Sensitive HPLC for Simultaneous Quantification of Lidocaine and Its Metabolites Monoethylyglycinexylidide and Glycinexylidide in Serum, Carol L. O'Neill* and Alphonse Poklis (Dept. of Pathol., Box 980165, Med. College of Virginia, Virginia Commonwealth Univ., Richmond, VA 23298-0165; * author for correspondence: fax 804-828-7722).

Recent advances in the treatment of liver diseases have increased the need for sensitive tests of hepatic function. Several studies have demonstrated that the conversion of lidocaine to its major metabolite, monoethylyglycinexylidide (MEGX), has emerged as a sensitive indicator for hepatic dysfunction [1–3]. Oellerich et al. [2] report that a MEGX concentration of 20 μg/L at 15 min after lidocaine dosing is the cutoff concentration for hepatic impairment. Shiffman et al. [3] demonstrated that MEGX concentrations can be used to monitor the severity of the histological condition of patients with chronic hepatitis and cirrhosis. Sensitivity of this assay is of utmost importance because the MEGX concentrations of these patients are usually <20 μg/L. A fluorescence polarization immunoassay kit (TDx FPIA; Abbott Labs., Chicago, IL) that is fast, sensitive, and easy to perform is available for MEGX determinations in serum. Unfortunately, this assay is very expensive and is not approved by the US Food and Drug Administration. Additionally, high serum bilirubin and an unknown compound found in a small percentage of patients will interfere with the assay [4]. Here we describe a sensitive HPLC procedure for determining lidocaine and its metabolites, MEGX and glycinexylidide (GX), in serum. MEGX and GX can be detected simultaneously at concentrations as low as 5 μg/L and accurately measured at concentrations as low as 10 μg/L.

In the extraction procedure 1.0 mL of serum, 50 μL of 1 mg/L tocainide (internal standard; Merck, Darmstedt, Germany), 0.5 mL of saturated borate buffer, and 3.0 mL of methylene chloride are vortex-mixed and centrifuged. The organic layer is transferred to a clean test tube and evaporated under a stream of dry nitrogen in a water bath at 40 °C. The sample is reconstituted in mobile phase (by vol, 10:90 acetoniitrite and 0.02 mol/L phosphoric acid containing 0.2 mL/L triethylamine) and injected onto the HPLC system: a Hewlett-Packard (Avondale, PA) Series 1050 chromatograph equipped with a Supelcosil (Belleville, PA) LC-8-DB (250 × 4.6 mm) column and a Whatman (Clifton, NJ) silica guard column. The ultraviolet detector is set at 205 nm for MEGX and GX and 263 nm for lidocaine. The pump flow is set at 1.7 mL/minute. Concentrations are calculated from a calibration curve prepared from 0, 0.5, 1.0, and 2.0 mg/L for lidocaine; 0, 0.5, 1.0, and 2.0 mg/L for MEGX; and 0, 0.5, 1.0, and 2.0 mg/L for GX. Quantification is based on the peak-height ratio.

The method allows for the simultaneous quantification of GX, MEGX, and lidocaine. A typical chromatogram is shown in Fig. 1A. Retention times for GX, tocainide, MEGX, and lidocaine are 7.9, 9.7, 10.9, and 17.2 min, respectively. A long analysis time is needed to avoid interference by an as-yet unidentified peak that elutes just after MEGX. Quantification of MEGX would be affected if the two peaks were not resolved. An example of this interference is shown in Fig. 1B.

The absolute recoveries are 87.7% and 53.3% for MEGX and GX (50 μg/L), respectively, and 82.2% for lidocaine (1.0 mg/L). Recovery of GX is lower because of its increased water solubility. The precision of the slope of the calibration curve for each analyte was calculated from analyses on 6 days. The percent CVs were 5.7%, 5.6%, and 6.7% for MEGX, GX, and lidocaine, respectively. A linear correlation was determined by analysis over 4 days of the calibration curves constructed from concentrations of 10, 25, 50, 100, and 300 μg/L for MEGX and GX and 0.2, 0.5, 1.0, 2.0, and 4.0 mg/L for lidocaine. The method is linear over these ranges with correlation coefficients >0.999.

The intra- and interrun precision was determined by analyzing serum samples to which the analytes were added in low and high concentrations. The low concentrations were 10 μg/L for MEGX and GX and 0.2 mg/L for lidocaine; the high concentrations were 100 μg/L for MEGX and GX and 2.0 mg/L for lidocaine. The intrarun precision (10 samples analyzed on the same day) was 8.9% and 2.2% for MEGX, 8.7% and 2.0% for GX, and 13.2% and 5.3% for lidocaine. The interrun precision (one sample of each concentration analyzed on 10 different days) was 11.3% and 4.6% for MEGX, 11.6% and 3.3% for GX, and 13.5% and 8.4% for lidocaine.

In patients' serum samples the concentration of lidocaine may be >100-fold the concentration of MEGX; therefore, the detector wavelength is changed from 205 nm to 263 nm after MEGX has eluted. This is necessary in our system because the absorbance of 1.0 mg/L lidocaine at 205 nm is 2.0, well above the absorbance of 1.5 that is generally considered the limit for ultraviolet analysis. As evidenced by the precision data, the change in wavelength does not adversely affect the precision of the lidocaine results.

The limit of detection (LOD), determined by analysis of serial dilutions of drug-free serum to which the analytes were added, is the lowest concentration at which a peak could consistently be detected by the integrator. The LODs for MEGX and GX are 5 μg/L and 0.1 mg/L for lidocaine. The LOD for MEGX and GX is lower than those previously reported [1, 5, 6] because we used ultraviolet detection at 205 nm. Oellerich et al. [1] did not...
report an LOD or limit of quantification (LOQ) for their HPLC method, but the lowest calibrator for MEGX was 25 μg/L. Chen et al. [6] reported an LOQ of 10 μg/L and an LOD of 8 μg/L for MEGX, but their assay cannot quantify lidocaine concentrations >2.0 mg/L.

Selectivity of the assay was determined by analyzing with the above procedure drugs routinely encountered in therapeutic drug monitoring. None of the following drugs interfered with the detection or quantification of the analytes of interest: disopyramide, salicylate, acetaminophen, ibuprofen, naproxen, caffeine, theophylline, carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, valproate, quinidine, flecainide, chloramphenicol, imipramine, desipramine, amitriptyline, nor- triptyline, doxepin, nordoxepin, fluoxetine, diazepam, nordiazepam, chlordiazepoxide, norchlordiazepoxide, procainamide, N-acetylpromacylamide, bupivacaine, and cyclosporin A.

A comparison of methods study was performed between the present HPLC (y) assay and the TDx FPIA (x). The serum MEGX concentrations for 65 patients were included in the comparison. Linear-regression analysis of these data yielded y = 2.689 + 0.956x (r = 0.977, Sres = 7.9 μg/L). There was no significant difference between the results from both assays (paired t-test, P = 0.534). We also evaluated the accuracy of the HPLC assay by analyzing the TDx controls by HPLC and the HLCX controls by TDx. All results were within 10% of their target values. Although FPIA is currently the method of choice for MEGX, there are some problems with the assay. Samples with high bilirubin concentrations can give apparent MEGX readings that increase with increasing bilirubin content [4]. If the bilirubin concentration is high enough, the sample cannot be analyzed because of excessive background fluorescence. The sample must then be diluted with drug-free serum and reanalyzed, but dilution does not always solve the problem. In addition, many other endogenous serum factors may interfere with the FPIA.

MEGX HPLC procedures are not as rapidly performed as FPIA assays, but they do offer several advantages over FPIA. HPLC assays are less expensive, especially if one considers the cost of repeating diluted samples with high background. The assay is not subject to interferences from bilirubin, other endogenous factors, or metabolites that may cross-react with the FPIA. Also, lidocaine, MEGX, and GX can be detected simultaneously, thereby avoiding the need to perform separate lidocaine tests on patients with low MEGX concentrations to ensure that the proper dose of lidocaine was administered. The present HPLC method for determining lidocaine and its metabolites MEGX and GX in serum has reliable, low LOD and LOQ values that are sufficient for further study of the relationship between MEGX or GX concentrations and the severity of hepatic disease.

References


Iron is transported by blood transferrin [1], which has two high-affinity binding sites to iron [2]; therefore, plasma or serum iron concentrations indicate the amount of transferrin-bound iron. Measurement of plasma or serum iron is an important clinical test for several diseases. However, interference of non-transferrin-bound iron has been reported in measurement of plasma or serum iron [3-5]. Although circulating ferritin has a relatively low iron content [6, 7], the saturation of plasma transferrin in patients with hyperferritinemia is influenced by the presence of ferritin iron [4]. We have studied the influence of ferritin on the serum concentration of iron measured by five different methods: three direct colorimetric methods, a constant-potential coulometric method, and the International Committee for Standardization in Haematology (ICSH) method [8].

For the direct colorimetric methods we used three different chemicals as chromogen: 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (Ferrozine) [9] ("Iron FZ"), Hoffmann-La Roche, Basel, Switzerland), 2-nitroso-5-(N-propyl-N-sulfopropylamino)phenol (nitroso-PSAP) ("Quick auto Neo Fe 7070"; Shino Test Co., Tokyo, Japan), and bathophenanthroline sulfonate (Batho) ("Fe B-Test Wako"); Wako Pure Chemical Industries, Osaka, Japan). For the constant-potential coulometric method, we used a Ferrochem II Analyzer (ESA, Bedford, MA). In the ICSH method, Batho was used as the chromogen, and serum samples were heated at 56 °C for 15 min with the deproteination reagent (trichloroacetic acid, thioglycollic acid, and HCl). The ferritin concentration in serum was measured by the ACS:180 ferritin assay (Ciba Corning Diagnostics, E. Walpole, MA) with the ACS:180 analyzer [10].

To examine the influence of ferritin, we measured serum iron before and after the absorption of ferritin with solid-phase anti-ferritin antibodies, as follows. We centrifuged 5 mL of "solid-phase" reagent from the ACS:180 ferritin assay (paramagnetic particles conjugated to anti-ferritin mouse monoclonal antibody) at 1000 g for 5 min at 4 °C, and then removed 4.5 mL of the supernate. Of the remaining "solid-phase" reagent (containing 0.2 mg of antibody) 60 μL was added to 1.0 mL of a patient's serum. After incubation for 1 h at room temperature, the ferritin-absorbed serum supernate was collected by centrifugation at 1700 g for 10 min. We found that >98% of ferritin protein was absorbed by this method, even from patients' sera in which the ferritin exceeded 10 000 μg/L.

Figure 1 shows the original serum iron concentration before immunosorption of ferritin and the percentage change of iron