A validated method for quantification of Suboxone® and its primary metabolites in authentic human breastmilk, maternal and infant plasma samples using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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INTRODUCTION

Untreated opioid use disorder (OUD) in pregnant women has been associated with increased risk of fetal growth restriction, preterm delivery, stillbirth, and more severe neonatal abstinence syndrome (NAS) [1]. Current opioid agonist treatments include methadone and Subutex®, along with the expanding use of a buprenorphine/naloxone combination product, Suboxone®. However, Suboxone® is not recommended for use in pregnant women due to its lacking safety information, as well as concerns of active Suboxone® components excreting into breastmilk postpartum. In particular, the naloxone component not only could have teratogenic potential that could result in birth defects but might also inadvertently reverse morphine treatment in breastfed infants with NAS [2]. The purpose of this research was to report concentrations of buprenorphine, naloxone, and their respective glucuronide metabolites detected in authentic clinical breastmilk, and maternal and infant plasma samples [4].

MATERIALS AND METHODS

Sample Preparation and Extraction - adapted from Swortwood *et. al* [3]

- 100 µL plasma or breastmilk
- 10 µL calibrator or QC mix
- 10 µL ISTD

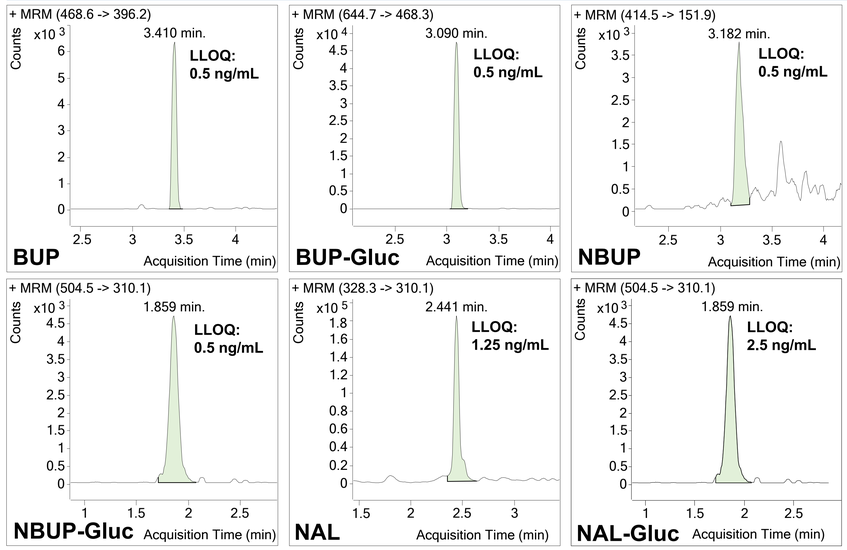
Protein precipitation

- Vortex and add 300 µL of ACN
- Centrifuge at 10,000 rpm at 4°C for 10 min. Transfer to culture tubes
- Vortex and add 2 mL of cold 0.1M phosphoric acid
- Rest samples in ice bath for 10 min

Solid-phase extraction

- Condition Phenomenex Strata-X-C 33 µm Polymeric Strong Cation (60 mg/3 mL) columns with 2 mL MeOH, 2 mL diH_2O, and 2 mL 0.1M phosphoric acid
- Load samples onto columns
- Wash with 2 mL diH_2O, 2 mL 0.1M acetic acid, and 2 mL MeOH
- Dry under vacuum for 5 min
- Elute with 3 mL DCM: IPA: NH_4OH (70:26:4, v/v/v)
- Evaporate under nitrogen at 35°C
- Reconstitute in 125 µL of 85 A:15 B

RESULTS & DISCUSSION



Legend		
Acronym	**Analyte**	**I.S**
BUP	Buprenorphine	BUP-d_4
BUP-Gluc	Buprenorphine-glucuronide	NBUP-Gluc-d_3
NBUP	Norbuprenorphine	NBUP-d_3
NBUP-Gluc	Norbuprenorphine-glucuronide	NBUP-Gluc-d_3
NAL	Naloxone	NAL-d_5
NAL-Gluc	Naloxone-glucuronide	NAL-Gluc-d_5

Figure 1. Quantifier transition for each analyte at respective LLOQ. Method was fully validated per ASB guidelines.

Table 1. Maternal breastmilk, plasma, and infant plasma concentration ranges of BUP, NBUP, NAL and their glucuronide metabolites 2.5 hours after Suboxone® sublingual dosing (i.e. estimated peak concentrations).
Concentrations with (*) symbol represent analyte detected in only one sample. N.D represent no detectable analyte.

Day	Matrix	Analyte					
		BUP	BUP-Gluc	NBUP	NBUP-Gluc	NAL	NAL-Gluc
2 (n=4)	Breastmilk	0.80 – 16.5	0.70 – 7.20	2.10 – 8.40	5.90 – 19.70	N.D	2.60 – 8.00
	Maternal Plasma	1.60 – 6.30	1.20 – 3.90	3.20 – 10.90	20.70 – 28.60	N.D	10.40 – 21.20
	Infant Plasma	N.D	N.D	N.D	N.D	N.D	N.D
3 (n=4)	Breastmilk	0.80 – 4.40	0.90*	1.10 – 4.20	2.10 – 4.20	N.D	3.00 – 3.30
	Maternal Plasma	1.70 – 4.70	0.90 – 2.60	2.00 – 10.70	25.70 – 40.50	N.D	12.10 – 18.00
	Infant Plasma	N.D	N.D	N.D	N.D	N.D	N.D
4 (n=4)	Breastmilk	1.20 – 5.40	0.50*	1.40 – 4.90	1.60 – 5.20	N.D	N.D
	Maternal Plasma	2.40 – 5.20	0.70 – 13.10	2.90 – 15.80	20.70 – 86.30	N.D	14.60 - 9.20
	Infant Plasma	N.D	N.D	N.D	N.D	N.D	N.D
14 (n=3)	Breastmilk	3.50 – 7.70	0.50*	2.00 – 4.40	1.00 – 11.60	N.D	4.30*
	Maternal Plasma	3.20 – 7.00	1.10 – 4.60	4.10 – 12.10	22.70 – 41.20	N.D	9.60 – 18.50
	Infant Plasma	N.D	N.D	0.95 and 5.42	N.D	N.D	N.D
30 (n=3)	Breastmilk	3.20 – 15.20	N.D	1.80 – 4.40	0.70 – 10.10	1.30*	3.90*
	Maternal Plasma	4.80 – 17.70	1.80 – 6.90	3.90 – 10.80	24.30 – 35.10	1.3*	19.00 – 35.20
	Infant Plasma	N.D	N.D	N.D	N.D	N.D	N.D

MATERIALS & METHODS

LC-MS/MS

- *Instrumentation*
  - Agilent 1290 Infinity II Liquid Chromatograph coupled to an Agilent 6470 Triple Quadrupole MS

Source Parameters	Value
Gas temperature (°C)	350
Gas flow (L/min)	8
Sheath gas temperature (°C)	400
Sheath gas flow (L/min)	12
Capillary voltage (V)	4500
Nebulizer (psi)	50
Nozzle (V)	500

- *Column and oven temperature:*
  - Restek Raptor Biphenyl column (100 × 2.1 mm, 2.7 µm) with matching guard held at 40°C
- *Mobile phase*
  - A: 0.1% formic acid in diH_2O
  - B: 0.1% formic acid in methanol
- *Gradient elution at 0.5 mL/min:*
  - 0.5 min hold at 15% B, Increase to 33% B in 1 min, Increase to 98% B in 2.6 min, Hold at 98% B for 2.5 min, Decrease to 15% B over 0.1 min, Re-equilibrate for 1.4 min (8 min total)

CONCLUSION

- Low [NBUP] in infant plasma support previous findings concluding minimal BUP exposure in utero and in breast-milk.
- NAL component contributes to decreased abuse potential of BUP for the mother. Although a low [NAL] was detected in a breastmilk sample, its poor oral bioavailability renders any ingestion by breastmilk inconsequential for the infant.
- Suboxone® is a safe medication for neonate and pregnant/nursing women with OUD.

REFERENCES

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