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Validation and application of a quantitative liquid chromatography tandem mass spectrometry assay for the analysis of rifapentine and 25-O-desacetyl rifapentine in human milk

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Abstract

A robust analytical method based on liquid chromatography coupled to tandem mass spectrometry was developed and validated to quantify rifapentine and 25-O-desacetyl rifapentine in human breast milk to aid in determining the breastfed infant risk to the excreted drug in human milk. Samples were extracted by a combination of protein precipitation and solid phase extraction using rifampicin-d3 as an internal standard. An Agilent® Poroshell 120 EC-C18 (4.6 mm × 50 mm, 2.7 μm) column was used for chromatographic separation employing an isocratic mobile phase consisting of acetonitrile: methanol: 0.1% formic acid (55/5/40, v/v/v) at a flow rate of 450 μL/min, and with a total run time of four minutes. Mass detection was on an AB Sciex API 4000 mass spectrometer using electrospray ionization in the positive mode and based on multiple reaction monitoring data acquisition. Rifapentine was accurately quantified across a concentration range of 2.00–2000 ng/mL and 25-O-desacetyl rifapentine from 4.00 to 2000 ng/mL. During validation, the inter- and intra-day accuracy and precision at the tested QC concentrations (N = 18) for rifapentine were between 97.4% and 100.6%, and 3.1% and 8.3%, respectively. The inter- and intra-day accuracy and precision for 25-O-desacetyl rifapentine were between 96.4% and 106.3%, and 6.7% and 11.8%, respectively. No significant matrix effects were observed, and the method

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I declare this work is original and has not been submitted to any other journal for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

was shown to be specific for rifapentine and 25-O-desacetyl rifapentine. Human milk samples (N = 22) generated during a phase I/II clinical trial were successfully analysed for rifapentine and 25-O-desacetyl rifapentine using this validated method. Concentrations for rifapentine and 25-O-desacetyl rifapentine in human milk samples (N = 22) ranged from 11.2-1180 ng/mL and 7.11–573 ng/mL, respectively.

Keywords

Tuberculosis; Rifapentine; Human milk; Liquid chromatography with tandem mass; spectrometry

1. Introduction

An estimated 3.2 million women were diagnosed with tuberculosis (TB) in 2019 [1]. A six-fold increase in perinatal deaths and a two-fold risk of premature birth and low birthweight have been associated with pregnant women with TB [1]. In Africa, TB rates were reported to be up to 10 times higher in pregnant women living with Human Immunodeficiency Virus (HIV) compared to those without HIV [2].

Rifapentine is an alternative first-line tuberculosis drug and its use with isoniazid as a three-month weekly regimen has been endorsed by the World Health Organization (WHO) for TB prevention in non-pregnant adults and children as an alternative to 6 months of isoniazid monotherapy. The use of rifapentine is currently not recommended in pregnant women by the WHO because of lack of safety data in this population [3].

Human milk is a complex matrix that consists of fats, proteins, carbohydrates, minerals, cells, and water [4]. The high fat content makes quantitative analysis challenging. Extensive sample clean-up is required to remove proteins and fatty components [5]. Drug transfer into human milk depends on many factors including lipid solubility, degree of ionization, volume of distribution, and protein binding [6]. High drug concentrations may lead to toxicity while sub-therapeutic drug concentrations may lead to the infant developing drug resistance. Clinical implications of some drugs excreted in human milk have been documented, e.g., the relative infant dose of ibuprofen was reported to be very low while the relative infant dose of lithium was reported to be relatively high (18–23%) [7,8]. Singh et al. reported isoniazid peak concentrations in human milk between 1.9 and 6.7 μg/mL and a relative infant dose of 1.2% [9].

The International Maternal Pediatric Adolescent AIDS Clinical Trial Network (IMPAACT) 2001 study (NCT02651259) was a Phase I/II, open-label, PK and safety study of 3HP among pregnant women with and without HIV, who had an indication for TB preventive therapy. An exploratory objective of IMPAACT 2001 was to determine how much rifapentine and 25-O-desacetyl rifapentine enters human milk from postpartum women receiving once-weekly rifapentine and isoniazid.

Rifapentine is metabolised by arylacetamide deacetylase to its less active metabolite 25-O-desacetyl rifapentine [10]. Rifapentine is known to be a potent inducer of CYP3A4 and a moderate inducer of CYP2C9 [11]. It interacts with warfarin, HIV-1 protease inhibitors

and reduces the efficacy of hormonal contraceptives [12,13]. Rifapentine was reported to significantly lower the area under the curve of bedaquiline and pretomanid [14,15].

The first step in determining whether or not rifapentine could provide potential benefit or harm to breastfeeding infants is to evaluate whether or not there are measurable concentrations in breast milk [16, 17]. To date, no assays have been developed to measure rifapentine and its 25-O-desacetyl metabolite in human milk. Olagunju et al. used a dried breast milk spots assay to quantify efavirenz [18]. However, many assays in other matrices such as plasma and dried blood spots have been published. An assay to measure rifapentine along with 13 other TB drugs in plasma has been reported by Lei et al., with a calibration range of 0.3–25.2 μg/mL [18]. Parsons et al. assayed rifapentine and its metabolite in dried blood spots where the concentration range of rifapentine and its metabolite were 50–80 000 ng/mL. Rifampicin-d3 was used as internal standard [19]. The limit of quantification of the human milk assay described here, were 2.00 ng/mL for rifapentine and 4.00 ng/mL for 25-O-desacetyl rifapentine.

A novel analytical method, based on liquid chromatography tandem mass spectrometry assay (LC-MS/MS) for the determination of rifapentine and 25-O-desacetyl rifapentine in human milk is described in this manuscript, as well as an application of the assay to measure rifapentine and 25-O-desacetyl rifapentine in human milk generated from the IMPAACT 2001 study.

2. Experimental

2.1. Collection of human milk samples and the application to a clinical study

Drug free human milk, donated by Milk Matters human milk bank, South Africa (HREC number 639/2019), was used to prepare test samples for method development, for the preparation of calibration standards (STDs) and quality control samples (QCs), and during validation procedures. Clinical samples were collected from women with and without HIV who were enrolled in IMPAACT 2001 and were continuing their rifapentine and isoniazid therapy in the post-partum period while breastfeeding their infants. Human milk was collected during the first weekly visit after delivery, three hours after the study drug dose; second weekly visit after delivery, six hours after the study drug dose; and 24 h after the study drug dose for the last dose visit.

The analytical method was employed for the analysis of human breast milk samples collected during a phase I/II trial of the pharmacokinetics, tolerability, and safety of once-weekly administered rifapentine and isoniazid in HIV-1-infected and HIV-1-uninfected pregnant and postpartum women with latent tuberculosis infection (IMPAACT 2001). Ethics Approval was granted by the University of Cape Town Faculty of Health Science Research Ethics Committee (HREC/REF: 373/2018). Each participant provided written informed consent.

2.2. Reagents and chemicals

Rifapentine reference material was donated by Sanofi (Bridgewater, New Jersey, United States) while 25-O-desacetyl rifapentine reference material and rifampicin-d3 were

purchased from Toronto Research Chemicals (North York, Ontario, Canada). Methanol and ascorbic acid were obtained from Sigma-Aldrich (Darmstadt, Germany), while 2-iso-propanol and formic acid were purchased from Merck (Darmstadt, Germany). Acetonitrile was purchased from Honeywell (Pittsburgh, USA). LC-MS/MS grade Millipore water was sourced in-house (Merck-Millipore, Germany).

2.3. Extraction procedure

Rifapentine and 25-O-desacetyl rifapentine were extracted from human milk by a combination of protein precipitation and SPE, with the protein precipitation portion being performed on ice. Human milk was thawed and vortexed for one minute, and 100 μL aliquoted into 1.5 mL microcentrifuge tubes. The appropriate working solution was added at 10 μL to the calibration standards and QCs, while 10 μL of blank solvent (methanol: water, 70:30, v/v) was added into blank, double blank and unknown samples. Methanol (250 μL) containing the internal standard, rifampicin-d3, at 250 ng/mL was added as a precipitation reagent, after which the sample was vortexed for 30 s and centrifuged for five minutes at 20 238 RCF. The supernatant (300 μL) was transferred into a 2 mL microcentrifuge tube containing 1.5 mL water and vortexed briefly. For SPE, 1 mL methanol was used to condition the C18 SPE Vac cartridges (Waters Sep-Pak, 50 mg), and 1 mL water for equilibration. The sample was loaded in two steps (900 μL added sequentially), eluting under positive pressure between the addition steps. The cartridges were washed with two volumes of 1 mL water: methanol (90:10, v/v) and two volumes 1 mL water: methanol (80:20, v/v). To elute the analytes, two volumes of 500 μL of methanol containing 50 μg/mL ascorbic acid was used. The eluent was dried under nitrogen at ~30 °C for approximately 30 min. The sample was reconstituted with 200 μL injection solvent comprising mobile phase A and B (45:55, v/v) and containing 20 μg/mL of ascorbic acid. Mobile phase A consisted of 0.1% formic acid in water: methanol (90:10, v/v) while mobile phase B was 0.1% formic acid in acetonitrile. Following vortex mixing for ~30 s, the samples were transferred to 96-well polypropylene plates and 5 μL injected for analysis.

2.4. Instrumentation

Reversed phase C18 columns have been used previously for the analysis of these analytes [18,20,21]. Different C18 columns were evaluated and a Poroshell 120 EC-C18 column (4.6 mm × 50 mm, 2.7 μm) at 30 °C gave the best resolution and separation. An Agilent 1200 HPLC (Agilent, CA, USA) was used. A sample volume of 5 μL was injected and the autosampler operated at ~8 °C. Chromatographic separation was achieved employing an isocratic mobile phase of water containing 10% methanol and 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) (45:55, v/v), at a flow rate of 450 μL/min for a run time of four minutes. A sample volume of 5 μL was injected and the autosampler was set to ~8 °C. Representative chromatograms of a subject sample are presented in Fig. 1.

Mass spectrometric detection of analytes eluting from the chromatographic system was performed on an AB Sciex API 4000 triple quadrupole mass spectrometer (AB Sciex™, Germany). Protonated ions of the analytes and internal standard was produced by electrospray ionization. Optimal ionization conditions were established by automated compound optimization and flow injection analysis (FIA). Optimal gas settings, employing

nitrogen as the nebulizing, turbo spray, curtain gas, and collision gas, were 50, 40, 30, and 6 psi, respectively. The optimal heated nebulizer temperature was 350 °C, the ion spray voltage was 5000 V, and the declustering and entrance potentials were 96 V and 10 V respectively, for both analytes. Optimal collision energy was found to be 31 eV, 21 eV, and 27 eV for rifapentine, 25-O-desacetyl rifapentine and rifampicin-d3 respectively, while optimal collision cell exit potentials were 42 V, 44 V, and 12 V for rifapentine, 25-O-desacetyl rifapentine and rifampicin-d3 respectively. Data acquisition was at unit resolution employing multiple reaction monitoring (MRM) of transition of the protonated precursor ions to the product ions at *m/z* 877.5–845.6 (Fig. 2A), *m/z* 835.4–803.5 (Fig. 2B), and *m/z* 827.4–795.6 for rifapentine, 25-O-desacetyl rifapentine and rifampicin-d3, respectively. The pause time between acquisition channels was set at 5 ms and dwell time at 80 ms. The instrument was controlled, and data captured and processed, by interfacing with a computer running Analyst® version 1.6.2 software (AB Sciex™, Germany).

2.5. Method validation

2.5.1. Preparation of calibration standards and quality controls—

Stock solutions of rifapentine and 25-O-desacetyl rifapentine were prepared in methanol at a concentration of 1 mg/mL. The stocks were diluted 1:1 with methanol to a concentration of 500 μg/mL and used to prepare working solutions by volumetric serial dilution with methanol: water (70:30, v/v) to the lowest working solution concentration (22.0 ng/mL and 44.0 ng/mL for rifapentine and 25-O-desacetyl rifapentine, respectively). Thirty-five microlitre aliquots of each working solution were stored in 1.5 mL microcentrifuge tubes at ~−80 °C. On the day of analysis, calibration standards were prepared by spiking 10 μL of each working solution into 100 μL of blank human breast milk to obtain concentrations of 2000, 1500, 750, 375, 180, 80.0, 35.0, 18.0, 8.00, 4.00, and 2.00 ng/mL for rifapentine, and at 2000, 1500, 750, 375, 180, 80.0, 35.0, 18.0, 8.00, and 4.00 ng/mL for 25-O-desacetyl rifapentine. Similarly, high, medium, low, and lowest limit of quantification (LLOQ) QCs (1600, 800, 5.00, 2.00 (for rifapentine) and 4.00 ng/mL (for 25-O-desacetyl rifapentine)) were prepared by spiking appropriate volumes of working solutions into blank human breast milk.

The accuracy and precision of the analytical method was validated across the full calibration ranges of both rifapentine and 25-O-desacetyl rifapentine. For this, three validation batches containing the QC samples in six-fold at high, medium, low, and at LLOQ, were analysed on three consecutive days to determine the intra- and inter-day accuracy and precision. The calibration curves were constructed using regressions of the peak-area ratio of rifapentine and 25-O-desacetyl rifapentine to the internal standard vs. nominal concentrations. Rifampicin-d3 was used as internal standard for both rifapentine and 25-O-desacetyl rifapentine.

2.5.2. Stock solution stability—

The stability of the stock solutions of rifapentine and 25-O-desacteyl rifapentine prepared in methanol was evaluated for four hours at room temperature and for ~68 days at ~−80 °C. This assessment was performed by diluting test and reference samples to 10 μg/mL in methanol and spectrophotometrically measuring the absorbance of the solutions in triplicate at 336 nm.

2.5.3. Working solution stability—Assessment of the stability of working solutions (WS) stored for ~24 h at ~−80 °C and on ice for 2 h, was done in both clear and amber tubes to also determine the effect of ambient light on the analytes. For this purpose, test WS's at different concentrations were diluted (5:95) and compared to the same concentrations of reference WS's prepared in injection solvent containing the internal standard. The assessments were made in six-fold and expressed as peak area ratios of analyte to internal standard as measured by LC-MS/MS using the validated analytical method.

2.5.4. Freeze-thaw stability—The stability of rifapentine and 25-O-desacetyl rifapentine under freeze-thaw conditions was assessed at two concentration levels by making use of low and high QC's (5.00 and 1600 ng/mL, respectively). The QCs were frozen and subjected to three consecutive freeze and thaw cycles. After freezing the QC's at ~−80 °C for at least 24 h, thawing was allowed for one hour at room temperature (~22 °C). The test QC's were analysed in six-fold with a freshly prepared valid calibration curve, using the validated analytical method, and the concentrations assessed for accuracy against the nominal QC concentrations.

2.5.5. Benchtop stability—The stability of rifapentine and 25-O-desacetyl rifapentine under normal working conditions on-bench was also assessed at low and high QC concentrations (5.00 and 1600 ng/mL, respectively). Preliminary stability assessments indicated degradation of both analytes when exposed to room temperature for prolonged periods. Therefore, the QC's frozen at ~−80 °C were thawed for one hour and then left on ice for 4.5 h (the ice was continually replenished). Analysis of the QC's were similar to the freeze-thaw stability assessment: in six-fold with a freshly prepared set of calibration standards to obtain the concentrations of the test QC's and compare these to the nominal concentrations.

2.5.6. On-instrument stability and reinjection reproducibility—The first validation batch was left in the autosampler at ~8 °C for the duration of the full validation, and then reinjected for analysis after ~24 and ~48 h. The peak area ratios of the reinjected high and low QC's (in six-fold) were then compared to those obtained during the first injection as an assessment of the stability of samples when kept in the autosampler and re-analysed approximately 24 and 48 h later.

2.5.7. Recovery—Peak area ratios of QC's at high, medium, and low concentrations (1600, 800, and 5.00 ng/mL, respectively) were compared to those of reference samples prepared by spiking extracted blank human breast milk at the expected QC concentrations. Recovery assessments were calculated by comparison of the QC's and reference samples in six-fold.

2.5.8. Matrix effects assessment—The method described by Matuszewski et al. [22,23] was used for the assessment of matrix effects. In short, aliquots from six different blank human breast milk sources were first extracted and the extracts then spiked with rifapentine and 25-O-desacetyl rifapentine at high, medium, and low concentrations (1600, 800, and 5.00 ng/mL, respectively) and with the internal standard at the same concentration for each (250 ng/mL). Necessary adjustments were made to compensate for dilution during

the extraction process. Using the resultant peak area ratios, a regression curve was generated for each blank source, and the differences in the gradients of the curves used to assess matrix effects.

2.5.9. Selectivity and carryover—The selectivity of the analytical method was assessed by the analysis of aliquots of six blank human breast milk sources, without the internal standard. Results were investigated to ensure that no endogenous components are present that could be mistakenly identified as rifapentine and 25-O-desacetyl rifapentine.

Carry over was assessed by inserting a double blank sample in the injection sequence immediately following the highest calibration standard. Results were then investigated for the presence of any analyte peaks in the double blank sample.

2.5.10. Effects of concomitantly administered medication—The first-line TB drug isoniazid and its metabolite, acetyl isoniazid, and antiretroviral drugs (efavirenz, nevirapine, lopinavir, and ritonavir) were spiked into high and low QC samples. These compounds were chosen since they were co-dosed during the associated clinical study, IMPAACT 2001. The relatively high concentration of 5 μg/mL for all the compounds were used, due to the lack of published data on the expected concentrations in human breast milk. The analyte/internal standard peak area ratios of the test samples were compared to those of high and low QC's (in six-fold) to calculate any quantitative differences.

3. Results and discussion

The calibration curves used for both analytes fit a quadratic (weighted by 1/x, x = concentration) regression across the respective concentration ranges of 2.00–2000 ng/mL for rifapentine and 4.00–2000 ng/mL for 25-O-desacetyl rifapentine. Representative calibration curves are presented in Fig. 3.

The combined accuracy (%Nom) and precision (CV%) statistics across all QC concentrations (N = 18) for rifapentine were between 97.4% and 100.6%, and 3.1% and 8.3%, respectively. For 25-O-desacetyl rifapentine the accuracy (%Nom) and precision (CV%) statistics were between 96.4% and 106.3%, and 6.7% and 11.8%, respectively. A summary of the combined accuracy and precision data of the three validation batches for all QCs is presented in Table 1.

Previously reported instability of rifapentine and 25-O-desacetyl rifapentine [19,21] has been compensated for by the addition of ascorbic acid during SPE and in the injection solution. Furthermore, protein precipitation was performed on ice. The results for the stability assessments are presented in Table 2. The stabilities of rifapentine and 25-O-desacetyl rifapentine at 1 mg/mL in methanol were tested at room temperature for four hours, and at ~− 20 °C and ~4 °C for 48 h. Percentage differences of less than 8.6% (for rifapentine) and 9.7% (for 25-O-desacetyl rifapentine) were found, with CVs(%) below 6.9% for both rifapentine and 25-O-desacetyl rifapentine, demonstrating short term stock solution stability. The stock solution of the internal standard was also prepared in methanol at 1 mg/mL and the stability thereof was tested on ice for four hours. A percentage

difference of 3.4%, with CVs (%) below 2.0%, demonstrated short term stock stability. Stock solutions of rifapentine and 25-O-desacetyl rifapentine at 1 mg/mL in methanol and stored at ~− 80 °C, were stable for up to 68 days, as proven by precision estimates (CV(%)) less than 3.0% and percentage differences, less than 7.0%.

Short-term working solution stability was proven for ~24 h at ~−80 °C and for up to two hours on ice at QC high and LLOQ working solution concentrations. The percentage difference between the peak area ratios of the reference and test solutions at the stated storage conditions, in clear and amber microcentrifuge tubes, were below 10% for rifapentine and 25-O-desacetyl rifapentine. This indicates that ambient light did not adversely affect rifapentine and 25-O-desacetyl rifapentine in working solutions prepared in methanol: water (70:30, v/v) over 2 h.

Freeze-thaw stability was proven over three cycles of thawing. The measured concentrations of the test QCs at high and low concentrations were all within 15% of the nominal concentrations. The percentage differences of both analytes, at both high and low QC levels, were less than 7% with CVs(%) below 11%. This proves that both rifapentine and 25-O-desacetyl rifapentine are stable in human breast milk on ice for at least 4.5 h.

The percentage difference between the initial and reinjected peak area ratios for rifapentine and 25-O-desacetyl rifapentine were greater than 15% for the low concentration of rifapentine after approximately 24 h, and greater than 15% for the high concentration for both rifapentine and 25-O-desacetyl rifapentine, and for the low concentration for rifapentine after approximately 48 h. Autosampler stability and reinjection reproducibility experiments indicate that no partial or entire batch reinjections may be performed for rifapentine and 25-O-desacetyl rifapentine and that the analytes are not stable in extracts for 24 and 48 h at ~8 °C. Dooley et al. covered the autosampler set at ~7 °C with foil to protect samples from light, which could have improved stability of the analytes in extracted matrix [24]. The effect of light on extracted samples was not tested for the current study.

Several extraction methods were evaluated, but initial protein precipitation extractions resulted in unacceptable ion suppression and resultant matrix effects, as well as poor recovery. A fatty residue was observed in samples that had been subjected to protein precipitation followed by liquid-liquid extraction using hexane, with large degrees of variability between samples from different lots of breastmilk tested. To overcome this, protein precipitation was used in conjunction with SPE to remove the maximum amount of endogenous matrix components. During SPE method development, the wash steps were optimized with as high a concentration of methanol as possible, without resulting in any elution of the analytes, to allow for the removal of these interfering substances as determined by evaluation of matrix effects.

The average percentage recovery across high, medium, and low concentrations of rifapentine and 25-O-desacetyl rifapentine was determined to be between 82.8% and 80.6%, with precision of between 1.3% and 4.2%. The final analytical method, combining protein precipitation and solid phase extraction, enabled extensive sample clean-up to remove proteins and fatty matrix components and proved to be reliable and selective. Applying

the assessment described by Matuszewski et al. [22,23], no significant matrix effects using human breast milk from six different sources was observed (Tables 3.1 and 3.2). The signal--to-noise ratio at LLOQ was above the minimum accepted criterion of 5 [25,26]. Fig. 4(A) and (B) show representative LLOQ with blank overlay chromatograms of rifapentine and 25-O-desacetyl rifapentine, respectively. This proves adequate sensitivity for the quantification of both analytes since the signal-to-noise ratio for both are well above the minimum accepted criterion of 5. The percentage accuracy for the LLOQ over the three validation days (N = 18) was 100.3% (CV(%) = 8.3) and 100.1% (CV(%) = 9.1) for rifapentine and 25-O-desacetyl rifapentine, respectively, well within acceptable limits. These figures furthermore graphically demonstrate selectivity for both analytes in the presence of the internal standard, since no peaks are present at the retention times of the analytes in the trace of the blank sample overlayed in the chromatograms. No carryover was observed during the validation.

The presence of concomitant medications at a concentration of 5 μg/mL had no significant influence on the resulting accuracy and precision estimates of rifapentine and 25-O-desacetyl rifapentine concentrations. The percentage difference across high and low levels were less than 3.7% and CVs(%) were below 6.5% when compared to the nominal concentrations for both rifapentine and 25-O-desacetyl rifapentine.

4. Application to a clinical pharmacokinetic study

The analytical method was used to successfully measure rifapentine and 25-O-desacetyl rifapentine concentrations in human breast milk samples in postpartum women taking the 3HP regimen. Rifapentine concentrations ranged from 11.2 to 1180 ng/mL while 25-O-desacetyl rifapentine concentrations ranged from 7.11 to 573 ng/mL (N = 22). Representative concentrations of rifapentine and 25-O-desacetyl rifapentine from human milk of IMPAACT 2001 participants are presented in Fig. 5. For context, median maternal plasma Cmin and Cmax values on dosing days were 1050 ng/mL and 27,400 ng/mL, respectively [27]. Since the study did not include all the information regarding daily intake of breastfeeding infants, no accurate concentrations of rifapentine and 25-O-desacetyl rifapentine to which such infants are exposed, can be presented here. The emphasis of the study is on the accuracy, robustness and therefore applicability of the analytical method to future studies which can address infant exposure more accurately. Accuracy (%Nom) of the quality control samples during clinical sample analysis were 100.1%, 95.2%, and 85.3% for rifapentine and 96.4%, 91.9%, and 87.8% for 25-O-desacetyl rifapentine at high (1600 ng/mL), medium (800 ng/mL) and low (5.00 ng/mL) concentrations, respectively.

5. Conclusion

Various methods were investigated to extract the analytes from the challenging human milk, with most of the results not being favourable when liquid-liquid extraction was used, even with highly non-polar solvents such as hexane. However, the current extraction procedure based on protein precipitation followed by SPE, resulted in a sensitive and reproducible analytical method for the quantification of rifapentine and 25-O-desacetyl rifapentine in human milk. An analytical method to quantify rifapentine and 25-O-desacetyl rifapentine

in human breast milk was developed and optimised to overcome matrix-related challenges and stability concerns surrounding the analytes. This method was successfully validated according to FDA and EMA guidelines [25,26] over the concentration ranges of 2.00 – 2000 ng/mL for rifapentine and 4.00 – 2000 ng/mL for 25-O-desacetyl rifapentine, and has proven to be robust and accurate when applied to matrix with variable protein and fat content from different individuals. The extraction process requires 100 μL of human milk and demonstrates good recovery of the analytes. The method has been applied to the analysis of samples from a clinical study, providing information on rifapentine and metabolite concentrations in human milk among women taking once weekly rifapentine for tuberculosis prophylaxis.

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References

[1]. WHO. Global tuberculosis report 2020: executive summary. 2020.

[2]. WHO. Tuberculosis in Women 2019 [cited 2019 July]. Available from: ⟨https://www.who.int/tb/publications/tb_women_factsheet⟩.pdf?ua=1.

[3]. WHO. Latent tuberculosis infection: updated and consolidated guidelines for programmatic management 2018 [Available from: ⟨https://www.who.int/tb/publications/2018/executivesummary_consolidated_guidelines⟩_ltbi.pdf.

[4]. Lawrence RA, Biochemistry of human milk. Breastfeeding, Elsevier, 2016, pp. 93–144.

[5]. Rezk N, White N, Bridges A, Abdel-Megeed M, Mohamed T, Moselhy S, et al. , Studies on antiretroviral drug concentrations in breast milk: Validation of a liquid chromatography–tandem mass spectrometric method for the determination of 7 anti-human immunodeficiency virus medications, Ther. Drug Monit 30 (5) (2008) 611. [PubMed: 18758393]

[6]. Fríguls B, Joya X, García-Algar O, Pallás C, Vall O, Pichini S, A comprehensive review of assay methods to determine drugs in breast milk and the safety of breastfeeding when taking drugs, Anal. Bioanal. Chem 397 (3) (2010) 1157–1179. [PubMed: 20386886]

[7]. Hale T, Abbey J, Baker T, Lactation pharmacology. Core curriculum for interdisciplinary lactation care, in: Campbell S, Lauwers J, Mannel R, Spencer B (Eds.), Interdisciplinary Lactation Care. Burlington, Massachusetts.: Jones & Bartlett Learning, 2019.

[8]. Riordan J, Wambach K. Breastfeeding and Human Lactation, third ed., Jones & Bartlett Learning, 2005.

[9]. Singh N, Golani A, Patel Z, Maitra A, Transfer of isoniazid from circulation to breast milk in lactating women on chronic therapy for tuberculosis, Br. J. Clin. Pharmacol 65 (3) (2008) 418–422. [PubMed: 18093257]

[10]. Nakajima AF, Kobayashi T, Watanabe Y, Nakajima A, Yokoi M, Human arylacetamide deacetylase is responsible for deacetylation of rifamycins: Rifampicin, rifabutin, and rifapentine, Biochem. Pharmacol 82 (2011) 1747–1756. [PubMed: 21856291]

[11]. Li AR, Rasmussen MK, Gorski A, Hall JC, Xu SD, Kaminski L, Cheng DL, Primary human hepatocytes as a tool for the evaluation of structure-activity relationship in cytochrome P450 induction potential of xenobiotics: Evaluation of rifampin, rifapentine and rifabutin, Chem. -Biol. Interact 107 (1–2) (1997) 17–30. [PubMed: 9402947]

[12]. Burman WG, K. Peloquin C Comparitive, pharmacokinetics and pharmacodynamics of the rifamycin antibacterials, Clin. Pharmacokinet 40 (5) (2001) 327–341. [PubMed: 11432536]

[13]. Baciewicz AC, Finch CR, Self CK, TH, Update on rifampin and rifabutin drug interactions, Am. J. Med. Sci 335 (2) (2008) 126–136. [PubMed: 18277121]

[14]. Winter HE E., Murray S, Erondu N, Ginsberg A, Rouse DJ, Severynse-Stevens D, Pauli E, Evaluation of the pharmacokinetic interaction between repeated doses of rifapentine or rifampin and a single dose of bedaquiline in healthy adult subjects, Antimicrob. Agents Chemother 59 (2) (2015) 1219–1224. [PubMed: 25512422]

[15]. Dooley KL, A.F. Park J-G Allen R Cramer Y Murray S Sutherland D Aweeka F Koletar SL Marzan F Bao J Savic R Haas DW; AIDS Clinical Trials Group A5306 Study Team. Phase I safety, pharmacokinetics, and pharmacogenetics study of the antituberculosis drug PA-824 with concomitant lopinavir-ritonavir, efavirenz, or rifampin, Antimicrob. Agents Chemother 58 (9) (2014) 5245–5252. [PubMed: 24957823]

[16]. Verstegen R, Anderson P, Ito S, Infant drug exposure via breast milk, Br. J. Clin. Pharmacol 128 (6) (2020) 795–804.

[17]. Ito S, Opioids in breast milk: Pharmacokinetic principles and clinical implications, J. Clin. Pharmacol 58 (2018) S151–S163. [PubMed: 30248201]

[18]. Olagunju A, Bolaji OO, Amara A, Waitt C, Else L, Soyinka J, et al. , Development, validation and clinical application of a novel method for the quantification of efavirenz in dried breast milk spots using LC-MS/MS, J. Antimicrob. Chemother 70 (2) (2015) 555–561. [PubMed: 25326089]

[19]. Parsons T, Marzinke M, Hoang T, Bliven-Sizemore E, Weiner M, Mac Kenzie W, et al. , Quantification of rifapentine, a potent antituberculosis drug, from dried blood spot samples using liquid chromatographic-tandem mass spectrometric analysis, Antimicrob. Agents Chemother 58 (11) (2014) 6747–6757. [PubMed: 25182637]

[20]. Lei QZ Y., Wang H, Zhou J, Lv X, Dang L, Zhu C, Simple and sensitive method for the analysis of 14 antituberculosis drugs using liquid chromatography/tandem mass spectrometry in human plasma, Rapid Commun. Mass Spectrom 34 (e8667) (2019) 1–8.

[21]. Winchester L, Podany A, Baldwin J, Robbins B, Fletcher C, Determination of the rifamycin antibiotics rifabutin, rifampin, rifapentine and their major metabolites in human plasma via simultaneous extraction coupled with LC/MS/MS, J. Pharm. Biomed. Anal 104 (2015) 55–61. [PubMed: 25481085]

[22]. Matuszewski B, Constanzer M, Chavez-Eng C, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC– MS/MS, Anal. Chem 75 (13) (2003) 3019–3030. [PubMed: 12964746]

[23]. Matuszewski B, Constanzer M, Chavez-Eng C, Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations, Anal. Chem 70 (5) (1998) 882–889. [PubMed: 9511465]

[24]. Dooley K, Bliven-Sizemore E, Weiner M, Lu Y, Nuermberger E, Hubbard W, et al. , Safety and pharmacokinetics of escalating daily doses of the antituberculosis drug rifapentine in healthy volunteers, Clin. Pharmacol. Ther 91 (5) (2012) 881–888. [PubMed: 22472995]

[25]. US_FDA, Draft guidance for industry: Bioanalytical method validation. US Department of Health and Human Services, Center for Drug Evaluation and Research, Rockville, MD, USA, 2018.

[26]. EMA European Medicines Agency: Guideline on bioanalytical method validation. 2011.

[27]. Mathad J, Savic R, Britto P, Jayachandran P, Wiesner L, Montepiedra G, et al. , Pharmacokinetics and safety of three months of weekly rifapentine and isoniazid for tuberculosis prevention in pregnant women, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am (2021).

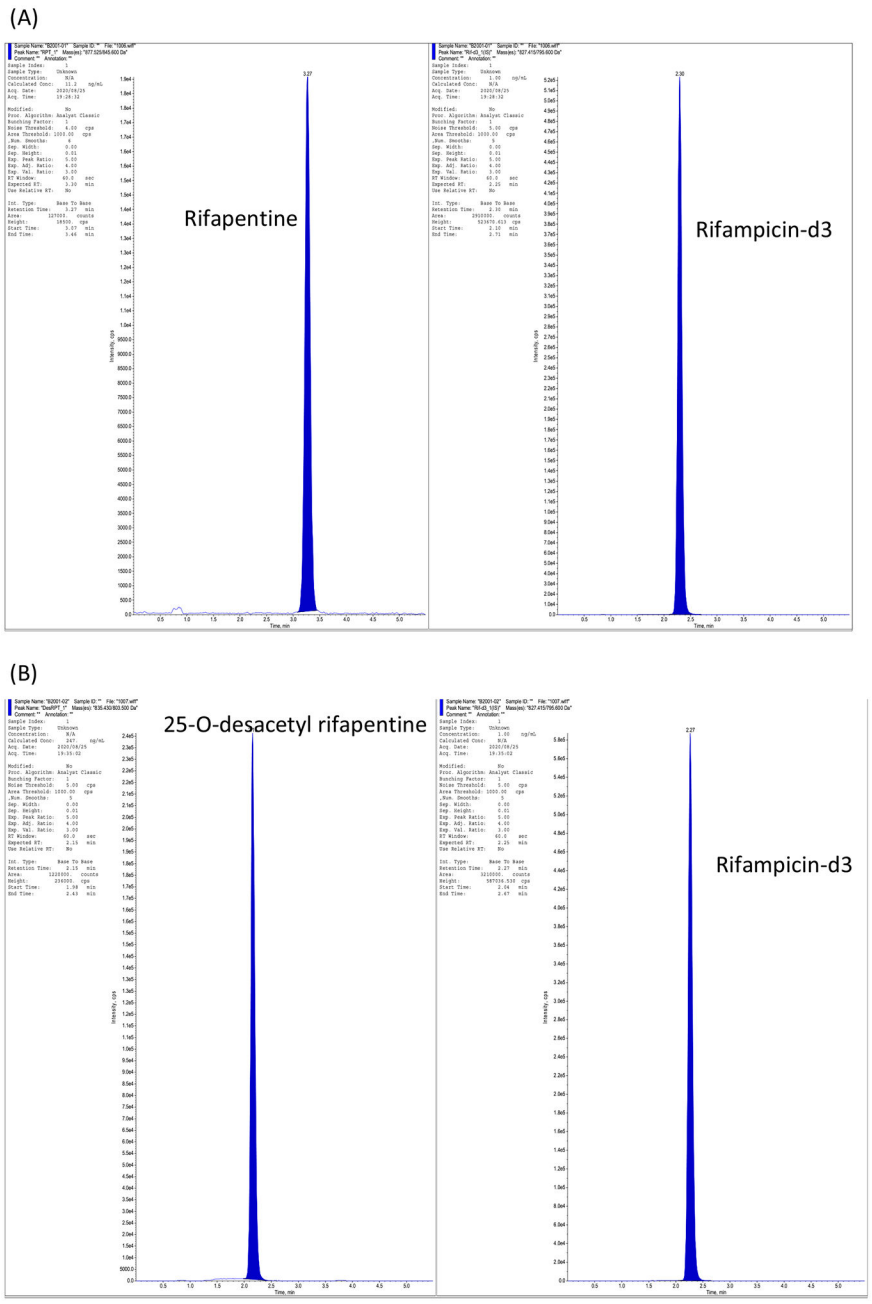

Fig. 1.

Representative chromatograms of rifapentine (A) and 25-O-desacetyl rifapentine (B) in human milk of an IMPAACT 2001 participant. The analyte chromatograms are shown on the left and the internal standard on the right.

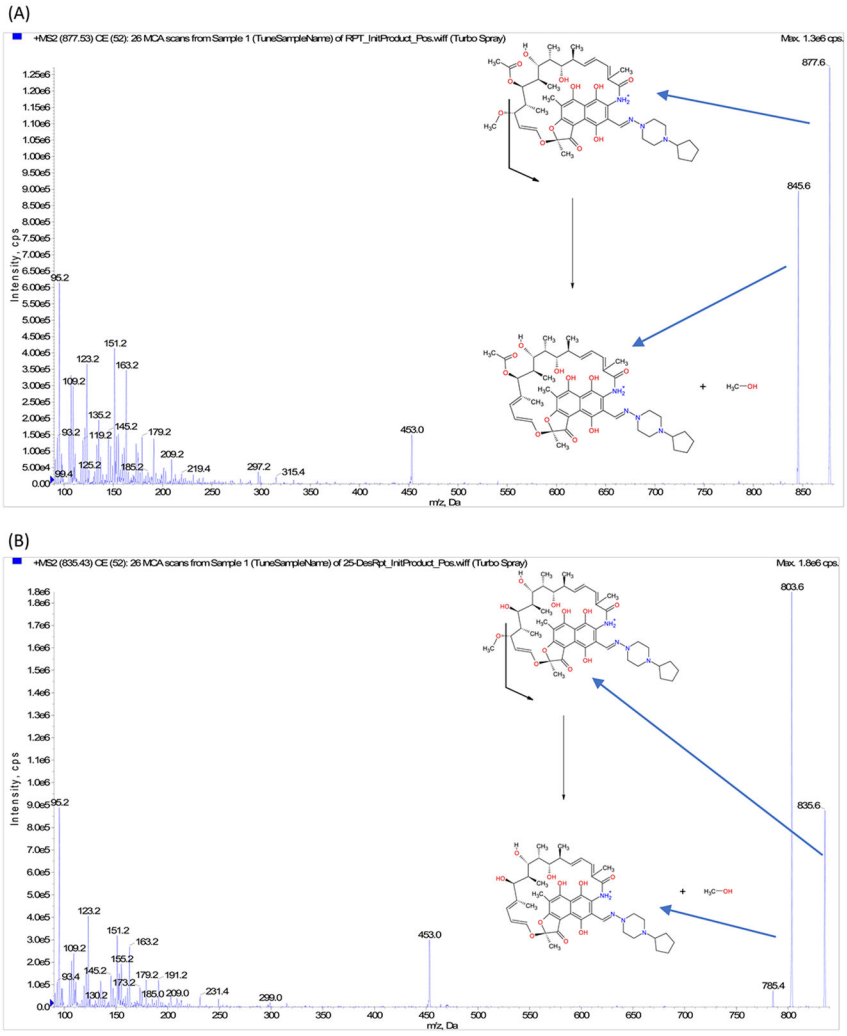

Fig. 2.

MS/MS spectra of (A) rifapentine and (B) 25-O-desacetyl rifapentine with the main fragment ions.

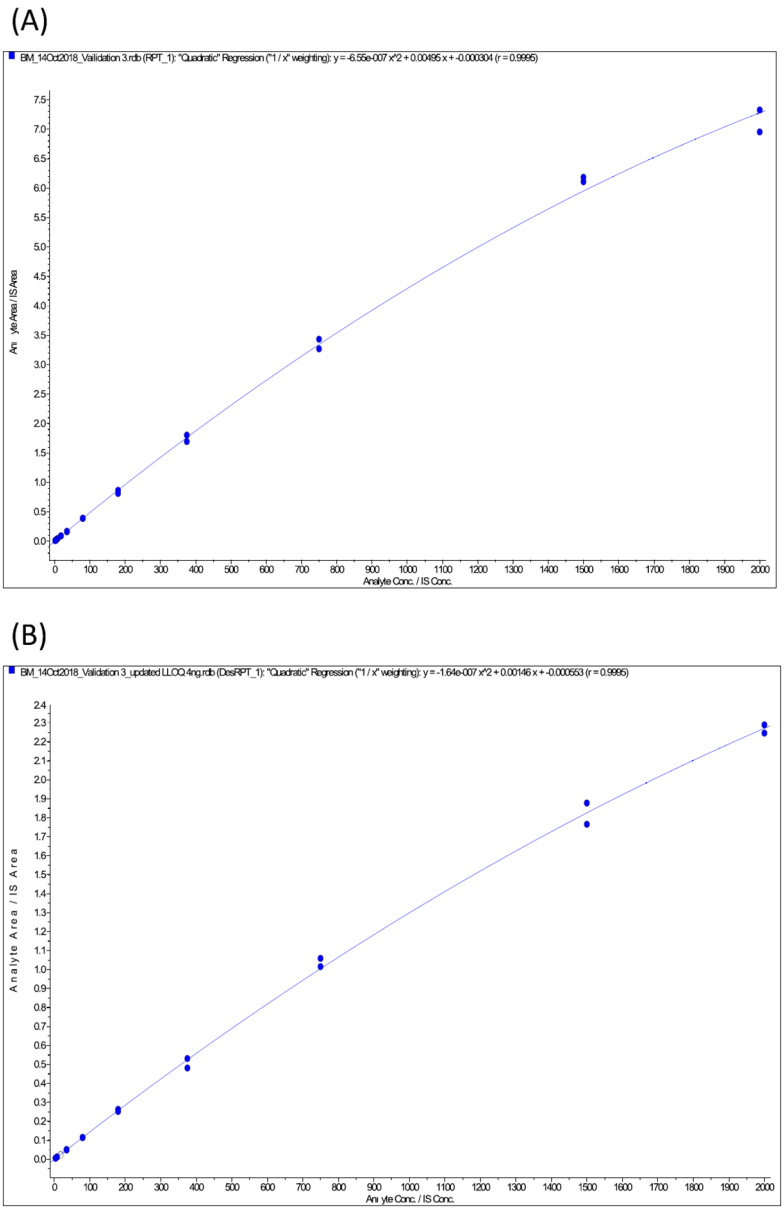

Fig. 3.

Representative calibration curves for rifapentine (A) and 25-O-desacetyl rifapentine (B) in human milk. The calibration curves range from 2.00 to 2000 ng/mL and 4.00–2000 ng/mL for rifapentine and 25-O-desacetyl rifapentine, respectively. They fit a quadratic regression weighted by 1/x, x = concentration. The regression equation is as follows $f(x) = ax^2 + bx + c$; rifapentine: $y = -6.65e\text{-}007\ x^2 + 0.00495x + 0.000304$ ($r = 0.9995$) and 25-O-desacetyl rifapentine: $y = -1.64e\text{-}007\ x^2 + 0.00146\ x + -0.000553$ ($r = 0.9995$).

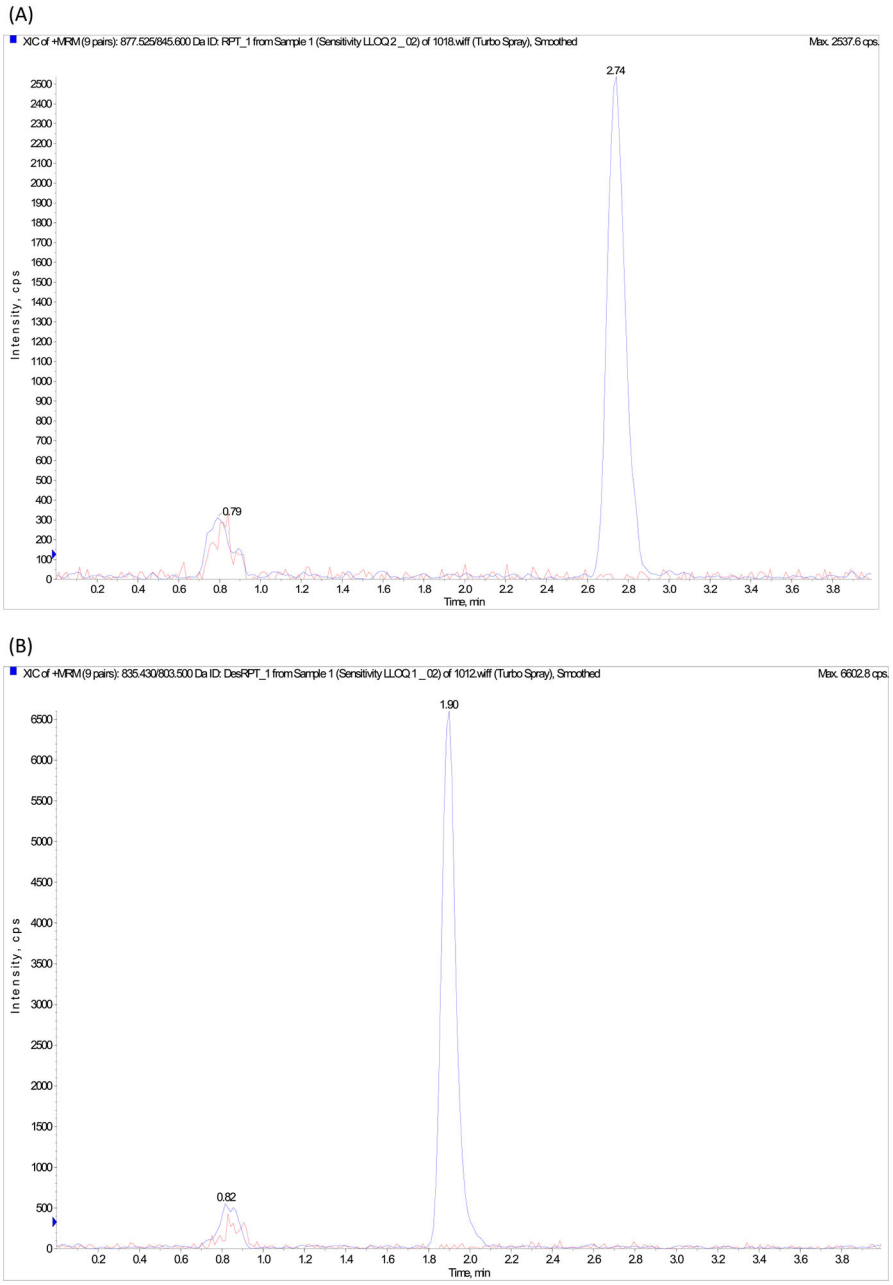

Fig. 4.

Raw LC-MS/MS chromatograms of rifapentine and 25-O-desacetyl rifapentine in human milk: (A) overlay of rifapentine LLOQ and blank, (B) overlay of 25-O-desacetyl rifapentine LLOQ and blank. The LLOQs are shown in blue and the blanks in red. The signal-to-noise ratio at LLOQ was 81.5 and 92.8 for rifapentine and 25-O-desacetyl rifapentine, respectively.

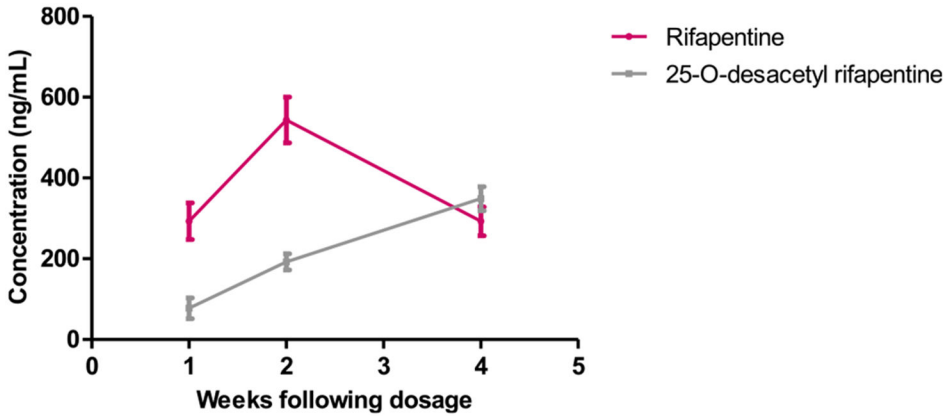

Fig. 5.

Representative concentrations of rifapentine and 25-O-desacetyl rifapentine in human milk vs. time profile of IMPAACT 2001 participants (N = 22). Data shows standard error of the mean for each week following dosage.

Table 1

Validation summary of rifapentine and 25-O-desacetyl rifapentine QCs.

	Rifapentine				25-O-desacetyl rifapentine			
	QCs							
	High	Medium	Low	LLOQ	High	Medium	Low	LLOQ
Nominal concentration (ng/mL)	1600	800	5.00	2.00	1600	800	5.00	4.00
Average	1586	804	4.87	2.01	1701	785	4.82	4.00
STDEV	56.1	24.9	0.277	0.166	113.7	74.6	0.567	0.365
CV(%)	3.5	3.1	5.7	8.3	6.7	9.5	11.8	9.1
%Accuracy	99.1	100.6	97.4	100.3	106.3	98.1	96.4	100.1
N	18	18	18	18	18	18	18	18

Table 2

Summary of stability results of rifapentine and 25-O-desacetyl rifapentine.

Rifapentine and 25-O-desacetyl rifapentine	
Stock solutions	In methanol: 68 days at ~80 °C
Working solutions	In methanol: water (70:30, v/v): 24 h at ~80 °C; on ice for 2 h
Freeze-thaw	3 cycles, thawed for one hour at room temperature
Benchtop	4.5 h on ice
On-instrument	No partial or entire batch reinjections may be performed

Table 3.1

Evaluation of matrix effects of rifapentine from six different human milk sources.

	High Concentration (1600 ng/mL) Peak Area Ratio	**Medium Concentration (800 ng/mL) Peak Area Ratio**	**Low Concentration (5.00 ng/mL) Peak Area Ratio**	**Area Ratio vs. Concentration Regression Slope**
Average	2.33	1.33	0.00887	0.00145
STDEV	0.104	0.0952	0.000578	0.0000651
CV(%)	4.5	7.2	6.5	4.5

Table 3.2

Evaluation of matrix effects of 25-O-desacetyl rifapentine from six different human milk sources.

	High Concentration (1600 ng/mL) Peak Area Ratio	**Medium Concentration (800 ng/mL) Peak Area Ratio**	**Low Concentration (5.00 ng/mL) Peak Area Ratio**	**Area Ratio vs. Concentration Regression Slope**
Average	1.81	1.11	0.00754	0.00113
STDEV	0.0216	0.0192	0.000282	0.0000135
CV(%)	1.2	1.7	3.7	1.2