Chromatographic Behavior of Underivatized Lidocaine and Metabolites in CGC

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Abstract: The chromatographic behavior in underivatized form of lidocaine and six of its metabolites was investigated using capillary gas chromatography (CGC). Ten fused silica capillary columns from different manufacturers covering a wide range of polarity were tested. On most of the used columns separation of the analytes was obtained; however, the performance with respect to peak symmetry varied extensively. An improvement in peak symmetry and resolution was observed using HP1 and Rtx-1 columns compared to HT5, despite the fact that all column types were nonpolar. The highly polar column (BPX70) could separate only five of seven compounds. Generally, the intermediate polar columns (DB17, HP50, and BPX35) showed the best performance with respect to peak symmetry, and at optimal conditions it took only 10 min to separate all the metabolites. The effect of different injector temperatures (200–350°C) on the stability of the compounds studied was also investigated. The minimum detectable quantities for lidocaine and its metabolites were between 30 and 400 fmol using nitrogen phosphorus detection.
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Key words: *capillary gas chromatography; lidocaine and metabolites; peak symmetry on different types of columns; injector temperature and thermal stability of compounds*

INTRODUCTION

The separation of pharmaceuticals by capillary gas chromatography (CGC) has developed considerably over the past decade through the introduction of new and selective stationary phases. Also, the more recent deactivated fused-silica columns now offer versatile chromatographic performance for many compounds in this area. The determination of polar compounds such as drug metabolites by CGC has in most cases been carried out after derivatization steps. Different derivatization procedures such as methylation, acylation, trimethylsilylation, trifluoroacylation, heptafluorobutylation, and reaction with tetrafluorobenzoyl chloride often used for metabolites have been reviewed [1–3]. However, reports on gas chromatography (GC) of underivatized metabolites are scarce. GC of hydroxylated metabolites of amitryptiline [4] and hydroxylated metabolites of lidocaine [5] have been reported, but the peaks of the metabolites were broad and or tailing compared to the peaks of parent compounds.

The aim of this study was to further examine the possibility of separating metabolites without derivatization and study parameters affecting the peak performance. Avoiding derivatization will be strongly advantageous in analytical issues at, e.g., structure elucidation, metabolic profiling, drug purity, and quantitative determination in bioanalysis. Another possibility may be determination of underivatized metabolites using CGC in combination with solid-phase microextraction (SPME). In this study, lidocaine and six of its metabolites, in underivatized form, *N*-dealkylated and hydroxylated (Figure 1), were used as model compounds.

Lidocaine is commonly used as a local anesthetic and as an antiarrhythmic agent. The major metabolite of lidocaine is 4-hydroxy-2,6-xylidine (4-OH-XY) which is in conjugated form in urine. Other metabolites found in humans are 2,6-dimethylaniline (2,6-xylidine), the monoethylglycinexylidide (MEGX), and glycinexylidide (GX). Small concen-

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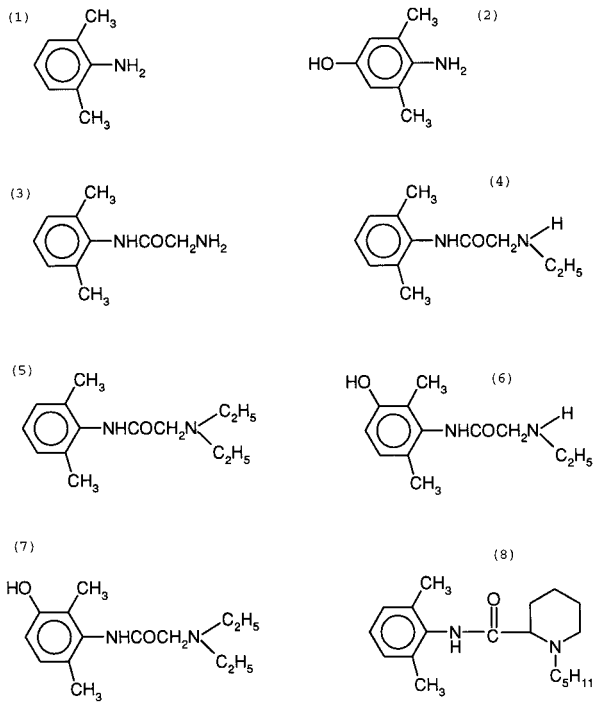

Figure 1. *The structure of lidocaine and its metabolites. (1) 2,6-xylidine; (2) 4-OH-2,6-xylidine; (3) GX; (4) MEGX; (5) Lidocaine; (6) 3-OH-MEGX; (7) 3-OH-lidocaine; (8) pentycaine.*

trations of 4-hydroxymonoethyl-gly-cinexylidide (4-OH-MEGX) and 4-hydroxyglycin-exylidide (4-OH-GX) were also found [6, 7].

The determination of lidocaine was performed by liquid chromatography and GC [6–13], spectrophotometry, and fluorometry [14]. The lidocaine metabolites were determined by liquid chromatography [6–8, 15]. GC analysis of lidocaine alone or together with its metabolites MEGX and GX has been reported elsewhere [6, 16]. Derivatization steps were necessary for the determination of 2,6-xylidine and 4-OH-XY by CGC. Rossi et al. [5] reported a GC method for GX, MEGX, 3-OH-MEGX, and 3-OH-lidocaine without derivatization using an HP1 capillary fused-silica column and GC-NPD. The peaks of GX, 3-OH-MEGX, and 3-OH-lidocaine showed some tailing, which agrees with our results. The HP1 column is not the best column for separation of these compounds, as will be shown below.

In the present study, analysis was carried out using different fused-silica columns from different manufacturers, covering a wide range of polarity to achieve good separation and peak symmetry. It is well known that the nature of the capillary column as well as the deactivation method is of fundamental importance for separation [17]. GC separation of lidocaine and its metabolites, in underivatized form, requires high-resolution GC with selective stationary phases and highly inert surfaces.

EXPERIMENTAL

Apparatus. The GC analysis was performed using a Hewlett-Packard model HP 5890 Series II equipped with an HP 7673A automatic injector and nitrogen-phosphorus and flame ionization detectors (NPD and FID). A ChemStation data system (HP 3365 Series II version A.04.02) was used for data processing.

Helium was used as carrier and as make-up gas. Helium, air, and hydrogen were of high-purity grade. All the gases were obtained from AGA (Lidingö, Sweden). The gas flow rates were measured using a digital flowmeter (J & W Scientific Fisons, CA, USA). The oven temperature was programmed for an initial hold of 2 min at 80°C, then an increase of 30°C min^{-1} until a temperature of 320°C was reached (for BPX70 from 90°C to 270°C at 30°C/min). The detector temperatures were 350°C and 300°C for FID and NPD respectively, and the injector temperature was kept at 250°C. The injector was operated at splitless mode, with the purge activation time adjusted to 1 min.

Columns. DB17 (50% phenyl dimethylpolysiloxane) was purchased from J & W Scientific (CA, USA). CP-Sil 5CP (dimethylpolysiloxane) was obtained from Chrompack Nederland BV (The Netherlands). Rtx-1 (dimethylpolysiloxane) was purchased from Restek Corporation (CA, USA). HT5 (5% phenyl-equivalent polysiloxane-carborane), HT8 (8% phenyl-equivalent polysiloxane-carborane), BPX35 (35% phenyl-equivalent polysilphenylene-siloxane), and BPX70 (70% cyanopropyl-equivalent polysilphenylene-siloxane) were purchased from SGE (TX, USA), while HP1 (dimethylpolylsiloxane), HP35 (35% diphenylmethylpolysiloxane), and HP50 (50% phenyl dimethylpolysiloxane) were obtained from Hewlett-Packard (Palo Alto, CA, USA). All columns used were of fused silica and were crosslinked (Table I).

Chemicals. Lidocaine, metabolites, and pentycaine (Figure 1) were supplied by the Department of Medicinal Chemistry, Astra Pain Control, Södertälje, Sweden, as its hydrochloride. Methanol LiChrosolv was obtained from Merck (Darmstadt, Germany).

Procedure. Stock solutions of the analytes in methanol were made in the concentration range 0.5–2.0 mM. When determining the retention factor, each compound was injected separately at least five times. The injection volume was 1.0 μL using an FID. For the study of asymmetry factors and mini-

Table I. *Columns specifications.*

Column	Length (m)	i.d. (mm)	Phase thickness (μm)	Max. temp. (°C)
HT5 (nonpolar)	12	0.33	0.1	480
HP1 (nonpolar)	15	0.32	0.25	320
CP-Sil CP5 (nonpolar)	25	0.32	0.25	320
Rtx-1 (nonpolar)	15	0.32	0.25	330
HT8 (nonpolar)	12	0.32	0.25	370
HP35 (intermediate polar)	15	0.32	0.1	320
HP50 (intermediate polar)	25	0.20	0.31	280
DB17 (intermediate polar)	30	0.25	0.5	280
BPX35 (intermediate polar)	25	0.32	0.1	370
BPX70 (highly polar)	15	0.32	0.25	290

mum detectable quantity (MDQ), the NPD was used. The concentrations of the analytes were in the range 10–200 nM, and each compound was injected separately at least three times. The injection volume was 1.0–2.0 μL. Retention factor (k'):

$$k' = (T_R - T_0)/T_0$$

$$T_0 = L/u$$

where T_R is the retention time of the compound, T_0 is the void time in minutes measured at 80°C, L is the column length, and u is the average mobile-phase linear velocity in the column. Peak symmetry (Asf):

$$Asf = b/a$$

where a and b are the baseline halfwidths (b is the latter half and a is the former half), measured from the perpendicular drawn through the peak maximum.

RESULTS AND DISCUSSION

Polar compounds such as amines, carboxylic acids, alcohols, and phenols are in most cases derivatized prior to their analysis by GC to shorten the analysis time and avoid peak tailing. Strong interaction between the solutes and column surface can, in some cases, lead to complete adsorption of these compounds. The surface inertness is an important parameter influencing column efficiency [18]. In capillary columns, there are different sites of adsorption. The first of these is attributed to metallic impurities (Na, Al, Ca, Fe, Mg, and Cu) found in the glass matrix. The metallic impurities can act as Lewis acids sites. The synthetic fused-silica materials are essentially free of metallic impurities. The second and most important adsorption site is attributed to the silica surface structure (hydroxy groups). The hydroxy groups (silanol groups) are available as proton donors for hydrogen bonding. Highly basic or acidic compounds are thus difficult to determine by GC. A completely deactivated surface is required or the compounds must be derivatized to nonpolar

Table II. *Asymmetry factors for lidocaine metabolites.*[a]

Compound	HT5[b]	HP1[b]	HT8[b]	CP-Sil 5CP[b]	Rtx1[b]	BPX35[c]	HP35[c]	HP50[c]	DB17[c]	BPX70[d]
Lidocaine	2.40	1.30	1.20	0.89	0.70	1.10	1.10	0.93	1.01	0.94
3-OH-lidocaine	4.50	1.60	1.33	0.70	0.80	1.40	1.07	0.80	0.98	None
MEGX	3.80	1.90	1.15	0.93	0.60	1.17	1.20	0.90	0.94	1.30
3-OH-MEGX	5.20	1.80	1.45	1.20	1.50	1.86	1.08	0.90	1.09	None
GX	4.30	2.82	1.45	1.15	1.10	1.38	0.83	0.90	1.10	2.38
2,6-Xylidine	4.50	2.60	3.70	3.00	2.60	1.90	1.50	1.16	1.04	1.11
4-OH-2,6-Xylidine	5.80	2.70	1.61	2.90	1.10	1.23	1.14	0.90	1.19	1.38

[a]Solute concentration: 10–200 nM.
[b]Nonpolar column.
[c]Intermediate polar column.
[d]polar column.
Temperature program (from 80°C to 300°C at 30°C/min; for BPX70, from 90°C to 270°C at 50°C/min).
Injector: splitless (250°C); detector: NPD (300°C).

Table III. *Retention factors of lidocaine and metabolites.*

Compound/Column	HT5	HP1	HT8	CP-Sil 5CP	Rtx-1	BPX35	HP35	HP50	DB17	BPX70
Lidocaine	9.93	6.75	9.72	6.00	9.50	4.11	5.95	5.05	4.10	2.66
3-OH-lidocaine	11.29	7.87	11.09	6.80	10.90	4.31	7.40	6.15	4.98	None
MEGX	9.59	6.37	9.44	5.73	9.10	3.67	5.84	4.98	4.01	3.00
3-OH-MEGX	10.96	7.56	10.70	6.60	10.50	4.64	6.93	6.08	4.92	None
GX	9.19	5.97	9.13	5.40	8.50	3.60	5.66	4.92	3.97	3.31
2,6-Xylidine	4.16	2.54	6.85	3.00	4.20	2.22	2.76	2.75	2.49	1.46
4-OH-2,6-Xylidine	7.32	4.58	7.22	4.38	6.74	3.03	4.42	3.94	3.30	2.41

Conditions: compound concentrations: 0.5–2.0 mM (injected volume 1 μL). Temperature program (from 80°C (2 min) to 300°C at 30°C/min; for BPX70, from 90°C to 270°C at 50°C/min). Injector: splitless (250°C); detector: FID (350°C).

products. Several types of deactivation method have been used to overcome adsorption problems. The different deactivating methods used for different capillary columns can affect the separation performance in different ways. These methods are often kept secret by the manufacturers, and fused-silica capillary tubing from different manufacturers may differ [17].

Peak symmetry. When the underivatized analytes were applied on different capillary columns, different effects on the peak symmetry were observed (Table II). The HT5 and BPX35 columns, both from the same manufacturer but having different deactivating methods, showed significant differences in peak performance. The HT5 column showed poor peak symmetry, especially for compounds con-

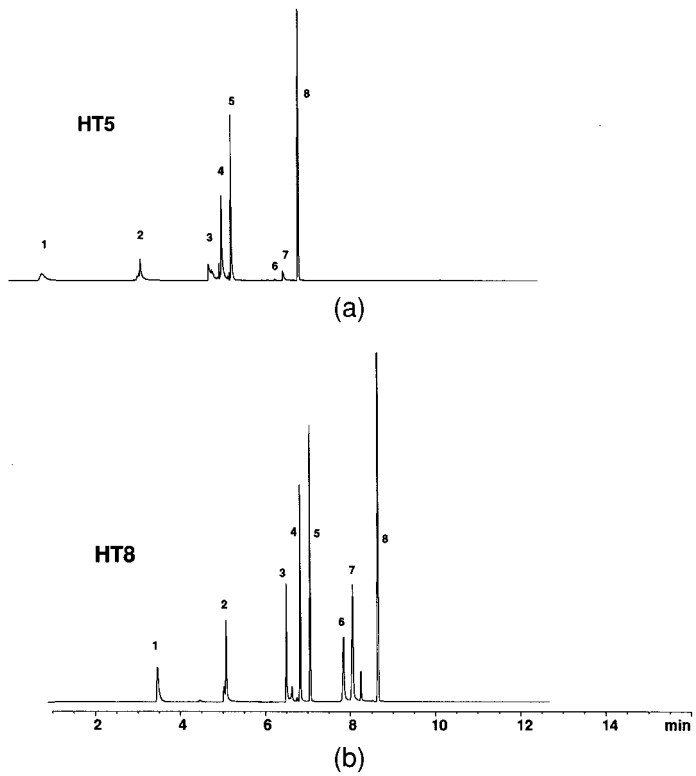

Figure 2. *Chromatograms of lidocaine and its metabolites on HT5 (12 m), HT8 (15 m), HP1 (15 m), CP-Sil 5CP (25 m), Rtx-1 (15 m), BPX35 (25 m), HP35 (15 m), BD17 (30 m), HP50 (25 m), and BPX70 (15 m) columns. Conditions: injected amount: 0.5–2.0 nmol. Columns are temperature-programmed from 80°C (2 min) to 300°C (4 min) at 30°C min^{-1}. Injector: splitless (250°C); detector: NPD (300°C). Peak identification: (1) 2,6-xylidine; (2) 4-OH-2,6-xylidine; (3) GX; (4) MEGX; (5) lidocaine; (6) 3-OH-MEGX; (7) 3-OH-lidocaine; (8) pentycaine.*

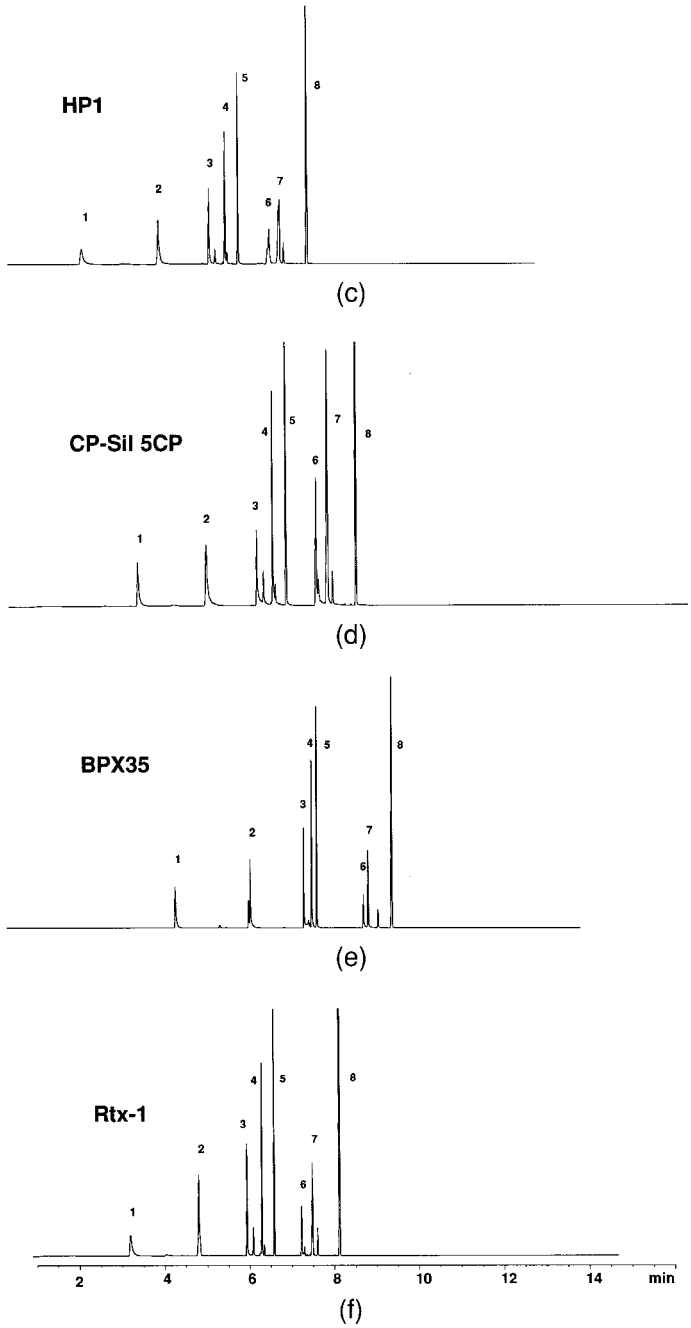

Figure 2. *Continued*

taining hydroxy or amine groups. The peak symmetry improved significantly using the HP50 column.

Rtx-1 and HT5 are both nonpolar but were obtained from different companies. Rtx-1 showed better peak symmetry than HT5 (Table II).

The intermediate polar columns DB17 and HP50 both showed excellent performance for all of the compounds studied. The peak symmetry was even better on DB17 and HP50 compared to HP35 and BPX35, the other intermediate polar columns (Table III). Figure 2 shows chromatograms for lidocaine and its metabolites on different columns (HT5, HP1, HT8, CP-Sil 5CP, Rtx-1, BPX35, HP35, HP50, and DB17). A low recovery for 3-OH-MEGX and 3-OH-lidocaine was observed on the HT5 column.

Comparing the HT5 and HT8 columns is of interest, since both columns have carborane in the backbone phases, but different deactivation methods were applied to them (as reported by the manufacturer). The HT8 column gave significant improve-

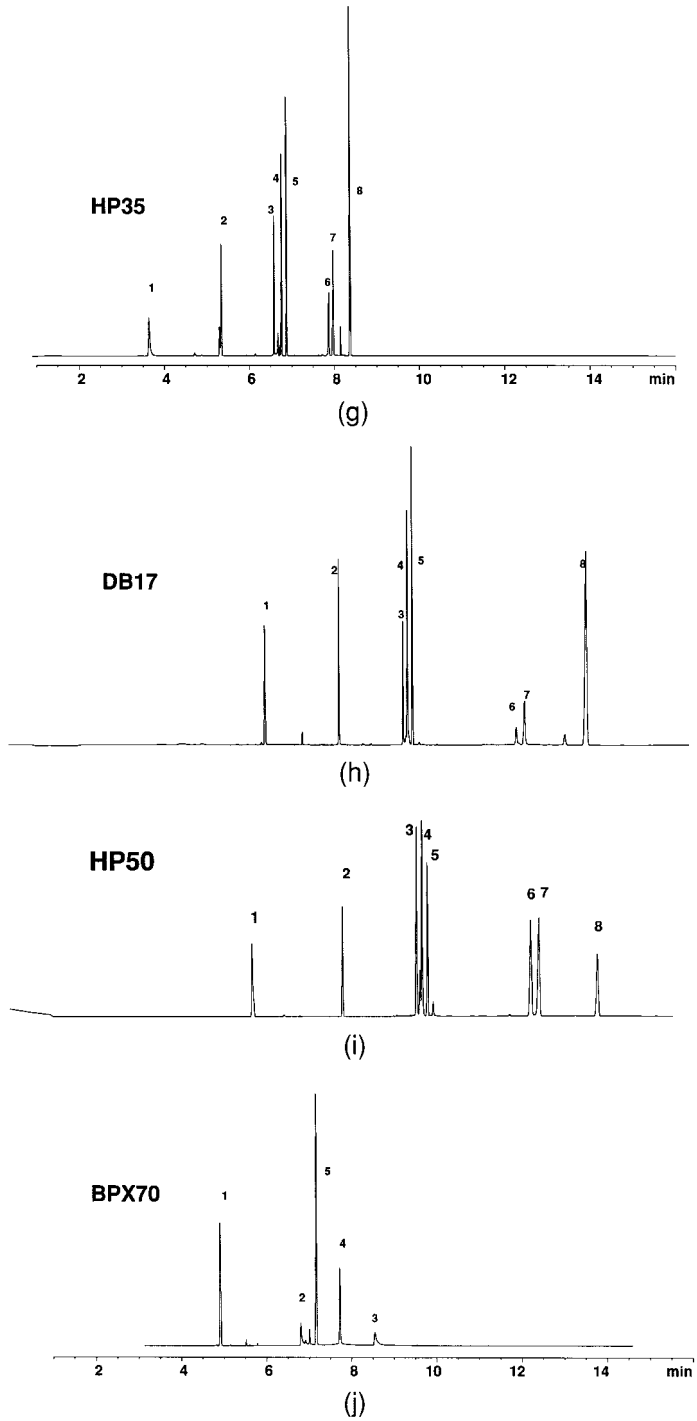

Figure 2. *Continued*

ment in peak shape compared to the HT5 column. PBX35 and HP35 columns showed a better peak symmetry for the compounds 2,6-xylidine and 4-OH-2,6-xylidine compared to the nonpolar columns (HT5, HT8, HP1, Rtx-1, and CP-Sil 5CP). The peak symmetry of lidocaine, GX, MEGX, 3OH-MEGX, and 3-OH lidocaine was improved using HT8, Rtx-1, and CP-Sil 5CP, compared to HP1 and HT5, although all columns had nonpolar phases. The chromatography of lidocaine and pentycaine (both are tertiary amines) was, as expected, acceptable on all columns tested.

Retention factors. The retention factor was increased for lidocaine and its metabolites on nonpo-

lar columns. This might be a result of a hydrogen-bond interaction between the metabolites and the capillary glass surface. The assumption is supported by the observation that the asymmetry factors were higher on the nonpolar than on the intermediate and polar columns. On the polar BPX70 column, the peaks of 3-OH-lidocaine and 3-OH-MEGX were missing, possibly due to too high retention, since the maximum column temperature was not sufficiently high, or possibly to strong adsorption. Generally, the analytes were resolved on almost all columns and the selectivity of the analytes was higher on the nonpolar columns compared to the polar ones. However, the best performance with respect to peak performance was obtained on the intermediate polar columns, DB17 and HP50.

Thermostability. The effect of different injector temperatures (200–350°C) on the peak area was investigated for all compounds (Figure 3). The optimum temperature was 250°C. The peak areas decreased when the injector temperature was 200°C. This may be because the compounds did not completely gasify and then adsorb on the injector liner. At high temperature (300–350°C), the peak areas also decreased, which is most probably due to the fact that the compounds broke down in the injector.

MDQ. The MDQ for NPD is defined as the mass flow rate of the detected sample which yields a response equal to twice the noise level. The MDQs for lidocaine and its metabolites are between 30 and 400 fmol (Table IV). The hydroxylated metabolites showed a reduced response compared with those which do not contain hydroxy groups. This might be due to the adsorption of these hydroxylated compounds in the GC system. Another possible explanation is that the hydroxy groups had a negative effect on the response of the NPD. It is believed that the background current in NPD is a result of the ionization of the vaporized alkali-metal atoms forming M^+ ions [19–21]. When the solutes enter the detector, the compounds containing nitrogen form cyano radicals, which then remove an electron from the atomized alkali-metal. The cyanide ions form hydrogen cyanide at the collector and thereby generate the output signal. The presence of functional groups such as OH will remove some electrons and compete with the cyano-radical molecules, which may reduce the response of these compounds.

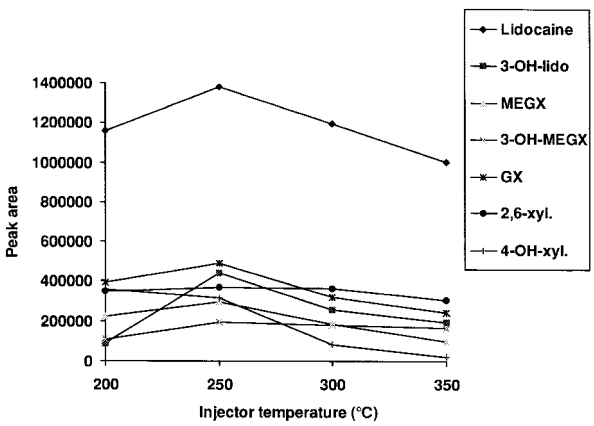

Figure 3. *Effect of different injector temperatures on the response of the compounds. Conditions: compound concentrations: 0.5–2.0 mM. Columns are temperature-programmed from 80°C to 340°C at 30°C min^{-1}. Detector: FID (350°C).*

Table IV. *MDQ of lidocaine and metabolites.*

Compound	Injected amount (fmol)
Lidocaine	30
3-OH-lidocaine	190
MEGX	40
3-OH-MEGX	390
GX	93
2,6-Xylidine	127
4-OH-2,6-Xylidine	170

Conditions: column: HP50 (intermediate polar), temperature program (from 80°C to 340°C at 30°C/min). Injector splitless (250°C); and detector: NPD (300°C).

CONCLUSIONS

This article clearly shows that it is possible to chromatograph underivatized metabolites of lidocaine with high peak performance using GC. Ten different columns were tested; on some columns peak tailing was observed, but other columns showed excellent performance with both narrow and symmetrical peaks. The optimal injection temperature was 250°C. At higher and lower temperatures, the peak area decreased. The MDQs for lidocaine and its metabolites were between 30 and 400 fmol using NPD. The results from this article demonstrate that CGC of underivatized metabolites may be a useful analytical tool in, e.g., structure elucidation (in combination with mass spectrometry), impurity testing, and quantitative determination. Work is in progress to apply a combination of this system and solid phase microextraction (SPME) for quantitative determination of lidocaine metabolites in biological samples.

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