Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 6, pp. 533–542, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

The determination of lidocaine and its metabolites by a direct injection LC method using large sample volumes of untreated plasma

N. DAOUD,* T. ARVIDSSON and K.-G. WAHLUND

Department of Analytical Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden

Abstract: Lidocaine and its *N*-dealkylated metabolites (glycylxylidide and monoethylglycylxylidide) have been determined at their therapeutic levels. The plasma samples were centrifuged and then injected directly into a liquid chromatograph containing a reversed-phase column with LiChrosorb RP-Select B as solid phase and 1-propanol-aqueous buffer containing decanesulphonate as eluent.

A pre-column venting plug technique was used, in which the chromatographic system consisted of two injector valves, a precolumn, a valve and a separation column. Lidocaine and its dealkylated metabolites were detected in the eluent by UV detection (210 nm) and the quantitations were performed by measurement of the peak areas of the samples and external standards.

Lidocaine and its metabolites were determined in the therapeutic range with a percentage recovery close to 100% and inter-assay precision (RSD) of 1.0–2.2%.

Keywords: *Reversed-phase liquid chromatography; direct injection; blood plasma; precolumn venting plug technique; lidocaine and its metabolites.*

Introduction

In a previous study [1] the authors introduced a precolumn venting plug technique for direct injection of untreated blood plasma into reversed-phase liquid chromatographic systems. With the direct injection procedure traditional work-up steps, such as extraction or precipitation, to separate the drug from plasma matrix components are omitted, i.e. the analytical method will require less labour intensity. The precision and accuracy of the assay are increased due to the exclusion of these sample pretreatment steps.

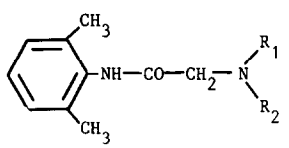
The main problem in applying an untreated plasma sample to the aqueous mobile phase has been thought to be the compatibility of the plasma proteins with the column system. Plasma proteins can become absorbed to the solid phase and also denatured or precipitated by components of the eluent. In the precolumn venting plug technique [1] the plasma sample is injected directly on to a very short precolumn while it is surrounded by plugs of a liquid (phosphate buffer pH 2.0) which protects it from contacting the

*On leave from the University of Baghdad, Iraq. To whom correspondence should be addressed.

organic modifier in the chromatographic eluent. This minimizes the denaturation of plasma proteins by the organic modifier and the unretained proteins can be washed out. The main part of the plasma proteins only come in contact with the precolumn and do not enter the separation column. The separation column was completely stable but the precolumn had to be changed at regular intervals. This technique allows the injection of large volume plasma samples.

In the present study lidocaine and its metabolites were determined using the precolumn venting plug technique to demonstrate its potential for the quantitation of low concentrations of drugs and metabolites which require large volumes of plasma samples to be applied to the chromatographic system.

Lidocaine has been used since the 1950s as a local anaesthetic and more recently has also been used for the treatment of cardiac arrhythmias. The two dealkylated metabolites of lidocaine, namely monoethylglycylxylidide (MEGX) and glycylxylidide (GX), possess some pharmacological and toxicological activity [2], and these compounds should therefore be considered in the design of analytical methods which are intended to be used for plasma level monitoring. The chemical structure of lidocaine and its *N*-dealkylated metabolites in plasma are given in Fig. 1.



	R_1	R_2
Lidocaine (L)	C_2H_5	C_2H_5
Monoethylglycylxylidide (MEGX)	H	C_2H_5
Glycylxylidide (GX)	H	H

Figure 1
Chemical structures of lidocaine and its *N*-dealkylated metabolites.

Several high-performance liquid chromatographic assays have been reported. In one method [3] lidocaine was extracted from plasma by a charcoal adsorption technique and the separation carried out on an octadecyl reversed-phase column. Solvent extraction of lidocaine from serum followed by injection of the extract into a straight-phase LC system has also been employed [4]. In other methods, the drug and its dealkylated metabolites were extracted from plasma by using ethylacetate [5] or methylene chloride [6] in the presence of an internal standard. Other extraction methods have used evaporation of the extraction solvents and dissolution of the residue in methanol followed by injection on to the reversed-phase LC system [7].

In the present study, the quantitation of lidocaine and its active metabolites MEGX and GX at therapeutic levels in 100 μl plasma samples have been determined by external standardization with quantitative recoveries close to 100% and a precision of 1.0–2.2% (RSD).

Experimental

Chemicals

Glycylxyldide (GX) and monoethylglycylxylidide (MEGX) were obtained from Astra (Södertälje, Sweden) and lidocaine was supplied from University Hospital (Uppsala, Sweden), all in the form of hydrochloride salts. 1-Propanol for HPLC quality and decanesulphonic acid sodium salt monohydrate were purchased from Fluka (Buchs, Switzerland). All other chemicals and reagents were of analytical grade. LiChrosorb RP-Select B (10 μm) was kindly supplied by E. Merck (Darmstadt, FRG).

Equipment

The pump was an LDC Solvent Delivery System 711-47 (Milton Roy minipump with pulse dampener, LDC, Riviera Beach, FL, USA). The sample injector was a Rheodyne Syringe Loading Injector 7120 (Rheodyne Inc., Berkeley, CA, USA) equipped with a 100 μl loop. A three-port valve Valco CV-3-HPax (Valco instruments Co., Houston, TX, USA) was used as a venting valve and a Valco-6-HPax injector equipped with a 2 ml loop was used as a plug fluid injector. The detector was a 655A variable wavelength UV monitor (Naka wards, Hitachi Ltd., Tokyo, Japan).

The separation column, 125 × 4.0 mm i.d., (Lichrocart HPLC-cartridge) was obtained from E. Merck (Darmstadt, FRG) packed with 7-μm LiChrosorb RP-Select B material. The precolumn, 10 × 3.2 mm i.d., was Lichroma tubes (316 stainless steel, Handy and Harman Tube Co., Norristown, PA, USA) constructed after modification of the fittings [7] and packed with 10-μm Lichrosorb RP-Select B. A home-made pressure regulator constructed according to ref. [8] was used to regulate the back-pressure in the venting line when the precolumn venting plug technique [1] was used.

Procedures

Blood plasma samples were prepared by adding 100 μl of a stock solution of the drug and its metabolites to 1000 μl carefully homogenized pooled blank plasma. Prior to injection of the blood plasma, it was carefully homogenized and centrifuged at about 5000 **g**.

The buffers were prepared by mixing 1.0 M sodium dihydrogen phosphate, 0.5 M disodium hydrogen phosphate or 1.0 M phosphoric acid, and diluted with deionized water to give pH 2.0, 3.0 and 6.0 with ionic strength equal to 0.1.

The mobile phases were prepared by mixing volumes of buffer and 1-propanol in the specified ratios. The counter ion (decanesulphonate) was added to this mixture in the specified concentrations.

The precolumns were slurry packed by using an ordinary LC pump with the support suspended in a mixture of methanol–dichloromethane (1:1, v/v).

External standards were prepared by dilution of a stock solution of lidocaine and its metabolites with buffer. The standard curves were constructed by plotting the peak area, measured by triangulation, of the standards versus their concentrations (mg/l).

Analytical method

The chromatographic system was set up according to Fig. 2. The precolumn was preceded by two injectors, the first for the plug fluid and the second for the plasma samples. A venting valve was situated between the precolumn and the separation column. The 2 ml of phosphate buffer (pH 2.0) contained in the loop of the plug fluid

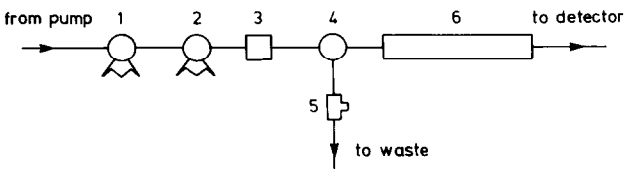


Figure 2
Precolumn venting plug technique system. 1 = Plug fluid injector (2 ml loop); 2 = sample injector (100 μl); 3 = precolumn, 10 × 3.2 mm i.d.; 4 = venting valve; 5 = laboratory-built pressure regulator [8]; 6 = separation column, 125 × 4.0 mm i.d.

injector is injected into the precolumn while the venting valve is maintained in the waste position. After the passage of 1.0 ml of eluent, the plasma sample (100 μl) is injected through the sample injector. After the passage of a further 1.10 ml of eluent the venting valve is switched to direct the eluent into the separation column.

The eluent consisted of 1-propanol–phosphate buffer, pH 3.0, (13.5:86.5, v/v) containing 1.0 mM of decanesulphonate and was maintained at a flow-rate of 0.9 ml/min. Detection was at 210 nm. The areas of the chromatographic peaks were measured by triangulation and the concentrations of lidocaine and its metabolites were obtained by comparison with external standards.

Results and Discussion

Regulation of retention and selectivity

The phase systems used in this study were reversed-phase liquid–solid systems with LiChrosorb RP-Select B as the solid phase, which is synthesized with the aim of giving symmetrical peaks for amine compounds. The eluents were aqueous solutions and the retention in such systems may be regulated by the addition of uncharged organic modifiers, by the pH of the mobile phase and by addition of hydrophobic ions acting as ion-pair reagents with the solutes. Initial experiments with LiChrosorb RP-8 as stationary phase showed tailing peaks. However, tailing was more severe at pH 6.0 than pH 2.0. LiChrosorb RP-select B showed no tailing at pH 2.0 but slight tailing at pH 6.0.

The choice of the mobile phase for direct plasma injections with the precolumn venting technique must make possible the flexible adjustment of retention conditions so that the solutes of interest can be resolved from disturbing peaks such as endogenous plasma components. This may require the presence of mobile phase components which selectively affect the retention of compounds of different nature, e.g. charge, type.

Lidocaine and its metabolites are cationic at neutral and acidic pH and their retention can be influenced by organic modifiers, pH as well as counter ions.

Initial experiments with mobile phases containing phosphate buffer (pH 2–6) and methanol, acetonitrile or 1-propanol gave interferences between the solutes and endogenous plasma peaks. Different concentrations of the organic modifiers and different pH did not improve the resolution enough.

Addition of organic sulphonate salts to the mobile phase may increase the resolution between the solutes and the early eluted endogenous components by selective interaction with the cationic solutes provided that the endogenous components are not cationic.

The mobile phase chosen for the analytical method contained buffer (pH 3.0), 1-propanol and decanesulphonate, and the pH, 1-propanol concentration and decane-

sulphonate concentration had been adjusted to resolve the drug and the metabolites from disturbing peaks such as endogenous plasma components. The specific effects of each three components on retention and resolution are discussed below.

pH-effect

Increase of the pH of the eluent often gives increasing binding of ammonium compounds to the stationary phase in reversed-phase systems [9]. Figure 3 gives the result when pH was changed in a mobile phase containing 1-propanol and decanesulphonate. The separation factor between lidocaine and the metabolite MEGX increased as the pH increased but pH 3.0 was chosen for the analytical method as a compromise between resolution and time. At this pH the solid phase also is chemically stable and peaks of ammonium compounds are symmetrical.

Effect of counter ion concentration

An increase in the concentration of the counter ion (decanesulphonate) gave increasing retention as illustrated in Fig. 4 and in accordance with well-known principles

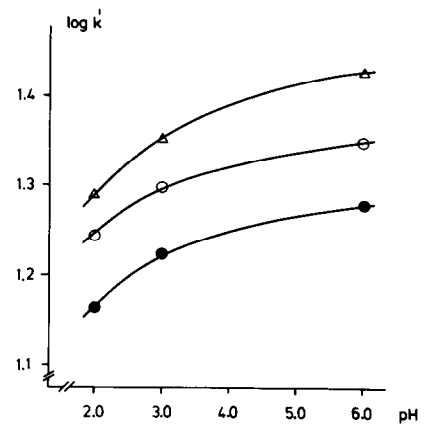


Figure 3
Regulation of retention by pH. Eluent: 1-propanol–phosphate buffer (1.6:8.4, v/v) containing 2 mM decanesulphonate; separation column: 7-μm LiChrosorb RP-Select B; precolumn: 10-μm LiChrosorb RP-Select B; sample volume: 100 μl; flow-rate: 0.9 ml/min. ● = GX, ○ = MEGX and △ = L.

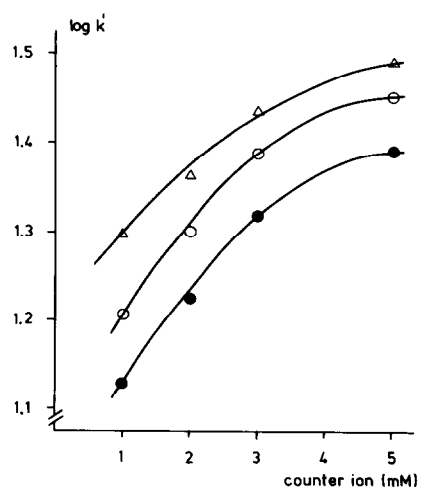


Figure 4
Regulation of retention by counter ion concentration. Eluent: 1-propanol–phosphate buffer (pH 3.0) (1.6:8.4, v/v) containing decanesulphonate; other conditions as in Fig. 3. ● = GX, ○ = MEGX and △ = L.

of ion-pair chromatography [10, 11]. At the lowest counter ion concentration (1 mM) the separation factor was maximal between lidocaine and the metabolite MEGX and still adequately high between the endogenous peaks and the first eluted metabolite (GX) peak to give enough resolution.

Disturbing peaks

The use of methanol or acetonitrile as modifiers in the regulation of retention gave many disturbing peaks from the endogenous plasma components that interfered with the solutes. The addition of counter ions (C8–C10 alkylsulphonates) to the mobile phase in order to increase the retention of the cationic solutes did not help significantly. Possibly the disturbing endogenous peaks have the same charge sign as the solutes of interest. Decanesulphonate C10 improved the situation slightly so that disturbing peaks interfered only with the first eluted metabolite (GX).

Replacing methanol or acetonitrile with 1-propanol in an eluent containing decanesulphonate gave better resolution between the endogenous peaks and the first eluted metabolite (GX), but a negative peak (system peak) appeared. System peaks [12, 13] can occur anywhere in a chromatogram and are caused by a disturbance of the distribution between the mobile and stationary phase of some component of the mobile phase which is sensed by the detector. The disturbance can occur as soon as the column is subjected to a change of the mobile phase composition, e.g. when a sample is injected. To decrease the probability of system peaks the mobile phase should contain as few components as possible.

The precolumn venting plug technique may produce more pronounced system peaks than ordinary sample injection, because of stripping of the mobile phase components from the precolumn by the buffer used as clean-up fluid. In the present system, the system peak did not interfere with the solutes but may very well in other cases. Therefore some understanding of its origin is necessary. The system peak is probably caused by decanesulphonate which should be highly adsorbed on the stationary phase [14]. This is indicated by the change in its retention when the concentration of sulphonate ion in the mobile phase was increased (Fig. 5). Also when the mobile phase contained no decanesulphonate, no system peak was observed.

Negative peaks also appeared depending on the 1-propanol quality. The 1-propanol for HPLC quality obtained from Fluka (Buchs, Switzerland) gave only one negative peak (Fig. 6), whereas 1-propanol for analysis that was obtained from E. Merck (Darmstadt, FRG) gave three negative peaks, and the last one interfered with the first eluted metabolite (GX). The reason behind this might be some impurities present in 1-propanol.

Effect of 1-propanol concentration

Based on the data of Figs 3 and 4, the final eluent for the separation of lidocaine and its metabolites was chosen to be 1-propanol–phosphate buffer (pH 3.0) containing 1 mM decanesulphonate. By increasing the content of 1-propanol in the eluent (Fig. 7), the retention of lidocaine and its *N*-dealkylated metabolites can be decreased leading to shorter separation time but on the other hand the content of 1-propanol in the eluent should be as low as possible in order to increase the stability of the system towards the plasma samples [7, 15]. However, eluent containing 13.5% of 1-propanol gave a suitable retention level and the solutes of interest did not interfere with the system peak (sulphonate ion) and GX was adequately resolved from the endogenous peaks (Fig. 8).

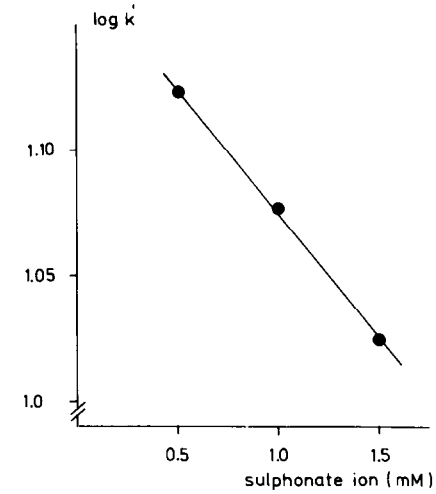


Figure 5
Influence of the sulphonate concentration on the retention of the system peak. Eluent: 1-propanol–phosphate buffer (pH 3.0) (1.4:8.6, v/v) containing decanesulphonate; other chromatographic conditions as in Fig. 3. Sample: phosphate buffer (pH 3.0, 100 μl); plug fluid; phosphate buffer (pH 2.0, μ = 0.1). Procedure according to Analytical method. ● = system peak.

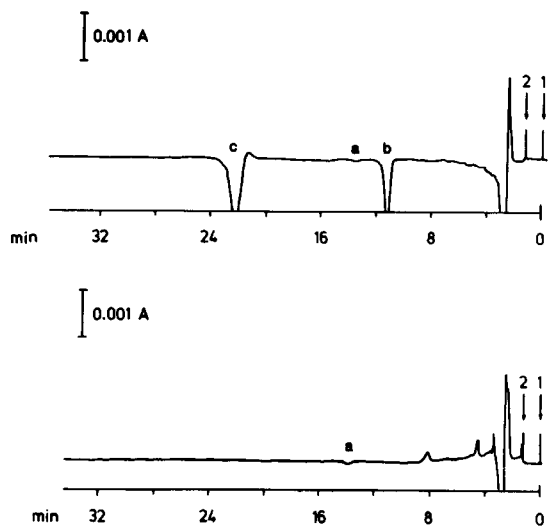


Figure 6
Influence of 1-propanol quality on system peak. Eluent: 1-propanol–phosphate buffer (pH 3.0) (1.35:8.65, v/v) containing 1 mM decanesulphonate; other chromatographic conditions as in Fig. 3. Sample: phosphate buffer (pH 3.0, 100 μl). Procedure according to Analytical method. 1 = Sample injection and 2 = venting valve switched. a, b and c = system peaks. a = system peak for decanesulphonate.

Stability of the system

The precolumn venting plug technique used here has been described previously [1]. The plasma sample, preceded and followed by 1.0 ml of a plug fluid (phosphate buffer), is loaded on to a short precolumn. The solutes are retained on the precolumn while the plug fluid washes out the proteins to waste. The eluent then transports the solutes into the separation column after switching the effluent of the precolumn from the waste to the separation column. This procedure prevents the separation column from becoming contaminated by plasma proteins and its life-time is not influenced by the plasma

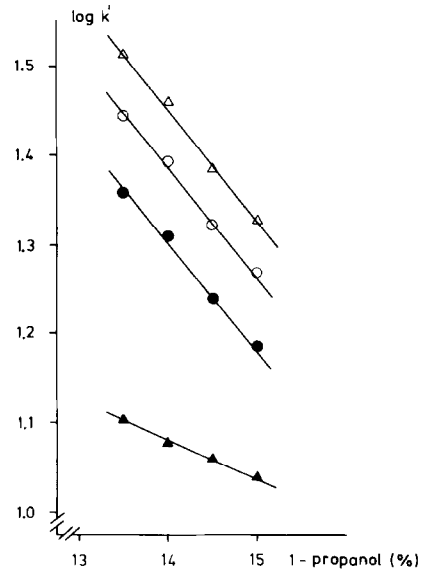


Figure 7
Regulation of retention by 1-propanol. Eluent: 1-propanol–phosphate buffer (pH 3.0) containing 1 mM decanesulphonate; other chromatographic conditions as in Fig. 3. ● = GX, ○ = MEGX, △ = L and ▲ = system peak from decanesulphonate.

samples. Instead, the short precolumn has to be exchanged at regular intervals. The performance has been shown to depend on the precolumn particle size, the concentration of organic solvent in the mobile phase, precolumn filters, pH and ionic strength of the plug fluid [1]. These parameters have been optimized to obtain as good stability of the precolumn as possible, i.e. use of 10 μm particles in the precolumn, phosphate buffer (pH 2.0, μ = 0.1) as plug fluid and a precolumn constructed with screen filters instead of frits [1].

With the chosen phase system 65 injections of 100 μl of plasma samples could be made before the initial values of the peak efficiency (N = 2400) and column back-pressure (55 bar) were changed by 10%. This is a reasonable limit for a change of the precolumn. The methodology is, however, so robust that it is possible to accept a larger change of these parameters. Under such conditions it is necessary to standardize at closer intervals to ensure accurate quantitation.

Detection

The effluent was detected at 210 nm in order to obtain high detector response of lidocaine and its metabolites because these solutes have maximum absorbance at 208 nm. The highest possible detector response should be chosen in order to decrease the volume of each plasma sample. This increases the number of plasma injections that can be made on each precolumn because their stability depends on the total volume of injected plasma [15].

Quantitation

Quantitation was performed by external standardization. The calibration curves were linear over the therapeutic concentration range with fitting equations as shown in Table 1 (Y = peak area in mm^2, X = concentration of the drug or its metabolites in mg/l). The correlation coefficients ranged between 0.9998–0.9999.

Precision studies were made on blank plasma to which each of the drug and its alkylated metabolites were added at two different concentration levels (Table 2).

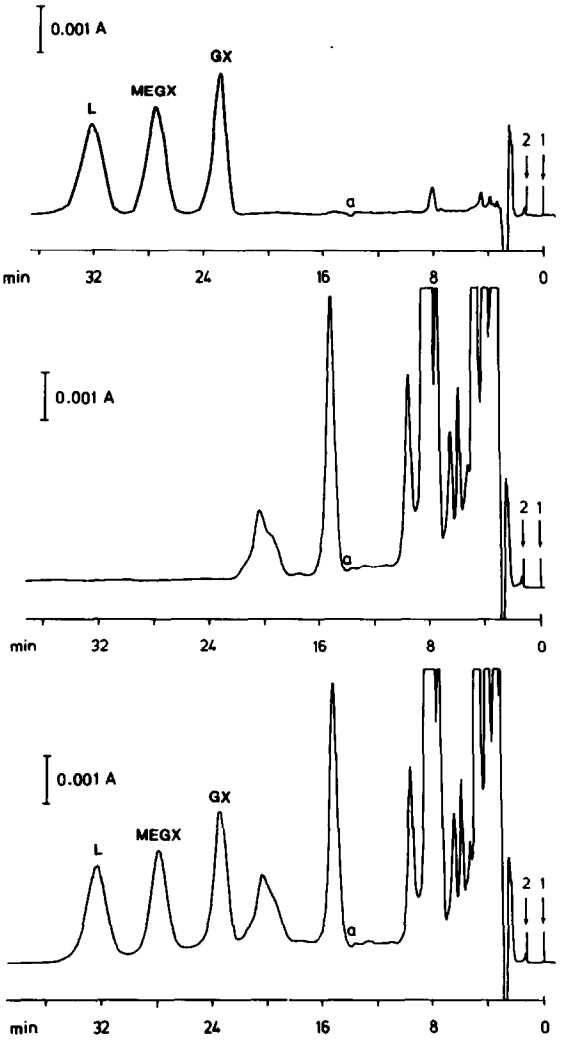


Figure 8
Standard, blank plasma and spiked plasma samples. Procedure according to Analytical method. Upper: standard sample. Middle: blank plasma. Lower: spiked plasma sample. Peaks: a = system peak from decanesulphonate; L (1.05 mg/l); GX (0.77 mg/l); MEGX (0.84 mg/l).

Table 1
Calibration curves

Compound	Equation	s.d. Slope	s.d. Intercept	Correlation coefficient	Concentration range (mg l^{-1})
GX	$Y = 1886\,X - 63.27$	7.62	51.43	0.9998	0.283–15.40
MEGX	$Y = 1716\,X - 66.33$	7.65	56.11	0.9998	0.307–16.73
Lidocaine	$Y = 1419\,X - 34.47$	5.47	50.20	0.9999	0.384–20.92

Table 2
The inter-day precision as indicated by calibration data obtained over a period of six days. Sample procedure according to analytical method

Compound	Added concentration (mg l^{-1})	Mean concentration (mg l^{-1})	Recovery (%)	RSD (%)	*n*
GX	0.280	0.276	98.5	2.0	20
	0.770	0.763	99.0	1.0	20
MEGX	0.304	0.305	100.3	2.2	20
	0.837	0.844	100.8	1.2	20
Lidocaine	0.380	0.382	100.5	1.7	20
	1.046	1.049	100.2	1.0	20

Lidocaine and its metabolites could be determined in the therapeutic range with an inter-assay precision (RSD) of 1.0–2.2%. The percentage recovery was 98.5–100.8%.

An example of the separation of lidocaine and its metabolites from endogenous compounds (Fig. 8) is shown for a spiked plasma sample.

Acknowledgement: We thank Professor Douglas Westerlund for valuable advice.

References

[1] N. Daoud, T. Arvidsson and K.-G. Wahlund, *J. Chromatogr.* **385**, 311–322 (1987).
[2] J. Blumer, J. M. Strong and A. J. Atkinson, *J. Pharmacol. Exp. Ther.* **186**, 31–36 (1973).
[3] R. F. Adams, F. L. Vandemark and G. Schmidt, *Clin. Chim. Acta* **69**, 515–524 (1976).
[4] P.-O. Lagerström and B.-A. Persson, *J. Chromatogr.* **149**, 331–340 (1978).
[5] R. L. Nation, G. W. Peng and W. L. Chiou, *J. Chromatogr.* **162**, 446–473 (1979).
[6] J. Hill, A. Roussin, J. Lelorier and G. Caille, *J. Pharm. Sci.* **69**, 1341–1342 (1980).
[7] P. K. Narang, W. G. Crouthamel, N. H. Carliner and M. L. Fisher, *Clin. Pharmacol. Ther.* **24**, 654–661 (1978).
[8] W. Lindner and H. Ruckendorfer, *Int. J. Environ. Anal. Chem.* **16**, 205–211 (1983).
[9] A. Tilly-Melin, M. Ljungcrantz and G. Schill, *J. Chromatogr.* **185**, 225–239 (1979).
[10] I. M. Johansson, *J. Liq. Chromatogr.* **4**, 1435–1457 (1981).
[11] J. H. Knox and R. A. Hartwick, *J. Chromatogr.* **204**, 3–21 (1981).
[12] L. Hackzell and G. Schill, *Chromatographia* **15**, 437–444 (1982).
[13] S. Levin and E. Grushka, *Anal. Chem.* **58**, 1602–1607 (1986).
[14] A. Sokolowski, *Chromatographia* **22**, 177–182 (1986).
[15] T. Arvidsson, K.-G. Wahlund and N. Daoud, *J. Chromatogr.* **317**, 213–226 (1984).

[*Received for review 26 August 1986*]