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Letter to the Editor

Creatinine

XIII. Micro high-performance liquid chromatographic assay of creatinine in biological fluids using fixed- or variable-wavelength UV detector

Sir,

Endogenous creatinine has been widely employed to estimate the glomerular filtration rate for the study of renal function or for modifying drug dosages in renal patients [1—4]. Earlier, a simple, micro high-performance liquid chromatographic (HPLC) method using a variable-wavelength UV detector (set at 215 nm) was developed in our laboratory [5] for the assay of creatinine in plasma, serum and urine. Since variable-wavelength detectors have been generally much more expensive to purchase and maintain than commonly available fixed-wavelength (such as 254-nm) UV detectors, it would then seem highly desirable to develop a method which can also utilize the fixed-wavelength detector for quantitation. The purpose of this communication is to report results of perfection of such a method. The sensitivity of the present assay is also higher than that reported earlier [5].

The previous method [5] involved quick deproteinization of plasma (0.01—0.1 ml) with 2.5 (2.0 is also sufficient; higher ratios may be used for lower volumes of sample in the present assay) volumes of acetonitrile, and injection of "protein-free" supernatant (50—100 μl) to the 25-cm cation-exchange column (Whatman Partisil PXS 10/25 SCX). The mobile phase was 0.1 *M* monobasic ammonium phosphate acidified to pH 2.6. In order to increase the absorption of creatinine at 254 nm [5] in the present assay the pH of mobile phase was adjusted to 4.8 with 0.5 *N* NaOH, and the resultant shorter retention time (also causing interferences from endogenous compounds), due to the basicity of creatinine [5], was then offset by using lower strengths (0.012—0.035 *M* dependent on columns used) of monobasic ammonium phosphate. With a flow-rate of 2—3 ml/min the creatinine retention time is usually 4—6 min. Using a sensitivity setting of 0.002 or 0.005 a.u.f.s. at 254 nm (Model 440 from Waters Assoc. or Model 160 from Beckman) the detection limit based on a signal-to-noise ratio of 3 could be estimated to be 0.4 μg/ml or 0.04 mg% which is much lower than the normal clinical range (0.3—1.5 mg%). For urine analysis (lower detector sensitivity settings required) a 0.1-ml sample was first diluted with 4

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ml of water—acetonitrile (20:80, v/v), and then centrifuged before injection to the column [5]. Coefficients of variation of response factors from spiked plasma (0.5—10 mg%) and urine (50—400 mg%) samples were 1.42 and 0.46%, respectively. Coefficients of variation for inter- or intra-assays of actual human plasma and urine samples were between 1.2 and 3.0%.

The specificity of the assay has been reported earlier [5]. This is further substantiated by the identical results in the analysis of 15 human urine samples obtained based on the present and previous [5] assays using two different wavelengths. No interferences with the creatinine analysis were found in our extensive studies of samples from humans [6, 7] and animals (dogs and rabbits used). Measurements of creatinine in serum samples containing either low [7] or high [8] levels of creatinine based on the conventional automated picrate method were found generally to result in an overestimate (as much as 30—60% in some samples) of creatinine when compared to our HPLC methods.

The creatinine eluted from the present mobile phase can also be quantitated by a variable-wavelength detector set at 235 nm (SpectroMonitor III from Laboratory Data Control, Riviera Beach, FL, U.S.A.), and the detection limit was found to be about 0.02 mg%. Our attempt to find a suitable internal standard has thus far been unsuccessful although a total of about 30 potential compounds have been tested. This is, however, not critical in view of the low variability of our method.

The present assay appears to offer some advantages in many respects over several other HPLC methods published. For example, in a recent publication [9] the method required 1 ml of serum sample, a more complicated gradient system and a variable-wavelength UV detector. The higher pH of mobile phase (up to 7.1) used may also shorten the life span of the column. Furthermore, the more "troublesome" [5] trichloroacetic acid was used in their deproteinization [9]. In this respect, our laboratory appears to be the first to extensively utilize acetonitrile for deproteinization in the HPLC analysis (refs. 5, 10—12, and many others). Its advantages over other commonly employed methods have been fully discussed [5]. The method of Brown et al. [13] involved direct injection of biological samples (may not be good for the column) and post-column reaction of creatinine with the picrate reagent. Furthermore, their detection limit was only 0.3 mg%. The method of Soldin and Hill [14] required a variable-wavelength UV detector (set at 200 nm) and a stabilization period of 4 h prior to analysis (less than 30 min is usually required in our assay). The method of Spierto et al. [15] required 0.5 ml of serum, a variable-wavelength UV detector (set at 236 nm), and a more time-consuming, expensive ultrafiltration procedure. No applications to urine analysis were shown in three [9, 13, 15] of the above four HPLC assays. It should be noted that the mobile phase employed in our assay also appears to be simpler and cheaper when compared to others [9, 13, 14], and no internal standard was used in most other assays [9, 13, 14].

It was reported recently from our laboratory that creatinine might be extensively secreted and reabsorbed simultaneously by renal tubules in both humans [16, 17] and animals [17, 18], and there might also be a significant nonrenal elimination in normal humans [18, 19] and normal rabbits [18]. Potential implications of these findings remain to be fully explored.

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