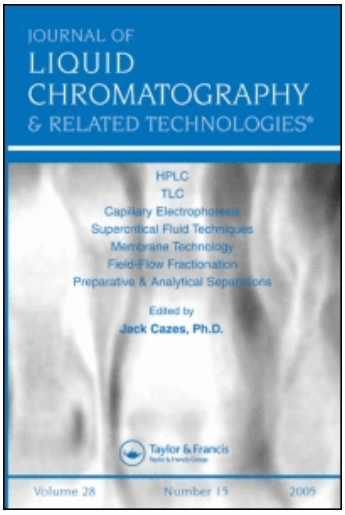

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Uric Acid in Human Serum: Reversed-Phase Liquid Chromatography with Electrochemical Detection

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To cite this Article Iriyama, Keiji , Yoshiura, Masahiko , Iwamoto, Takeo , Hosoya, Tatsuo , Kono, Hideo and Miyahara, Tadashi(1983) 'Determination of Uric Acid in Human Serum: Reversed-Phase Liquid Chromatography with Electrochemical Detection', Journal of Liquid Chromatography & Related Technologies, 6: 14, 2739 — 2746

To link to this Article: DOI: 10.1080/01483918308064943

URL: http://dx.doi.org/10.1080/01483918308064943

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(14), 2739-2746 (1983)

DETERMINATION OF URIC ACID IN HUMAN SERUM: REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

A method for the simultaneous determination of uric acid in human serum by reversed-phase high-performance liquid chromatography with electrochemical detection has been developed. Human serum (0.5 ml) was mixed with 0.5 ml of 0.2 N perchloric acid solution and the mixture was centrifuged at 3,000 g for 20 min. An aliquot (10 µl) of the supernatant (deproteinized human serum) was injected into the chromatographic system employed in this study. The assay limit for quantitation was about 10 pg for uric acid. Complete separation of uric acid was achieved in about 8 min under the present chromatographic conditions.

INTRODUCTION

As described elsewhere (1), high-performance liquid chromatography (HPLC) may be an obvious candidate as a reference method for the determination of serum uric acid. Different separation principles and detectors have been used for determining uric acid (UA) in serum by aid of HPLC. Ion-exchange columns have been

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used, coupled with ultraviolet detection (2) or electrochemical detection (3). Reversed-phase high-performance liquid chromatography (RPHPLC) has been also used with ultraviolet detection (4). Recently, we have developed a method for the determination of UA and catecholamines in rat serum and brain by RPHPLC with electrochemical detection (5).

The object of this paper is to report a simple, rapid, selective and highly sensitive method for the determination of UA in human serum by RPHPLC with electrochemical detection (ECD).

MATERIALS AND METHODS

UA was purchased from Wako Pure Chemicals, Tokyo, Japan. All other chemicals used in this study were the same ones as used in our previous report (6). All buffers and aqueous solutions were prepared with glass-distilled deionized water.

Serum samples prepared from healthy adult men were stored at $-80^{\circ}C$ until use. Standards were prepared by appropriately diluting a stock UA solution (1mg/ml) with water. The stock solution was prepared as follows. An aliquot (0.05 ml) of 0.05 N NaOH was dropped into 10 mg of UA for dissolving it completely because of its low solubility in pure water. A 0.1 N HCl (9.95 ml) was added into the alkaline UA solution.

Samples were analyzed using a reversed-phase partition mode of HPLC. A JASCO-HPLC, TRIROTAR III, was used throughout this

work. The instrument was fitted with an electrochemical detector (Model ECP-1, Kotaki Inc., Funabashi, Chiba, Japan). A Finepak SIL C18 column (ø 4.6 x 250 mm, JASCO, Tokyo, Japan) was used for the separation of UA. 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). The flow rate was 0.5 ml/min. Ten microliters of each sample was injected into the RPHPLC-ECD system. The electrochemical detector was set at +800 mV vs. the silver/silver chloride reference electrode, as Pachela et al. (2) reported that the onset potential of UA was about +330 mV vs. the silver/silver chloride reference electrode under their electrochemical conditions and also that an electrochemical detector was set at +800 mV. Fig. 1 shows the standard curve for high-performance liquid chromatographic determination of UA under the present chromatographic conditions. As shown here, the minimum detectable quantity is about 10 pg for UA. UA was quantitated by comparing the peak height in the respective chromatogram with value from a standard curve.

RESULTS AND DISCUSSION

Recently, we have found (5) that alumina can adsorb UA as well as catecholamines and also that the adsorption of UA onto alumina is not always quantitative. These findings suggest that the so-called alumina treatment procedure recently optimized (6) is not useful for extraction and preliminary purification of UA in biological materials. Therefore, we aimed to develop a pretreatment in

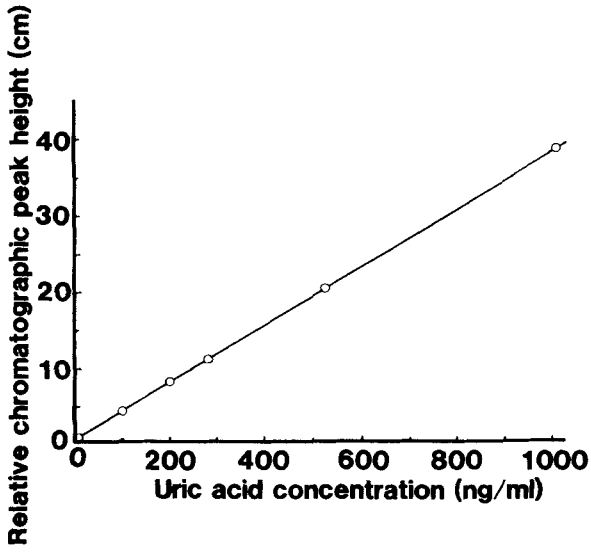

Figure 1. Calibration curve for uric acid under the present chromatographic conditions.

extracting procedure for UA of biological materials. In addition, we have found (5) that concentrations of UA in rat serum and brain are greater than those of any other electrochemically active components in both biological materials. From the above observation, we assumed that simply deproteinized serum might be able to be injected into the RPHPLC-ECD system for the determination of UA in serum without any interferences.

Deproteinization of human serum was achieved as follows: mix vigorously 0.5 ml of human serum with an equal volume of 0.2 M perchloric acid solution, and then centrifuge at 3,000 g for 20 min. An aliquot (10 μl) of the simply deproteinized serum was injected into the RPHPLC-ECD system.

Figure 2 shows such a reversed-phase high-performance liquid chromatogram. Peak X in the chromatogram has been found to be

Figure 2. The typical reversed-phase high-performance liquid chromatogram, obtained by injecting 10 μl of the deproteinized human serum into the RPHPLC-ECD system. Ten microliters of the serum was injected into a column (Finepak SIL C18, JASCO) by using a microsyringe. The eluent was 0.2 M phosphate buffer (KH_2PO_4-H_3PO_4, pH 2.0). The column temperature was maintained at 35°C. The flow rate was 0.5 ml/min. Eluate from RPHPLC was electrochemically monitored by aid of an electrochemical detector under the potentiostatic condition (+800 mV vs. Ag/AgCl. The sensitivity of the detector was set at 64 nA full scale. For further explantions, see the text.

UA after the further co-elution of a mixture of UA and the deproteinized serum. As shown in Fig. 2, the method for the determination of UA in serum, which has been developed in this study, is not subject to interferences in other methods. The phosphotungstic acid method is subject to interference resulting from endogenous

nonurate chromogens, nutrients, and drugs (7). Urikase methods based on spectrophotometry are also not without problems, in that the specificity of the absorbance measurement is less than that of the enzyme, and several interferences have been noted (8). Content of UA in 0.5 ml of healthy human serum, as shown in Fig. 2, was found to be 21.6 μg (43.2 μg/ml). Since the sample preparation did not involve any transfer, the value for UA amount might be nearly absolute, indicative of an endogenous quantity.

As described above, we have developed a practical method for the determination of UA in human serum by RPHPLC with ECD. In addition, most recently, we have developed a method for the determination of xanthine and hypoxanthine by RPHPLC with ECD (Iwamoto, Yoshiura, and Iriyama, to be published in Jikeikai Med. J. with any other related experimental results). Xanthine, hypoxanthine, and UA are themselves produced either as a result of the breakdown of cellular material in toto, the turnover of nucleic acids in the cells, or as a result of the intermediary metabolism of various purine nucleotide derivatives. Modern biochemical investigators have found purine metabolism in general to be of great theoretical interest, and in addition to the problem there is hope the study of this metabolic system will provide answers to a number of ancilary enigmas of biochemistry. The biochemical and clinical importance of UA in gout and several other desease states was discussed by Balis (9) and Glynn et al. (10). As described by Glynn et al. (10), previous epidemiologic studies of UA have been limited by a cross-sectional design which precludes a determination of factors predictive changes in UA levels. Furthermore, some studies have not controlled for the

health status and drug intake of their populations. We believe that the well-known separation power of RPHPLC, combined with current state of the art in electrochemical monitoring will circumvent some of the problems presently encountered in the analysis of UA of biological and clinical importance. Because of its simplicity and applicability to small sample volumes, this method is useful in basic biomedical research. For example, we have determined concentrations of UA in rat tissues (e.g. brain, heart, stomach, and ren) according to the procedures developed in this study (Yoshiura, Iwamoto, and Iriyama, to be published elsewhere). This method can be applied for studying the change of UA levels in mammalian tissues.

REFERENCES

1. Ingebresten, O.C., Borgen, J., and Farstad, M., "Uric acid determinations: reversed-phase liquid chromatography with ultraviolet detection compared with kinetic and equilibrium adaptations of the uricase method". Clin. Chem., 28, 496-498, 1982.

2. Pachla, L.A., Kissinger, P.T., Yu, L., Watson, F., Pragay, D., Chilcote, M.E., Weiner, L.M., and Pennick, B.R., "Measurement of serum uric acid by liquid chromatography", Clin. Chem., 25, 1847-1852, 1979.

3. Milner, J.A. and Perkins, E.G., "Determination of uric acid in biological fluids by high-pressure liquid chromatography", Anal. Biochem., 88, 560-565, 1978.

4. Brown, N.D., Kintzios, J.O., and Koetitz, S.E., "Determination of hypoxanthine, xanthine, and uric acid in biological fluids by ion-pair high-performance liquid chromatography", J. Chromatogr., 177, 170-173, 1979.

5. Iwamoto, T., Yoshiura, M., and Iriyama, K., "Uric acid determination: reversed-phase liquid chromatography with electrochemical detection", Jikeikai Med. J., 30, in press.

6. Iriyama, K., Yoshiura, M., and Iwamoto., "Determination of catecholamines in rat tissues by high-performance liquid chromatography with electrochemical detection", Jikeikai Med. J., 30, 35-44, 1983.

7. Geisinger, K.R., Batsakis, J.G., and Bauer, R.C., "Serum uric acid", Amer. J. Clin. Pathol., 72, 330-336, 1979.

8. Young, D.S., Pestaner, L.C., and Gilberman, V., "Effects of drugs on clinical laboratory tests", Clin. Chem., 21, 1D-432D, 1975.

9. Balis, M.E., "Uric acid metabolism in man", Adv. Clin. Chem., 18, 213-246, 1976.

10. Glynn, R.J., Canpion, E.W., and Silbert, J.E., "Trends in serum uric acid levels", Arthr.Rheum., 26, 87-93, 1983.