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High-performance liquid chromatography–tandem electrospray mass spectrometry for the determination of lidocaine and its metabolites in human plasma and urine

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Abstract

A sensitive, selective and accurate high-performance liquid chromatographic–tandem mass spectrometric assay was developed and validated for the determination of lidocaine and its metabolites 2,6-dimethylaniline (2,6-xylidine), monoethylglycinexylidide and glycinexylidide in human plasma and urine. A simple sample preparation technique was used for plasma samples. The plasma samples were ultrafiltered after acidification with phosphoric acid and the ultrafiltrate was directly injected into the LC system. For urine samples, solid-phase extraction discs (C_{18}) were used as sample preparation. The limit of quantification (LOQ) was improved by at least 10 times compared to the methods described in the literature. The LOQ was in the range 1.6–5 nmol/l for the studied compounds in plasma samples. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Lidocaine; 2,6-Xylidine; Monoethylglycinexylidide; Glycinexylidide

1. Introduction

Lidocaine is a widely used amide-type local anaesthetic. It also has antiarrhythmic effects and is used as a therapeutic agent in the treatment of cardiac disorders. The major metabolites of lidocaine are 4-hydroxy-2,6-xylidine (4-OH-XYL), 2,6-xylidine, monoethylglycinexylidide (MEGX) and glycinexylidide (GX) [1–4]. It has been suggested that the production of the lidocaine metabolite MEGX can be used as a dynamic marker for liver function in liver transplantated patients [5].

The determination of lidocaine has been performed by liquid and gas chromatography (GC), spectrophotometry and fluorometry [1–12]. The lidocaine metabolites were determined by liquid chromatography (LC) [3–6,13]. Gas chromatographic analysis of lidocaine alone or together with its metabolites MEGX and GX has also been reported [3,14]. Derivatization is often necessary for the determination of 2,6-xylidine and 4-OH-XYL by capillary

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GC. Rossi et al. [2] have reported a GC method for underivatized GX, MEGX, 3-OH-MEGX and 3-OH-lidocaine using an HP-1 fused-silica capillary column and GC–nitrogen–phosphorus detection (NPD). In previous work we reported on gas chromatographic separation of underivatized lidocaine and its metabolites using different capillary fused-silica columns and GC–NPD [15].

The aim of this study was to investigate a simple sample preparation technique for lidocaine and three of its metabolites (GX, MEGX and 2,6-xylidine) and to improve the limit of quantification (LOQ) using reversed-phase LC and tandem mass spectrometric detection. The detection limits reported using LC–MS, GC–NPD and GC–MS were about 20 nmol/l for lidocaine and about 50 nmol/l for the metabolites after liquid–liquid extraction or solid-phase extraction from the plasma samples [2,6,16].

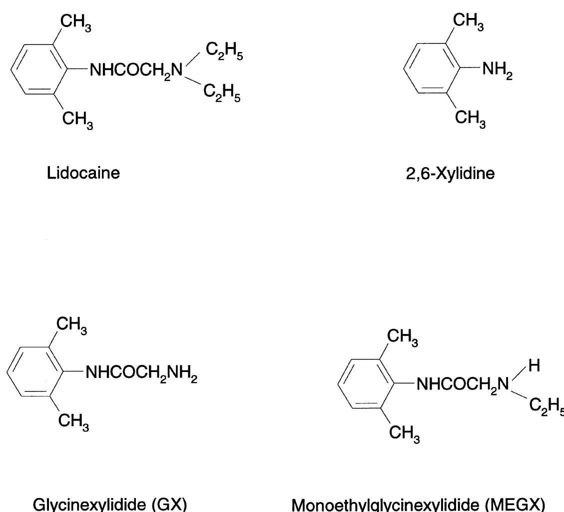


Fig. 1. The structures of lidocaine and its metabolites.

2. Experimental

2.1. Chemicals

Lidocaine, metabolites (2,6-xylidine, GX and MEGX) and lidocaine-D3 (internal standard, I.S.) were supplied by the Department of Medicinal Chemistry, AstraZeneca (Södertälje, Sweden) as hydrochlorides. Acetonitrile (gradient grade), methanol LiChrosolv and formic acid (puriss >99%) were obtained from Merck (Darmstadt, Germany). The structures of lidocaine and its metabolites are shown in Fig. 1.

2.2. Apparatus

The high-performance liquid chromatography (HPLC) apparatus included two pumps, Shimadzu LC10Advp, Shimadzu (Kyoto, Japan) and an autosampler, CTC-Pal, was obtained from CTC Analytics (Zwingen, Switzerland). The mass spectrometric instrument was a QII Z-spray mass spectrometer from Micromass UK (Manchester, UK) and the chromatographic data system was MassLynx version 3.1. An ultrafiltration kit Centrisart I (M_r 20 000 cut off) obtained from Sartorius (Goettingen, Germany) was used for ultrafiltration of the plasma samples. Solid-phase extraction discs (SPEC, C18AR, 15 mg, 3 ml) were obtained from Ansys (CA, USA).

2.3. Sample preparation

2.3.1. Plasma samples

Ultrafiltration. The plasma sample was thawed and carefully homogenized using a whirl mixer. A volume of 500 μl plasma sample was mixed with 75 μl of 2 *M* phosphoric acid in an ultrafiltration kit, Centrisart I (M_r 20 000 cut off). A 50-μl volume of internal standard solution (3.5 μm) and 125 μl of 10 m*M* formic acid were added and then centrifuged at 3500 rpm at 25°C for 20 min. The ultrafiltrate was directly injected (30 μl) into the chromatographic system.

2.3.2. Urine samples

Solid-phase extraction. A 0.5-ml volume of urine was perfused through a solid-phase disc (C_{18}). The disc was washed with 0.5 ml phosphate buffer (pH 7.4), followed by elution of the analytes with 0.8 ml 18% acetonitrile in 10 m*M* aqueous trifluoroacetic acid (TFA). The eluate was diluted 1:1 with mobile phase A (see below) and 30 μl was injected into the chromatographic system.

2.4. Analytical procedure

2.4.1. Chromatographic system

The analytical column was a YMC basic, 150×3.0 mm, 3 μm, and was purchased from YMC Europe (Schermbeck, Germany). An optiguard (C_8, 10×1 mm) obtained from Optimize Technologies (OR, USA) was used as a guard column. A VICI Valco C4W valve (VICI Valco Instrument, Houston, TX, USA) was used as gate valve between the analytical column and the mass spectrometer.

Gradient HPLC was used with a mixer volume of 0.2 ml. Mobile Phase A was 0.1% formic acid in acetonitrile–water (0.5:99.5) and mobile phase B contained 0.1% formic acid in acetonitrile–water (80:20). The gradient started from 0.5% of mobile phase B up to 80% from 0 to 8 min and then from 8 to 10 min isocratic at 80% of mobile phase B, and at 10.1 min mobile phase B was set at 0.5% again. The flow-rate was 0.35 ml/min. For the sake of system stability the next injection was performed after 14 min.

All experiments were conducted using a triple quadrupole mass spectrometric instrument (Micromass) equipped with a Z-electrospray interface (ESI) operated in the positive ion mode. The source block and desolvation temperatures were 150°C and 250°C, respectively. Nitrogen was used as both drying and nebulizing gas, while argon was used as collision gas. The eluate from the analytical column was introduced into the ESI source after 3 min using the Valco valve. The data were collected using MassLynx version 3.1. All calculations were based on peak area ratios. Prior to each batch of analysis, a test sample containing all the metabolites was analyzed in order to check the sensitivity and to set integration parameters.

The selection of operating protonated ions is shown in Fig. 2. The scan mode was multiple reaction monitoring using the precursor ion at m/z (M+1) (m/z: 235, 238, 122, 207, 179) and after collisional dissociation the product ions 86, 86, 105, 58 and 122 were used for the quantification of lidocaine, lidocaine-D3 (I.S.), 2,6-xylidine, MEGX and GX, respectively.

2.4.2. Validation

Each calibration curve consist of seven calibration points covering the range from 1.6 to 810 nmol/l for lidocaine in plasma (20–10 000 n*M* for urine samples), from 2 to 200 nmol/l for GX and MEGX (20–2000 n*M* for urine samples) and from 5 to 200 nmol/l for 2,6-xylidine (50–2000 n*M* for urine samples). The peak area ratios for lidocaine, 2,6-xylidine, GX and MEGX and the internal standard (lidocaine-D3) were measured and a standard curve without the zero concentration was constructed.

3. Results and discussion

3.1. Method development

The aim of the present study was to develop a method to determine lidocaine and some of its major metabolites in urine and plasma samples. A simple sample preparation, short separation time and a low quantification limit were considered when the study started.

The aim for sample preparation method was to remove interferences from the biological sample and it should be also reproducible with a high recovery involving a minimum number of working steps. For plasma samples different types of sample preparation were tested. It was difficult to obtain high recovery for the metabolites using liquid–liquid extraction. Solid-phase extraction gave both high recovery and good chromatography but it took more time and more steps compared to ultrafiltration. Using ultrafiltration high recovery was obtained after acidification of the sample. Phosphoric acid was added to the plasma to minimize the protein binding. After ultrafiltration the sample was directly injected into the chromatographic system. No interfering peaks and no damage on the LC or MS systems was observed using ultrafiltration as a sample preparation. Since ultrafiltration is very simple to use it became the method of choice for plasma samples.

Direct injection of urine samples onto the LC–MS–MS system showed that high variation in the response and a bad linear coefficient was obtained. This is may due to the high salt concentration in urine samples. The presence of salt can decrease the formation of the ions and deteriorate electrospray ionization process as well. A rapid and simple solid-

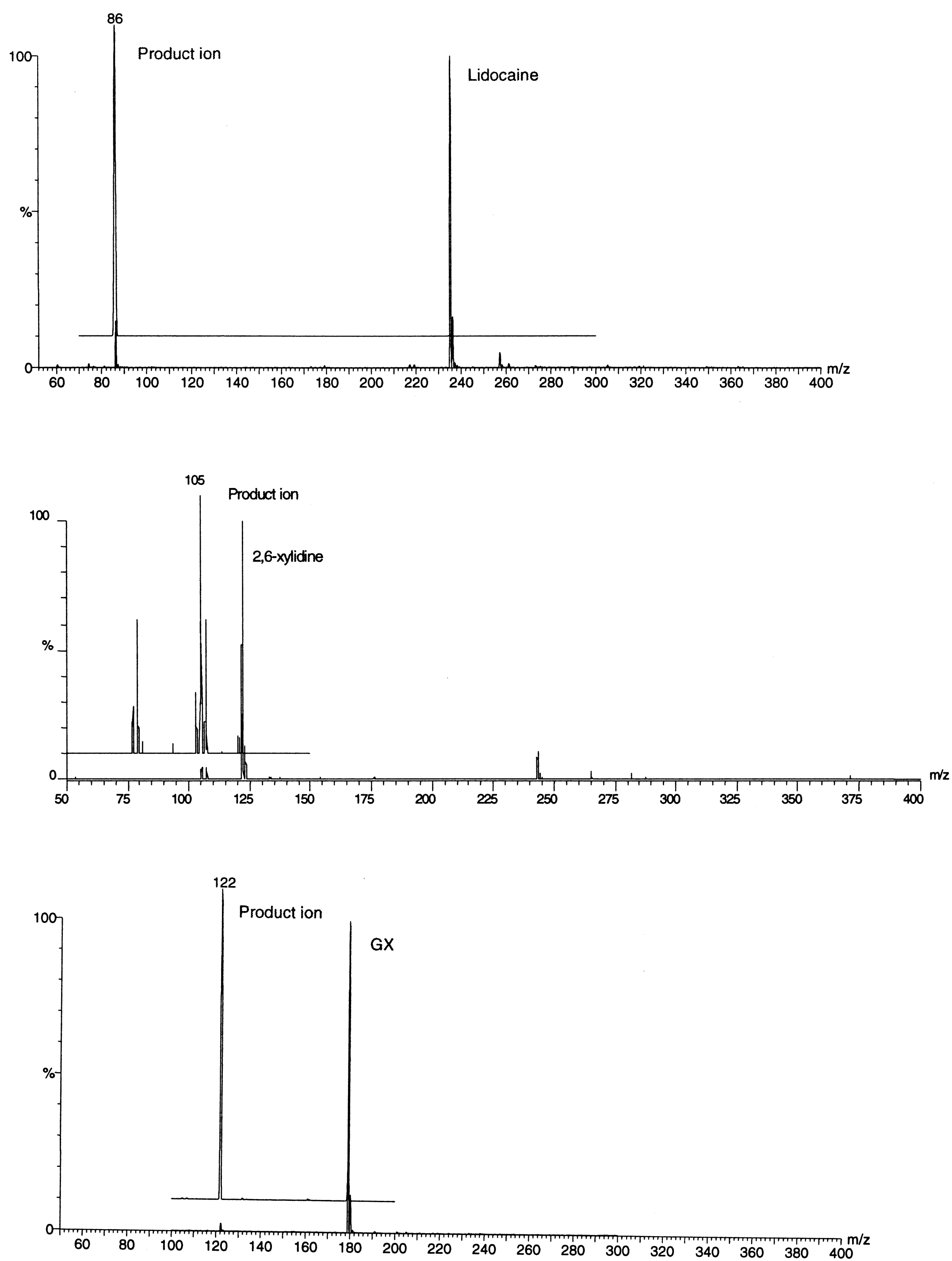


Fig. 2. Mass spectra of precursor and product ions of lidocaine, 2,6-xylidine, GX and MEGX.

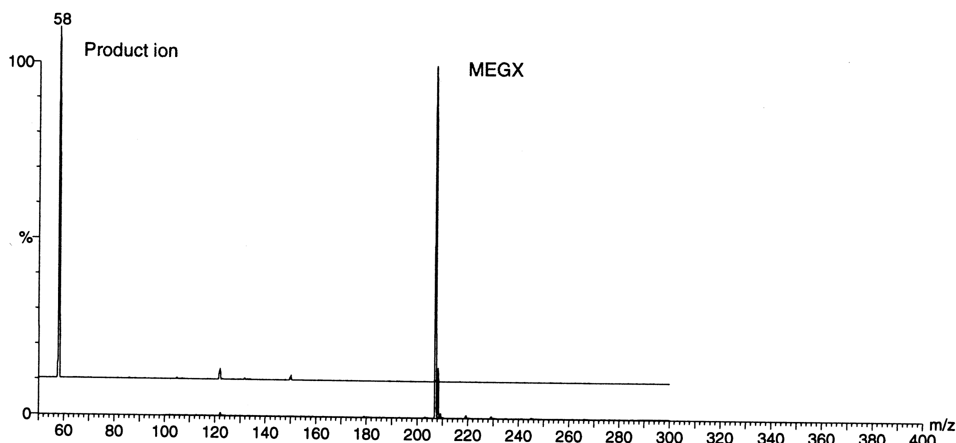


Fig. 2. (*continued*)

phase extraction was used for the urine samples to remove urine salts.

Gradient reversed-phase HPLC was used to give a short analysis time. The solutes were not completely baseline separated; however, the selectivity of MS–MS detection made it easy to obtain accurate results. Different mobile phases were screened. A low pH (2–3) was necessary to protonate the analytes and obtain short retention times (analytes pK_a values from 3.7 to 7.8). The effect of LC eluent composition on MS response was also studied. The ionization efficiency of an analyte can be affected by the presence of competing buffer electrolytes or biological components in the eluent [17]. Optimal assay performance will depend on balancing the highly individual requirements for both LC performance and electrospray ionization efficiency. The influence of the formic acid and ammonium formate as mobile phases was investigated. Higher MS response was obtained using formic acid compared to ammonium formate.

All the ions corresponding to the protonated analytes ($[M-H]^+$) were chosen as precursor ions. The MS conditions were optimized by tuning the instrument with studied analytes to obtain a maximum response and stable product ions. The effect of the biological matrix on the MS response was investigated by comparing the responses of spiked plasma samples and direct injection of analytes in mobile phase at different concentrations. No significant changes in the MS response were observed.

There is a continuous discussion on how to optimize the various steps and how to balance the steps towards each other in LC–MS assays. Currently a number of different approaches are evaluated in order to optimize LC–MS assays for various purposes. One path goes toward parallel sample preparation schemes [18] in order to improve sample throughput for large series of samples. Another path goes toward generic methods that require little time for method set-up, such as protein precipitation and turbulent flow chromatography [19]. This paper is investigating another way to optimize an assay. By using a very simple sample preparation scheme and spending a little more time on the chromatographic analysis, it was possible to develop an assay with a high degree of integrity and a short time for method establishment.

3.2. Selectivity

No significant interfering peaks were detected at the retention times of the studied solutes when running a mixture of the metabolites and human blank plasma or urine from six different persons. This is due to the high selectivity achieved when operating with the triple quadrupole system (Figs. 3 and 4).

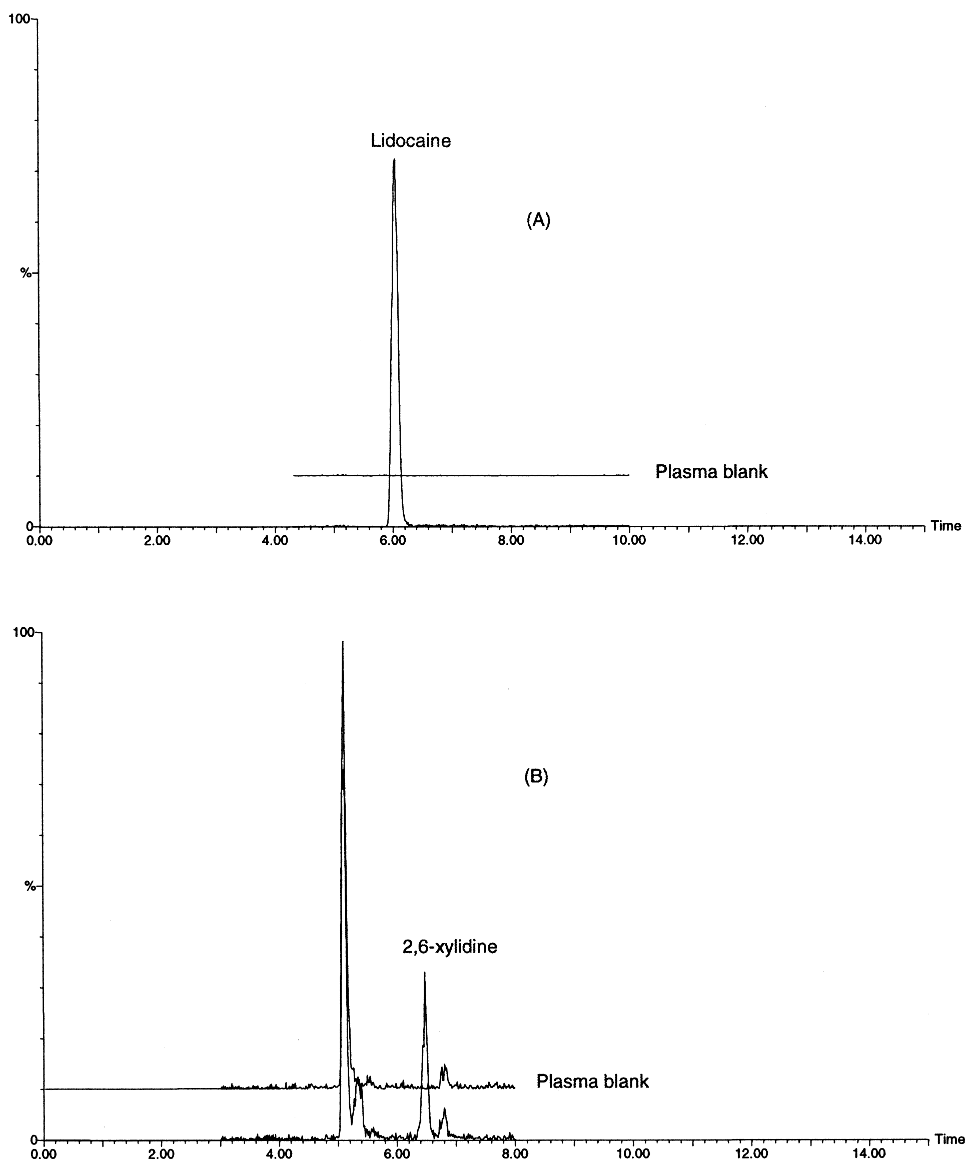
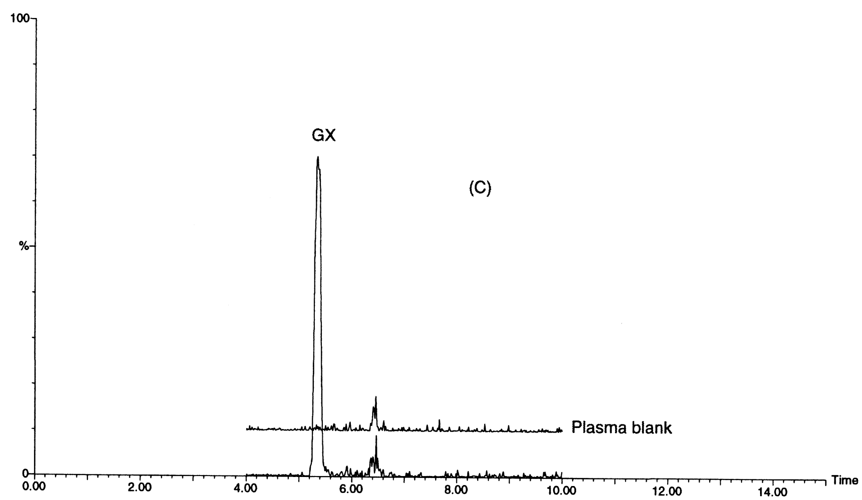
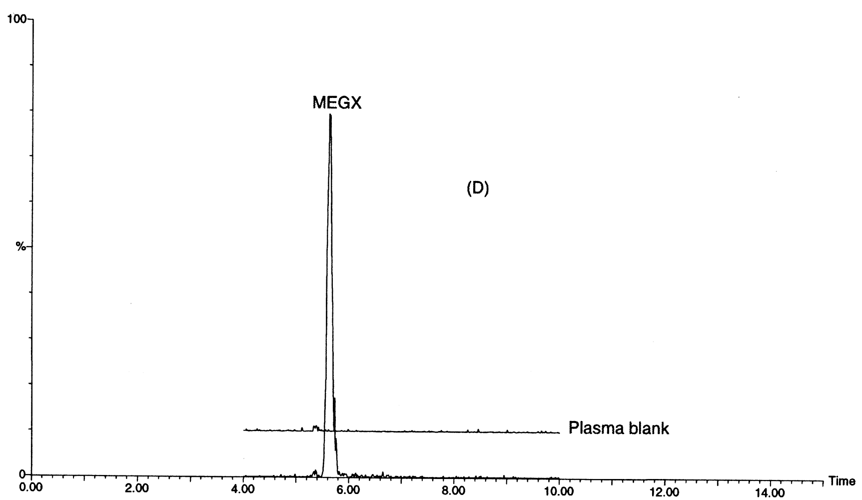


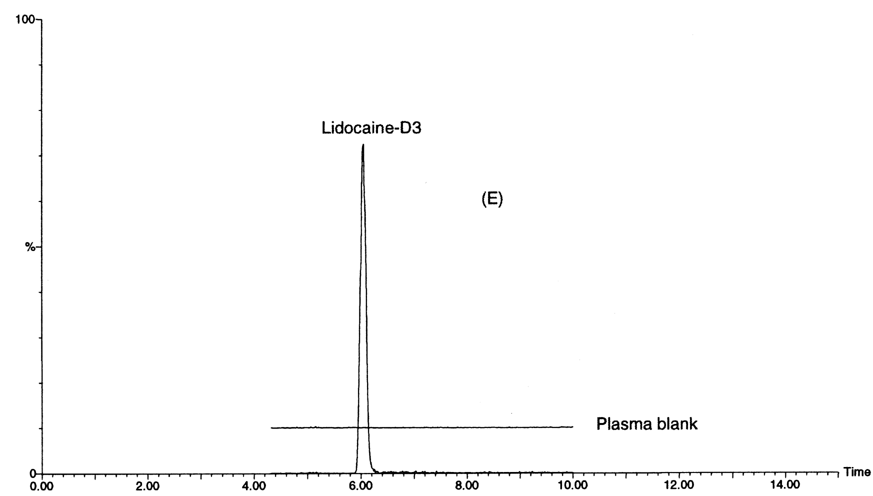
Fig. 3. Chromatograms obtained from extracted blank plasma and from blank plasma spiked with analytes (20 n*M* each) and I.S. (175 n*M*): (A) lidocaine, (B) 2,6-xylidine, (C) GX, (D) MEGX and (E) lidocaine-D3 (I.S.).

3.3. Linearity

Calibration curves were typically described by the formula $y=ax+b$ for lidocaine and $y=cx^2+ax+b$ for the metabolites, where y is the peak area ratio, x is the concentration and a, b are the slope and intercept, respectively. The regression equation was weighted $(1/x)$. The linear regression correlation coefficients, R^2, intercepts and slopes are given in Tables 1 and 2. The back-calculated values of the calibration points agreed closely with the theoretical concentrations (Tables 3 and 4). No deviation beyond $\pm10\%$ of the nominal concentrations was observed.







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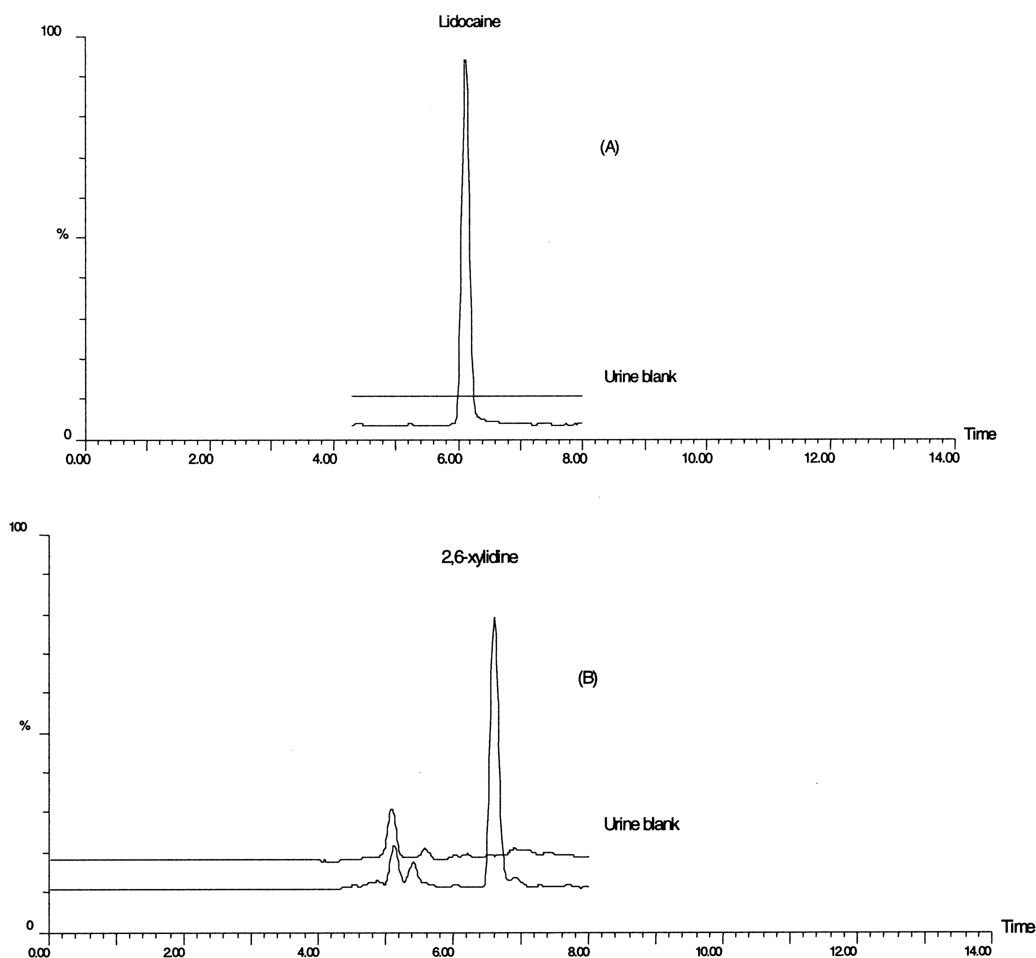

Fig. 4. Chromatograms obtained from extracted blank urine and from blank urine spiked with analytes (100 n*M* each) and I.S. (175 n*M*): (A) lidocaine, (B) 2,6-xylidine, (C) GX, (D) MEGX and (E) lidocaine-D3 (I.S.).

3.4. Accuracy and precision

The accuracy was determined by the percentage of the ratio [(found/theoretical concentration)−1] for human plasma and urine quality control samples, low (L), medium (M) and high (H) within the range of the calibration curve. The variations were from −0.3 to 1.6% (−2.5 to 3.5%, urine samples) for lidocaine, from −1.3 to 8.1% (−9.1 to −5.2%, urine samples) for 2,6-xylidine, from 1.4 to 1.9% (−12 to −5.5%, urine samples) for GX and from −6.4 to 4.1% (−8.4 to −6.4%, urine samples) for MEGX (Tables 5 and 6).

The precision was determined by the relative standard deviation (RSD) of the intra- and inter-assay variations at three different concentrations for quality control samples in human plasma and urine. The precision was consistently about 1–7.0% (plasma samples) and 1.9–5.3% (urine samples) for the intra-assay ($n=6$). The data of inter-assay variation of the precision ($n=18$) were in the range 1.8–13% (plasma samples) and 3.3–7.8% (urine samples). The precision results are summarized in Tables 5 and 6.

3.5. Recovery and limit of quantification

The recovery was determined by comparing the peak area after extraction at two different concen-

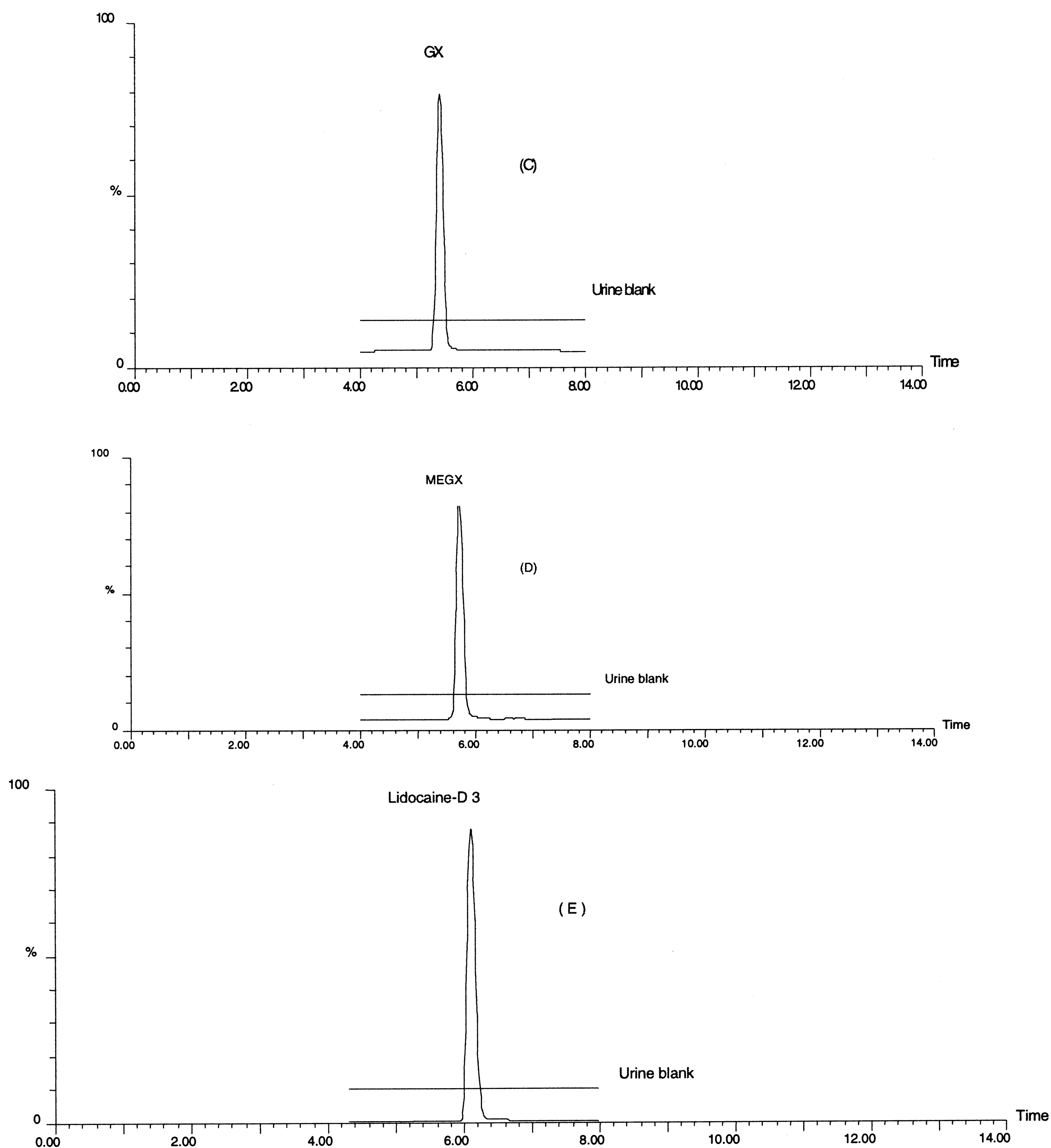

Fig. 4. (*continued*)

tration levels (low- and high-quality control samples), with the peak area obtained after adding the concentrations to an extracted blank plasma. The recoveries were 98% for lidocaine, 88% for GX, 96% for MEGX and 99% for 2,6-xylidine ($n=4$). For urine, the recoveries were 89%, 97%, 96% and 92% for lidocaine, GX, MEGX and 2,6-xylidine, respectively ($n=4$).

Table 1
Regression parameters for the calibration curves for plasma samples

Solute	Batch	Curvature c $(\cdot 10^{-4})$	Slope (a)	Intercept (b)	R^2
Lidocaine[a]	1		1.0894	−0.1722	1.0000
	2		1.0675	0.01916	1.0000
	3		1.0594	−0.07852	1.0000
	4		1.0343	0.14340	0.9998
	5		1.0361	0.02284	0.9999
2,6-Xylidine[b]	1	0.7532	0.06963	−0.08306	0.9997
	2	0.8037	0.0599	0.04683	0.9992
	3	1.155	0.0520	−0.02769	0.9987
	4	1.149	0.0536	−0.04639	0.9995
	5	0.2630	0.0807	0.06422	0.9999
GX[b]	1	1.272	0.0827	0.00635	0.9997
	2	0.9617	0.0747	0.03971	0.9999
	3	1.129	0.0690	−0.02990	0.9991
	4	1.269	0.0699	0.01515	0.9998
	5	1.681	0.3062	0.05439	0.9996
MEGX[b]	1	3.333	0.2170	−0.07053	1.0000
	2	2.827	0.2004	0.05694	0.9995
	3	3.784	0.1567	0.01004	0.9993
	4	2.309	0.1771	−0.05171	0.9997
	5	3.653	0.4319	−0.1170	1.0000

[a] First-order equation: $y=ax+b$ (lidocaine).
[b] Second-order equation: $y=cx^2+ax+b$ (2,6-xylidine, GX and MEGX).

Table 2
Regression parameters for the calibration curves for urine samples

Solute	Batch	Curvature c $(\cdot 10^{-5})$	Slope (a)	Intercept (b)	R^2
Lidocaine[a]	1		0.8227	5.753	0.9977
	2		0.9043	−0.8399	1.0000
	3		0.7925	5.524	0.9968
	4		0.8418	3.073	0.9960
2,6-Xylidine[b]	1	−0.905	0.1326	−0.3497	0.9987
	2	1.306	0.1050	0.4094	0.9998
	3	−1.224	0.1576	−2.484	0.9949
	4	0.957	0.1309	0.09695	0.9998
GX[b]	1	−3.713	0.6591	−2.690	0.9994
	2	3.200	0.5846	−0.2933	0.9997
	3	−5.160	0.7001	−4.589	0.9988
	4	8.109	0.6532	−0.8782	0.9998
MEGX[b]	1	−5.708	0.7229	−1.175	0.9997
	2	1.587	0.6436	0.1300	0.9998
	3	−9.158	0.7252	−3.771	0.9988
	4	−1.422	0.6657	−0.6984	0.9999

[a] First-order equation: $y=ax+b$ (lidocaine).
[b] Second-order equation: $y=cx^2+ax+b$ (2,6-xylidine, GX and MEGX).

Table 3
Back-calculated values of the calibration of the plasma samples

Solute	Concentration (n*M*)	Mean (n=5)	RSD (%)	Mean accuracy (%)
Lidocaine	1.60	1.60	3.3	−1.1
	4.05	4.05	4.1	0.0
	8.10	8.1	1.0	0.5
	20.1	20.1	3.5	−1.1
	82.0	82.0	2.2	1.5
	405	405	1.1	0.1
	809	809	0.5	−0.2
2,6-Xylidine	5.00	5.16	6.3	3.2
	10.0	10.1	5.6	1.1
	15.0	14.9	5.2	−0.5
	25.0	24.3	4.5	−2.9
	50.0	48.7	4.4	−2.7
	100	103	2.4	2.5
	200	199	0.4	−0.3
GX	2.00	1.93	6.3	−3.3
	5.00	5.19	9.7	3.8
	10.0	10.1	7.6	1.4
	25.0	24.6	4.1	−1.6
	50.0	49.7	2.6	−0.6
	100	101	3.0	0.6
	200	200	0.5	0.0
MEGX	2.00	2.05	5.9	2.3
	5.00	4.96	8.2	−0.9
	10.0	10.1	6.6	0.6
	25.0	24.8	2.1	−0.8
	50.0	48.6	2.7	−2.8
	100	102	1.6	2.2
	200	199	0.3	−0.3

Table 4
Back-calculated values of the calibration of the urine samples

Solute	Concentration (n*M*)	Mean (n=4)	SD	RSD (%)	Mean accuracy (%)
Lidocaine	20.0	18.1	2.34	13	−9.4
	50.0	46.9	1.58	3.4	−6.3
	100	103	2.38	2.3	2.5
	250	267	7.89	3.0	6.7
	1000	1054	59.6	5.7	5.4
	5000	5220	172	3.3	4.4
	10 000	9712	229	2.4	−2.9
2,6-Xylidine	50.0	53.1	3.15	5.9	6.2
	100	95.0	4.11	4.3	−5.0
	150	151	3.30	2.2	0.8
	250	246	14.0	5.7	−1.6
	500	483	25.5	5.3	−3.5
	1000	1038	54.8	5.3	3.8
	2000	1984	23.2	1.2	−0.8
GX	20.0	21.7	1.48	6.8	8.2
	50.0	48.0	1.02	2.1	−4.1
	100	97.0	4.30	4.5	−3.3
	250	243	13.1	5.4	−2.9
	500	503	10.9	2.2	0.5
	1000	1018	34.9	3.4	1.8
	2000	1990	15.8	0.8	−0.5
MEGX	20.0	21.3	0.746	3.5	6.4
	50.0	48.9	0.574	1.2	−2.3
	100	96.5	1.67	1.7	−3.5
	250	245	11.2	4.6	−1.9
	500	500	8.06	1.6	−0.1
	1000	1019	30.7	3.0	1.9
	2000	1988	16.4	0.8	−0.6

Table 5
Intra- and inter-assay precision and accuracy in plasma

Compound	Concentration (n*M*)	Intra-assay		Inter-assay		Mean accuracy, % (n=18)
		Mean (n=6)	RSD (%)	Mean (n=18)	RSD (%)	
Lidocaine	4.05	4.20	4.0	4.1	3.8	1.3
	81.0	82.1	1.0	82.3	2.6	1.6
	608	599	1.0	606	1.8	−0.3
2,6-Xylidine	10.0	10.5	4.0	10.8	6.8	8.1
	50.0	48.9	3.0	49.4	4.2	−1.3
	150	147	2.0	151	3.0	0.5
GX	5.0	5.24	7.0	5.09	10	1.9
	25.0	25.3	6.0	25.4	4.5	1.7
	150	149	3.0	152	3.9	1.4
MEGX	5.0	5.15	5.0	4.68	13	−6.4
	25.0	26.7	2.0	26.0	4.9	4.1
	150	152	4.0	154	3.4	2.4

Table 6
Intra- and inter-assay precision and accuracy in urine

Compound	Concentration (n*M*)	Intra-assay		Inter-assay		Mean accuracy, % (n=18)
		Mean (n=6)	RSD (%)	Mean (n=18)	RSD (%)	
Lidocaine	50.0	50.0	3.2	48.8	4.8	−2.5
	1000	967	1.9	1035	5.2	3.5
	7500	7136	4.5	7315	4.6	−2.5
2,6-Xylidine	100	95	5.3	93.2	6.0	−6.8
	500	466	4.7	454	4.9	−9.1
	1500	1441	4.6	1422	4.4	−5.2
GX	50.0	44.6	3.2	43.8	3.3	−12
	250	258	3.8	236	7.8	−5.5
	1500	1360	4.2	1343	4.4	−10
MEGX	50.0	46.9	4.9	46.7	5.9	−6.7
	250	242.3	3.0	229	5.4	−8.4
	1500	1405	5.0	1404	4.8	−6.4

Table 7
Limit of quantification (LOQ) of lidocaine and its metabolites

Compound	LOQ (n*M*)		Mean (n=10)		RSD (%)		Signal-to-noise ratio (S/N)	
	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine
Lidocaine	1.6	20.0	1.67	17.7	4.5	3.4	34	269
2,6-Xylidine	5.0	50.0	5.4	52.8	6.9	4.3	16	71
GX	2.0	20.0	1.92	20.3	9.6	4.3	19	148
MEGX	2.0	20.0	2.1	19.3	7.8	4.6	44	194

The limit of quantification (LOQ) in plasma was set at 1.6 nmol/l for lidocaine, 2.0 nmol/l for GX and MEGX and 5 nmol/l for 2,6-xylidine. The signal-to-noise ratios at the LOQ were 34, 19, 44 and 16 for lidocaine, GX, MEGX and 2,6-xylidine, respectively (Table 7). Due to the high concentration level of the solutes in the urine samples, the LOQ was set at 20 nmol/l for lidocaine, GX and MEGX and 50 nmol/l for 2,6-xylidine (Table 7).

3.6. Carry-over

The carry-over was tested by injecting the mobile phase after the highest standard concentration. The observed carry-over was less than 20% of the LOQ.

4. Conclusions

A HPLC–MS–MS method for the assay of lidocaine and its metabolites 2,6-xylidine, GX and MEGX has been developed and validated. The acceptance criteria for the study validation were well in line with the international criteria [20]. The results showed that the method is selective and accurate. The LOQ was, as compared to prior work, improved by a factor of ten for lidocaine, 2,6-xylidine, MEGX

and GX. In addition, the results indicated that the method is suitable for determination of lidocaine and its metabolites in plasma and urine samples.

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