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Note

A simple, rapid and sensitive method for the determination of rat serum uric acid by reversed-phase high-performance liquid chromatography with electrochemical detection

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The biochemical and clinical importance of uric acid (UA) in gout and several other disease states has been discussed [1]. UA in serum is routinely determined either by a spectrophotometric procedure with phosphotungstate [2], or by adaptation of the enzymic method with uricase [3]. These techniques have problems as discussed elsewhere [4]. High-performance liquid chromatography (HPLC) is now an accepted technique as a reference method for the determination of UA. HPLC methods employing ion-exchange columns coupled with electrochemical detection (ED) [4] or ultraviolet detection (UVD) [5] have been proposed. Recently, reversed-phase liquid chromatography with UVD has been studied [6, 7]. However, there has been no report on a method for the determination of UA by reversed-phase high-performance liquid chromatography (RP-HPLC) with ED.

In this note, we describe a simple, rapid and sensitive method for the determination of UA in rat serum by RP-HPLC—ED.

MATERIALS AND METHODS

A JASCO-HPLC, Model Trirotar III with a 25 cm × 4.6 mm I.D. stainless-steel column packed with Fine Sil C_{18} (particle size, 10 μm; JASCO, Tokyo, Japan), coupled to an electrochemical detector (Model ECP-1, Kotaki, Funabashi, Japan) was employed in this study. The flow-rate was 0.5 ml/min. The column temperature was always kept at 35°C. The mobile phase was 0.2 *M* phosphate buffer (KH_2PO_4—H_3PO_4, pH 2.0). Unless otherwise stated, 10 μl of each sample solution were injected into the RP-HPLC—ED system.

UA was purchased from Wako Pure Chemicals, Tokyo, Japan. Any other chemicals used in this study were the same as used in previous reports [8—10].

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All buffers and aqueous solutions were prepared in glass-distilled deionized water.

An aliquot (0.05 ml) of 0.05 *M* sodium hydroxide solution was dropped onto 10 mg of UA, and 9.95 ml of 0.1 *M* hydrochloric acid were then added to the dissolved UA solution. The stock solution thus prepared was stored at 4°C. Appropriate dilution of the stock solution with 0.1 *M* hydrochloric acid solution was done just before use.

Whole rat blood was collected after decapitation; the serum was obtained by centrifugation for 15 min at 4000 *g* and stored at −80°C until use. A 1.0-ml volume of 2% sulphosalicylic acid (SSA) solution was added to 1.0 ml of rat serum. The mixture was frozen at −80°C and then thawed at room temperature. The mixture was centrifuged at 3000 *g* for 20 min. The supernatant was injected into the chromatographic system employed in this study. The concentration of UA in rat serum pool was ascertained by the standard addition technique following the procedure of Pachla et al. [4].

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of UA in aqueous solution containing only UA as an electrochemically active component except for (a) peak component(s) near the void volume. In this case, the electrochemical detector was set at +800 mV vs. the silver—silver chloride reference electrode. Fig.

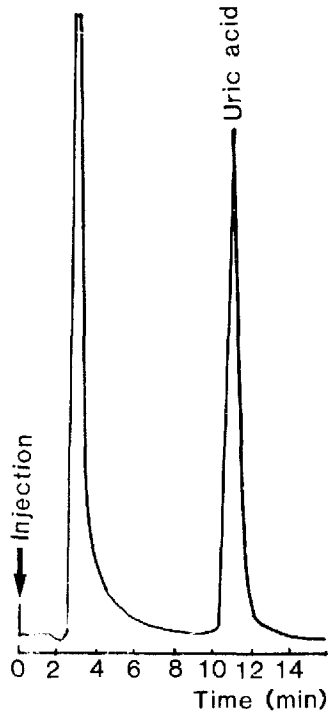
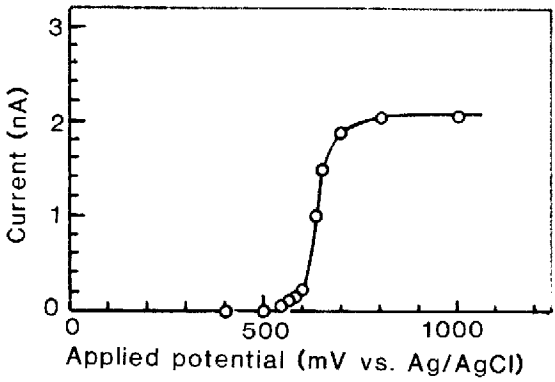


Fig. 1. The typical reversed-phase high-performance liquid chromatogram of uric acid in an artificially prepared sample solution under the present chromatographic conditions. For explanation of the chromatographic conditions, see text.



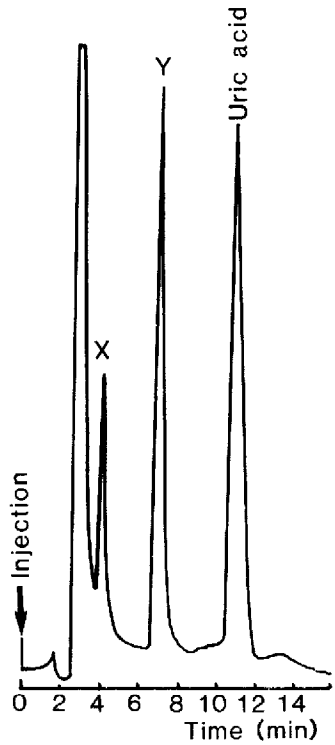


Fig. 2. Hydrodynamic voltammogram for uric acid obtained using the present chromatographic system by repeated injection of 1 ng of uric acid at different electrochemical detector potentials.

Fig. 3. The typical reversed-phase high-performance liquid chromatogram of rat serum after deproteinization under the present chromatographic conditions. For further explanations, see text.

2 illustrates a hydrodynamic voltammogram obtained for UA in the chromatographic system employed in this study. As shown in Fig. 2, the onset potential of UA oxidation is about +550 mV. Thus, the electrochemical detector was set at +800 mV vs. the silver—silver chloride reference electrode.

Fig. 3 shows a typical chromatogram for rat serum after the simple treatment described in the previous section. In the chromatogram, two unidentified peaks (peaks X and Y) are observed. It has been found that the retention time for the peak X component is the same as that for norepinephrine. Peak Y has not yet been identified. When an UA solution and deproteinized rat serum were co-chromatographed, it was found that only the UA peak was enhanced corresponding to the peak height of UA added to the rat serum. The minimum detectable quantity was about 10 pg under the present chromatographic conditions [11]. The content of UA in 1 ml of rat serum was found to be 4.9 ng.

UA standard solutions added to rat serum were analyzed with good precision at concentrations comparable to those in biological samples. The recoveries of UA in different standard solutions added to twenty different rat serum samples prepared on four different days were found to be 98.5 ± 1% under the present experimental conditions.

Excellent precision of retention time for UA was always obtained in routine analysis over a six-day period for 50 different rat serum samples, probably due to the fact that the retention time of UA is not affected by the sample matrix. However, the calibration graphs were obtained before and after the UA determination for the separation study in our laboratory. Both calibration graphs always coincided well with each other. Repeated injections of UA gave an average precision (R.S.D.) of less than 2%.

As described above, we have developed a simple, rapid and sensitive method for the determination of UA by RP-HPLC—ED. Because of its simplicity and applicability to small sample volumes (e.g. 0.1 ml of rat serum), this method is useful in biochemical and basic medical research. The method developed in this study has been used in determining UA in 0.1 ml of human serum, 0.1 ml of human cerebrospinal fluid, and 5 mg of rat brain [12]. As it has been revealed that this method is not subject to interferences encountered in other methods, it should be an attractive alternative to the colorimetric and enzymatic methods now used.

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