Journal of Pharmaceutical and Biomedical Analysis 95 (2014) 61–67

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Multi-matrix assay of the first melatonergic antidepressant agomelatine by combined liquid chromatography-fluorimetric detection and microextraction by packed sorbent

Maria Addolorata Saracino[a], Laura Mercolini[a], Giorgia Carbini[a], Vittorio Volterra[a], Antonio Lucio Quarta[b], Mario Amore[c], Maria Augusta Raggi[a,*]

[a] Department of Pharmacy and Biotechnology, Alma Mater Studiorum – University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[b] Department of Mental Health, Local Health Unit ASL, C.S.M. Parma Nord – SPOI, Via Suor Maria 3, Colorno, 43052 Parma, Italy
[c] Department of Neuroscience, Section of Psychiatry, University of Genova, Largo Rosanna Benzi 10, 16132 Genova, Italy

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 8 February 2014
Accepted 12 February 2014
Available online 21 February 2014

Keywords:
Agomelatine
Saliva
Plasma
Dried blood spots
Liquid chromatography-fluorescence detector

ABSTRACT

A rapid and reliable analytical method has been developed to quantify the melatonergic antidepressant agomelatine in three matrices, and namely saliva, plasma and dried blood spots. The method is based on the use of liquid chromatography with fluorimetric detection exploiting the native fluorescence of agomelatine. For saliva and plasma samples an original microextraction by packed sorbent procedure was implemented obtaining satisfactory extraction yield of the analyte (always higher than 89%) and a good clean-up of the matrices. On the contrary, agomelatine was extracted from dried blood spots by suitable solvent microwave-assisted extraction and injected into chromatographic system. Satisfactory results in terms of sensitivity, linearity, precision, selectivity and accuracy were obtained. Thus, the developed method seems to be suitable for therapeutic drug monitoring of depressed patients under agomelatine therapy.

1. Introduction

Major Depressive Disorder (MDD) has been estimated to be the fourth major cause of disability worldwide and may become second only to cardiovascular diseases by around 2020 [1]. Accordingly, the antidepressants are among the most prescribed ones worldwide, and held the ninth place in the global ranking of drug classes by revenue [2]. Although the social, clinical and economical relevance of the phenomenon, less than 50% of all patients treated with the currently available antidepressants show full remission, with a large number of subjects showing relapsing symptoms [3]. One reason for this failure is the still incomplete knowledge about the pathogenic mechanisms of depression and about the inner actions of available antidepressants, including First Generation Antidepressants as well as Second Generation Antidepressants [4]. Agomelatine (N-[2-(7-methoxy-1-naphthyl)ethyl]acetamide, AGO, Fig. 1), a melatonin (MLT, Fig. 1) analogue, is an innovative antidepressant that works by targeting both melatonergic (M_1/M_2) and serotonergic (5-HT_{2C}) receptors. It is designed to counteract the disturbed biological rhythms and abnormal sleep patterns associated with depressive disorders [5,6]. In fact, many depressed patients display disrupted circadian rhythms and AGO favours their resynchronization by virtue of its distinctive pharmacological profile: the melatonergic agonism favours sleep quality, onset and architecture; while the blockade of 5-HT_{2C} receptors also participates in its promotion of restorative slow-wave sleep and modulation of circadian rhythms [7]. Moreover, by avoiding 5-HT_{2A} stimulation, AGO shows a more favourable side-effect profile compared Selective Serotonin Reuptake Inhibitors, concerning sexual functioning, weight-gain and gastro-intestinal disturbances [8]. After oral administration the peak plasma concentration is reached within 1–2 h, while the mean terminal half-life is of 2–3 h. In the therapeutic dose-range (25–50 mg day^{-1}), AGO systemic exposure seems to increase proportionally with dose, but at the higher doses a

Abbreviations: MDD, Major Depressive Disorder; AGO, agomelatine; MLT, melatonin; IS, internal standard; DBS, dried blood spot; MEPS, microextraction by packed sorbent; MAE, microwave assisted extraction; SPE, solid phase extraction; LLE, liquid–liquid extraction; TDM, therapeutic drug monitoring.

* Corresponding author at: Laboratory of Pharmaco-Toxicological Analysis, Department of Pharmacy and Biotechnology (FaBiT), Via Belmeloro 6, 40126 Bologna, Italy. Tel.: +39 051 2099739; fax: +39 051 2099740.
E-mail address: mariaaugusta.raggi@unibo.it (M.A. Raggi).

http://dx.doi.org/10.1016/j.jpba.2014.02.005
0731-7085/

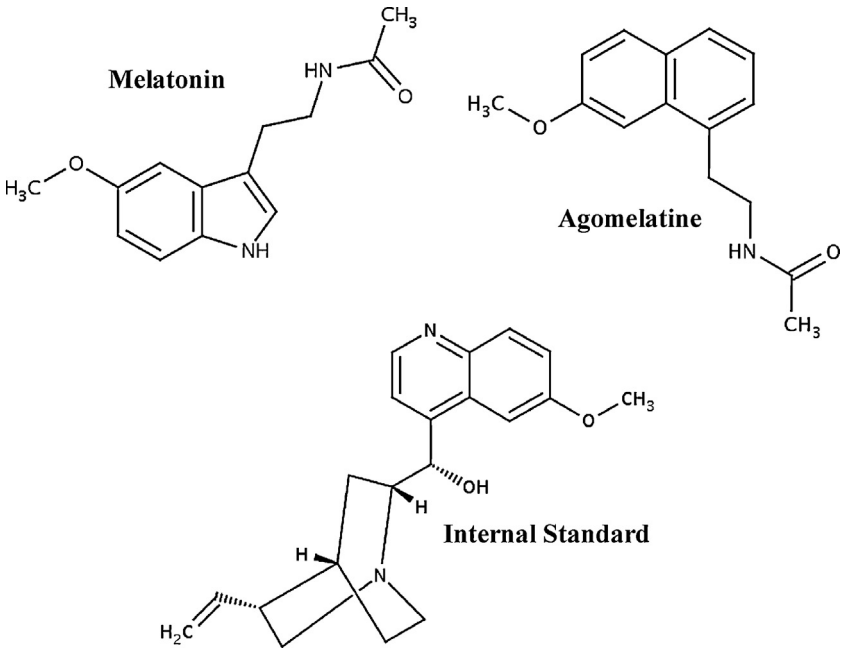

Fig. 1. Chemical structures of agomelatine, melatonin, and quinidine (used as internal standard).

saturation of the first pass effect occurs. There are currently no data that show increased toxicity in patients with high systemic plasma (or blood) concentrations of AGO; while the potential for hepatotoxicity is the major drawback during AGO treatment [9]. The most common adverse reactions were dizziness, headache, upper abdominal pain and hepatic impairment [10]. In the last few years, the therapeutic drug monitoring (TDM) of several psychotropic drugs, and particularly antidepressants [11], has proven to be of notable value for the assessment of the compliance of patients in following the therapy and in avoiding severe adverse effects caused by wrong dosages of the drugs or their interaction with other drugs. For these reasons, a constant monitoring of the drug levels in suitable matrices is often advisable, especially during polypharmacy. The assay of saliva specimens is an increasing area of research with implications for basic and clinical purposes and offers stress-free and non-invasive sampling. On the other hand, being whole blood is the optimal matrix for the drug analysis, the use of dried blood spot (DBS) technique could be also an attractive approach for TDM.

To the best of our knowledge, only two methods have been reported in the literature for the analysis of AGO: both of them are based on the use of a high throughput liquid–liquid extraction (LLE) method for determination of AGO in human plasma samples by means of liquid chromatography coupled with mass spectrometric detection in the positive ionization mode [12,13]. No liquid chromatography-fluorimetric detection (HPLC-F) assay for the determination of AGO in biological matrices different from plasma has been described so far. Therefore, the aim of the present study was to develop and fully validate an HPLC-F method for the analysis of AGO in specific matrices, and namely saliva and DBSs as well as plasma samples. An original microextraction by packed sorbent technique has been implemented for the clean-up of both saliva and plasma samples, while a rapid microwave assisted extraction (MAE) with a suitable solvent has been developed for DBSs.

2. Experimental

2.1. Chemicals and solutions

Acetonitrile and methanol for HPLC, potassium dihydrogen phosphate (KH_2PO_4), 2N sodium hydroxide (NaOH) and triethylamine were obtained from Sigma Aldrich (St. Louis, MO, USA). Agomelatine and quinidine sulfate salt dihydrate (used as the Internal Standard, IS, Fig. 1), was also purchased from Sigma Aldrich. Ultrapure water (18.2 MΩ cm), obtained by means of a MilliQ apparatus by Millipore (Milford, MA, USA), was used.

Methanolic stock solutions of AGO and IS (1 mg mL^{-1}) were prepared by dissolving 2 mg of pure substance in 2 mL of methanol. Working standard solutions of the analyte and the IS were prepared daily by diluting the primary stock solutions with the mobile phase. Calibration standards were prepared by diluting the appropriate working solutions with blank saliva, plasma and DBS from healthy volunteers. DBS samples were obtained by a puncture on a finger with a single-use lancet and transferring a drop of blood on the FTA® card. As soon as the blood was absorbed by the paper, a known aliquot of a standard solution containing the analyte and the IS was carefully transferred onto each spot; the spots were then dried and subjected to extraction procedure.

2.2. Sample collection

Blood and saliva samples were obtained from some patients (1 men, age = 62 years; 4 women, mean age = 48 years) from the Department of Mental Health (Local Health Unit ASL, C.S.M. Parma Nord – SPOI) from Parma (Italy) and subjected to oral therapy with Valdoxan® (daily doses of 25 mg for at least 1 week). The biological samples were usually drawn early in the morning from fasting patients (2–3 h after the last drug administration).

Blood (3 mL) was stored in glass tubes containing ethylenediaminetetraacetic acid (EDTA) as the anticoagulant, then centrifuged (within 2 h from collection) at 4000 rpm for 15 min at 5 °C; the supernatant (plasma) was then transferred to polypropylene tubes and stored at −20 °C until LC analysis.

Unstimulated saliva samples (0.5 mL) were collected from patients half an hour after blood sampling, with a disposable plastic pipette. It was put into polypropylene tubes and stored frozen at −20 °C until the time of LC analysis, when they were centrifuged at 4000 rpm for 15 min at 5 °C; the assays were carried out on the clear supernatant.