Validation of an assay method for lidocaine, prilocaine and tetracaine using UV detection high-performance liquid chromatography

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INTRODUCTION

Limited options for immediate pain relief in cases of traumatic skin injuries are available. Currently, peri-lesional lidocaine injection being the primary method. This approach is often challenging for medical personnel to administer and can cause discomfort for patients.

To address these issues, researchers have formulated a novel topical anesthetic combining lidocaine, prilocaine, and tetracaine.

Develop and validate a High-Performance Liquid Chromatography (HPLC) method to initiate stability studies and biopharmaceutical assessments of this new formulation.

MATERIALS & METHODS

ANALYTICAL VALIDATION

Mobile phase
Acetonitrile/PBS* (40/60)
**Phosphate-buffered saline 40mM at pH = 7*

Stationary phase
Kinetex® Column C18
2.6µm ; 150mm x 4.6mm

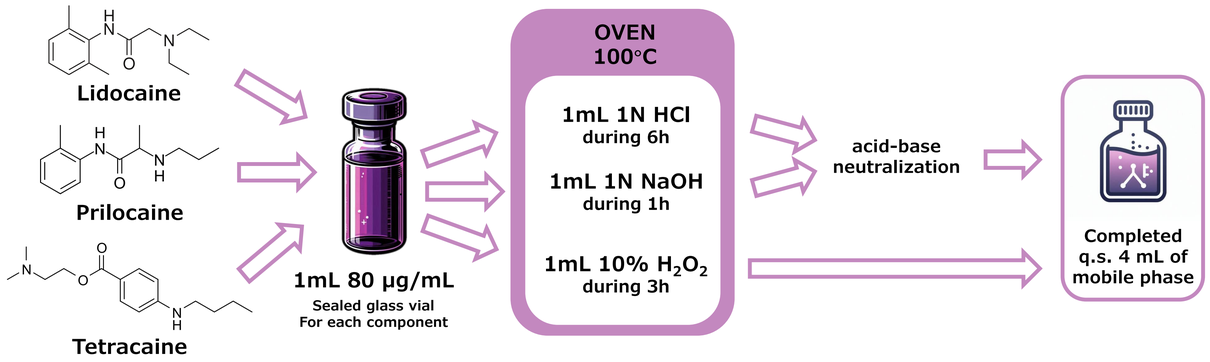
HPLC Shimadzu®
Flow rate 1 mL/min
Injection 20 µL
Thermostated 25.0 °C

UV detector
220 nm and 330 nm

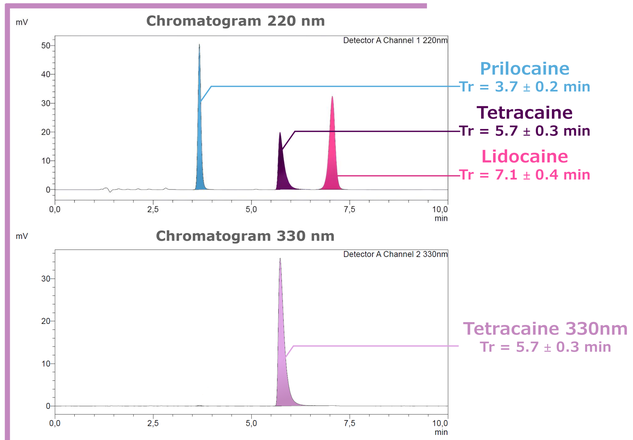
Concentration
Target 20 µg/mL
Range 12 – 28 µg/mL

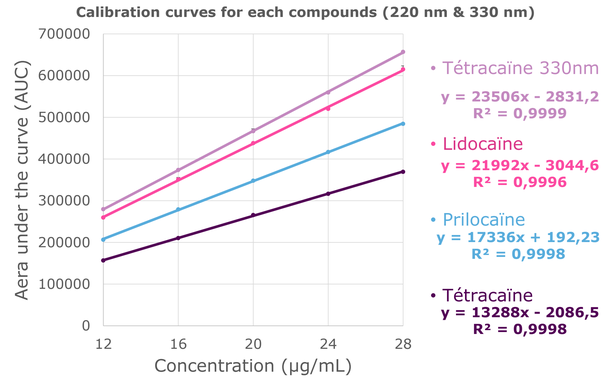
→ **Specificity** (Standard solutions for each compounds), **linearity, precision and accuracy**
In accordance with the recommendations of the International Conference on Harmonisation (ICH)

FORCED DEGRADATION



RESULTS & DISCUSSION





Linearity: the method was linear in range concentration (3 repetitions, n= 15) (R^2 > 0.999) (p-value < 0.05)

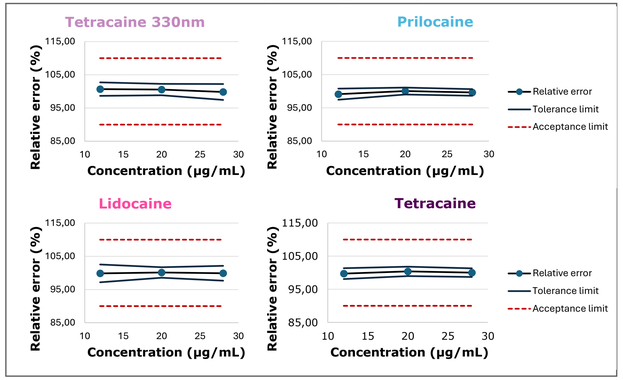
Specificity: chromatographic peak separation was achieved (R > 1.5)

Precision and accuracy of calibration curves

Calculated concentration ± SD (µg/mL)	Accuracy (%)	Calculated concentration ± SD (µg/mL)	Accuracy (%)
Tetracaine 330nm		**Prilocaine**	
12.02 ± 0.16	**100.1 ± 1.3**	11.90 ± 0.16	**99.1 ± 1,3**
16.02 ± 0.07	**100.1 ± 0.4**	16.09 ± 0.19	**100.6 ± 1.2**
19.98 ± 0.23	**99.9 ± 1.2**	20.05 ± 0.05	**100.3 ± 0.2**
23.92 ± 0.12	**99.7 ± 0.5**	24.03 ± 0.29	**100.1 ± 1.2**
28.07 ± 0.10	**100.2 ± 0.3**	27.92 ± 0.12	**99.7 ± 0.4**
Lidocaine		**Tetracaine**	
11.95 ± 0.18	**99.6 ± 1.5**	11.94 ± 0.15	**99.5 ± 1.2**
16.10 ± 0.22	**100.6 ± 1.4**	15.99 ± 0.03	**100.0 ± 0.2**
20.06 ± 0.03	**100.3 ± 0.2**	20.14 ± 0.11	**100.7 ± 0.5**
23.79 ± 0.07	**99.1 ± 0.3**	23.98 ± 0.11	**99.9 ± 0.5**
28.10 ± 0.38	**100.4 ± 1.4**	27.95 ± 0.12	**99.8 ± 0.4**

Intermediate precision: the coefficient of variation was less than 1.52% (3 days: n = 27)
Precision & accuracy: Recovery rates ranged between ~99% and ~101%

Accuracy profiles (β = 90%)



Percentage of product degraded

	Lidocaine	Prilocaine	Tetracaine
Acid	**3 %**	**4 %**	**98 %**
Base	**2 %**	**1 %**	**100 %**
Oxydative	**78 %**	**32 %**	**66 %**

No degradation products were detected at the retention times of the parent compounds

CONCLUSION

The analytical method has been **successfully validated** according to the criteria outlined in the ICH guideline Q2 (R1). This validated method allows for the **accurate and precise quantification of lidocaine, prilocaine and tetracaine**. With this robust analytical tool, researchers can now proceed with stability studies and biopharmaceutical assessments of the novel topical formulation.