Pharmacokinetics of Ketamine in Lactating Women

by

Aditi Dhananjay Shahane

Bachelor of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, 2020

Submitted to the Graduate Faculty of the

School of Pharmacy in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2022

UNIVERSITY OF PITTSBURGH

SCHOOL OF PHARMACY

This thesis was presented

by

Aditi Dhananjay Shahane

It was defended on

December 2, 2022

and approved by

Dr. Grace Lim, Associate Professor, MD, MS, Anesthesiology and Peri-operative Medicine, School of Medicine, Magee Women's Research Institute

Dr. Imam Shaik, PhD, Assistant Professor, Pharmacy and Therapeutics, School of Pharmacy

Dr. Raman Venkataramanan, PhD, Professor, Pharmaceutical Sciences, School of Pharmacy, Professor, Pathology, School of Medicine

Thesis Advisor: Dr. Raman Venkataramanan, PhD, Professor, Pharmaceutical Sciences, School of Pharmacy, Professor, Pathology, School of Medicine

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Aditi Dhananjay Shahane, MS

University of Pittsburgh, 2022

Ketamine is a unique dissociative non-opioid analgesic with a better side-effect profile than opioids. It is commonly prescribed peri-operatively for moderate to severe, acute, and chronic pain management. According to recent guidelines published in 2018, use of ketamine is to be avoided during pregnancy. This is due to lack of data regarding safety of ketamine in this patient population. However, there is an increasing interest in using ketamine for pain management using multimodal pain management strategy after cesarean deliveries. It is essential to confirm the safety and efficacy of ketamine in this patient population. Unfortunately, there is lack of data regarding maternal pharmacokinetics of ketamine during pregnancy and post-partum. Literature on drug exposure to the infant during lactation is also unavailable. In the current study, we aimed to gather more data on lactating maternal pharmacokinetics as well as the amount of drug that is excreted in human milk and is available for consumption by the infant.

A clinical study was performed to evaluate ketamine pharmacokinetics and assessing infant drug exposure when ketamine is intravenously administered to lactating women. Plasma and human milk samples were collected from 8 subjects at specific timepoints during and after continuous intravenous ketamine infusion at 0.05 - 0.1 mg/kg/hr for 12 hours (NCT04037085). An UPLC-MS/MS assay methodology was successfully developed and validated for measuring ketamine, norketamine and dehydronorketamine concentrations in human milk samples. Non-compartmental analysis (NCA) and two-compartmental modeling was performed using WinNonlin for ketamine to estimate various maternal plasma pharmacokinetic parameters. Two compartmental behavior of ketamine was confirmed. To assess the infant drug exposure, the Milk

to plasma ratio (M/P ratio) and % Relative Infant Dose (%RID) for ketamine were determined to be 3.64 and 0.0135% respectively. As % RID is < 10% threshold, low transfer of ketamine through human milk is expected. We conclude that it is safe to administer sub-anesthetic doses of short-term ketamine infusions in lactating women.

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Preface

I would like to express my deepest gratitude to my academic advisor and mentor, Dr. Raman Venkataramanan for his guidance and support throughout my masters. I am extremely grateful to have been his student and will cherish his academic teachings and life lessons throughout my life.

I would like to thank Dr. Grace Lim for serving on my committee and for her valuable inputs throughout the project. I would also like to thank Dr. Imam Shaik for his significant contributions towards this project and for teaching me the basics of bioanalytical research and pharmacokinetic analysis.

I would like to thank Wenchen Zhao for his contribution in analysis of plasma samples and for patiently training me in sample handling and LC-MS/MS operation. I would like to thank all the Venkat lab members for extending a helping hand whenever needed and creating a healthy work environment.

I am thankful to all my friends and my family all over the world for their unwavering faith in me. Most importantly, I would like to thank my parents, grandparents, and my brother for their unconditional love and endless support throughout my graduate journey.

1.0 Introduction

1.1 Cesarean Delivery

Cesarean delivery is a major surgical procedure for delivery of fetus [1]. Worldwide, C-section deliveries have increased from 7% in 1990 to 21% in 2021. One in every five of all childbirths use C-section for delivery [2]. Almost 25 million cesarean surgeries are performed every year worldwide. According to the latest report by Centre for Disease Control (CDC) in 2020, in the United States, 31.8 % of total births were by cesarean delivery [3]. Cesarean deliveries are thus, one of the very commonly performed surgical interventions in the United States.

1.1.1 Pain Management after C-section Deliveries

Pain following cesarean deliveries is one of the most unwanted clinical outcomes. In a study conducted by Eisenach, *et.al.*, each year nearly 500,000 women in the United States suffer from severe post-partum pain. Within tertiary care centers in the US, it was reported that one in five women after cesarean delivery and one in thirteen women after vaginal delivery experience severe acute pain. Severity of the pain was found to impact persistent pain, increasing the depression risk and has undesirable effects on routine activities and sleep as well. For ensuring proper care and good bonding with the newborn and getting back to activities of daily living, it is important for the mother to receive adequate care to manage post-partum pain [4]. Post-surgical recovery needs like breastfeeding and newborn care are also extremely important and can be adversely impacted if adequate analgesia is not maintained [5].

1.1.2 Difficulties associated with Pain Management

In clinics, opioids are primarily indicated for pain management owing to their analgesic and anesthetic properties. Unfortunately, they are also frequently abused because of their addictive euphoric effects and their use is continued to avoid severe withdrawal symptoms.

Opioid treatment is a double-edged sword where even though the drug is clinically effective, the adverse effects, tolerance and withdrawal symptoms associated with them must be managed with extreme caution. Unchecked use of opioids has resulted in the current 'opioid epidemic' in the United States. From 2010 to 2017, there was a 131% increase in the opioid-related diagnosis at delivery in women [6].

'Opioid Use Disorder' (OUD) is prevalent in all ages and genders. Unfortunately, pregnant women are not an exception. According to CDC's 2019 report, 7% of women reported using prescription opioid medication during pregnancy. 1 in 5 of these women stated that they obtained the opioids from a non-healthcare setting, or their use was not for pain relief [7].

Another point of concern when consuming or administering opioids during pregnancy is the possible long-term effects on maternal and child health. When the pregnant mother consumes opioids, the fetus can also be exposed to it which can lead to 'Neonatal Abstinence Syndrome (NAS) in the infant. Children suffering from NAS have to begin treatment immediately after birth.

Use of 'multimodal approach' is needed to ensure that opioid consumption by the patient is minimized. Adjunctive analgesic medications such as paracetamol or NSAIDs can help reduce the opioid dose and consequently the adverse effects associated with opioid use.

1.1.3 Multimodal Approach for Pain Management in C-section

The Procedure-Specific Postoperative Pain Management (PROSPECT) initiative provides recommendations for pain management strategies for common yet painful surgeries. Peri-operative and post-operative pain management for cesarean surgeries have separate guidelines which was published in 2020. This guideline recommends a multimodal approach for pain management which makes use of combination of two or more analgesics in combination with specific surgical procedures to maximize pain alleviation [8].

Before delivery, the mother is administered epidural or intrathecal long-acting opioid (morphine or dimorphine) along with paracetamol for pain relief. For management of intra-operative post-delivery pain, intravenous paracetamol, intravenous NSAIDs, intravenous dexamethasone along with regional anesthesia techniques (in case intra-thecal morphine has not been administered) are recommended. Post-partum or post-delivery pain management recommendations include oral/intravenous paracetamol with oral/intravenous NSAIDs, and using opioids as rescue option [9].

1.1.4 Physiological Changes during Pregnancy and its effect on Pharmacokinetics

Body composition and physiology changes immensely during pregnancy as well as post-partum. These physiological changes affect the pharmacokinetic parameters during absorption, distribution, metabolism, and elimination of drugs [10].

1.1.4.1 Absorption

Absorption can be defined as the movement of a drug from the site of administration into the blood circulation. Gastric pH increases during pregnancy, which can change drug ionization and affect absorption of certain drugs. Decrease in intestinal motility is observed during pregnancy which could lower drug absorption and bioavailability. However, increased blood flow and cardiac output in pregnancy could lead to higher extent of absorption. Gastrointestinal changes have not been documented to significantly change drug absorption during pregnancy when drug is given orally [11].

1.1.4.2 Distribution

Distribution refers to the characteristic of the drug to go to different sites within the body from systemic circulation.

Cardiac output, stroke volume increases during early pregnancy and remains high until delivery. The total body water and body fat increases during pregnancy as well, which could result in higher volume of distribution for hydrophilic drugs and lipophilic drugs respectively [12, 13]. Additionally, uterine blood flow increases during pregnancy and additional feto-placental compartment is added as well. This increases the apparent volume of distribution and can result in drug accumulation [11].

Serum albumin and alpha-1-acid glycoprotein levels decrease during pregnancy, which could lead to increased free fraction of drug in the blood and lead to increased drug distribution [14, 15].

1.1.4.3 Metabolism

Drug metabolism can be defined as chemical biotransformation of the administered drug by various enzymes to a more hydrophilic molecule to facilitate its elimination. These metabolic enzymes are present in different locations within the body like blood, gut, intestine, liver, kidney, and placenta.

Activity of certain hepatic metabolic enzymes like CYP3A4, CYP2A6, CYP2D6, CYP2C9 and UGT1A4 increase, while CYP1A2 and CYP2C19 activity appears to decrease during pregnancy. In such situations, dose adjustments might be necessary [11, 16-19].

1.1.4.4 Excretion

Irreversible removal of drugs from the body is termed as excretion. Kidney is one of the major organs responsible for excretion of drugs. Renal excretion through urine depends on glomerular filtration rate, tubular secretion and reabsorption. Pregnancy results in an increase in the GFR, which leads to increased clearance of certain drugs like ampicillin and sulbactum [20, 21].

1.2 Ketamine

1.2.1 Ketamine Pharmacology

Ketamine is a cyclohexanone derivative represented as (2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one). Ketamine (CI-581) was first synthesized by Calvin Stevens (Parke-Davis and Co.) in 1962 during the search for a substitute for phencyclidines. Ketamine

reported lesser adverse effects as compared to phencyclidines and hence was introduced clinically in 1970 [22]. Physicochemical properties of ketamine are summarized in the Table 1 [23]. Currently ketamine is commonly used in clinical practice as an analgesic and anesthetic.

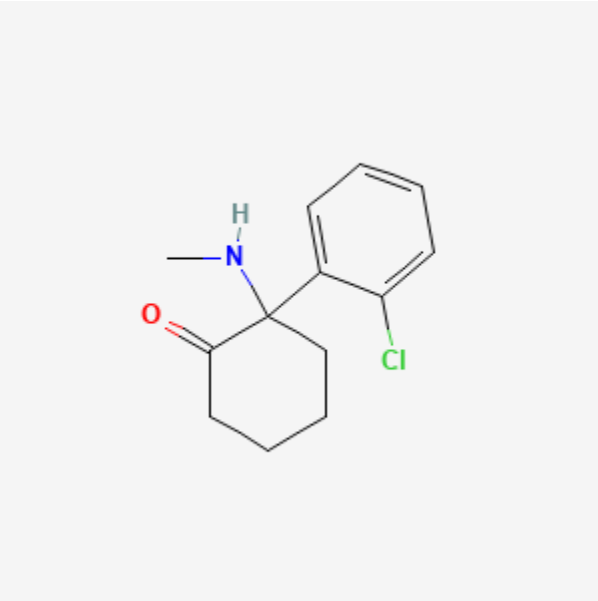


Figure 1 Structure of ketamine

Table 1 Physicochemical properties of ketamine

Sr. No.	Physicochemical Property	Value
1.	Molecular weight	237.72 M
2.	Molecular Formula	C13H16ClNO
2.	Color and Appearance	White crystals
3.	Solubility	Solubility in water (20g/100mL), Freely soluble in Methanol and Alcohol.
4.	Melting Point	262 – 263 °C
5.	Log P	3.2
6.	pH (10% aq. solution)	3.5
7.	pKa	7.5
8.	Isomers	S-ketamine and R-ketamine

Due to the unique solubility and Log P profile of ketamine, it can be administered by various routes – intravenous (bolus, infusion), intramuscular, intranasal, rectal, and as an elixir. It crosses the blood brain barrier and results in CNS depression.

Another distinctive property of ketamine is its chirality. The C_2 atom in the molecule produces two stereoisomers. These two enantiomers (shown in Figure 2) show similar chemical and physical properties. However, their affinities towards the drug receptors (which are also optically active)

are dissimilar which makes them clinically different. Higher affinity of S(+)ketamine toward specific receptors makes it 3 – 4X more potent analgesic than R(-)ketamine and racemic mixture [22].

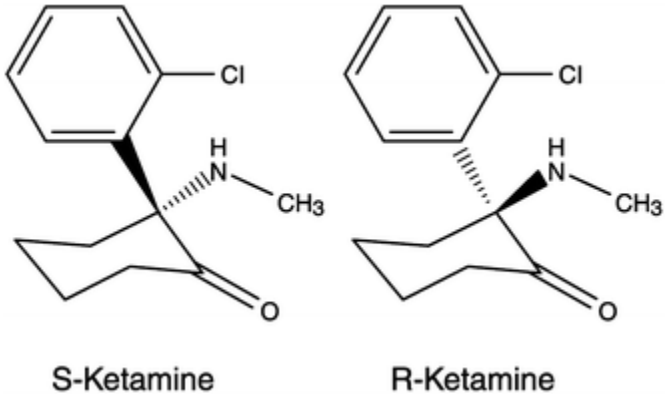


Figure 2 Enantiomers of ketamine : S(+)ketamine and R(-)ketamine

Analgesic and anesthetic activity of ketamine is because of glutamate receptors antagonism, non-competitive inhibition of NMDA receptors, opioid receptor activation, nicotinic and muscarinic acetylcholine receptors inhibition and sodium channel blocking. Analgesic activity of ketamine occurs primarily due to its antagonistic activity on NMDA receptor with a minor contribution from opioid receptor agonism. The anesthetic activity is seen due to its effect in blocking of the sodium channels.

Amnesia, a well-known side-effect of the drug, is possibly due to its activity on muscarinic receptors.

Ketamine differs from other anesthetic agents as it preserves the respiratory function, increases blood pressure and heart rate due to its sympathomimetic effects. Additionally, does not impact gut motility. Thus, as compared to opioids, where there is a risk for respiratory and cardiovascular

depression, ketamine has a better side-effect profile. It is also preferred in asthmatic and hemodynamically unstable patients.

Ketamine is characterized by a wide therapeutic range and overdose due to death is very unlikely. Adverse effects like hypertension, tachycardia, increased pulmonary pressures have been reported due to sympathomimetic activity of ketamine. The drug also has been recreationally abused since 1970's due to its psychedelic effects like induction of a dream-like state, hallucinations, changed perception of time, feelings of invulnerability, etc. [22].

1.2.2 Pharmacokinetics of Ketamine

In 1975, the first clinical study on pharmacokinetics of ketamine in man was published. Ketamine is an old drug and the pharmacokinetic profile of the drug in healthy adults has been well-researched.

When given intravenously, ketamine showed a two-compartment behavior with distribution half-life of ~10 minutes and elimination half-life of ~2.5 hours [24]. The volume of distribution and clearance in healthy adults was reported to be 3 L/kg and 15-20 ml/kg/min respectively. Plasma protein binding of ketamine was reported to be 20-50%.

Ketamine shows immediate onset of action (30 sec) when given intravenously, within 1-5 min intramuscularly, 5 – 10 min intranasally, and 15 – 20 min when administered orally. Upon extravascular administration, its bioavailability varies. Intramuscular injection has a bioavailability of 93%, intranasal has 25-50%, however oral bioavailability is reported to be 20-25% [25].

In mass-balance studies conducted in healthy adults, the five-day average recovery of tritium labelled ketamine was found to be 91% in urine and 3% in feces [26]. Ketamine is

metabolized in the liver; 2% of the drug is excreted unchanged through urine, 2% is excreted in as norketamine, 16% as dehydronorketamine and 80% as glucuronic acid conjugates. It undergoes N-demethylation by CYP3A4 and minor contributions by CYP2B6 and CYP2C9 to generate the major biologically active metabolite - norketamine [27]. Norketamine is further converted to hydroxynorketamine by CYP2B6, and this can undergo further non-enzymatic conversion to dehydronorketamine. Both, hydroxynorketamine and dehydronorketamine have been proven to be active only preclinical studies [28]. Norketamine and hydroxynorketamine are further converted into glucuronide conjugates [29, 30]. Detailed pathway of ketamine metabolism is depicted in Figure 3.

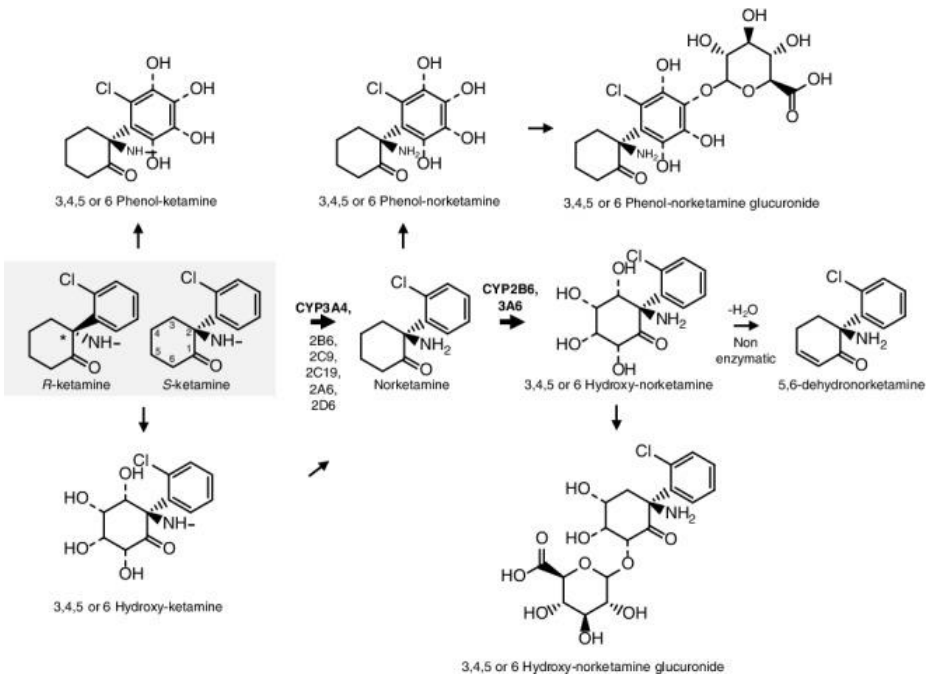


Figure 3 Metabolism pathway of ketamine

1.2.3 Ketamine Indications for Pain Management

In 2018, the American Society of Regional Anesthesia and Pain Medicine (ASRA), the American Academy of Pain Medicine (AAPM), and the American Society of Anesthesiologists (ASA) published guidelines for acute and chronic pain management using ketamine.

The recommendations are briefly summarized as -

1. Peri-operative use of subanesthetic doses of intravenous bolus and infusion ketamine for procedures associated with severe post-operative pain.
2. Recommended as a peri-operative adjunct analgesic for opioid tolerant patients.
3. Use of ketamine infusions for short-term pain management of Chronic Regional Pain Syndrome (CRPS) based on moderately available clinical evidence.
4. Patients experiencing mild pain are not benefited from ketamine therapy.

   Ketamine use is to be avoided in pregnancy, severe hepatic impairment, severe cardiovascular disease, psychosis and patients with increased intracranial pressure [31-33].

1.2.4 Potential of Ketamine as Adjuvant Analgesic during Cesarean Delivery and Post-partum

Currently, use of ketamine as an adjuvant analgesic for intra-operative pain alleviation is not recommended due to limited evidence of its benefits, concerns for side-effects and influence on mother-child bonding [9]. Some of the concerning side-effects of ketamine include nausea, vomiting, dizziness, hallucinations, bad dreams, and blurred vision [34].

A randomized double-blind placebo-controlled trial was conducted in healthy women to evaluate the efficacy of low-dose ketamine in multimodal post-cesarean analgesia. No additional benefit of using low-dose ketamine along with bupivacaine, morphine or fentanyl was found in the subjects. However, a lower pain score two weeks post-delivery was reported by subjects who received ketamine [35].

Another randomized study conducted in Turkey by Caliskan, *et.al.* explored the effect of persistent low-dose ketamine and fentanyl as adjuvants to bupivacaine spinal anesthesia treatment in cesarean delivery. Adjuvant low-dose IV ketamine provided a longer lasting analgesic effect post-operatively as compared to fentanyl. Additionally, consumption of analgesics was also lower in ketamine group as compared to other groups [36, 37].

1.2.5 Ketamine Pharmacokinetics during Pregnancy and Post-partum

The earliest study evaluating safety of ketamine in mothers and their infants during labor and delivery was published in 1972. 14 pregnant women and 18 control non-pregnant women were enrolled in this study. Two doses of ketamine were studied. An IV bolus loading dose of 2.2 mg/kg or 1.5 mg/kg followed by a continuous infusion of 0.11 mg/kg/min or 0.08 mg/kg/min respectively was administered to expectant mothers. Maternal and fetal blood samples were analyzed for ketamine and its metabolites. 60% of the ketamine maternal plasma concentration was observed in fetal cord blood. Thus, placental transfer of ketamine during labor and delivery has been confirmed [38].

Subsequent studies evaluating use of ketamine during pregnancy for its analgesic and anesthetic potential were conducted, but the primary aim of these studies was to determine efficacy and monitor side-effects and adverse effects [39].

There is still a lack of data documenting ketamine pharmacokinetics during pregnancy. Additionally, no data is available evaluating ketamine transfer through maternal milk to the infant during lactation.

1.3 Proposed Clinical Study to study Ketamine Pharmacokinetics in Lactating Women

A prospective, open label, interventional, clinical study was conducted in women weaning from breastfeeding. The study was conducted at University of Pittsburgh Medical Centre, Montefiore – Clinical and Translational Research Centre and was approved by University of Pittsburgh IRB (STUDY 18120046) and National Institute of Health (NCT04037085). Eight lactating women volunteers received 0.03 – 0.1 mg/kg/hr ketamine infusion for 12 hours. Infusions were started at 0.1 mg/kg/hr (max: 8mg/hr) with delays for 1 hour if side effects were reported, and resumption of the infusion at 0.05 mg/kg/hr (4mg/hr if started at max 8mg/hr) after full symptom resolution (Figure). This dosing resulted in limited side effects (lightheadedness, euphoria) and no serious adverse events.

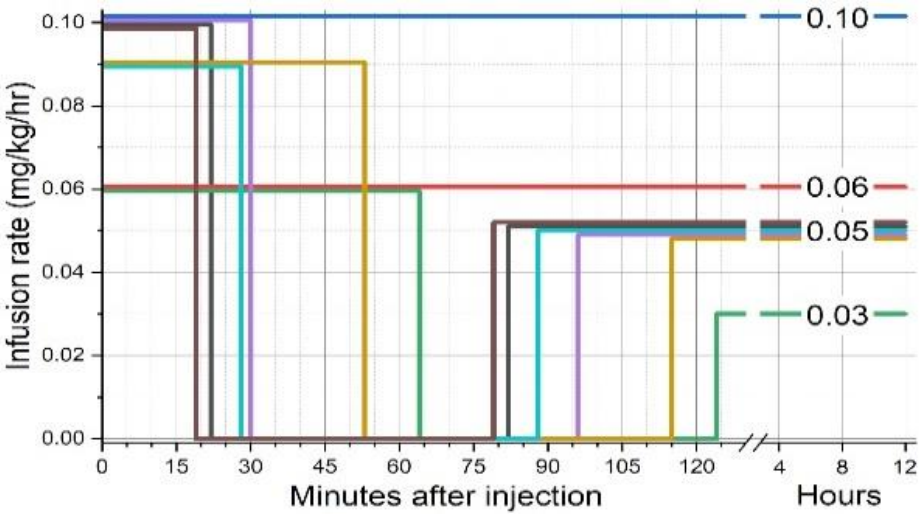


Figure 4 Infusion rates over time of each lactating volunteer (N=8) receiving ketamine

Maternal blood samples (~2mL) were collected at 8, 10, 12,12.5, 13, 14, 16 and 20 hours after start of infusion. Plasma was separated out, aliquoted and stored at -80°C until analysis. The entire human milk expressed by the subjects was collected from the start of the infusion until 12 hours after stopping the infusion. Time and volume of milk samples collected was noted and samples were frozen until analysis. Safety parameters were monitored during the entire study duration.

2.0 Development of UPLC-MS/MS Assay Method for Simultaneous Quantification of Ketamine and its metabolites – Norketamine and Dehydronorketamine in Human Milk.

A UPLC-MS/MS assay methodology was developed and validated for quantifying ketamine, norketamine and dehydronorketamine concentrations in the human milk samples obtained from the 8 post-partum women in the clinical study. FDA's Bioanalytical Method Validation Guidance [40] was followed for developing and validating the assay.

2.1 Chemicals and Reagents

Stock solutions of +/- ketamine hydrochloride, +/- norketamine hydrochloride, dehydronorketamine hydrochloride, ketamine-D4 hydrochloride and norketamine-D4 hydrochloride were purchased from Cerilliant (St. Louis, MO). LC-MS grade acetonitrile and LC-MS grade water were obtained from Fischer Scientific (Fair Lawn, NJ, USA). Blank human milk was procured from Mid-Atlantic Mothers Milk Bank (Pittsburgh, PA, USA).

2.2 Standards

The primary stock solutions for 1 mg/mL +/- ketamine hydrochloride (Ket) and +/- Norketamine hydrochloride (Norket) were prepared in methanol and the stock solution for 100 ug/mL Dehydronorketamine hydrochloride (DHNK) was prepared in acetonitrile. The internal

standard primary stock solutions of 100 ug/mL ketamine-D4 hydrochloride and 100 ug/mL +/- Norketamine-D4 hydrochloride were prepared in methanol. These solutions were obtained from Cerilliant (St. Louis, MO). The working stocks for standards and quality control samples were prepared by diluting these primary stock solutions. A working stock solution containing mix of ketamine HCl 1 ug/mL and norketamine HCl 1 ug/mL and dehydronorketamine HCl 0.1 ug/mL was prepared. The working solution of internal standard mix containing 5 ng/mL of each internal standard was prepared in acetonitrile. Stocks of calibration curve standards and quality control samples were prepared by spiking blank human milk with known volume of working solution using serial dilution and stored as aliquots and then used for preparing standard curves. The concentration range for the calibration curves for ket and norket was 1 – 100 ng/mL, and for DHNK was 0.1-10 ng/mL. Four quality controls (QC) 2, 20, 40 and 80 ng/mL concentrations were selected for ket and norket over the range of the standard curve. The QC concentrations were 10-fold lower for DHNK. The working standards, spiked milk standards and quality control samples were stored at -80°C. The working stock for internal standard solution was stored at 4°C.

2.3 Sample Preparation

To 50µL of blank human milk 450µL of Acetonitrile containing 5ng/mL internal standard solution was added. The contents were thoroughly vortexed and then centrifuged at 21,000g for 12 mins at 10°C. 450µL of resultant supernatant was transferred to a clean glass tube and dried under air stream. The dried contents were reconstituted into 100µL initial mobile phase. The solution was centrifuged again at 21000g for 12 mins at 10°C and the supernatant was transferred

to total recovery vials. 3µL was injected onto the UPLC column and analyzed using a mass spectrometer.

2.4 Chromatographic Conditions

Waters Acquity UPLC system equipped with Acquity UPLC BEH RP18 1.7 µm, 2.1 × 100 mm column, combined with a Waters guard column (289002078) was used for separation of analytes. The LC method consisted of a gradient mobile phase of solvent A (95% 2mM ammonium acetate + 5% acetonitrile + 0.1% formic acid) and solvent B (95% acetonitrile and 5% water with 2mM ammonium acetate and 0.1% formic acid) for elution of the analyte. The gradient started at 100% of solvent A which was held for 1.0 minute and then changed to 80% solvent A and 20% solvent B which was kept constant for the next 3 minutes. The solvent composition then changed to 65% solvent A and 35% solvent B over 0.1 minute and then to 30% solvent A and 70% solvent B which was maintained for 1 minute. The gradient then returned to the initial solvent condition of 100% solvent A, achieving the initial base line. The total run time was 7 minutes, and the flow rate was set to 0.3 mL/min.

2.5 Mass Spectrometric Conditions

The separated analytes were analyzed using Multiple Reaction Monitoring (MRM) on Waters Zevo TQ-S Mass Spectrometer (Waters, Milford, MA, USA) in positive electron spray

ionization mode. The settings for this instrument were as follows: capillary voltage 1.5kV, desolvation temperature 500°C, cone gas flow 150 L/h, desolvation gas flow 1000 L/h; argon pressure 0.15 mL/min, and nitrogen pressure 7 bar. Details of the MS conditions for the analytes are presented in Table 2. MassLynx software version 4.2 was used for operating the instrument, to collect and process the data.

Table 2 The cone voltage and collision energy settings in UPLC-MS/MS for ketamine, norketamine, dehydronorketamine, ketamine-d4 (IS) and norketamine-d4 (IS).

	Parent m/z	Daughter m/z	Dwell (sec)	Cone Energy (V)	Collision Energy (V)
ketamine	237.8405	124.994	0.038	60	26
NK	223.900	125.010	0.038	48	22
DHNK	221.900	141.830	0.038	54	24
ketamine-D4 (IS)	241.900	128.910	0.038	66	20
NK-D4 (IS)	227.900	128.960	0.038	50	22

2.6 Bioanalytical Method Validation

2.6.1 Standard Curve Linearity

Blank human milk spiked with working standard solutions were used for preparing the standards for the calibration curve and the quality controls. The concentration range used for ketamine and norketamine was 1 - 100 ng/mL and for dehydronorketamine was 0.1 - 10 ng/mL. The response for a sample was calculated as the ratio of peak area of the analyte to the peak area

of internal standard. Response of the sample on y-axis was plotted against the theoretical concentration value on x-axis for each analyte separately. The slope, intercept, and co-relation co-efficient (R^2) of standard curve was obtained by performing linear regression analysis with a weighting of 1/x. Standard concentrations within ≤ 15 % deviation as compared to theoretical value were accepted. For the LLOQ, ≤ 20 % deviation was termed acceptable.

2.6.2 Lower Limit of Quantification

LLOQ was set to be the lowest concentration of the analyte giving a response 5 folds higher than the response from blank sample. A deviation of ≤ 20 % from the theoretical value was termed acceptable.

2.6.3 Accuracy and Precision

The accuracy and precision of the assay were determined using the quality control samples of concentrations 2, 20, 40, and 80 ng/mL. Samples were run in triplicates for calculating the variability of the response within the same day or the intraday variation. The samples were also injected on five different days to calculate the inter-day variation.

Accuracy of the assay method was calculated as % difference of the measured concentration from the theoretical concentration.

$$\% \, Deviation = \left[\frac{(ConcM - ConcT)}{(ConcT)}\right] x \, 100$$

Where;

ConcM = Measured concentration,

ConcT = Theoretical concentration

The precision of the assay method was reported as % co-efficient of variation (% CV).

$$\%\ Coefficient\ of\ Variation = \frac{(Standard\ Deviation)}{(Mean)}\ x\ 100$$

2.6.4 Recovery

The recovery of each analyte in QCs with concentrations of 2, 20, 40 and 80 ng/mL was computed by comparing the peak area from neat samples to the peak area of the analyte when it was spiked in the human milk before extraction. Recovery of IS was also simultaneously checked at a single concentration of 5 ng/mL. Results were expressed as % Recovery.

2.6.5 Matrix Effect

For evaluating the matrix effect, the peak area of the analyte in the solvent acetonitrile (neat sample) was compared to the peak area of the analyte samples spiked with known concentration of analyte post extraction. Four quality control samples with concentrations 2, 20, 40 and 80 ng/mL and 5 ng/mL IS each were analyzed. Results were expressed in terms of % matrix effect (% ME).

$$\%\ Matrix\ Effect\ (\%\ ME) = \frac{(Response\ from\ matrix)}{(Response\ from\ neat)}\ x\ 100$$

2.6.6 Stability

The stability of ketamine, norketamine and dehydronorketamine in human milk was evaluated at four concentrations 2, 20, 40 and 80 ng/mL (n=3) under three different conditions. The milk samples spiked with known concentrations of drug mixture mentioned above were stored for 24 hours at room temperature, 4°C and frozen at - 80°C and then thawed at room temperature. Freshly prepared standard curve and quality control samples were used for comparison.

2.7 Results

2.7.1 Mass Spectral Analysis

The five analytes; ketamine, norketamine, dehydronorketamine, ketamine-d4 and norketamine-d4 were infused into the mass spectrometer in the + ESI mode and the mass-to-charge transition ratios (*m/z* ratio) from parent molecule to daughter ion were noted. The instrument settings were optimized and set to 1.5 kV capillary voltage, 500 °C desolvation temperature, 150 L/h cone gas flow, 1000 L/h desolvation gas flow, 0.15 mL/min argon gas pressure and 7 bar nitrogen gas pressure.

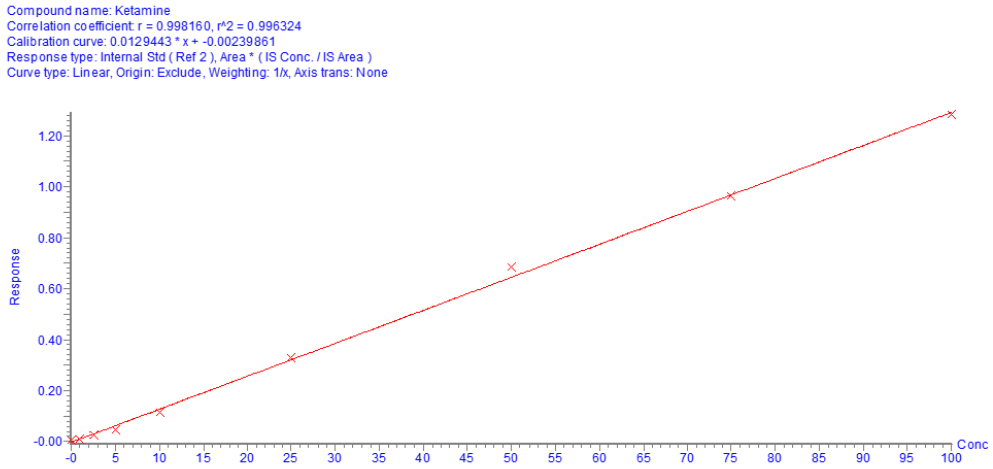
2.7.2 Separation and Relative Retention Time

The retention times for ketamine, ketamine-d4, norketamine, norketamine-d4 and dehydronorketamine were 2.91 min, 2.90 min, 2.87 min, 2.86 min, and 2.72 min respectively. The total run time for each sample was 7 minutes.

2.7.3 Linearity

The response of a compound (the ratio of peak area of sample to the peak area of the IS) was plotted on the y-axis against the concentration on x-axis. The response was found to be linearly proportional to the concentration for ketamine, norketamine and dehydronorketamine in the standard curve range (R^2 = 0.998, R^2 = 0.997, and R^2 = 0.998). Representative standard curves showing linearity of assay are shown in Figure 5.

a)



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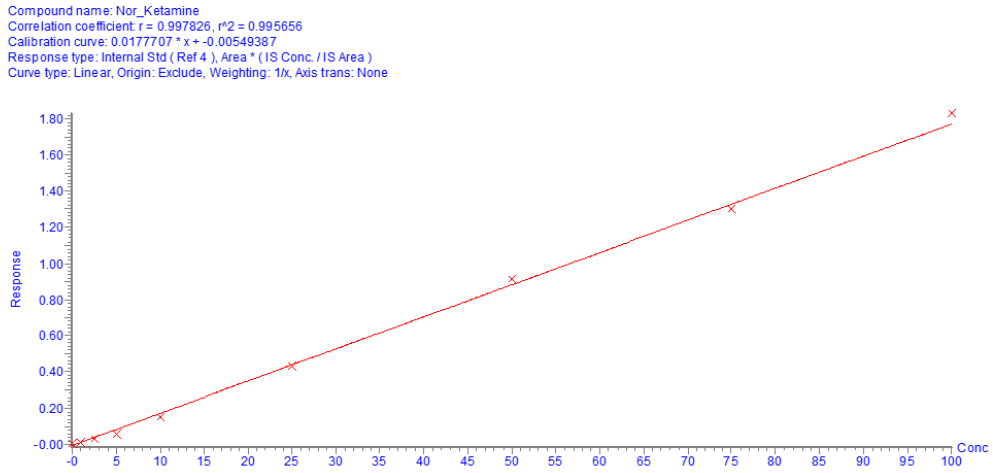


c)

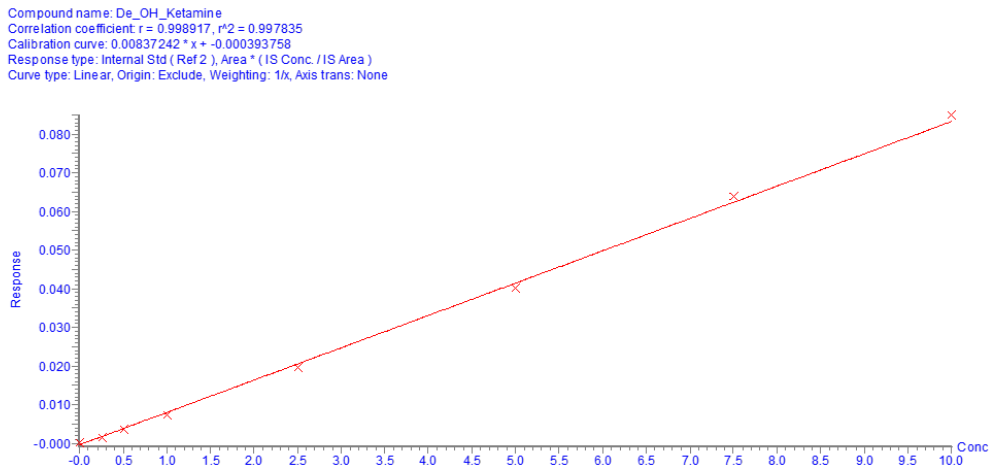


Figure 5 Representative validation standard curves for a) ketamine b) norketamine and c) dehydronorketamine respectively.

2.7.4 Precision and Accuracy

Precision and accuracy of inter-day and intra-day variation were assessed and accepted if the % CV was ≤ 15%. Detailed estimates with mean ± SD, % CV and % bias for all QC samples are presented in Table 3. All the measured concentrations were within the acceptable criteria and did not deviate more than 15% of the theoretical concentrations. The % CV was <15%.

Table 3 Intra-day and inter-day precision and accuracy in human milk.

	Added concentration (ng/mL)	Intra-day (%) (N = 3)			Inter-day (%) (N = 5)		
		Mean ± S.D.	CV (%)	Bias (%)	Mean ± S.D.	CV (%)	Bias (%)
ketamine	2	1.90 ± 0.00	0.00	-5.00	1.98 ± 0.11	5.53	-1.00
	20	16.47 ± 0.31	1.86	-17.67	17.60 ± 0.92	5.22	-12.00
	40	36.87 ± 0.21	0.56	-7.83	39.16 ± 2.23	5.68	-2.10
	80	73.00 ±0.26	0.36	-8.75	80.24 ± 5.98	7.45	0.30
Norket	2	1.93 ± 0.06	2.99	-3.33	1.94 ± 0.09	4.61	-3.00
	20	15.83 ± 0.06	0.36	-20.83	17.20 ± 0.75	4.39	-14.00
	40	36.07 ± 0.21	0.58	-9.83	38.48 ± 2.18	5.66	-3.80
	80	74.30 ± 0.85	1.15	-7.13	80.76 ± 5.55	6.87	0.95
DHNK	0.2	0.20 ± 0.00	0.00	0.00	0.20 ± 0.00	0.00	0.00
	2	1.83 ± 0.06	3.15	-8.33	1.82 ± 0.04	2.46	-9.00
	4	3.50 ± 0.00	0.00	-12.50	3.78 ± 0.23	6.03	-5.50
	8	7.57 ± 0.12	1.53	-5.42	7.90 ± 0.41	5.14	-1.25

2.7.5 Recovery

Recovery was expressed as % recovery. It was computed by comparing the peak area of analyte from neat samples to peak area of analyte in milk samples spiked with known concentration of analyte pre-extraction. The % recoveries at 2, 20, 40 and 80 ng/mL are depicted in Table 4.

2.7.6 Matrix Effect

No matrix effect was observed for all three compounds and the responses obtained from the QC milk samples were close to 100% of the response obtained from neat samples. The % ME of ket and norket quality controls with concentrations 2, 20, 40 and 80 ng/mL was assessed to be 101.9%, 95.5%, 95.2% and 97.9% and 122.6%, 118.9%, 114.8% and 106.6% respectively. For DHNK, the matrix effect for quality control samples with concentrations of 0.2, 2, 4 and 8 ng/mL was analyzed to be 111.2%, 106.1%, 105% and 108.2% respectively. The % matrix effect at 0.2, 2, 4 and 8 ng/mL are summarized in Table 4. Representative chromatograms for blank neat samples, drug-spiked neat samples, blank human milk samples and drug-spiked human milk samples are shown in Figures 6, 7, 8, and 9 respectively.

Table 4 Recovery and matrix effect in human milk

	Added concentration (ng/mL)	Recovery (%) (N = 3)		Matrix Effect (%) (N = 3)	
		Mean ± S.D.	CV (%)	Mean ± S.D.	CV (%)
Ketamine	2	97.34 ± 1.49	1.53	101.85 ± 2.43	2.39
	20	99.33 ± 1.23	1.24	95.45 ± 1.39	1.46
	40	100.55 ± 1.18	1.18	95.17 ± 1.22	1.29
	80	100.04 ± 0.44	0.44	97.92 ± 0.59	0.61
Norket	2	95.13 ± 1.01	1.06	122.58 ± 1.20	0.98
	20	97.57 ± 1.39	1.42	118.94 ± 1.26	1.06
	40	100.87 ± 0.70	0.69	114.75 ± 1.49	1.30
	80	99.96 ± 0.34	0.34	106.60 ± 0.71	0.66
DHNK	0.2	94.39 ± 2.13	2.26	111.24 ± 3.12	2.81
	2	97.44 ± 2.03	2.08	106.13 ± 2.38	2.24
	4	99.86 ± 1.51	1.51	105.03 ± 2.03	1.93
	8	98.77 ± 0.69	0.70	108.21 ± 2.22	2.05

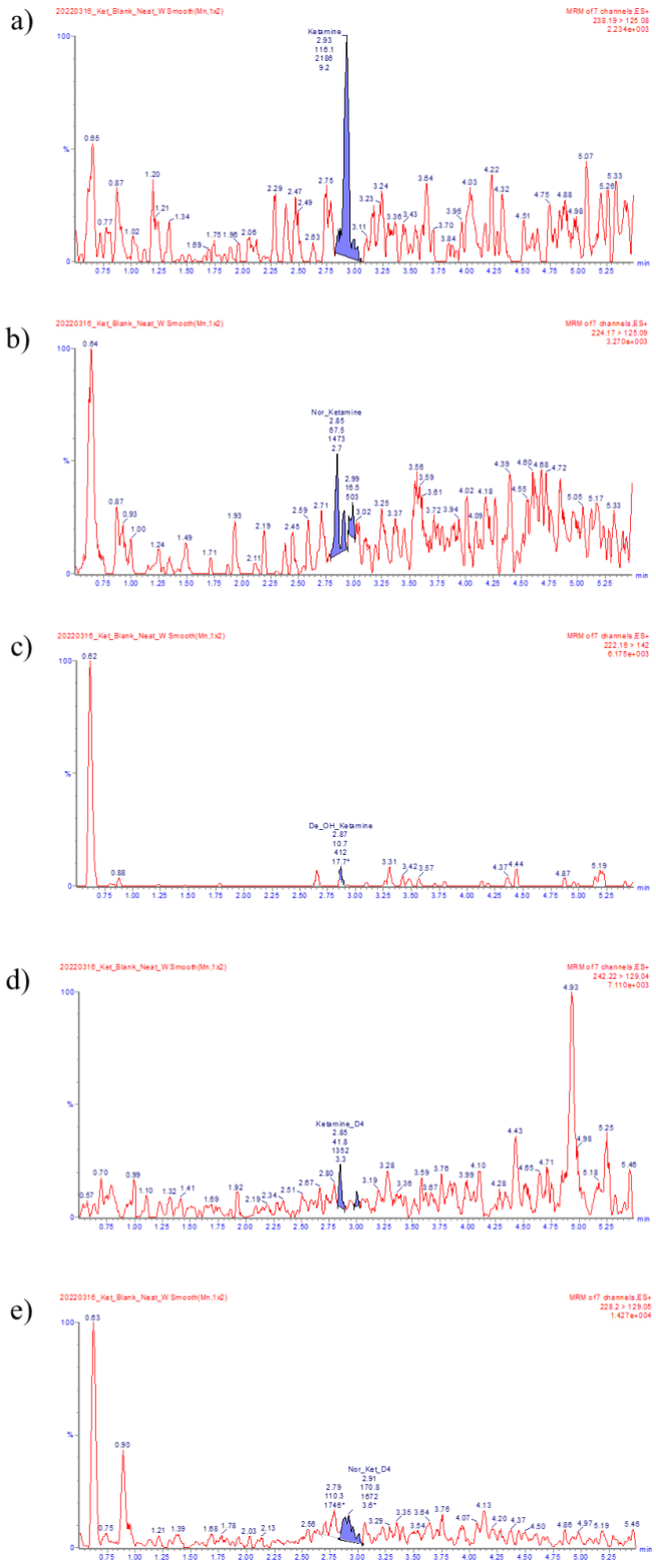


Figure 6 Representative neat (blank) chromatograms for a) ketamine b) norketamine c) dehyrdonorketamine d) ketamine-d4 and e) norketamine-d4 respectively.

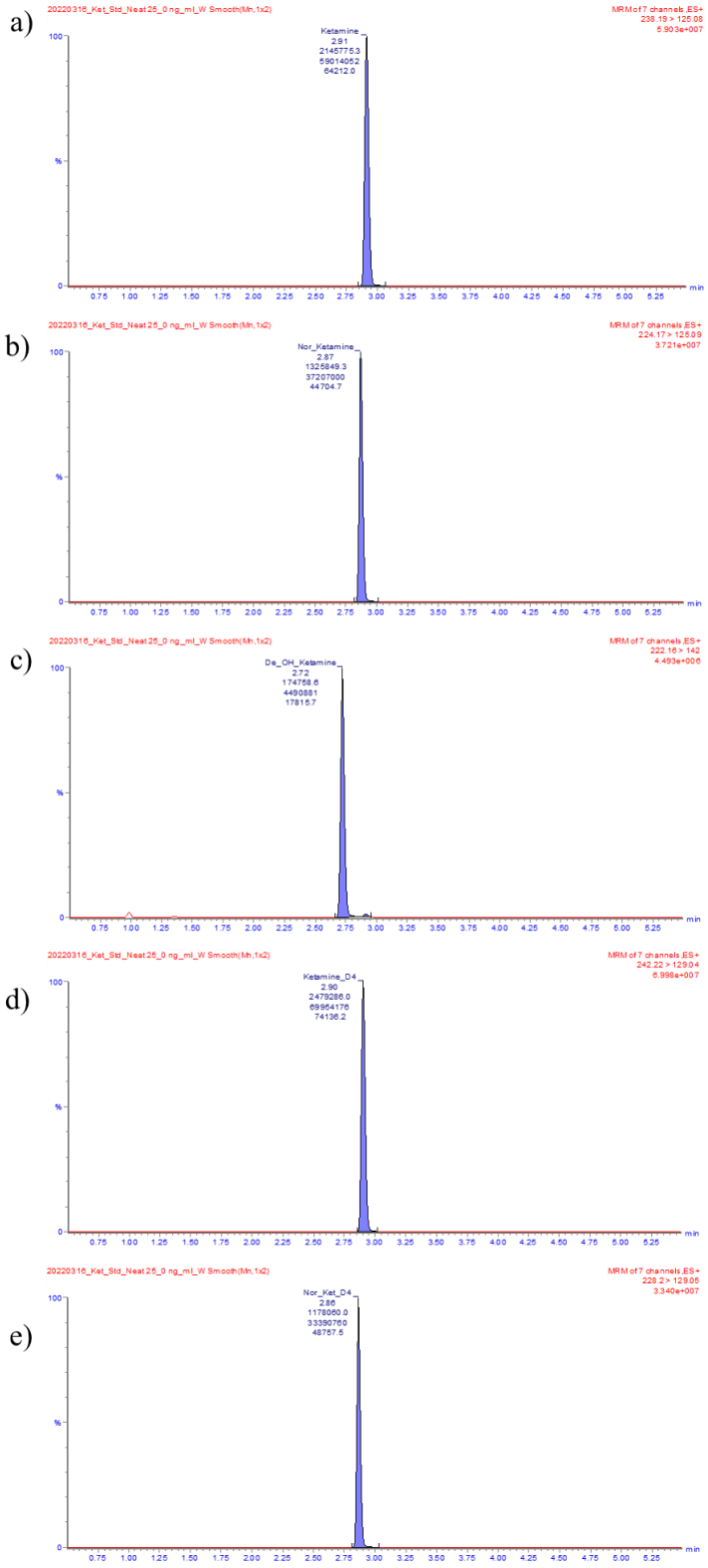


Figure 7 Representative chromatograms for neat solutions containing a) ketamine (25 ng/mL) b) norketamine (25 ng/mL) c) dehydronorketamine (2.5 ng/mL) d) ketamine-d4 (IS)) and e) norketamine-d4 (IS) respectively.

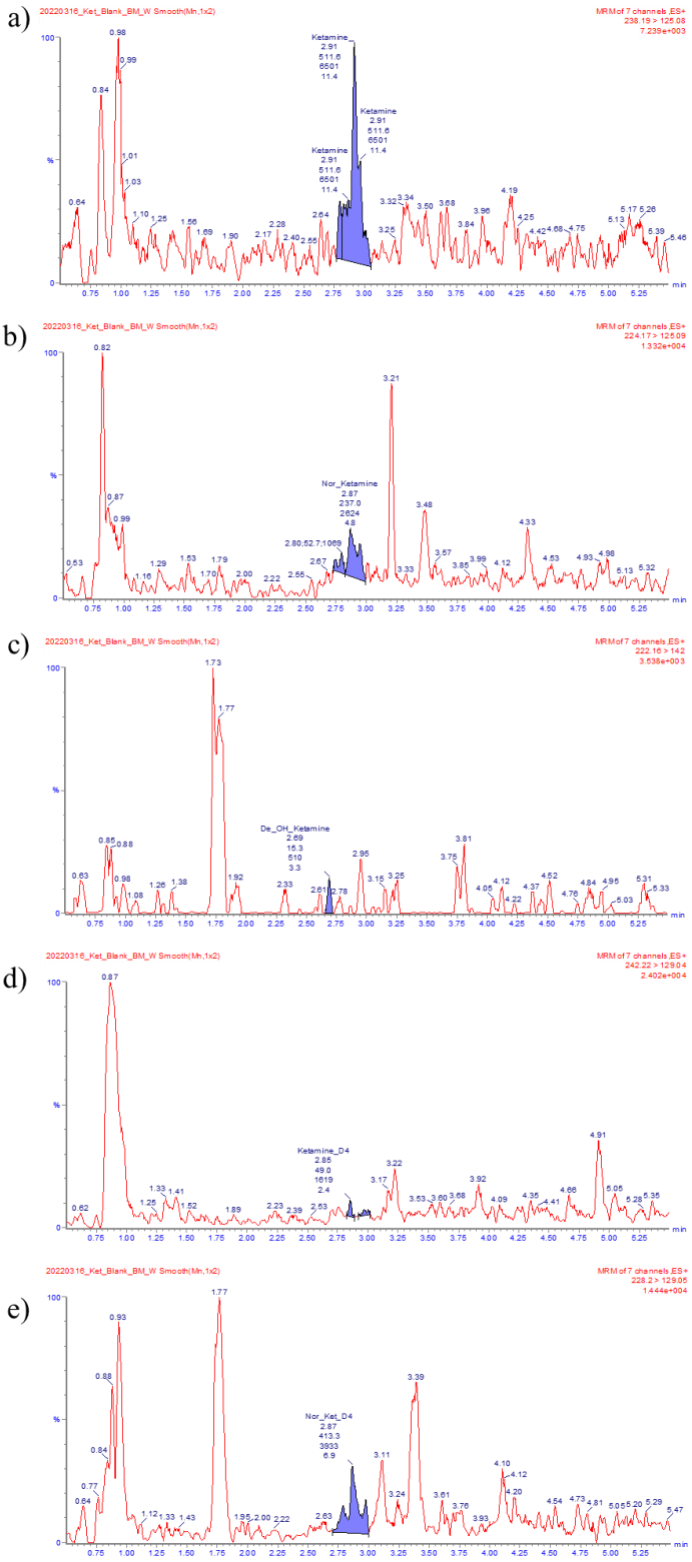


Figure 8 Representative chromatograms from blank human milk a) ketamine b) norketamine c) dehydronorketamine d) ketamine-d4 (IS) and e) norketamine-d4 (IS) respectively.

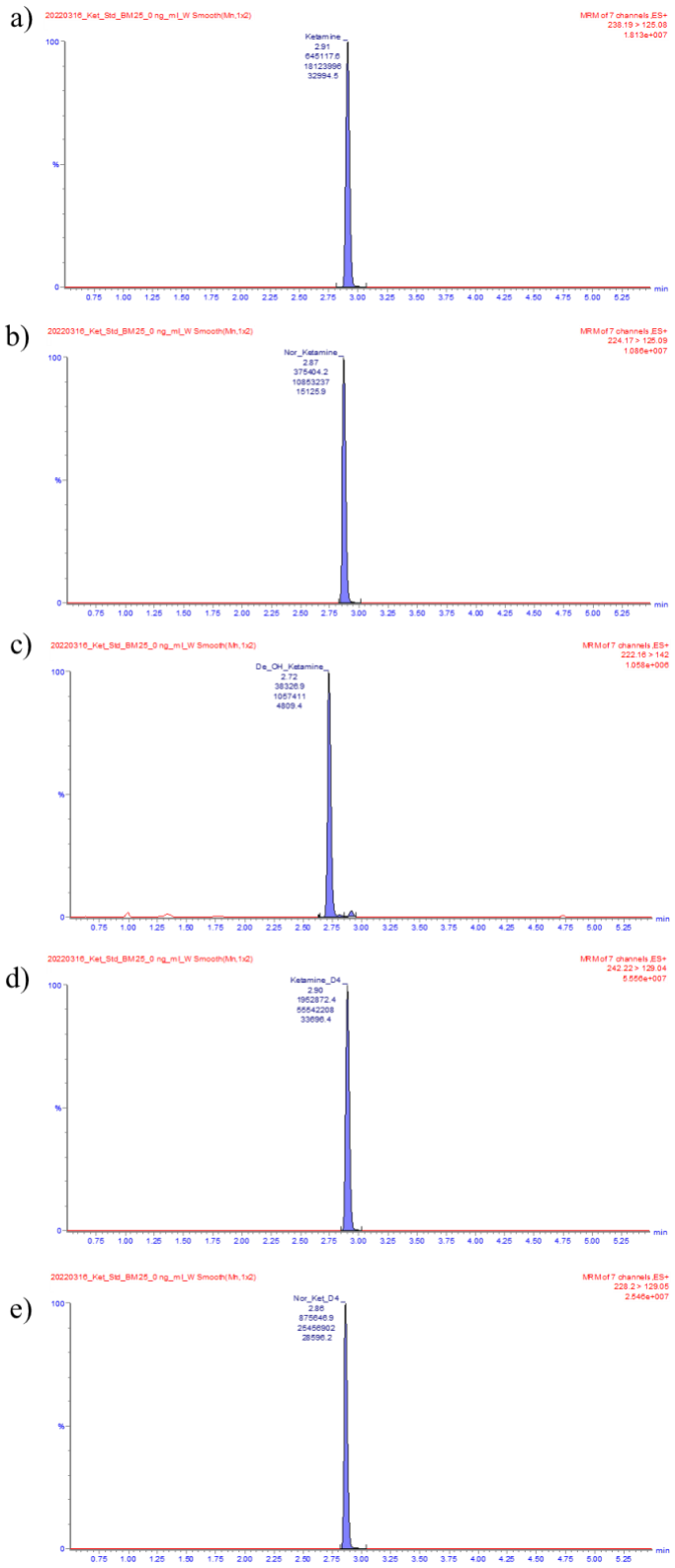


Figure 9 Representative chromatograms for human milk spiked with a) ketamine (25 ng/mL) b) norketamine (25 ng/mL) c) dehydronorketamine (2.5 ng/mL) d) ketamine-d4 (IS) e) norketamine-d4 (IS) respectively.

2.7.7 Stability

The % CV was reported <15% for ket, norket and DHNK for all stability conditions. No significant difference was observed for ket for the three stability conditions (Bias < 15%). However, the metabolites norket and DHNK did show significant change (Bias > 15 %) in the estimated concentration under all three stability conditions indicating that they are temperature sensitive, and the clinical samples should be stored in -80°C and thawed immediately prior to analysis. The stability data is presented in Table 5.

Table 5 Stability data in human milk stored at 4°C for 24 hours, room temperature for 24 hours and three times freeze at -80°C and thaw at room temperature.

	Ref conc. (ng/mL)	Analysis after three freeze-thaw cycles at -80°C and RT respectively (N = 3)			Analysis after 24 hours at 4°C (N = 3)			Analysis after 24 hours thawing at RT (N = 3)		
		Mean ± S.D.	CV (%)	Bias (%)	Mean ± S.D.	CV (%)	Bias (%)	Mean ± S.D.	CV (%)	Bias (%)
Ket	2	1.90 ± 0.00	0.00	-5.00	1.97 ± 0.06	2.94	-1.67	1.97 ± 0.06	2.94	-1.67
	20	16.83 ± 0.12	0.69	-15.83	17.33 ± 0.06	0.33	-13.33	17.73 ± 0.15	0.86	-11.33
	40	35.80 ± 0.14	0.40	-10.50	36.93 ± 0.38	1.03	-7.67	40.67 ± 0.21	0.51	1.67
	80	80.90 ± 0.89	1.10	1.13	72.57 ± 0.65	0.90	-9.29	77.27 ± 0.76	0.99	-3.42
Norket	2	1.87 ± 0.06	3.09	-6.67	1.97 ± 0.06	2.94	-1.67	2.00 ± 0.00	0.00	0.00
	20	15.87 ± 0.15	0.96	-20.67	16.47 ± 0.12	0.70	-17.67	16.60 ± 0.00	0.00	-17.00
	40	33.80 ± 0.00	0.00	-15.50	33.93 ± 0.12	0.34	-15.17	38.23 ± 0.21	0.54	- 4.42
	80	77.93 ± 0.72	0.93	-2.58	70.30 ± 1.00	1.42	-12.13	74.60 ± 0.60	0.80	- 6.75
DHNK	0.2	0.20 ± 0.00	0.00	0.00	0.20 ± 0.00	0.00	0.00	0.20 ± 0.00	0.00	0.00
	2	1.47 ± 0.06	3.94	-26.67	1.60 ± 0.00	0.00	-20.00	1.60 ± 0.00	0.00	-20.00
	4	2.80 ± 0.14	5.05	-30.00	2.90 ± 0.00	0.00	-27.50	3.03 ± 0.06	1.90	-24.17
	8	6.03 ± 0.06	0.96	-24.58	6.07 ± 0.06	0.95	-24.17	6.50 ± 0.10	1.54	-18.75

2.8 Clinical Sample Analysis

The newly developed and validated assay methodology was used to estimate the concentrations of ket, norket and DHNK in human milk samples collected from eight subjects in the clinical study. Samples with concentrations higher than the standard curve range were diluted

and analyzed again. The concentration ranges for ket, norket and DHNK in human milk samples were found to be 3.2 – 219.2 ng/mL, 1 – 136.8 ng/mL, and 0.1 – 3.7 ng/mL respectively.

Representative chromatograms for five compounds for patient 8 taken 12 hours after starting ketamine infusion are shown in Figure 10. Human milk concentration versus time profiles of ket, norket and DHNK for 8 subjects are shown in Figure 11.

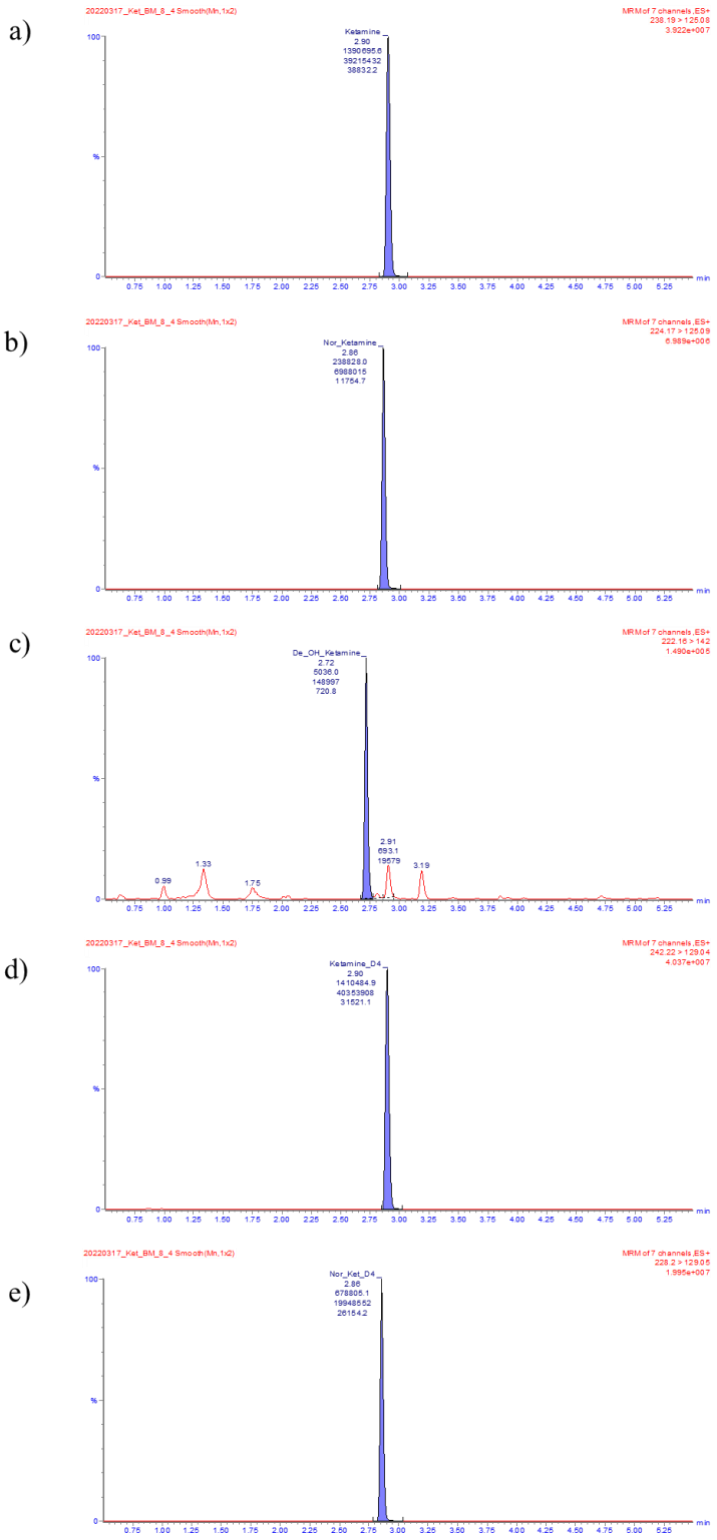


Figure 10 Chromatograms for a) ketamine (79.1 ng/mL) b) norketamine (21.5 ng/mL) c) dehydronorketamine (0.4 ng/mL) d) ketamine-d4 (IS) e) norketamine-d4 (IS) respectively of human milk sample obtained at 12 hr (T12) after starting ketamine infusion in subject 8.

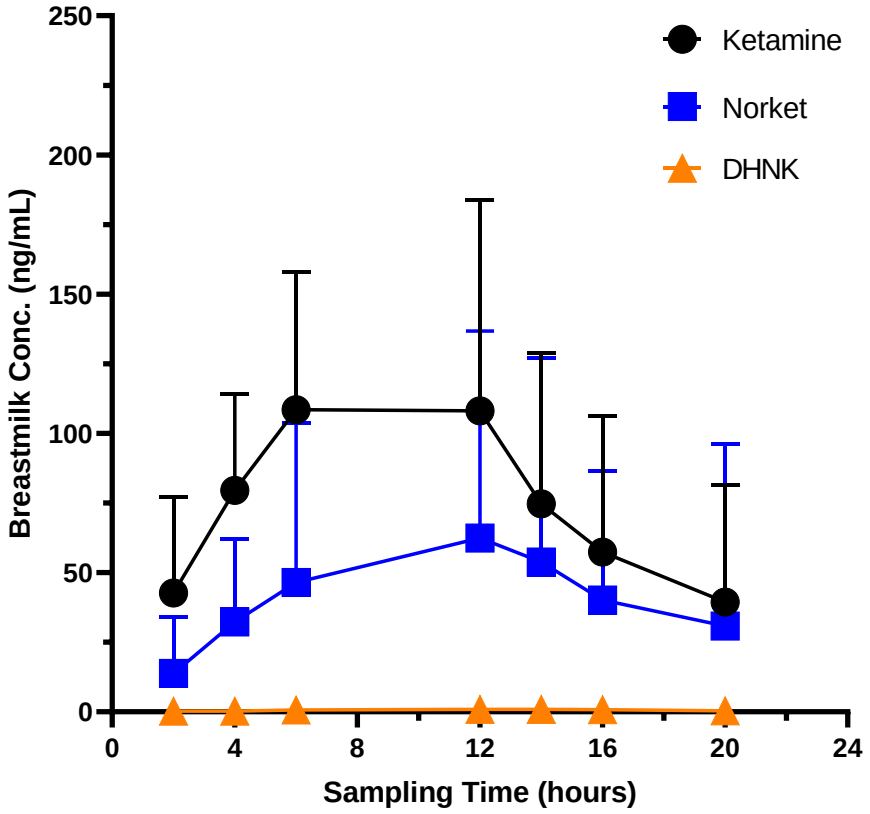


Figure 11 Human milk concentrations (ng/mL) versus time (hours) profile for ketamine, norketamine and dehydronorketamine (DHNK) during and post 12-hour 0.1 mg/kg/hr IV infusion in 8 subjects. Data presented as Mean and SD.

2.9 Conclusion

A reproducible and sensitive UPLC-MS/MS assay for simultaneously estimating ketamine and its two metabolites – norketamine and dehydronorketamine concentrations in human milk were using protein precipitation methodology and successfully developed and validated. Deuterated ketamine and norketamine were used as internal standards. The developed method uses

a simple sample processing technique and low sample volume and can be used efficiently for analysis of multiple patient samples obtained from clinical studies.

3.0 Pharmacokinetic Analysis of Acquired Clinical Data

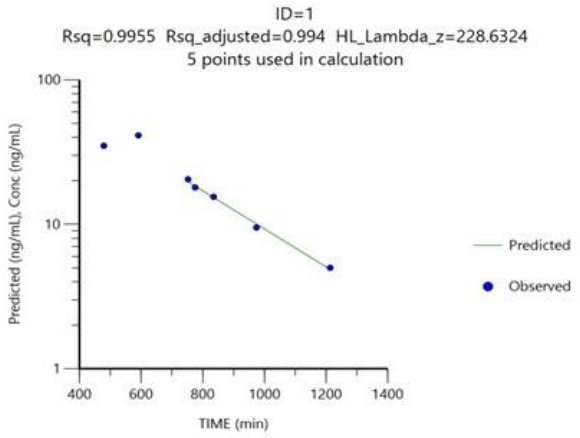
3.1 Clinical data

Plasma samples were obtained from 8 post-partum women after 12 hours of 0.1 mg/kg/hr continuous IV infusion of ketamine. Plasma samples were analyzed using a validated UPLC-MS/MS assay methodology developed in our laboratory (unpublished data).

3.2 Estimation of Pharmacokinetic Parameters using Non-Compartmental Analysis

3.2.1 Methodology for Performing Non-Compartmental Analysis

Non-Compartmental Analysis was performed using Phoenix Winnonlin 8.3.4 (Certara, Princeton, NJ). Pharmacokinetic parameters - $AUC_{0-\infty}$, $t_{1/2}$, Cmax, CL and Vss were calculated. $AUC_{0-\infty}$ was determined using linear trapezoidal method and terminal phase of the plasma-concentration curve was used for estimating the disposition half-life. Slope selection performed for individual subjects is shown in Figure 12.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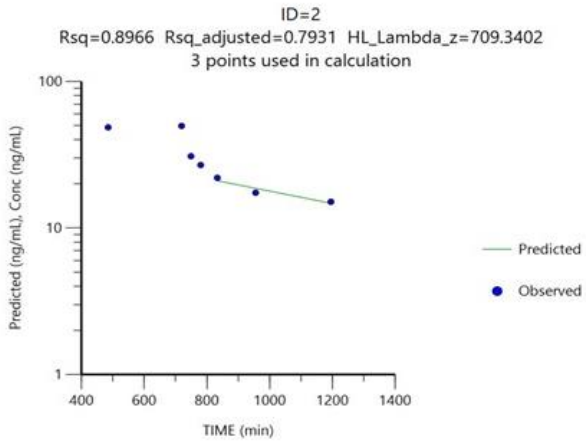
Predicted (ng/mL), Conc (ng/mL)
TIME (min)
Predicted
Observed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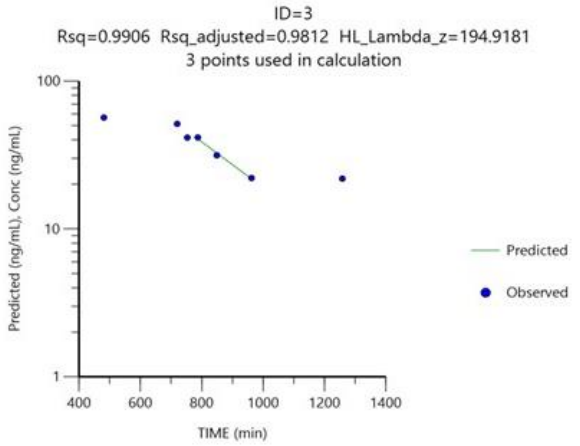
Rsq=0.8966 Rsq_adjusted=0.7931 HL_Lambda_z=709.3402
3 points used in calculation
Predicted (ng/mL), Conc (ng/mL)
TIME (min)
Predicted
Observed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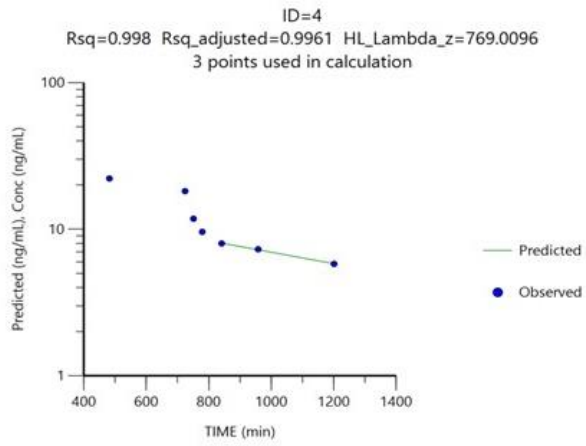
Predicted (ng/mL), Conc (ng/mL)
TIME (min)
Predicted
Observed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TIME (min)
Predicted
Observed

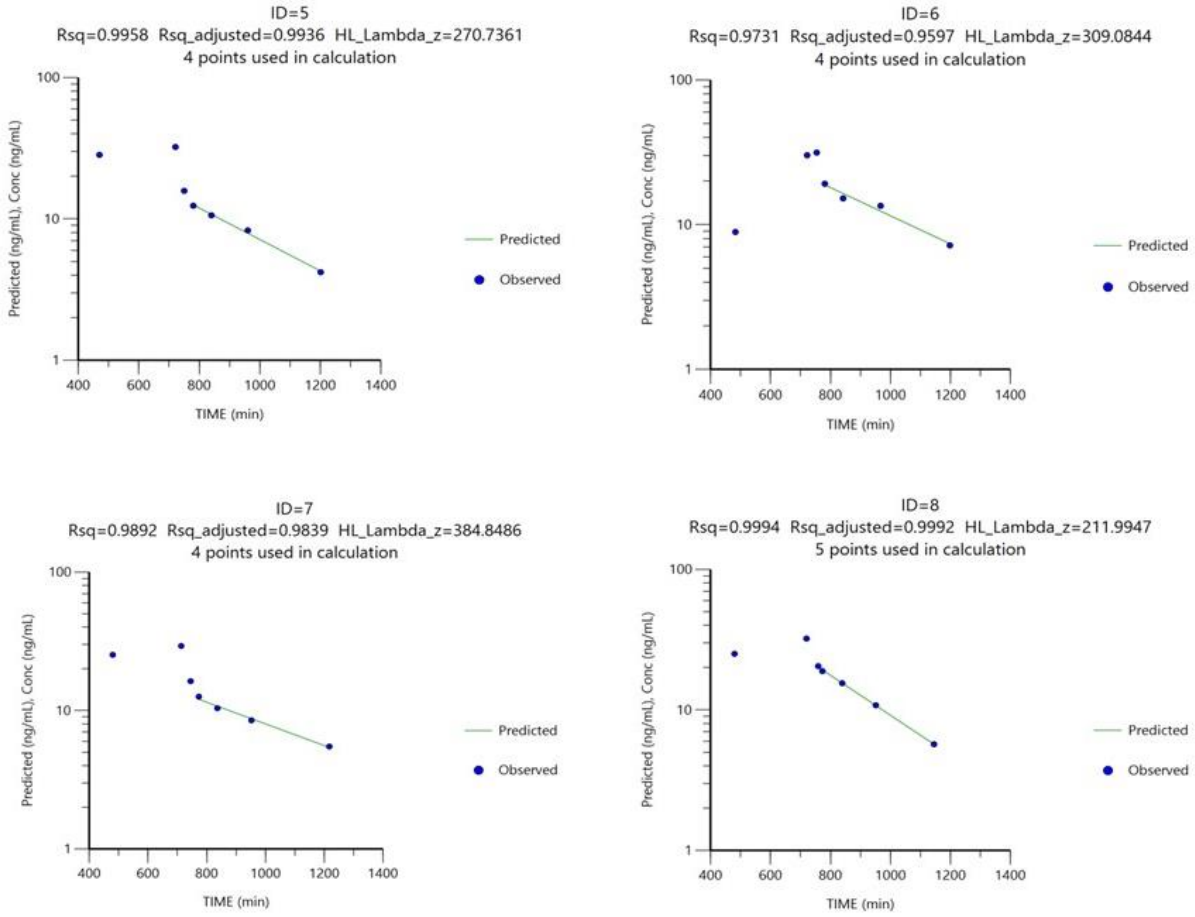


Figure 12 Points selected for calculating slope from terminal elimination phase in individual subjects using non-compartmental analysis.

3.2.2 Results

The mean $AUC_{0-\infty}$ was determined to be 27.29 ± 12.91 ug.min/mL. The elimination half-life was 385 ± 227 min, the clearance (CL) was 3.7 L/min and volume of distribution at steady

state was 2021 ± 1131 L. Results obtained from non-compartmental analysis are presented in Table 6.

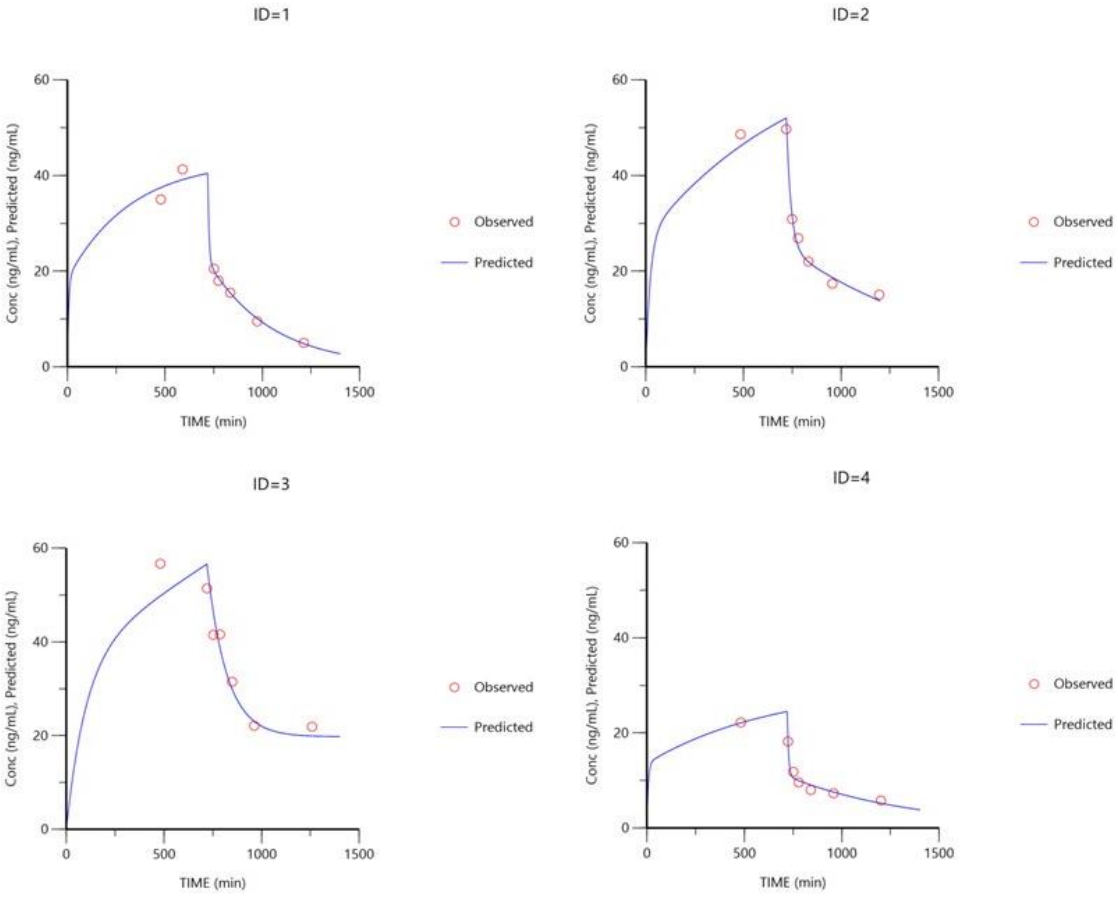
Table 6 Pharmacokinetic parameters for individual subjects after non-compartmental analysis.

ID	Half-life λ_z (min)	Tmax (min)	Cmax (ng/mL)	$AUC_{0-\infty}$ (min*ug/mL)	Vz (L)	CL (L/min)	Vss (L)
1	228.63	591.00	41.30	24.2	557.53	1.69	550.29
2	709.34	719.00	49.70	48.44	4009.5	3.92	3105.23
3	194.92	481.00	56.70	47.46	1133.96	4.03	1752.37
4	769.01	482.00	22.20	20.41	5250.91	4.73	3815.23
5	270.74	721.00	32.30	20.38	1082.68	2.77	948.89
6	309.08	754.00	31.50	16.94	2158.78	4.84	2731.69
7	384.85	713.00	29.30	20.30	2516.7	4.53	2058.31
8	211.99	720.00	32.20	20.16	1031.86	3.37	1205.79
Mean ± S.D.	384.82 ± 227.41	648.63 ± 113.21	36.90 ± 11.47	27.29 ± 12.91	2217.74 ± 1653.09	3.74 ± 1.08	2020.97 ± 1130.77

3.3 Estimation of Pharmacokinetics Parameters using Compartmental Analysis

3.3.1 Methodology for Performing Two-Compartmental Analysis

Phoenix WinNonlin 8.3.4 (Certara, Princeton, NJ) software was used to conduct two-compartmental modelling to estimate pharmacokinetic parameters of ketamine. Similar to NCA, $AUC_{0-\infty}$ was calculated using linear trapezoidal method and terminal portion was used to estimate the elimination half-life. Ketamine infusion rate of 0.1 mg/kg/hr for 720 minutes was used for all 8 subjects. Points used for estimation of elimination half-life for individual subjects are shown in Figure 13. $AUC_{0-\infty}$, elimination half-life, MRT, Vss, CL, Cmax were reported.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ID=2
ID=3
ID=4
Conc (ng/mL), Predicted (ng/mL)
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40
60
0
500
1000
1500
Observed
Predicted

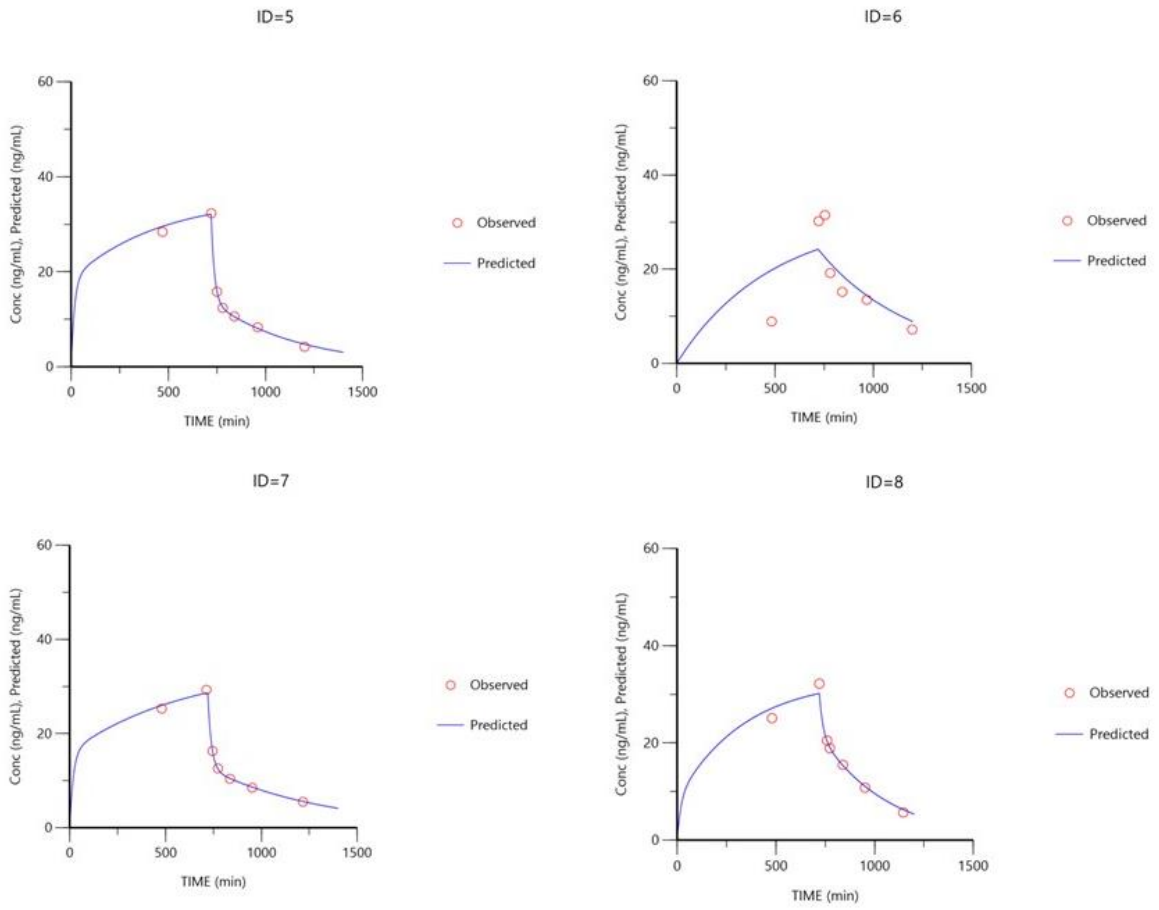

Figure 13 Predicted ketamine plasma profile in individual subjects using two-compartmental analysis.

3.3.2 Results

Patient 3 was excluded while performing calculations due to extremely low clearance values. The mean elimination half-life of ketamine was estimated to be 364 ± 121 min. The area under the plasma concentration time curve ($AUC_{0-\infty}$) was 28.48 ± 9.98 ug.min/mL, clearance (CL) was 3.1 ± 1.1 L/min, and volume of distribution at steady state (V_{ss}) was 1143 ± 653 L. Results from two-compartmental modelling are summarized in Table 7.

Table 7 Pharmacokinetic parameters for individual subjects (ID 1-8) after two-compartmental modeling

ID	$AUC_{0-\infty}$ (min*ug/mL)	β half-life (min)	CL (L/min)	Cmax (ng/mL)	MRT (min)	Vss (L)
1	31.08	225.96	1.32	40.45	189.48	249.37
2	50.0	560.83	3.8	52.03	503.11	1909.75
3	136289.23	4783206.90	0.001	56.61	6899363.90	9689.23
4	21.51	451.14	4.49	24.49	357.88	1607.11
5	25.68	314.73	2.20	32.14	228.28	502.24
6	22.52	336.52	3.64	24.24	481.83	1754.51
7	24.66	421.53	3.73	28.68	332.07	1238.79
8	23.96	236.93	2.84	30.19	259.73	738.80
Mean ± S.D.	28.48 ± 9.98	363.95 ± 121.13	3.15 ± 1.09	33.17 ± 9.95	336.05 ± 121.50	1142.94 ± 653.06

3.4 Conclusion

The $AUC_{0-\infty}$, CL, and elimination/beta half-life obtained by non-compartmental analysis and two-compartmental model were found to be similar. The V_{ss} estimated using non-compartmental analysis was found to be 2-fold higher than the two-compartmental model.

4.0 Estimation of Infant Drug Exposure

4.1 Milk to Plasma Ratio

4.1.1 Significance

Milk to Plasma ratio (M/P Ratio) aids in the assessment of partitioning tendency of a drug molecule between human milk and plasma. M/P ratio for a drug can be estimated by comparing average concentrations obtained at similar sampling timepoints in plasma and human milk. M/P ratio greater than 1 indicates higher partitioning of drug into the milk as compared to plasma. While a M/P ratio lower than 1 indicates that more amount of drug is present in the plasma as compared to milk at the specified time window [41-43].

4.1.2 Method

The M/P ratio for ketamine, norketamine and dehydronorketamine were determined by dividing the concentrations in milk by the concentrations in plasma only if the sampling timepoints were within +/- 30 minutes of each other.

$$MP\ Ratio = \frac{Avg\ conc.\ in\ maternal\ milk\ \left(\frac{ng}{mL}\right)}{Avg\ conc.\ in\ maternal\ plasma\ \left(\frac{ng}{mL}\right)}$$

4.1.3 Results

Of total 56 plasma samples and 55 human milk samples collected from 8 subjects, only 29 samples qualified the condition for a similar (+/- 30 minutes) sampling time window. The mean of M/P ratios in 8 subjects for ketamine, norketamine and dehydronorketamine were 3.64 ± 1.55, 1.71 ± 0.48, and 0.09 ± 0.07 respectively.

4.1.4 Conclusion

Higher M/P ratio (3.64) for the parent drug ketamine indicates greater predisposition of the molecule to partition into milk than plasma.

M/P ratio of 1.71 for norketamine signifies almost 2-fold inclination towards milk and plasma matrices. For dehydronorketamine, M/P ratio is low (0.09), indicating little to no tendency for partitioning into milk.

4.2 Percent Relative Infant Dose

4.2.1 Significance

Percent Relative Infant Dose (% RID) is an estimate of drug exposure to the infant when the drug is administered to the mother. For this clinical study, % RID would be the percent of dose the infant would be theoretically exposed to via transfer into breastmilk.

% RID value ≤ 10 % is considered low [44].

Cumulative Infant Dose (CID) is calculated to estimate the % RID separately for each compound. CID represents the collective amount of drug and its metabolites theoretically consumed by the infant through breast milk when ketamine is administered to the mother.

4.2.2 Method

Cumulative Infant Dose (CID) was calculated in a stepwise manner to determine the % RID. For the two metabolites – norketamine and dehydronorketamine, CID was determined in terms of molar equivalents of the parent drug – ketamine.

Steps for calculating CID -

1. Find the amount of individual compound in milk at each timepoint.
2. Summation of the amount of individual compound in milk at each timepoint to get the total amount of compound consumed by the infant.

$$Cumulative\ Infant\ Dose\ (ng) = \Sigma\ [\ Drug\ concentration\ in\ milk\ at\ specific\ timepoint\ \left(\frac{ng}{mL}\right) * Volume\ of\ milk\ collected\ at\ that\ timepoint\ (mL)\ ]$$

For calculating % RID, CID was divided by the dose given to the mother and then multiplied by 100. % RID was determined separately for the parent drug (ketamine) and collectively for ketamine and its metabolites.

$$RID\ (\%) = \frac{Cumulative\ infant\ dose\ via\ breast\ milk\ \left(\frac{mg}{\frac{kg}{day}}\right)}{maternal\ dose\ \left(\frac{mg}{\frac{kg}{day}}\right)}\ x\ 100$$

4.2.3 Results

The Cumulative Infant Doses (CID) determined for individual subjects for ketamine, ketamine molar equivalents of norketamine and dehydronorketamine are given in Table 8.

The mean % RID for ketamine only was found to be 0.0135% and the % RID for ketamine and its metabolites together was found to be 0.0195%. Results for % RID for individual subjects are summarized in Table 9. Amount (ng) of ketamine, norketamine and DHNK versus time (hours) in milk for 8 subjects are shown in Figure 14.

Table 8 Cumulative Infant Dose for ketamine and metabolites

	Infant Dose		
Patient ID	**Ketamine (ng)**	**Norketamine (ng)**	**Dehydronorketamine (ng)**
1	1169.72	772.75	16.96
2	7963.80	4785.75	48.58
3	555.77	328.40	3.10
4	9016.90	3508.84	34.96
5	9096.90	5956.07	80.75
6	49655.90	18862.98	182.09
7	3943.80	1750.54	18.55
8	7305.30	2571.14	48.47

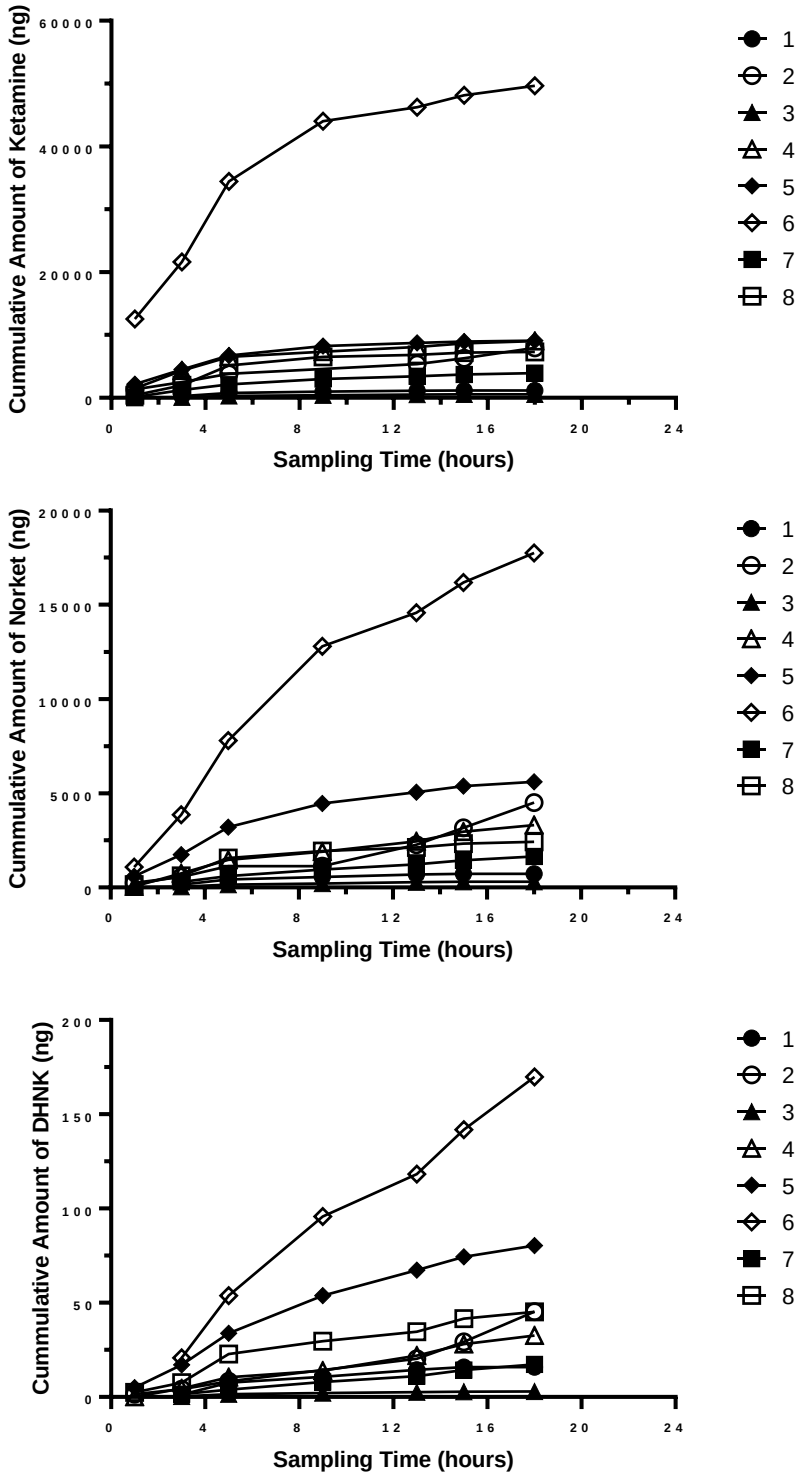

Figure 14 Cummulative amount (ng) of ketamine, norket and DHNK versus sampling time (hours) in human milk of 8 subjects.

Table 9 % Relative Infant Dose for ketamine only and ketamine with it's metabolites

Patient ID	Maternal Dose (mg)	%RID ketamine only	% RID of Ket and ketamine Equivalent NKET and DHNK
1	40.9	0.0029	0.0048
2	189.8	0.0042	0.0067
3	191.4	0.0003	0.0005
4	96.6	0.0093	0.0130
5	56.5	0.0161	0.0268
6	82	0.0606	0.0838
7	92	0.0043	0.0062
8	68	0.0107	0.0146

4.2.4 Conclusion

The % RID values for ketamine only as well as ketamine with its metabolites was determined to be well below the 10% threshold indicating very low transfer of drug to the infant through milk.

5.0 Discussion

Till date, studies assessing pharmacokinetics of ketamine and metabolites in lactating women or studies quantifying ketamine exposure to infant during breastfeeding have not been conducted. Due to insufficient data ketamine is not recommended as a part of multimodal analgesia regimen as well. Therefore, as previously discussed in Section 1.2.5, there was a need to conduct a clinical study to evaluate ketamine pharmacokinetics in lactating population. A clinical study was proposed and part one of the study was successfully completed in UPMC-Montefiore, Pittsburgh, PA. Maternal plasma and breast milk samples were collected from women exclusively bottle-feeding or women weaning from breastfeeding at specific timepoints.

To analyze ketamine and metabolite concentrations in the breast milk samples, a specific and reproducible assay was required. Most of the publications reporting data from milk samples look at extraction of ketamine from the matrix, without its metabolites. Researchers from University of Tehran have developed a method utilizing electro membrane extraction (EME) combined with HPLC-UV for quantifying ketamine in milk, human plasma and wastewater [45], [46]. Another published method quantifies ketamine in Holstein cow milk using liquid-liquid extraction and HPLC-UV detection [47]. Publication by Lopez-Garcia *et. al*. uses protein precipitation and LC-MS/MS for simultaneous measurement of 40 psychoactive drugs including ketamine in human milk, cow milk and various milk matrices like fresh, powdered, whole and skimmed milk [48]. It is important to note that none of the above-mentioned assays measure the primary active metabolite of ketamine – norketamine or the other metabolites which are also shown to be active pre-clinically.

Currently, there is only one published LC-MS/MS method published by Wolfson *et. al*. for quantifying ketamine, norketamine, hydroxynorketamine and dehydronorketamine concentrations in human milk [49]. However, this method uses solid phase extraction for extraction which involves multiple time-consuming steps, is expensive and requires large sample volumes [50].

Our lab has developed a novel UPLC-MS/MS method for quantifying ket, korket and DHNK concentrations in human plasma (unpublished data). We decided to optimize this pre-existing method to develop an assay for human milk as matrix. Milk contains high lipid and protein content as compared to plasma. Hence, the goal was to develop an efficient method for extracting all three analytes while ensuring good recovery and minimal matrix effect. The newly developed method was specific, sensitive, and reproducible. The validated assay showed linear response over the range of 1 – 100 ng/mL for ket and norket and 0.1 - 10 ng/mL for DHNK. The inter-day and intra-day percent co-efficient of variations were <15% and well within the acceptable limits. Recovery was close to 100% for ket, norket, DHNK as well as both the internal standards - ket-D4 and norket-D4. One of the disadvantages of using protein precipitation is poor sample cleanup, leading to higher possibility of matrix effect. But no matrix effect was observed for all analytes and internal standards. The stability of all three analytes was tested under different temperature conditions. There was no significant effect of conditions on ket. However, instability of metabolites, norket and DHNK was noted. Norket and DHNK showed significant (>15% bias) change in response after storing at different temperatures. Thus, to prevent drug concentration changes in the sample, we recommend thawing the sample for analysis just before processing.

In conclusion, the validated assay can be successfully implemented for quantifying ketamine, norketamine and dehydronorketamine concentrations in human milk samples.

Plasma concentrations of ket, norket and DHNK were also successfully quantified using a validated UPLC-MS/MS method developed in our lab. Most importantly, we could establish plasma pharmacokinetic profiles for ket, norket and DHNK for all the 8 subjects from the clinical study. Critical pharmacokinetic parameters like CL, Vss, T1/2, $AUC_{0-\infty}$, MRT and Cmax were calculated using non-compartmental analysis as well as two-compartmental modeling. The values were found to be similar for both analyses. One of the limitations was that, even though plasma concentrations of patient 3 were comparable to other subjects, the PK parameters were not similar and hence, we had to exclude patient 3 during the analysis. Additionally, ketamine exhibited two-compartmental behavior similar to published literature [24]. Table 10 presents a comparison of the major pharmacokinetic parameters in healthy adults (non-pregnant population), pregnant women, and our study participants. The clearance, volume of distribution and half-life of ketamine calculated using a two-compartment model were found to be higher in the study participants than in healthy (non-pregnant) adults.

Table 10 Comparison of ketamine pharmacokinetics in non-pregnant adults and lactating women in our study [38, 51-54]

	Non-pregnant adults	**Study subjects - Two compartment values (Mean and S.D.)**
Clearance (mL/min/kg)	13 – 35	36.1 ± 9.8
Volume of Distribution (L/kg)	3 - 8	12.3 ± 5.1
Half-life (hr)	2.5 - 3	6.1 ± 2.0

Ketamine presented a higher M/P ratio of 3.64 while the metabolites norket and DHNK showed a lower M/P ratio of 1.71 and 0.09. This would indicate a higher tendency for ketamine as compared to norket or DHNK to partition into milk. However, M/P ratio value cannot be assessed by itself, and the maternal drug clearance must be taken into consideration before making conclusions.

$$\% \, RID = \frac{MP \; ratio}{Cl \; maternal} x \; Milk \; intake \; x \; 100$$

Where;

% RID = Percent relative infant dose

MP ratio = Milk to plasma ratio

Cl maternal = Maternal total body clearance (ml/kg/min)

F = 1

A good measure of infant drug exposure, clinically useful in risk assessment for a drug would be the % RID. % RID was found to be well-within the acceptable 10% limit for all three compounds. The known physiochemical properties of ketamine like large volume of distribution, high protein binding, short half-life, high ionization at physiological pH, low pKa and low lipid solubility also fit with the observed low % RID values [55]. In our study, the % RID, M/P ratio and mean weight normalized clearance of ketamine were found to be 0.0135%, 3.64 and 36.13 ml/min/kg respectively. These results match the published infant exposure predictions as shown in Figure 15.

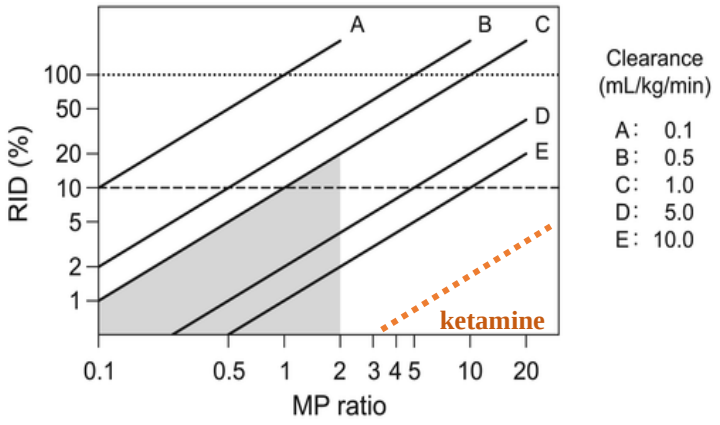

Figure 15 Relative infant dose (RID) and milk-to-(maternal) plasma concentration (MP) ratio. (adapted from Verstegen *et.al.* [56])

RID is infant dose of drug via milk (mg/kg/d) expressed as a percentage of a daily weight-adjusted maternal dose of the drug. RID 100% (thin dotted line) is equivalent to a therapeutic dose of the mother per body weight. RID of 5–10% is usually considered a reference point (dashed line) in the risk assessment because 5–10% of a therapeutic dose per weight is likely to be pharmacologically inconsequential for dose-dependent effects. If breastfed infants have pathologically low function of drug eliminating organs, RID needs to be interpreted with caution. The graph shows RID–MP ratio relationship in logarithmic scale according to 5 categories of drug clearance in the mother (A–E); the higher clearance of the drug, the more rightward shift of RID–MP ratio relation. The shaded area shows a domain in the RID–MP ratio space, where most drugs fall (i.e. clearance of >1 mL/kg/min and MP ratio <2). A given MP ratio is related to different RIDs depending on drug clearance. For clinical risk assessment, MP ratio should not be compared between drugs with different clearance values.

In conclusion, our study fills an important knowledge gap in pharmacokinetics and infant exposure of ketamine in lactating women. These results also contribute to understanding the safety of peri-operative low-dose ketamine infusions in postpartum women.

6.0 Future Directions

Additional work is needed to completely understand infant exposure of ketamine through human milk in lactating women. The current study was one part of a proposed clinical study. It consisted of only 8 subjects to evaluate the preliminary safety, understand pharmacokinetics, and access risk for drug exposure to the infant when ketamine infusion is administered peri-operatively as a low-dose. The pharmacokinetics of ketamine as well as the infant exposure may differ upon increasing ketamine dose and this needs to be evaluated in the future. Additionally, there is a recent meta-analytical publication looking into three-compartmental behavior of ketamine which we would like to explore in the future in our study as well [57]. Furthermore, to understand infant drug exposure, we need to study the effect of changing milk composition (seen during the lactation period) on M/P ratio and % RID.

Part-two of the proposed clinical study is a randomized controlled trial which will help us better understand the inter-patient and intra-patient pharmacokinetic and infant exposure variability. We plan to utilize the currently developed assay methodology for analyzing the human milk samples collected from this study. A larger patient population would confirm our current observations and help us recognize safety and efficacy of ketamine in mother and infant as a peri-operative analgesic in lactating women.

Bibliography

1. Sung Sharon, M.H. *Cesarean Section*. 2022; Available from: https://www.ncbi.nlm.nih.gov/books/NBK546707/.
2. Organization, W.H. *Caesarean section rates continue to rise, amid growing inequalities in access*. 2021; Available from: https://www.who.int/news/item/16-06-2021-caesarean-section-rates-continue-to-rise-amid-growing-inequalities-in-access.
3. Control, C.f.D., *Births – Method of Delivery (U.S.)*, N.C.f.H. Statistics, Editor. 2020.
4. Eisenach, J.C., et al., *Severity of acute pain after childbirth, but not type of delivery, predicts persistent pain and postpartum depression.* PAIN, 2008. **140**(1): p. 87-94.
5. Kerai, S., K.N. Saxena, and B. Taneja, *Post-caesarean analgesia: What is new?* Indian Journal of Anaesthesia, 2017. **61**(3): p. 200-214.
6. Hirai, A.H., et al., *Neonatal Abstinence Syndrome and Maternal Opioid-Related Diagnoses in the US, 2010-2017.* JAMA, 2021. **325**(2): p. 146-155.
7. Jean Y. Ko, P.D.V.D.A., MPH; Sarah C. Haight, MPH; Brian Morrow, MA; Shanna Cox, MSPH; Beatriz Salvesen von Essen, MPH; Andrea E. Strahan, PhD; Leslie Harrison, MPH; Heather D. Tevendale, PhD; Lee Warner, PhD; Charlan D. Kroelinger, PhD; Wanda D. Barfield, MD, *ital Signs: Prescription Opioid Pain Reliever Use During Pregnancy — 34 U.S. Jurisdictions.* Morbidity and Mortality Weekly Report (MMWR), 2020 **69**((28)): p. 897–903.
8. Veef, E. and M. Van de Velde, *Post-cesarean section analgesia.* Best Practice & Research Clinical Anaesthesiology, 2022. **36**(1): p. 83-88.
9. Roofthooft, E., et al., *PROSPECT guideline for elective caesarean section: updated systematic review and procedure-specific postoperative pain management recommendations.* Anaesthesia, 2021. **76**(5): p. 665-680.
10. MM, C., *Physiologic and Pharmacokinetic Changes in Pregnancy.* Frontiers Pharmacology, 2014. **5**: p. 65.
11. Feghali, M., R. Venkataramanan, and S. Caritis, *Pharmacokinetics of drugs in pregnancy.* Semin Perinatol, 2015. **39**(7): p. 512-9.
12. Qasqas, S.A., et al., *Cardiovascular pharmacotherapeutic considerations during pregnancy and lactation.* Cardiol Rev, 2004. **12**(4): p. 201-21.
13. Pirani, B.B., D.M. Campbell, and I. MacGillivray, *Plasma volume in normal first pregnancy.* J Obstet Gynaecol Br Commonw, 1973. **80**(10): p. 884-7.
14. Erman, A., et al., *Enhanced urinary albumin excretion after 35 weeks of gestation and during labour in normal pregnancy.* Scand J Clin Lab Invest, 1992. **52**(5): p. 409-13.
15. Cheung, C.K., T. Lao, and R. Swaminathan, *Urinary excretion of some proteins and enzymes during normal pregnancy.* Clinical chemistry, 1989. **35 9**: p. 1978-80.
16. Hebert, M.F., et al., *Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study.* Clin Pharmacol Ther, 2008. **84**(2): p. 248-53.

17. Tracy, T.S., et al., *Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy.* American Journal of Obstetrics and Gynecology, 2005. **192**(2): p. 633-639.
18. Brazier, J.L., et al., *Pharmacokinetics of caffeine during and after pregnancy.* Dev Pharmacol Ther, 1983. **6**(5): p. 315-22.
19. Tsutsumi, K., et al., *The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans.* Clin Pharmacol Ther, 2001. **70**(2): p. 121-5.
20. Davison, J.M. and W. Dunlop, *Renal hemodynamics and tubular function normal human pregnancy.* Kidney Int, 1980. **18**(2): p. 152-61.
21. Chamberlain, A., et al., *Pharmacokinetics of ampicillin and sulbactam in pregnancy.* Am J Obstet Gynecol, 1993. **168**(2): p. 667-73.
22. Sinner, B. and B.M. Graf, *Ketamine.* Handb Exp Pharmacol, 2008(182): p. 313-33.
23. Information, N.C.f.B., *PubChem Compound Summary for CID 3821, Ketamine.* 2022.
24. Wieber, J., et al., *Pharmacokinetics of ketamine in man.* Anaesthesist, 1975. **24**(6): p. 260-3.
25. Pai, A. and M. Heining, *Ketamine.* Continuing Education in Anaesthesia Critical Care & Pain, 2007. **7**(2): p. 59-63.
26. Chang, T., et al., *Metabolic disposition of tritium labelled ketamine ketalar ci 581 in normal human subjects.* Clinical research, 1970. **18**: p. 597.
27. Chang, T. and A.J. Glazko, *Biotransformation and disposition of ketamine.* Int Anesthesiol Clin, 1974. **12**(2): p. 157-77.
28. Zanos, P., et al., *Ketamine and Ketamine Metabolite Pharmacology: Insights into Therapeutic Mechanisms.* Pharmacol Rev, 2018. **70**(3): p. 621-660.
29. Karch, S.B., *Karch's Pathology of Drug Abuse*. 3rd ed. 2002, London, U.K.: CRC Press. 467-471.
30. Dinis-Oliveira, R.J., *Metabolism and metabolomics of ketamine: a toxicological approach.* Forensic Sciences Research, 2017. **2**(1): p. 2-10.
31. Schwenk, E.S., et al., *Consensus Guidelines on the Use of Intravenous Ketamine Infusions for Acute Pain Management From the American Society of Regional Anesthesia and Pain Medicine, the American Academy of Pain Medicine, and the American Society of Anesthesiologists.* Regional Anesthesia & Pain Medicine, 2018. **43**(5): p. 456-466.
32. Cohen, S.P., et al., *Consensus Guidelines on the Use of Intravenous Ketamine Infusions for Chronic Pain From the American Society of Regional Anesthesia and Pain Medicine, the American Academy of Pain Medicine, and the American Society of Anesthesiologists.* Regional Anesthesia & Pain Medicine, 2018. **43**(5): p. 521-546.
33. Orhurhu VJ, R.J., Ly N, et al. *Ketamine In Acute and Chronic Pain Management.* 2022; Available from: https://www.ncbi.nlm.nih.gov/books/NBK539824/.
34. Bell, R.F., et al., *Peri-operative ketamine for acute post-operative pain: a quantitative and qualitative systematic review (Cochrane review).* Acta Anaesthesiologica Scandinavica, 2005. **49**(10): p. 1405-1428.
35. Bauchat, J.R., et al., *Low-dose ketamine with multimodal postcesarean delivery analgesia: a randomized controlled trial.* Int J Obstet Anesth, 2011. **20**(1): p. 3-9.
36. Sen, S., et al., *The persisting analgesic effect of low-dose intravenous ketamine after spinal anaesthesia for Caesarean section.* European Journal of Anaesthesiology, 2005. **22**(7): p. 518-523.

37. Carvalho, B. and A.J. Butwick, *Postcesarean delivery analgesia.* Best Practice & Research Clinical Anaesthesiology, 2017. **31**(1): p. 69-79.
38. Little, B., et al., *Study of ketamine as an obstetric anesthetic agent.* Am J Obstet Gynecol, 1972. **113**(2): p. 247-60.
39. White, P.F., W.L. Way, and A.J. Trevor, *Ketamine--its pharmacology and therapeutic uses.* Anesthesiology, 1982. **56**(2): p. 119-36.
40. Administration, U.S.F.a.D., *Bioanalytical Method Validation Guidance for Industry*, Center for Drug Evaluation and Research and C.f.V. Medicine, Editors. 2018.
41. Ito, S. and G. Koren, *A novel index for expressing exposure of the infant to drugs in breast milk.* British Journal of Clinical Pharmacology, 1994. **38**(2): p. 99-102.
42. Ito, S., *Drug Therapy for Breast-Feeding Women.* New England Journal of Medicine, 2000. **343**(2): p. 118-126.
43. Begg, E.J., *3 - Determinants of drug transfer into human milk*, in *Drugs and Human Lactation (Second Edition)*, P.N. Bennett, Editor. 1996, Elsevier Science B.V.: Amsterdam. p. 47-58.
44. Anderson, P. and J. Sauberan, *Modeling drug passage into human milk.* Clinical Pharmacology & Therapeutics, 2016. **100**(1): p. 42-52.
45. Asadi, S. and S. Nojavan, *Two-step voltage dual electromembrane extraction: A new approach to simultaneous extraction of acidic and basic drugs.* Anal Chim Acta, 2016. **923**: p. 24-32.
46. Tabani, H., et al., *Introduction of agarose gel as a green membrane in electromembrane extraction: An efficient procedure for the extraction of basic drugs with a wide range of polarities.* J Chromatogr A, 2017. **1497**: p. 47-55.
47. Sellers, G., et al., *Pharmacokinetics of ketamine in plasma and milk of mature Holstein cows.* J Vet Pharmacol Ther, 2010. **33**(5): p. 480-4.
48. López-García, E., et al., *Simultaneous LC-MS/MS determination of 40 legal and illegal psychoactive drugs in breast and bovine milk.* Food Chem, 2018. **245**: p. 159-167.
49. Wolfson, P., et al., *The Pharmacokinetics of Ketamine in the Breast Milk of Lactating Women: Quantification of Ketamine and Metabolites.* J Psychoactive Drugs, 2022: p. 1-5.
50. Medvedovici, A., E. Bacalum, and V. David, *Sample preparation for large-scale bioanalytical studies based on liquid chromatographic techniques.* Biomed Chromatogr, 2018. **32**(1).
51. White, P.F., et al., *Comparative pharmacology of the ketamine isomers. Studies in volunteers.* Br J Anaesth, 1985. **57**(2): p. 197-203.
52. Geisslinger, G., et al., *Pharmacokinetics and pharmacodynamics of ketamine enantiomers in surgical patients using a stereoselective analytical method.* Br J Anaesth, 1993. **70**(6): p. 666-71.
53. Fanta, S., et al., *Population pharmacokinetics of S-ketamine and norketamine in healthy volunteers after intravenous and oral dosing.* Eur J Clin Pharmacol, 2015. **71**(4): p. 441-7.
54. Peltoniemi, M.A., et al., *Ketamine: A Review of Clinical Pharmacokinetics and Pharmacodynamics in Anesthesia and Pain Therapy.* Clinical Pharmacokinetics, 2016. **55**(9): p. 1059-1077.
55. Breitzka, R.L., T.L. Sandritter, and F.K. Hatzopoulos, *Principles of drug transfer into breast milk and drug disposition in the nursing infant.* J Hum Lact, 1997. **13**(2): p. 155-8.
56. Verstegen, R.H.J., P.O. Anderson, and S. Ito, *Infant drug exposure via breast milk.* British Journal of Clinical Pharmacology, 2022. **88**(10): p. 4311-4327.

57. Kamp, J., et al., *Ketamine Pharmacokinetics.* Anesthesiology, 2020. **133**(6): p. 1192-1213.