Determination of Olanzapine and Desmethylolanzapine in the Plasma of Schizophrenic Patients by Means of an Improved HPLC Method with Amperometric Detection

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Key Words

Column liquid chromatography
Amperometric detection
Olanzapine in plasma
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Summary

An improved HPLC method with electrochemical detection has been developed for the determination of olanzapine and its main metabolite, desmethylolanzapine, in human plasma. Chromatographic separation and analysis were performed on a C18 reversed-phase column with a mixture of methanol, acetonitrile, and pH 3.7 phosphate buffer as mobile phase; 2-methylolanzapine was used as internal standard. Careful pretreatment of the plasma samples was implemented by means of solid phase extraction (SPE).

Response was linearly dependent on concentration and precision was satisfactory over the concentration range 0.5-75.0 ng mL⁻¹ for both analytes. The limit of detection was 0.2 ng mL⁻¹ for both analytes. Application to plasma samples of patients treated with Zyprexa tablets gave good results. Because of its sensitivity and selectivity, and the need for small plasma samples, this method seems to be a useful tool for clinical monitoring.

Introduction

Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine, Figure 1a, is an atypical antipsychotic drug recently introduced commercially by Eli Lilly. It was introduced in Italy at the end of 1998 and its use is now widespread for the therapy of schizophrenic patients, particularly those who do not respond to classical neuroleptics. Olanzapine is metabolized mainly in the liver [1], the main metabolite being the 10-N-glucuronide. The cytochrome P450 system is involved in the formation of other metabolites: the isofoms CYP1A2 and CYP2D6 are involved in the formation of 4'-N-desmethylolanzapine (Figure 1b) and 2-hydroxymethylolanzapine [2], respectively. Further investigation of the metabolism of olanzapine would be very important because its bioavailability and pharmacokinetics are not completely understood [3]. Olanzapine is administered as Zyprexa tablets, usually at very low dosages (2-20 mg day⁻¹) [4] and the resulting plasma concentrations of olanzapine and its metabolites are in the few nanograms per milliliter range; very sensitive monitoring methods are, therefore, needed.

Although several papers describing the analysis of olanzapine can be found in the literature [3, 5-12], only one describes the simultaneous determination of olanzapine and desmethylolanzapine (in rat plasma).

Figure 1. The chemical structures of (a) olanzapine, (b) desmethylolanzapine, and (c) 2-methylolanzapine (I. S.).
methylolanzapine, and 2-methylolanza-
pine were 1 mgmL 1 in methanol, and
were stable for at least two months when
stored at −20 °C. Working solutions were
prepared every day by dilution of the stock solutions with the mobile phase.

**Apparatus and Chromatographic Conditions**

HPLC was performed with a Varian (Harbor City, CA, USA) model 9002 isocratic pump and an Antec (DB Leiden, The Netherlands) Decade amperometric de-
tector (glassy carbon working electrode, Ag/AgCl reference electrode, and 316 stainless-steel auxiliary electrode). The analytical cell was set at +800 mV. Compounds were separated on a 150 mm × 4.6 mm i.d., 5 µm particle, Microsorb Rainin C18 reversed-phase column protected with a 4 mm × 3.0 mm i.d., 5 µm particle, Phenomenex SecurityGuard C18 precolumn. The mobile phase was a mixture of methanol (11%), acetonitrile (9.7%), and 8.9 mmol L−1 phosphate buffer (79.3%), containing 7.18 mmol L−1 triethylamine. The final apparent pH of the mixture was adjusted to 3.7 with phosphoric acid. The flow rate was 0.7 mL min −1 and the injection loop was 20 µL. The mobile phase was filtered through Sartorius (Goettingen, Germany) nylon filters (diameter 47 mm, pore size 0.2 µm). The column and the analytical cell were thermostatted at 30 °C.

**Method Validation**

**Extraction Yield**

Analyte standard solutions (25 µL) of three different concentrations (to furnish plasma concentrations of 6, 12.5, or 50 ng 

**Precision**

The assays described under “Extraction Yield” were repeated six times within the same day to determine the repeatability (intraday precision) of the method and six times on different days to determine the intermediate precision (interday precision) of the method. These assays were