Journal of Pharmaceutical Analysis 2014;4(2):153–158

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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ORIGINAL ARTICLE

Determination of gouty arthritis' biomarkers in human urine using reversed-phase high-performance liquid chromatography

Lei-Wen Xiang[a,b], Jing Li[a], Jin-Ming Lin[a], Hai-Fang Li[a,*]

[a]*Beijing Key Laboratory of Microanalysis Methods and Instrumentation, Department of Chemistry, Tsinghua University, Beijing 100084, China*
[b]*Institute of Food and Fermentation Industries, Fuqing Branch of Fujian Normal University, Fuqing 350300, China*

Received 10 May 2013; accepted 30 November 2013
Available online 12 December 2013

KEYWORDS

Gouty arthritis;
Creatinine;
Uric acid;
Hypoxanthine;
Xanthine;
High-performance liquid chromatography

Abstract Creatinine, uric acid, hypoxanthine and xanthine are important diagnostic biomarkers in human urine for gouty arthritis or renal disease diacrisis. A simple method for simultaneous determination of these biomarkers in urine based on reversed-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detector was proposed. After pretreatment by dilution, centrifugation and filtration, the biomarkers in urine samples were separated by ODS-BP column by elution with methanol/50 mM NaH_2PO_4 buffer solution at pH 5.26 (5:95). Good linearity between peak areas and concentrations of standards was obtained for the biomarkers with correlation coefficients in the range of 0.9957–0.9993. The proposed analytical method has satisfactory repeatability (the recovery of data in a range of creatinine, uric acid, hypoxanthine and xanthine was 93.49–97.90%, 95.38–96.45%, 112.46–115.78% and 90.82–97.13% with standard deviation of $<5\%$, respectively) and the limits of detection (LODs, S/N ≥ 3) for creatinine, uric acid, hypoxanthine, and xanthine were 0.010, 0.025, 0.050 and 0.025 mg/L, respectively. The established method was proved to be simple, accurate, sensitive and reliable for the quantitation of gouty arthritis' biomarkers in human urine samples. The ratio of creatinine to uric acid was found to be a possible factor for assessment of gouty arthritis.

1. Introduction

In clinical diagnosis, the uric acid concentration in gouty arthritis patients has been evaluated on (a) decreased destruction of uric acid, (b) overproduction of uric acid, and (c) an abnormality in the kidney excretion of uric acid [1]. Uric acid is the hepatic product of purine metabolism. After primary filtration by the kidney, the metabolic uric acid is reabsorbed into the blood circulation system or

*Corresponding author. Tel./fax: +86 10 62797463.
E-mail address: lihaifang@mail.tsinghua.edu.cn (H.-F. Li).
Peer review under responsibility of Xi'an Jiaotong University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jpha.2013.11.003

secreted into the urine [2]. The symptoms of gouty might be abnormal metabolism of kidney excretion of uric acid. In addition, creatinine is one of the most widely used biomarkers of kidney function [3]. Therefore, the study of simultaneous determination of uric acid and creatinine for diagnosis of gouty arthritis has been of importance.

The conventional method for the determination of these biomarkers in plasma and urine is based on the enzymatic conversion of urate to allantoin using uricase followed by colorimetric measurement [4,5]. However, this method includes unstable reagents and suffers from interferences such as ascorbic acid and dopamine, which are present in biological fluids. In the past decades, different chromatographic methods were applied to determine uric acid or uric acid and creatinine, including ion-exchange liquid chromatography [6], paired-ion liquid chromatography [7], size-exclusion liquid chromatography [8], liquid chromatography/mass spectrometry [9–11], high-performance liquid chromatography (HPLC) [5,12–19], column-switching liquid chromatography [20], hydrophilic interaction chromatography [21] and capillary electrophoresis [22–27].

In human body, uric acid is the end-metabolic product of adenine and guanine as shown in Fig. 1A. Adenine is catalyzed by adenase to hypoxanthine, which is catalyzed by xanthine oxidase undergoing xanthine and finally to uric acid. Xanthine can also be produced from guanine by guanase catalysis [1]. Creatinine is excreted from creatine and phosphocreatine and this process occurs at fractional rates of 0.016 and 0.03 per day for creatine and phosphocreatine, respectively. It leads to the irreversible, non-enzymatic dehydration and loss of phosphate from phosphocreatine as shown in Fig. 1B. The amount of creatinine in the urine is proportional to the amount of creatine and creatine phosphate present in the human body [28]. Creatinine is one of the most widely used markers of renal function. Changes in urinary creatinine content can indicate the renal problems [3]. Herein, creatinine, uric acid, hypoxanthine and xanthine are important diagnostic biomarkers in human urine for gouty arthritis or renal disease.

However, to the best of our knowledge, methods for simultaneous determination of uric acid, creatinine, xanthine and hypoxanthine in urine still have not been reported. Sometimes the spot concentration of uric acid does not represent the real level in the body because of the effect of biological clock. The 24 h monitoring uric acid is not applicable for clinical diagnosis. Therefore, the combination of uric acid, creatinine, hypoxanthine and xanthine is more instructional for correct and integrated diagnosis of gouty arthritis. In our study, a chromatographic method for simultaneous determination of these four biomarkers in urine was proposed. A simple pretreatment to remove the protein interferents was performed for real urine samples before chromatographic analysis. The samples were pretreated by dilution, centrifugation and filtration, and then were analyzed with HPLC. Urine samples from healthy and gouty volunteers were gathered. It was found in this study that the ratio of creatinine to uric acid may be considered as an additional assessment factor for diagnosis of gouty arthritis.

2. Experimental

2.1. Chemicals and reagents

Creatinine, uric acid, hypoxanthine and xanthine were purchased from Sigma-Aldrich (USA). Methanol supplied by J&K Scientific Ltd. (USA) was of HPLC grade. Sodium dihydrogen phosphate and sodium hydroxygen obtained from Beijing Chemical Reagent Company (China) were used for preparing HPLC buffer solutions. All chemicals were dissolved with de-ionized water.

Creatinine standard (200 mg/L) was freshly prepared in water. Uric acid standard (200 mg/L) was dissolved in basic aqueous solution at a pH value of 10.35 to increase the solubility. Hypoxanthine and xanthine standards (200 mg/L, respectively)

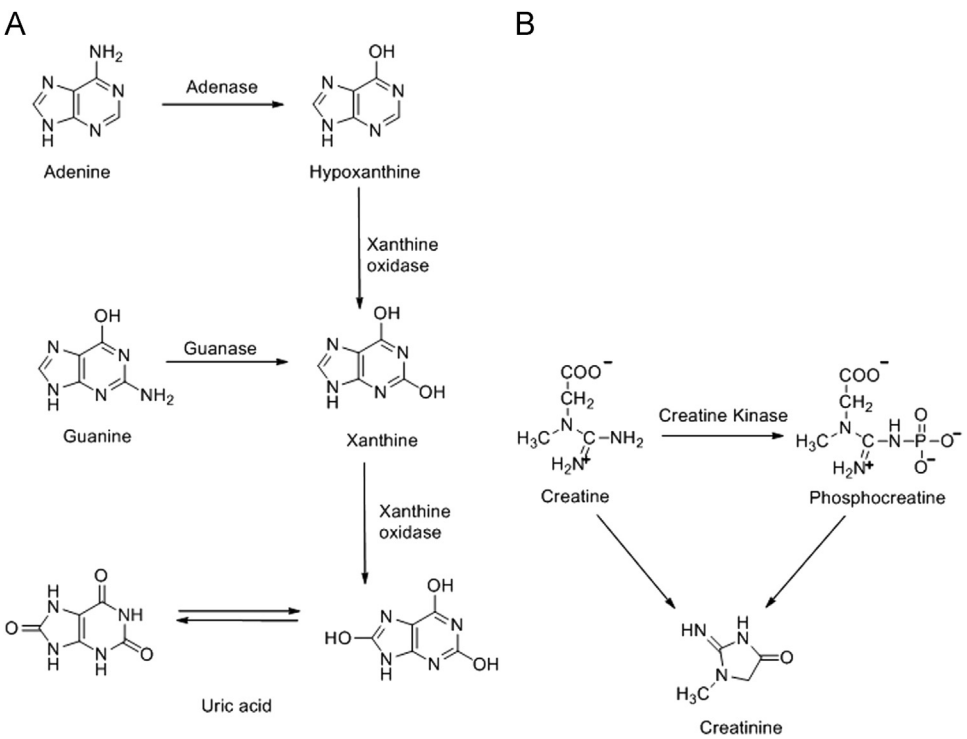

Fig. 1 Chemical structures of uric acid, creatinine, hypoxanthine and xanthine.