



# Simultaneous quantification of lidocaine and prilocaine in human plasma by LC-MS/MS and its application in a human pharmacokinetic study

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## ABSTRACT

**Objective:** The aim of the work was to develop and validate a simple, sensitive and selective Liquid chromatography with Mass spectroscopic method for simultaneous quantification of lidocaine and prilocaine in human plasma.

**Design and methods:** Analytes and the internal standards from human plasma were extracted by using solid-phase extraction technique using Waters Oasis® HLB 1 cc (30 mg) cartridges. The reconstituted samples were chromatographed on Phenomenex Kinetex EVO 4.6\*100 mm 2.6 μ 100A column by using a mixture of acetonitrile and 5 mM ammonium acetate buffer (80:20, v/v) as the mobile phase at a flow rate of 0.6 mL/min.

**Results:** The method was validated over the concentration range of 0.10–201.80 ng/mL for lidocaine and 0.10–201.66 ng/mL for prilocaine. The calibration curve obtained was linear.

**Conclusion:** Method validation was performed as per FDA guidelines and the results met the acceptance criteria. A run time of 3.0 min for each sample, make it possible to analyze more than 350 human plasma samples per day. The proposed method was found applicable for pharmacokinetic studies.

## 1. Introduction

Lidocaine is a very selective amino amide linked local anesthetic, which is widely used in treatment of ventricular arrhythmias. The analgesic effects are attributed to the drug induced blockade of receptors of nociceptive transmission like G-protein-coupled receptors. It is dealkylated by CYP3A4 to monoethylglycinexyidide, which has longer half-life than parent moiety [1,2]. Prilocaine is the prototypic amino amide type local anesthetic, which is prescribed for the epidural analgesia and peripheral nerve block. O-toluidine, metabolite of

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prilocaine is capable of oxidizing hemoglobin to methemoglobin, but is correlated with methemoglobinemia with dose exceeding 600 mg. In injectable form, often it is used in dentistry and for regional anesthesia [3,4]. Fig. 1 illustrates the chemical structures of lidocaine and prilocaine.

Synergism of amino amides with similar effects is rationale for successful pain management than a monotherapy. Numerous clinical trials on fixed dose combinations of lidocaine and prilocaine have demonstrated their highly effectiveness in alleviating pain, associated with medical procedures than a monotherapy. Oraquix® and EMLA®, a new combination therapy of lidocaine and prilocaine is permitted by US FDA for the treatment [5,6].

Very few HPLC, LC-MS and GC-MS methods have been reported for the estimation of lidocaine [7–15] and prilocaine [16–20] either independently or in pharmaceutical formulations and in biological samples. Currently, one HPLC method with UV detection [21] and one LC-MS/MS [22] method are only known for simultaneous determination of lidocaine and prilocaine in plasma samples. HPLC method utilizes liquid-liquid extraction with diethyl ether using bupivacaine as internal standard for simultaneous quantification of the drugs along with its metabolite [21]. LC-MS/MS method quantified lidocaine and prilocaine in the mouse plasma after an intact skin application of EMLA® (2.5% prilocaine and 2.5% lidocaine) [22].

Sensitive and quick bioanalytical methods are in greater demand for simultaneous quantification of lidocaine and prilocaine for pharmacokinetic and bioequivalence applications in humans. The current study describes a selective and sensitive method, which uses simple solid-phase extraction technique for sample preparation, followed by LC coupled with electrospray ionization-tandem mass spectrometry for simultaneous quantitation of lidocaine and prilocaine in human plasma. This assay method is evaluated by analyzing various samples from the healthy male volunteers, following topical application of lidocaine and prilocaine in a clinical pharmacokinetic study.

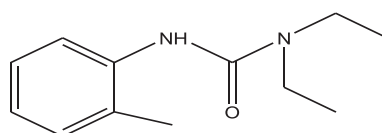
## 2. Experimental

### 2.1. Chemicals and reagents

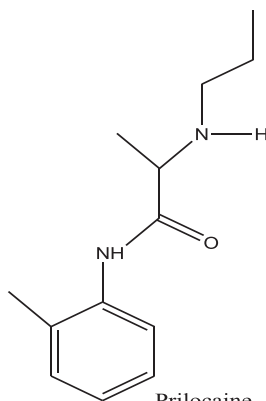
The reference samples of lidocaine (99.8%), prilocaine hydrochloride (99.8%) lidocaine-d6 hydrochloride (IS1) (99.1%) and prilocaine-d7 hydrochloride (IS2) (98.5%) were purchased from TLC Pharmaceutical Standards Ltd, (Ontario, Canada). Milli-Q water purification system (Millipore, Bangalore, India) was used to prepare water for LC-MS/MS analysis. Acetonitrile and methanol (HPLC grade) were purchased from J.T Baker, Phillipsburg, USA. Analytical grade acetic acid and ammonium acetate were purchased from Merck, Mumbai, India. Oasis® HLB 1cc (30 mg) solid phase extraction cartridges were procured from Waters corporation, Milford, Massachusetts, USA. Deccan Pathological Lab (Hyderabad, India) supplied control K2-EDTA human plasma sample.

### 2.2. Instrumentation and chromatographic conditions

A Nexera X2 UHPLC system coupled with LCMS-8060 system (Shimadzu Corporation Kyoto, Japan) controlled by LabSolutions



Lidocaine



Prilocaine

Fig. 1. Chemical structures of lidocaine and prilocaine.

workstation consisting of a Kinetex 2.6  $\mu$  EVO 100A, 4.6\*100 mm (Phenomenox, Torrance, CA,USA) column was used for the study. Column was maintained at 40 °C and processed sample aliquots (2  $\mu$ L) were injected into it. The mobile phase containing of acetonitrile and 10 mM ammonium acetate in Milli-Q water (80:20, v/v) was delivered with a flow rate of 0.6 mL/min into ESI (electrospray ionization) chamber of mass spectrometer. MS-MS detection was done in positive ion mode for quantitation of both the analytes and internal standards using a Shimadzu LC-MS equipped with a Turboionspray™ interface at 300 °C and DL Temperature 250 °C. The CID gas was set at 270 KPa. The source parameters namely the nebulizing Gas Flow was 3.00 L/min, heating Gas Flow was 10.00 L/min and drying Gas Flow was 10.00 L/min. The compound parameters namely, the Q1 pre-bias, Q3 pre-bias, collision energy (CE) were 12, 17, 10 V for lidocaine, 18, 17, 22 V for IS1, 26, 18,15 V prilocaine and 18, 19, 16 V for IS2. MRM (Multiple-reaction monitoring mode) was employed for detection of the ions, by checking the transition pairs of  $m/z$  235.10 precursor ion to the  $m/z$  86.15 for lidocaine;  $m/z$  241.30 precursor ion to the  $m/z$  86.15 for IS1;  $m/z$  221.20 precursor ion to the  $m/z$  86.15 product ion for prilocaine; and  $m/z$  228.10 precursor ion to the  $m/z$  86.15 product ion for IS2. Quadrupoles Q1 and Q3 were fixed on unit resolution. LabSolutions was used to process the analysis data obtained.

### 2.3. Preparation of standard solutions

Primary stock solutions of lidocaine (1000 $\mu$ g/mL), prilocaine (1000  $\mu$ g/mL), lidocaine-d6 (1000  $\mu$ g/mL) and prilocaine-d7 (1000  $\mu$ g/mL) were prepared in HPLC grade methanol and were kept at 2–8 °C. Working standard solutions of lidocaine and prilocaine were prepared by appropriate dilution of primary stock solutions using diluents (methanol and Milli-Q water, 60:40, v/v).

### 2.4. Preparation of calibration curve standards and quality control samples

Control human plasma of 950  $\mu$ L was spiked with 25  $\mu$ L working standard solutions of each analyte for calibration samples. A set of eight non-zero concentrations of calibration curve (CC) standards ranging from 0.10 to 201.80 ng/mL for lidocaine and 0.10–201.66 ng/mL for prilocaine were prepared. Quality control (QC) samples were prepared by spiking control human plasma in bulk with lidocaine and prilocaine at suitable concentrations. The QC samples prepared for lidocaine were 0.10 (LLOQ), 0.30 (LQC), 5.01 (MQC1), 85.93 (MQC2) and 171.87 ng/mL (HQC) and for prilocaine were 0.10 (LLOQ), 0.30 (LQC), 5.08 (MQC1), 86.32 (MQC2) and 172.65 ng/mL (HQC). All the samples were kept at  $-70 \pm 5$  °C for further use.

### 2.5. Sample processing

200  $\mu$ L aliquot of human plasma sample was mixed with 20  $\mu$ L of the internal standards mixture working solution (0.50  $\mu$ g/mL lidocaine-d6 and prilocaine-d7). To this, 200  $\mu$ L of 0.1% formic acid buffer solution and vortexed to ensure mixing of contents. The sample mixture was loaded onto Oasis® HLB 1 cc (30 mg) extraction cartridge that was pre-conditioned with 1 mL of methanol followed by 1 mL water. The extraction cartridge was washed with 1 mL of water and followed by 1 mL of 5% methanol in water and allowed to drying under positive pressure for approximately 1 minute. The analytes were eluted with 1 mL of mobile phase, vortexed and loaded the sample into auto sampler vials and 2  $\mu$ L of sample was injected into LC-MS/MS system.

### 2.6. Method validation

As per USFDA guidelines, a complete validation of the method was carried out [23]. The parameters validated were accuracy, precision, linearity, selectivity, sensitivity, matrix effect, dilution integrity, stability and recovery. Six blank human plasma matrix samples were analyzed to assess selectivity. Six spiked LLOQ samples were used to establish sensitivity. Matrix effect was assessed for both the analytes with internal standards at LQC and HQC concentration levels. Linearity was observed for lidocaine and prilocaine in the concentration range of 0.10–201.80 and 0.10–201.66 ng/mL, respectively. At four different QC levels, intra-day precision and accuracy were estimated by analyzing six replicates in the same day and on five different days. Recovery of lidocaine and prilocaine at LQC, MQC2 and HQC levels was determined by comparing the peak areas of analytes in extracted samples with that of non-extracted samples. Dilution integrity test was done at three times the LLOQ concentration of both the analytes. Different stability studies such as auto sampler, reinjection, bench top, wet extract, freeze-thaw and long term stabilities were completed at LQC and HQC levels for both the analytes.

#### 2.6.1. Regulatory requirements and compliance with ethical standards

The study was carried out in accordance with the provisions of the current version of the “CDSCO Schedule Y and its amendments, ICMR Ethical Guidelines for Biomedical Research on Human Subjects, ‘ICH Guidelines for Good Clinical Practice’, and Declaration of Helsinki”.

The protocol and the informed consent form (ICF) used to obtain informed consent of study participants healthy Indian male subjects ( $n = 12$ ) were reviewed by an Ethics Committee. The study protocol commenced only after obtaining the written approval from the Ethics Committee.

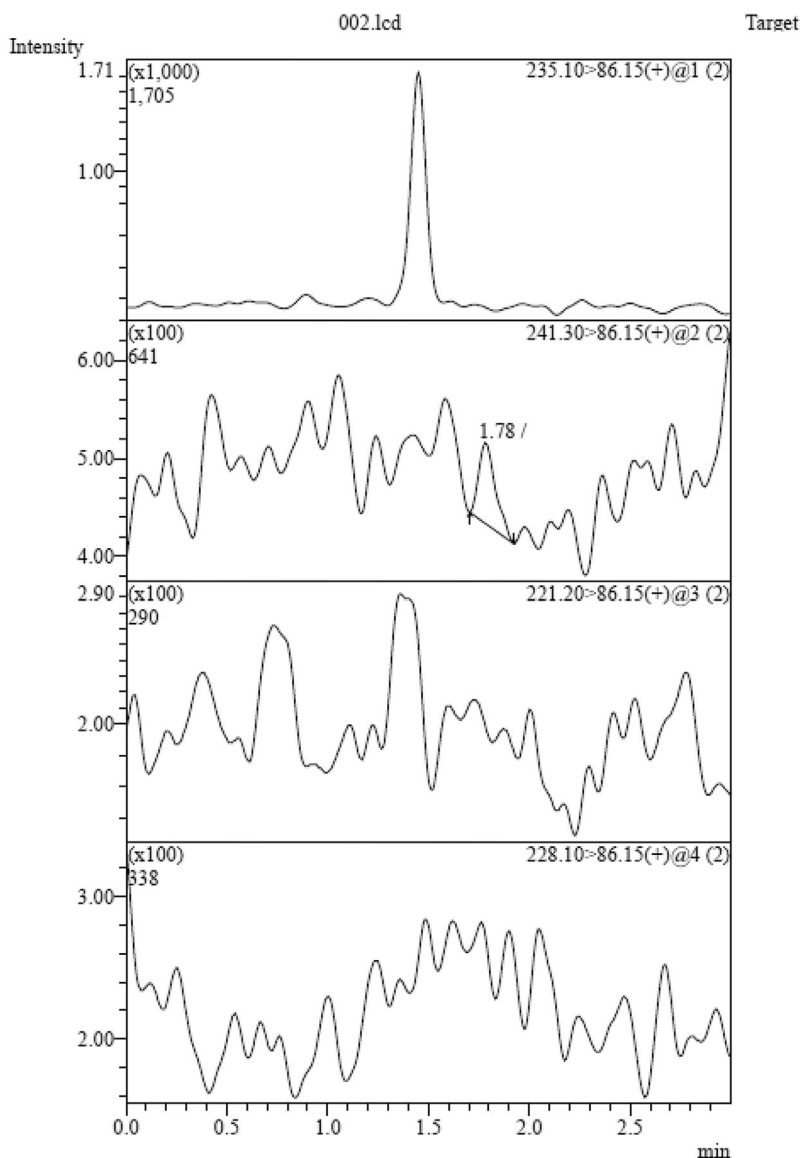
The study participants were informed before the initiation of the study through an oral briefing of the purpose, procedures to be carried out, potential hazards and rights of the volunteers during the course of the study. Participants understood and signed the consent forms voluntarily, prior to the study and specific procedures.

The data identifying each study-participant by name was kept confidential and accessible only to the quality assurance auditor during audits, and if necessary, to the Ethics Committee and various regulatory authorities.

## 2.7. Pharmacokinetic study design

Lidocaine (2.5%) and prilocaine (2.5%) cream was applied topically after observing overnight fast of at least 10 hours. Lidocaine and prilocaine cream 50 g was applied dermally over 400 cm<sup>2</sup> on thigh for 4 hours at which time the excess drug was removed. Cream was applied within 5 min of specified drug administration time. Blood samples (4 mL) were collected before application of the cream and at 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 15, 18, 24 and 36 h after application in K2-EDTA vacutainer collection tubes (BD, Franklin, NJ, USA). The tubes were centrifuged to collect the plasma at 3200rpm for 10 min at 4 °C. The subject plasma samples were stored at  $-70 \pm 5$  °C until their analysis. Plasma concentration-time profiles of lidocaine and prilocaine were analyzed using WinNonlin software Version 5.1 by non-compartmental model.

An incurred sample re-analysis (ISR) was also conducted, selecting the seven subject samples (2 samples from each subject) near  $C_{max}$  and the elimination phase. The variability (% difference) between original value and ISR values were within  $\pm 20\%$ .



**Fig. 2.** Typical MRM chromatograms of lidocaine and prilocaine in human blank plasma. As shown in Fig. 3, no significant interference was observed with retention times of lidocaine and prilocaine, when working concentration of lidocaine-d6 and prilocaine-d7 were injected.

### 3. Results

#### 3.1. Mass spectrometry

Good response was observed in positive ionization mode than in negative ionization mode. MRM technique was used for quantitation. The most sensitive precursor ion transitions were observed from  $m/z$  235.10 to 86.15 for lidocaine, from  $m/z$  241.30 to 86.15 for lidocaine-d6, from  $m/z$  221.20 to 86.15 for the prilocaine and from  $m/z$  228.10 to 86.15 for the prilocaine-d7.

#### 3.2. Method development

Several trails were conducted to optimize mobile phase composition. Several combinations of organic solvents and buffers with variable contents were tried on C18 columns of different makes such as Hypersil, Hypurity advance, Chromolith, Zorbax, Kromasil, Kinetex and Inertsil. Finally, good resolution, response and symmetric peak shapes were obtained with mobile phase of acetonitrile and 10 mM ammonium acetate (80:20, v/v) on Kinetex EVO 100A (4.6 × 100mm, 2.6 μm) column. The retention times of lidocaine and prilocaine were 2.9 and 1.9 min, made 3.0 min as the suitable run time.

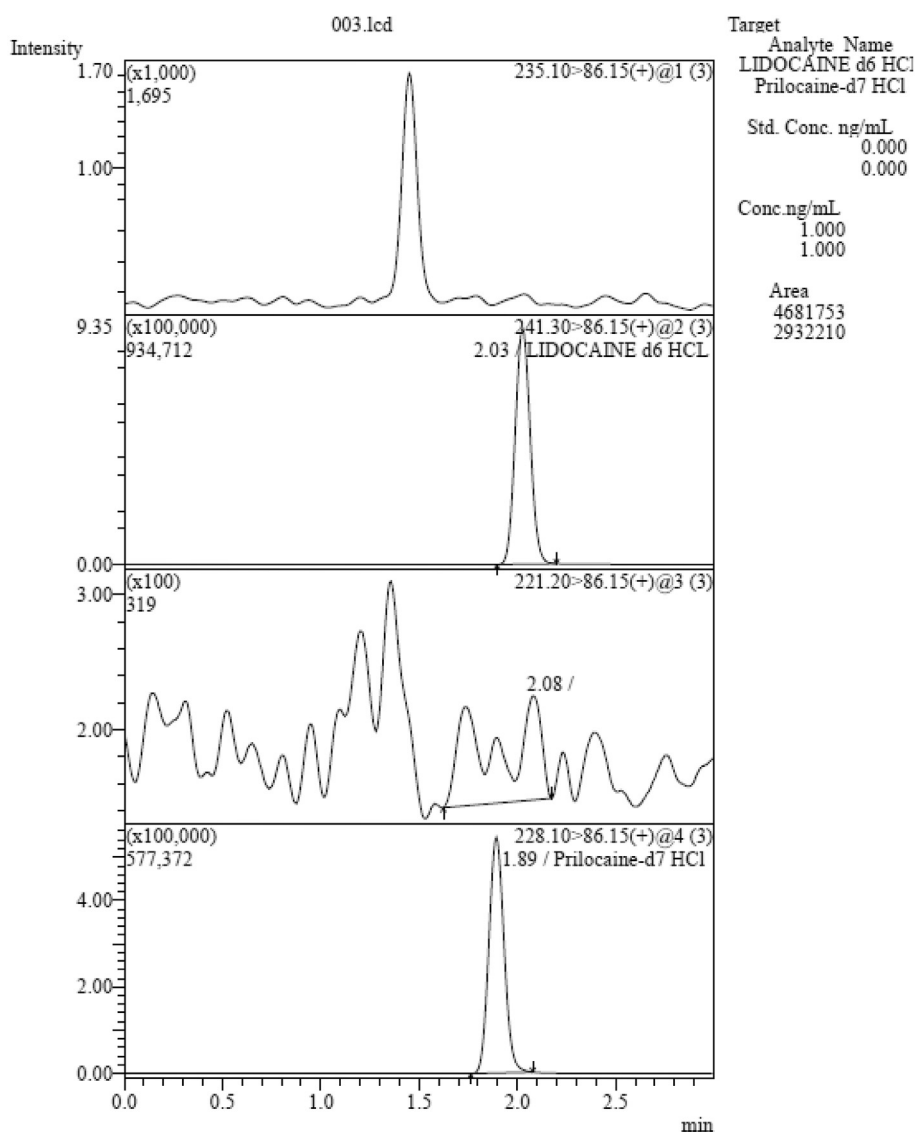


Fig. 3. Typical MRM chromatograms of lidocaine and prilocaine in human blank plasma with internal standard.

### 3.3. Selectivity and specificity chromatography

The degree of interference by endogenous plasma constituents with the analytes and IS was assessed by inspection of chromatograms derived from processed blank plasma sample. As shown in Fig. 2, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free plasma at the retention time of the analytes and Internal standards.

### 3.4. Sensitivity

At LLOQ QC level, the precision and accuracy were found to be 5.30% and 99.01% for lidocaine, 6.99% and 100.98% for prilocaine. Typical MRM chromatograms of LLOQ QC in human plasma of lidocaine and prilocaine is shown in Fig. 4.

### 3.5. Matrix effect

In eight batches including lipemic and hemolysed plasma, the matrix effect observed was not significant for lidocaine and prilocaine at LQC and HQC levels. For lidocaine, the precision for IS normalized matrix factor at LQC and HQC level was found to be 1.36% and 0.48% respectively and IS normalized factor was 0.96 for LQC and 1.00 for HQC. For prilocaine, the precision for IS normalized matrix factor at LQC and HQC level was found to be 2.95% and 0.57% respectively and IS normalized factor was 1.00 for LQC and 1.00 for HQC.

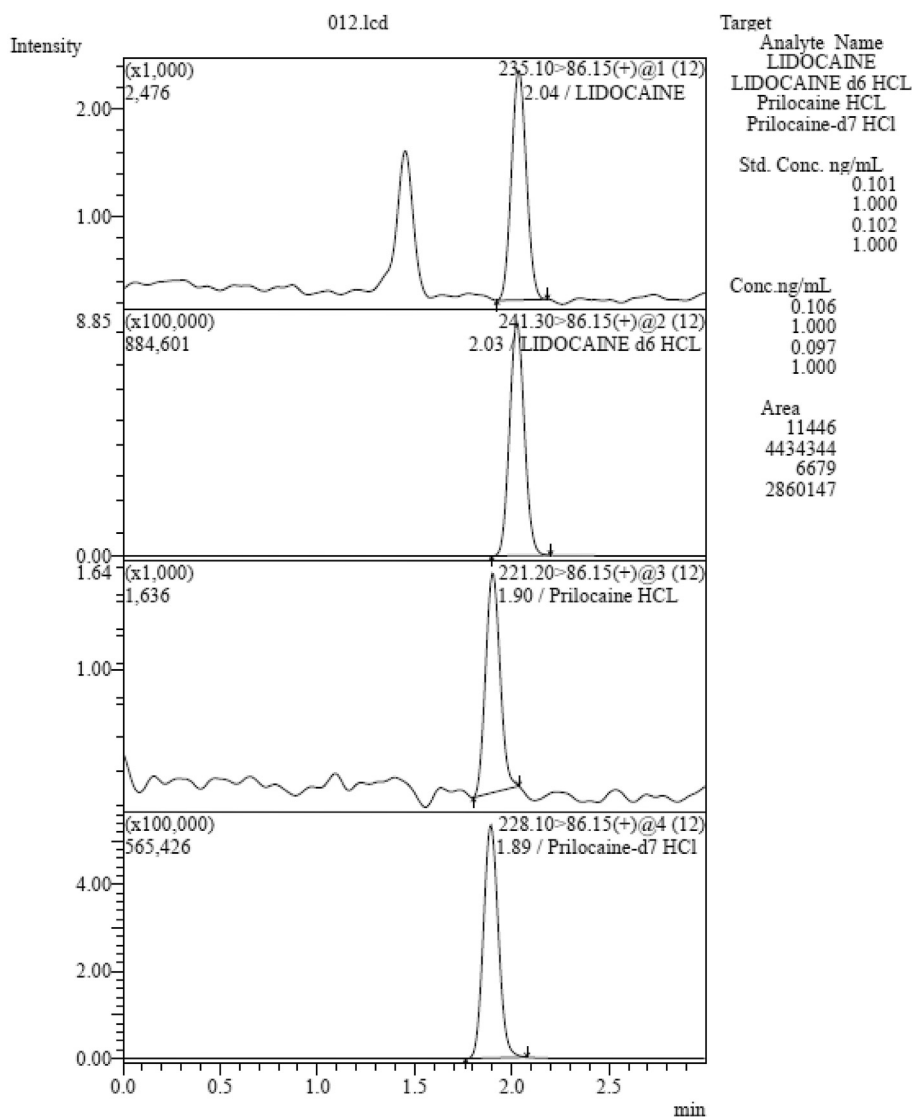


Fig. 4. Typical MRM chromatograms of lidocaine and prilocaine LLOQ with internal standard.

**Table 1**

Precision and accuracy of the method for determining lidocaine and prilocaine in plasma samples.

Analyte	Concentration added (ng/mL)	Intra-batch precision and accuracy range (4 from each batch)		Inter-day precision and accuracy (n = 24; 6 from each batch)	
		Precision range (%)	Accuracy range (%)	Precision (%)	Accuracy (%)
Lidocaine	0.10	3.33–6.12	96.04–101.98	5.30	99.01
	0.30	0.74–2.69	88.26–104.36	7.37	93.29
	85.93	1.95–3.33	91.52–106.87	6.91	96.54
	171.87	1.25–3.17	92.42–106.34	6.34	96.63
Prilocaine	0.10	2.20–6.80	95.10–106.86	6.99	100.98
	0.30	2.16–4.03	88.04–97.01	5.09	92.69
	86.32	1.82–3.82	93.30–107.98	6.53	98.27
	172.65	1.03–3.09	92.04–105.49	5.66	97.28

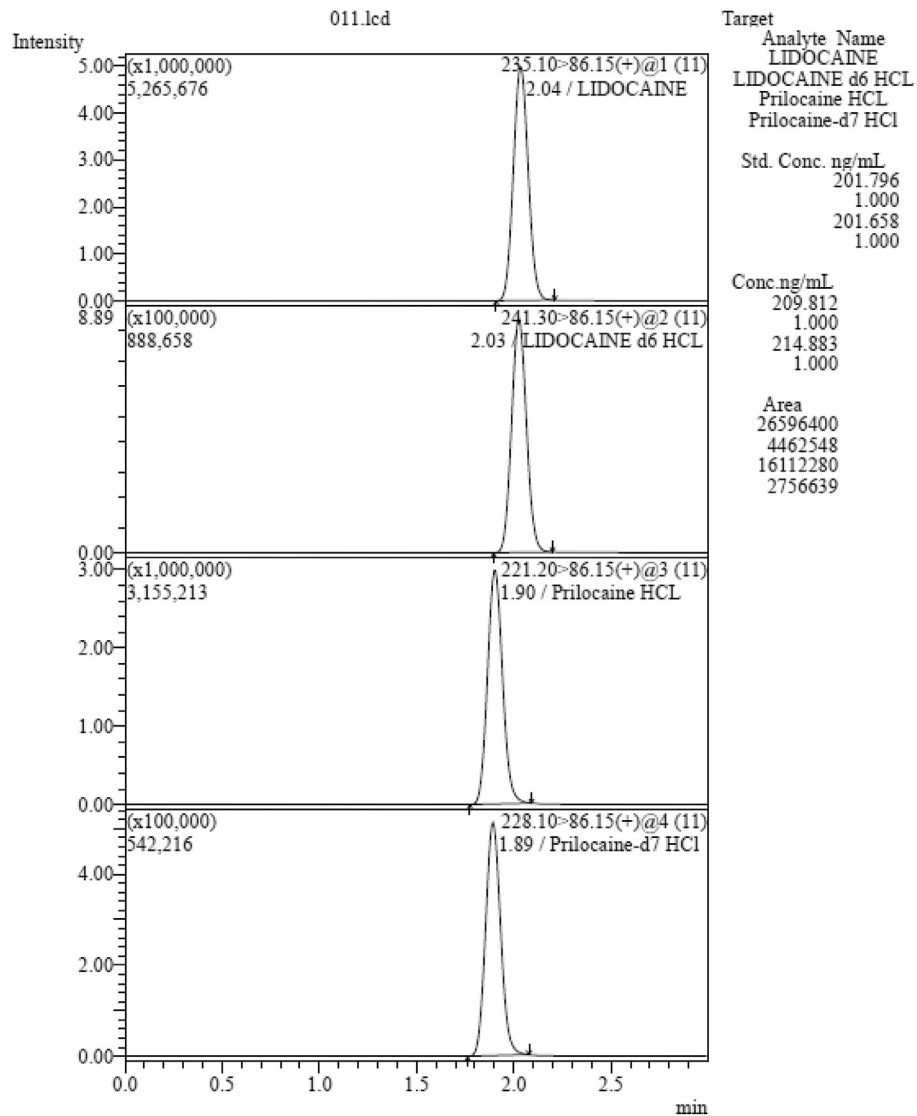


Fig. 5. Typical MRM chromatograms of lidocaine and prilocaine ULOQ with internal standard.

### 3.6. Linearity

Eight-point calibration curve was plotted over the concentration range of 0.10–201.80 ng/mL for lidocaine and 0.10–201.66 ng/mL for prilocaine. The mean regression coefficient,  $R^2$  was found to be  $\geq 0.99$  for both the analytes proving a linear relationship between concentration and peak area.

### 3.7. Precision and accuracy

Table 1 summarizes the data for intra-day and inter-day precision and accuracy for lidocaine and prilocaine. The results were found to be within acceptable limits in both the occasions. Typical MRM chromatograms of ULOQ in human plasma of lidocaine and prilocaine is shown in Fig. 5.

### 3.8. Extraction efficiency

The recoveries of analytes and internal standards after solid phase extraction with Oasis® HLB 1cc (30 mg) cartridges were good and reproducible. The mean overall recoveries of lidocaine, prilocaine, lidocaine-d6 and prilocaine-d7 were 89.76%, 90.75%, 90.37 and 90.70%, respectively.

### 3.9. Dilution integrity

Dilution integrity was performed by using three times of ULOQ concentration (606.582 ng/mL for lidocaine and 609.342 ng/mL for prilocaine). The samples were diluted in 1:4 ratio and analysed using dilution factor “5”. For lidocaine: The % accuracy and CV % for 1:4 dilutions were found to be 101.18% and 0.68% which criteria are within the acceptance. For prilocaine: The % accuracy and CV % for 1:4 dilutions were found to be 99.78% and 0.63% which criteria are within the acceptance.

### 3.10. Stability studies

In various stability studies, namely bench top (16 h), auto sampler (51 h), freeze-thaw (5 cycles), reinjection (24 h), wet extract (45 h at room temperature), whole blood (60 min) and long-term stabilities at  $-70^\circ\text{C}$  for 30 days, the mean % nominal values of the analytes were found to be within  $\pm 15\%$  of the predicted concentrations for the analytes at their LQC and HQC levels (Table 2). Thus, the obtained results were within the acceptable limits during the entire validation.

### 3.11. Pharmacokinetic study results

To verify the sensitivity and selectivity of this method in a real-time situation, the proposed method was used to determine lidocaine and prilocaine in human plasma samples collected from healthy male volunteers ( $n = 12$ ). The mean plasma concentrations vs time profile of lidocaine and prilocaine are shown in Fig. 6 and the pharmacokinetic data is listed in Table 3.

The study results were authenticated by conducting the Incurred Sample reanalysis (ISR). The differences in concentrations between

**Table 2**  
Stability samples result for lidocaine and prilocaine in human plasma ( $n = 6$ ).

Stability test	Lidocaine				Prilocaine			
	Concentration added (ng/mL)	Mean $\pm$ SD (ng/mL)	Accuracy/Stability (%)	Precision (%)	QC (spiked concentration, ng/mL)	Mean $\pm$ SD (ng/mL)	Accuracy/Stability (%)	Precision (%)
Autosampler stability (at $10^\circ\text{C}$ for 51 h)	0.30	$0.31 \pm 0.02$	103.02	2.76	0.30	$0.30 \pm 0.03$	98.34	10.78
	171.87	$180.42 \pm 3.54$	104.98	1.85	172.65	$175.95 \pm 2.73$	101.91	1.55
Wet extract stability (at room temperature for 45 h)	0.30	$0.30 \pm 0.00$	98.99	1.29	0.30	$0.28 \pm 0.01$	93.69	2.52
	171.87	$184.16 \pm 2.11$	107.15	1.15	172.65	$177.99 \pm 2.16$	103.09	1.21
Bench top stability (16 h)	0.30	$0.29 \pm 0.01$	98.66	2.76	0.30	$0.29 \pm 0.01$	94.68	2.63
	171.87	$176.98 \pm 3.28$	102.98	1.85	172.65	$173.95 \pm 3.72$	100.76	2.14
Freeze-thaw stability (five cycles)	0.30	$0.29 \pm 0.01$	97.32	3.00	0.30	$0.29 \pm 0.01$	94.68	3.37
	171.87	$178.84 \pm 2.55$	104.06	1.42	172.65	$177.92 \pm 1.45$	103.06	0.82
Reinjection stability (24 h)	0.30	$0.26 \pm 0.01$	88.26	3.88	0.30	$0.29 \pm 0.01$	95.35	4.25
	171.87	$167.17 \pm 4.78$	97.27	2.86	172.65	$170.03 \pm 4.31$	98.48	2.54
Long-term Stability (at $-70^\circ\text{C}$ for 30 days)	0.30	$0.30 \pm 0.01$	100.34	1.84	0.30	$0.28 \pm 0.01$	93.02	4.57
	171.87	$179.56 \pm 2.76$	104.48	1.54	172.65	$172.79 \pm 2.88$	100.08	1.67



## Comparative Linear Plot of Mean Plasma Lidocaine Vs Prilocaine Concentrations versus Time

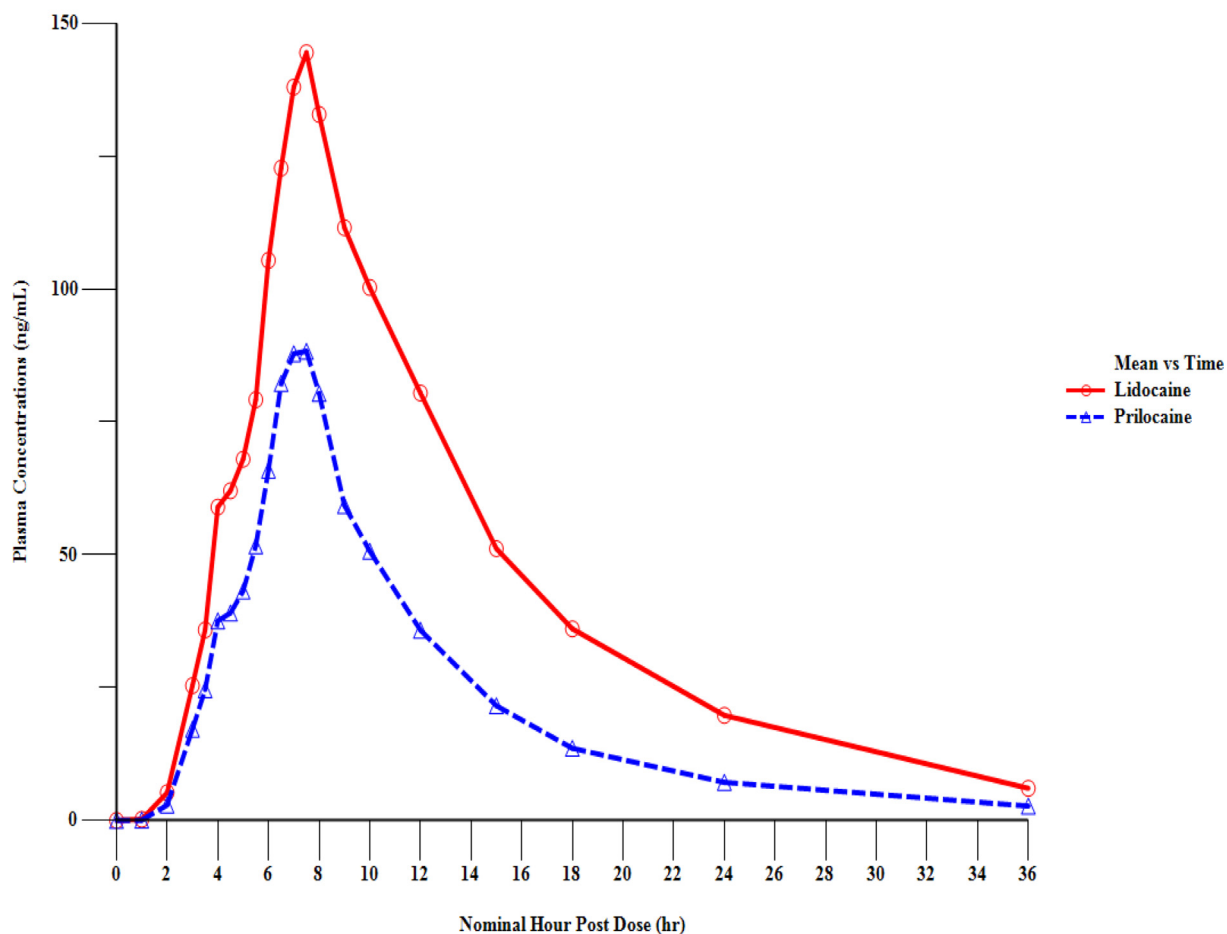


Fig. 6. Mean plasma concentration-time profile of lidocaine and prilocaine in human plasma following administration single dose of lidocaine 2.5% and prilocaine 2.5% cream to healthy volunteers (n = 12).

**Table 3**  
Pharmacokinetic parameters of lidocaine and prilocaine (n = 12, Mean  $\pm$  SD).

Parameter	Lidocaine	Prilocaine
$t_{max}$ (h)	8.00 $\pm$ 1.49	7.21 $\pm$ 0.45
$C_{max}$ (ng/mL)	158.33 $\pm$ 69.41	97.87 $\pm$ 41.51
$AUC_{0-t}$ (ng h/mL)	1524.14 $\pm$ 657.21	767.36 $\pm$ 209.22
$AUC_{0-inf}$ (ng h/mL)	1585.57 $\pm$ 690.22	797.22 $\pm$ 217.09
$t_{1/2}$ (h)	6.99 $\pm$ 0.74	7.59 $\pm$ 1.53
Kel ( $h^{-1}$ )	0.10 $\pm$ 0.01	0.09 $\pm$ 0.02

the ISR and the initial values for all the tested samples were less than 12.24% for lidocaine and 10.80% for prilocaine (Table 4). The ISR results encourage the proposed method for its use in Pharmacokinetic application.

#### 4. Conclusion

We described a new LC-MS/MS based rapid, simple, specific and sensitive assay protocol for simultaneous quantification of lidocaine

**Table 4**  
Incurred samples re-analysis data of lidocaine and prilocaine.

Sample No	Analyte Name	Original Concentration (ng/mL)	ISR Concentration (ng/mL)	Mean of original and ISR concentration	Variability (%)
1	Lidocaine	182.225	187.598	184.912	2.91
2		175.912	187.819	181.866	6.55
3		4.827	5.067	4.947	4.85
4		189.497	190.55	190.024	0.55
5		4.039	3.989	4.014	1.25
6		85.294	90.102	87.698	5.48
7		71.817	77.05	74.434	7.03
8		3.873	3.933	3.903	1.54
9		75	79.206	77.103	5.46
10		6.882	7.009	6.946	1.83
11		157.297	171.984	164.641	8.92
12		132.075	143.046	137.561	7.98
13		3.052	3.45	3.251	12.24
14		173.176	170.491	171.834	1.56
15		202.407	205.423	203.915	1.48
16		3.158	3.262	3.21	3.24
17		175.252	177.278	176.265	1.15
18		197.836	202.691	200.264	2.42
19		4.089	4.106	4.098	0.41
20		56.461	59.117	57.789	4.60
21		4.049	3.971	4.01	1.95
22		53.843	53.87	53.857	0.05
23		57.519	58.383	57.951	1.49
24		96.695	99.751	98.223	3.11
1	Prilocaine	149.421	148.586	149.004	0.56
2		139.613	141.392	140.503	1.27
3		3.632	3.697	3.665	1.77
4		142.075	140.953	141.514	0.79
5		2.906	2.955	2.931	1.67
6		64.423	66.454	65.439	3.10
7		45.873	51.11	48.492	10.80
8		2.091	2.097	2.094	0.29
9		46.929	48.937	47.933	4.19
10		2.74	2.904	2.822	5.81
11		118.81	126.191	122.501	6.03
12		96.813	102.31	99.562	5.52
13		2.938	3.217	3.078	9.06
14		103.13	99.242	101.186	3.84
15		118.732	123.574	121.153	4.00
16		2.312	2.392	2.352	3.40
17		112.766	113.888	113.327	0.99
18		130.382	131.859	131.121	1.13
19		3.131	3.191	3.161	1.90
20		35.912	38.613	37.263	7.25
21		2.276	2.286	2.281	0.44
22		34.673	34.979	34.826	0.88
23		35.402	37.474	36.438	5.69
24		60.552	64.082	62.317	5.66

$$\text{Variability (\% difference)} = \frac{| \text{ISR value} - \text{Original value} |}{\text{Mean of ISR value and original value}} \times 100.$$

and prilocaine in human plasma. As per recent FDA guidelines, the procedure was fully validated. The method proved suitable for pharmacokinetic studies in humans. Solid phase extraction, cost effectiveness and sample turnover rate of 3.0 min per sample make it attractive procedure for high-throughput bioanalysis of lidocaine and prilocaine. Based on the validation results, we can conclude that the method developed is applicable for bioequivalence and therapeutic drug monitoring studies.

#### Conflict of interest

Authors have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plabm.2019.e00129>.

## References

- [1] N. John, A. Lynda, A.E. Mark, A brief history of local anesthesia, *IJHNS* 7 (2016) 29–32.
- [2] M.H. Holmdahl, Xylocaine (lidocaine, lignocaine), its discovery and godrd's contribution to its clinical use, *Acta. Anaesthesiol. Scand. Suppl.* 113 (1998) 8–12.
- [3] G. Kerstin, A.H. Ulrich, M. Christoph, Hemoglobin adducts of the human bladder carcinogen *o*-toulidine after treatment with the local anesthetic prilocaine, *Toxicology* 229 (2007) 157–164.
- [4] V. Adams, J. Marely, C. Mc Carroll, Prilocaine induced methanoglobinamia in a medicinally compromised patient. Was this an inevitable consequence of the dose administered? *Br. Dent. J.* 203 (2007) 585–587.
- [5] T. Anna, O. Arne, R.E. Thoma, A Systematic review of lidocaine-prilocaine cream (EMLA) in the treatment of acute pain in neonates, *J. Pediatr.* 101 (1998) 1–9.
- [6] M.M. Buckley, P. Benfield, Eutectic lidocaine/prilocaine cream. A review of the topical anesthetic/analgesic efficacy of a eutectic mixture of local anesthetics (EMLA), *Drugs* 46 (1993) 126–151.
- [7] V.G. Santosh, D.I. Ashwini, Development and validation of RP-HPLC method for simultaneous estimation of benzoxonium chloride and lidocaine hydrochloride, *WJPLS* 3 (2017) 99–102.
- [8] S.A. Nada, W.A. Nouruddin, M. Hamed, et al., Determination of thiomersal, lidocaine and phenylephrine in their ternary mixture, *J. Chromatogr. Sep. Tech.* 4 (2013) 1–6.
- [9] T.S. Belal, M.M. Bedair, A.A. Gazy, et al., Validated selective HPLC DAD Method for the simultaneous determination of diclofenac sodium and lidocaine hydrochloride in presence of four of their related substances and potential impurities, *Acta Chromatogr.* 27 (2015) 477–493.
- [10] M.A.N. Hamdah, P. Matthew, S.G. James, et al., A high-performance liquid chromatography assay method for the determination of lidocaine in human serum, *Pharmaceutics* 52 (2017) 1–8.
- [11] B.M. Dhananjaya, M. Khushbu, M. Priyanka, Stability indicating analytical method for the simultaneous estimation of lidocaine and nifedipine in the combined dosage form, *Der Pharma Chem.* 10 (2018) 1–16.
- [12] R.J. Eduardo, V.L.B.B. Maria, M.M. Juliana, HPLC assay of lidocaine in *in vitro* dissolution test of the poloxmer 407 gels, *Braz. J. Pharm. Sci.* 38 (2002) 107–111.
- [13] L. Zivanovic, M. Zecevic, S. Markovic, et al., Validation of liquid chromatographic method for analysis of lidocaine hydrochloride, dexamethasone acetate, calcium dobesilate, buthyl hydroxylanisol and degradation product hydroquinone in suppositories and ointment, *J. Chromatogr. A.* 1088 (2005) 182–186.
- [14] G. Alexander, N. Alexandra, Y. Irina, et al., Development of a HPLC-MS/MS method for simultaneous determination of nifedipine and lidocaine in human plasma, *J. Pharm. Biomed. Anal.* 131 (2016) 13–19.
- [15] Z. Chik, T.D. Lee, D.W. Holtz, Validation of high-performance liquid chromatography-mass spectrometric method for the analysis of lidocaine in human plasma, *J. Chromatogr. Sci.* 44 (2006) 262–265.
- [16] S.M. Janina, J. Linda, G. Ingrid, et al., Development of an HPLC method for determination of related impurities in prilocaine substance, *Chromatographia* 75 (2012) 329–336.
- [17] S. Stockmann, E. Spies, H. Gehring, et al., Evaluation and application of a liquid chromatographic method for prilocaine analysis in human plasma, *Clin. Lab.* 59 (2013) 17–132.
- [18] W. Robin, D. Peter, C. Hugh, et al., Determination of prilocaine in human plasma samples using high-performance liquid chromatography with dual electrode electrochemical detection, *J. Chromatogr.* 526 (1990) 215–222.
- [19] L.S. Meredith, T.S. James, Stability indicating HPLC assays for the determination of prilocaine and procaine drug combinations, *J. Pharm. Biomed. Anal.* 30 (2002) 49–58.
- [20] K. Yucel, A. Alptug, Development and validation of gas chromatography-mass spectroscopy method for determination of prilocaine HCl in human plasma using internal standard methodology, *Biomed. Chromatogr.* 21 (2007) 1077–1082.
- [21] J. Klein, D. Fernandes, M. Gazarian, et al., Simultaneous determination of lidocaine, prilocaine and the prilocaine metabolite *o*-toluidine in plasma by high-performance liquid chromatography, *J. Chromatogr. B.* 655 (1994) 83–88.
- [22] A.M. Alaa, M. Kamal, B.K. Samuel, et al., A pharmacokinetic study of a topical anesthetic (EMLA®) in mouse soft tissue laceration, *Dent. Traumatol.* 28 (2012) 483–487.
- [23] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Rockville, MD, USA, 2013.