Validation of an HPLC/MS/MS Bioanalytical Method for the Quantitative Analysis of Lidocaine from Rat and Mini-Pig Plasma

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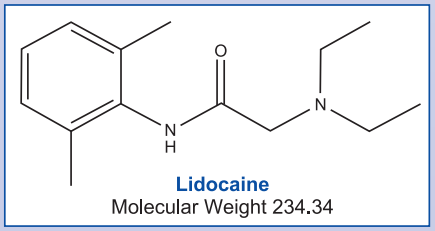
Overview

- **Purpose** - Develop and validate an HPLC/MS/MS method to determine concentrations of Lidocaine from mini-pig and rat plasma
- **Methods** – Protein precipitation and HPLC/MS/MS (API4000)
- **Results** – Concentrations range from 10 – 10,000 ng/mL with accuracies and precision better than 15% using HPLC/MS/MS

Introduction

Lidocaine was one of the first in class amino-amide type local anesthetics. Lidocaine is commonly used to relieve skin irritations and injected during dental and minor surgeries. As lidocaine formulations and delivery techniques are improved, the need to measure systemic levels of lidocaine in plasma is warranted.

Previous HPLC or LC/MS/MS assays required run times >10 minutes and/or laborious extraction procedures for the analysis of lidocaine from biological fluids.1 Here we report on a simple preparation method coupled with LC/MS/MS to provide an accurate and precise assay with a 5 minute run time for the determination of lidocaine from rat and mini-pig plasma.



Lidocaine
Molecular Weight 234.34

Methods

Extraction

- Lidocaine extracted from plasma using methanol precipitation
- HPLC/MS/MS was used in the postive ion mode with the Turbolonspray™ source (API4000)

HPLC

- Gradient HPLC using (MEOH/acetone 9/1) with 1% formic acid and water with 1% formic acid mobile phases
- Flow rate = 0.8 mL/minute
- HSC18 2.1x50 mm (Supelco)
- Column heated to 50°C

Mass Spectrometry

- Sciex API4000 operating in MRM mode
- ESI
- Positive ion mode
- MRM transitions for Lidocaine 235.5 ➔ 85.8

Table 1. Typical Standard Curve and QC Results for the HPLC/MS/MS Analysis of Lidocaine in Mini-Pig and Rat Plasma.

QC Level (ng/mL)	Intra-assay Accuracy and Precision (% ± %CV)	Inter-assay Accuracy and Precision (% ± %CV)
Mini-Pig		
10.0	106 ± 7.1	100 ± 13.7
30.0	94.1 ± 3.4	107 ± 12.0
500	98.0 ± 2.9	109 ± 9.1
8000	97.0 ± 1.8	104 ± 9.5
10000	93.9 ± 2.2	103 ± 11.7
Rat		
10.0	88.0 ± 13.2	96.6 ± 12.5
30.0	89.5 ± 5.9	101 ± 7.6
500	93.5 ± 5.0	102 ± 6.4
8000	101 ± 3.9	98.5 ± 4.7
10000	98.0 ± 2.5	97.1 ± 7.7

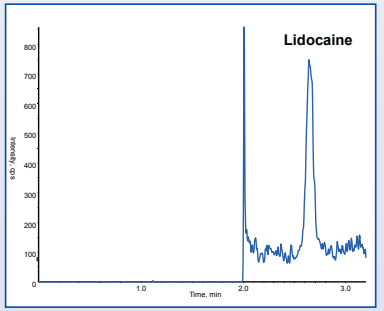
All standard curves gave correlation coefficients >0.995 and had accuracies better than ± 5%
Lidocaine d_{10} was used as the internal standard

Table 2. Short-Term Stability Results for the HPLC/MS/MS Analysis of Lidocaine in Mini-Pig and Rat Plasma.

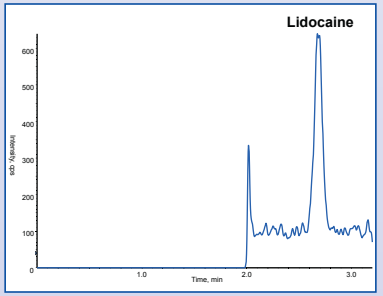
Freeze Thaw Stability (% Difference from Control)	Bench-Top Stability (Hours)	Extract Stability (Hours)
Mini-Pig		
± 11.6%	> 53	> 48
Rat		
± 9.6%	> 53	> 23

Table 3. Additional Validation Results for the HPLC/MS/MS Analysis of Lidocaine from Mini-Pig and Rat Plasma.

Lidocaine Recovery (%)	Matrix Factor (MF, CV)	100% Hemolyzed Plasma (% Diff. from Control)	50x Dilution QC (% Difference from Control)
Mini-Pig			
100-108	1.08, 3.2%	±6.9%	-5.3%
Rat			
95.7-104	0.967, 3.4%	±16.0%	3.5%



HPLC/MS/MS Chromatogram from the Analysis of a Mini-Pig Plasma Sample Fortified with 10.0 ng/mL of Lidocaine



HPLC/MS/MS Chromatogram from the Analysis of a Rat Plasma Sample Fortified with 10.0 ng/mL of Lidocaine

Conclusions

- Developed and validated an HPLC/MS/MS method to quantify Lidocaine in mini-pig and rat plasma
- To date over 300 mini-pig and rat plasma samples have been analyzed for Lidocaine in support of multiple PK studies

References

1. Maes, et. al. *J Chromatogr B* 852:180-87