Available online at www.sciencedirect.com

SciVerse ScienceDirect

Chinese Chemical Letters 23 (2012) 715–718

CHINESE CHEMICAL LETTERS

www.elsevier.com/locate/cclet

Determination of zidovudine using anion exchange chromatography with integrated pulsed amperometric detection

Yu Chen [a], Yu Xiu Liu [b], Zhi Dong Chen [b,*], Mei Lan Chen [c], Yan Zhu [d]

[a] *Hangzhou Vocational and Technical College, Hangzhou 310018, China*
[b] *School of Chemistry and Chemical Engineering, Changzhou University, Changzhou 213164, China*
[c] *College of Biology and Environment Engineering, Zhejiang Shuren University, Hangzhou 310015, China*
[d] *Department of Chemistry, Zhejiang University, Hangzhou 310028, China*

Received 15 February 2012
Available online 11 May 2012

Abstract

A rapid and practical method for direct detection of zidovudine in high performance anion exchange chromatography (HPAEC) has been developed with integrated pulsed amperometric detection (IPAD). Dionex AS18 (250 mm × 2 mm) and AG18 (50 mm × 2 mm) columns and 11 mmol/L NaOH solution were used for separation. Multi-step potential waveform parameters were optimized to maximize the signal-to-noise ratio (S/N). Utilizing an optimized waveform, the repeatability (intra-day) precision and intermediate (inter-day) precision are obtained with relative standard deviation (RSD) of 1.88, 2.27, respectively. The limit of quantification (LOQ) and limit of detection (LOD) were found to be 9.70, 3.0 ng/mL, respectively, with correlation coefficients of 0.9992 over concentration range 0.01–10 μg/mL. The present method was successfully applied to the determination of zidovudine in human plasma. The recoveries of plasma sample spiked by 0.7 μg/mL, 2.7 μg/mL obtained were 95.3–101.5%, with relative standard deviation (RSD) of 2.54%, 2.21%, respectively.

Keywords: Zidovudine; AIDS; Integrated pulsed amperometry; High performance anion exchange chromatography; Plasma

The first agents shown to be safe and effective for the treatment of patients infected with human immunodeficiency virus (HIV) were nucleoside reverse transcriptase inhibitors (NRTI). 3-Azido-3-deoxythymidine (Zidovudine, AZT) is the first one of them used in the management of AIDS and AIDS-related complex [1].

A variety of analytical methods has been developed for the determination of AZT in biological samples, most of them have been achieved using high-performance liquid chromatography with UV [2–7], HPLC–MS/MS [8,9] and HPLC–MS [10–12] were also developed for the determination. Ratio-spectra derivative spectrophotometric method [13], micellar electrokinetic chromatography [14] high performance thin layer chromatographic [15], fluorescence spectroscopy [16], and immunoassay [17] have also been reported for AZT.

The LC/UV methods were used to quantitative zidovudine alone or analyze it simultaneously with several NRTIs or to follow its degradation products. However in all the LC/UV methods, plasma volume requirement was high, chromatographic run time was longer and sensitivity was inadequate for pharmacokinetic studies in novel drug

* Corresponding author.
E-mail address: czd_chen@yahoo.com.cn (Z.D. Chen).

1001-8417/$ – see front matter
http://dx.doi.org/10.1016/j.cclet.2012.04.009

formulations. Immunoassays are sensitive enough but are not selective and specific methods. LC with mass spectrometric detection was widely applied to determination in biological fluids. Being highly sensitive methods, nevertheless still require sophisticated equipments that were not available in many laboratories.

Herein a rapid and validated method for the quantification of zidovudine in plasma was reported. AZT is a polar compound, and is present as an anion under alkaline conditions because of its p*K*a is 9.68 [18]. Therefore, it can be separated by anion-exchange chromatography; moreover, for the groups it contains, it is expected to be directly oxidized at gold electrodes under alkaline conditions. Pulsed amperometric detection was developed for polar aliphatic compounds which can be oxidized at electrodes. In this study, integrated pulsed amperometric detection (IPAD) was applied to detect AZT in high performance anion exchange chromatography (HPAEC). It was demonstrated that this method was a rapid and simple one for the determination of AZT in real sample.

1. Experimental

A DX-600 ion chromatography system consisting of a GP50 gradient pump and a LC 25 chromatography oven was connected with an ED50A electrochemical detector was used in this study. Dionex AS18 (250 mm × 2 mm) and AG18 (50 mm × 2 mm) columns packed with anion-exchange resin were used as the separation columns. The concentration of NaOH solution used as eluent was 11 mmol/L. The analysis was performed at 30 °C with the flow rate set at 0.25 mL/min. In all analyses, 10 μL was injected. The detection was carried out by a pulsed amperometry cell equipped with a working gold electrode and a combined pH–Ag/AgCl reference electrode.

Aliquots of 400 μL plasma were diluted with 400 μL water in a 4 mL polypropylene tube. 800 μL acetonitrile was then added. The mixtures were vortex mixed for about 60 s then centrifuged at 10,000 rpm for 10 min, clear supernatant were pipetted and was evaporated under stream of nitrogen at about 30 °C to almost dryness and the volume was completed to 400 μL with water. The sample was then loaded to the solid phase extraction (SPE) cartridge. The cartridge was preconditioned by washing with 10 mL water, 5 mL acetonitrile then another 10 mL water. After loading the sample, the cartridge was washed with 2 mL water then dried by using airflow under vacuum. The sample was filtered through a 0.45 μm syringe filter then diluted 1:100 with water, 10 μL was directly injected to the system.

2. Results and discussion

Waveform optimization was performed by injecting a standard solution of zidovudine and plotting the amperometric signal-to-noise ratio as a function of the parameter to be optimized. The waveform consists of three distinct regions divided by adsorption/initiation, E1, E2; current integration, E3, E4; and cleaning/activation, E5, E6. E1, E2 and E3 are factors affecting the detection sensitivity, and therefore were especially examined.

First, E1 was varied from −0.6 to −0.25 V in 0.05 V increments, the maximum response for zidovudine was −0.5 V, a potential value of −0.5 V gave the best compromise and was chosen as for E1. With E1 at −0.50 V, E2 was evaluated between −0.45 and −0.25 V, the maximum responses for zidovudine were about −0.40 V, a preferred value for E2 was −0.40 V. With E1 at −0.50 V and E2 at −0.40 V, the optimization of the E3 in the range from 0.00 to 0.08 V. The optimal value of E3 was found to be 0.01 V.

The potential step duration also exerted a strong influence on the amperometric response. With E1, E2 and E3 at their optimal values, T1, T3 were optimized by a series of separate experiments. When T1 was varied from 20 to 60 ms in 10 ms increments, the maximum responses for zidovudine was 30 ms, and was chosen as for T1·T3 was modified from 200 to 300 ms, it was found that 260 ms had the optimal signal-to-noise ratio for detection response and baseline stability.

The following working pulse potentials and durations were used for detection: E1 = −0.50 V (T1 = 30 ms); E2 = −0.40 V (T2 = 160 ms); E3 = 0.01 V (T3 = 260 ms); E4 = −0.4 V (T4 = 90 ms); E5 = 0.55 V (T5 = 10 ms); E6 = −0.20 V (T6 = 10 ms). The output range on the detector was set to 1 μA.

The calibration curve was constructed by using the chromatographic peak areas from triplicate injection of standards at six increasing concentrations in the 0.01–1 μg/mL range. The response for the tested compound was linear ($R^2 > 0.9992$) in the concentration range studied with the calibration curve $y = 0.9887x + 0.0836$.

The repeatability (intra-day) precision was performed by analyzing reference standard solution of a concentration of 0.1 μg/mL during 1 day, and intermediate (inter-day) precision was performed at different days under the same