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29 **The use of dried blood spots for quantification of 15 antipsychotics and 7 metabolites with**
30 **ultra-high performance liquid chromatography – tandem mass spectrometry**

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63 **Abbreviations**

64 7-hydroxy-N-desalkyl-quetiapine, 7OH-NDA-QUE; 7-hydroxy-quetiapine, 7OH-QUE;
65 amisulpride, AMI; antipsychotic, AP; aripiprazole, ARI; asenapine, ASE; bromperidol, BRO;
66 clozapine, CLO; dehydro-aripiprazole, DARI; dried blood spot, DBS; dynamic multiple-
67 reaction monitoring, dMRM; electrospray ionisation, ESI; haloperidol, HAL; hematocrit, HCT;
68 hydroxy-iloperidone, HILO; iloperidone, ILO; internal standard, IS; levosulpiride, LSUL; liquid
69 chromatography, LC; lower limit of quantitation, LLOQ; lurasidone, LUR; methyl *tert*-butyl
70 ether, MTBE; mass spectrometry, MS; multiple-reaction monitoring, MRM; N-demethyl-
71 clozapine, NDM-CLO; N-demethyl-olanzapine, NDM-OLA; olanzapine, OLA; paliperidone,
72 PAL; pipamperone, PIP; quetiapine, QUE; reduced haloperidol, RHAL; retention time, RT;
73 risperidone, RIS; sertindole, SER; stable isotope labeled internal standard, SIL-IS; therapeutic
74 drug monitoring, TDM; ultra-high performance liquid chromatography-tandem mass
75 spectrometry, UHPLC-MS/MS; zuclopenthixol, ZUC;

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87 **1. Introduction**

88 Antipsychotics (APs) are used for treatment of psychotic symptoms in patients with
89 schizophrenic, schizophreniform, schizoaffective, psycho-organic and bipolar disorders [1-4].

90 A combination of psychotherapy and pharmacotherapy can improve symptoms significantly.

91 However, monitoring of these APs in serum or plasma is often recommended. Therapeutic

92 drug monitoring (TDM) can aid in finding the right therapy, explaining non-response,

93 pharmacokinetic interactions or poor response [5, 6].

94 Especially in psychiatric populations, classical venous blood sampling is often experienced as

95 unpleasant and even frightening. The interest in alternative sampling techniques, like dried

96 blood spots (DBS), has consequently increased. DBS sampling is a micro-sampling technique

97 where a drop of capillary blood is spotted on special filter paper. This technique has been

98 used routinely since the 1960s, when Guthrie described a method to detect phenylketonuria

99 in newborns [7]. DBS sampling has a lot of advantages, including ease of sampling, less

100 invasive and inexpensive sampling, transport and storage [8, 9]. Since only very small

101 volumes of blood are collected (typically between 10 and 80 μ l), interest in DBS methods has

102 increased in the last two decades due to the availability of more sensitive analytical

103 techniques [8, 10, 11].

104 Interest in DBS sampling for TDM has recently increased [11, 12]. DBS methods have been

105 described for monitoring of e.g. antidepressants, antiretroviral drugs, antibiotics,

106 antiepileptic drugs, chemotherapeutic agents, antimycotics and immunosuppressants [12].

107 Some DBS methods for multiple drugs including one or two APs are already published [13-

108 15]. No analytical method for determination of multiple AP using DBS has been reported yet.

109 Analyzing multiple APs in one method makes it possible to monitor polymedicated patients
110 and to analyze clinical samples of different patients containing any of these APs in one batch.
111 We aimed to develop a fast and easy to perform DBS method for quantification of 16 APs
112 and 8 metabolites using a highly sensitive ultra-high performance liquid chromatography-
113 tandem mass spectrometry (UHPLC-MS/MS) technique. Selection of these 16 APs was based
114 on their importance on the worldwide market and includes the newer APs like asenapine,
115 iloperidone and lurasidone. Metabolites showing pharmacological activity or helping in
116 interpretation of TDM data were also selected. Since paliperidone (9-hydroxy-risperidone) is
117 both a parent compound and a metabolite of risperidone, the total amount of compounds
118 included in the method is 23. Except for bromperidol and levosulpiride, deuterated internal
119 standard (IS) were used for each individual compound [16].

120

121 **2. Experimental**

122 **2.1 Chemicals and reagents**

123 7-hydroxy-N-desalkyl-quetiapine dihydrochloride (7OH-NDA-QUE), 7-hydroxy-quetiapine
124 (7OH-QUE), amisulpride (AMI), aripiprazole (ARI), asenapine (ASE), bromperidol (BRO),
125 clozapine (CLO), dehydro-aripiprazole hydrochloride (DARI), haloperidol (HAL), hydroxy-
126 iloperidone (HILO), iloperidone (ILO), levosulpiride (LSUL), lurasidone hydrochloride (LUR), N-
127 demethyl-clozapine (NDM-CLO), N-demethyl-olanzapine (NDM-OLA), olanzapine (OLA),
128 paliperidone (PAL), pipamperone dihydrochloride (PIP), quetiapine hemifumarate (QUE),
129 reduced haloperidol (RHAL), risperidone (RIS), sertindole (SER) and zuclopenthixol succinate
130 salt (ZUC) were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada).
131 The stable isotope labelled internal standards (SIL-IS) 7OH-NDA-QUE-d₈ dihydrochloride,
132 7OH-QUE-d₈, AMI-d₅, ARI-d₅, ASE-¹³C₃, CLO-d₈, DARI-d₈ hydrochloride, HAL-d₄, HILO-d₄,

133 ILO-d₃, LUR-d₈ hydrochloride, NDM-CLO-d₈, NDM-OLA-d₈, OLA-d₈, PAL-d₄, PIP-d₁₀
134 dihydrochloride, QUE-d₈ fumarate, RHAL-d₄, RIS-d₄, SER-d₄ and ZUC-d₄ succinate salt were
135 also purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada).
136 Acetonitrile, acetic acid, formic acid, methanol and methyl *tert*-butyl ether (ethanol
137 stabilized) (MTBE) were from Merck (Darmstadt, Germany). All chemicals were of LC quality.

138

139 **2.2 Standards**

140 Methanolic stock solutions of 7OH-NDA-QUE, 7OH-QUE, AMI, ASE, BRO, HAL, RHAL, LUR,
141 LSUL, PIP, QUE, SER and ZUC were prepared at a concentration of 1 mg/ml. ARI, CLO, DARI,
142 HILO, ILO, NDM-CLO, NDM-OLA, OLA, PAL and RIS stock solutions were prepared in
143 acetonitrile at a concentration of 1 mg/ml. Working solutions of each analyte (100, 10 and
144 1 µg/ml) were prepared by further dilution of the stock solutions with acetonitrile.
145 Methanolic stock solutions of 7OH-NDA-QUE-d₈, 7OH-QUE-d₈, AMI-d₅, ASE-¹³C₃, HAL-d₄,
146 RHAL-d₄, LUR-d₈, PIP-d₁₀, QUE-d₈, SER-d₄ and ZUC-d₄ were prepared at a concentration of
147 100 µg/ml. ARI-d₈, CLO-d₈, DARI-d₈, HILO-d₄, ILO-d₃, NDM-CLO-d₈, NDM-OLA-d₈, OLA-d₈, PAL-
148 d₄ and RIS-d₄ stock solutions were prepared in acetonitrile at a concentration of 100 µg/ml.
149 A working solution containing a mixture of all SIL-IS was prepared in acetonitrile by dilution
150 of the stock solutions. The final concentration of the deuterated compounds ranged
151 between 5 and 150 ng/ml, i.e. in the range of calibration level 3 or level 4 of the non-
152 deuterated compounds.

153 The calibration standards consisted of a mixture of the working solutions containing the 23
154 analytes at 7 concentration levels. The chosen calibration ranges cover both the defined
155 therapeutic ranges and the suprathreshold concentrations [5]. The quality control (QC)
156 standards were also prepared as a mixture from the different working solutions at 3

157 concentration levels (QC low, QC mid and QC high). All solutions were stored at -20°C.
158 Twenty µl of the calibration and QC standards were spiked to 180 µl of human whole blood
159 to yield final concentrations as shown in table 1. Although we realize that addition of 10 %
160 organic solvent to blood is suboptimal and may affect blood properties to some extent, this
161 was not problematic as our procedure utilizes complete spots for analysis.

162

163 **2.3 DBS sample collection**

164 As known, the hematocrit (HCT) is identified as the single most important parameter
165 influencing the spread of blood on DBS cards, affecting the spot formation, spot size, drying
166 time, homogeneity, the robustness and reproducibility of these assays [8, 17, 18]. In order to
167 overcome this problem, it was decided to analyze the entire spot instead of using discs
168 generated by punching only a part of the DBS. Hence, all issues concerning the spreading of
169 blood could be avoided [19]. However, the influence of the HCT on other parameters, like
170 matrix effects (ME), remained to be investigated [8].

171 Patient DBS samples were collected as follows: the preferred finger was disinfected with a
172 70% isopropanol cloth, air-dried and warmed for a few minutes. With the help of a single-
173 use automatic lancet (Accu-Chek® Safe-T-Pro Plus, Roche Diagnostics, Mannheim, Germany),
174 the fingertip was pricked. The first drop of blood was wiped off, since it contains an
175 important amount of tissue fluid [12]. The second drop was collected in a 25-µl precision
176 capillary 'end to end' (Hirschmann Laborgeräte, Eberstadt, Germany). Once entirely filled,
177 the capillary was placed in the center of a marked circle on FTA™ DMPK-C Cards (GE
178 Healthcare, Freiburg, Germany) until the capillary was completely emptied.

179

180 **2.4 Sample preparation and extraction**

181 Twenty-five μl of whole blood was spotted onto DMPK-C cards. In line with commonly
182 applied drying times described in literature [12, 19], these were left to dry for at least 3h at
183 room temperature. The whole DBS was excised and collected into a 2-ml Eppendorf tube
184 (Eppendorf AG, Hamburg, Germany). Extraction was performed by adding 450 μl of
185 methanol, 150 μl of methyl *tert*-butyl ether (MTBE) and 12.5 μl of the IS working solution.
186 After shaking the samples during 5 min on an Eppendorf MixMate (Eppendorf AG), the
187 extract was transferred to a 1.5-ml Eppendorf tube and evaporated to dryness under a
188 gentle stream of nitrogen at 40°C. The samples were reconstituted in 50 μl of aqueous
189 ammonium acetate (10 mM)/acetonitrile (9:1; v/v), vortexed for 30 s and centrifuged for 2
190 min at 10 000 x *g*. After transferring the extracts to an autosampler vial, a volume of 10 μl
191 was injected into the UHPLC-MS/MS system.

192

193 **2.5 Instrumentation and analytical method**

194 Samples were analyzed on an Agilent 1290 Infinity LC system (Agilent Technologies, Santa-
195 Clara, California, U.S.A.) coupled with an Agilent 6460 Triple Quadrupole mass spectrometer
196 (MS) run in Jetstream® electrospray ionization (ESI) mode. Data were acquired and analyzed
197 with Masshunter Workstation Software version B.06.00 (Agilent Technologies). The LC
198 system was optimized for rapid resolution.

199 Separation was achieved using an Agilent SB C₁₈ reversed phase column (2.1 x 50 mm, 1.7
200 μm) (Agilent Technologies) with column oven temperature at 40°C. Gradient elution was
201 performed at a flow rate of 0.5 ml/min with 10 mM aqueous ammonium acetate at pH 3.7
202 (A) and acetonitrile (B) using the following program: starting conditions 10 % B; increase to
203 75 % B between 0 and 2.5 min; further increase to 95 % B between 2.5 and 3 min; retain 95%
204 B between 3 and 4.5 min; go back to initial conditions from 4.6 to 6.

205 The MS conditions were optimized as follows: Jetstream ESI technology, positive mode,
206 nebulizer gas: nitrogen, sheat gas temperature: 400°C, sheat gas flow: 12 L/min, nebulizer
207 pressure: 50 psi, capillary voltage: 3000 V, and nozzle voltage: 0 V.

208 The MS was operated in dynamic multiple-reaction monitoring (dMRM) mode, monitoring 3
209 ion transitions for each analyte in their specific retention time (RT) window ($RT \pm 0.25$ min).

210 The mass spectrometric conditions for each analyte are identical those of a previous method
211 for simultaneous determination of the same 16 APs and 8 metabolites in serum
212 (Supplemental data table 1) [16].

213

214 **2.6 Validation**

215 The following validation parameters were investigated according to in-house guidelines,
216 which are based upon the international guidelines of EMA (European Medicines Agency) and
217 FDA (Food and Drug Administration)[20, 21], modified by specific recommendations for
218 forensic and clinical toxicology [22, 23]: selectivity, linearity, precision, accuracy, recovery,
219 matrix effects, stability and incurred sample reanalysis.

220 Selectivity was evaluated by analyzing blank blood from eight different sources, two zero
221 samples (blank blood + mix of SIL-IS) and two samples spiked with only analytes and no SIL-
222 IS. Carryover was tested by injecting the highest concentration of the calibration curve,
223 followed by two blank injections and should not exceed 20 % of the lower limit of
224 quantitation (LLOQ) (n=2).

225 A seven-point calibration curve was analyzed using IS-corrected areas on each of four
226 consecutive days. At each of these four days, duplicates of QC samples at LLOQ, low,
227 medium and high concentration levels were analyzed. Intra-, interday-precision and accuracy
228 were determined using an ANOVA-calculation as described by Wille et al. [23] Accuracy and

229 precision data were acceptable when the % bias respectively % coefficient of variation (%
230 CV) was lower than 15 % (20 % for LLOQ).

231 Extraction recovery (ER) and matrix effects (ME) were determined at two concentration
232 levels (QC low and QC high) using whole blank blood from six volunteers, spiked before and
233 after extraction according to the post-extraction addition technique as described by
234 Matuszewski et al [24]. ME are calculated as the percent ratio of peak areas of the analytes
235 spiked after extraction and the blood free solution prepared in acetonitrile. ME were also
236 evaluated at low and high HCT (19.2 and 67.0 %, respectively). Relative ME were calculated
237 as the percent ratio of the IS corrected peak areas of the analytes spiked after extraction and
238 the blood free solution. ER is calculated as the percent ratio of the IS corrected peak areas of
239 the analytes spiked before and after extraction. % CV of the relative ME should not exceed
240 15 %.

241 Stability was evaluated at QC low and QC high (n=3) after 1 day, 1 week and 1 month at
242 different storage conditions (room temperature, 4°C and -18°C). DBS were stored in zip-
243 closure bags with desiccant. Finally, stability of the processed samples on the autosampler
244 was determined by analyzing the extracts of QC low and QC high (n=3) after 6 and 12 h,
245 respectively. Concentrations of all stability samples were calculated based on the daily
246 calibration curves. The concentration of the stability samples had to be within 90-110 % of
247 the mean of that of the control samples, and the 90 % confidence interval (CI) of the stability
248 samples had to be within 80-120 % of the mean concentration of the control samples [22,
249 23].

250 Incurred sample reanalysis was performed on DBS samples of 20 different patients with a
251 time interval of 1 to 3 months between initial analysis and reanalysis. Data were acceptable

252 when the % difference between the results was within ± 20 % of their mean for two-thirds of
253 the samples [20].

254

255 **3. Results & discussion**

256 **3.1 Filter paper selection, extraction procedure and detection**

257 Two types of filter paper were tested, Whatman 903 paper (GE Healthcare, Freiburg,
258 Germany) and Whatman FTATM DMPK-C Cards (GE Healthcare). Both filter papers are
259 cellulose cards not containing protein denaturing agents, which are known to cause ME [18].
260 After extraction of samples on Whatman 903 paper, higher matrix effects were seen in
261 comparison with the DMPK-C cards. Consequently, DMPK-C cards were selected for further
262 method development.

263 Different extraction solvents were evaluated: methanol, acetonitrile and MTBE, as well as
264 mixtures of these solvents: methanol: acetonitrile at 1:1 (v/v), 3:1 (v/v) and 1:3 (v/v) and
265 methanol: MTBE at 1:1 (v/v), 3:1 (v/v) and 1:3 (v/v). In literature, methanol, acetonitrile or
266 mixtures of both are preferable for extraction because they cause protein denaturation and
267 precipitation [12, 25]. Acetonitrile would yield a higher recovery and less matrix effects than
268 methanol [9]. MTBE was also tested since it was selected as optimal extraction solvent for
269 our serum method [16]. Highest recoveries were achieved with a 600 μ l methanol: MTBE
270 (3:1, v/v). Water had to be avoided as extraction solvent, since it is known to cause stability
271 problems for olanzapine [16, 26, 27] and it increases the interference from endogenous
272 compounds [12, 25].

273 Chromatographic conditions and MS parameters were adopted from our serum method for
274 quantification of APs [16]. Only the source parameters of the MS had to be reevaluated in

275 order to increase sensitivity of the MS. Since absolute amounts in DBS are lower than in
276 serum, optimal sensitivity of the detector is mandatory.

277

278 **3.2 Validation experiments**

279 Analysis of eight different blank samples revealed no interference from endogenous
280 compounds nor from filter paper components. The response was less than 20 % of the LLOQ
281 at the mass transitions of the APs and less than 5 % of the response of the IS. For zero
282 samples, the response of the IS was less than 20 % of LLOQ at the transitions of APs. The
283 method proved to be highly selective. No carryover was observed. Injecting blanks right after
284 the highest calibrators yielded signals lower than 20 % of the LLOQ for all compounds.
285 Linearity was evaluated by analyzing four calibration curves on four consecutive days.
286 Unweighted and 1/x weighted linear regression were statistically and visually evaluated.
287 Inclusion of the zero value in the 95 % CI of the y-intercept indicates absence of constant
288 error, and a correlation coefficient of 0.99 or higher was pursued. If a linear curve without
289 1/x weighting would be used, standard error estimation would be biased. For all compounds,
290 linear regression (1/x weighting) provided the best fit, with r^2 of 0.99 or higher for 16 of the
291 23 analytes and a zero value in the 95 % CI for all compounds except for NDM-OLA. In
292 comparison, unweighted linear regression resulted in an r^2 higher than 0.99 for only 9 of the
293 23 compounds and a zero value in the 95 % CI for only 20 of the 23 analytes.

294 Heteroscedasticity was proven for all compounds by plotting residuals versus nominal
295 concentrations. Consequently, 1/x weighted linear regression was applied for all
296 compounds. Accuracy was evaluated using EMA criteria, which state that the back-calculated
297 concentration should be within 15 % of the nominal value (20 % at LLOQ). This was fulfilled
298 for all compounds, except for OLA and NDM-OLA and for PIP at LLOQ. All calibration curves

299 were linear in the proposed range, except for OLA and its metabolite NDM-OLA. These
300 compounds were not detected at the lowest concentration of the calibration curve and
301 back-calculated concentrations were often aberrant. The underlying cause likely is the
302 instability of these compounds in aqueous medium, since the extract was reconstituted in
303 aqueous ammonium acetate [16, 26, 27]. As a result, OLA and NDM-OLA were excluded from
304 further analysis.

305 On the other hand, if a mean calibration curve is generated from all calibration curves,
306 aberrancies are seen for LUR. As a consequence, daily calibration is necessary for LUR. The
307 LLOQ, defined as the lowest concentration of the calibration curve, could be accepted for all
308 analytes, except for PIP. This implicates that PIP concentrations lower than 50 ng/ml (a
309 subtherapeutic concentration) cannot be quantified reliably.

310 Precision and accuracy were determined at four concentration levels (LLOQ, QC low, QC mid
311 and QC high), analyzed in duplicate on four consecutive days. Accuracy (% bias), intraday
312 precision (repeatability) and interday precision (intermediate precision) were calculated
313 from data obtained with ANOVA-analysis (Table 2) [23]. Except for PIP at LLOQ and QC low
314 and for LUR at QC low, all accuracy data were within the acceptance criteria (bias \leq 15%, for
315 LLOQ \leq 20%). Both intraday and interday precision are acceptable when the CV (%) is lower
316 than 15 % (20 % for LLOQ). Only for LUR, aberrancies were seen for both intraday and
317 interday precision at QC low and QC mid. EMA criteria were fulfilled for all other
318 compounds.

319 An overview of all ME and ER is given in Table 3. The ER (IS corrected) varied between 28.7 %
320 for 7OH-NDA-QUE and 84.5 % for PIP, with a median ER of 66.4 % for all compounds. Due to
321 the use of a more apolar extraction solvent, ER was limited but sufficiently high for this
322 method. The absolute median ME was 66.1 % (range 8.8 to 100.4 %). Ion suppression is seen

323 when ME are below 100 %, ion enhancement when ME are above 100 %. For most
324 compounds, a significant amount of ME is seen with the DBS method. However, these ME
325 are almost completely compensated by the use of SIL-IS (Supplemental data Figure 1). When
326 calculating the IS corrected ME, a median ME of 98.8 % (range 86.2 to 125.8 %) was
327 obtained. For all compounds, CV (%) of the IS corrected ME was lower than 8.5 % and
328 fulfilled the criteria (< 15 %). Besides the DBS, also filter paper may contribute to high ME.
329 Moreover, both methanol and MTBE are known to extract endogenous blood lipids and
330 induce a high ME too. Polar lipids like glycerophosphocholines are soluble in methanol, while
331 non-polar lipids like cholesterol, cholesterol esters and triacylglycerols are soluble in MTBE.
332 Presence of lipids in the extract can have an influence on sensitivity, selectivity and
333 reproducibility of results. Lipids would be less extracted when using acetonitrile [9].
334 Consequently, high ME are expected when using a combination of both methanol and MTBE.
335 However, when acetonitrile was used, lower recoveries were seen and ME were comparable
336 with methanol.

337 ER and ME values can be influenced by the HCT. HCT levels are normally about 41-50 % for
338 men and 36-44 % for women [8, 28]. Since ER and ME were tested on blood samples of six
339 different volunteers, the HCT of these volunteers was determined and were found within
340 this normal range. According to literature, a range of 28-67 % HCT would cover the majority
341 of adult human blood samples [18]. Therefore, ME were also tested on samples with low
342 (19.2 %) and high HCT (67.0 %). Adjustment of HCT was made by removing or adding plasma
343 to a whole blood sample of one of the volunteers. No difference was seen between the
344 calculated ME at low, normal and high HCT (Supplemental data Figure 2).

345 Stability experiments were conducted in a way that represents the actual storage conditions
346 and handling of the samples during the study. Stability was evaluated when storing DBS

347 samples during 1 day, 1 week and 1 month at different storage temperatures (room
348 temperature, 4°C and -18°C). Results were compared with samples analyzed directly after
349 drying for 3 h at room temperature (Table 4). All compounds were stable during 1 month at
350 the 3 storage temperatures. The stability studies revealed no difference between storage at
351 room temperature, 4°C and -18°C.

352 Stability of the extracted samples was also evaluated. Extracted samples were measured
353 after 6 and 12 h while residing in the autosampler at room temperature. The acceptance
354 criteria were not fulfilled for DARI, HILO, LSUL, LUR and ZUC at QC low and for 7-OH-NDA-
355 QUE and LUR at QC high. For 7-OH-NDA-QUE, LSUL and HILO, the deviation from the ratio of
356 means could be accepted, while the 90 % CI did not meet the criteria. Only for LUR, both the
357 deviation from the ratio of means and the 90 % CI did not meet the criteria. In Figure 1, a
358 decrease in concentration is only seen for LUR at both QC low and QC high. After 12 h, the
359 concentration of LUR was decreased with 32.3 % at QC low and 25.2 % at QC high. Since LUR
360 is not stable in the processed sample, this can explain the aberrancies in linearity, precision
361 and accuracy. The longer it takes for an extracted sample to be analyzed on the instrument,
362 the lower the concentration of LUR will be.

363 Capillary DBS samples from 20 psychiatric patients were reanalyzed within 1 to 3 months
364 after the initial analysis. These 20 capillary samples contained 52 different antipsychotics
365 and metabolites. The criterium for incurred sample reanalysis was fulfilled, since the %
366 difference between the results was within 20 % of their mean for all compounds in all
367 samples, except for 7-OH-NDA-QUE.

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369

370

371 **3.3 Clinical application**

372 Applicability of the DBS method was evaluated on samples, collected from patients with
373 schizophrenia or bipolar disorder, within the framework of a large clinical study. Patients had
374 to be in 'steady-state' condition (reached after 5-7 half-lives of the drug), which was
375 translated as an unchanged dose of the antipsychotic in the last 7 days before sample
376 collection. From every patient, we collected capillary DBS samples, as well as serum and
377 whole blood just prior to the morning dose of the AP (trough or minimal concentration). In
378 this study, overall, 10 of the antipsychotics and 6 of their metabolites could be quantified in
379 the DBS samples. Figure 2 illustrates 4 chromatograms of capillary DBS samples from 4
380 patients treated with one or more AP(s). Patient A was treated with aripiprazole (20
381 mg/day), patient B with both amisulpride (400 mg/day) and clozapine (500 mg/day), patient
382 C with risperidone I.V. (62.5 mg/2 ml every 14 days) and patient D was treated with
383 amisulpride (200 mg/day) and quetiapine (1400 mg/day). As can be seen in Figure 2, all
384 antipsychotics were found in the DBS samples from these patients. Table 5 shows data for 10
385 antipsychotics, quantified in serum, blood and DBS from 10 different patients.
386 Concentrations found in blood and DBS are quite similar. For some compounds,
387 concentrations in serum differ more, since they are influenced by the blood:plasma(serum)
388 distribution of the antipsychotics. Obviously, a large dataset, with sufficient coverage of each
389 of the antipsychotics, is required to confirm the correlation between the different matrices,
390 together with the clinical interpretation of the results.

391

392 **4. Conclusion**

393 The UHPLC-MS/MS method for analysis of APs and metabolites using DBS overall meets the
394 requirements of both FDA and EMA for 15 out of the 16 APs and 7 out of the 8 metabolites

395 selected [20, 21]. Only OLA and NDM-OLA were rejected from the method, likely owing to
396 their instability in the aqueous reconstitution solvent. Since LUR was not stable in the
397 extracted DBS sample, analysis by UHPLC-MS/MS must be performed as quickly as possible
398 for this compound. The short run time of our method (6 min) is highly beneficial in this
399 respect. This DBS method has high potential in TDM of APs and can be a valuable alternative
400 to the classic venous blood withdrawal currently used for monitoring.

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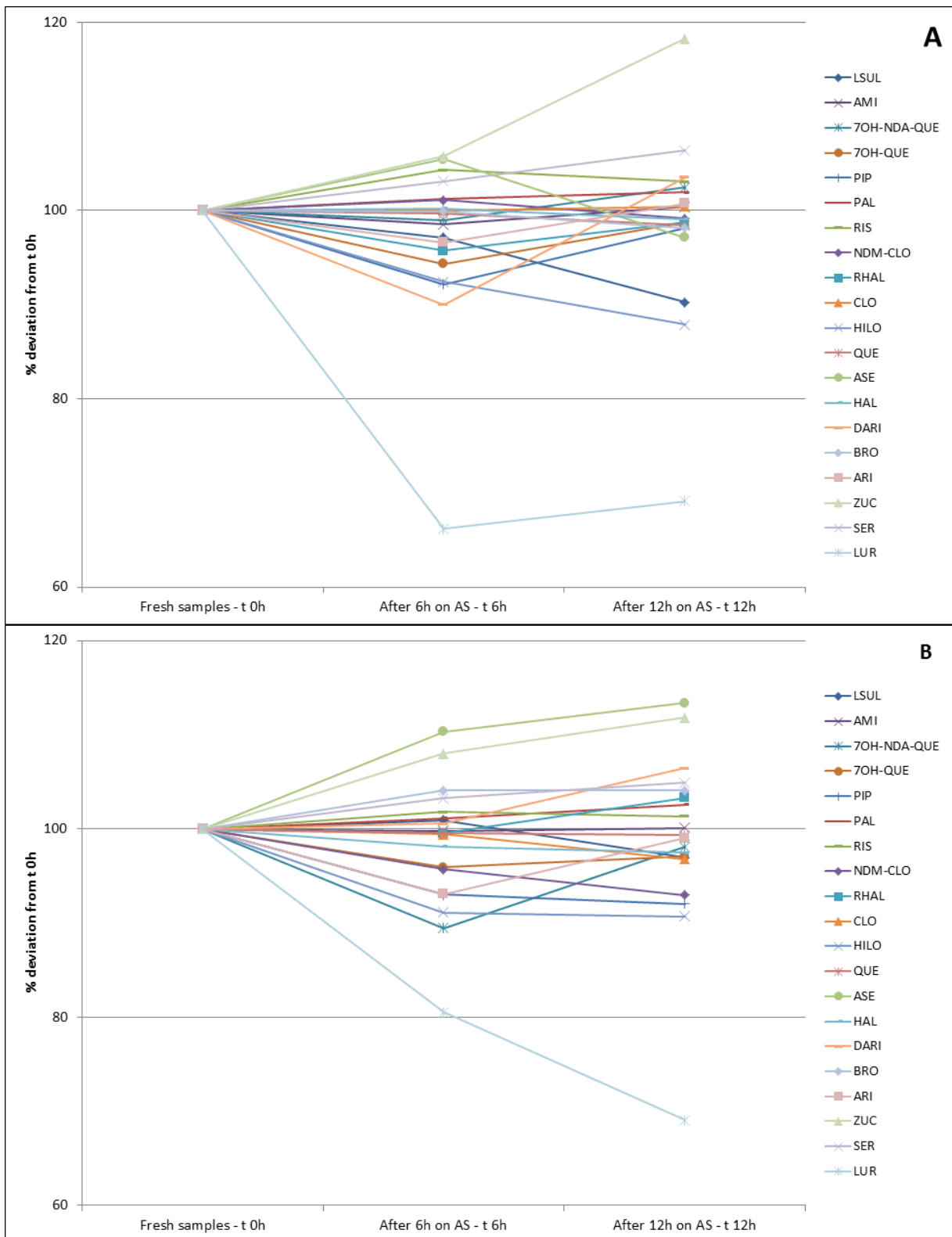
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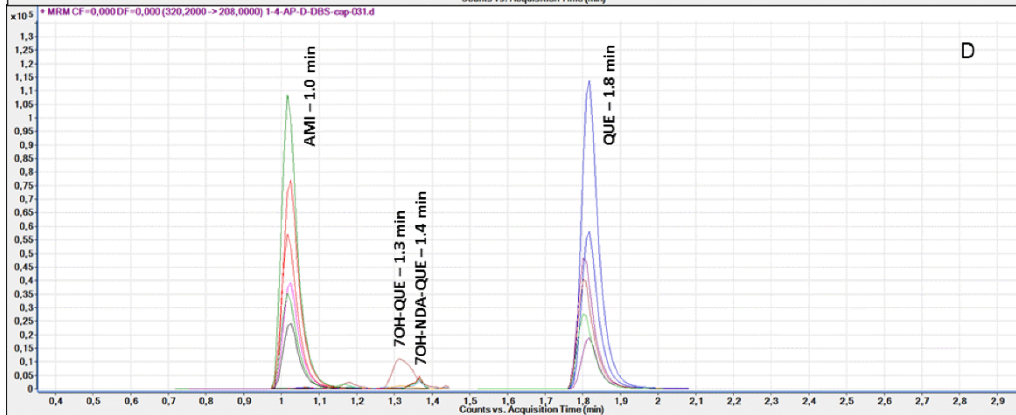
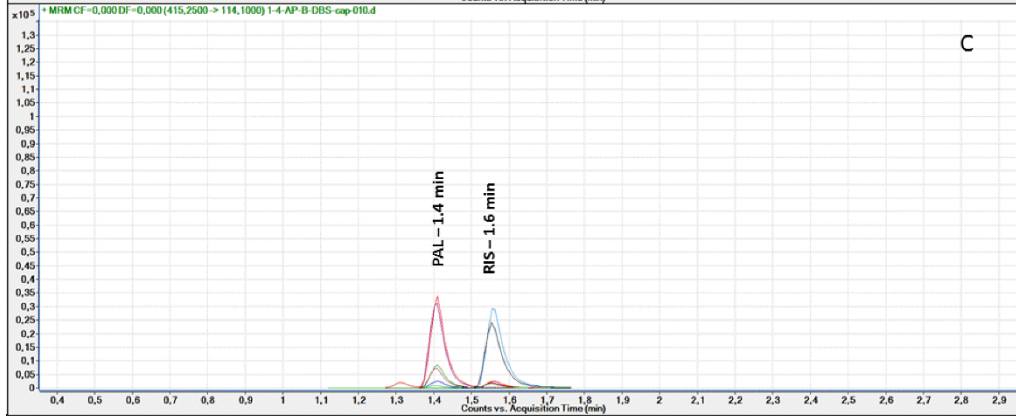
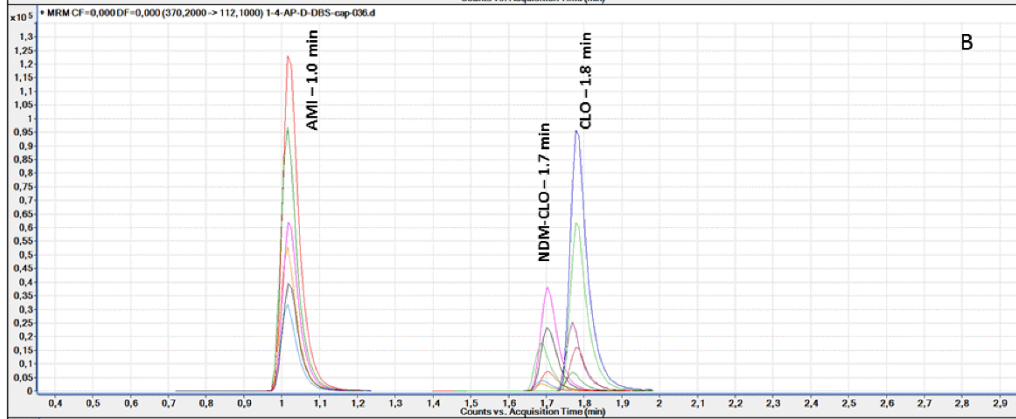
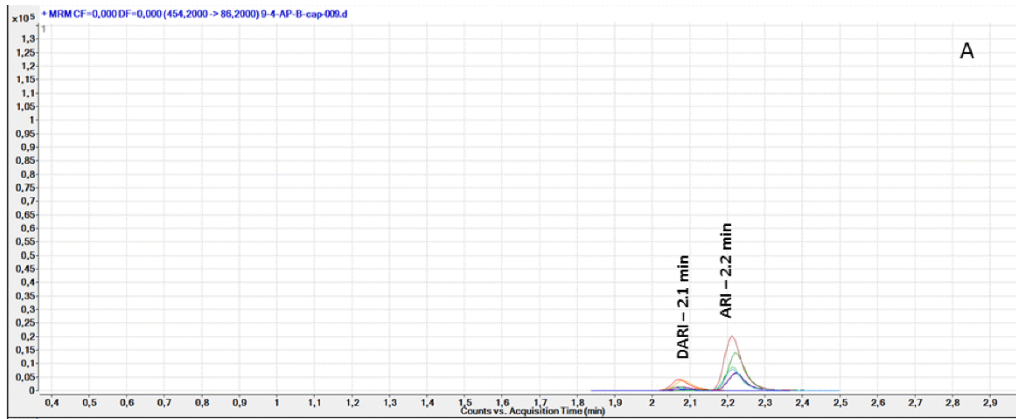
506 **Figure 1** : Stability of the extracted dried blood spot samples, spiked with QC low (A) and QC high (B), while residing on the autosampler during 12 h (n=4). Stability was calculated as
 507 high (B), while residing on the autosampler during 12 h (n=4). Stability was calculated as
 508 deviation from fresh samples.



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511 **Figure 2:** Representative chromatograms of capillary DBS samples obtained from 4 patients
512 treated with multiple APs. MRM transitions of all antipsychotics and their deuterated IS are
513 presented. (A) ARI and DARI (found concentrations were 184 and 180 ng/ml, respectively);
514 (B) AMI, CLO and NDM-CLO (found concentrations were 270, 457 and 449 ng/ml,
515 respectively); (C) PAL and RIS (found concentrations were 12 and 16 ng/ml, respectively); (D)
516 AMI, QUE, 7OH-NDA-QUE and 7OH-QUE (found concentrations were 147, 152, 14 and 13
517 ng/ml, respectively).



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522 **Table 1:** Dried blood spot concentrations of calibration standards and quality control

523 samples of all analytes

Analyte	Abbreviation	Calibrations standards (ng/ml)							Internal quality control samples (ng/ml)		
		L1	L2	L3	L4	L5	L6	L7	QC low	QC med	QC high
Haloperidol	HAL	0.5	1	2.5	5	10	25	50	1.5	7.5	35
Reduced haloperidol	RHAL	0.5	1	2.5	5	10	25	50	1.5	7.5	35
Iloperidone	ILO	0.5	1	2.5	5	10	25	50	1.5	7.5	35
Hydroxy iloperidone	HILO	0.5	1	2.5	5	10	25	50	1.5	7.5	35
Asenapine	ASE	1	2.5	5	10	25	50	100	3	20	75
Bromperidol	BRO	1	5	10	15	30	60	100	3	25	80
7-hydroxy quetiapine	7OH-QUE	1	5	10	15	30	60	100	3	25	80
7-hydroxy N-desalkyl quetiapine	7OH-NDA- QUE	1	5	10	15	30	60	100	3	25	80
N-demethylolanzapine	NDM-OLA	1	5	10	15	30	60	100	3	25	80
Risperidone	RIS	1	5	10	25	50	75	150	3	35	100
Zuclopenthixol	ZUC	1	5	10	50	100	150	300	3	75	225
Paliperidone	PAL	1	10	25	50	100	150	300	3	75	225
Olanzapine	OLA	1	10	25	50	100	150	300	3	75	225
Sertindole	SER	5	10	50	100	150	200	400	15	125	300
Lurasidone	LUR	5	25	50	100	250	500	1000	15	200	750
Pipamperone	PIP	10	50	150	300	500	750	1000	30	400	850
Dehydro-aripiprazole	DARI	10	50	100	300	500	750	1000	30	350	850
Amisulpride	AMI	10	50	100	150	300	600	1200	30	250	900
N-demethylclozapine	NDM-CLO	10	50	100	200	500	750	1500	30	350	1150
Quetiapine	QUE	10	50	250	500	750	1000	1500	30	650	1250
Aripiprazole	ARI	20	50	250	500	750	1000	1500	60	650	1250
Clozapine	CLO	50	100	250	500	750	1000	1500	150	650	1250
(Levo)sulpiride	LSUL	50	100	250	500	750	1000	1500	150	650	1250

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Table 2: Accuracy and precision data for all analytes at four concentration levels.

Analyte	LLOQ			QC low			QC mid			QC high		
	Precision intraday	Precision interday	Accuracy bias (%)	Precision intraday	Precision interday	Accuracy bias (%)	Precision intraday	Precision interday	Accuracy bias (%)	Precision intraday	Precision interday	Accuracy bias (%)
	CV (%)	CV (%)		CV (%)	CV (%)		CV (%)	CV (%)		CV (%)		
7OH quetiapine	7.77	7.77	8.25	8.00	8.00	-8.75	4.55	5.78	-2.1	7.07	7.76	0.80
7OH-N-desalkyl quetiapine	9.29	12.01	16.13	6.75	8.04	-3.04	11.04	11.05	-5.75	10.47	11.48	12.8
Amisulpride	3.53	3.95	1.00	3.72	4.40	1.54	5.40	5.40	-5.78	4.91	4.95	3.76
Aripiprazole	4.01	4.65	4.75	3.93	4.88	-0.69	7.50	7.50	-5.89	2.94	4.09	-1.61
Asenapine	9.24	10.75	11.63	6.37	7.22	-5.25	8.06	8.37	-5.81	2.98	3.87	-2.07
Bromperidol	2.95	5.89	-2.50	4.80	5.20	-1.96	7.70	7.70	-1.80	2.55	3.60	-0.73
Clozapine	6.01	6.46	-2.60	3.85	4.06	9.47	5.39	5.39	-7.02	6.13	6.98	2.02
Dehydro- aripiprazole	5.39	5.96	10.25	3.93	4.80	6.71	5.58	5.58	-7.00	4.06	4.39	2.79
Haloperidol	6.56	7.40	2.75	6.02	6.67	1.92	11.22	11.22	-3.60	3.34	4.30	-1.25
Hydroxy-iloperidone	6.18	6.76	8.50	5.72	5.72	4.17	6.31	6.66	-6.53	2.76	4.49	-0.68
Iloperidone	10.63	10.63	6.25	5.49	5.60	3.58	4.16	4.16	-3.83	7.20	7.20	3.10
Levosulpiride	6.87	6.87	-2.47	3.10	3.21	7.29	5.75	5.75	-5.78	3.02	3.92	3.60
Lurasidone	14.24	16.23	14.70	15.61	16.32	17.33	16.24	16.24	-6.54	12.75	12.75	-3.64
Norclozapine	5.00	5.00	4.50	3.92	4.05	5.63	8.11	8.11	-7.01	4.35	4.35	0.32
Paliperidone	4.50	5.35	9.62	3.57	4.14	0.37	7.33	7.33	-5.75	2.36	3.18	1.79
Pipamperone	18.91	18.91	-20.25	6.79	7.38	15.58	6.69	7.18	2.41	7.77	8.12	-13.15
Quetiapine	5.03	6.42	6.88	3.47	3.77	0.71	6.46	6.46	-5.03	2.95	3.41	1.78
Reduced haloperidol	4.03	5.66	3.75	6.79	7.04	4.67	11.02	10.40	-3.97	5.03	5.03	-3.79
Risperidone	5.80	5.80	5.13	3.28	4.04	2.08	8.91	8.91	-6.93	3.60	4.30	-0.17
Sertindol	4.18	4.35	12.78	5.38	5.62	1.83	9.98	9.98	-5.08	5.90	6.20	1.10
Zuclopenthixol	7.51	8.76	-6.50	7.14	7.21	-4.50	8.71	8.71	-7.00	2.92	3.18	-3.36

Table 3: Recoveries, matrix effects and their respective 95% confidence intervals (CI) obtained with dried blood spot samples of different sources (n = 6) spiked with 'QC low' and 'QC high' concentrations. The CV of the internal standard (IS) corrected matrix effects were < 15 % for all compounds.

Analyte	Recovery (n = 6)				Matrix effects (n=6)				IS corrected matrix effects (n=6)					
	QC low		QC high		QC low		QC high		QC low		CV	QC high		CV
	mean (median) (%)	95% CI	mean (median) (%)	95% CI	mean (median) (%)	95% CI	mean (median) (%)	95% CI	mean (median) (%)	95% CI		mean (median) (%)	95% CI	
7-OH-N-desalkylquetiapine	33.2 (32.6)	28.0 - 38.4	28.7 (27.4)	25.4 - 32.0	8.8 (8.4)	6.9 - 10.7	19.0 (16.7)	12.1 - 25.9	100.7 (95.5)	82.8 - 118.6	8.5	125.8 (130.4)	112.7 - 138.9	5.5
7-OH-quetiapine	34.2 (33.6)	28.3 - 40.1	31.8 (31.9)	28.6 - 35.0	10.1 (10.1)	7.4 - 12.8	21.5 (18.4)	12.5 - 30.5	98.3 (100.1)	88.1 - 108.5	4.9	112.7 (112.8)	105.9 - 119.8	3.2
Amisulpride	62.3 (62.1)	61.0 - 63.6	65.0 (64.1)	60.1 - 69.9	91.4 (92.2)	86.6 - 96.2	96.4 (95.8)	91.9 - 100.9	93.2 (94.4)	89.9 - 96.5	1.6	97.5 (98.9)	91.9 - 103.1	2.7
Aripiprazole	69.6 (70.2)	65.8 - 73.4	70.7 (68.1)	65.7 - 75.7	53.3 (52.3)	44.6 - 62.0	65.3 (67.0)	60.4 - 70.2	93.5 (93.8)	90.7 - 96.3	1.4	97.2 (98.9)	91.1 - 103.3	3.0
Asenapine	61.8 (51.8)	44.7 - 78.9	63.4 (62.5)	54.5 - 72.3	60.5 (63.9)	54.2 - 66.8	59.4 (59.8)	55.9 - 62.9	99.2 (103.9)	85.4 - 113.0	6.6	97.3 (94.4)	90.3 - 105.3	3.4
Bromperidol	68.1 (69.7)	64.0 - 72.2	68.0 (67.8)	63.7 - 72.3	57.2 (57.3)	51.6 - 62.8	66.9 (66.1)	61.2 - 72.6	97.5 (98.0)	91.8 - 103.2	2.7	107.2 (109.6)	101.2 - 113.2	2.8
Clozapine	54.3 (54.0)	52.3 - 56.3	58.8 (58.0)	55.1 - 62.5	52.4 (51.1)	46.9 - 57.9	74.4 (72.2)	68.2 - 80.6	95.8 (95.8)	92.8 - 98.8	1.4	98.4 (99.6)	93.2 - 103.6	2.5
Dehydro-aripiprazole	63.6 (64.2)	58.6 - 68.6	70.1 (70.2)	66.4 - 73.8	39.3 (37.9)	33.4 - 45.2	52.5 (53.5)	48.8 - 56.2	100.8 (101.5)	96.1 - 105.5	2.2	103.6 (103.9)	98.3 - 108.9	2.5
Haloperidol	72.3 (73.2)	66.7 - 77.9	70.9 (68.0)	65.3 - 76.5	57.3 (58.3)	54.5 - 60.1	68.8 (67.4)	62.8 - 74.8	97.9 (98.2)	93.7 - 102.1	2.0	110.4 (114.2)	102.0 - 118.8	3.8
Hydroxy-iloperidone	68.3 (68.3)	66.7 - 69.9	68.1 (68.7)	63.4 - 72.8	84.5 (84.9)	79.9 - 89.1	100.4 (97.5)	93.3 - 107.5	92.6 (91.8)	87.6 - 97.6	2.5	98.3 (97.8)	91.5 - 105.1	3.2
lloperidone	69.3 (69.8)	64.7 - 73.9	71.0 (69.9)	65.4 - 76.6	64.7 (64.9)	62.2 - 67.2	77.2 (74.8)	71.0 - 83.4	101.9 (101.1)	99.6 - 104.2	1.1	111.6 (112.3)	104.9 - 118.3	3.0
Levosulpiride	68.9 (69.4)	66.5 - 71.3	69.9 (68.7)	64.1 - 75.7	91.7 (92.4)	89.5 - 93.8	90.6 (90.2)	88.1 - 93.1	93.5 (93.5)	91.1 - 95.9	1.2	91.7 (93.2)	85.9 - 97.5	2.9
Lurasidone	44.6 (47.0)	39.2 - 50.0	58.5 (58.6)	54.9 - 62.1	58.4 (59.2)	49.6 - 67.2	62.8 (59.5)	50.3 - 75.3	104.1 (104.8)	95.4 - 112.8	4.1	96.7 (97.2)	87.2 - 106.2	4.6
N-desmethyl-clozapine	48.4 (48.0)	46.8 - 50.0	49.5 (49.6)	46.6 - 52.4	42.0 (40.6)	36.1 - 47.9	73.3 (70.2)	67.1 - 79.5	95.0 (95.5)	92.4 - 97.6	1.3	105.9 (105.8)	99.8 - 112.0	2.8
Paliperidone	65.0 (63.3)	60.9 - 69.1	65.0 (63.1)	61.1 - 68.9	82.3 (82.5)	78.0 - 86.6	96.3 (94.5)	91.6 - 101.0	97.1 (97.5)	95.5 - 98.7	0.8	103.7 (104.5)	97.9 - 109.5	2.7
Pipamperone	64.0 (63.3)	62.2 - 65.8	84.5 (84.9)	81.6 - 87.4	85.8 (86.4)	80.2 - 91.4	95.3 (95.3)	90.5 - 100.1	99.1 (100.0)	96.8 - 101.4	1.1	108.4 (109.5)	102.9 - 114.1	2.5
Quetiapine	70.2 (70.5)	67.8 - 72.6	72.8 (72.2)	69.3 - 76.3	87.2 (87.6)	81.2 - 93.2	97.9 (97.0)	93.9 - 101.9	94.3 (94.4)	91.9 - 96.7	1.2	97.7 (98.4)	92.7 - 103.7	2.4
Reduced haloperidol	74.7 (75.4)	68.9 - 80.5	66.4 (65.6)	58.5 - 74.3	83.8 (83.8)	79.2 - 88.4	98.1 (98.6)	93.6 - 102.6	86.2 (86.3)	84.2 - 88.2	1.0	111.1 (114.4)	102.4 - 119.8	3.9
Risperidone	67.8 (67.4)	66.4 - 69.2	68.5 (66.7)	64.7 - 72.3	89.5 (89.2)	84.7 - 94.3	97.6 (95.9)	93.0 - 102.2	96.6 (97.0)	93.9 - 99.3	1.3	103.4 (105.3)	97.7 - 109.1	2.7
Sertindole	66.4 (66.0)	60.5 - 72.3	65.2 (65.5)	61.5 - 68.9	55.3 (54.3)	45.4 - 65.2	46.0 (48.2)	40.8 - 51.2	104.0 (104.5)	100.6 - 107.4	1.6	103.7 (105.3)	96.9 - 110.5	3.2
Zuclopenthixol	62.8 (63.5)	57.1 - 68.5	69.9 (69.5)	63.7 - 76.1	46.6 (47.1)	36.6 - 56.6	42.5 (41.7)	33.7 - 51.3	102.8 (100.8)	97.4 - 108.2	2.6	103.2 (104.3)	93.5 - 112.9	4.6

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

Table 4 : Stability of the analytes in dried blood spots at QC low (n=3) and QC high (n=3) after storage at room temperature, 4°C and -18°C for 1 month. Stability was calculated as deviation from samples analyzed directly after drying for 3 h at room temperature.

Analyte	QC low (mean %)			QC high (mean %)		
	1 m at RT	1 m at 4°C	1 m at -18°C	1 m at RT	1 m at 4°C	1 m at -18°C
7-OH-N-desalkylquetiapine*	102.1	92.7	97.3	125.5	104.7	99.6
7-OH-quetiapine	109.4	94.4	106.2	115.9	92.7	100.6
Amisulpride	117.7	118.7	116.3	116.7	113.8	119.3
Aripiprazole	109.0	109.9	110.0	104.4	110.3	112.1
Asenapine	106.3	99.3	90.7	108.0	109.2	109.4
Bromperidol	111.6	102.7	97.9	108.0	104.6	106.6
Clozapine	98.5	96.7	100.0	95.8	90.5	93.5
Dehydro-aripiprazole	108.1	107.1	105.6	113.8	112.0	113.0
Haloperidol	109.8	110.4	110.5	105.1	108.2	112.1
Hydroxy-iloperidone	115.5	113.1	108.9	110.0	112.7	121.5
Iloperidone	109.5	107.2	113.5	110.3	112.5	113.5
Levosulpiride	111.9	114.1	113.2	119.0	125.1	118.8
Lurasidone	106.9	106.4	107.1	101.1	108.3	107.0
N-desmethyl-clozapine	104.1	100.8	107.7	103.9	100.6	111.1
Paliperidone	110.3	107.8	112.3	116.5	118.0	119.0
Pipamperone	104.9	106.4	105.9	100.3	105.1	102.2
Quetiapine	109.1	116.5	113.2	109.4	119.0	111.9
Reduced haloperidol	103.3	106.0	112.5	103.5	112.2	111.9
Risperidone	110.9	110.7	112.5	105.7	112.5	107.2
Sertindole	104.0	115.2	114.5	109.7	112.2	109.3
Zuclopenthixol	114.4	107.9	110.4	109.4	107.6	112.4

* stability calculated as deviation from QC low after 1 day

Table 5: Serum, whole blood and capillary DBS concentrations of 10 antipsychotics found in samples of 10 different patients.

Antipsychotic	Serum concentration (ng/ml)	Whole blood concentration (ng/ml)	Capillary DBS concentration (ng/ml)
Amisulpride	213.0	229.2	269.8
Aripiprazole	363.8	247.0	264.1
Bromperidol	4.1	4.4	4.7
Clozapine	484.5	467.1	456.8
Haloperidol	1.8	1.1	2.0
Paliperidone	24.8	18.4	20.9
Pipamperone	136.7	156.2	244.3
Quetiapine	85.2	57.9	59.8
Risperidone	19.4	12.3	11.6
Zuclopenthixol	4.3	4.5	4.6

Supplemental Data 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		
<i>Dehydro-aripiprazole</i>	446.1	285.1	18	100.0	176	2.0
		98.1	42	34.5		
		84.1	62	4.5		
<i>Dehydro-aripiprazole-d8</i>	454.2	293.1	22	100.0	214	2.0
		102.1	46	33.2		
		86.2	66	5.2		
Asenapine	286.1	166.0	34	100.0	172	1.9
		229.0	18	100.6		
		215.0	30	63.4		
Asenapine- ¹³ C ₃ ,d ₃	290.1	229.0	22	100.0	172	1.9
		166.0	34	128.6		
		215.0	30	64.0		
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		
<i>N-desmethylclozapine</i>	313.1	192.0	42	100.0	172	1.6
		270.0	22	57.3		
		227.0	26	17.2		
<i>N-desmethylclozapine-d8</i>	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		
<i>Reduced haloperidol</i>	378.2	149.0	26	100.0	166	1.7
		109.0	58	61.4		
		342.1	18	11.7		
<i>Reduced haloperidol-d4</i>	382.2	149.0	26	100.0	166	1.7
		109.0	54	61.4		
		346.1	22	12.1		
lloperidone	427.2	261.1	26	100.0	196	1.9
		190.0	42	83.4		
		233.1	30	76.1		
lloperidone-d3	430.2	261.1	26	100.0	196	1.9
		190.0	42	80.0		
		233.1	30	73.6		

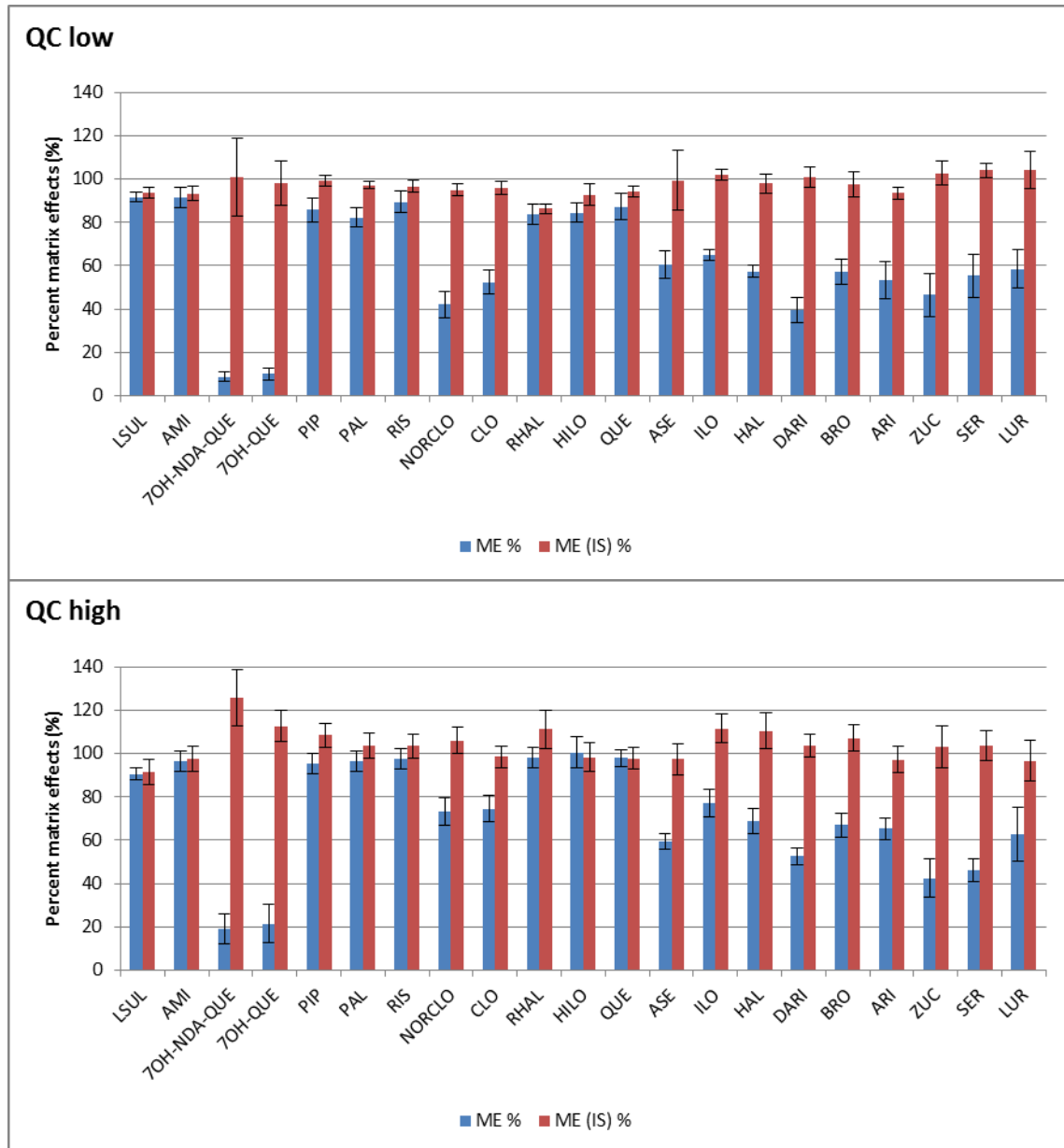
<i>Hydroxy-iloperidone</i>	429.2	261.1	18	100.0	196	1.7
		190.0	42	33.2		
		233.1	30	30.2		
<i>Hydroxy-iloperidone-d4</i>	433.3	261.1	18	100.0	196	1.7
		190.0	42	45.8		
		233.1	30	33.9		
Lurasidone	493.3	166.1	42	100.0	260	2.7
		120.1	66	40.2		
		177.0	46	35.3		
Lurasidone-d8	501.3	166.1	46	100.0	260	2.7
		120.1	66	41.9		
		181.6	46	4.8		
Levosulpiride*	342.2	112.1	22	100.0	188	0.5
		110.1	42	30.0		
		214.0	30	20.0		
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		
<i>N-desmethyloanzapine</i>	299.1	198.0	38	100.0	176	0.8
		256.0	22	83.5		
		213.0	26	63.3		
<i>N-desmethyloanzapine-d8</i>	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		
<i>7-hydroxy quetiapine</i>	400.2	269.0	18	100.0	172	1.1
		237.1	42	20.9		
		295.0	22	14.2		
<i>7-hydroxy quetiapine-d8</i>	408.2	274.1	22	100.0	196	1.1
		302.1	26	25.9		
		241.1	42	24.6		
<i>7-hydroxy N-desalkyl quetiapine</i>	312.1	226.0	26	100.0	172	1.2
		164.0	62	98.5		
		208.0	38	72.5		
<i>7-hydroxy N-desalkyl quetiapine-d8</i>	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		
Sertindole	441.2	113.1	30	100.0	188	2.4
		71.2	54	13.6		
Sertindole-d4	445.2	117.1	34	100.0	188	2.4
		73.2	58	15.1		
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*

Supplemental data Figure 1: Left: Mean absolute ME and mean IS corrected ME obtained by extraction of dried blood spots containing blank whole blood spiked with QC low (n = 6). Right: Mean absolute ME and mean IS corrected ME obtained by extraction of dried blood spots containing blank whole blood spiked with QC high (n = 6). The vertical bars represent the 95 % confidence interval. Analytes in the x-axis are sorted based on the elution sequence.



Supplemental data Figure 2: Absolute and IS corrected ME obtained by extraction of dried blood spots containing blank whole blood with low (19.2 %), normal (range 36.0-50.0 %) and high hematocrit (67.0 %) spiked with QC low and QC high.

