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HPLC assay of Lidocaine in plasma with solid phase extraction and UV detection

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Abstract

A sensitive and reliable method based on solid-phase extraction and reversed-phase liquid chromatography was developed and validated for the quantitation of Lidocaine (Lid) in dog plasma. Phenacemide was used as an internal standard (IS) in the extraction which employed C_{18} solid-phase extraction cartridges. The washing and eluting solutions were 2 ml acetonitrile-pH 9.0 phosphate buffer (10:90 v/v) and 0.5 ml acetonitrile-pH 4.0 phosphate buffer (40:60 v/v), respectively. The eluent obtained from the cartridge was directly analyzed on a reversed-phase ODS column with UV detection at 210 nm. A clean chromatogram and high sensitivity were achieved at this wavelength. The mobile phase was acetonitrile and pH 5.9 phosphate buffer (20:80 v/v). The retention times were 6.4 and 7.2 min for Lid and IS, respectively, at a flow rate of 1.0 ml min^{-1}. The mean absolute recovery was 96.6% ($n=9$) with a CV of 3.8% for Lid and 81.7% with CV of 2.5% ($n=3$) for IS. The limit of quantitation was 20 ng ml^{-1}, with the intra- and inter-day precisions ($n=5$) of 4.4 and 3.4%, respectively, and the intra- and inter-day accuracies ($n=5$) of -4.3 and -5.0%, respectively. For the analyses of Lid in spiked plasma samples at 20, 100 and 200 ng ml^{-1}, the overall mean intra- and inter-day precisions ($n=15$) were 3.9 and 4.9%, respectively, and the overall mean intra- and inter-day accuracies ($n=15$) were -3.7 and -4.6%, respectively. The correlation coefficients for calibration plots in the range 20–1000 ng ml^{-1} in plasma were typically higher than 0.998. The suitability of the method was demonstrated by the study in a beagle dog receiving a low intravenous dose of Lid.

Keywords: Lidocaine; Local anesthetic; Plasma assay; Reversed-phase liquid chromatography; Solid-phase extraction

1. Introduction

Lid is the most widely used local anesthetic. It is also used for the regional management of major pain, via either central (spinal and epidural) or peripheral administration [1,2]. Non-invasive topi-

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Table 1
Extraction recovery

Concentration (ng mg^{-1})	20	100	200	I.S.
Recovery (%)[a]	97.8 ± 1.4	98.9 ± 4.4	93.1 ± 2.0	81.7 ± 2.0

[a] Mean ± SD ($n = 3$).

cal administration of local anesthetics is preferred in clinics for the relief of local pain, owing to its convenience of application as well as reducing adverse effects. Pharmacokinetic studies of such topical delivery systems require sensitive and reliable analytical methods for the analysis of Lid in blood.

Many studies have been reported on the analysis of Lid in biological samples. The high performance liquid chromatographic (HPLC) method using UV detection has been described most often due to its many advantages. However, since Lid contains no chromophore, which absorbs light strongly in the visible or near-UV regions, the detection wavelength employed for this compound must be close to 200 nm, and thus an efficient and highly selective sample preparation procedure is required for the sensitive analysis of Lid in biological samples.

Sample preparation procedures for Lid in plasma described in the literature are primarily achieved by liquid–liquid extraction methods carried out by the extraction of the drug using organic solvent added to alkalinize plasma samples, which is usually followed by re-extraction with an acidic solution [3–7]. Some of these methods produced high recovery and sensitivity. One report indicated that the limit of quantification approached 20 ng ml^{-1} in plasma [8]. However, the liquid phase extraction methods are usually time-consuming and labor-intensive due to the numerous steps involved, such as centrifugation, solvent evaporation and redissolution. Many hours are required to complete a batch of samples. At the same time, large amounts of environmentally hazardous solvents are consumed, which leads to additional expense and labor. Direct injection of plasma samples has also been reported [9], however, the sensitivity and specificity were not sufficient enough to detect drug levels in lower nanogram ranges and were thus deemed unsuitable for pharmacokinetic studies. Only one study [10] was reported employing the solid-phase extraction method, but as electro–chemical detection was used, the extraction conditions may not be suitable for the HPLC analysis with UV detection. This method has not been widely adopted for Lid analysis, probably due to limited availability of the instrument. Fluorescence detection for Lid was also rarely reported [11] due to the necessity of post column derivatization. In this study, we developed a sensitive and reliable HPLC method using solid phase extraction and low wavelength UV detection of Lid at 210 nm.

2. Experimental

2.1. Materials and instrumentation

Lid was purchased from Sigma (St. Louis, MO); Phenacemide (I.S.) was supplied by Abbott Laboratories (North Chicago, IL). Monoethylglycylxylidide (MEGX) and glycylxylidide (GX) were kindly supplied by Astra (Westboro, MA). Acetonitrile (HPLC grade) was from J.T. Baker (Phillipsburg, NJ).

The liquid chromatographic system consists of a pump (Beckman Model 110-A, Beckman Instru-

Table 2
Intra-day precision[a] and accuracy[b]

Concentration (ng ml^{-1})	20	100	200
Measured (ng ml^{-1})[c]	19.1 ± 0.8	95.1 ± 4.7	196.0 ± 4.3
Precision	4.4%	5.0%	2.2%
Accuracy	−4.3%	−4.9%	−2.0%

[a] Precision = SD/Mean × 100.
[b] Accuracy = (measured − theoretical)/theoretical × 100.
[c] Mean ± SD ($n = 5$).

Table 3
Inter-day precision and accuracy

Concentration (ng ml^{-1})	20	100	200
Measured (ng ml^{-1})[a]	19.0 ± 0.7	93.7 ± 4.6	205.1 ± 13.0
Precision	3.4%	4.9%	6.3%
Accuracy	−5.0%	−6.3% +2.6%	

[a] Mean ± SD ($n = 5$).

ments, Cotati, CA) with a 50 μl sample loop, a guard column (Perisorb, RP-18, P.J. Cobert Associates, St. Louis, MO), a reversed phase analytical column (Prodigy 5u ODS, 150 × 4.6 mm, Phenomenex, Torrance, CA), a variable wavelength UV detector (Spectro Monitor III, model 1204A, Laboratory Data Control, Riviera Beach, FL) and an integrator (Shimadzu C-R3A Chromatopac, Kyoto, Japan). The Vac-Elut manifold and the Bond-Elut cartridges (CN, C_8 and C_{18}, 1 ml 100 mg^{-1}) used in the solid phase extraction were obtained from Varian Analytical Supplies (Harbor City, CA). A Millipore water purification system (Continental Water Systems Corp. EL Paso, TX) was used for the filtration of the buffer solutions and mobile phase. The Millipore filter membranes (Type GS, 0.22 μm) were obtained from Millipore Corp. (Bedford, MA).

The stock standard solutions of Lid and IS at concentrations of 0.30 and 0.38 mg ml^{-1}, respectively were prepared separately with a mixture of acetonitrile–water (30:70 v/v). Working standard solutions were prepared from the stock solutions by dilution with HPLC water and were used within 1 week after preparation. All standard solutions were stored in sealed bottles at 4°C.

2.2. Solid phase extraction

Blank and dosed dog blood specimens were centrifuged at 2500 rpm for 10 min with EDTA as an anti-coagulant and 0.5 ml of plasma was used for solid phase extraction. C_{18} cartridges (100 mg ml^{-1}) were preconditioned by 2 ml of acetonitrile, followed by 2 ml of water. A small amount of water was left in the cartridge to prevent the sorbent from drying before loading the samples. 25 μl of working standard solution containing 98 ng internal standard was added to 0.5 ml of plasma, which was then diluted to 2 ml by HPLC water. After a brief vortex, the sample was transferred onto the preconditioned C_{18} cartridge. Low vacuum (< 3 in. Hg) was used to moderate the extraction speed at ~0.5 ml min^{-1}. Drawing and dispensing of the sample were performed slowly and with caution in order to avoid the formation of air bubbles in the cartridge which could hinder the plasma sample from freely passing through the sorbent bed. After all of the plasma sample was passed through the cartridge, the glass culture tube used as the container of the sample was thoroughly rinsed with 0.5 ml of water which was also loaded onto the cartridge. After loading of samples, the cartridge was washed four times, each time with 0.5 ml of washing solution consisting of acetonitrile–pH 9.0 phosphate buffer (10:90 v/v). The inner wall of the cartridge was carefully rinsed while washing to achieve maximum elimination of the endogenous plasma components adsorbed on the inner wall of a cartridge and to prevent them from being present in the chromatogram. The washing speed was also adjusted to ~0.5 ml min^{-1}. At the end of washing, high vacuum (10–15 in. Hg) was applied so as to remove the remaining solvent. The washed cartridge was eluted with 0.5 ml of acetonitrile-pH 4.0 phosphate buffer (40:60 v/v). Slow elution was achieved by using low vacuum (3–3.5 in. Hg), which was critical to high recovery. At the end of elution, high vacuum (10–15 in. Hg) again was applied to recover all the eluting solution. After the vortex, 50 μl of the effluent was injected onto the column for quantitation. The C_{18} cartridges were found to be reusable for at least twice with the same efficiency under the procedure described. After initial use, the cartridges were reconditioned with 2 ml of acetonitrile followed by 2 ml of water.

2.3. Chromatographic conditions

The mobile phase was acetonitrile-pH 5.9 phosphate buffer (20:80 v/v), which was degassed prior to use and delivered at a flow rate of 1.0 $mlmin^{-1}$. The UV detection was carried out at 210 nm with the absorbence setting at 0.01 a.u.f.s.

3. Results and discussion

3.1. Selection of sorbent for solid-phase extraction

At normal biological pH, Lid exists mainly as a unionized form, therefore, nonpolar interaction was considered a primary mechanism of retention of Lid. Among the nonpolar sorbents commercially available, cyanopropyl (CN), octyl (C_8) and octadecyl (C_{18}) sorbents were evaluated for Lid in terms of adsorption and desorption properties. After 0.5 ml of a standard aqueous solution (SAS) containing 50 ng Lid and 98 ng IS was introduced onto each cartridge, the effluent was collected and directly injected onto the HPLC column through a 50 μl sample loop. To find out how the analytes were retained by each sorbent, the peak heights (h_e) were compared with those obtained by direct injection of the SAS without any treatment (h_o). Smaller values of h_e/h_o suggested that more analytes were retained on the cartridges. The preliminary results showed that Lid was poorly retained by CN cartridge, which was attributed to the weak lipophilicity of CN sorbent. The strongest bonding occurred between C_{18} sorbent and the analytes, which led to the highest recovery of the drug after extraction from plasma samples, while allowing the use of stronger washing solutions to achieve cleaner extraction. From spiked plasma samples, less than 20% of Lid was retained on CN cartridge which was finally recovered by eluting, compared to $\sim 80\%$ on C_8 cartridge and $> 95\%$ on C_{18} cartridge under the same conditions used. Based on these results, C_{18} cartridge was selected for the extraction of Lid in this procedure. The pH of the sample solution that was injected onto the HPLC column had a large influence on the shape of the peaks. A lower pH (< 5.5) resulted in narrower and more symmetrical peaks.

3.2. Optimization of washing and eluting solutions

3.2.1. Extracting analytes from SAS with acetonitrile and water

After 0.5 ml of the SAS as described above was introduced to the C_{18} cartridges, 2 ml of acetonitrile–water mixtures in different ratios (5:95–60:40 v/v) was added. The effluent was directly injected onto HPLC, and the obtained peak height h'_e was also compared with h_o to find out the eluting efficiency of each eluent. Higher h'_e/h_o values represented higher eluting efficiencies. The analytes could not be detected in the effluent when the eluents contained less than 15% (v/v) acetonitrile. The eluting efficiency increased as the acetonitrile content in the eluents increased and reached the maximum value when acetonitrile exceeded 50% (v/v) in the eluent.

3.2.2. Extracting analytes from SAS with acetonitrile and phosphate buffer

To study the influence of pH of the eluents on eluting efficiency, eluents consisting of 40% acetonitrile and 60% (v/v) phosphate buffer in the pH range 3.0–10.0 were prepared. After introduction of 0.5 ml of the SAS to the cartridge, eluting efficiencies of 0.5 ml of each of those mixtures were measured as described in the previous section. Results showed that eluting efficiency increased as the pH of the eluents decreased due to ionization of Lid in acidic environment. To achieve a selective extraction of a drug with high recovery from biological samples, eluting efficiency of the washing solution should be lower as long as it was capable of eliminating interfering components, while that of the eluting solution should be as high as possible once no significant interference appeared. Therefore, eluents with a higher pH (> 8.0) would be preferred as the washing solutions, while a lower pH (< 5.0) would be more suitable for the eluting solutions. When the mixtures of acetonitrile and pH 4.0 phosphate buffer were investigated as the eluting solution, high recoveries ($> 95\%$) were obtained with the eluents containing 40% (v/v) or more acetonitrile. As discussed earlier in this paper, due to the effect of the pH of the injected sample on the peak shape, a lower pH (< 5.5) was also

preferred for the eluting solutions. When up to 2 ml of the mixtures of acetonitrile and pH 9.0 phosphate buffer were applied after loading of 0.5 ml of the SAS onto C_{18} cartridge, no Lid could be detected in the effluents if acetonitrile was less than 15% (v/v) in the mixture. Thus, acetonitrile and pH4.0 phosphate buffer (40:60 v/v) was initially chosen as eluting solution, and acetonitrile-pH9.0 phosphate buffer (15:85 v/v) as washing solution.

3.2.3. Extracting analytes from spiked plasma with acetonitrile and phosphate buffer

When 0.5 ml of plasma samples spiked with 50 ng Lid and 98 ng IS was extracted with the above washing and eluting solutions on C_{18} cartridges, the recovery of Lid decreased by $\sim 10\%$, which could partly be caused by the elution of Lid during washing because the loss in recovery was greatly reduced by lowering the acetonitrile content in the washing solution from 15 to 10% (v/v). One possible reason might be interaction of Lid to unknown plasma components that were eluted during washing. The originally selected eluting solution was shown to be effective for the extraction of Lid from plasma samples, thus no further adjustment was made. The volume of the eluting solution also affected the recovery. The smallest volume by which more than 90% Lid could be recovered over the working concentration range was 0.5 ml when 100 mg cartridge was used. Thus, the washing and eluting solutions finally selected for this method were 2 ml acetonitrile-pH 9.0 phosphate buffer (10:90 v/v) and 0.5 ml acetonitrile-pH 4.0 phosphate buffer (40:60 v/v), respectively.

3.3. Optimization of mobile phase

Acetonitrile was chosen as the organic modifier in the mobile phase because of its low absorbance at 210 nm. In this study, we found that both pH and acetonitrile content of the mobile phase had a significant effect on the peak shape and retention times of the analytes. Mobile phases containing various amounts of acetonitrile and phosphate buffers with pH values of 3.0–8.0 were investigated. Lower pH (3.0–4.0) and a higher content of acetonitrile ($>40\%$) both led to the earlier elution of Lid which caused overlap of Lid peak and some peaks of plasma origin. Higher pH (7.0–8.0) or lower content of acetonitrile ($<15\%$) prolonged retention times but resulted in broad peaks. Although a small temperature fluctuation had no significant effect on the retention times of both Lid and IS, a large degree of column temperature change (>5°C) would lead to changes in retention times, and shorter retention times of both analytes were observed at higher column temperature. Thus, the retention times of Lid and IS could be optimized by adjusting the mobile phase in terms of acetonitrile content and pH value or by column temperature control. To run the analysis at ambient temperature (25°C), the mobile phase finally chosen consisted of 20% acetonitrile and 80% pH 5.9 phosphate buffer (0.05 M). At the flow rate of 1.0 ml min^{-1}, the retention times for Lid and the internal standard were 6.4 and 7.2 min, respectively.

3.4. Calibration and linearity

Calibration plots over the concentration range 20–1000 ng ml^{-1} in plasma were measured on three separate days within 1 week. The standard samples for calibration were prepared by spiking 0.5 ml of blank plasma with 98 ng IS and 25 μl of Lid stock standard solutions of different concentrations. The concentrations of the standard samples were 10, 20, 40, 50, 100, 200, 500, and 1000 ng ml^{-1} plasma for Lid and 196 ng ml^{-1} plasma for internal standard. Three replicate samples were prepared and analyzed for each concentration. The calibration graphs obtained by plotting the peak-height ratios (Lid/IS) against the concentrations of Lid added to the blank plasma were highly linear over the range 20–1000 ng ml^{-1}, and the correlation coefficient for each calibration graph invariably exceeded 0.998. The slopes of three standard graphs obtained on three separate days had a coefficient of variation (CV) of 7%. The mean regression equation is $y = 92.6\ x - 1.67$, where y (ng ml^{-1}) is the concentration of Lid in plasma, and x is the peak height ratio (h_{Lid}/h_{IS}).

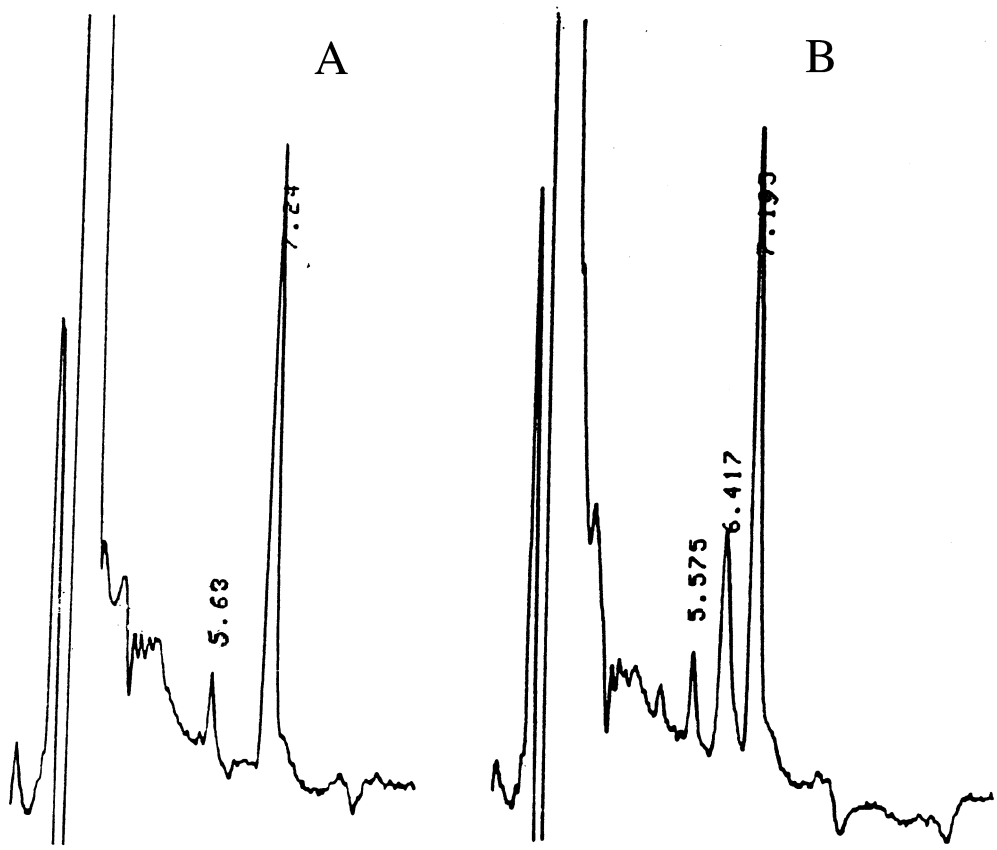

Fig. 1. HPLC elution profile for extracted plasma samples from a beagle dog. (A) blank plasma spiked with I.S.; (B) dog plasma taken 1 h after an intravenous bolus injection of 0.94 mg kg^{-1} Lid-HCl. Lid concentration was 63.5 ng ml^{-1}.

3.5. Recovery

The extraction efficiency of Lid by this method was determined at concentrations of 20, 100, 200 ng ml^{-1} in plasma. The recovery of the internal standard was measured at 196 ng ml^{-1}. Spiked plasma samples of 0.5 ml at each concentration were extracted and analyzed in three replicates under the described conditions. The peak area was compared to the mean peak area resulting from the analyses of three reference standards containing corresponding levels of Lid and IS in the eluting solution. As shown in Table 1, high recoveries were obtained at all concentrations. The overall mean absolute recovery was 96.6% with a CV of 3.8% ($n = 9$) for Lid, and 81.7% with CV of 2.5% ($n = 3$) for internal standard.

3.6. Precision and accuracy

The precision and accuracy of the extraction procedure and chromatography were established by analyses of the three quality control (QC) samples (20, 100 and 200 ng ml^{-1} plasma) with the obtained regression equation. The intra-day precision and accuracy for each of the three QC samples were checked by replicate analysis ($n = 5$) on the same day. The inter-day precision and accuracy ($n = 5$) were evaluated by analyses of the QC samples on five consecutive days. Tables 2 and 3 showed that the assay was highly reproducible with low intra- and inter-day variation. For the three concentrations investigated, the precision and accuracy were less than 6.3%.

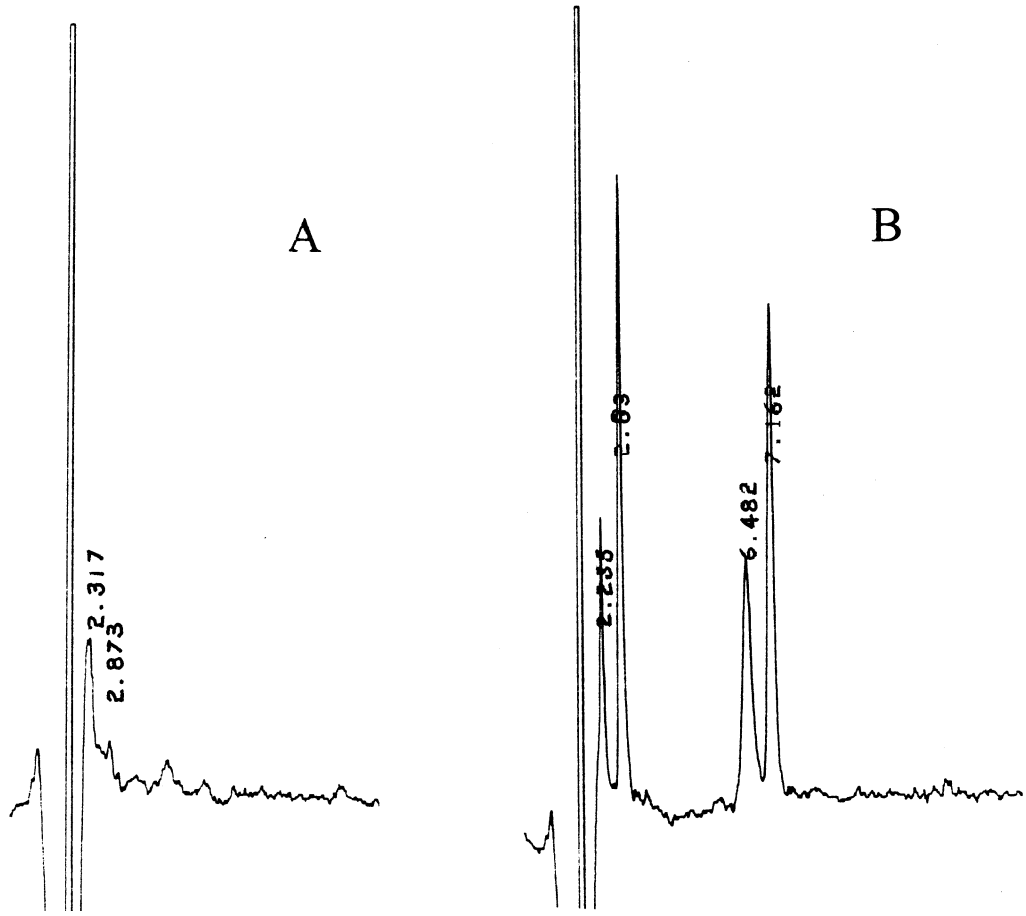

Fig. 2. Specificity of the assay under the chromatographic condition. (A) chromatogram of blank eluting solvent; (B) eluting solvent spiked with Glycinexylidide (GX), Monoethylglycinesylidide (MEGX), Lidocaine and Phenacemide (I.S.). Their retention times were 2.2, 2.8, 6.4, 7.2 min, respectively.

Under the chromatographic conditions used, and with the injection frequencies of 60–80 samples per week, the efficiency of the analytical column remained unchanged for at least 1 year.

3.7. Limit of quantitation

The limit of quantitation was set at the lowest measurable Lid concentration with acceptable inter-day and intra-day precision and accuracy. When 0.5 ml plasma was analyzed, the lower limit of quantitation of Lid by this method was 20 ng ml^{-1}. At this concentration, the intra- and inter-day precisions were 4.4 and 3.4%, respectively, and the intra- and inter-day accuracies were −4.3 and −5.0%, respectively. The detection limit, based on the 3:1 peak height ratio of Lid over noise equalled 10 ng ml^{-1}, meaning that the Lid peak could be detected when 500 pg in 50 μl was injected onto the column.

3.8. Specificity of the assay

The specificity of the assay was examined by analyzing blank dog plasma samples. Fig. 1 shows that no endogenous interference was encountered after extraction. The possibility of interference from its two main metabolites MEGX and GX was excluded by determining their retention times under the same chromatographic conditions (Fig. 2).

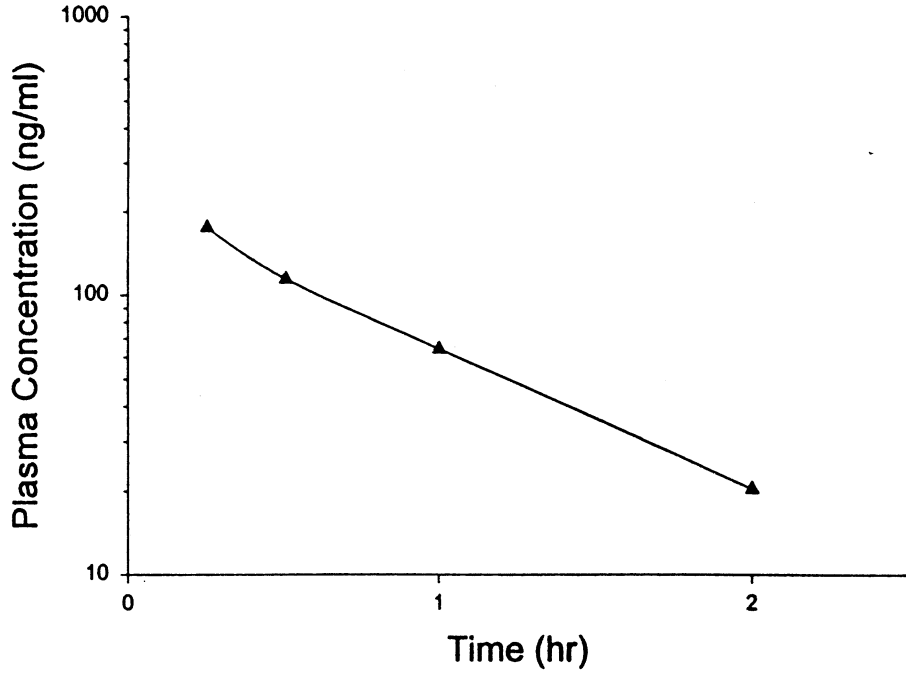

Fig. 3. Plasma drug concentration vs. time after intravenous administration of 0.94 mg kg^{-1} of Lidocaine hydrochloride in a female beagle dog.

3.9. Application

The application of the assay was tested in a beagle dog which received a low dosage of Lid HCl (0.94 mg kg^{-1}) by I.V. bolus. Blood samples were taken 0, 15, 30, 60, 120, 180 and 240 min after injection. As shown in Fig. 3, the drug plasma concentrations dropped rapidly with a half life of 0.86 h after the injection.

4. Conclusions

A simple and sensitive method of assaying Lid in dog plasma has been developed and validated. The solid phase extraction of the drug from plasma using the C_{18} cartridge was selective and efficient and thus allowed the sensitive analysis of Lid by UV detection at 210 nm. The overall sensitivity, linearity, precision and recovery were adequate for pharmacokinetic studies of Lid. Additionally, it is possible that the sensitivity could be further improved by the use of more plasma samples, larger sample injection volume or condensation of the effluent after extraction.

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