analytes evaluated in oral fluid-buffer solution (Quantisal device) at four concentration levels.

Analyte	LLOQ (n=5)			QC low (n=5)			QC mid (n=5)			QC high (n=5)		
	Precision intraday	Precision interday	Accuracy	Precision intraday	Precision interday	Accuracy	Precision intraday	Precision interday	Accuracy	Precision intraday	Precision interday	Accuracy
	CV (%)	CV (%)	Bias (%)	CV (%)	CV (%)	Bias (%)	CV (%)	CV (%)	Bias (%)	CV (%)	CV (%)	Bias (%)
7OH quetiapine*	5.73	5.77	3.44	5.74	5.50	-7.00	2.87	2.87	-7.30	3.32	3.35	5.75
7OH-N-desalkyl quetiapine*	13.75	13.63	16.25	3.33	3.45	-4.08	4.30	4.41	-6.40	3.48	3.48	1.66
Amisulpride	2.57	3.58	13.00	2.41	2.52	9.17	2.91	2.91	-6.03	2.07	2.53	8.14
Aripiprazole	3.45	3.36	17.75	5.47	5.47	3.79	2.61	2.83	-6.30	2.93	2.94	4.91
Bromperidol	14.71	15.28	5.50	4.38	4.51	3.17	2.98	3.46	-2.29	3.58	3.85	2.66
Clozapine	5.13	5.23	-2.58	1.48	2.40	12.08	6.20	6.20	-7.90	1.81	2.08	0.98
Haloperidol	13.10	14.04	12.75	4.20	4.15	9.33	1.93	2.26	-5.70	5.43	5.54	3.57
Norclozapine*	2.52	3.12	3.98	3.44	3.46	4.75	3.24	3.66	-6.26	2.78	3.38	5.70
Norolanzapine*	9.98	9.75	11.50	9.64	9.61	-5.67	3.60	3.60	0.77	0.88	2.74	6.75
Olanzapine*	4.55	4.63	11.25	4.30	4.72	-6.08	2.12	2.13	-1.10	2.95	3.11	6.37
Paliperidone	14.73	16.17	20.00	3.54	3.72	1.50	4.10	4.35	-9.00	1.97	1.92	5.62
Pipamperone	4.90	4.84	3.18	2.90	2.94	**15.56**	2.49	2.63	-1.35	2.39	2.39	-4.24
Quetiapine*	1.78	3.52	11.7	2.96	3.07	6.00	2.83	2.83	-7.07	1.42	1.96	6.18
Reduced haloperidol*	3.93	4.19	6.50	4.71	5.07	13.50	9.12	9.02	-6.23	6.94	7.02	4.07
Risperidone	6.73	7.29	17.50	3.28	3.33	4.67	2.11	2.64	-7.36	4.19	4.28	4.70
Zuclopenthixol*	4.62	4.61	6.75	1.61	1.99	3.83	3.15	3.37	-6.80	1.10	2.20	3.84

* L2 was evaluated as LLOQ (L1 did not fulfill the criteria)

Table 3: Absolute and internal standard (IS) corrected matrix effects and their respective 95% confidence intervals (CI) obtained with oral fluid of 5 different sources spiked with 'QC low' and 'QC high' concentrations. The % CV of the IS corrected matrix effects were < 15% for all compounds, except for reduced haloperidol at QC low and quetiapine at QC high.

	Matrix effects (n=5)				**IS corrected matrix effects (n=5)**					
	QC low		**QC high**		**QC low**			**QC high**		
Analyte	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**	**CV (%)**	**Mean (%)**	**95% CI**	**CV (%)**
7OH-N-desalkylquetiapine	95.1	85.0-105.1	103.4	100.1-106.8	100.8	86.3-115.2	11.6	102.0	96.7-107.4	4.3
7OH-quetiapine	101.9	96.1-107.8	111.7	108.4-115.0	95.7	83.1-108.3	10.6	105.5	99.2-111.7	4.8
Amisulpride	105.6	101.8-109.5	96.4	92.8-99.9	107.8	100.6-114.9	5.4	106.6	102.7-110.5	2.9
Aripiprazole	108.8	104.0-113.6	99.6	96.9-102.4	109.3	100.6-117.9	6.4	106.1	101.8-110.4	3.2
Bromperidol	111.4	108.1-114.7	105.3	102.3-108.3	108.6	99.3-117.9	6.9	107.4	103.1-111.8	3.3
Clozapine	111.8	106.2-117.3	99.7	92.7-106.7	110.4	101.0-119.8	6.9	109.1	103.6-114.5	4.0
Haloperidol	120.0	117.6-122.4	103.1	98.3-108.0	116.9	107.4-126.4	6.6	105.3	98.7-111.8	5.0
N-desmethylclozapine	82.0	75.6-88.3	92.3	87.2-97.3	105.2	95.7-114.6	7.2	102.8	96.2-109.4	5.2
N-desmethylolanzapine	91.1	80.8-101.4	84.6	82.0-87.2	131.5	110.1-152.9	13.1	96.1	91.7-100.6	3.7
Olanzapine	94.5	89.1-100.0	101.9	97.0-106.9	99.2	89.0-109.4	8.3	106.2	102.5-109.9	2.8
Paliperidone	108.0	104.3-111.7	89.8	85.6-93.0	109.4	101.5-117.4	5.9	112.2	107.8-116.6	3.2
Pipamperone	104.9	101.4-108.4	99.3	95.4-103.2	102.2	95.3-109.1	5.5	99.5	93.7-105.3	4.7
Quetiapine	108.3	103.6-113.1	90.8	61.5-120.1	107.1	99.1-115.0	6.0	97.8	78.1-117.6	**16.3**
Reduced haloperidol	108.4	98.3-118.5	91.9	74.4-109.5	104.5	59.9-149.2	**34.4**	94.3	82.8-105.9	9.3
Risperidone	111.6	107.2-116.0	104.2	101.9-106.5	110.5	99.5-121.5	8.0	105.6	100.7-110.5	3.7
Zuclopenthixol	100.8	96.6-105.0	98.1	95.6-100.6	111.5	102.5-120.4	6.5	115.0	107.8-122.1	5.0

Table 4: Absolute recovery from the collection pad (ER_{pad}) and 95% confidence interval (CI) obtained with Quantisal collection devices spiked with 1, 0.5 and 0.1 ml of neat oral fluid from 3 different sources with 'QC low' and 'QC high' concentrations. Samples were analyzed after one day of interaction between the collection pad, buffer and oral fluid.

	1 ml neat oral fluid				0.5 ml neat oral fluid				0.1 ml neat oral fluid			
	QC low		QC high		QC low		QC high		QC low		QC high	
Analyte	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**	**Mean (%)**	**95% CI**
7OH-N-desalkylquetiapine	39.1	18.9-59.3	37.4	30.7-44.2	42.6	35.1-50.0	34.8	17.5-52.1	44.8	6.2-83.5	12.6	-2.5-27.6
7OH-quetiapine	40.3	25.0-55.7	40.5	28.2-52.8	37.8	30.2-45.4	36.4	21.0-51.9	23.1	5.1-41.1	15.7	-3.4-34.8
Amisulpride	36.5	14.7-58.3	37.0	25.1-48.9	26.4	2.6-50.2	25.4	13.7-37.1	13.2	-2.9-29.3	8.4	-3.3-20.1
Aripiprazole	13.5	8.6-18.4	25.3	18.1-32.6	11.0	9.3-12.7	19.6	8.7-30.4	5.2	0.6-9.9	5.0	-1.6-11.3
Bromperidol	27.5	17.2-37.7	35.4	26.2-44.7	29.3	22.3-36.3	31.2	14.8-47.5	16.7	4.6-28.8	12.0	-5.4-29.4
Clozapine	36.7	24.3-49.1	52.7	45.8-59.7	35.4	27.8-43.0	54.9	35.8-74.0	22.9	0.9-45.0	27.4	-5.9-60.8
Haloperidol	32.5	20.5-44.5	38.1	24.3-51.8	29.2	26.1-32.2	33.6	16.7-50.5	24.2	-9.5-57.8	14.0	-2.8-30.9
N-desmethylclozapine	34.0	9.8-58.3	39.2	32.0-46.5	34.7	24.5-45.0	36.6	20.4-52.9	23.8	9.1-38.6	13.7	-0.1-27.5
N-desmethylolanzapine	94.7	8.6-180.8	31.3	25.3-37.2	84.6	-3.1-172.3	30.2	12.7-47.6	96.8	41.7-151.9	8.6	2.7-14.5
Olanzapine	40.8	31.3-50.3	39.6	31.0-48.2	43.0	38.1-48.0	35.1	20.3-50.0	34.3	5.2-63.3	14.0	-2.4-30.3
Paliperidone	37.9	31.5-44.3	47.9	38.7-57.1	36.4	23.5-49.3	46.4	30.1-62.8	24.9	5.7-44.0	20.8	-3.9-45.5
Pipamperone	37.7	24.5-50.8	49.3	40.7-57.9	36.1	31.3-41.0	48.8	31.3-66.4	23.6	5.1-42.0	21.7	-1.6-44.9
Quetiapine	35.1	26.3-43.9	48.3	38.9-57.7	35.1	27.0-43.1	49.2	31.6-66.7	22.6	2.5-42.7	25.3	-8.0-58.6
Reduced haloperidol	31.0	27.3-34.6	53.7	47.3-60.1	29.4	15.8-43.0	56.5	36.0-76.9	32.1	-6.5-70.7	29.6	-1.7-60.9
Risperidone	37.1	30.0-44.3	39.8	28.9-50.6	36.0	25.1-46.9	35.4	19.0-51.8	27.1	5.7-48.5	14.4	-4.9-33.7
Zuclopenthixol	19.2	13.7-24.7	29.0	23.1-34.9	13.5	11.3-15.7	22.9	9.1-36.8	-	-	6.2	-1.0-13.3

687 **Table 5:** Mean, median, standard deviation (SD), 25 and 75% percentiles (Q1 and Q3) and range of oral fluid (OF) to serum ratios, together with the
688 regression equation and correlation coefficient (R^2) of all antipsychotics found in patient samples (n=79). Samples which contained less than 200 µl of neat
689 oral fluid (NOF) were excluded from the calculations (n=10). For every antipsychotics the pKa and the % protein binding (Pb) is also given.

Antipsychotic	pKa	Protein binding (%)	n	N° samples < 200 µl NOF*	Mean	Median	SD	Q1	Q3	Range	Regression line	R^2
Amisulpride	9.4	17	10	1	13.42	6.01	19.03	3.68	12.43	1.04-68.66	y = 5.24x + 377.77°	0.68°
Aripiprazole	7.6	> 99	15	1	0.21	0.15	0.11	0.12	0.32	0.07-0.39	y = 0.12x + 16.18	0.53
Bromperidol	-	90	2	2	-	-	-	-	-	1.25-4.71	-	-
Clozapine	3.7 ; 7.6	95	15	1	2.75	1.50	3.84	1.04	2.16	0.53-15.57	y = 1.87x + 197.68	0.13
N-desmethylclozapine		-	15	1	3.69	2.15	3.91	1.60	2.91	0.97-14.07	y = 3.99x - 27.60	0.23
Haloperidol	8.3	90	7	0	6.28	4.17	3.93	3.60	8.09	2.36-14.05	y = 3.68x + 4.99$^+$	0.64$^+$
Reduced haloperidol		-	7	0	28.50	13.53	33.30	9.62	26.00	7.48-107.27	y = 12.24x + 18.83$^+$	0.78$^+$
Olanzapine	5.0 ; 7.4	93	20	3	6.44	3.25	6.39	1.68	9.97	0.16-21.62	y = 0.20x + 128.08	0.00
N-desmethylolanzapine		-	19	2	3.93	2.6	3.80	1.37	4.36	0.50-13.16	y = 0.69x + 15.31	0.03
Paliperidone	2.6 ; 8.2	74	21	1	1.75	1.30	1.36	0.78	2.34	0.19-5.12	y = 0.91x + 24.10	0.14
Pipamperone	4.2 ; 8.0	-	2	0	-	-	-	-	-	1.26-15.76	-	-
Quetiapine	3.3 ; 6.8	83	25	5	1.54	1.33	0.96	0.78	1.86	0.30-4.26	y = 1.71x - 17.89	0.49
7OH-N-desalkylquetiapine		-	24	5	1.85	1.48	1.29	1.09	2.09	0.64-6.65	y = 4.77x - 17.01	0.62
7OH-quetiapine		-	23	5	5.35	4.76	3.20	2.28	7.11	1.54-13.20	y = 8.67x - 15.62	0.64
Risperidone	3.1 ; 8.2	89	11	3	2.53	2.27	1.88	1.05	2.95	0.91-7.77	y = 0.98x + 9.29	0.45
9-hydroxyrisperidone		74	9"	3	1.69	1.43	1.51	0.74	1.81	0.39-5.64	y=0.78x + 13.29	0.27
Zuclopenthixol	3.4 ; 6.1	98	4	1	0.66	0.73	0.30	0.51	0.88	0.20-0.99	y = 0.69x - 0.65$^+$	0.44$^+$

° One sample was determined as outlier (serum 213 ng/ml; oral fluid 14 624 ng/ml)
$^+$ Data are not reliable since only a limited number of concentrations were available.
" Two patients were taking both risperidone and paliperidone, these data were excluded from the calculations.

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Supplemental digital content

Table 1: Mass spectrometric conditions of all analytes including MRM transitions, collision energy (CE), qualifier/quantifier ratio, fragmentor voltage (FV), retention time (RT) used for UHPLC-MS/MS.

Analyte	Precursor ion (m/z)	Product ion (m/z)	CE (V)	Ratio (%)	FV (V)	RT (min)
Amisulpride	370.2	242.0	26	100.0	188	1.0
		196.0	42	51.2		
		112.1	22	34.4		
Amisulpride-d5	375.2	242.0	26	100.0	188	1.0
		196.0	42	51.2		
		117.1	26	33.1		
Aripiprazole	448.2	285.1	22	100.0	228	2.1
		98.1	38	44.3		
		176.1	30	41.8		
Aripiprazole-d8	456.2	293.1	26	100.0	220	2.1
		176.0	30	41.6		
		102.1	42	34.5		
Bromperidol*	420.1	165.0	22	100.0	172	2.0
		123.0	46	74.6		
		402.0	14	8.0		
Clozapine	327.1	270.0	18	100.0	172	1.7
		192.0	46	75.4		
		164.0	90	21.9		
Clozapine-d8	335.2	275.1	22	100.0	172	1.7
		192.0	50	80.4		
		164.0	90	35.2		
N-desmethylclozapine	313.1	192.0	42	100.0	172	1.6
		270.0	22	57.3		
		227.0	26	17.2		
N-desmethylclozapine-d8	321.2	192.0	46	100.0	172	1.6
		275.1	22	27.6		
		227.0	30	13.8		
Haloperidol	376.2	165.0	22	100.0	172	1.9
		123.0	42	122.1		
		95.1	82	53.3		
Haloperidol-d4	380.2	165.0	22	100.0	172	1.9
		123.0	42	113.2		
		95.1	82	48.2		
Reduced haloperidol	378.2	149.0	26	100.0	166	1.7
		109.0	58	61.4		
		342.1	18	11.7		
Reduced haloperidol-d4	382.2	149.0	26	100.0	166	1.7
		109.0	54	61.4		
		346.1	22	12.1		
Olanzapine	313.2	256.0	18	100.0	176	0.9
		198.0	42	28.0		
		169.0	42	14.4		
Olanzapine-d3	316.2	256.0	18	100.0	176	0.9
		198.0	42	27.7		
		169.0	46	15.8		
N-desmethylolanzapine	299.1	198.0	38	100.0	176	0.8
		256.0	22	83.5		
		213.0	26	63.3		
N-desmethylolanzapine-d8	307.2	198.0	38	100.0	176	0.8
		213.0	26	56.0		
		169.0	42	40.5		
Paliperidone	427.2	207.1	26	100.0	176	1.4
		110.0	46	26.2		
		82.1	58	7.3		
Paliperidone-d4	431.2	211.1	26	100.0	176	1.4
		114.1	46	24.8		

		179.0	46	3.0		
Pipamperone	376.2	165.0	26	100.0	166	1.3
		123.0	50	69.6		
		291.1	14	35.9		
Pipamperone-d10	386.3	165.0	26	100.0	166	1.2
		123.0	54	67.8		
		291.1	14	40.5		
Quetiapine	384.2	253.0	18	100.0	172	1.8
		221.1	38	52.0		
		279.1	22	15.8		
Quetiapine-d8	392.2	226.1	38	100.0	172	1.8
		257.7	22	69.2		
		286.1	22	46.7		
7-hydroxy quetiapine	400.2	269.0	18	100.0	172	1.1
		237.1	42	20.9		
		295.0	22	14.2		
7-hydroxy quetiapine-d8	408.2	274.1	22	100.0	196	1.1
		302.1	26	25.9		
		241.1	42	24.6		
7-hydroxy N-desalkyl quetiapine	312.1	226.0	26	100.0	172	1.2
		164.0	62	98.5		
		208.0	38	72.5		
7-hydroxy N-desalkyl quetiapine-d8	320.2	226.0	26	100.0	172	1.2
		164.0	62	79.7		
		208.0	42	45.0		
Risperidone	411.2	191.1	26	100.0	188	1.5
		82.1	66	8.3		
		110.0	54	7.3		
Risperidone-d4	415.3	195.1	26	100.0	188	1.5
		73.2	66	7.4		
		114.1	54	6.8		
Zuclopenthixol	401.2	230.9	38	100.0	188	2.4
		221.0	58	94.2		
		169.0	42	82.8		
Zuclopenthixol-d4	405.2	221.0	58	100.0	188	2.4
		231.0	34	94.9		
		104.1	26	76.8		

** IS used for bromperidol: haloperidol-d4; IS used for levosulpiride: amisulpride-d5*

Table 2: Absolute and internal standard (IS) corrected recovery from the oral fluid matrix (ER_{matrix}) and 95% confidence interval (CI) obtained with 3 ml buffer solution of the Quantisal collection devices and 1 ml of neat oral fluid (n=3) spiked with 'QC low' and 'QC high' concentrations.

	QC low (n=3)				QC high (n=3)			
Analyte	**Mean ER %**	**95% CI**	**Mean ER (IS) %**	**95% CI**	**Mean ER %**	**95% CI**	**Mean ER (IS) %**	**95% CI**
7OH-N-desalkylquetiapine	54.1	21.0-87.2	83.7	30.6-136.8	61.3	53.3-69.2	94.0	83.2-104.9
7OH-quetiapine	60.0	50.8-69.2	91.8	89.2-94.3	62.4	58.8-66.0	88.8	76.8-100.9
Amisulpride	26.2	23.6-28.8	94.3	80.4-108.3	39.2	35.7-42.8	100.0	90.9-109.1
Aripiprazole	60.5	51.1-69.9	85.3	73.1-97.5	68.3	63.5-73.0	84.9	73.8-96.0
Bromperidol	59.6	53.0-66.2	84.4	81.4-87.4	62.3	61.1-63.6	85.1	73.2-96.9
Clozapine	67.7	64.4-71.0	87.8	81.9-93.7	76.7	70.0-83.4	91.9	87.2-96.6
Haloperidol	53.6	46.0-61.1	75.8	68.8-82.8	63.3	60.6-66.0	86.4	73.5-99.3
N-desmethylclozapine	52.2	36.7-67.8	99.6	81.0-118.1	53.6	46.1-61.0	92.1	84.1-100.2
N-desmethylolanzapine	73.0	54.3-91.7	89.1	79.2-99.0	76.6	64.2-89.0	90.2	72.3-108.1
Olanzapine	58.6	42.6-74.5	91.9	68.4-115.3	63.0	56.2-69.8	84.8	74.8-94.7
Paliperidone	56.0	47.7-64.4	82.5	73.2-91.9	68.2	67.0-69.3	88.5	73.7-103.3
Pipamperone	58.1	48.3-67.8	83.8	75.8-91.8	72.2	70.2-74.2	107.4	97.9-116.9
Quetiapine	63.9	56.0-71.9	82.1	71.5-92.8	72.4	71.0-73.8	86.8	83.3-90.3
Reduced haloperidol	63.4	59.0-67.9	75.2	68.9-81.5	86.6	56.1-117.1	92.0	69.6-114.4
Risperidone	56.3	45.2-67.4	83.7	65.3-102.0	62.4	58.4-66.3	88.4	78.4-98.4
Zuclopenthixol	61.8	58.9-64.7	86.6	82.6-90.6	69.3	68.7-70.0	82.7	67.9-97.5

Table 3: Comparison between antipsychotic (AP) oral fluid to serum concentration ratios obtained from patient samples with a neat oral fluid (NOF) volume of > 0.2 ml and samples with a NOF amount of > 0.5ml (indicated with *).

AP	NOF > 200 µl (n)	NOF > 500 µl (n)*	Mean	Mean *	Median	Median *	SD	SD*	Q1	Q1*	Q3	Q3*	Range	Range*	Regression equation	Regression equation*	R^2	R^2*
AMI	10	5	13.4	5.4	6.0	4.8	19.0	3.5	3.7	3.3	12.4	6.4	1.0-68.7	1.0-11.3	y = 5.24x + 377.77°	y = 17.21x - 1603.70	0.68 °	0.94 °
ARI	15	10	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1-0.4	0.1-0.3	y = 0.12x + 16.18	y = 0.11x + 8.78	0.53	0.91
BRO	2	0	-	-	-	-	-	-	-	-	-	-	1.3-4.7	-	-	-	-	-
CLO	15	6	2.8	1.4	1.5	1.3	3.8	0.6	1.0	1.0	2.2	1.6	0.5-15.6	0.6-2.6	y = 1.87x + 197.68	y = 1.21x - 17.96	0.13	0.79
NDM-CLO	15	6	3.7	1.9	2.2	1.9	3.9	0.6	1.6	1.5	2.9	2.3	1.0-14.1	1.0-2.7	y = 3.99x - 27.60	y = 1.77x + 8.96	0.23	0.77
HAL	7	5	6.3	6.3	4.2	4.2	3.9	4.0	3.6	3.7	8.1	6.2	2.4-14.1	3.5-14.1	y = 3.68x + 4.99+	y = 3.51x + 6.45	0.64 +	0.63 +
RHAL	7	5	28.5	17.0	13.5	13.5	33.3	9.5	9.6	11.7	26.0	17.1	7.5-107.3	7.6-34.9	y = 12.24x + 18.83+	y = 11x + 28.67	0.78 +	0.75 +
OLA	20	14	6.4	5.2	3.3	2.6	6.4	4.9	1.7	1.5	10.0	8.3	0.2-21.6	0.2-14.6	y = 0.20x + 128.08	y = 0.96x + 74.43	0.00	0.01
NDM-OLA	19	12	3.9	2.9	2.6	2.0	3.8	3.3	1.4	1.2	4.4	2.9	0.5-13.2	0.5-13.2	y = 0.69x + 15.31	y = 0.25x + 9.42	0.03	0.05
PAL	21	14	1.8	1.8	1.3	1.8	1.4	1.2	0.8	0.8	2.3	2.6	0.2-5.1	0.2-4.3	y = 0.91x + 24.10	y = 1.33x + 12.19	0.14	0.22
PIP	2	1	-	-	-	-	-	-	-	-	-	-	1.3-15.8	-	-	-	-	-
QUE	25	15	1.5	1.4	1.3	1.2	1.0	0.8	0.8	0.7	1.9	1.8	0.3-4.3	0.4-3.0	y = 1.71x - 17.89	y = 0.91x + 21.45	0.49	0.35
7OH-NDA-QUE	24	15	1.9	1.7	1.5	1.2	1.3	1.4	1.1	0.9	2.1	2.0	0.6-6.7	0.6-6.7	y = 4.77x - 17.01	y = 6.42x - 25.03	0.62	0.77
7OH-QUE	23	15	5.4	4.7	4.8	3.9	3.2	2.7	2.3	2.3	7.1	6.5	1.5-13.2	1.5-10.6	y = 8.67x - 15.62	y = 1.11x + 8.30	0.64	0.16
RIS	11	6	2.5	2.9	2.3	2.4	1.9	2.3	1.1	1.2	3.0	3.0	0.9-7.8	0.9-7.8	y = 0.98x + 9.29	y = 1.48x + 6.08	0.45	0.47
9OH-RIS	9"	4"	1.7	1.8	1.4	0.9	1.5	2.0	0.7	0.6	1.8	1.6	0.4-5.6	0.4-5.6	y=0.78x + 13.29	y = 1.05x + 8.47	0.27	0.32
ZUC	4	3	0.7	0.7	0.7	0.9	0.3	0.3	0.5	0.5	0.9	0.9	0.2-1.0	0.2-1.0	y = 0.69x - 0.65+	y = 0.75x - 1.08	0.44 +	0.40 +

° One sample was determined as outlier (serum 213 ng/ml; oral fluid 14 624 ng/ml)
[+] Data are not reliable since only a limited number of concentrations were available.
'' Two patients were taking both risperidone and paliperidone, these data were excluded from the calculations.

Figure 1: Box-Whiskerplots of the comparison between antipsychotic oral fluid to serum concentration ratios obtained from patient samples with a neat oral fluid (NOF) volume of > 0.2 ml and samples with a NOF amount of > 0.5ml (indicated with *). The boxes represent the median, 25 and 75% percentiles, the whiskers represent the range. Graph A gives an overview of the calculated plots, while graph B is a focused on the lower oral fluid to serum ratios.