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Highly sensitive bioassay of lidocaine in human plasma by high-performance liquid chromatography–tandem mass spectrometry

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Abstract

A very sensitive HPLC–tandem mass spectrometric (LC–MS–MS) method for assaying lidocaine in human plasma was set up and fully validated. Lidocaine and an internal standard (bupivacaine) were extracted from 1 ml of alkalinized plasma with *tert*.-butylmethyl ether, back-extracted to a H_3PO_4 acidified solution and injected into a C_{18} column. Acetonitrile–26 mmol/l ammonium acetate, pH 4.5 (70:30, v/v) was the mobile phase at a flow-rate of 1 ml/min. The effluent was detected by PE Sciex API 365 LC–MS–MS system in positive ion mode. Ionisation was performed using an atmospheric pressure chemical ionization ion source operating at 400°C. The multi reaction monitoring transition 235→86 was monitored. Linearity was ascertained in the 0.2–30 ng/ml range with a limit of quantitation of 0.2 ng/ml. Intra- and inter-assay precision and accuracy were ≤3.8%. The high sensitivity and specificity achieved by the method allowed concentrations of lidocaine to be measured in plasma of healthy subjects topically treated with lidocaine (5% ointment) on normal skin over a 32-h period after dosing.

Keywords: Lidocaine; Bupivacaine; Anaesthetics

1. Introduction

Lidocaine is a widely used local anaesthetic, characterized by a fast onset and an intermediate persistence of activity [1,2]. Like other local anaesthetics, at relatively high plasma concentrations lidocaine possesses relevant systemic adverse effects, mainly on the central nervous and cardiovascular systems. When used for topical application, its absorption from the intact skin is poor, but, when applied to damaged skin, the systemic absorption could be more effective [1].

A very sensitive bioassay of lidocaine was set up and validated in order to monitor systemic concentrations of the drug after topical application of a 5% ointment to the intact skin of healthy volunteers.

Previously reported methods utilizing HPLC with UV [3–5] or fluorimetric [6] detection, GC with thermoionic specific [7] or with chemical ionisation–mass spectrometric detection [8] claimed limits of quantitation (LOQs) from 10 to 100 ng/ml, which proved to be insufficient for our purpose.

2. Materials and methods

2.1. Chemicals

Working standards of lidocaine hydrochloride, and bupivacaine hydrochloride, used as internal standard (I.S.), were purchased from Sigma (St. Louis, MO, USA). Solvents and reagents, HPLC or analytical grade, were supplied by Carlo Erba (Milan, Italy), Merck (Darmstadt, Germany) and BDH (Poole,

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UK). Gases for triple–quadrupole were produced by nitrogen and air generators (N_2 purity 99.999, air dew point −60°C) manufactured by Claind (Lenno, Italy).

2.2. Apparatus

The HPLC apparatus included two pumps (series 200 Micro Pump), an autosampler (series 200 Autosampler) and a detector (triple–quadrupole API 365 LC–MS–MS, PE-Sciex), all from Perkin-Elmer (Foster City, CA, USA).

2.3. Extraction procedure

A 1-ml volume of plasma, 20 μl of I.S. solution (5 μg/ml in methanol), 250 μl of 2 mol/l NaOH and 5 ml of *tert*.-butylmethyl ether were placed in a 12-ml screwcap tube. The tube was shaken for 15 min using a rotating mixer at 32 rpm and then centrifuged at 1500 *g* for 5 min. The tube was frozen at −80°C for 15 min and the organic phase was transferred into a conical centrifuge test tube containing 250 μl of a 17 mmol/l H_3PO_4 solution. The tube was vortexed for 30 s and then centrifuged as before. The supernatant was aspired under vacuum and the remaining solution was transferred into an autosampler vial. A 10-μl volume was injected into the column.

2.4. Analytical procedure

A Luna C_{18} 3 μm, 75×4.6 mm I.D. analytical column from Phenomenex (USA) was used. The analytical column was protected by a guard column cartridge filled with Hypersil (10×3.2 mm I.D.) C_{18}, 5 μm, from Hichrom (Theale, UK). The mobile phase was acetonitrile–26 mmol/l ammonium acetate at pH 4.5 (70:30, v/v) used at the flow-rate of 1.0 ml/min. The run lasted 3 min.

An API 365 triple–quadrupole MS–MS PE-Sciex mass spectrometer equipped with a heated nebulizer interface was used.

The eluate from the column was introduced directly into the atmospheric pressure chemical ionization (APCI) source, where the liquid is sprayed into a gently heated vaporizer. The rapid desolvation and vaporization of the droplets minimize thermal decomposition and preserve molecular identity.

The data were collected using the PE-Sciex proprietary software program SAMPLE CONTROL, version 1.3. Peak height were integrated by the PE-Sciex proprietary software program MACQUAN, version 1.5. All calculations were based on chromatographic peak height ratios for the selected reaction monitoring LC–MS precursor-product ion transitions with lidocaine and the I.S.

Under these conditions lidocaine is not chromatographically separated from the I.S. The retention time for lidocaine and bupivacaine is about 1.25 min.

3. Results

3.1. Selection of operating protonated ions

Fig. 1 shows the protonated ions of lidocaine and bupivacaine used in this investigation. The product ion scans for the above two analytes are depicted in Fig. 2.

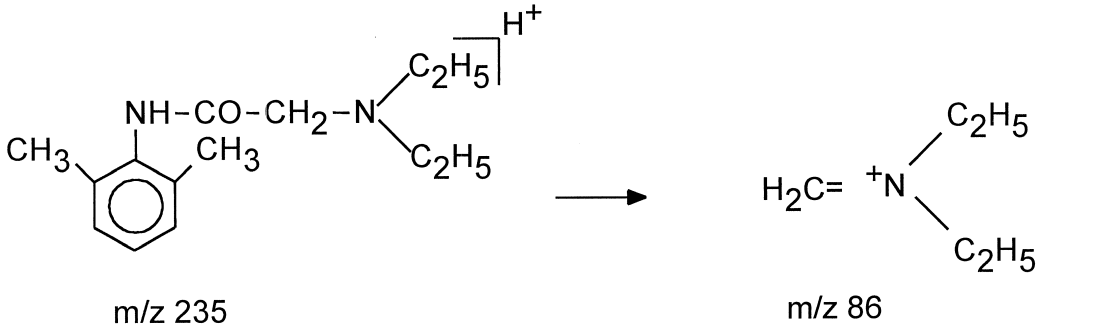
All the ions corresponding to the protonated analytes ($[M-H]^+$) were chosen as precursor ions. For lidocaine, the fragment ion at 86 m/z was selected for the assay. Bupivacaine was monitored through the protonated ion at 140 m/z.

Fig. 3 shows representative chromatograms obtained from extracted blank plasma and from blank plasma spiked with lidocaine at concentrations of 0.2 and 30 ng/ml.

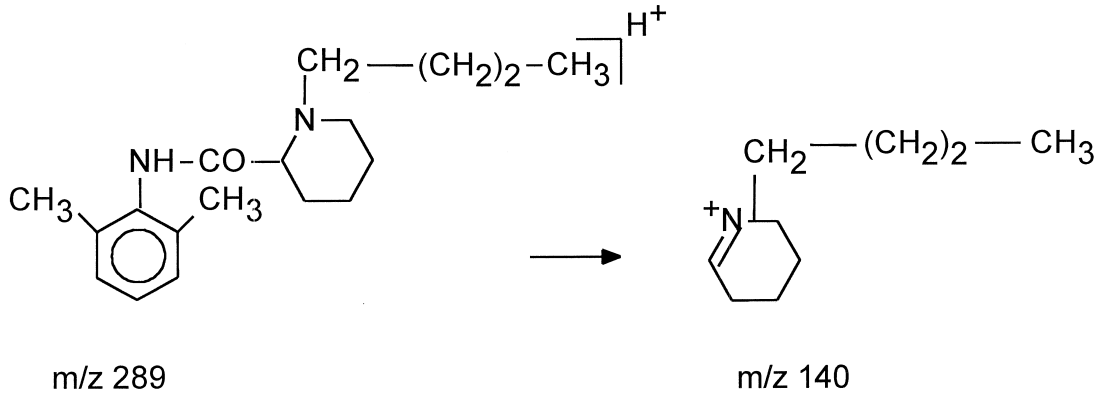
3.2. Calibration curve

The calibration covered the range from 0.2 to 30 ng/ml of plasma with seven calibrators. The chromatographic data were automatically processed for peak-height ratios of the analytes to the I.S. and fitted to a weighted ($1/y$) linear regression.

Mean results obtained from five curves are summarized in Table 1. The mean accuracy of back-calculated concentrations ranged from −5.0% (at the lowest calibration point) to +4.2% and the precision was ≤5.3%. The mean coefficient of determination (r) was 0.9998.



LIDOCAINE



BUPIVACAINE

Fig. 1. Product ions of protonated lidocaine and bupivacaine.

3.3. Intra-assay, inter-assay and limit of quantitation

Table 2 summarizes mean values, SD, precision and accuracy of intra-assay, inter-assay tests and limit of quantitation. Intra-assay precision for all samples analyzed was on average $\leq$2.5% and accuracy ranged from $-$1.3 to 2.3% of the nominal concentration. Mean values of inter-assay precision were $\leq$3.8% and accuracy ranged from $-$0.1 to 1.3%. The limit of quantitation proved to be as low as 0.2 ng/ml with precision and accuracy of 10.0 and 0.0%, respectively. The limit of detection proved to be 0.015 ng/ml.

3.4. Specificity

No significant interfering peaks were found at the retention time of lidocaine and the I.S. This was mainly due to the high specificity achieved operating with the triple–quadrupole system.

3.5. Stability

Table 3 lists the stability data of lidocaine in autosampler vials, in plasma and after three freeze–thaw cycles. No significant change was detected in the measurements of lidocaine concentration in plasma stored 24 h at room temperature, in extracted

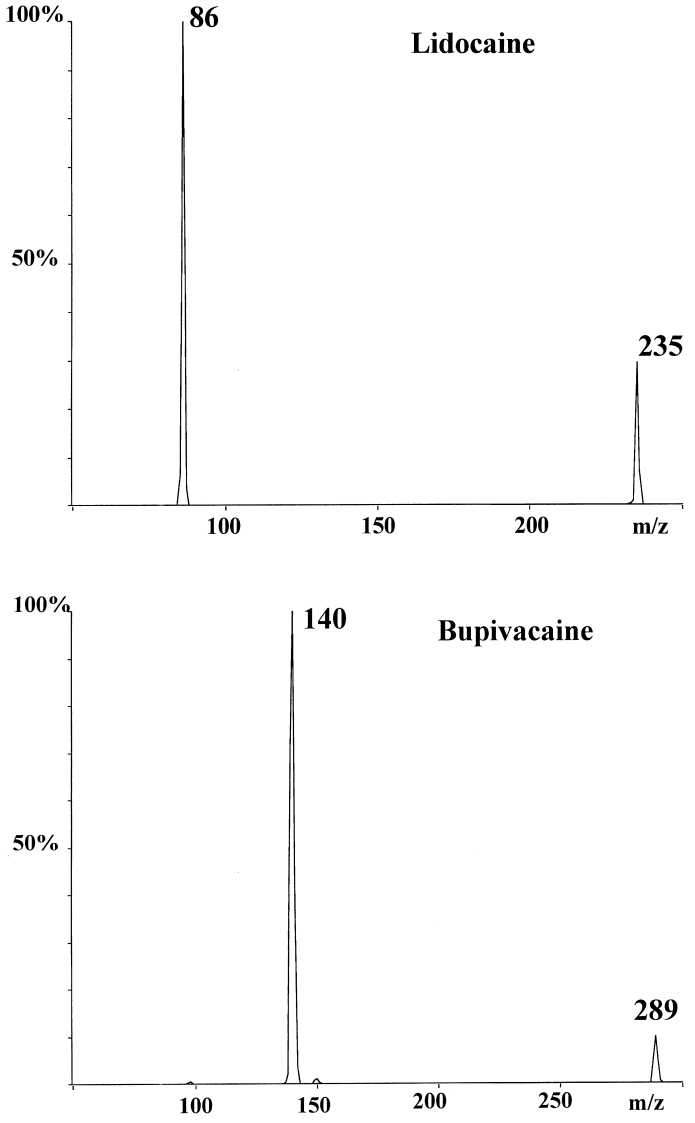


Fig. 2. Product ion scans of lidocaine and bupivacaine.

plasma samples stored in final solution at room temperature for at least 24 h in autosampler vials before injection, as well as when three freeze–thaw cycles were tested.

3.6. Recovery

The lidocaine recovery in the concentration range investigated was on average 93.2%. The extraction yield of the I.S. from plasma was 89.8% with an RSD of 1.8% at the concentration of 100 ng/ml.

3.7. Bioavailability measurement

Eighteen healthy subjects (nine males and nine females) were treated with 5 g of a 5% lidocaine ointment (Ortodermina), corresponding to 250 mg of active ingredient, on a 100-cm^2 area of a forearm, with an occlusive dressing which was removed after

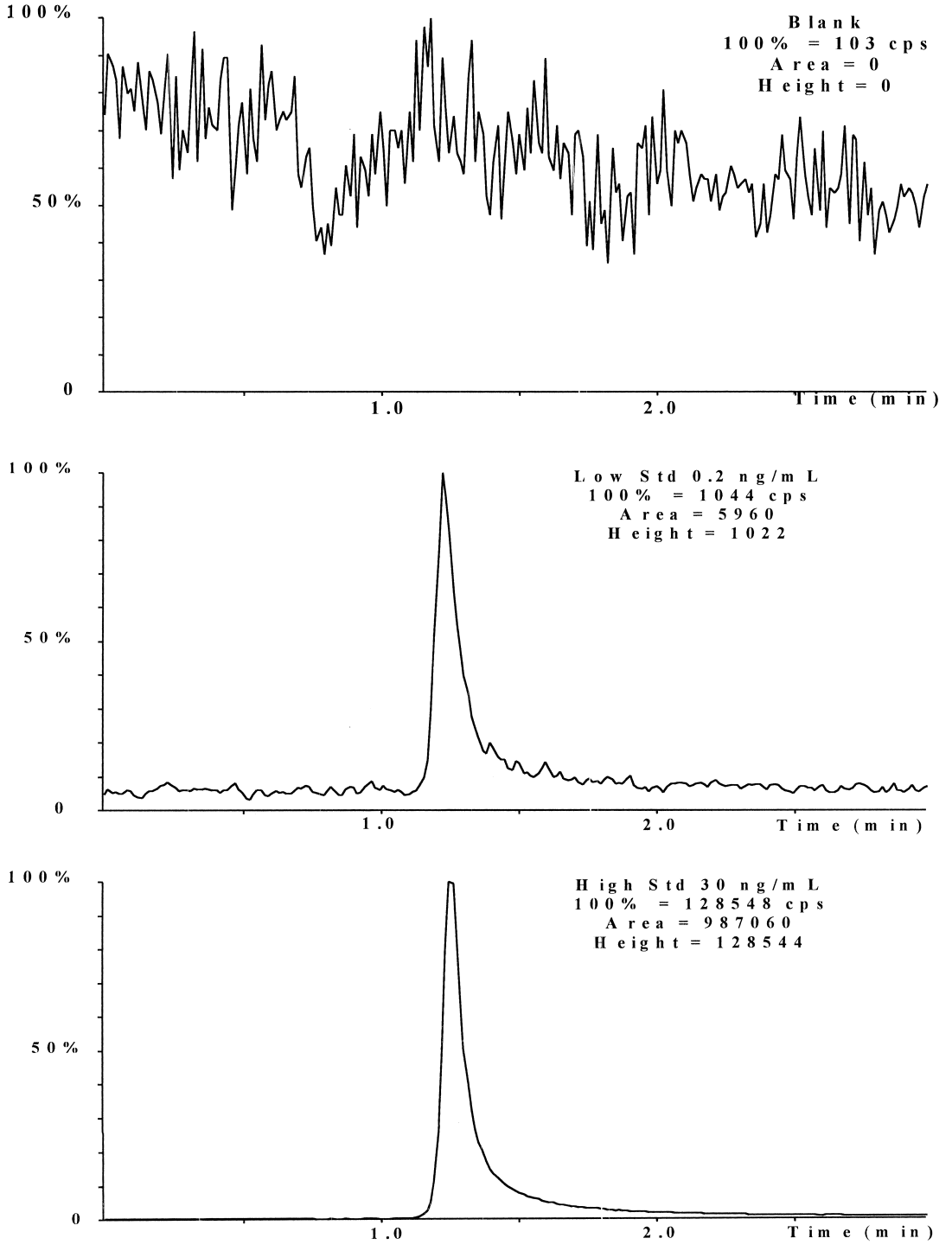


Fig. 3. Representative chromatograms obtained from extracted blank plasma and plasma spiked with 0.2 and 30 ng/ml of lidocaine. Note: 100% of the scale was considered the highest peak. In order to compare the three graphs, panel 1 is ≃10 times more expanded than panel 2, and ≃1000 times than panel 3.

Table 1
Mean values of back calculated calibration curves and calibration parameters

	Theoretical concentrations (ng/ml)							Calibration curve parameters		
	30	10	5	2	1	0.5	0.2	Slope $\cdot 10^{-3}$	Intercept $\cdot 10^{-4}$	r
	Back-calculated concentrations (ng/ml)									
n	5	5	5	5	5	5	5	5	5	5
Mean	29.87	9.94	5.21	1.98	1.01	0.52	0.19	7.5405	4.0268	0.9998
SD	0.21	0.18	0.07	0.03	0.01	0.01	0.01	0.4281		
Precision (%)	0.7	1.8	1.3	1.5	1.0	1.9	5.3	5.68		
Accuracy (%)	−0.4	−0.6	+4.2	−1.0	+1.0	+4.0	−5.0			

Table 2
Mean precision and accuracy of intra- and inter-assay and LOQ

	Intra-assay: QCs (ng/ml)			Inter-assay: QCs (ng/ml)			LOQ (ng/ml)
Concentrations added	20	4	0.8	20	4	0.8	0.2
n	6	6	6	18	18	18	6
Mean	20.33	4.09	0.79	19.99	4.05	0.80	0.20
SD	0.50	0.09	0.02	0.51	0.09	0.03	0.02
Precision (%)	2.5	2.2	2.5	2.6	2.2	3.8	10.0
Accuracy (%)	+1.6	+2.3	−1.3	−0.1	+1.3	0.0	0.0

45 min. Lidocaine concentrations were measured in timed plasma samples over 32 h after the application. The mean plasma concentrations showed an unexpected sustained behaviour (Fig. 4) which on average increased until 24 h. Individual peak concentrations ranged from 2.3 to 40.4 ng/ml and were reached from 1 to 28 h after administration. A relatively high variability is thus involved in the transdermal absorption of lidocaine, with RSD values from 60 to 100% for plasma concentrations, 69%

Table 3
Results of stability tests carried out on lidocaine assay; values expressed in percentage: 100% is the nominal concentration added

		Concentrations (ng/ml)		
		20	4	0.8
In autosampler vials at room temperature for 24 h	n	4	4	4
	Mean	102.9	97.4	96.0
	SD	3.52	0.46	1.54
	Precision (%)	3.4	0.5	1.6
	Difference (%)	+2.9	−2.6	−4.0
In plasma, after three freeze–thaw cycles	n	4	4	4
	Mean	102.6	100.9	97.6
	SD	0.50	0.77	2.64
	Precision (%)	0.5	0.8	2.7
	Difference (%)	+2.6	+0.9	−2.4
In plasma, after 24 h at room temperature	n	4	4	4
	Mean	103.4	103.0	97.6
	SD	1.17	2.87	2.00
	Precision (%)	1.1	2.8	2.0
	Difference (%)	+3.4	+3.0	−2.4

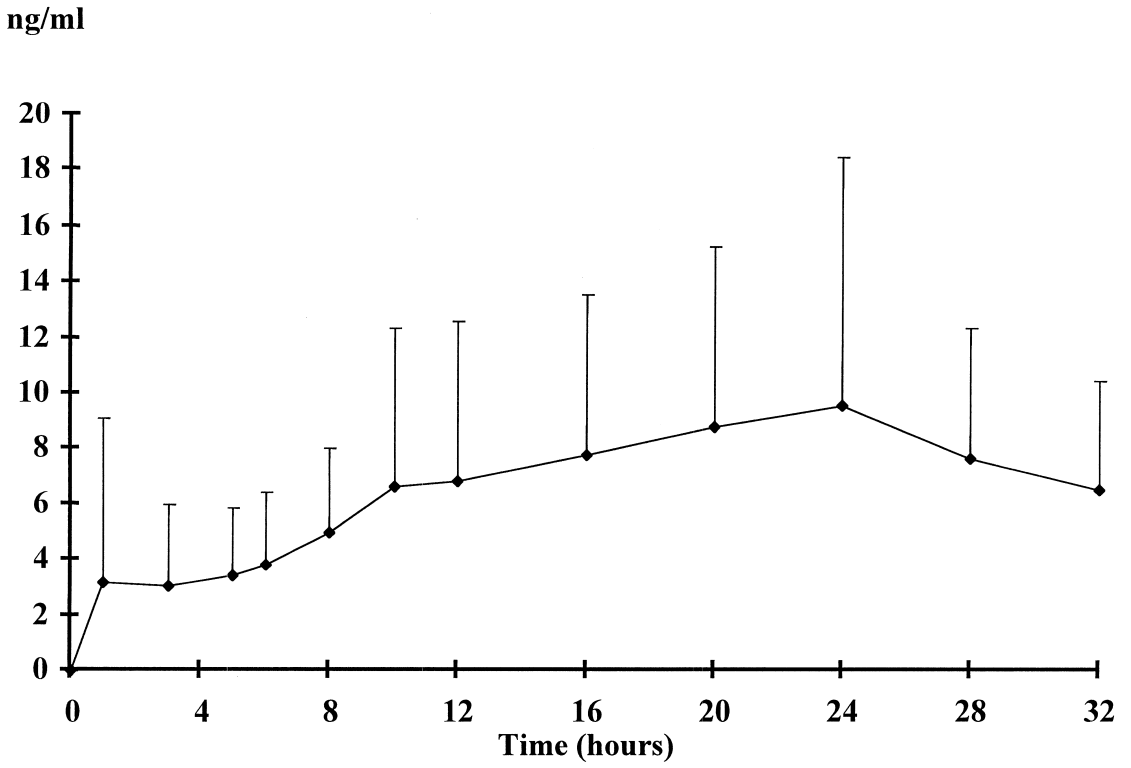


Fig. 4. Plasma concentration–time behavior of lidocaine after topical administration of 250 mg on 100 cm^2 of intact skin in healthy volunteers. Mean values of 18 findings. Vertical bars are standard deviation.

for C_{max}, 52% for t_{max} and 58% for trapezoidal area under plasma concentration-time curve. Considering that the inter-assay and intra-assay precision and accuracy of the method were $\leq$3.8%, the above in vivo variability must be ascribed to differences in the rate and extent of transdermal absorption.

The few samples encountered with concentrations $>$30 ng/ml were diluted with blank human plasma to 1:1 (v/v) and reassayed.

4. Discussion and conclusions

The need to quantify low plasma concentrations of lidocaine after topical application on intact skin compelled us to set up and validate a specific and very sensitive analytical method. From a pilot trial we ascertained that the expected lidocaine concentrations would range from $\leq$1 to $\leq$20 ng/ml. These concentrations in addition proved to fluctuate, showing a first peak at 5–8 h and a further increasing plateau peaking at 24 h, which called for a blood sampling period longer than 24 h.

Table 4 lists the main analytical characteristics of our method and some other previously published methods. With all these methods a high recovery was found e.g. 77– 89% [4,6] and 99–100% [3].

Most methods are addressed to the research field, namely a metabolic characterization in animals or in humans, considering that the lidocaine metabolite monoethylglycinexylidide is regarded as hepatotoxic [5]. Sensitivities achieved by these authors are enough for their purposes. Most authors have used an I.S. of the same therapeutic class as lidocaine. However, in therapeutic drug monitoring an I.S. not belonging to this class is preferable or a quantitation without I.S. should be validated.

The method set up by us reaches a recovery of 93% and possesses the highest sensitivity compared to other methods. The time-course of the analysis

Table 4
Overview of the main analytical characteristics of some methods for the assay of lidocaine in plasma

Ref.	I.S.	Matrix/ extraction/ recovery	Analytics	LOD/LOQ Calibration range (ng/ml)
This paper	Bupivacaine	Human plasma LLE[a] Recovery 93%	LC–MS–MS m/z 86 analyte m/z 140 I.S. Time course 3 min	LOD 0.015 LOQ 0.20 Range 0.20–30 Range ratio 150
[7]	Bupivacaine	Rat plasma SPE Recovery 99%	Capillary GC–TSD Time course 10 min	LOD 5 LOQ 5 Range 100–2000 Range ratio 20
[4]	Etidocaine	Human plasma LLE Recovery 78–83%	HPLC–UV λ 210 nm Time course 15 min	LOQ 10 Range 10–2000 Range ratio 200
[5]	Tocainide	Human serum LLE Recovery 82%	HPLC–UV 263 nm Time course 20 min	LOD 100 LOQ 2100 Range 200–4000 Range ratio 20
[3]	Bupivacaine	Piglet plasma LLE Recovery 99–100%	HPLC–UV λ 210 nm Time course 10 min	LOD 4 LOQ 20 Range 20–1000 Range ratio 50
[6]	*N*-Methylephedrine	Dog plasma; human plasma LLE and derivatization with 9-fluorenylmethylchloroformiate Recovery 77 – 89%	HPLC–Fluorescence λ_{ex} 254 nm λ_{em} 313 nm	LOD 1 LOQ 25 Range 25–200 Range ratio 8
[8]	Mepivacaine	Human plasma LLE	Capillary GC–MS–CI m/z 235 analyte m/z 247 I.S.	LOQ 50 Range 50–1200 Range ratio 24

[a] LLE = liquid–liquid extraction; SPE = solid-phase extraction; LC = liquid chromatography; MS = mass spectrometry; MS–MS = tandem mass spectrometry; GC = gas chromatography; TSD = thermoionic specific detection; UV = ultraviolet; LOD = limit of detection; LOQ = limit of quantitation; CI = chemical ionization.

was only 3 min, without a hindrance to specificity, which was fully guaranteed by the triple–quadrupole detection system.

With additional validation, our method could be employed in assaying:

(i) bupivacaine using lidocaine as an I.S.

(ii) either lidocaine or bupivacaine or both the drugs in therapeutic monitoring, working without an I.S., or selecting another I.S.

(iii) concentrations higher than 30 ng/ml, performing the extraction from less than 1 ml of plasma

(iv) lidocaine metabolites due to the high specificity arising from the tandem mass spectrometry.

In conclusion, the method described in this paper is suitable for bioavailability studies with lidocaine involving low drug concentrations, as it possesses as a very low sensitivity (LOQ = 0.2 ng/ml) and both intra-assay and inter-assay precision and accuracy $\leq$3.8%. The speed of the analytical run allows series of about 100–120 samples to be managed daily by a skillful operator. Thus, in spite of the high cost of the LC–MS–MS apparatus, the cost of the routine assay is not prohibitive. The good stability of lidocaine in blood at room temperature and after three freeze–thaw cycles and storage in final solution simplifies the precautions needed for laboratory manipulations during the analytical procedures.

The validation of our method meets international

requirements for bioassays addressed to bioavailability investigations [9].

References

[1] L. Juhlin, G. Hägglund, H. Evers, Acta Derm. Venereol. 69 (1989) 18.
[2] L. Juhlin, H. Evers, F. Broberg, Acta Derm. Venereol. 60 (1980) 544.
[3] J. Klein, D. Fernandes, M. Gazarian, G. Kent, G. Koren, J. Chromatogr. B 665 (1994) 83.
[4] H. Lofti, J. Debord, M.F. Dreyfuss, P. Marquet, M. Ben-Rhaiem, P. Feiss, G. Lachâtre, Ther. Drug Monit. 19 (1997) 160.
[5] C.L. O'Neal, A. Poklis, Clin. Chem. 42 (1996) 330.
[6] A. Sintov, R. Siden, R.J. Levy, J. Chromatogr. 496 (1989) 335.
[7] S.S. Rossi, A.C. Moore, L.R. Hagey, T.L. Yaksh, S.R. Chaplan, Ther. Drug Monit. 19 (1997) 179.
[8] G. Karlaganis, J. Bircher, Biomed. Environ. Mass. Spectrom. 14 (1987) 513.
[9] V.P. Shah, K.K. Midha, S. Dighe et al., J. Pharm. Sci. 81 (1992) 309.