Ultrafast bioanalytical analysis using the Echo® MS system - two case studies

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ABSTRACT

Lopinavir and ritonavir, which are frequently co-administered protease inhibitors, were analyzed in human plasma on the Echo® MS system after preparation using an automatable liquid-liquid extraction method without an internal standard and were able to reach a lower limit of quantitation of 0.5 ng/mL for each compound. Alprazolam, a commonly prescribed benzodiazepine, was also analyzed in human plasma, and prepared using a similar liquid-liquid extraction method, this time with a labeled internal standard. The same 0.5 ng/mL lower limit of quantification was reached. In both cases samples were analyzed at a rate of one sample every two seconds, providing accurate data, at relevant sensitivities at an ultra-high throughput manner.

INTRODUCTION

Protease inhibitors (PIs) are a class of anti-viral drugs that prevent viral replication by selectively binding to viral proteases and inhibiting their function. The development of PI-based therapies has been of enormous benefit to people infected with HIV. Used in combination with other drugs, PIs have dramatically reduced the number of people who become ill from HIV-related opportunistic infections or who die from AIDS. Unfortunately, the effectiveness of protease inhibitors can fade over time. Mutations during viral replication can result in viruses that produce new, different proteases that are not targeted by current PI therapies.[1] The best way to avoid this drug resistance is to reduce or stop HIV replication. With less HIV replication, there is less of a chance of a new strain that is resistant to anti-HIV drugs. To keep HIV levels as low as possible, PIs are typically taken in combination with at least two other anti-HIV drugs. Such combination therapies are referred to as highly active antiretroviral therapy (HAART). Lopinavir and ritonavir are two protease inhibitors that are often used as part of a fixed-dose combination and serve as the model compounds in this study.

Benzodiazepines are often used to treat anxiety, insomnia and central nervous system disorders. They belong to the class of depressant drugs, acting on GABA receptors to dampen neural pathway excitation and consequently provide a calming effect.[1] They are among the most common prescribed psychiatric medications. However, benzodiazepines are often accompanied by other drugs of abuse and detected alongside opioids in many overdose cases and fatalities. Alprazolam is the most prescribed benzodiazepine for anxiety and panic disorders.[3] As such, rapid, high-throughput detection of alprazolam in plasma is critical to forensic and clinical toxicology. LC-MS analysis strategies can be time consuming to develop and implement as they typically require lengthy and costly solid-phase extraction (SPE) sample preparation. Acoustic Ejection Mass Spectrometry (AEMS) offers a clear alternative for analysis of alprazolam in plasma.

Acoustic Ejection Mass Spectrometry (AEMS), as implemented in the Echo MS system with a SCIEX Triple Quad 6500+ mass spectrometer, offers clear benefits for quantification of lopinavir and ritonavir in human plasma. Requiring minimal sample preparation and no chromatographic separation, it provides high sample throughput without sacrificing robustness or reproducibility.

MATERIALS AND METHODS

Sample Preparation:

Lopinavir and ritonavir (Sigma Aldrich) were spiked into human plasma (BioIVT) samples in the range of 0.5 ng/mL to 250 ng/mL each. Samples were processed using a liquid-liquid extraction method as follows. 0.5 mL of di-isopropyl ether was added to 0.1 mL aliquots of spiked plasma. Samples were vortexed for 1 minute followed by centrifugation at 12,000 rpm for 5 minutes. 0.4 mL of supernatant liquid was collected and dried under a nitrogen stream. Samples were reconstituted in 100 µL of 25% v/v methanol in water and transferred to a 384-well plate for analysis by AEMS. Plates are available from Beckman Coulter Life Sciences.

Alprazolam was spiked into human plasma in the range of 0.5 ng/mL to 250 ng/mL along with 50 ng/mL of internal standard (alprazolam D5). Samples were processed using a liquid-liquid extraction method as follows. 1mL of di-isopropyl ether was added to 0.3 mL aliquots of spiked plasma. Samples were vortexed for 1 minute followed by centrifugation at 12,000 rpm for 5 minutes. 0.8 mL of supernatant liquid was collected and dried under a nitrogen stream. Samples were reconstituted in 100 µL of 25% v/v methanol in water for analysis.

Table 1. Optimized compound parameters for the analysis.

Name	Q1 / Q3 (m/z)	DP (V)	CE (V)	CXP (V)
Ritonavir	721.3/296.1	30	28	10
Lopinavir	629.3/447.2	30	22	10
Alprazolam	309.2 / 205.1	55	61	9
Alprazolam D5	314.2 / 210.0	55	55	9

Table 2. Optimized source conditions for the analysis.

Source parameters	Value	Source parameters	Value
Curtain gas (psi)	25	CAD gas (psi)	9 (Alprazolam) 10 (Ritonavir - Lopinavir)
Ion source gas 1 (psi)	90	Ion spray voltage (V)	5000
Ion source gas 2 (psi)	70	Source temperature (°C)	350

Acoustic ejection method :
Methanol was used as carrier solvent in this assay at 425 µL/min. 50nL sample volumes were ejected into the mass spectrometer for the analysis Alprazolam and 0.1%v/v formic acid was added to methanol for Ritonavir – Lopinavir assay.

Data processing:
Data processing was performed using SCIEX OS software. A calibration curve was generated with six replicates at each concentration level to evaluate ejection reproducibility and accurately determine the lower limit of quantification (LLOQ).

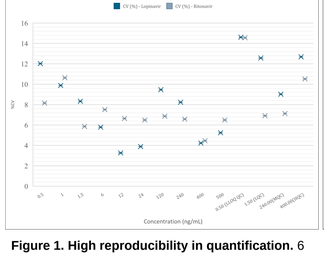

Figure 1. High reproducibility in quantification. 6 replicates at each concentration level of lopinavir and ritonavir in extracted human plasma, along with 6 replicates at quality control levels, showed excellent %CVs, below 15% at all levels.

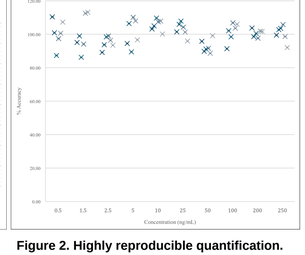

Figure 2. Highly reproducible quantification. 6 replicates at each concentration level of alprazolam in extracted human plasma showed excellent % accuracy with %CVs below 15% at all levels.

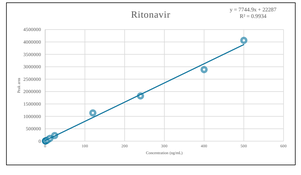

Figure 3. Calibration curve for quantification of ritonavir in human plasma using the Echo MS system.

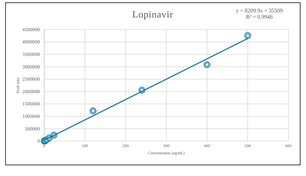

Figure 4. Calibration curve for quantification of lopinavir in human plasma using the Echo MS system.

Table 3. Quantification summary for ritonavir analysis by the Echo MS system.

Actual Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)	CV (%)	N
0.50	0.49	97.37	8.15	6
1.00	1.06	106.14	10.63	6
1.50	1.48	98.51	5.86	6
6.00	5.56	92.66	7.51	6
12.00	13.24	110.31	6.64	6
24.00	26.72	111.34	6.49	6
120.00	134.81	112.34	6.86	6
240.00	215.70	89.88	6.58	6
400.00	341.23	85.31	4.46	6
500.00	480.76	96.15	6.49	6
Quality Control				
0.50 (LLOQ QC)	0.46	91.68	14.55	6
1.50 (LQC)	1.45	96.36	6.91	6
240.00 (MQC)	248.37	103.49	7.12	6
400.00 (HQC)	429.18	107.30	10.52	6

Table 4. Quantification summary for lopinavir analysis by the Echo MS system.

Actual Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)	CV (%)	N
0.50	0.50	100.02	12.02	6
1.00	1.01	101.05	9.88	6
1.50	1.46	97.35	8.33	6
6.00	5.68	94.62	5.79	6
12.00	13.62	113.52	3.26	6
24.00	26.49	110.37	3.89	6
120.00	133.63	111.36	9.46	6
240.00	225.31	93.88	8.23	6
400.00	342.02	85.51	4.23	6
500.00	466.56	93.31	5.24	6
Quality Control				
0.50 (LLOQ QC)	0.49	98.51	14.61	6
1.50 (LQC)	1.41	94.31	12.57	6
240.00 (MQC)	242.70	101.13	9.02	6
400.00 (HQC)	416.81	104.20	12.68	6

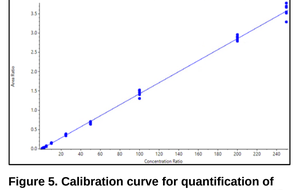

Figure 5. Calibration curve for quantification of alprazolam in human plasma using the Echo MS system. Good linearity and reproducibility were observed for the samples ejected in six replicates at each concentration level.

Table 5. Quantification summary for Alprazolam analysis by the Echo MS system.

Actual Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)	CV (%)	N
0.50	0.50	100.63	8.04	6
1.50	1.50	100.02	10.78	6
2.50	2.38	95.02	3.87	6
5.00	5.04	100.87	8.36	6
10.00	10.56	105.55	3.33	6
25.00	25.70	102.78	4.10	6
50.00	46.33	92.67	4.31	6
100.00	101.41	101.41	5.67	6
200.00	201.35	100.67	2.24	6
250.00	250.94	100.38	4.83	6

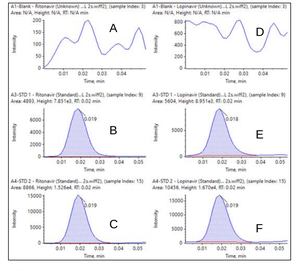

Figure 6. XICs for transition for ritonavir and lopinavir using the Echo MS system. Blank, 0.5 ng/mL and 1.5 ng/mL (A,B,C for ritonavir and D,E,F for lopinavir, respectively) are shown.

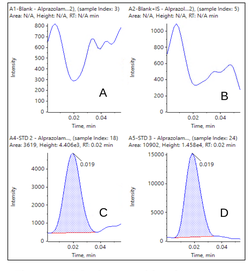

Figure 7. XICs for transition for alprazolam. Double blank and blank from extracted plasma (A and B), LLOQ at 0.5 ng/mL (C) and 1.5 ng/mL (D).

CONCLUSIONS

The Echo MS system produced very sensitive, accurate and reproducible results for the quantitative analysis. Lopinavir and ritonavir both showed very good reproducibility and accuracy over the calibration range of 0.5 to 500 ng/ml in human plasma, indicating that this method could be used for routine analysis of these compounds without an internal standard. Alprazolam showed good linearity and reproducibility as well in the same concentration range, showing that incorporation of a labeled internal standard, while it may not be necessary, can provide equivalent, and possibly slightly more reproducible data. The extremely short analysis times (2 sec/sample) enabled rapid generation of quantitative data for high numbers of samples.

REFERENCES

- Baghla R, Kern R, Ultra-fast, Accurate and simultaneous quantification of ritonavir and lopinavir in human plasma, SCIEX technical note.
- Pearson M, Baghla R, Kern R Ultra-fast quantification of alprazolam in human plasma extracts, SCIEX technical note

TRADEMARKS/LICENSING