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Short Communication

High-performance liquid chromatographic method for the simultaneous determination of monoethylglycinexylidide and lignocaine

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ABSTRACT

An accurate and sensitive high-performance liquid chromatographic method with UV detection was developed for the simultaneous measurement of monoethylglycinexylidide (MEGX) and lignocaine in human plasma and serum, using organic solvent extraction and trimethoprim (TMP) as an internal standard. The mean recoveries for MEGX, TMP and lignocaine were 86.1 ± 3.7, 98.3 ± 1.8 and 77.0 ± 4.7%, respectively (n = 6). The relative standard deviations for MEGX concentrations of 10 and 200 ng/ml were <4% and for lignocaine concentrations of 200 and 1200 ng/ml they were <8%.

INTRODUCTION

Lignocaine is widely used as a local anaesthetic agent and also as a therapeutic agent in the treatment of certain cardiac dysrhythmias. Recently it has been suggested that the production of the lignocaine metabolite monoethylglycinexylidide (MEGX) could be used as an index of liver function [1]. In this role it has been proposed that the plasma or serum concentration of monoethylglycinexylidide might be useful in evaluating liver function in potential liver transplant donors and in the assessment of patients before and after liver transplantation [1–3]. Current analytical methods for the measurement of MEGX use either fluorescence polarization immunoassay (TDx, Abbott Diagnostics Division, North Chicago, IL, USA) [1] or high-performance liquid chromatography (HPLC). However, the HPLC assays for MEGX using UV detection which have been reported [4–13] are not as sensitive as the currently available TDx assay (detection limit 10 ng/ml).

In this paper we describe a sensitive and selective HPLC–UV assay which uses trimethoprim (TMP) as the internal standard. This assay is able to measure concentrations of MEGX and lignocaine as low as 10 and 100 ng/ml, respectively, in plasma and serum.

EXPERIMENTAL

Chromatographic system

The HPLC system consisted of a Model 510 pump (Millipore, Bedford, MA, USA), a Model 480 variable-wavelength UV detector set at 205 nm (Millipore), a Model 7125 manual injector

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equipped with a 100-μl loop (Rheodyne, Cotati, CA, USA), a μBondapak C_{18} (10-μm) column (300 × 3.9 mm I.D.) (Millipore) and a Type 3066 pen recorder (Yokogawa Electric, Tokyo, Japan) with a chart speed of 20 cm/h. Chromatography was performed at room temperature using a mobile phase consisting of acetonitrile–0.05 *M* potassium dihydrogenphosphate buffer (pH 4.0) (14:86) at a flow-rate of 1.3 ml/min.

Materials

Lignocaine and MEGX were gifts from Astra Pharmaceuticals (Södertälje, Sweden). Trimethoprim (TMP) was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and dichloromethane were of HPLC grade (Malinckrodt Australia, Clayton, Australia). Potassium dihydrogenphosphate, orthophosphoric acid and sodium hydroxide were of analytical-reagent grade. The water used was obtained form a Milli-Q water purification system (Millipore).

Methods

To 0.5 ml of plasma or serum were added 50 μl of TMP (1.5 μg/ml) and 40 μl of 0.25 *M* sodium hydroxide solution. The samples were vortex mixed for 10 s (Sybron, Maxi Mix II) and then 4 ml of dichloromethane were added. The mixture was vortex mixed for a further 5 s and then placed on a rotating mixer (25 rpm) for 5 min. Following centrifugation (1000 *g* for 5 min), the aqueous layer was removed by aspiration and the organic phase was tranferred into a clean tube and evaporated under a gentle stream of air at 40°C. The residue was reconstituted in 300 μl of 0.05 *M* potassium dihydrogenphosphate (pH 2.8) and a 100-μl aliquot was injected into the HPLC system. Peak heights were measured manually and the ratios were used to calculate MEGX and lignocaine concentrations based on calibration graphs prepared from drug-free plasma spiked with lignocaine and MEGX.

RESULTS

The calibration graphs were linear over the ranges 10–250 ng/ml for MEGX and 100–1500 ng/ml for lignocaine, with correlation coefficients of >0.999. The inter-assay relative standard deviations (R.S.D.) for MEGX at 200, 100 and 10 ng/ml were 1.8, 1.4 and 3.9%, respectively (n = 9), and for lignocaine at 1200, 500 and 200 ng/ml they were 1.7, 1.7 and 2.6%, respectively (n = 9). The intra-assay R.S.D. values for MEGX at 200, 100 and 10 ng/ml were 0.3, 0.8 and 1.7%, respectively (n = 9), and for lignocaine at 1200, 500 and 200 ng/ml they were 2.0, 1.8 and 7.1%, respectively (n = 9). The mean recoveries for MEGX, TMP and lignocaine were 86.1 ± 3.7, 98.3 ± 1.8 and 77.0 ± 4.7%, respectively (n = 6), over the

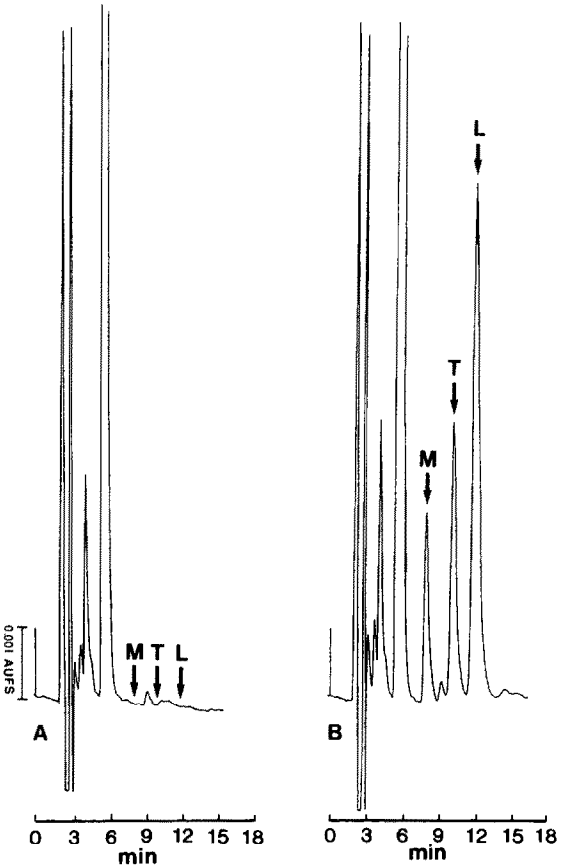

Fig. 1. Chromatograms of (A) predose serum from a liver transplant patient and (B) serum from the same patient 15 min after an intravenous bolus injection of 50 mg of lignocaine (measured MEGX and lignocaine concentrations were 100 and 670 ng/ml, respectively). Peaks M = MEGX; T = TMP (internal standard, 250 ng/ml); L = lignocaine.

range of concentrations used for the calibration graphs.

Typical chromatograms from the serum samples of a liver transplantation recipient are shown in Fig. 1. The retention times of MEGX, TMP and lignocaine were 7.5, 9.6 and 11.3 min, respectively.

DISCUSSION

The method is reproducible and sensitive for both lignocaine and MEGX. Proelss and Townsend [8] reported that the optimum recoveries for MEGX and lignocaine were achieved in the pH range 9.0–9.7. In that study sodium carbonate or sodium hydroxide was used to adjust the pH of plasma or serum samples to within the range of 8.0–11.5. In our study, sodium carbonate produced interfering peaks in the chromatogram. Using the amount of sodium hydroxide suggested in their assay, a much higher pH than that stated [8] was reached in the samples. In our study pH was critical and at pH > 11 there was a significant decrease in MEGX recovery and a very variable recovery for lignocaine. By using 40 μl of 0.25 *M* sodium hydroxide solution per 0.5 ml of plasma or serum sample, a final pH between 9.5 and 10.0 was achieved.

By including a 5-s vortex mixing step after addition of the organic solvent during extraction, the reproducibility of lignocaine recovery was greatly improved. Following extraction, 0.05 *M* potassium dihydrogenphosphate (pH 2.8) was used to redissolve lignocaine, MEGX and TMP; if mobile phase was used, a much longer time was required for reconstitution of the residue, probably owing to the basic nature.

Previously reported HPLC–UV assays which simultaneously measured lignocaine and its metabolite(s) used variously ethylmethylglycinexylidide (EMGX) [4,5,10,12], procaine hydrochloride [6,7], tocainide [1,13] and *p*-chlorodisopyramide [8,9] as internal standards. In our study, EMGX and procaine hydrochloride were not available and neither tocainide nor *p*-chlorodisopyramide was suitable for the assay. Trimethoprim proved most suitable as an internal standard in this assay, having a retention time between those of MEGX and lignocaine and a good recovery when extracted from plasma and serum.

The detection limits of MEGX in the previously reported HPLC–UV assays for the simultaneous determination of lignocaine and its metabolite(s) were more than 20 ng/ml [4–13]. In those reported studies, the between- and within-day R.S.D. values at this low concentration of MEGX were not reported. In our assay, we chose a signal-to-noise ratio of 3:1 as the minimum clearly measurable limit for peak height. The inter- and intra-assay R.S.D. values for an MEGX concentration of 10 ng/ml in plasma or serum were $<4\%$.

A highly sensitive MEGX assay is essential if HPLC is to be the method of choice for the analysis of samples collected as part of the assessment of liver function in patients before and after liver transplantation. Our assay is being used in our laboratory for studies on lignocaine metabolism in liver transplantation patients.

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REFERENCES

1 M. Oellerich, E. Raude, M. Burdelski, M. Schulz, F. Schmidt, B. Ringe, P. Lamesch, R. Pichlmayr, H. Raith, M. Scheruhn, M. Wrenger, Ch. Wittekind, B. Klawunn, A. Knoke, B. Mues and M. Farle, *J. Clin. Chem. Clin. Biochem.*, 25 (1987) 845.

2 M. Oellerich, M. Burdelski, H. Lautz, M. Schulz, F. Schmidt and H. Herrmann, *Ther. Drug Monit.*, 12 (1990) 219.

3 T. J. Schroeder, D. A. Gremse, M. E. Mansour, A. W. Theuerling, M. E. Brunson, F. C. Ryckman, F. J. Suchy, I. Penn, J. W. Alexander, A. J. Pesce, M. R. First and W. F. Balistreri, *Transplant. Proc.*, 21 (1989) 2299.

4 P. K. Narang, W. G. Crouthamel, N. H. Carliner and M. L. Fisher, *Clin. Pharmacol. Ther.*, 24 (1978) 654.

5 F. A. Luzzi, T. L. Wenger, J. K. Klinger, A. Barchowsky and H. C. Strauss, *J. Chromatogr.*, 311 (1984) 291.

6 R. Kawai, S. Fujita and T. Suzuki, *J. Pharm. Sci.*, 74 (1985) 1219.

7 Y. K. Tam, S. R. Tawfik, J. Ke and R. T. Coutts, *J. Chromatogr.*, 423 (1987) 199.

8 H. F. Proelss and T. B. Townsend, *Clin. Chem.*, 32 (1986) 1311.
9 H. R. Angelo, J. Bonde, J. P. Kampmann and J. Kastrup, *Scand. J. Clin. Lab. Invest.*, 46 (1986) 623.
10 K. Kushida, K. Oka, T. Suganuma and T. Ishizaki, *Clin. Chem.*, 30 (1984) 637.
11 R. L. Nation, G. W. Peng and W. L. Chiou, *J. Chromatogr.*, 162 (1979) 466.
12 J. Hill, A. Roussin, K. Lelorier and G. Caille, *J. Pharm. Sci.*, 69 (1980) 1341.
13 R. Verbesselt, T. B. Tjandramaga and P. J. De Schepper, *Ther. Drug Monit.*, 13 (1991) 157.