



RESEARCH ARTICLE

A SIMPLE AND SENSITIVE METHOD FOR THE DETERMINATION OF BACLOFEN IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY TANDEM-MASS SPECTROMETRY

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A simple and sensitive method for determining baclofen in human plasma using ethanol precipitation followed by LC/MS/MS detection is described. Methyl paraben was used as internal standard. This method is more cost effective and simple compared to the existing SPE method. Chromatographic separation was achieved on a C-18 column (100 × 2.1 mm and 3.5 μm) with a gradient elution of mobile phase consisting of 10 mM ammonium formate containing 0.1% formic acid and acetonitrile. The method is linear over a range of 5.0 to 500.0 ng/ml concentration with an extraction efficiency of about 95% and is particularly suitable for pharmacokinetic studies.

Key words: Baclofen, Tandem-mass spectrometry, Method validation.

INTRODUCTION

Baclofen is used as a skeletal muscle relaxant and in the treatment of reversible spasticity resulting from multiple sclerosis (Sweetman, 2002). Baclofen is rapidly absorbed from gastrointestinal tract and the peak plasma concentration is achieved within about 2 h. A sensitive and selective bio-analytical method is required for determining the drug concentration in the plasma. Different methods were proposed for the determination of baclofen in plasma. A number of HPLC methods (Millerioux *et al* 1996; Ban *et al* 2005; Spahn *et al* 1988; Tosunoglu and Ersoy, 1995) and capillary electrophoresis methods (Chiang *et al* 2000; Kowalski *et al* 2004; Chang and Yang, 2003) have been used for separation. UV detection commonly used in HPLC has low sensitivity and also require lengthy extraction, cleanup procedures for analysis of plasma samples. HPLC and capillary electrophoresis techniques with pre-column derivatization followed by fluorescence detection are also used. O-phthalaldehyde-*tert*-

butanethil (Ban *et al* 2005), 4-chloro-7-nitro-benzofuran (Tosunoglu and Ersoy, 1995), naphthalene-2, 3-dicarboxaldehyde (Chiang *et al* 2000) and anthracene-2, 3-dicarboxaldehyde (Chang and Yang, 2003) are used as derivatizing agents.

Compared to the extensive HPLC and capillary electrophoresis methods, a very few LC-tandem MS detection methods are reported for the determination of baclofen and other drugs in biological fluids (Flardh and Jacobson, 1999; Miksa and Poppenga, 2003; Chhabra *et al* 2012). A solid-phase extraction followed by tandem mass spectrometry is used to determine baclofen in human plasma (Flardh and Jacobson, 1999) and in bovine serum (Miksa and Poppenga, 2003).

The aim of the present work was to develop and validate a simple, sensitive and selective method for determination of baclofen in human plasma using protein precipitation followed by LC/MS/MS detection and its application to pharmacokinetic studies.