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DEVELOPMENT AND VALIDATION OF LC-MS/MS ASSAY METHODS TO DETERMINE UBROGEPANT AND ATOGEPANT IN HUMAN BREAST MILK

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PURPOSE

Ubrogepant is a calcitonin-gene-related peptide (CGRP) receptor antagonist for the acute treatment of migraine, while atogepant is a potent, selective, competitive oral CGRP receptor antagonist for the preventive treatment of episodic migraine in adults.

Studies in pregnant women have shown that migraines often improve or disappear during pregnancy but tend to reappear within one to four weeks post-partum, making migraines a concern in lactating women.

The pharmacokinetics of ubrogepant and atogepant in lactating women and their excretion into breast milk have not been evaluated clinically. Consequently, the lack of evidence regarding infant exposure to ubrogepant or atogepant through breastfeeding may prevent patient usage of these drugs.

OBJECTIVE

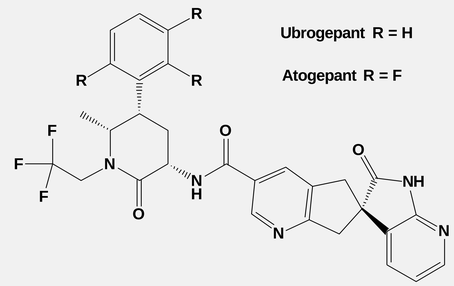
The objective of this study was to develop a novel LC-MS/MS bioanalytical method to support the evaluation of the pharmacokinetic attributes associated with the excretion of ubrogepant and atogepant in breast milk.

METHOD

- Calibration curves in human breast milk were prepared for ubrogepant and atogepant (1.00 - 1000 ng/mL).
- Samples extracted by protein precipitation with stable-labeled internal standards (d3) via the addition of acetonitrile (CAN).
- Chromatographic separation was performed in reversed-phase mode with a Waters XBridge C18 (2.1 x 30 mm, 3.5 µm) analytical column set at 50°C with a mobile phase of acetonitrile and 0.02% acetic acid (aq).
- Following isocratic elution (30% ACN at 1000 µL/min), phospholipids were flushed from the column at a high flow rate with 95% ACN at 1500 µL/min.
- Parent ions were formed by positive ion electrospray on a SCIEX API 5000 with MRM transitions *m/z* 550.2 > 264.1 and 604.2 > 264.1 for ubrogepant and atogepant, respectively.

RESULTS

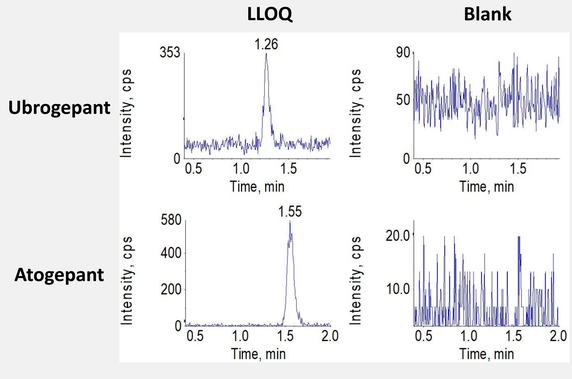
Figure 1. Structures of Ubrogepant and Atogepant



Chromatography

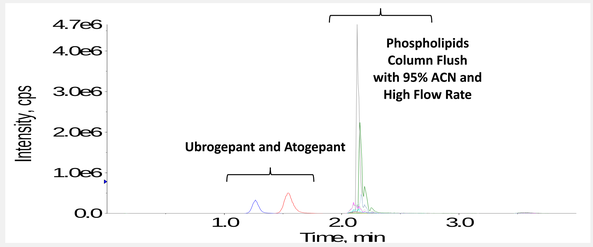
A novel, sensitive, and selective LC-MS/MS method was developed and validated for the determination of ubrogepant and atogepant in human breast milk. This high-throughput chromatography showed the elution of atogepant and ubrogepant at 1.26 and 1.55 minutes, respectively (***Figure 2***).

Figure 2. Chromatogram of Ubrogepant and Atogepant LLOQ and Blank



The lysophosphatidylcholines and phosphatidylcholines, which are notorious for suppressing MS signal, were demonstrated to be well separated from the analytes and were flushed out of the column at each injection to avoid late elution interference and allow method reproducibility and robustness (***Figure 3***).

Figure 3. Chromatogram of Ubrogepant, Atogepant, and Phospholipids



Evaluations

The extraction recovery was quantitative for both ubrogepant and atogepant. Calibration curves ranging from 1.00 - 1000 ng/mL were generated using a weighted ($1/x^2$) linear least-squares regression for each analyte. Precision and accuracy (%CV ≤9.9%, %bias between -9% and 8%, n=18) are summarized in ***Table 1.*** Specificity, matrix effect (n=3) in ***Tables 2 and 3,*** and matrix factor all meet acceptance criteria according to the ICH Guideline M10, based upon controls from six individual breast milk donors.

Table 1. Precision and Accuracy of Ubrogepant and Atogepant

Nominal Conc.	LLOQ QC 1.00 ng/mL	Low QC 3.00 ng/mL	Mid QC 500.00 ng/mL	High QC 750.00 ng/mL
	Ubrogepant			
Mean	1.08	2.99	492.27	728.07
%CV	5.6	3.7	3.8	4.3
%Bias	8.0	-0.3	-1.5	-2.9
	Atogepant			
Mean	0.91	2.92	504.99	749.95
%CV	9.9	5.1	1.7	3.6
%Bias	-9.0	-2.7	1.0	0.0

Acceptance Criteria: %CV ≤15% (LLOQ ≤20%); %Bias ±15%. (LLOQ Bias ±20%).

Table 2. Matrix Effect Evaluation of Ubrogepant

Donor	1	2	3	4	5	6
	Matrix Effect Ubrogepant					
Nominal Conc.	Low QC 3.00 ng/mL					
Mean	2.79	2.82	2.79	2.95	2.71	2.91
%CV	2.2	2.8	4.3	1.0	4.1	1.0
%Bias	-7.0	-6.0	-7.0	-1.7	-9.7	-3.0
Nominal Conc.	High QC 750.00 ng/mL					
Mean	747.31	709.10	708.63	721.31	702.60	733.21
%CV	1.2	1.0	5.7	2.8	2.6	3.0
%Bias	-0.4	-5.5	-5.5	-3.8	-6.3	-2.2

Acceptance Criteria: %CV ≤ 15%; %Bias ±15%.

Table 3. Matrix Effect Evaluation of Atogepant

Donor	1	2	3	4	5	6
	Matrix Effect Atogepant					
Nominal Conc.	Low QC 3.00 ng/mL					
Mean	2.86	2.83	2.96	2.64	2.84	2.66
%CV	0.3	9.9	4.7	14.4	6.3	9.8
%Bias	-4.7	-5.7	-1.3	-12.0	-5.3	-11.3
Nominal Conc.	High QC 750.00 ng/mL					
Mean	656.07	673.69	687.25	652.97	672.70	664.87
%CV	4.8	3.7	4.8	12.0	8.7	3.7
%Bias	-12.5	-10.2	-8.4	-12.9	-10.3	-11.4

Acceptance Criteria: %CV ≤ 15%; %Bias ±15%.

Stability

Ubrogepant and atogepant were stable in breast milk for at least 25 hrs at 4°C, 240 days for ubrogepant and 83 days for atogepant at -80°C and four freeze-thaw cycles; post-extraction stability was established for 168 h at 4°C.

CONCLUSION

The method developed for the quantitation of ubrogepant and atogepant in human breast milk was successfully validated and demonstrated to be specific, precise, and accurate, making it suitable for clinical sample analysis.

ALTASCIENCES

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