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# Ultra-high-pressure liquid chromatography tandem mass spectrometry determination of antidepressant and anxiolytic drugs in neonatal meconium and maternal hair

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ABSTRACT

A procedure based on ultra-high-pressure liquid chromatography tandem mass spectrometry has been developed for the determination of 22 antidepressant and anxiolytic drugs ad metabolites in the three consecutive maternal hair segments representing the pregnancy trimesters and paired neonatal meconium samples. After hair washing with methyl alcohol and diethyl ether and subsequent addition of internal standards, hair samples were treated with 500 μl VMA-T M3 reagent for 1 h at 100 °C. After cooling, 100 μl M3 extract were diluted with 400 μl water and a volume of 10 μl was injected into chromatographic system. Meconium samples were firstly treated with 1 ml methyl alcohol and the organic layer back-extracted twice with 1.5 ml of a mixture of ethylacetate:hexane (80:20, v/v). Chromatographic separation was achieved at ambient temperature using a reverse-phase column and a linear gradient elution with two solvents: 0.3% formic acid in acetonitrile and 5 mM ammonium formate pH 3. The mass spectrometer was operated in positive ion mode, using multiple reaction monitoring via positive electrospray ionization. The method was linear from the limit of quantification (0.05–1 ng/mg hair and 5–25 ng/g meconium depending on analyte under investigation;) to 10 ng/mg hair and 1000 ng/g meconium, with an intra- and inter-assay imprecision and inaccuracy always less than 20% and an analytical recovery between 66.6% and 95.3%, depending on the considered analyte and biological matrix. Using the validated method, **7** mothers were found positive to one or more hair segments and 5 meconium samples were found positive to one or more antidepressant and anxiolytic drugs, assessing prenatal exposure to these drugs following maternal consumption in one or more pregnancy trimesters.



## 1. Introduction

Psychiatric disorders are equally common during pregnancy as among non-pregnant women [1]. The principal disorders encountered during pregnancy include major depression, anxiety disorders, bipolar affective disorder, and schizophrenia [2], and many of these conditions are treated with antidepressant or anxiolytic medications [3]. Some treated women discontinue their therapy once pregnancy is recognized, either on their own or at the advice of a clinician in order to minimize exposure of the fetus to these psychoactive drugs [4], while others continue to use throughout pregnancy.

The most commonly psychotropic drugs used during pregnancy are selective serotonin reuptake inhibitors (5.1%), benzodiazepine or benzodiazepine-like medicines (3.9%) [1]. These substances are able to pass the placental barrier, and may potentially influence fetal and brain development [3]. It is possible that exposure to prenatal antidepressants or anxiolytic medications may disturb neurotransmitter systems in the brain and have long-lasting consequences on neurodevelopment in the offspring. As all medications

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**Table 1**
Ultra-performance liquid chromatography tandem mass spectrometry parameters for the multiple reaction monitoring (MRM) acquisition mode.

| Analyte | Retention time (min) | MRM transitions | | | | | |
|---|---|---|---|---|---|---|---|
| | | Quantification | | | Confirmation | | |
| | | *m/z* | CV (V) | CE (eV) | *m/z* | CV (V) | CE (eV) |
| Norclozapine | 4.26 | 313.2 > 192.1 | 32 | 40 | 313.2 > 227.1 | 32 | 28 |
| Clozapine | 4.33 | 327.1 > 192.2 | 35 | 40 | 327.1 > 270.2 | 35 | 23 |
| Venlafaxine | 433 | 278.2 > 58.1 | 25 | 18 | 278.2 > 121.2 | 25 | 18 |
| Quetiapine | 4.40 | 384.1 > 221.1 | 35 | 38 | 384.1 > 253.0 | 35 | 24 |
| Citalopram | 4.49 | 325.2 > 109.1 | 40 | 24 | 325.2 > 58.2 | 40 | 24 |
| Paroxetine | 4.61 | 330.2 > 70.1 | 40 | 29 | 330.2 > 192.2 | 40 | 20 |
| Duloxetine | 4.83 | 298.2 > 154.1 | 12 | 8 | 298.2 > 44.1 | 12 | 8 |
| Imipramine | 4.85 | 281.2 > 86.1 | 35 | 20 | 281.2 > 57.8 | 35 | 30 |
| Norfluoxetine | 4.90 | 296.0 > 134.1 | 15 | 6 | 296.0 > 30.6 | 15 | 10 |
| Norsertraline | 5.04 | 292.2 > 158.9 | 8 | 28 | 292.2 > 275.1 | 8 | 16 |
| Fluoxetine | 5.05 | 310.1 > 148.2 | 20 | 8 | 310.1 > 44.2 | 20 | 12 |
| Sertraline | 5.16 | 306.2 > 159.0 | 14 | 30 | 306.2 > 275.1 | 14 | 14 |
| Medazepam | 5.20 | 271.19 > 207.1 | 30 | 26 | 271.19 > > 91.1 | 30 | 26 |
| Amitriptyline | 5.24 | 278.3 > 105.1 | 30 | 24 | 278.3 > 91.1 | 30 | 24 |
| Oxazepam | 5.31 | 287.1 > 241.2 | 35 | 20 | 287.1 > 269.1 | 35 | 15 |
| Lorazepam | 5.41 | 321.0 > 275.1 | 30 | 20 | 321.0 > 229.1 | 30 | 20 |
| Alprazolam | 5.43 | 309.2 > 205.2 | 50 | 43 | 309.2 > 281.2 | 50 | 30 |
| Clonazepam | 5.43 | 316.1 > 214.1 | 55 | 39 | 316.1 > 270.1 | 55 | 24 |
| Clomipramine | 5.53 | 315.1 > 86.1 | 35 | 18 | 315.1 > 58.2 | 35 | 28 |
| Nor-diazepam | 5.59 | 271.1 > 140.0 | 50 | 35 | 271.1 > 165.1 | 50 | 25 |
| Lormetazepam | 5.88 | 335.1 > 177.1 | 40 | 40 | 335.1 > 289.2 | 40 | 25 |
| Diazepam | 6.1 | 285.1 > 193.2 | 50 | 32 | 285.1 > 154.1 | 50 | 28 |
| Promethazine (IS) | 4.65 | 285.1 > 86.2 | 35 | 25 | | | |
| Oxazepam-d5 (IS) | 5.31 | 292.1 > 246.1 | 35 | 20 | | | |
| Lorazepam-d4 (IS) | 5.39 | 325.2 > 279.1 | 34 | 24 | | | |
| Alprazolam-d5 (IS) | 5.4 | 314.2 > 210.1 | 34 | 42 | | | |
| Diazepam-d5 (IS) | 6.07 | 290.2 > 198.2 | 50 | 34 | | | |

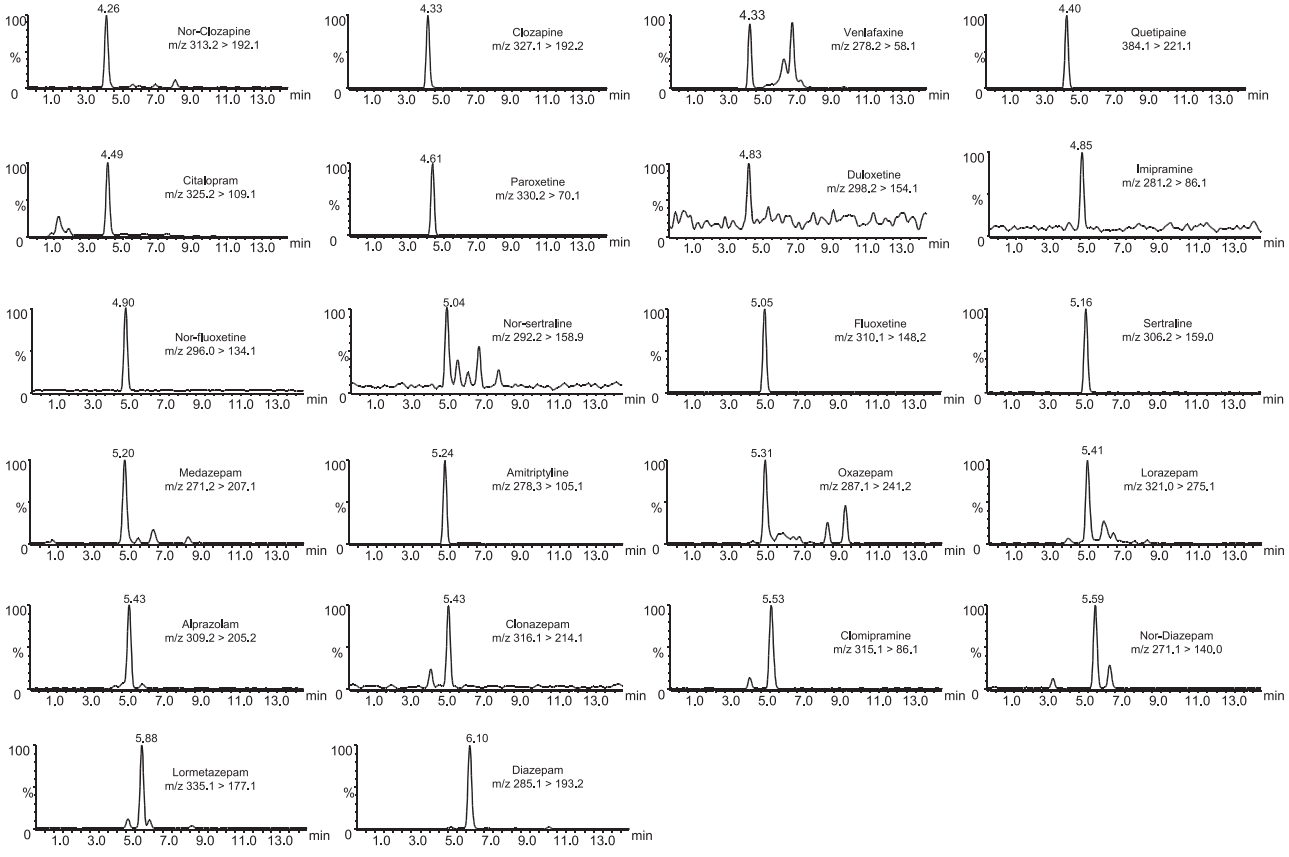


**Fig. 1.** UHPLC-MS/MS chromatogram of drug-free hair pool spiked with 1 ng/mg analytes under investigation.