



**HAL**  
open science

# Simultaneous Quantification of Psychotropic Drugs in Human Plasma and Breast Milk and its Application in Therapeutic Drug Monitoring and Peripartum Treatment Optimization

Marguerite Le Marois, Natalia Doudka, Eleni Tzavara, Laurence Delaunay, Sylvie Quaranta, Olivier Blin, Raoul Belzeaux, Romain Guilhaumou

## ► To cite this version:

Marguerite Le Marois, Natalia Doudka, Eleni Tzavara, Laurence Delaunay, Sylvie Quaranta, et al.. Simultaneous Quantification of Psychotropic Drugs in Human Plasma and Breast Milk and its Application in Therapeutic Drug Monitoring and Peripartum Treatment Optimization. *Therapeutic Drug Monitoring*, 2024, 46 (2), pp.227-236. 10.1097/FTD.0000000000001136 . hal-04891756

**HAL Id: hal-04891756**

**<https://hal.science/hal-04891756v1>**

Submitted on 16 Jan 2025

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Simultaneous Quantification of Psychotropic Drugs in Human Plasma and Breast Milk  
and its Application in Therapeutic Drug Monitoring and Peripartum Treatment  
Optimization**

**Marguerite Le Marois<sup>a,b,c</sup>**, MPharm, MS; **Natalia Doudka<sup>a</sup>**, MS; **Eleni Tzavara<sup>d,e,f</sup>**, MD,  
PhD; **Laurence Delaunay<sup>a</sup>**, BS; **Sylvie Quaranta<sup>g</sup>**, PharmD, PhD; **Olivier Blin<sup>a,b</sup>**, MD, PhD;  
**Raoul Belzeaux<sup>c,f,h</sup>**, MD, PhD; **Romain Guilhaumou<sup>a,b</sup>**, PharmD, PhD.

- a. Service de Pharmacologie clinique et Pharmacovigilance, Hôpital de la Timone, 264 rue Saint Pierre, 13385 Marseille Cedex 5, France;
- b. Aix Marseille Université, Institut de neurosciences des systèmes, Inserm UMR 1106, Marseille 13385, France;
- c. Aix Marseille Univ, CNRS, Inst Neurosci Timone, Marseille, France;
- d. Pôle de Psychiatrie, Hôpital Sainte-Marguerite, AP-HM, Marseille, France;
- e. Université Paris Cité, CNRS, Integrative Neuroscience and Cognition Center, 75006 Paris, France;
- f. Fondation FondaMental, Créteil, France;
- g. Laboratoire de Pharmacocinétique et Toxicologie, Hôpital de la Timone, 264 rue Saint Pierre, 13385 Marseille Cedex 5, France;
- h. Pôle Universitaire de Psychiatrie, CHU de Montpellier, France.

Corresponding author: Romain Guilhaumou, PharmD, PhD. Service de Pharmacologie Clinique et Pharmacovigilance, APHM, Institut de neurosciences des systèmes, Inserm UMR 1106, Université d'Aix-Marseille, 13385 Marseille Cedex 5, France

Tel: +33.491.38.75.65

Fax: +33.491.38.78.83

E-mail: [romain.guilhaumou@ap-hm.fr](mailto:romain.guilhaumou@ap-hm.fr)

Conflict of Interest

None declared.

Source of funding

None declared.

Acknowledgements

The authors would like to thank Dr Pastol and Dr des Robert for their expertise and for providing blank milk samples.

## **ABSTRACT**

**Background.** Therapeutic drug monitoring (TDM) is recommended for several psychotropic drugs, particularly in sensitive situations such as the peripartum period. This study aimed to develop an ultra-high-performance liquid chromatography-tandem spectrometry method for the simultaneous quantification of 14 psychotropic drugs in human plasma and four in breast milk.

**Methods.** The samples were precipitated with methanol containing the stable isotope-labelled analogs. Chromatographic separation was performed using a Phenomenex Luna Omega Polar C18 column. Detection was performed using a triple-quadrupole mass spectrometer equipped with an electrospray ionization interface. The method was fully validated in plasma according to the European Guidelines on Bioanalytical Method Validation and partially validated in breast milk by determining the intra-day precision and accuracy, linearity, lower limit of quantification, and matrix effect.

**Results.** The correlation coefficients of the calibration curves were greater than 0.99. Coefficients of variation ranged from 3.05–14.66 % and 0.62–14.90 % for internal standard-normalized matrix effect, 1.4–14.1 % and 2.1–10.4 % for intra-day precision, and 3.2–13.9 % and 4.1–9.6 % for inter-day precision, in plasma and milk, respectively. The relative error in accuracy did not exceed  $\pm 15$  % for any analyte. The method was successfully applied clinically to measure the concentrations of psychotropic drugs in 952 plasma samples, among which, 43 % of the concentrations were out of the therapeutic range, and 13 breast milk samples, with calculated relative infant doses ranging from 0.32–8.18%.

**Conclusion.** To the author's knowledge, this is the first routine technique validated for the quantification of psychotropic drugs in both plasma and breast milk, allowing treatment optimization and prevention of adherence issues, including those in breastfeeding patients.

**Keywords:** Antipsychotics; Antidepressants; Breastfeeding; Mass spectrometry;  
Psychiatric disorders.

1           **INTRODUCTION**

2           Psychiatric disorders are severe conditions for which pharmacotherapy, either alone or  
3 in combination with psychotherapy, remains the predominant line of treatment.

4 Psychopharmacology has revolutionized the management of mental disorders since 1950, and  
5 currently, there are many compounds with proven clinical efficacy. However, issues such as  
6 patient adherence, treatment resistance, drug interactions, and toxicity remain challenges in  
7 everyday practice.

8           Therapeutic drug monitoring (TDM) helps address these issues and provide optimum  
9 customized treatment. TDM, which traditionally involves measuring drug concentrations in  
10 blood or other body fluids, is recommended for many psychotropic drugs at different levels,  
11 ranging from “strongly recommended” to “potentially useful”.<sup>1</sup> Indications for TDM  
12 include treatment initiation, dosage modification, occurrence of adverse reactions, lack  
13 of therapeutic effects, adherence monitoring, and situations that can alter pharmacokinetics,  
14 such as comorbidities, pregnancy, or drug interactions.<sup>1,2</sup>

15           Pregnancy and breastfeeding are typical indications for measuring the plasma  
16 concentrations of psychoactive drugs.<sup>1</sup> The peripartum period, from pregnancy to 1 year after  
17 delivery, is a high-risk period for the relapse of schizophrenia and recurrent mood disorders.<sup>2</sup>  
18 Individuals tend to discontinue their treatment during this period fearing safety issues, which  
19 may cause pernicious effects.

20           Importantly, during peripartum, the choice to breastfeed is a sensitive and difficult  
21 decision for, both, the mother and physician. The World Health Organization recommends  
22 exclusive breastfeeding during the first six months of life.<sup>3</sup> Breastfeeding is the ideal way of  
23 feeding and may be critical in creating an intimate bond between the mother and child.  
24 However, psychotropic drugs easily diffuse in breast milk, thus posing risks for the infant.<sup>4-6</sup>

25 Therefore, monitoring psychotropic drug concentrations in breast milk may be useful for  
26 mothers who intend to breastfeed,<sup>7</sup> since it is a non-invasive and feasible method to estimate  
27 the infant's exposure and prevent toxicity. Moreover, reassuring the mother of breastfeeding  
28 safety during treatment may enhance her adherence<sup>8</sup> and thus prevent relapse during a  
29 vulnerable period.

30 Recently, liquid chromatography-tandem mass spectrometry (LC–MS/MS) has  
31 become a reference method for quantifying antipsychotics and antidepressants.<sup>9–13</sup> To  
32 achieve this, various mass spectrometry analytical protocols have been developed for different  
33 biological fluids,<sup>11–16</sup> including blood and milk. However, no common LC–MS/MS protocol  
34 has been described for quantifying psychotropic drugs in, both, maternal plasma and breast  
35 milk.

36 Consequently, the purpose of this study was two-fold. First, the study aimed to  
37 develop a sensitive, specific, and rapid ultra-performance LC–MS/MS method for quantifying  
38 multiple psychotropic drugs in plasma and breast milk. For plasma analysis, 14 psychotropic  
39 drugs, including drugs frequently prescribed with a strong recommendation level of evidence  
40 from the TDM Consensus,<sup>1</sup> were included. Among these, the following four psychotropic  
41 drugs with strong levels of recommendation for TDM (levels 1 or 2) and frequently  
42 administered during the ‘peripartum period’ were selected for analyzing breast milk:  
43 aripiprazole, escitalopram, olanzapine, and quetiapine. Information on their use during  
44 breastfeeding remains limited, although low excretion in milk is expected, according to the  
45 Drugs and Lactation Database (LactMed).<sup>18</sup> Second, the study aimed to apply and validate  
46 this analytical method in a psychiatric population suitable for TDM and treatment  
47 optimization during the peripartum period.

48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72

## **MATERIALS AND METHODS**

### **Chemicals and Reagents**

Aripiprazole, clomipramine, clozapine, duloxetine, haloperidol, methylphenidate, mirtazapine, O-desmethylvenlafaxine, risperidone, venlafaxine, and fluoxetine-d6 were purchased from Euromedex (Souffelweyersheim, France); amisulpride, escitalopram, olanzapine, amisulpride-d5, clozapine-d8, quetiapine-d8, and venlafaxine-d6 from Santa Cruz Biotechnology (Dallas, TX, USA); N-desmethylclomipramine and 9-OH-risperidone from Sigma-Aldrich (L'Isle-d'Abeau Chesnes, France). Quetiapine was provided by LGC Standard (Luckenwalde, Germany), haloperidol-d4 by Cerilliant (Darmstadt, Germany), and N-desmethylozapine by Interchim (Montlucon, France). The European Directorate for Quality of Medicines and HealthCare (Strasbourg, France) supplied fluoxetine. All the other chemicals listed as follows were commercially available analytical grade materials: methanol from Biosolve (Dieuze, France), acetonitrile and formic acid from Fisher Scientific (Illkirch-Graffenstaden, France), and ammonium formate from MP Biomedicals (Illkirch-Graffenstaden, France). Milli-Q water (Millipore Milli-Q Gradient A10, France) prepared from demineralized water was used throughout the study. Ultrapure argon (>99.998 % purity) obtained from Air Products (Rognac, France) was used as the collision gas. Nitrogen was used for desolvation and was provided by a ZEFIRO 35 LC-MS nitrogen generator (FDGSi, Evry, France). "Normal Plasma," (HYPHEN BioMed, France), a drug-free freeze dried citrated human plasma, was used for method development and validation. The drug-free (blank) milk samples used for method validation were obtained from the Maternity Hospital La Conception, Marseille.

### **Chromatographic and Spectrometric Conditions**



73 LC-MS/MS analysis was performed using an Acquity ultra performance liquid  
74 chromatography (UPLC) H-Class system (Waters Corporation, Milford, MA, USA) and a  
75 triple-quadrupole mass spectrometer (Quattro Premier XE; Waters Corporation) equipped  
76 with an electrospray ionization (ESI) interface. Data were acquired using the PC-based  
77 MassLynx V4.1 software and processed using QuanLynx V4.1 quantification software  
78 (Waters Corporation, Milford, MA, USA).

79

### 80 **Liquid chromatography**

81 The UPLC system was equipped with a quaternary solvent manager, sample manager,  
82 flow-through needle, and column heater. Chromatography was performed on a Phenomenex  
83 Luna Omega Polar C18 column (internal diameter: 100 mm x 2.1 mm ID; particle size: 1.6  
84  $\mu\text{m}$ ; pore size:  $100 \times 10^{-10}$  m). The column and autosampler temperatures were maintained at  
85 40 and 10 °C, respectively. Chromatography was performed using gradient elution with a  
86 binary mobile phase composed of phase A containing 10 mM ammonium formate in  
87 water/acetonitrile 95/5 (v/v) and mobile phase B containing acetonitrile. The mobile phase  
88 was delivered at a flow rate of 0.5 mL/min and the total analytical run time was 8.5 min.  
89 Gradient conditions were as follows: 0 to 0.6 min, isocratic 0 %B, 0.6 to 5.0 min linear; 0–70  
90 % B, 5.0 to 5.1 min linear; 70–0 % B, 5.1 to 8.5 min, isocratic 0 %B.

91

### 92 **Mass spectrometry**

93 The mass spectrometry detector was equipped with an ESI source operating in multi-  
94 reaction monitoring using the positive ion mode. The precursor ions and the two fragment  
95 ions of each compound were monitored. The mass detector parameters were as follows:  
96 capillary voltage, 3.5 KV; desolvation gas flow (nitrogen), 850 L/h; cone gas, flow 25 L/h;

97 gas temperature, 350 °C; source temperature, 120 °C. The fragmentation transitions,  
98 fragmentor voltages, and collision energies are listed in Table S1.

99

### 100 **Preparation of Stock Solutions, Calibration Curve, and Quality Control**

101 Stock solutions were prepared in acetonitrile for olanzapine and in methanol for the  
102 other molecules, at a final concentration of 1.0 mg/mL. Internal standards (IS) were prepared  
103 in methanol to yield a final concentration of 1.0 mg/mL for amisulpride-d5, clozapine-d8,  
104 venlafaxine-d6, and quetiapine-d8, and 0.1 mg/mL for haloperidol-d4 and fluoxetine-d6. All  
105 stock solutions were stored below -20 °C.

106 For quantification in plasma, 50 µg/mL working solutions for 10 molecules  
107 (amisulpride, aripiprazole, clomipramine, clozapine, fluoxetine, norclozapine,  
108 norclomipramine, O-desmethyl-venlafaxine, quetiapine, and venlafaxine) were prepared in  
109 methanol from the stock solution and 5.0 µg/mL working solutions were prepared for six  
110 compounds (9-OH-risperidone, duloxetine, escitalopram, mirtazapine, olanzapine, and  
111 risperidone). Separate solutions were prepared for methylphenidate and haloperidol at 5.0 and  
112 0.5 µg/mL, respectively. All working solutions were stored at -20 °C. The first working  
113 solution was used to prepare the calibration curve spiking solutions by serial dilution with  
114 methanol (Table S2).

115 Secondary working solutions were used to prepare the following spiking solutions for  
116 quality control (QC) preparation: 50.0 µg/mL for 10 compounds, 5.0 µg/mL for six  
117 compounds and methylphenidate, and 0.5 µg/mL for haloperidol. Working IS solutions were  
118 prepared by diluting the stock solution with methanol to obtain a final concentration of 5  
119 µg/mL for amisulpride-d5, clozapine-d8, fluoxetine-d6, quetiapine d8, and venlafaxine-d6,  
120 and 2 µg/mL for haloperidol-d4. Seven non-zero samples were prepared by spiking blank

121 plasma with an appropriate amount of working solution to obtain calibration standard  
122 concentrations (Table S3).

123 For quantification in breast milk, working solutions at 4 µg/mL for aripiprazole and  
124 quetiapine were prepared in methanol from the stock solution, and at 0.5 µg/mL for  
125 escitalopram and olanzapine. A working solution was used to prepare calibration curve  
126 spiking solutions by serial dilution in methanol, yielding concentrations of 200, 400, 800,  
127 1600, 2000, 3200, and 4000 ng/mL for aripiprazole and quetiapine, and 25, 50, 100, 200, 250,  
128 400, and 500 ng/mL for escitalopram and olanzapine. A secondary working solution was used  
129 to prepare the following spiking solutions for QC: 3000 ng/mL aripiprazole and quetiapine  
130 and 375 ng/mL escitalopram and olanzapine. Working IS solutions were prepared by diluting  
131 the stock solution with methanol to obtain a final concentration of 500 ng/mL for clozapine-  
132 d8 and quetiapine-d8 and 200 ng/mL for haloperidol-d4. Seven non-zero samples were  
133 prepared by spiking blank breast milk with the appropriate amount of working solution to  
134 obtain calibration standard concentrations of 20, 40, 80, 160, 200, 320, and 400 ng/mL for  
135 aripiprazole and quetiapine, and 2.5, 5.0, 10.0, 20.0, 25.0, 40.0, and 50.0 ng/mL for  
136 escitalopram and olanzapine. The lowest concentration on the calibration curve was defined  
137 as the low limit of quantification (LLOQ). QC samples were prepared as follows: low QC  
138 (LQC), 60 ng/mL for aripiprazole and quetiapine and 7.5 ng/mL for escitalopram and  
139 olanzapine; medium QC (MQC), 100 ng/mL for aripiprazole and quetiapine and 12.5 ng/mL  
140 for escitalopram and olanzapine; high QC (HQC), 300 ng/mL for aripiprazole and quetiapine  
141 and 37.5 ng/mL for escitalopram and olanzapine.

142

### 143 **Sample Preparation**

144 Twenty microliters of the working IS solution was added to human plasma and breast  
145 milk samples (0.2 mL) placed in microcentrifuge vials (Eppendorf, Hamburg, Germany), and

146 20 µL of working IS solution was added. The samples were precipitated using the following  
147 volumes of methanol: 1000 µL for blank, 980 µL for patient samples, and 960 µL for  
148 calibration and QC samples. After vortexing for 25–30 s, the samples were centrifuged at  
149 19,745 g for 10 min. Plasma (150 µL) or milk (100 µL) supernatant, containing all molecules,  
150 was then transferred into an autosampler vial. Finally, 5 µL of solution was injected into the  
151 LC-MS/MS system for analysis.

152

### 153 **Validation Procedures**

154 The assay was validated according to the European Guidelines for Bioanalytical  
155 Method Validation.<sup>19</sup>

#### 156 **Full validation in plasma**

157

##### 158 *Linearity and sensitivity*

159 Linearity was estimated by assaying calibration curves in six independent runs,  
160 consisting of a blank plasma sample, a zero sample, and the following seven calibrator plasma  
161 concentrations: 0.75, 1.5, 3.0, 6.0, 7.5, 12, and 15 ng/mL for haloperidol; 2.5, 5, 10, 20, 25,  
162 40, and 50 ng/mL for methylphenidate; 7.5, 15, 30, 60, 75, 120, and 150 ng/mL for 9-OH-  
163 risperidone, duloxetine, escitalopram, mirtazapine, olanzapine, and risperidone; and 50, 100,  
164 200, 400, 500, 800, and 1000 ng/mL for the others molecules. Different regression models  
165 and weighting factors were tested to select the best fit. The back-calculated concentrations of  
166 the calibration standards had to be within 15 % of the nominal value, except 20 % for the  
167 LLOQ level. At least 75 % of the calibration standards had to fulfil these criteria. Blank and  
168 zero samples were not retained when calculating calibration curve parameters. The LLOQ  
169 was determined by analyzing six replicates in the same run. A precision of less than 20 % and  
170 an accuracy of 80–120 % were allowed for the LLOQ.

171

172 *Accuracy and precision*

173 The intra-day precision (repeatability) was assessed by running six replicates at four  
174 concentrations (LLOQ, LQC, MQC, and HQC) on the same day under the same operating  
175 conditions, and the inter-day precision was estimated on six different days using the same  
176 instrument. The precision at each concentration was expressed as the relative standard  
177 deviation (or coefficient of variation (CV)) and should not exceed 15 %, except for the  
178 LLOQ, where it should not exceed 20 %.

179 Accuracy was calculated in terms of bias and expressed as the percentage deviation of  
180 the mean calculated concentration and the corresponding nominal concentration. The mean  
181 value should be within 85–115 % of the nominal concentration, except for the LLOQ, where  
182 it should be 80–120 % of the nominal concentration.

183

184 *Extraction recovery*

185 The extraction recovery (%) of the analytes, was determined for three different  
186 concentrations (LQC, MQC, and HQC) by comparing the peak areas of the three extracted  
187 spiked samples with those of three pre-extracted samples at the same concentrations.

188

189 *Matrix effect*

190 Matrix effect (MF) was evaluated for each analyte and IS by calculating the ratio of  
191 the peak area in the presence of the matrix (blank matrix spiked after extraction with analyte)  
192 to the peak area in the absence of the matrix (diluted stock solution) at the same  
193 concentration. Six matrix batches were spiked at LQC and HQC concentrations and IS was  
194 added to all the samples. The IS-normalized MF was calculated as the ratio of the MF of the  
195 analyte to that of the IS. The CV of the IS-normalized MF should not exceed 15 %.

196

197 *Dilution integrity*

198 Dilution integrity was assessed by spiking the samples at concentrations above the  
199 HQC. These samples were diluted with a blank matrix in five replicates per dilution factor  
200 (1/2 and 1/5) for each analyte, except for haloperidol (triplicates per dilution factor). The  
201 dilution integrity was considered adequate when the difference in concentration did not  
202 exceed 15 %.

203

204 *Carryover*

205 Carryover was assessed by injecting a blank sample immediately after a high-  
206 concentration sample. The criterion was that the peak area in the blank sample should not  
207 exceed 20 % of the LOQ for the compounds or 5 % for the IS.

208

209 *Selectivity*

210 The ion chromatograms of six plasma samples obtained from different patients were  
211 compared with those of the corresponding standard plasma samples to investigate the  
212 interference from endogenous plasma compounds. These patients were treated for psychiatric  
213 disorders with lithium or valproic acid (n=6).

214

215 *Stability*

216 Stability studies were performed in duplicate at three different concentrations (LQC,  
217 MQC, and HQC). The samples reached the pharmacological laboratory within 4 h of  
218 collection. They were then centrifuged and immediately stored at -35 °C for a maximum of 48  
219 h. Benchtop stability was determined after 4 h at room temperature (20–24 °C) and was  
220 compared with that of freshly spiked plasma samples. Long-term stability was assessed after

221 storage of QC samples at -80 °C for 2 months. The freeze/thaw stability was determined after  
222 two freeze/thaw cycles (-35 °C to room temperature). The analyte was considered stable in  
223 the matrix when the difference in concentration between the fresh and stability-testing  
224 samples did not exceed 15 %.

225

### 226 **Partial validation in breast milk**

227 In the case of matrix modification (i.e., plasma to breast milk), the European  
228 Medicines Agency (EMA) guidelines recommend partial validation, which includes at least  
229 the determination of intraday precision and accuracy. MF was included to verify its effect on  
230 the analytical process. Linearity was also determined by analyzing calibration curves in six  
231 independent runs, consisting of a blank milk sample, a zero sample, and the following seven  
232 calibrator concentrations in breast milk: 20, 40, 80, 160, 200, 320, and 400 ng/mL for  
233 aripiprazole and quetiapine and 2.5, 5.0, 10, 20, 25, 40, and 50 ng/mL for escitalopram and  
234 olanzapine. The LLOQ was also evaluated.

235

### 236 **Clinical Application**

237 The clinical applicability of the present method was evaluated by analyzing plasma  
238 samples collected over one year from 596 patients and breast milk samples collected for over  
239 8 months from seven patients treated with one or several psychotropic drugs.

240 Observed steady states through concentrations in plasma were compared to TDM  
241 references ranges.<sup>1</sup>

242 The Relative Infant Dose (RID), as a percentage, could be calculated from patients'  
243 breast milk samples (Supplementary Data S4),<sup>5,6</sup> which allowed the estimation of an infant's  
244 exposure to the drug and thus toxicity prevention. An RID < 10 % is generally considered

245 safe. Infant exposure was evaluated as follows: minimal, RID below 2 %; low, RID between  
246 2–5 %; moderate, RID between 5–10 %; and “high” with an RID above 10 %.<sup>5</sup>

247

248 Informed consent and ethical approval were waived because of the retrospective  
249 collection of anonymous routine clinical data from TDM, and this work was exempt from  
250 review board approval.

251

## 252 **RESULTS AND DISCUSSION**

### 253 **Method Validation**

#### 254 **Linearity and sensitivity**

255 Least-squares linear regression was performed using a weighting factor  $1/X$ , since  
256 there was no significant difference on accuracy and precision using a weighting factor  $1/X^2$ .  
257 The blank calibrator was not included in the regression. In plasma, the assay was linear over  
258 0.75–15 ng/mL for haloperidol; 2.5–50 ng/mL for methylphenidate; 7.5–150 ng/mL for 9-  
259 hydroxy-risperidone, duloxetine, escitalopram, mirtazapine, olanzapine, and risperidone; and  
260 50–1000 ng/mL for the others molecules. In milk, the assay was linear over the range of 20–  
261 400 ng/mL for aripiprazole and quetiapine and 2.5–50 ng/mL for escitalopram and  
262 olanzapine. A linear relationship between the concentration and the response of the instrument  
263 was observed over the measured concentration range. The coefficient of determination ( $r^2$ ) of  
264 all calibration curves was higher than 0.99 for the 18 analytes (14 drugs and four metabolites)  
265 in plasma and the four analytes in milk.

266 In plasma, back-calculated concentrations over six runs did not deviate more than 14.9  
267 % from the nominal concentrations for the non-zero calibration standards. The LLOQ was  
268 established as follows: 0.75 ng/mL for haloperidol with precision and accuracy of 7.6 and  
269 98.7 %, respectively; 2.5 ng/mL for methylphenidate with precision and accuracy of 9.7 and



270 100.0 %; 7.5 ng/mL for duloxetine, escitalopram, mirtazapine, olanzapine, risperidone, and 9-  
271 OH-risperidone, with precision and accuracy ranging from 4.7–8.3 % and 100.8–109.5 %; and  
272 50 ng/mL for the other molecules, with precision and accuracy ranging from 3.8–10.1 % and  
273 98.4–108.0 %.

274 In milk, the back-calculated concentrations over six runs did not deviate more than  
275 15.1 % from the nominal concentrations for the non-zero calibration standards. The LLOQ  
276 was established at 20 ng/mL for aripiprazole and quetiapine, with precisions of 5.4 and 7.2 %,  
277 and accuracies of 93.1 and 103.7 %, respectively. The LLOQ was established at 2.5 ng/mL  
278 for escitalopram and olanzapine, with precisions of 9.2 and 6.2 %, and accuracies of 101.4  
279 and 111.5 %, respectively.

280

### 281 **Accuracy and precision**

282 In plasma, the intra-day accuracy and precision ranged from 86.5–113.6 % and 1.4–  
283 14.1 %, and the inter-day accuracy and precision ranged from 89.4–113.4 % and 3.2–13.9 %,   
284 respectively (Table 1). In breast milk, the intra-day accuracy and precision ranged from 85.4–  
285 114.2 % and 2.1–10.4 %, and the inter-day accuracy and precision ranged from 86.9–109.4 %  
286 and 4.1–9.6 %, respectively (Table 2). The presented method provided adequate accuracy and  
287 precision for the quantifying all 18 compounds in human plasma samples and aripiprazole,  
288 escitalopram, olanzapine, and quetiapine in breast milk.

289

### 290 **Extraction recovery**

291 All 18 compounds showed acceptable mean extraction recoveries from plasma,  
292 ranging from 64.87–103.49 %, with a precision below 15 % at the three QC concentrations.

293

### 294 **Carryover**

295 The blank plasma chromatogram of the sample injected after a highly spiked sample  
296 showed a signal lower than 20 % of the LLOQ at the retention times of the 18 analytes.  
297 Therefore, carryover effect was excluded from the analysis.

298

### 299 **Matrix effect**

300 The MF in plasma was evaluated by comparing the area obtained from a blank plasma  
301 extract spiked with psychotropic drugs at LQC and HQC concentrations versus a direct  
302 injection of diluted stock solution at the same concentrations. The IS-normalized MF value  
303 ranged from 0.85–1.33 and the CV ranged from 3.05–14.66 %. In milk, the MF was evaluated  
304 by comparing the area obtained from three blank milk extracts spiked with the four drugs at  
305 LQC and HQC concentrations versus the direct injection of diluted stock solution at the same  
306 concentrations. The IS-normalized MF values ranged from 0.89–1.10 %, and the CV ranged  
307 from 0.62–14.90 %. No MFs were observed at the LQC or HQC concentrations for all 18  
308 analytes in plasma or for the four analytes in milk.

309

### 310 **Dilution integrity**

311 Concentration differences between undiluted and diluted samples ranged from -12.2–  
312 14.02 % (1/5 dilution) and -5.81–13.65 % (1/2 dilution). Dilution integrity was confirmed for  
313 all molecules, except for the 1:2 dilution factor of escitalopram, which showed a  
314 concentration difference of -16.37 %, which was marginally below the accepted difference  
315 ( $\pm 15$  %) defined by the EMA guidelines. However, this is unlikely to affect escitalopram  
316 TDM in clinical practice.

317

318 **Selectivity**

319 After injecting the plasma samples from different patients treated for psychiatric  
320 disorders with lithium or valproic acid (n=6), no significant interference was observed at the  
321 same retention time for each molecule.

322

323 **Stability**

324 In bench-top stability analyses, the mean percentage changes in the peak area ranged  
325 from -14.26–14.59 %, which suggested that all the analytes were stable for 4 h at room  
326 temperature. Analyte concentrations after 2 months at -80 °C or after two freeze-thaw cycles  
327 did not show significant deviations from the nominal concentrations (Table 3).

328

329 **Clinical Application**

330 The validated method was successfully applied in 2020 for the TDM of 14  
331 psychotropic drugs in 596 patients (952 samples) (Table 4). Among these, the concentrations  
332 of 239 samples were below the therapeutic range and 170 were above, indicating that 43 % of  
333 the concentrations were outside the therapeutic range. Moreover, the high standard deviations  
334 observed in the concentrations demonstrated the important inter-individual pharmacokinetic  
335 variability of these molecules. In summary, these results confirm the importance of TDM in  
336 psychotropic treatment.

337 This method was successfully applied to measure psychotropic drug concentrations in  
338 13 breast milk samples from seven patients between February and October 2020. Three  
339 patients were treated with aripiprazole (5 or 10 mg/day). In two patients, aripiprazole  
340 concentrations in milk were 9.6 and 28.6 ng/mL, with corresponding RIDs of 0.57 and 2.15  
341 %, respectively, indicating minimal to low exposure of the infant. The third patient presented  
342 with undetectable concentrations of aripiprazole in both milk and maternal blood, suggesting

343 adherence. Four patients were treated with escitalopram, with daily dosages from 1.5 (an  
344 unusually low dosage for one patient) to 15 mg. Among these, three patients had milk  
345 concentrations ranging from 1.1–100 ng/mL, with the calculated RIDs varying from 0.32 to  
346 4.50 %, indicating minimal to low infant exposure. The fourth patient, who was treated daily  
347 with 5 mg of escitalopram, presented a milk concentration of 33.27 ng/ml and an RID of 8.18  
348 %, corresponding to a moderate exposure of her infant to the drug. Thus, close monitoring of  
349 the infant and milk concentrations is advised, if breastfeeding was pursued. Finally, one  
350 patient treated with 100 mg of quetiapine per day showed its absence in milk and a  
351 concentration in maternal blood that was lower than that in the therapeutic range; either  
352 control of treatment adherence or a change in the dosing regimen is recommended. In  
353 conclusion, RID calculations allowed close monitoring of infant exposure to the mother's  
354 treatments through milk, preventing any toxicity issues. It reassures patients and their  
355 physicians about breastfeeding safety and thus may help improve patient adherence to  
356 treatment.

357

## 358 **CONCLUSION**

359 Psychotropic drugs are widely prescribed, often for prolonged durations. However,  
360 they are frequently associated with drug exposure issues, partly owing to their high inter- and  
361 intra-variability in pharmacokinetics, resulting in narrow therapeutic ranges, low therapeutic  
362 response, or adverse effects, and thus adherence difficulties. This study aimed to validate a  
363 sensitive, specific, and rapid routine ultra-performance LC–MS/MS method for the  
364 simultaneous quantification of 14 psychotropic drugs in plasma. This method was  
365 successfully applied to samples collected from 596 patients in 2020.

366 The study addresses a specific issue in women treated with psychotropic drugs during  
367 the peripartum period. To prevent toxicity in infants from exposure through breastfeeding, the

368 same technique was validated in breast milk for four molecules frequently prescribed to this  
369 population. The measured aripiprazole and escitalopram concentrations in breast milk samples  
370 from patients were low, in accordance with the information available in the LactMed  
371 database,<sup>18</sup> except in one patient whose infant's exposure to escitalopram was moderate.  
372 Monitoring concentrations in breast milk allowed us to reassure patients and prescribers of  
373 infant exposure and breastfeeding safety or to adapt breastfeeding recommendations when  
374 necessary. It also helped detect probable adherence issues.

375         To our knowledge, this is the first routine technique for quantifying psychotropic  
376 drugs in both plasma and breast milk. Moreover, this method is suitable for TDM in a  
377 psychiatric population, including breastfeeding women.

## REFERENCES

- 378 1. Hiemke C, Bergemann N, Clement HW, et al. Consensus Guidelines for Therapeutic  
379 Drug Monitoring in Neuropsychopharmacology: Update 2017. *Pharmacopsychiatry*.  
380 2018;51(1-02):9-62. doi:10.1055/s-0043-116492
- 381 2. Belzeaux R, Sanguinetti C, Murru A, et al. Pharmacotherapy for the peripartum  
382 management of bipolar disorder. *Expert Opin Pharmacother*. 2019;20(14):1731-1741.  
383 doi:10.1080/14656566.2019.1626826
- 384 3. Breastfeeding. <https://www.who.int/westernpacific/health-topics/breastfeeding>.  
385 Accessed March 24, 2023.
- 386 4. Sprague J, Wisner KL, Bogen DL. Pharmacotherapy for depression and bipolar  
387 disorder during lactation: A framework to aid decision making. *Semin Perinatol*.  
388 2020;44(3):151224. doi:10.1016/j.semperi.2020.151224
- 389 5. Kaplan YC, Erol-Coskun H. Safety Parameters and Risk Categories Used for  
390 Psychotropic Drugs in Pregnancy and Lactation. In: Uguz F, Orsolini L, eds. *Perinatal*  
391 *Psychopharmacology*. Cham: Springer International Publishing; 2019:37-53.  
392 doi:10.1007/978-3-319-92919-4\_3
- 393 6. Larsen ER, Damkier P, Pedersen LH, et al. Use of psychotropic drugs during  
394 pregnancy and breast-feeding. *Acta Psychiatr Scand Suppl*. 2015;(445):1-28.  
395 doi:10.1111/acps.12479
- 396 7. Menon SJ. Psychotropic medication during pregnancy and lactation. *Arch Gynecol*  
397 *Obstet*. 2008;277(1):1-13. doi:10.1007/s00404-007-0433-2
- 398 8. Pastol J, Le Marois M, Guilhaumou R, Blin O, Belzeaux R. Measuring drug  
399 concentrations in breast milk to improve therapeutic monitoring and patient adherence in  
400 bipolar disorder: A case report. *Aust N Z J Psychiatry*. 2022;56(1):96.  
401 doi:10.1177/00048674211010340
- 402 9. Hasselstrøm J. Quantification of antidepressants and antipsychotics in human serum  
403 by precipitation and ultra high pressure liquid chromatography-tandem mass spectrometry. *J*  
404 *Chromatogr B Analyt Technol Biomed Life Sci*. 2011;879(1):123-128.  
405 doi:10.1016/j.jchromb.2010.11.024
- 406 10. Cao Y, Zhao F, Chen J, et al. A simple and rapid LC-MS/MS method for the  
407 simultaneous determination of eight antipsychotics in human serum, and its application to  
408 therapeutic drug monitoring. *J Chromatogr B Analyt Technol Biomed Life Sci*.  
409 2020;1147:122129. doi:10.1016/j.jchromb.2020.122129
- 410 11. Degreef M, van Nuijs ALN, Maudens KE. Validation of a simple, fast liquid  
411 chromatography-tandem mass spectrometry method for the simultaneous quantification of 40  
412 antidepressant drugs or their metabolites in plasma. *Clin Chim Acta Int J Clin Chem*.  
413 2018;485:243-257. doi:10.1016/j.cca.2018.06.047
- 414 12. Qi Y, Liu G. Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry  
415 for Simultaneous Determination of Antipsychotic Drugs in Human Plasma and Its Application

- 416 in Therapeutic Drug Monitoring. *Drug Des Devel Ther.* 2021;15:463-479.  
417 doi:10.2147/DDDT.S290963
- 418 13. Saar E, Beyer J, Gerostamoulos D, Drummer OH. The analysis of antipsychotic drugs  
419 in human matrices using LC-MS(/MS). *Drug Test Anal.* 2012;4(6):376-394.  
420 doi:10.1002/dta.1337
- 421 14. López-García E, Mastroianni N, Postigo C, et al. Simultaneous LC-MS/MS  
422 determination of 40 legal and illegal psychoactive drugs in breast and bovine milk. *Food*  
423 *Chem.* 2018;245:159-167. doi:10.1016/j.foodchem.2017.10.005
- 424 15. Salazar FR, D'Avila FB, de Oliveira MH, Ferreira PL, Bergold AM. Development and  
425 validation of a bioanalytical method for five antidepressants in human milk by LC-MS. *J*  
426 *Pharm Biomed Anal.* 2016;129:502-508. doi:10.1016/j.jpba.2016.07.047
- 427 16. Weisskopf E, Panchaud A, Nguyen KA, et al. Stereoselective determination of  
428 citalopram and desmethylcitalopram in human plasma and breast milk by liquid  
429 chromatography tandem mass spectrometry. *J Pharm Biomed Anal.* 2016;131:233-245.  
430 doi:10.1016/j.jpba.2016.08.014
- 431 17. Weisskopf E, Panchaud A, Nguyen KA, et al. Simultaneous determination of selective  
432 serotonin reuptake inhibitors and their main metabolites in human breast milk by liquid  
433 chromatography-electrospray mass spectrometry. *J Chromatogr B Analyt Technol Biomed*  
434 *Life Sci.* 2017;1057:101-109. doi:10.1016/j.jchromb.2017.04.039
- 435 18. Drugs and Lactation Database (LactMed®). National Institute of Child Health and  
436 Human Development; 2006.
- 437 19. European Guideline on Bioanalytical Method Validation.  
438 [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf)  
439 [method-validation\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf). Accessed March 24, 2023.
- 440