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Short communication

Determination of 2,3-benzodiazepine derivatives in rat plasma by high-performance liquid chromatography

M. Rizzo[a,*], G. De Sarro[b], R. Gitto[c], M. Zappalà[c], A. Chimirri[c]

[a]*Chair of Chemistry, School of Pharmacy, University of Catanzaro, 88021 Catanzaro, Italy*

[b]*Chair of Pharmacology, Department of Experimental and Clinical Medicine, School of Medicine, University of Catanzaro, Catanzaro, Italy*

[c]*Department of Medicinal Chemistry, School of Pharmacy, University of Messina, Messina, Italy*

Abstract

A simple high-performance liquid chromatographic method with ultraviolet detection at 240 nm for determination of a novel AMPA/kainate antagonist 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-2,3-benzodiazepine (2,3-BZ 6), and its derivatives in rat plasma is described. The procedure involves a fast extraction of the drugs from the plasma spiked with an internal standard. The samples are applied to a pre-packed glass column and drugs are eluted using ethyl acetate. A linear response was observed over the examined concentration range. The lower limit of detection of 2,3-BZ 6 was 5.5 ng/ml. The assay has been used to determine the time course of plasma levels of the 2,3-benzodiazepine derivatives in Sprague–Dawley rats.

Keywords: Benzodiazepines

1. Introduction

Recently, we synthesized a series of 1-aryl-3,5-dihydro-7,8-dimethoxy-2,3-benzodiazepines (2,3-BZs), chemically similar to GIKY 54266 that were proven to possess anticonvulsant activity in various models of seizures acting as non-competitive antagonists at the α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor [1,2]. The most active compound of the series was 1-(4′-aminophenyl)-derivative (2,3-BZ 6), which showed lower toxicity and longer lasting action than GIKY 52466.

We developed a simple high-performance liquid chromatographic (HPLC) assay to determine 2,3-BZ 6 and its derivatives 2,3-BZ 6Me, 2,3-BZ 5 and 2,3-BZ 5Me (Fig. 1) in rat plasma. The method was suitable for pharmacokinetic studies of 2,3-BZs in Sprague–Dawley rats.

2. Experimental

2.1. Chromatography

A Beckman System Gold 125 HPLC system with a 166 UV detector was used; detection was monitored at 240 nm. A Partisil 10 ODS (250×4.6 mm I.D., particle diameter 10 μm) column with an ODS guard column was used. The mobile phase was CH_3CN–0.01 *M* CH_3COONa (35:65, v/v, pH 5.25) at a flow-rate of 2 ml/min.

*Corresponding author. Tel.: +39-961-391-131; fax: +39-961-391-490.

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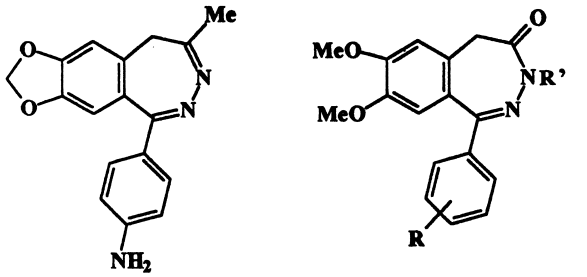

Fig. 1. Structures of GYKI 52466 and 2,3-BZs.

2.2. Extraction

A 0.5-ml aliquot of plasma was mixed with 0.1 ml of 2 *M* NaOH and 0.1 ml of 2,3-BZ 2Me (10 μg/ml) as I.S. was added. The sample was applied to Extrelut 1 (E. Merck, Darmstadt, Germany), a prepacked glass column. After 10 min ethyl acetate (6 ml) was added. The eluate was collected and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.1 ml of mobile phase and was injected into the chromatographic system.

3. Results

3.1. Linearity

A linear response was observed over the examined concentration range (0.05–2 μg/ml). The linear regression coefficient is 0.996 for 2,3-BZ 6 ($y=0.64x+0.118$); 0.969 for 2,3-BZ 6Me ($y=0.91x+0.004$); 0.970 for 2,3-BZ 5 ($y=1.34x+0.013$) and 0.999 for 2,3-BZ 5Me ($y=2.36x-0.005$), respectively.

3.2. Detection and recovery

The lower limit of detection was 5.5 ng/ml 2,3-BZ 6, 6.5 ng/ml for 2,3 BZ 6Me, 7 ng/ml for 2,3-BZ 5 and 8.5 ng/ml for 2,3-BZ 5Me. The calculated extraction recovery from plasma ranged from 74.36 to 93.75%.

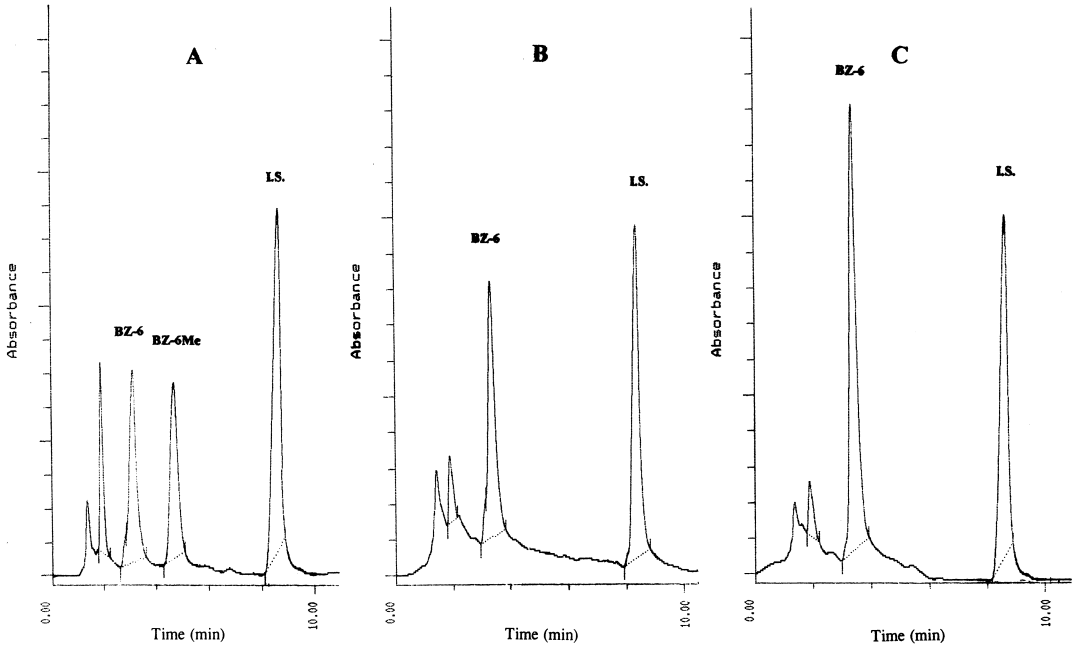

Fig. 2. Representative chromatograms of rat plasma spiked with 2,3-BZ 6 (0.5 μg/ml), 2,3-BZ 6Me (0.5 μg/ml) and I.S. (1 μg/ml) (A), and plasma sample obtained 15 min (B) and 45 min (C) after i.p. administration of 2,3-BZ 6 to rats.

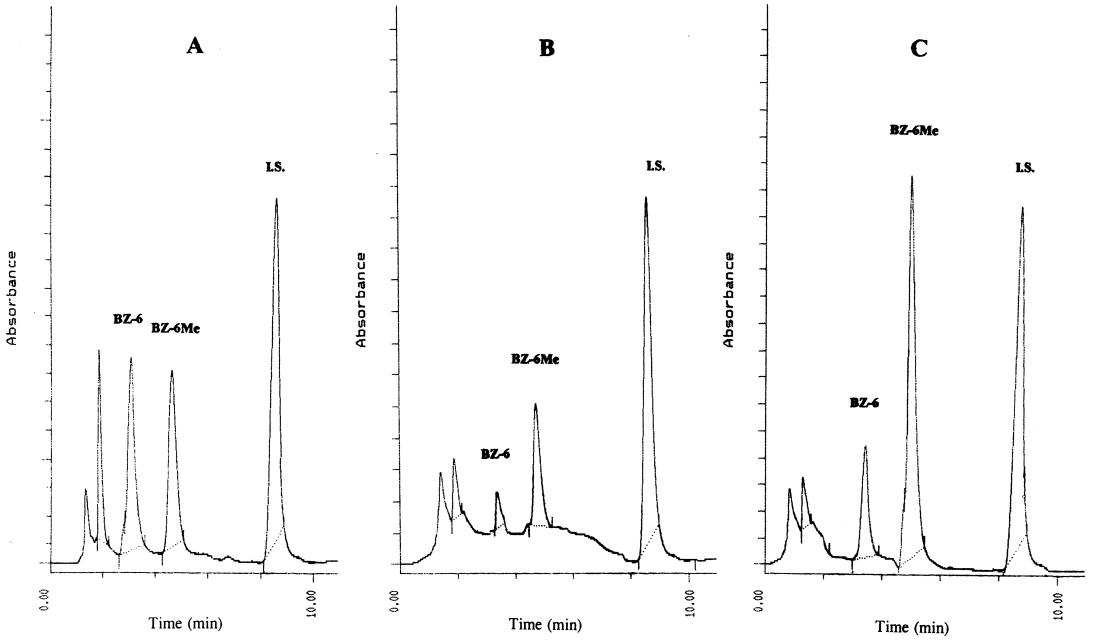

Fig. 3. Representative chromatograms of rat plasma spiked with 2,3-BZ 6 (0.5 μg/ml), 2,3-BZ 6Me (0.5 μg/ml) and I.S. (1 μg/ml) (A) and plasma sample obtained 15 min (B) and 45 min (C) after i.p. administration of 2,3-BZ 6Me to rats.

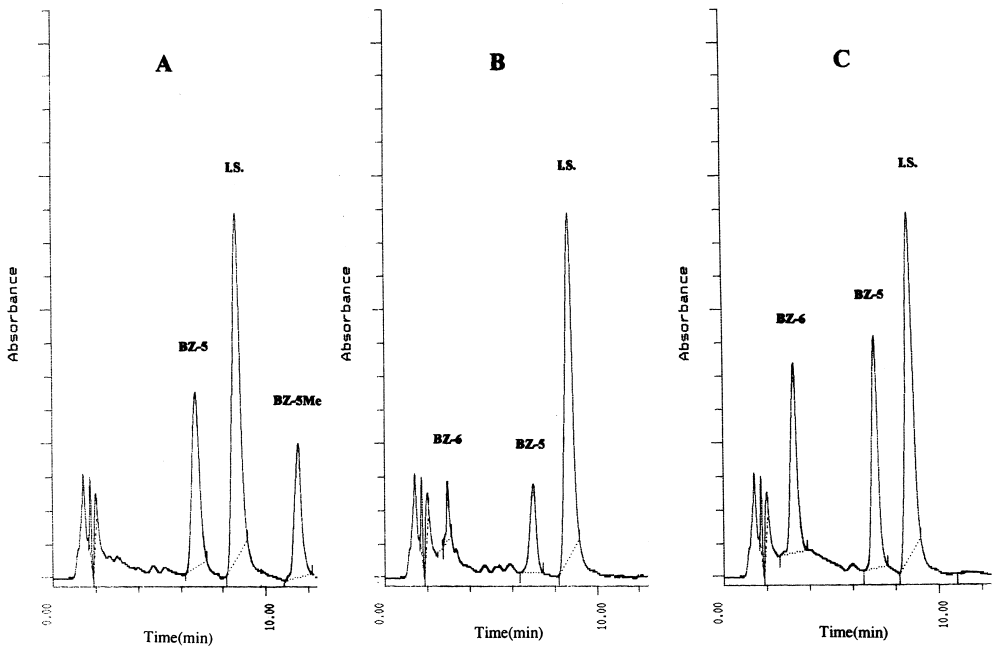

Fig. 4. Representative chromatograms of rat plasma spiked with 2,3-BZ 5 (0.5 μg/ml), 2,3-BZ 5Me (0.5 μg/ml) and I.S. (1 μg/ml) (A), and plasma sample obtained 30 min (B) and 75 min (C) after i.p. administration of 2,3-BZ 5 to rats.

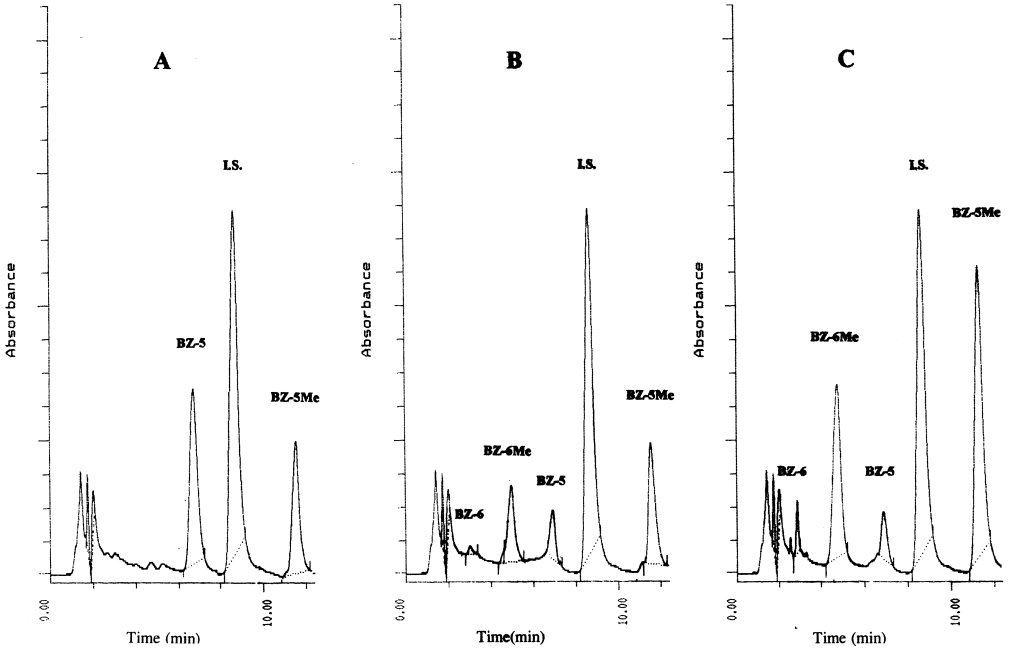

Fig. 5. Representative chromatograms of rat plasma spiked with 2,3-BZ 5 (0.5 μg/ml), 2,3-BZ 5Me (0.5 μg/ml) and I.S. (1 μg/ml) (A), and plasma sample obtained 30 min (B) and 75 min (C) after i.p. administration of 2,3-BZ 5Me to rats.

3.3. Precision and accuracy

The intra-assay accuracy for all compounds ranged from 92 and 105.5%, whereas the intra-assay precision ranged from 0.59 to 8.16%. The inter-assay precision was 2.17 to 11.9% in rat plasma.

3.4. Chromatograms

Fig. 2 shows the representative chromatograms of 2,3-BZs and I.S. after administration of 2,3-BZ 6.

Fig. 3 shows the representative chromatograms of 2,3-BZs and I.S. after administration of 2,3-BZ 6Me.

Fig. 4 shows the representative chromatograms of 2,3-BZs and I.S. after administration of 2,3-BZ 5.

Fig. 5 shows the representative chromatograms of 2,3-BZs and I.S. after administration of 2,3-BZ 5Me.

4. Conclusions

This report describes the methodology and validation of a HPLC–UV assay for the simultaneous determination of 2,3-BZ 6, 2,3-BZ 6Me, 2,3-BZ 5 and 2,3-BZ 5Me in rat plasma. The method is rapid and simple. The extraction procedures demonstrate excellent efficiency. The sensitivity of the method allowed to study the pharmacokinetics of these 2,3-BZs. Demethylation of 2,3-BZ 6Me and 2,3-BZ 5Me was indicated, as described before [3]. Moreover, our study suggests that 2,3-BZ 6Me, 2,3-BZ 5 and 2,3-BZ 5Me are prodrugs of 2,3BZ 6, in which they are biotransformed in vivo through different metabolic pathways.

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