Measurement of Lidocaine and 2,6-DMA in Minipig Plasma, Skin, and Dermal Tapes Using UHPLC with Electrospray MS/MS

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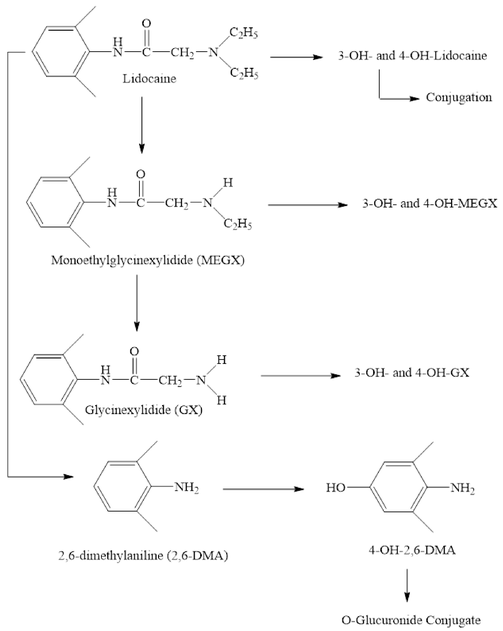
INTRODUCTION

Lidocaine, or 2-diethylaminoaceto-2,6-xylidide, was synthesized in 1943 and has been utilized as a local anesthetic agent. Medical indications for lidocaine include short-term emergency control of ventricular arrhythmias, post-herpetic neuralgia pain, localized neuropathic pain, chronic low back pain, and intravenous infusion for management during surgical recovery. Lidocaine metabolism has been investigated in several species. In humans, more than 80% of the lidocaine dose is excreted in urine as the glucuronide conjugate of 4-OH-2,6-xylidine (4-OH-2,6-dimethylaniline), as shown in the metabolic pathway below. The pathway also indicates the presence of an intermediate metabolite, 2,6-xylidine, or 2,6-dimethylaniline (2,6-DMA).

Reported in this poster are sensitive LC-MS/MS methods developed to measure lidocaine and 2,6-DMA with application to transdermal studies.

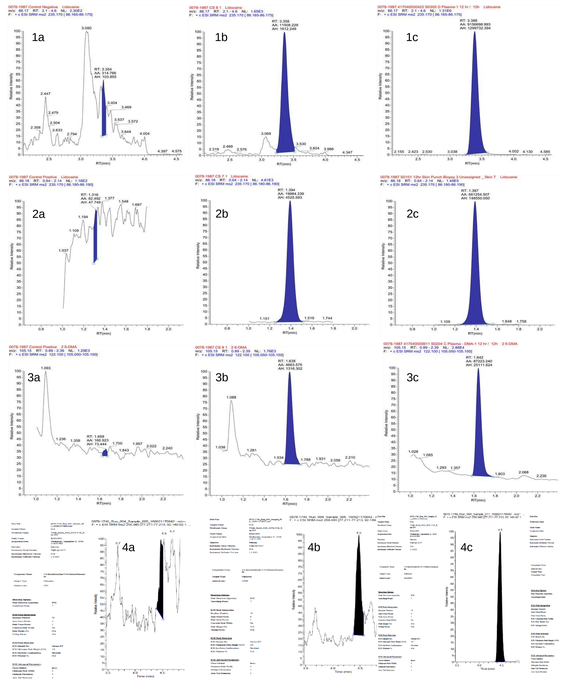


METHODS

The methods for lidocaine in minipig plasma, tissue biopsies, and dermal tapes utilized mixed mode/SCX solid phase extraction as clean-up procedure. Tissue biopsy homogenization and stratum corneum extraction from dermal tapes were optimized for extraction efficiencies. Chromatographic separations were conducted on Waters BEH C18 or Waters HSS T3 columns with detection by positive electrospray ionization in SRM mode. The lower quantitation limits were of 25 pg/mL in plasma, 15 ng/g in tissue, and 5 ng/tape for lidocaine.

2,6-DMA was measured in plasma by ultrafiltration, and in skin tissue homogenates by ultrafiltration and further derivatization using 4-methoxybenzoyl chloride to form the corresponding benzamide derivative. Chromatographic separations were conducted on a Waters BEH C18 column with detection by positive electrospray ionization in MRM mode. The lower quantitation limits for 2,6-DMA in plasma and the benzamide derivative of 2,6-DMA in skin tissue homogenate was 200 pg/mL (or 0.60 ng/g tissue). Due to the absence of pertinent metabolism in stratum corneum, the dermal tape assay was not developed for 2,6-DMA.

REPRESENTATIVE CHROMATOGRAMS



Chromatograms from plasma extracts of (1a) double blank, (1b) 25 pg/mL lower limit calibration standard and (1c) an incurred sample (105 ng/mL) using the conventional SPE lidocaine method

Chromatograms of lidocaine from tissue homogenates of (2a) zero blank, (2b) 3.0 µg/mL lower limit calibration standard, and (2c) an incurred sample (101 µg/mL) using the high range tissue method

Chromatograms of 2,6-DMA from minipig plasma, from (3a) zero blank, (3b) 0.200 ng/mL lower limit calibration standard and (3c) an incurred sample (3.85 ng/mL)

Chromatograms of 2,6-DMA as the 4-methoxybenzamide derivative in skin biopsy tissue homogenates (4a) zero blank, (4b) 0.200 ng/mL calibration standard (LLOQ) and (4c) 500 ng/mL calibration standard (ULOQ)

DATA SUMMARY

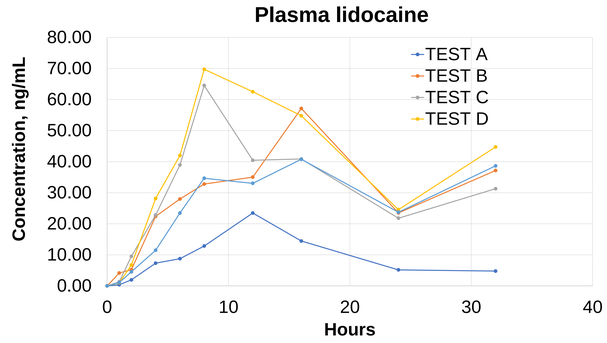
Matrix	K_3EDTA plasma	K_3EDTA plasma	Biopsy (high range)	Biopsy (low range)	Dermal tape
Extraction	Standard SPE	Microelution SPE	Standard SPE	Standard SPE	Standard SPE
ULOQ	25 ng/mL	25 ng/mL	300 µg/gm	15.0 µg/gm	5.0 µg/tape
LLOQ	25 pg/mL	50 pg/mL	3 µg/gm	15.0 ng/gm	5.0 ng/tape
Sample volume	100 µL	100 µL	50 µL	50 µL	50 µL
P&A, low limit	4.0%, -2.8%	2.7%, 3.2%	3.7%, 2.3%	5.9%, 8.0%	2.6%, 4.0%
P&A, low	2.8%, 0.0%	3.0%, -2.3%	2.3%, 1.3%	5.6%, 5.8%	4.2%, 4.7%
P&A, mid	2.1%, 2.7%	1.1%, 1.1%	3.3%, 2.4%	5.3%, 4.4%	1.7%, 2.9%
P&A, high	2.3%, 0.0%	1.8%, -3.2%	2.0%, -0.9%	4.6%, -1.8%	2.8%, 4.3%
Over range dil.	DF = 10	nt	nt	nt	DF = 10
Benchtop, RT	21.6 hr	nt	22.7 hr	21.5 hr	27.3 hr
Freeze/thaw	3 cycles, -20/-80 °C	nt	3 cycles, -20/-80 °C	4 cycles, -20 °C 2 cycles, -80 °C	3 cycles, -20/-80 °C
Whole blood	> 2 hr, RT	nt	na	na	na
Long-term	127 days, -20/-80 °C	nt	120 days, -20/-80 °C	121 days, -20/-80 °C	3 days
Hemolysis	No effect	nt	na	na	na
Recovery	93.5-95.2%	87.2-99.2%	92.4-98.0%	78.2-79.6%	97.1-101.2%
Extract stability	92 hr	nt	149 hr	204 hr	59 hr
Reinj. repro.	67 hr	nt	138 hr	186 hr	49 hr
Selectivity	10/10 < 20% LLOQ	10/10 < 20% LLOQ	10/10 < 20% LLOQ	10/10 < 20% LLOQ	10/10 < 20% LLOQ
Spiked selectivity at low QC, P&A	1.9%, +2.1% n = 10	3.5%, +1.1% n = 10	1.2%, +2.7% n = 10	7.6%, +5.8% n = 10	2.4%, -2.0% n = 10
Regression	Linear, $1/x^2$	Linear, $1/x^2$	Linear, $1/x^2$	Linear, $1/x^2$	Linear, $1/x^2$

P&A: precision and accuracy, Reinj. repro.: Reinjection reproducibility, DF: dilution factor, Overrange dil.: over range dilution
nt: not tested, na: not applicable,

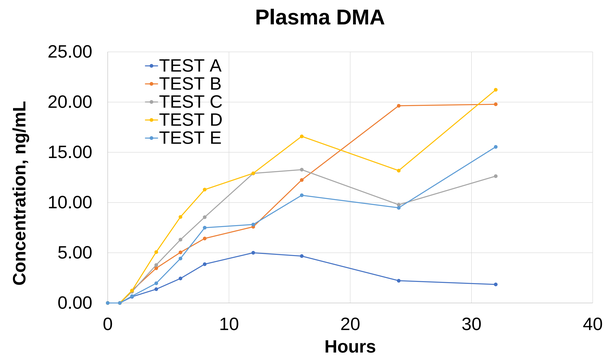
RESULTS

Method development and optimization for each analyte and matrix resulted in robust methods that were validated to regulatory standards in all cases except 2,6-DMA in tissue homogenates for which a partial validation was conducted.

The methods have supported several pilot studies to determine the relative bioavailability of lidocaine in transdermal formulations. For example, in a study evaluating formulations of several topically applied lidocaine-containing patches in female Gottingen minipigs, 5 formulations were administered to 10-15 kg minipigs over 32 hours. Ten blood draws were collected per animal as well as single-point collection of skin punch biopsies and dermal tapes 12 hours post dosing. The methods described provided plasma pharmacokinetic concentration-time plots over 32 hours of dosing for both lidocaine and 2,6-DMA. The amounts of lidocaine measured per tape ranged from 0.98 to 54.4 µg/tape and lidocaine tissue concentrations were measurable in all but one sample and ranged from 6.1 to 203 µg/gm.



Plasma lidocaine concentrations measured after administration of various dermal patches for 12 hours, each point representing the average from 5 animals per test lot.



Plasma 2,6-DMA concentrations for the same specimens

CONCLUSION

A collection of novel methods for minipig plasma and skin as well as dermal tape were developed to determine the concentrations of lidocaine and 2,6-DMA. Quantitation limits were approximately 7-fold lower than previously reported for lidocaine and 3-fold lower for 2,6-DMA.