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Journal of Chromatography B, 726 (1999) 185–194

JOURNAL OF CHROMATOGRAPHY B

Simultaneous determination of local anesthetics including ester-type anesthetics in human plasma and urine by gas chromatography–mass spectrometry with solid-phase extraction

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Received 7 April 1998; received in revised form 29 October 1998; accepted 29 October 1998

Abstract

The present study describes the simultaneous determination of seven different kinds of local anesthetics and one metabolite by GC–MS with solid-state extraction: Mepivacaine, propitocaine, lidocaine, procaine (an ester-type local anesthetics), cocaine, tetracaine (an ester-type local anesthetics), dibucaine (Dib) and monoethylglycinexylidide (a metabolite of lidocaine) were clearly separated from each other and simultaneously determined by GC–MS using a DB-1 open tubular column. Their recoveries ranged from 73–95% at the target concentrations of 1.00, 10.0 and 100 μg/ml in plasma, urine and water. Coefficients of variation of the recoveries ranged from 2.3–13.1% at these concentrations. The quantitation limits of the method were approximately 100 ng/ml for monoethylglycinexylidide, propitocaine, procaine, cocaine, tetracaine and dibucaine, and 50 ng/ml for lidocaine and mepivacaine. This method was applied to specimens of patients who had been treated with drip infusion of lidocaine, and revealed that simultaneous determination of lidocaine and monoethylglycinexylidide in the blood and urine was possible.

Keywords: Mepivacaine; Propitocaine; Lidocaine; Procaine; Cocaine; Tetracaine; Dibucaine; Monoethylglycinexylidide

1. Introduction

Local anesthetics (LAs) are drugs that are mainly used to reversibly block nerve function. They are currently analyzed by HPLC [1,2], GC–MS [3–5], enzyme immunoassays and dry chemistry methods. In addition, simultaneous detection of different LAs is possible using HPLC [1,2,6,7] and GC–MS [5,8–13], as well as the REMEDi HS system, a commercial on-line HPLC for broad-spectrum drug identification [7].

Due to the similar structure of LAs, simultaneous determination of five LAs was performed by GC–nitrogen phosphorus detection (NPD) using an open tubular column (17 m×0.2 mm×0.33 μm (film thickness), cross-linked methyl silicone) [11], but it was not successful by GC–MS. We demonstrated here, that seven different kinds of LAs including ester-type LAs and monoethylglycinexylidide (MEGX), a metabolite of lidocaine (Lid), were simultaneously determined by GC–MS with solid-phase extraction and that simultaneous determination

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0378-4347/99/$ – see front matter
PII: S0378-4347(98)00510-6

of Lid and MEGX by GC–MS was possible in the blood and urine of patients with acute myocardial infarction.

2. Experimental

2.1. Chemicals

Propitocaine (prilocaine; Prop) hydrochloride, Lid hydrochloride, mepivacaine (Mep) hydrochloride, bupivacaine (Bup) hydrochloride and MEGX hydrochloride were kindly provided by Astra-Japan (Osaka, Japan). Tetracaine (amethocaine; Tet) hydrochloride was kindly provided by Kyorin (Tokyo, Japan). Procaine (Proc) hydrochloride, cocaine (Coc) hydrochloride and dibucaine (cinchocaine, Dib) hydrochloride were purchased from Dai-ichi Pharmacy (Tokyo, Japan), Takeda (Osaka, Japan) and Teikoku Kagaku (Osaka, Japan), respectively. The other chemicals (analytical grade) were purchased from Wako (Osaka, Japan).

2.2. Preparation of standard solution

Bup was used as the internal standard (IS) in this study. The stock solutions of LAs were prepared at the concentration of 5 mg/ml in ethanol solution for Bup, Tet, Coc and Dib, and in 75% ethanol aqueous solution for Proc, Mep and MEGX. Then the following working solutions were made with ethanol; 0.5, 1.0, 2.5, 5, 10, 25, 50 and 100 μg/ml. For a recovery test, solutions of each LA (1.00, 10.0 and 100 μg/ml) were prepared with plasma, urine and water. To block esterase activity in the plasma, neostigmine bromide (final 0.3 m*M*; Wako) was mixed before the addition of Proc and Tet to the plasma.

2.3. Administration of lidocaine to patients with acute myocardial infarction

2.3.1. Case 1

Lid was administered to a 57-year-old male for treatment of acute myocardial infarction as follows: 1, administration start time (0)–58 h, dose 40 mg/h; 2, 58–64.3 h, 20 mg/h; 3, 64.3–72 h, 0 mg/h; 4, 72–86.5 h, 60 mg/h; 5, 86.5 h, administration was stopped.

2.3.2. Case 2

Lid was administered to a 55-year-old male as follows: 1, administration start time (0)–264 h, dose 100 mg/h; 2, 264–300 h, 80 mg/ml; 3, 300–327 h, 60 mg/ml; 4, 327–343 h, 30 mg/ml; 5, 343 h, administration was stopped.

2.3.3. Case 3

Lid was constantly administrated to a 52-year-old male 60 mg/h from the start to at least 100 h.

Samples were collected at the time described in Table 5. Informed consent for the sample collection was obtained from the patients before collection in every case.

2.4. Extraction of local anesthetics

An Extrelut® column (Merck, Darmstadt, Germany) was used due to its excellent drug extraction ability [14]. Aliquots (0.2 ml) of sample specimens were mixed with the I.S. solution (5.0 μg/10 μl) for the I.S. method. In the external standard method, the I.S. was not added in this step. The specimens were alkalized with 20 μl (for plasma), 35 μl (for urine) or 5 μl (for water) of 1 *M* sodium hydroxide. The pH of the sample mixtures was checked (about 10–11), and they were then applied to an aliquot (0.7 g) of Extrelut® column. After 30 min at room temperature, the elution procedure was performed with 10 ml of dichloromethane-2-propanol solution (85:15, v/v). The eluate was dried with nitrogen stream at 50°C. For the I.S. method, the residue was reconstituted with 100 μl of ethanol for GC–MS analysis. For the external standard method, the residue was dissolved in 100 μl of 50 μg/ml Bup ethanol solution. The total time, necessary for these procedures, was about 3 h.

2.5. Apparatus and analytical conditions

A model of JMS DX303 and DA5000 GC–MS system (JEOL, Tokyo, Japan) was used. The GC-column employed was a DB-1 column (0.53 mm×15 m×1.5 μm, J&W Scientific, Folsom, CA, USA). The column temperature was programmed to increase from 140–300°C at the rate of 16°C/min and then remained at 300°C for 10 min. The injection,

separator and inlet temperatures were set at 260, 270 and 275°C, respectively. The flow-rate of the carrier gas (He) was 15 ml/min. Electron impact (EI) and positive ion detection modes were used. Acceleration and ionization voltages, ionization current, and conversion dynode voltage were 3 kV, 70 eV, 0.3 mA and −10 kV, respectively. Scan and selected ion monitoring modes were used for qualitative and quantitative analysis, respectively. The following ions (m/z) were recorded for the quantitative analysis of MEGX (58), Prop (86), Lid (86), Proc (86), Mep (98), Coc (82), Tet (58), Bup (IS, 140) and Dib (86).

Analysis with the REMEDi HS system (Bio–Rad, Hercules, CA, USA) was also performed for comparison, as previously described [7].

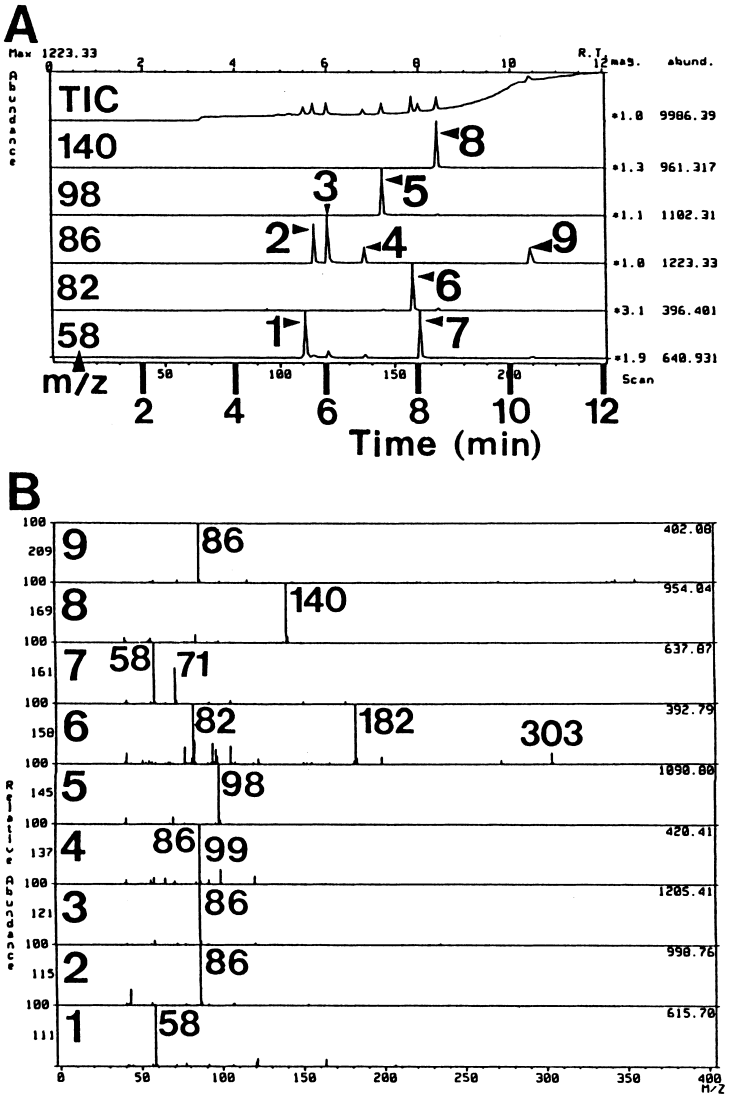

Fig. 1. Total ion, mass chromatograms (A) and mass spectra (B) of seven local anesthetics (LAs), MEGX and the internal standard (IS) (each 100 μg/ml) spiked. 1, Monoethylglycinexylidide (MEGX); 2, propitocaine (Prop); 3, lidocaine (Lid); 4, procaine (Proc); 5, mepivacaine (Mep); 6, cocaine (Coc); 7, tetracaine (Tet); 8, bupivacaine (Bup, I.S.); 9, dibucaine (Dib). Analytical conditions are described in the text.

2.6. Statistical analysis

Statistical analysis was performed using StatView® program (ABACUS Concepts, Barkley, CA, USA).

3. Results

Mass chromatograms and mass spectra for eight different kinds of LAs, Prop, Lid, Proc, Mep, Coc, Tet, Bup (as I.S.) and Dib as well as MEGX are shown in Fig. 1. These LAs and MEGX were clearly separated at each of the following retention times (min); MEGX (5.5), Prop (5.7), Lid (6.0), Proc (6.8), Mep (7.2), Coc (7.85), Tet (8.0), Bup (8.4) and Dib (10.4).

For quantitative analysis, calibration curves for these substances in ethanol solution were prepared, and each of them demonstrated a linear dependency between the peak area ratio to the I.S. peak area and concentrations in the range of 0.5–100 μg/ml (Table

Table 1
Relationship between concentration and peak area ratio in calibration curves for the seven local anesthetics and MEGX in three between-run tests

Substance	Test number	Regression line[a]	S.D. slope[b]	Correlation coefficient
MEGX[c]	1	$Y=-0.0218+0.0134X$	0.00039	0.991
	2	$Y=-0.0230+0.0132X$	0.00039	0.991
	3	$Y=-0.0232+0.0132X$	0.00045	0.988
	Mean	$Y=-0.0227+0.0133X$	0.00023	0.989
Propitocaine	1	$Y=-0.0576+0.0338X$	0.00068	0.996
	2	$Y=-0.0551+0.0337X$	0.00078	0.994
	3	$Y=-0.0552+0.0336X$	0.00055	0.997
	Mean	$Y=-0.0559+0.0337X$	0.00038	0.996
Lidocaine	1	$Y=-0.0566+0.0510X$	0.00075	0.998
	2	$Y=-0.0551+0.0507X$	0.00099	0.996
	3	$Y=-0.0568+0.0507X$	0.00083	0.997
	Mean	$Y=-0.0508+0.0508X$	0.00048	0.997
Procaine	1	$Y=-0.0253+0.0187X$	0.00059	0.989
	2	$Y=-0.0197+0.0187X$	0.00041	0.995
	3	$Y=-0.0245+0.0186X$	0.00075	0.983
	Mean	$Y=-0.0232+0.0187X$	0.00034	0.989
Mepivacaine	1	$Y=-0.0505+0.0403X$	0.00045	0.999
	2	$Y=-0.0500+0.0406X$	0.00067	0.997
	3	$Y=-0.0527+0.0406X$	0.00063	0.997
	Mean	$Y=-0.0511+0.0405X$	0.00033	0.998
Cocaine	1	$Y=-0.0129+0.0130X$	0.00020	0.997
	2	$Y=-0.0118+0.0130X$	0.00024	0.996
	3	$Y=-0.0124+0.0130X$	0.00023	0.997
	Mean	$Y=-0.0124+0.0130X$	0.00013	0.997
Tetracaine	1	$Y=-0.0299+0.0154X$	0.00038	0.993
	2	$Y=-0.0298+0.0154X$	0.00048	0.990
	3	$Y=-0.0317+0.0156X$	0.00029	0.996
	Mean	$Y=-0.0305+0.0155X$	0.00022	0.993
Dibucaine	1	$Y=-0.0396+0.0197X$	0.00062	0.989
	2	$Y=-0.0396+0.0196X$	0.00041	0.995
	3	$Y=-0.0391+0.0196X$	0.00045	0.994
	Mean	$Y=-0.0394+0.0196X$	0.00028	0.993

[a] Peak area ratio (substance/IS); X, concentration of substance (0.5–100 μg/ml).
[b] S.D. slope, standard deviation for slope of regression line, significant differences in all LAs and MEGX were observed at the level of $p>0.05$ among three between-run tests by student's t-test.
[c] MEGX, monoethylglycinexylidide.

1). In three between-run analyses, three different regression lines for the relationship between concentration and peak area ratio in calibration curves showed almost the same slope (Table 1). Significant differences using student's *t*-test for slope of regression lines in all LAs and MEGX were found at the level of $p>0.05$ among three between-run analyses (Table 1).

The recoveries of LAs and MEGX using the external method are shown in Table 2. When compared to the target concentration of 1.00 μg/ml, they ranged from 87 to 95% in water, from 78 to 86% in urine, and from 73 to 85% in plasma. In the case of the target concentration of 10.0 μg/ml, the recoveries ranged from 92–97% (water), from 86–94% (urine) and from 81–90% (plasma). In 100 μg/ml, those ranged from 96–99% (water), from 87–95% (urine) and from 83–90% (plasma).

Coefficients of variation (C.V.s) of the recovery at the concentration of 1.00 μg/ml were 4.1–13.1% in the plasma, urine and water. C.V.s at the target concentration of 10.0 and 100 μg/ml were 2.3–9.4%, and 3.1–8.8%, respectively (Table 2).

Furthermore, within-run and between-run precision values were obtained by GC–MS with I.S. (Tables 3 and 4). In within-run precision, C.V.s of the measured values at the concentrations of 1.00, 10.0 and 100 μg/ml in plasma, urine and water were 4.1–11.3%, 3.5–9.4% and 2.7–8.3%, respectively (Table 3). For day-to-day precision, C.V.s at con-

Table 2
Recoveries of seven local anesthetics and MEGX in plasma, urine and water by GC–MS using the external standard method and their variations

Substance	Water		Urine		Plasma	
	MV±S.D.[a]	C.V. (%)[b]	MV±S.D.	C.V. (%)	MV±S.D.	C.V. (%)
Target concentration (1.00 μg/ml)						
MEGX	0.91±0.053	5.9	0.80±0.044	5.5	0.74±0.097	13.1
Propitocaine	0.93±0.097	10.5	0.81±0.067	8.3	0.78±0.079	10.1
Lidocaine	0.95±0.039	4.1	0.85±0.068	8.0	0.80±0.068	8.5
Procaine	0.88±0.095	10.9	0.79±0.084	10.7	0.78±0.066	8.5
Mepivacaine	0.93±0.050	5.4	0.86±0.059	6.9	0.85±0.065	7.6
Cocaine	0.87±0.081	9.3	0.78±0.077	9.8	0.75±0.060	8.0
Tetracaine	0.87±0.112	12.9	0.80±0.087	10.8	0.74±0.071	9.6
Dibucaine	0.91±0.064	7.0	0.85±0.051	6.0	0.73±0.060	8.2
Target concentration (10.0 μg/ml)						
MEGX	9.25±0.39	4.2	8.69±0.68	7.8	8.06±0.52	8.5
Propitocaine	9.53±0.38	3.9	8.88±0.57	6.4	8.14±0.49	6.0
Lidocaine	9.24±0.70	7.5	8.65±0.40	4.6	8.53±0.60	7.0
Procaine	9.35±0.55	5.8	8.82±0.68	7.7	7.96±0.74	9.3
Mepivacaine	9.63±0.44	4.6	9.35±0.22	2.3	8.95±0.47	5.2
Cocaine	9.51±0.68	7.2	8.55±0.38	4.4	8.26±0.45	5.5
Tetracaine	9.35±0.59	6.3	8.73±0.61	7.0	8.31±0.78	9.4
Dibucaine	9.68±0.69	7.1	9.01±0.60	6.7	8.32±0.70	8.4
Target concentration (100 μg/ml)						
MEGX	97.5±3.32	3.4	90.2±3.89	4.3	82.8±5.58	6.7
Propitocaine	97.4±3.48	3.6	92.2±5.72	6.2	86.1±6.01	7.0
Lidocaine	98.6±3.03	3.1	92.5±6.14	6.6	90.3±3.09	3.4
Procaine	97.9±7.41	7.6	89.9±7.90	8.8	85.2±7.18	8.4
Mepivacaine	98.3±3.83	3.9	95.3±4.92	5.2	89.7±7.10	7.9
Cocaine	96.1±4.93	5.1	88.4±5.78	6.5	87.2±5.07	5.8
Tetracaine	97.1±4.60	4.7	87.3±5.40	6.2	88.8±5.82	6.6
Dibucaine	97.3±5.61	5.8	90.1±3.28	3.6	88.7±6.86	7.7

[a] MV±S.D., mean value (μg/ml)±S.D. ($n=6$).
[b] C.V. (%), coefficient of variation (%).

Table 3
Within-run precision for measured values of seven local anesthetics and MEGX in plasma, urine and water by GC–MS using the internal standard method and their variations

Substance	Water		Urine		Plasma	
	MV±S.D.[a]	C.V. (%)[b]	MV±S.D.	C.V. (%)	MV±S.D.	C.V. (%)
Target concentration (1.00 μg/ml)						
MEGX	1.01±0.068	6.7	1.00±0.088	8.8	1.00±0.086	8.6
Propitocaine	1.00±0.096	9.6	1.00±0.090	9.0	0.99±0.070	7.1
Lidocaine	1.00±0.075	7.5	0.99±0.064	6.5	0.99±0.099	10.0
Procaine	0.99±0.101	10.2	0.96±0.088	9.1	0.96±0.106	11.3
Mepivacaine	1.03±0.073	7.1	0.95±0.093	9.8	0.96±0.058	6.0
Cocaine	1.00±0.089	8.9	0.97±0.097	10.0	0.96±0.092	9.6
Tetracaine	1.02±0.084	8.2	1.01±0.076	7.6	0.97±0.081	8.3
Dibucaine	1.02±0.059	5.8	1.05±0.068	6.5	1.01±0.041	4.1
Target concentration (10.0 μg/ml)						
MEGX	10.01±0.35	3.5	10.03±0.44	4.4	10.02±0.52	5.2
Propitocaine	9.67±0.44	4.6	9.63±0.67	7.0	10.06±0.56	5.6
Lidocaine	10.22±0.47	4.6	9.75±0.47	4.8	9.82±0.68	6.9
Procaine	9.91±0.56	5.7	9.56±0.58	6.1	9.73±0.66	6.8
Mepivacaine	9.88±0.39	3.9	9.83±0.59	6.0	10.38±0.44	4.2
Cocaine	10.10±0.50	5.0	10.04±0.75	7.5	9.71±0.66	6.8
Tetracaine	10.28±0.71	6.9	9.50±0.89	9.4	9.46±0.77	8.1
Dibucaine	9.73±0.51	5.2	9.52±0.58	6.1	9.82±0.62	6.3
Target concentration (100 μg/ml)						
MEGX	98.2±2.93	3.0	101.0±3.04	3.0	97.3±4.23	4.3
Propitocaine	100.9±3.50	3.5	99.3±5.74	5.8	100.2±3.49	3.5
Lidocaine	101.8±2.77	2.7	100.8±3.49	3.5	99.0±3.94	4.0
Procaine	99.3±4.20	4.2	99.2±6.61	6.7	95.8±5.78	6.0
Mepivacaine	98.1±6.39	6.5	99.4±5.72	5.8	97.4±6.00	6.2
Cocaine	98.8±7.21	7.3	98.1±6.12	6.2	98.3±5.51	5.6
Tetracaine	99.3±7.62	7.7	97.3±4.51	4.6	97.9±6.32	6.5
Dibucaine	98.1±3.58	3.6	97.0±8.02	8.3	98.1±4.13	4.2

[a] MV±S.D., mean value (μg/ml)±S.D. (n=6).
[b] C.V. (%), coefficient of variation (%).

centrations of 1.00, 10.0 and 100 μg/ml were 9.7–13.2%, 8.1–12.1% and 8.9–12.6%, respectively (Table 4).

The detection limits of LAs and MEGX by GC–MS were approximately 80 ng/ml for MEGX, Prop, Proc, Coc, Tet and Dib, and they were 40 ng/ml for Lid, Mep and Bup. The quantitation limits of the method were about 50 ng/ml for Lid and Mep, and 100 ng/ml for MEGX, Prop, Proc, Coc, Tet and Dib.

Fig. 2 shows the mass spectra of MEGX and Lid in serum and urine specimens from a patient with ventricular arrhythmia, revealing the same pattern as those in Fig. 1. Concentrations of Lid and MEGX in the serum and urine from three different patients with acute myocardial infarction are shown in Table 5, and compared to those by the REMEDi system. The relationship between measured values of Lid and MEGX calculated by the GC–MS method (X) and those by the REMEDi system (Y) in serum and urine was as follows: Lid, $Y=1.387\,X-0.563$, S.D. slope=0.031, $r=0.996$ ($n=18$); MEGX, $Y=0.944\,X-0.006$, S.D. slope=0.059, $r=0.970$ ($n=18$). S.D. slope represents standard deviation for the slope of the regression line; r shows the correlation coefficient

Table 4
Day-to-day precision for measured values of seven local anesthetics and MEGX in plasma, urine and water by GC–MS using the internal standard method and their variations

Substance	Water		Urine		Plasma	
	MV±S.D.[a]	C.V. (%)[b]	MV±S.D.	C.V.(%)	MV±S.D.	C.V.(%)
Target concentration (1.00 μg/ml)						
MEGX	1.00±0.115	11.5	1.01±0.126	12.5	0.96±0.127	13.2
Propitocaine	1.00±0.107	10.7	0.99±0.114	11.5	0.99±0.128	12.9
Lidocaine	1.01±0.098	9.7	0.99±0.108	10.9	1.00±0.109	10.9
Procaine	0.98±0.113	11.5	0.98±0.121	12.3	0.97±0.115	11.9
Mepivacaine	1.00±0.106	10.6	1.01±0.123	12.2	0.99±0.109	11.0
Cocaine	0.97±0.109	11.2	0.96±0.112	11.7	0.98±0.116	11.8
Tetracaine	1.01±0.120	11.9	0.98±0.117	11.9	0.97±0.122	12.6
Dibucaine	1.00±0.103	10.3	0.99±0.117	11.8	0.98±0.107	10.9
Target concentration (10.0 μg/ml)						
MEGX	9.71±0.99	10.2	9.65±1.06	11.0	9.83±1.12	11.4
Propitocaine	9.84±0.89	9.0	9.61±1.02	10.6	9.72±1.03	10.6
Lidocaine	10.03±0.92	9.2	10.22±0.83	8.1	10.10±0.97	9.6
Procaine	10.32±1.05	10.2	9.94±1.10	11.1	10.03±1.06	10.6
Mepivacaine	10.03±1.01	10.1	9.88±1.05	10.6	9.65±1.04	10.8
Cocaine	10.21±1.09	10.7	10.43±1.15	11.0	10.04±1.06	10.6
Tetracaine	9.95±1.11	11.2	9.55±1.10	11.5	9.74±1.07	11.0
Dibucaine	9.64±1.01	10.5	9.93±1.20	12.1	9.54±1.12	11.7
Target concentration (100 μg/ml)						
MEGX	96.1±10.9	11.3	98.3±11.5	11.7	95.4±12.0	12.6
Propitocaine	99.2±11.1	11.2	102.9±10.4	10.1	102.5±11.2	10.9
Lidocaine	100.1±8.9	8.9	103.1±9.5	9.2	101.3±10.3	10.2
Procaine	102.4±10.6	10.4	101.1±11.2	11.1	98.6±10.8	11.0
Mepivacaine	100.9±10.8	10.7	99.1±10.2	10.3	102.8±10.1	9.8
Cocaine	98.7±11.3	11.5	102.0±10.9	10.7	99.6±11.4	11.4
Tetracaine	99.8±10.8	10.8	97.3±11.9	12.2	96.5±11.7	12.1
Dibucaine	97.2±9.6	9.9	96.6±9.1	9.4	99.8±9.3	9.3

[a] MV±S.D., mean value (μg/ml)±S.D. (n=12).
[b] C.V. (%), coefficient of variation (%).

(Table 5). The results demonstrate a close relationship between the measured values by GC–MS and those by REMEDi.

4. Discussion

LAs are widely used in medical practice, and Lid is employed for the treatment of ventricular arrhythmia. Furthermore, both Lid and Prop are used as EMLA (eutectic mixture of local anesthetics) cream to relieve venepuncture pain in children [15]. An active metabolite of Lid, MEGX has been reported to significantly contribute to the toxic effect of Lid [16]. Concerning drug (LAs) screening, it is advantageous to use the same analytical method to determine blood/serum/urine concentrations of various kinds of LAs. For example, concentrations of Lid and MEGX have been simultaneously determined by GC–NPD in Lid associated deaths [17,18].

Regarding simultaneous qualitative analysis of LAs, several studies have used GC(–MS) [5,8,10,11] and HPLC [1,2] due to the possibility of separating LAs with structural similarities. Simultaneous quali-

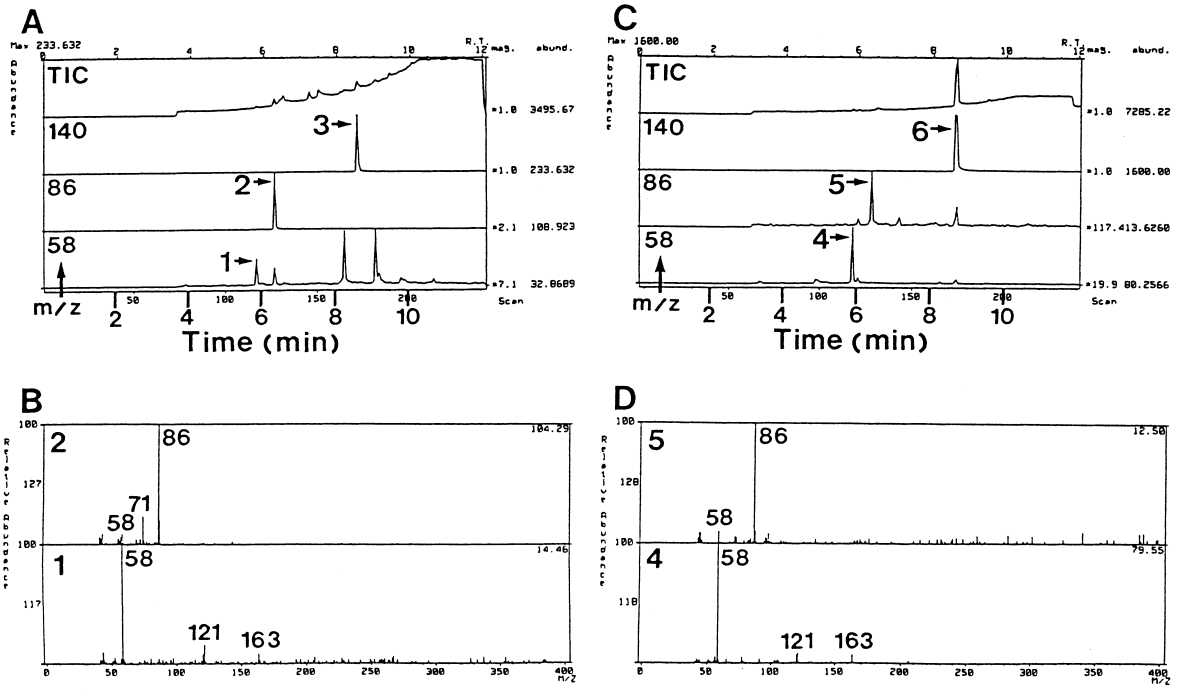

Fig. 2. Total ion, mass chromatograms (A and C), and mass spectra (B and D) of Lid and MEGX in the extracts from a patient's (No. 2 in Table 5) serum (A and B) and urine (C and D). 1 (MEGX 1.80 μg/ml), 2 (Lid 3.53 μg/ml), 4 (MEGX 8.41 μg/ml), 5 (Lid 0.362 μg/ml); 3 and 6, Bup (IS).

tative analyses of LAs by GC(–MS) have been performed using SPB-1 or Hp-17 columns with ten compounds [5]; using an OV-17 column with five compounds [8]; 2% OV-101 column with five compounds [10]; and an open tubular column (cross-linked methyl silicone) with seven compounds [11]. In the present study, seven different LAs including ester-type LAs as well as MEGX were separated using a DB-1 column.

Mass spectra of LAs (Prop, Proc, Lid, Mep, Tet, Bup and Dib) shown by our data were almost the same as those reported by Seno et al. [5], and those of MEGX and Coc corresponded to those by Liu et al. [4] and Welch et al. [19]. Therefore, EI mass spectra data in our study are useful for the identification of LAs. The reliability of qualitative analysis by GC–MS is better than that by the GC–NPD method; for example, with GC–NPD using a HP-1 column, caffeine and carbamazepine could not be distinguished from MEGX and Bup, respectively, due to the similarities in retention times [12], but they were independently identified by GC–MS.

In precision tests, three between-run analyses showed very good reproducibility for these calibration curves, since the student's *t*-test showed no significant differences for the slope of the calibration lines among three between-run tests in all LAs and MEGX. Therefore, quantitative analysis for LAs and MEGX by GC–MS was carried out under good precision.

As an example of simultaneous quantitative analysis, Björk et al. [11] reported that five kinds of LAs were quantitated with two internal standards (mesocaine and pentycaine) by GC–NPD. Their recoveries ranged from 96–108% and the C.V.s were from 1.5–13.8%. In this study, the accuracy of the measured values in the within-run analysis ranged from 95–105% and the C.V.s were from 2.7–11.3%, demonstrating the same accuracy as that by Björk et al. [11]. Furthermore, recoveries of the LAs and

Table 5
Measured values of lidocaine and its active metabolite, MEGX in serum and urine from three patients with acute myocardial infarction

Case	Sex	Age (year)	Specimen (number)		Time[a] (h)	Concentration (μg/ml) of			
						Lidocaine		MEGX	
						GC–MS	REMEDi*	GC–MS	REMEDi*
1	Male	57	Serum	(1)	54	3.11	3.00	0.245	0.256
				(2)	66	1.59	1.76	0.232	0.219
			Urine	(1)	59	18.0	29.9	7.23	5.30
				(2)	66	18.8	28.3	4.79	6.41
				(3)	78	54.6	75.3	9.51	7.16
				(4)	83	43.6	57.6	14.0	11.8
2	Male	55	Serum	(1)	269	3.53	4.54	1.80	1.68
				(2)	291	5.95	4.29	3.71	1.61
				(3)	315	3.19	3.31	1.63	1.45
				(4)	336	2.23	1.86	0.954	0.916
				(5)	338	1.03	1.52	0.821	0.713
			Urine	(1)	319	0.692	0.587	11.6	11.8
				(2)	338	0.362	0.352	8.41	7.49
				(3)	343	0.437	0.458	6.67	7.18
3	Male	52	Serum	(1)	46	2.53	3.17	0.256	0.495
				(2)	70	2.32	2.94	0.270	0.386
			Urine	(1)	75	11.7	13.6	9.58	10.5
				(2)	99	7.89	9.28	11.9	12.9

[a] Time, sample collection time after hospitalization; *REMEDi, these measured values were, in part, data from our previous study [7]. The regression line for Lid, $Y=1.387\ X-0.563$, S.D. slope=0.031, $n=18$, $r=0.996$, $t=44.4$, $p<0.0001$; the line for MEGX, $Y=0.944\ X-0.0064$, S.D. slope=0.059, $n=18$, $r=0.970$, $t=15.9$, $p<0.0001$; Y, the measured values by REMEDi; X, the measured values by GC–MS; S.D. slope, standard deviation for slope of regression line; n, sample number; r, correlation coefficient; t, t value for regression slope; p, p value for regression slope.

MEGX by GC–MS ranged from 73–95% at the target concentrations of 1.00, 10.0 and 100 μg/ml in plasma, urine and water, showing good results that can be applicable to practical analysis of LAs.

Moreover, plasma concentrations of Proc and Tet, ester-type LAs, were quantitated in this study by adding neostigmine to the specimens. If neostigmine was not added, Pro and Tet were readily hydrolyzed into 4-aminobenzoic acid by esterases in the plasma [20]. Terada et al. [13] reported that three ester-type LAs were analyzed by GC–NPD after heptafluorobutyryl derivation at the detection limit of 60–70 pg. Here, with GC–MS, Proc and Tet were detected at about 80 ng/ml without derivation, like other LAs and MEGX, indicating that this method is effective for several types of LAs and demonstrates relatively good sensitivity.

Antiarrhythmic effects of Lid and its active metabolites, MEGX and glycinexylidide (GX), are well known [15,21,22]. MEGX and GX possess approximately 83% and 10% of Lid antiarrhythmic activity, respectively [22]. When Lid is administered to a patient, the main antiarrhythmic effects are due to both Lid and MEGX. Therefore, their concentrations should be measured simultaneously to prevent toxic, adverse effects. In this study, Lid and MEGX concentrations in the serum and urine of three patients corresponded well to those of the REMEDi system, since the correlation coefficients were 0.996 (Lid) and 0.970 (MEGX). These findings indicated that they were at the same levels as those reported by Narang et al. [21] and that this GC–MS method may be useful for drug monitoring of both Lid and MEGX.

Furthermore, although we did not analyze autopsied specimens, this GC–MS method may be

applicable for specimens in medico-legal practices and as a useful tool for forensic toxicology and drug monitoring.

Acknowledgements

The authors thank Mr. K. Uchiyama, Astra-Japan and Mr. M. Sakanoue, Kyorin for providing standard chemicals.

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