Journal of Chromatography A, 1216 (2009) 30–35

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Study of cloud point extraction and high-performance liquid chromatographic determination of isoniazid based on the formation of isonicotinylhydrazone

Zhi-ming Zhou, Dao-yuan Zhao, Jing Wang, Wei-jun Zhao, Ming-min Yang*

College of Science, Nanjing Agricultural University, Nanjing, Jiangsu Province 210095, China

ARTICLE INFO

Article history:
Received 10 June 2008
Received in revised form
12 November 2008
Accepted 12 November 2008
Available online 18 November 2008

Keywords:
Isoniazid
Isonicotinylhydrazone
Urine sample
Cloud point extraction (CPE)
High-performance liquid chromatography
Langmuir isotherm

ABSTRACT

Isoniazid (INH) reacted with p-dimethylaminobenzaldehyde (DABD) in the presence of trichloroacetic acid to give isonicotinylhydrazone (INZ) having λ_{max} 365 nm. Cloud point extraction (CPE) is carried out to extract INH and IHZ in aqueous solutions using surfactant poly(ethylene glycol) 4000 (PEG4000), respectively. Langmuir model is used to study the adsorption behaviors of the two solutes on micelles of PEG4000. A linear correlation is found between variation of PEG4000 concentration required for feed concentration of the two solutes and used to predict PEG4000 concentration required for extracting INH and IHZ in CPE procedure. The results calculated show that, for a desired recovery level of 90%, only can IHZ be sufficiently extracted by PEG4000. In this experiment, the feed concentration of PEG4000 is defined by above-mentioned correlation, and the effects of other operating parameters, e.g., concentration of salt, pH and centrifugation time on extraction of PEG4000-IHZ system have also been studied in detail. The proposed CPE method coupled with HPLC-UV system is successfully used for the determination of INH in urine sample.

1. Introduction

Isoniazid (INH) is efficient in the treatment of tuberculosis. However, prolonged administration of this drug may be accompanied by side effects with neurotoxic manifestation [1]. The determination of INH level in human body fluid and pharmaceutical formulations is vital. In recent years, there are many reports concerning INH analysis. Spectrophotometry [2–4] is available for simple qualitative analysis of INH, but many other drugs and sample matrix may interfere with the analytical results. HPLC techniques are fast and efficient for qualitative analysis of INH. These assays used UV, fluormetric, or electrochemical detectors [5–8]. But the sensitivity of these methods is not very good. Under the reported chromatographic conditions, the methods are only sensitive enough to determine INH in the $\mathrm{mg\,L^{-1}}$ range. The tandem MS coupled with chromatographic has been reported for quantification of INH [1,9], the sensitivity of methods is in the range $10^{-3}\,\mathrm{mg\,L^{-1}}$, but the reported methods require expensive instruments. To increase sensitivity, extraction techniques have been used for pre-concentration of solute. But, traditional extraction methods not only involve the use of toxic organic solvents but also need too much time.

When concentration of an aqueous solution containing a non-ionic surfactant is higher than its critical micelle concentration (CMC), the solution can be separated into two phases. One phase contains most surfactant micelle and is called the “coacervate” phase. The other phase is dilute phase. It is found that solutes are solubilized into the interior or outer palisade layer of the micelles. Because the coacervate phase is a concentrated micelle solution, any organic solute in the water will tend to solubilize in the micelles of coacervate phase. This technique is known as cloud point extraction (CPE) [10]. Cloud point extraction has been introduced as a promising new separation and extraction technique, as an alternative to toxic organic solvents. Besides, the coacervate phase itself is compatible with micelles or hydro-organic mobile phase of HPLC system. The technique is effective for extracting solute, especially hydrophobic solute, from original matrix [11–15].

Since the micelles in water have both hydrophobic and hydrophilic parts, and the hydrophilic part surrounding the hydrophobic core, the micelles have the ability to solubilize the hydrophobic compounds into their interior. This type of interaction can be expressed by Langmuir isotherm [16].

However, the hydrophility of INH is so powerful that the micelles have not sufficient ability to solubilize INH moleculars into their interior or outer palisade layers. In this paper, the problem was overcome by a reaction of INH and p-dimethylaminobenzaldehyde (DABD) to give isonicotinylhydrazone (IHZ) with weak polar. After IHZ was extracted by CPE procedure, the coacervate phase was

---

* Corresponding author. Fax: +86 25 84395255.
E-mail address: y7mm452002@yahoo.com.cn (M.-m. Yang).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.11.028

determined with HPLC system, there was a linear relationship between concentration of INH and the signal of IHZ, the amount of INH was defined on the signal of IHZ. The method was sensitive enough to determine INH at the level of 2×10^{-3} mg L^{-1}.

In order to design a more effective CPE process and meet the economy of the CPE process, the surfactant amount required should be predicted. The performance of PEG4000-INH and PEG4000-IHZ systems was investigated at a fixed temperature, respectively. The equilibrium solubilization data of two systems followed Langmuir type isotherm. M.K.Purkait et al. reported a linear correlation between the surfactant concentrations required and various feed solute concentration when the concentration level of solute in the dilute phase was fixed [17]. However, the concentration of solute in the dilute phase is changed with initial solute and surfactant concentrations [18,19]. It is reported that, beyond the surfactant concentration, the surfactant partition coefficient (defined as the ratio of the concentration of surfactant in the coacervate phase to that in dilute phase) remains almost constant with increasing surfactant concentration [20]. As the partition coefficient remains unchanged, the ratio of solute concentration in the two phases should be almost constant. Consequently, the extent of extraction should become nearly constant. Therefore, when the amount of surfactant required was calculated with Langmuir isotherm by fixing the desired extraction efficiency or fixing solute concentration level in the dilute phase, respectively, it is obviously that the former one is more reasonable.

In this study, with the knowledge of initial INH concentration, the desired recovery level of INH, the calculated isotherm constants *m* and *n*, Langmuir isotherm was developed to quantify the amounts of PEG4000 required in PEG4000-INH and PEG4000-IHZ systems, respectively. According to the calculated results, only in PEG4000-IHZ system can IHZ be efficiently extracted, and, a CPE method coupled with HPLC-UV was proposed to determinate INH in urine.

2. Experimental

2.1. Reagents and solutions

INH was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Stock solution containing 100 μg mL^{-1} of isoniazid was prepared with distilled water and stored at 4 °C.Trichloroacetic acid was obtained from LingFeng Reagent Co. (Shanghai, China), and diluted with distilled water to 0.2 mol L^{-1}. DABD was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). PEGs (molecular masses 600, 1000, 4000 and 20 000) were obtained from Guangdong Guanghua Chemical Factory (Guangdong, China), and dissolved with distilled water to obtain stock solution of 30% (w/v). Na_2SO_4, $(NH_4)_2SO_4$, $MgSO_4$, $ZnSO_4$ were purchased from Nanjing Reagent Plant (Jiangsu, China). Acetonitrile was of HPLC-grade, and all other chemicals were of analytical grade.

2.2. Apparatus

All analysis was performed on a chromatographic system (Shimadzu, Tokyo, Japan) consisted of two delivery pumps (model LC-20AT), a photodiode array detector (model SPD-M20A) and a system controller. The stationary phase column was a LiChrospher C18 (250 mm × 4.6 mm, 5 μm particles). A centrifugal machine from Changsha Pingfan Instrument and Meter Co. (Hunan, China) was used to accelerate phase separation. The ultrasonication from Kunshan Ultrasonic Instrument Plant (Jiangsu, China) was used to degassing.

2.3. Preparation of phase diagrams

The phase behaviors for PEG-salt systems were studied at 25 °C by observing the salt concentration required for clarification of the solution or the onset of turbidity. Na_2SO_4 was used as phase-separation salt, and PEG600, PEG1000, PEG4000 and PEG20 000 were selected as phase-separation polymers. To prepare phase diagrams of PEGs- Na_2SO_4 systems, the PEGs of 20% (w/v) were put into a centrifugal tube, Na_2SO_4 of known concentration was then added dropwise to the tube and the mixtures were vortexed after addition of each drop of Na_2SO_4 until a cloudy appearance was noted and a two-phase system was ultimately obtained. The composition of these mixtures was determined. Then, water was added dropwise to the tube to get a clear one-phase system and more Na_2SO_4 was added again to form a two-phase system. Cycles for the formation of two-phase system were repeated to obtain the final phase diagram.

2.4. Derivatization experiments

1.0 mL volume of standard solution of isoniazid and 0.5 mL of 0.2 mol L^{-1} DABD were taken in a 10.0 mL centrifugal tube, opportune amount of 0.2 mol L^{-1} trichloroacetic acid solution and enough water were added so that the final solution was 3.0 mL and pH at 2. The solution was kept for 5 min for complete formation of IHZ.

2.5. Adsorption character studies

A series of solutions were prepared with a certain concentration of PEG4000 and different concentration solutes. 1.0 g Na_2SO_4 was added to the solutions. After Na_2SO_4 was dissolved, the solutions were centrifuged at 3000 rpm for 5 min in order to complete phase separation. The volumes of the coacervate phases were measured and concentrations of solute in dilute phase were detected by HPLC method at room temperature (25 °C).

2.6. Cloud-point extraction procedure

3.0 mL solution prepared as in Section 2.4 was adjusted with NaOH solution of 0.2 mol L^{-1} to pH 7.0, followed by the addition of 0.5 mL of 30% (w/v) PEG4000 and 1.0 g Na_2SO_4. The mixture was diluted to 5.0 mL, and then was stirred for 5 min in a vortex. The complete separation of the two phases was achieved by centrifugation for 5 min at 3000 rpm. The lower water phase was carefully removed by using a syringe with a long needle that passed through the upper coacervate phase. The coacervate phase was left in the tube and diluted to 0.5 mL with mobile phase. Therefore, a pre-concentration of 10 times was finally obtained.

2.7. Pre-concentration of isoniazid from urine samples

Urine samples of a tubercular patient were collected 10 h after administration of the drugs (50 mg). Aliquots of 5 mL of urine samples were diluted to 50 mL with distilled water. The dilute urine sample was filtered through 0.45 μm membrane and then treated according to the procedures described in Sections 2.4 and 2.6. After phase separation, a 20-μL volume of coacervate phase was injected into the HPLC system.

2.8. Liquid chromatographic analysis

A 20-μL volume of the coaceravate phase was injected into the HPLC system for analysis. In the study of the partition behaviors of INH, the mobile phase consisted of acetonitrile-water (5:95, v/v), the flow rate was of 0.8 mL min^{-1} and the column effluent was monitored at 264 nm. In the determination of IHZ, the mobile