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, felodipine, chloramphenicol, imipramine, desipramine, amitriptyline, nortriptyline, doxepin, nordoxepin, fluoxetine, diazepam, nordiazepam, chloridiazepoxide, norchloridiazepoxide, procardinamide, N-acetylsalicylamide, bupicvinic, 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 \( y = 2.689 + 0.956x \) (\( r = 0.977, S_{xy} = 7.9 \mu g/L \)). 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 HPLC 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-phenylsulfonyl) 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 deproteinization 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 1000g 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 1700g 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 immunabsorption of ferritin and the percentage change of iron...
These data suggest that the direct colorimetric method using Ferrozine as chromogen is more satisfactory and accurate in measurement of transferrin-bound iron of patients with high concentrations of ferritin. Therefore, measured values of serum iron in patients with high ferritin concentrations should be carefully evaluated when the constant-potential coulometric method and the ICSH method are used.

References

Interference by Calcium in the Measurement of Urinary “Microalbumins,” Kevin Taylor, Terry S. Eley, Shirley P. Thompson, and Gerald A. Maguire (Dept. of Clin. Biochem., Addenbrooke’s Hosp., Cambridge, CB2 2QR, UK; * author for correspondence: fac 44-1223-216862; e-mail gam1004@cuis.cam.a.uk)

Albumin in urine at concentrations greater than those found in the urine of healthy people (~20 mg/L) but less than the detection limit of commonly used dip-stick tests for urine protein (~250 mg/L) is referred to as “microalbuminuria.” The presence of urinary “microalbumin” in otherwise healthy diabetics identifies those patients likely to develop diabetic nephropathy [1].

The most commonly used methods for measuring microalbumin are immunoturbidimetry and immunonephelometry [2]. In the course of an investigation into the possible value of urinary microalbumin measurements for detection of symptomatic preeclampsia [3], we observed anomalous behavior of urines with high calcium concentrations. This led us to investigate the effect of calcium on the immunoturbidimetric assay of albumin. Here we report that, when phosphate buffer is used, urine calcium at high (but physiological) concentrations can interfere in immunoturbidimetric microalbumin measurements. We investigated the nature of this interference and describe ways of overcoming it.