Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 824 (2005) 327–332
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of thiopental in urine sample with high-performance liquid chromatography using iodine–azide reaction as a postcolumn detection system

Robert Zakrzewski *, Witold Ciesielski

Department of Instrumental Analysis, University of Łódź, Pomorska 163, 90-236 Łódź, Poland

Received 25 April 2005; accepted 19 July 2005

Abstract

The reaction between iodine and azide ions induced by thiopental was utilized as a postcolumn reaction for chromatographic determination of thiopental. The method is based on the separation of thiopental on an Nova-Pak® CN HP column with an acetonitrile–aqueous solution of sodium azide as a mobile phase, followed by spectrophotometric measurement of the residual iodine ($\lambda = 350$ nm) from the postcolumn iodine–azide reaction induced by thiopental after mixing an iodine solution containing iodide ions with the column effluent containing azide ions and thiopental. Chromatograms obtained for thiopental showed negative peaks as a result of the decrease in background absorbance. The detection limit (defined as S/N = 3) was 20 nM (0.4 pmol injected amount) for thiopental. Calibration graphs, plotted as peak area versus concentrations, were linear from 40 nM. The elaborated method was applied to determine thiopental in urine samples. The detection limit (defined as S/N = 3) was 0.025 nmol/ml urine. Calibration graphs, plotted as peak area versus concentrations, were linear from 0.05 nmol/ml urine. Authentic urine samples were analyzed, thiopental was determined at nmol/ml urine level.

Keywords: Iodine–azide reaction; Thiopental; HPLC

1. Introduction

Thiopental, 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid is an ultrashort-acting intravenous anesthetic [1]. It is frequently used clinically in the treatment of intensive care patients suffering from severe head injuries and in the management of intracranial hypertension [2]. A fatal case involving mixtures of chemicals including thiopental [3], a suicide by thiopental injection [4,5] and drug-facilitated sexual assault [6] were reported. Monitoring its concentration in body fluids is important also for the optimization of pharmacotheraphy. Although thiopental is typically excreted in urine at high percentage in metabolized form, there remains about 1% of the unchanged drug which can be highly useful for screening and identification of the thiopental [7–9].

High-performance liquid chromatography has been well established as an analytical technique for determination of a wide range of drugs in variety of complex matrices. Several high-performance liquid chromatographic methods have already been reported for determination of thiopental in plasma or serum using UV detection around $\lambda = 280$ nm [10–16] or mass spectrometry [8]. Main practical problems in sample treatment prior to drug analysis in biological fluids are related with matrix elimination, which has to carried out as preclean chemistry protocol in order to avoid sticking the column by protein and to obtain low process blanks which demand mobile phase systems of high purity and good sample clean-up of the biological sample. The postcolumn photochemical reaction was also applied for determination of the drug but it cannot be detected with sufficient sensitivity

* Corresponding author. Tel.: +48 42 635 58 08; fax: +48 42 635 58 08.
E-mail address: robzak@chemul.uni.lodz.pl (R. Zakrzewski).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.07.028

[17]. The described photoreactor does not decrease column efficiency.

The iodine–azide reaction induced selectively by sulphur(II) compounds is widely used for their determination by varied analytical techniques such as measurement of nitrogen evolved during the reaction, coulometry, flow injection analysis, titrimetry, enthalpimetry, and kinetic methods with amperometric or spectrophotometric detection. Lately, the iodine–azide reaction has been applied as postcolumn reaction detection system [18,19]. The determination is based on the separation of sulphur(II) compounds with chromatographic column and then measurement ($\lambda = 350$ nm) of the unreacted iodine in the iodine–azide reaction. The reaction takes place only in the presence of a sulphur(II) compound (selective induction). When constant concentrations of iodine solution in a postcolumn reagent and azide ions in the mobile phase are supplied to the HPLC system, a constant absorbance is maintained and recorded as a background from iodine absorption. The signal decreases when a sulphur(II) compound appears in the sample, due to consumption of iodine in the iodine–azide reaction. The induction activity is detected as a negative peak photometrically at $\lambda = 350$ nm. The peak area are proportional to the amount of the sulphur(II) compound.

For the determination of thiopental in urine samples, it is necessary to use a selective and sensitive detection system such as iodine–azide reaction because of the low level of thiopental present in urine and also to prevent interference from the complex sample matrix. In this study, we describe a simple, rapid, reliable and specific method for determination of thiopental (as a standard solution and in urine sample) based on high-performance liquid chromatography and iodine–azide reaction as the detection system which combines both the specificity and selectivity of chromatographic separation and iodine–azide reaction as the postcolumn detection system. The sample preparation we present has two advantages: it is a one step process and no internal standard is required.

2. Experimental

2.1. Chromatographic system

A flow diagram of the chromatographic system used in this study is shown in Fig. 1. The analytical column was a Nova-Pak® CN HP (150 mm × 3.9 mm i.d., 5 μm, Waters). The flow-rate was 1 ml/min at ambient temperature. Eluate from the Postcolumn Reaction Module (Waters) was monitored at a wavelength of $\lambda = 350$ nm using a variable wavelength LC spectrophotometer (Waters 2487 Dual λ). The mobile phase delivery system was a Multisolvent Delivery System Model 600E (Waters). Test samples were applied to the HPLC column with a Rheodyne 7725i injector. Reagent Manager (Waters) was used as a single-piston, pulse-dampened pumping system for postcolumn reagent delivery to the Postcolumn Reaction Module (the reaction tube, 4 m × 0.25 mm i.d.) (Waters). The temperature of the postcolumn iodine–azide reaction was controlled by temperature control system (Waters). The chromatographic system and recorder were connected with busSaT/In Module (Waters). The chromatograms were integrated with Millennium^{32} software (Waters).

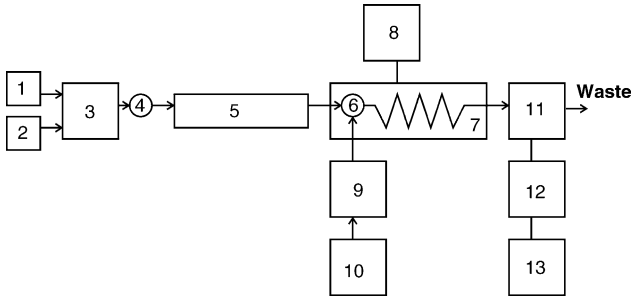

Fig. 1. Flow diagram system with iodine–azide procedure detection. (1): sodium azide solution, (2): acetonitrile, (3): pump, (4): injector valve, (5): analytical column, (6): mixing tee, (7): postcolumn reaction module, (8): temperature control system, (9): pump, (10): iodine solution in potassium iodide solution, (11): LC spectrophotometer, (12): busSaT/In module and (13): computer.

2.2. Chemicals and solutions

Thiopental was purchased from Biochemie (Kundl, Austria). Sodium azide, hydrochloric acid, arsenic(III) oxide, iodine, potassium iodide, ethylenediaminetetraacetic acid disodium salt (EDTA), HPLC-grade methanol and acetonitrile were obtained from Aldrich (Steinheim, Germany) or LAB-SCAN Analytical Sciences (Dublin, Ireland).

All the solutions were freshly prepared daily. The water used in preparation of solutions was triple distilled and then degassed.

2.2.1. Solution

A stock thiopental solution: 100 μmol thiopental was dissolved in 1 ml 1 M sodium hydroxide solution and diluted to 100 ml with water. Working standard thiopental solutions (20 nM–1 μM) were prepared by appropriate serial dilution of stock solution with water. A sodium azide solution: 1.5 g sodium azide was dissolved in water and hydrochloric acid was added to obtained pH 7.8 and then was adjusted to 0.5 l with water. An iodine solution: 6.3 g iodine and 20 g potassium iodide were dissolved and were adjusted with water to 0.5 l. To 200 μl of the solution mentioned above 0.657 g of potassium iodide was added and diluted with water to 0.2 l.

2.3. Sample collection of urine and processing

To 4 ml of urine, 0.75 ml 0.1 M EDTA and specified amount of thiopental were added and then diluted with methanol to 10 ml. The sample was processed further as in Section 2.4.