Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 154 (2016) 1–7

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Review Article

Resonance light scattering determination of 6-mercaptopurine coupled with HPLC technique

Ai Ping Li, Jing Dong Peng *, MingQiong Zhou, Jin Zhang

Chemistry and Chemical Engineering, Southwest University, Beibei District, Chongqing, PR China

ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form 9 August 2015
Accepted 20 September 2015
Available online 25 September 2015

Keywords:
Resonance light scattering
HPLC
6-mercaptopurine

ABSTRACT

A simple, fast, costless, sensitive and selective method of resonance light scattering coupled with HPLC was established for the determination of 6-mercaptopurine in human urine sample. In a Britton–Robinson buffer solution of pH 5.5, the formation of coordination complex between 6-mercaptopurine and metal palladium (II) led to enhance the RLS intensity of the system. The RLS signal was detected by fluorescence detector at $\lambda_{ex} = \lambda_{em} =$ 315 nm. The analytical parameters were provided by the coupled system, the linear of 6-mercaptopurine response from 0.0615 to 2.40 μg L^{-1} and the limit of detection (S/N = 3) was 0.05 μg L^{-1}. The presented method has been applied to determine 6-mercaptopurine in human urine samples which obtained satisfactory results. Moreover, the reaction mechanism and possible reasons for enhancement of RLS were fully discussed.

Contents

1. Introduction

6-mercaptopurine ($C_5H_4N_4S$,MPH) belongs to nucleic acid metabolism antagonist. It could competitively inhibit xanthenes' transformation

Abbreviations: RLS, resonance light scattering; HPLC, high performance liquid chromatography; MPH, 6-mercaptopurine; H_2L^+, cation; HL, molecule; L^-, anion.

* Corresponding author.
E-mail address: hxpengjd@swu.edu.cn (J.D. Peng).

http://dx.doi.org/10.1016/j.saa.2015.09.010
1386-1425/

process and the mutual transformation of complex objects between purine and the proliferation of tumors. It is used for maintenance treatment of acute leukemia and also effective to chronic myeloid leukemia and a variety of other tumors. It has also been used to treat malignant mole, choriocarcinoma, polycythemia, psoriatic arthritis, and inflammatory bowel disease. Unfortunately, taking this drug is easy to cause bone marrow suppression reaction, gastrointestinal suppression reaction and other adverse effects of suppression. Moreover, treatment of leukemia with mercaptopurine often causes drug resistance [1,2].

A literature survey reveals many reports dealing with the detection and quantification of 6-mercaptopurine using various analytical techniques individually. They particularly include fluorescence detection [3–6], chemiluminescence detection [7–9], electrochemical detection [10–12], high performance liquid chromatography determination [13–16], Mass Spectrometry determination [17], CE [18] and Raman assays [19]. Unfortunately, 6-mercaptopurine only has a few native fluorescence and the species that could be derivatized are limited. For normal fluorescence assays, they always have a low selectivity because it does not have separation function and thus they have limited applications. The electrochemical determination of MPH has limited their application in the real samples due to their poor repeatability and complex electrode modification process. Various methods had been incorporated into high performance liquid chromatography to detect MPH in plasma and urine. However, most of them had been involved more complicated extraction or derivatization procedures. The Mass Spectrometry instrument is too expensive though it has low detection limit. HPLC and MS are always used together to detect MPH. Although it is selective and sensitive, it always requires expensive equipments and toxic solvents and often involves complex sample pretreatments. CE and Raman assays also suffer from the defects as HPLC and MS. Therefore, how to develop a simple, fast, costless and reliable assay of MPH has been a challenge for analytical researchers.

Resonance light scattering (RLS) is an unorthodox elastic light-scattering with the two basic characteristics of appreciable electronic couplings which are in essence resulting from the mutual interactions between/among some types of chromophores to finally produce the scatters and sufficiently enlarged volumes [20]. Since Pasternack et al. [21] recommended RLS technique on science and successfully studied aggregation of biological macromolecule with small molecules, RLS has been used extensively in analytical chemistry. In this work, we develop a resonance light scattering coupled with HPLC method for the determination of 6-mercaptopurine. The quantitative analysis is benefiting from RLS signal enhancement of coordination complex due to the interaction between 6-mercaptopurine and palladium (II) to form the scatter in acid medium. The reaction systems above are rarely used in analytic chemistry. It is so simple that no complicated derivatization procedure was involved and it is more sensitive, and selective than other methods.

2. Experimental

2.1. Apparatus

A Shimadzu liquid chromatography (Shimadzu, Japan) equipped with DGU-20A5R degassing unit, two LC-20AD pumps, and RF-20A fluorescence detector was used. A PCD-C3000 post-column instrument was purchased from Tian Mei Da Scientific Instruments Co., Ltd. (Shenyang, China) consisting of two RLS-01 pumps, a room temperature reactor, a heating and cooling monitor. Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used to gain the static RLS spectra. UV-8500 absorption spectrophotometer (Tianmei, Shanghai) was used to gain absorption spectra. A PENFOR27 Fourier transform infrared spectroscopy (Bruker, Germany) was employed to record infrared spectra. The surface of coordinate complex was observed by scanning electron microscopy (SEM, S-4800, Hitachi, Tokyo, Japan) at an acceleration voltage of 30 kV. The pH measurements were made with a model PHS-FE20 pH meter (Mettler-Toledo Instruments Co. Ltd., Shanghai). Double distilled water was prepared by a Millipore SZ-93 system (Shanghai Ya rong Biochemical Apparatus Co., Ltd.).

2.2. Reagents and materials

6-mercaptopurine and adenine were purchased from Aladdin Reagent, palladium chloride ($PdCl_2$) was attain from Reagent Factory (Shanghai, China). Britton–Robinson buffer solution with different pH was prepared by mixing the mixed acid (composed of 0.04 mol L^{-1} H_3PO_4, HAc and H_3BO_3 with 0.2 mol L^{-1} NaOH) in proportion which were purchased from Chuan dong Chemical Industry (Chongqing, China). HPLC grade methanol was purchased from Kermel (Tianjin, China). Double distilled water was used throughout. Analytical reagent grade hydrochloric acid (HCl) was purchased from Chemistry Reagent Factory (Chongqing, China). All reagents were filtered through a 0.2 μm pore size filter membrane (Millipore, Bedford, MA, USA).

2.3. Sample preparation, collection and pretreatment

6-mercaptopurine and adenine standards were weighed accurately and dissolved in 0.2 mol L^{-1} NaOH to prepare a stock solution of 1 mg mL^{-1}. The standard solutions were stored at 4 °C in darkness. A 500 μg mL^{-1} stock solution of $PdCl_2$ (Shanghai Reagent Factory, China) was prepared by dissolving 0.5 g $PdCl_2$ in suitable amount of concentrated hydrochloric acid and diluting to 1 L with water. Working solutions were freshly prepared by diluting the corresponding stock solution. Britton—Robinson buffer solution with different PHs was prepared by mixing the mixed acid (composed of 0.04 mol L^{-1} H_3PO_4, HAc, and H_3BO_3 with 0.2 mol L^{-1} NaOH) in proportion. All stock solutions were kept at 0–4 °C during the experiment.

Human urine samples were collected from healthy volunteer, and the analysis was conducted immediately after the sample collection. Adding 1.5 mL urine samples in a dry centrifuge tube, and then acetonitrile of twice volume was added to precipitate protein. The standard addition was carried out by spiking a 200 μL 6-mercaptopurine and adenine standard solution to urine clear supernatant which was vortex-mixed for 40 s and centrifuged for 10 min at 8000 r min^{-1}. The organic phase was separated and evaporated to dryness at 40 °C in a vacuum drying oven, then reconstituted with 100 μL mobile phase. All samples were filtered through a 0.22 μm pore size filter membrane.

5 mL 0.01 mol L^{-1} $PdCl_2$ and 0.01 mol L^{-1} 6-mercaptopurine were mixed in pH 5.5, after standing for 3 h; the yellow precipitate was centrifuged off. The supernatant liquid was discarded. Vacuum drying at 35 °C for 24 h was done to get the solid sample of the product and the FTIR spectra of 6-mercaptopurine and the product were obtained, respectively.

2.4. The RLS–HPLC hyphenated instrument

The instrumental set-up is schematically illustrated in Fig. 1. After injection of the sample solution the 20 μL injection volume, the HPLC separation was achieved at 30 °C on a reverse phase column of Synergi Hydro-RP (250 mm × 4.6 mm; 4 mm), with 25:75 (v/v) methanol–water as mobile phase, the flow rate was 0.3 mL min^{-1}. From pump B, the BR buffer solution flowed into the reactor through one T-shaped interface at a rate of 0.1 mL min^{-1}. It provided an appropriate acid medium for 6-mercaptopurine anion. Meanwhile, in pump A, palladium chloride solution as the probe reagent flowed into the reactor at a rate of 0.15 mL min^{-1}. The three streams mixed in heated reactor, which would be maintained at 30 °C, the separated components from the chromatography column would associate with metal palladium (II) to form coordination complexes in the reacting tube. Finally, the fluid flowed into the detector by scanning synchronously and the RLS detection was monitored at $\lambda_{ex} = \lambda_{em} = 315$ nm.