Validation of an HPLC/MS/MS Bioanalytical Method for the Quantitative Analysis of Loxapine, Amoxapine, 7-OH-Loxapine and 8-OH-Loxapine in Human Plasma

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Overview

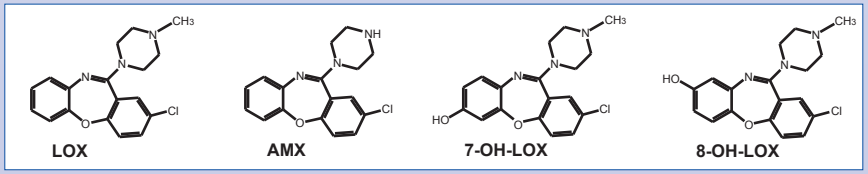
- **Purpose** - Develop and validate an HPLC/MS/MS method to determine concentrations of Loxapine, Amoxapine, 7-OH-Loxapine and 8-OH-Loxapine in human plasma
- **Methods** – 96 well-plate SPE ion-exchange extraction and HPLC/MS/MS (API4000)
- **Results** – Range from 0.05 - 50 ng/mL with accuracies and precision better than +/-15% using HPLC/MS/MS

Introduction

Loxapine (LOX) is a dibenzoxazepine neuroleptic drug used mainly for the treatment of schizophrenia. Loxapine is metabolized to amoxapine (AMX), 7-OH-Loxapine (7-OH-LOX) and 8-OH-Loxapine (8-OH-LOX).

As new therapeutic areas and routes of administration are explored for LOX, therapeutic concentrations could be achieved with a <1 mg dose of LOX. At these dose levels, LOX and metabolites could be near 0.05 ng/mL in human plasma 24 hours post dose.

Previous HPLC assays quantified LOX and metabolites but limits of quantitation >3 ng/mL were achieved. These assays also suffered from poor precision (>20% RSD) and long run times (>10 minutes/sample). Here we report on an accurate and precise LC/MS/MS assay for the determination of LOX, AMX, 7-OH-LOX and 8-OH-LOX from human plasma.



Methods

Extraction

- Acidify plasma sample
- 96 well plate SPE using weak-cation exchange cartridges
- Elute with methanol containing formic acid, then dry down
- Reconstitute in water/acetonitrile

HPLC

- Gradient HPLC using acetonitrile and water with 1% formic acid.
- Flow rate = 0.5 mL/minute
- HSC18 2.1x50 mm (Supelco)
- Twenty-Five µL injections

Mass Spectrometry

- Sciex API4000 operating in MRM mode
- ESI
- Positive ion mode
- MRM transition s for LOX, AMX, 7-OH-LOX and 8-OH-LOX
  - 328.4 → 270.5 (LOX)
  - 314.6 → 270.5 (AMX)
  - 344.4 → 286.5 (7-OH-LOX)
  - 344.4 → 286.5 (8-OH-LOX)

Table 1. Typical Standard Curve and QC Results for the HPLC/MS/MS Analysis of Loxapine, Amoxapine, 7-OH-Loxapine and 8-OH-Loxapine from Human Plasma.

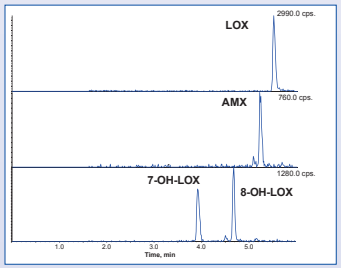
QC Level (ng/mL)	Intraassay Accuracy and Precision (%±%RSD)	Intraassay Accuracy and Precision (%±%RSD)
Loxapine		
0.0500	87.2 ± 6.6	*
0.150	92.1 ± 6.8	100 ± 3.1
5.00	104 ± 0.6	105 ± 1.9
40.0	94.9 ± 2.2	94.0 ± 2.8
50.0	95.9 ± 2.9	*
Amoxapine		
0.0500	92.8 ± 2.8	*
0.150	92.1 ± 3.9	98.4 ± 3.2
5.00	100 ± 2.5	105 ± 3.5
40.0	98.3 ± 1.9	98.9 ± 4.4
50.0	104 ± 1.4	*
7-OH Loxapine		
0.0500	95.1 ± 6.8	*
0.150	94.3 ± 6.3	101 ± 4.6
5.00	102 ± 2.5	103 ± 3.6
40.0	93.7 ± 2.0	94.4 ± 2.4
50.0	98.1 ± 4.2	*
8-OH Loxapine		
0.0500	88.6 ± 5.2	*
0.150	89.9 ± 5.0	98.2 ± 3.0
5.00	100 ± 1.6	102 ± 2.7
40.0	94.2 ± 1.4	96.8 ± 1.2
50.0	100 ± 1.7	*

All standard curves gave correlation coefficients >0.99 and had accuracies better than 7%

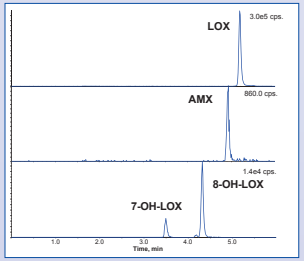
Table 2. Short-Term Stability Results for the HPLC/MS/MS Analysis of Loxapine, Amoxapine, 7-OH-Loxapine and 8-OH-Loxapine from Human Plasma.

Freeze Thaw Stability (% Difference from Control)	Benchtop Stability (Hours)	Extract Stability (Hours)
Loxapine		
±4%	> 6	> 45
Amoxapine		
±6%	> 6	> 45
7-OH-Loxapine		
±2%	> 6	> 45
8-OH-Loxapine		
±3%	> 6	> 45

HPLC/MS/MS Chromatogram from the Analysis of a Human Plasma Sample Fortified with 0.05 ng/mL of Loxapine, Amoxapine, 7-OH-Loxapine and 8-OH-Loxapine



HPLC/MS/MS Chromatogram from the Analysis of a Human Plasma Sample from a Patient Dosed with Loxapine



Conclusions

- Developed and validated an HPLC/MS/MS method to quantify Loxapine and its metabolites, Amoxapine, 7-OH-Loxapine and 8-OH-Loxapine from human plasma with an LLOQ of 0.05 ng/mL
- Method supports PK studies for Loxapine in clinical trials

References

- Hue B, et. al. *Therapeutic Drug Monitoring* 20:335-339, (1998)
- Cheung SW, et. al. *J Chromatogr* 564:213-221, (1991)