Journal of Pharmaceutical and Biomedical Analysis 88 (2014) 36–44

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Quantification of typical antipsychotics in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry for therapeutic drug monitoring

Julieta Gradinaru [a], Astrid Vullioud [a], Chin B. Eap [a,b], Nicolas Ansermot [a,*]

[a] Unit of Pharmacogenetics and Clinical Psychopharmacology, Center for Psychiatric Neurosciences, Department of Psychiatry, Lausanne University Hospital, Hospital of Cery, 1008 Prilly-Lausanne, Switzerland
[b] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland

ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form 29 July 2013
Accepted 30 July 2013
Available online 7 August 2013

Keywords:
Antipsychotics
Protein precipitation
UHPLC-MS/MS
Method validation
Therapeutic drug monitoring

ABSTRACT

A selective and sensitive method was developed for the simultaneous quantification of seven typical antipsychotic drugs (*cis*-chlorprothixene, flupentixol, haloperidol, levomepromazine, pipamperone, promazine and zuclopenthixol) in human plasma. Ultra-high performance liquid chromatography (UHPLC) was used for complete separation of the compounds in less than 4.5 min on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm; 1.7 μm), with a gradient elution of ammonium formate buffer pH 4.0 and acetonitrile at a flow rate of 400 μl/min. Detection was performed on a tandem quadrupole mass spectrometer (MS/MS) equipped with an electrospray ionization interface. A simple protein precipitation procedure with acetonitrile was used for sample preparation. Thanks to the use of stable isotope-labeled internal standards for all analytes, internal standard-normalized matrix effects were in the range of 92–108%. The method was fully validated to cover large concentration ranges of 0.2–90 ng/ml for haloperidol, 0.5–90 ng/ml for flupentixol, 1–450 ng/ml for levomepromazine, promazine and zuclopenthixol and 2–900 ng/ml for *cis*-chlorprothixene and pipamperone. Trueness (89.1–114.8%), repeatability (1.8–9.9%), intermediate precision (1.9–16.3%) and accuracy profiles (<30%) were in accordance with the latest international recommendations. The method was successfully used in our laboratory for routine quantification of more than 500 patient plasma samples for therapeutic drug monitoring. To the best of our knowledge, this is the first UHPLC-MS/MS method for the quantification of the studied drugs with a sample preparation based on protein precipitation.

1. Introduction

Typical antipsychotics (first generation), developed many years ago, are still widely used for treatment of schizophrenia and other neuropsychiatric diseases, despite synthesis and prescription of atypical antipsychotics (second generation). The typical antipsychotics studied in this work were the butyrophenones haloperidol (HALO) and pipamperone (PIPAM), the thioxanthenes *cis*-chlorprothixene (*cis*-CHLO), flupentixol (FLU) and zuclopenthixol (ZUCLO) and the phenothiazines levomepromazine (LEVO) and promazine (PROMA) (Fig. 1).

Taking into account the considerable inter-individual variability of plasma concentrations of these drugs, therapeutic drug monitoring (TDM) should be used for efficient control of the medication [1]. According to the last update of expert consensus [1], routine TDM is strongly recommended for HALO, recommended for FLU and useful for *cis*-CHLO, LEVO, PIPAM and ZUCLO. Therapeutic reference ranges have been proposed for HALO (1–10 ng/ml), FLU (1–10 ng/ml), *cis*-CHLO (20–300 ng/ml), LEVO (30–160 ng/ml), PIPAM (100–400 ng/ml), ZUCLO (4–50 ng/ml) [1] and PROMA (10–50 ng/ml) [2].

A large number of analytical methods have been described for the quantification of typical antipsychotics alone or in combination with other psychotropic drugs in human plasma or serum. Several methods using gas chromatography (GC) coupled with nitrogen phosphorus [3], mass spectrometry (MS) [4,5] or tandem mass spectrometry (MS/MS) [6] detection have been proposed, as well as methods using high performance liquid chromatography (HPLC) coupled with fluorescence [7,8], ultraviolet [9–12], MS [13–15] or MS/MS [16–20] detection. Sample treatment was mostly performed by liquid–liquid extraction (LLE) [3,4,7,9,10,13,14,16,17] or solid-phase extraction (SPE) [5,6,8,11,12,15,18]. More recently, due

* Corresponding author. Tel.: +41 21 643 65 09; fax: +41 21 643 64 44.
E-mail address: nicolas.ansermot@chuv.ch (N. Ansermot).

0731-7085/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2013.07.041

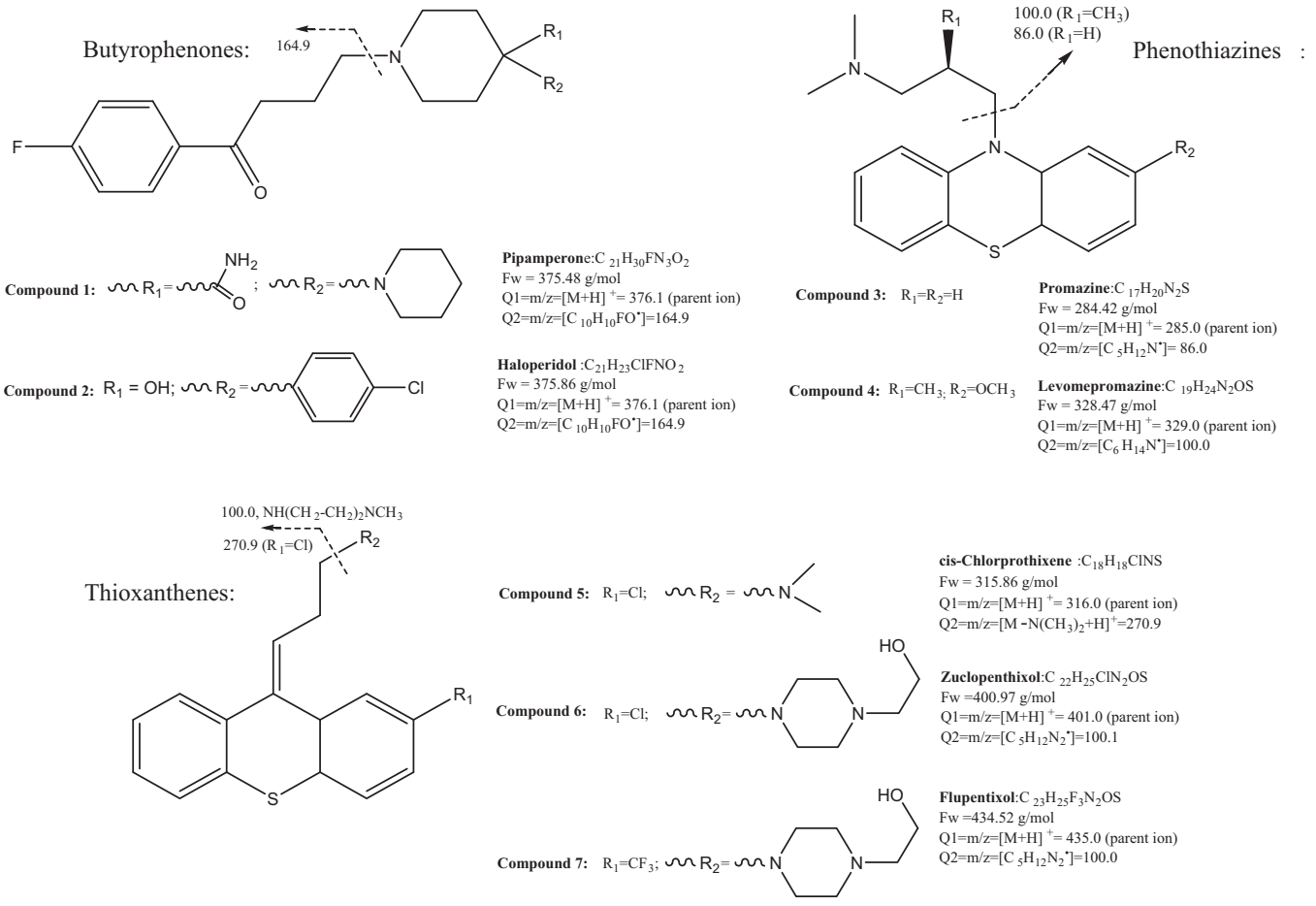

Fig. 1. Chemical structures of the seven typical antipsychotics used in the present study with their respective proposed fragmentation patterns. Fw: formula weight; Q1 (quadrupole 1): parent ion; Q2 (quadrupole 2): daughter ion.

to an increase of selectivity and sensitivity with MS/MS, sample preparation can be simplified with a simple protein precipitation (PP) step [19,20].

A new generation of separation techniques, ultra-high performance liquid chromatography (UHPLC), permits to increase sensitivity, resolution and speed of separation [21]. UHPLC combined to the high selectivity and sensitivity of MS/MS has become the "gold standard" for quantitative analysis of drugs. As far as we know, no UHPLC-MS/MS method has been published for the quantification of the studied drugs in human plasma or serum with a sample preparation based on PP. Only one UHPLC-MS/MS method has been described, but with a sample preparation performed by LLE [22]. Among the 62 psychotropic drugs simultaneously analyzed, 17 drugs (including 3 from our library) did not pass validation criteria due to the high number of monitored transitions. Furthermore, only 5 stable isotope-labeled internal standards (SIL-IS) were used for the 62 compounds, which could decrease the analytical precision.

Over the last decade the number of samples for TDM of psychotropic drugs has dramatically increased in our laboratory, indicating the necessity to develop rapid and economic methods for simultaneous multi-drug quantification. The scope of the present study was to develop and validate a selective and sensitive method to determine seven typical antipsychotics (*cis*-CHLO, FLU, HALO, LEVO, PIPAM, PROMA and ZUCLO) in human plasma. To the best of our knowledge, this is the first UHPLC-MS/MS method for the quantification of these drugs with a sample preparation performed by simple PP. The present work was based on previous methods developed successfully in our laboratory for the analysis of other drugs [23–25].

2. Experimental

2.1. Chemicals and biologicals

Reference standards were purchased from the following companies: *cis*-CHLO hydrochloride, FLU dihydrochloride, PIPAM dihydrochloride and PROMA hydrochloride from Toronto Research Chemicals (Toronto, Canada), LEVO hydrochloride and ZUCLO dihydrochloride from LGC Standards (Molsheim, France) and HALO base from Sigma–Aldrich (Buchs, Switzerland).

The SIL-IS *cis*-chlorprothixene-d6 hydrochloride (*cis*-CHLO-d6), flupentixol-d4 dihydrochloride (FLU-d4), levomepromazine-d6 hydrochloride (LEVO-d6), pipamperone-d10 dihydrochloride (PIPAM-d10) and promazine-d6 hydrochloride (PROMA-d6) were purchased from Toronto Research Chemicals and haloperidol-d4 base (HALO-d4) and zuclopenthixol-d4 succinate (ZUCLO-d4) from LGC Standards. All other chemicals and biologicals were obtained as described previously [25].

2.2. Stock and working solutions

Analytes were separated into three groups according to the clinical requirements regarding range of measurement. Two independent batches by separate weighing were prepared, one for the calibration standards and the other one for the validation standards. Stock solutions of each analyte at 1 mg/ml (calculated as base) were prepared in methanol (MeOH) and stored at −20 °C. Further dilutions were made in MeOH to prepare the multi-level calibration and validation standards in plasma (see Section 2.6.3).