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Short communication

Development and validation of a bioanalytical method for five antidepressants in human milk by LC–MS

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ABSTRACT

The use of medications during lactation is a common practice; however, pharmacological treatments impose serious doubts to both professionals and nursing mothers regarding the safety of drugs used during this period. Most of drugs are excreted in breast milk and there is great variability in the amount of analytes that can be received by the infant. Dilemmas about breastfeeding arise most commonly in relation to postpartum depression. Depression is a major clinical problem during the postpartum period and the vulnerability to onset or recurrence of depressive symptoms increases the possibility of psychotropic drug use during lactation. Selective inhibitors of serotonin reuptake are commonly prescribed for the treatment of depressive disorders, including fluoxetine, sertraline, citalopram, and paroxetine. A validated bioanalytical method using liquid chromatography coupled to mass spectrometry was developed and validated for determination of antidepressants in human milk following protein precipation. The bioanalytical method was successfully applied to assess milk samples from nursing mothers. From found concentrations, infant absolute (4.36–12.26 μg/kg/day) and relative dose (0.60–2.90%,) were estimated and low values were obtained indicating safe use during laction. However, other factors such as complemantary feeding and hepatic or renal disorders in the infant should be considered.

1. Introduction

Breastfeeding is an essential physiological process with health and social benefits. It provides nutrition, immunological and many other bioactive factors and can prevent many diseases in the infant. The World Health Organiztion (WHO) and Pediatric Academics around the world have endorsed breast milk as the ideal form of nutrition for the newborn and they emphasize the physical and emotional benefits for the baby. They also reccomend exclusive breastfeeding for the first six months of life and to continue breastfeeding for at least the first year of life [1,2].

The need for pharmacological treatment is common in the postpartum period. Postpartum women often take either prescription or over-the-coutner medications. Many of these mothers are advised to stop breasfeeding or avoid drug therapy based on information obtained from product literature [3,4].

There is a substantial incidence of immediate postapartum disorders, such as depression, and it has focused attention on the dilemma regarding the use of psychotropic medications in postpartum women. Depression that is not treated can compromise the infant's development, breastfeeding, and other essential needs [1].

Selective serotonin reuptake inhibitors (SSRIs) are often the first choice for the treatment of depression, especially in the postpartum period. This group includes fluoxetine, sertraline, citalopram, and paroxetine [1,3–5]. In Brazil, these drugs were the most frequently prescibed among the antidepressants [6]. Bupropion is an atypical antidepressant that acts as a dopaminergic and noradrenergic reuptake inhibitor. It is an effective therapy for smoking cessation and relapse prevention and can be particularly useful in preventing postpartum relapse [7].

Data from breast milk studies are important to defining the risk-benefit assessment of antidepressant therapy in nursing women. Breast milk is an unconventional matrix that has been used to assess infant exposure to various drugs. It has an advantage of easy and non-invasive collection, although extraction of drugs is an analytical challenge because of its high protein/fat content and changing composition during postpartum period [1,3,4].

Many studies have been published for the determination of antidepressants in plasma and human milk. Quantification method for breast milk was mainly liquid chromatography with ultraviolet detection and liquid–liquid extraction or solid phase extraction technique as an extraction procedure [8–16]. Single drug deter-

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mination was established in these studies; only one reported simultaneous determination of antidepressants in human milk [15]. A bupropion study in breast milk using LC–MS as a quantification method has been reported [16]. Moreover, multi-drug methods for determination of antidepressants and other psychotropic drugs in plasma have been generated using LC–MS [17–19]. Therefore, a liquid chromatography-mass spectometry method to measure and detect five antidepressants (fluoxetine, sertraline, citalopram, paroxetine, and bupropion) in human milk after protein precipitation as a cleaning and extraction procedure was developed and validated. This method was applied to analyze breast milk samples collected from the milk bank of two hospitals in Rio Grande do Sul, Brazil. The aim of this paper is to contribute an alternative method to analyze human milk samples and provide data on drug therapy during breastfeeding.

2. Material and methods

2.1. Chemicals and reagents

Fluoxetine (FLU), sertraline (SER), citalopram (CIT), paroxetine (PAR), and bupropion (BUP) were obtained from Pharmanostra Ltda (Campinas, Brazil). Atenolol, used as an internal standard, was obtained also from same company. Methanol, acetonitrile, and acetic acid used in the analyses were from Merck® (Frankfurt, Germany) and ammonium acetate from FMaia Ind e Com (Cotia, SP). All solvents were of analytical grade. Ultrapure water was obtained using a Mili-Q Plus system from Milipore (Bedford, MA, USA).

2.2. Human milk

Samples of human milk were obtained from mothers in the lactation period that were taking antidepressants and agreed to participate in this study. The samples were collected at two hospitals – Santa Casa de Caridade de Bagé and Hospital Materno Infantil Presidente Vargas, Porto Alegre, Brazil, where the volunteers were being treated. A nurse supervised collection of the samples, which were stored at $-20 \pm 2\,^{\circ}C$. Drug-free milk was collected from volunteer mothers and donated by Santa Casa de Caridade de Bagé, Brazil.

2.3. Standard solutions, work solutions, and quality control samples

Stock solutions of fluoxetine, sertraline, citalopram, paroxetine, bupropion, and atenolol (IS) at 1 mg/mL were prepared in acetonitrile. Work solution of IS was prepared by diluting stock solution to 1 μg/mL in acetonitrile. Work solutions of the antidepressant compounds were freshly prepared at 1, 2, 4, 8, 20, and 40 μg/mL in water. All solutions were stored at $-20 \pm 2\,^{\circ}C$. Quality control samples were prepared in 1 mL of drug-free milk. For fluoxetine, citalopram, and bupropion, quality control concentrations were prepared at 15 ng/mL (low quality control – LQC), 160 ng/mL (middle quality control – MQC), 240 ng/mL (high quality control – HQC), and 640 ng/mL (dilution quality control – DQC). Sertraline and paroxetine were prepared at 60 ng/mL (LQC), 300 ng/mL (MQC), 450 ng/mL (HQC), and 1200 ng/mL (DQC). Dilution quality samples were diluted to 320 and 600 ng/mL, respectively. Lower limit of quantification (LLOQ) was prepared at 5 ng/mL (fluoxetine, citalopram, and bupropion) and 20 ng/mL (sertraline and paroxetine). Calibration standards were made by spiking drug-free milk with suitable amounts of work solutions at concentrations of 5, 10, 20, 40, 80, 160, and 320 ng/mL (fluoxetine, citalopram, and bupropion) and 20, 40, 80, 120, 200, 400, and 600 ng/mL (sertraline and paroxetine). Drug-free milk used for calibration standards and quality control samples was collected from a pool of 15 donors.

2.4. Sample preparation

A 250 μL volume of milk sample (blank, quality control, calibrators, and real samples) was transferred to a 1.5 mL polypropilene conical tube and 50 μL of IS (1 μg/mL) were added. Acetonitrile (700 μL) ice-cold ($-20\,^{\circ}C$) was added to the tube. This mixture was vortexed for 1 min and centrifuged at 14000 rpm for 15 min at $4\,^{\circ}C$. After this procedure, the supernatant was filtered in 0.22 μm PTFE 13 mm Millex directly to a vial.

2.5. Instruments

The LC system consisted of an Agilent 1260 Infinity Series instrument equipped with a G1311 B quarternary pump, a G1329 B auto sampler, G1314 F UV/VIS detector, and a G1316A thermostatizer coupled to an Agilent 6120 B series quadrupole mass detector. Analytical data were acquired and analyzed using Chem Station software (v. 1.4.1), all from Agilent Technologies (Palo Alto, CA, USA). An Eppendorf 5430 R (Hamburg, Germany) centrifuge was used to prepare all samples.

2.6. Liquid chromatography-mass spectometry (LC–MS)

LC optimal conditions were achieved with a Nucleosil C8 column (5 μm, 150 × 4.6 mm Macherey Nagel, Düren, Germany) protected by a C8 guard column (4 × 2.0 mm, Phenomenex, Torrance, CA, USA). Temperature for analyses was set at $35\,^{\circ}C$. The mobile phase was composed of 20 mM ammonium acetate pH 6.0 (adjusted with 0.1% acetic acid) and acetonitrile (25:75 v/v) in isocratic condition with a flow rate of 0.7 mL/min. Injection volumes were 10 μL.

Single ion monitoring was performed for quantitative analyses. Mass detector was operated with an electrospray ionization source in positive mode (ESI+) and the following parameters were set: nebulizer pressure at 55 psi, drying gas flow at 12L/min, drying gas temperature at $350\,^{\circ}C$, and capillary voltage at 1000 V. Gain value was kept at 1. Quantification and confirmation ions (m/z) were monitored for each compound with different fragmentation voltage. Ions monitored for fluoxetine were 310 and 148 (m/z), sertraline 306 and 275 (m/z), citalopram 325 and 109 (m/z), paroxetine 330 and 192 (m/z) and finally for bupropion 240 and 184 (m/z), Fragmentation voltage was set at 70 V for bupropion and paroxetine and 100 V for fluoxetine, sertraline and citalopram. IS monitored ions (m/z) were 267 and 145 with fragmentor voltage at 100 V. Fig. 1 represents the proposed fragmentation for the analytes fluoxetine, sertraline, citalopram, paroxetine, and bupropion.

2.7. Bioanalytical method validation

Validation was performed according to the Guideline on Bioanalytical Method Validation by the European Medicines Agency (2012) [20]. The assessed parameters were: selectivity, sensitivity, linearity, precision, accucary, carryover, matrix effect, and stability of analytes.

Selectivity of the method was evaluated by analyzing six blank milk samples from different donors. Results obtained were compared with those obtained from samples spiked with analytes at the lower limit of quantification (LLOQ) concentration. In addition, samples added with other psychotropic drugs, like diazepam, bromazepam, chlorpromazine, levopromazine, sulpiride, amitriptyline, and nortriptyline, observed in the treatment of donor patients, were tested to assess selectivity.

Carryover was tested during validation, injecting blank samples three times, one time before and two times after analyzing