Journal of Chromatography B, 1017–1018 (2016) 136–144

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Development and validation of a liquid chromatography-tandem mass spectrometric assay for quantitative analyses of triptans in hair

Daniele Vandelli[a,*], Federica Palazzoli[a], Patrizia Verri[a], Cecilia Rustichelli[b], Filippo Marchesi[a], Anna Ferrari[c], Carlo Baraldi[c], Enrico Giuliani[d], Manuela Licata[a], Enrico Silingardi[a]

[a] Department of Diagnostic Medicine, Clinical and Public Health, Unit of Legal Medicine-Forensic Toxicology Laboratory, University of Modena and Reggio Emilia, via del Pozzo, 71, 41124 Modena, Italy
[b] Department of Life Sciences, University of Modena and Reggio Emilia, via G. Campi, 103, 41125 Modena, Italy
[c] Department of Diagnostic, Clinical and Public Health Medicine, Unit of Medical Toxicology, Headache and Drug Abuse Centre, University of Modena and Reggio Emilia, via del Pozzo, 71, 41124 Modena, Italy
[d] Clinical and Experimental Medicine Doctorate School, University of Modena and Reggio Emilia, via del Pozzo, 71, 41124 Modena, Italy

ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 19 February 2016
Accepted 23 February 2016
Available online 27 February 2016

Keywords:
Triptans
HPLC–MS/MS
Hair samples
Migraine
Overuse

ABSTRACT

Triptans are specific drugs widely used for acute treatment of migraine, being selective $5HT_{1B/1D}$ receptor agonists. A proper assumption of triptans is very important for an effective treatment; nevertheless patients often underuse, misuse, overuse or use triptans inconsistently, i.e., not following the prescribed therapy. Drug analysis in hair can represent a powerful tool for monitoring the compliance of the patient to the therapy, since it can greatly increase the time-window of detection compared to analyses in biological fluids, such as plasma or urine. In the present study, a liquid chromatography-tandem mass spectrometric (LC–MS/MS) method has been developed and validated for the quantitative analysis in human hair of five triptans commonly prescribed in Italy: almotriptan (AL), eletriptan (EP), rizatriptan (RIZ), sumatriptan (SUM) and zolmitriptan (ZP). Hair samples were decontaminated and incubated overnight in diluted hydrochloric acid; the extracts were purified by mixed-mode SPE cartridges and analyzed by LC–MS/MS under gradient elution in positive multiple reaction monitoring (MRM) mode. The procedure was fully validated in terms of selectivity, linearity, limit of detection (LOD) and lower limit of quantitation (LLOQ), accuracy, precision, carry-over, recovery, matrix effect and dilution integrity. The method was linear in the range 10–1000 pg/mg hair, with R^2 values of at least 0.990; the validated LLOQ values were in the range 5–7 pg/mg hair. The method offered satisfactory precision (RSD <10%), accuracy (90–110%) and recovery (>85%) values. The validated procedure was applied on 147 authentic hair samples from subjects being treated in the Headache Centre of Modena University Hospital in order to verify the possibility of monitoring the corresponding hair levels for the taken triptans.

1. Introduction

Migraine is an idiopathic recurring disorder consisting of headache attacks lasting 4–72 h, of moderate to severe intensity, associated with nausea, photo-and phonophobia that affects 10% of the global adult population [1]. The prevalence of this pathology is highest in women and in people between the ages of 25 and 55 [2]. Migraine patients who suffer from recurrent attacks are a population at risk of overuse and abuse of analgesic medications [3].

Triptans are selective agonists at 5-hydroxytryptamine 1B/1D ($5\text{-}HT_{1B/1D}$) receptor subtypes and are recommended as first-line drugs for the treatment of migraine attacks (acute treatment) in patients suffering from moderate-severe migraine [4]. Sumatriptan (SUM) was the first to be marketed, at the beginning of the '90s. Even if it is fast absorbed orally, its bioavailability is only 14% and it has a short half-life, about 2 h. Six other triptans have been later introduced: zolmitriptan (ZP), naratriptan, rizatriptan (RIZ), eletriptan (EP), almotriptan (AL), and frovatriptan, which have greater oral bioavailability, longer plasma half-life, higher lipophilicity, and greater potency and affinity for 5-HT1B/1D receptors [5]. Concerning lipophilicity, EP is the more lipophilic and SUM is the less lipophilic among the serotonin $5HT_{1B/1D}$ receptor agonists [6]. Triptans are homogeneous in their mechanism of

* Corresponding author.
E-mail address: daniele.vandelli@unimore.it (D. Vandelli).

http://dx.doi.org/10.1016/j.jchromb.2016.02.031
1570-0232/

action and only minor differences in the efficacy of oral triptans for migraine have been reported [7]. In particular, all triptans have a selective vasoconstrictor action on dilated cranial blood vessel and act on trigeminal nerve terminals reducing the release of inflammatory factors; the main differences concern pharmacokinetics and metabolism [5].

Triptans are the most commonly prescribed class of medications for acute migraine treatment in the specialty care [8]. However, the regular and frequent use of triptans, like of any other symptomatic analgesics, can cause chronic migraine and medication-overuse headache (MOH) [9,10]. MOH is a chronic daily headache due to overuse of acute medications [11]; in particular, according to the diagnostic criteria of the International Classification of Headache Disorders, 3rd edition (beta version) (ICHD-3 beta) "overuse" is defined by "regular intake of one or more triptans in any formulations, on 10 or more days per month for more than three months" [1]. MOH causes enormous suffering, very high social costs and is difficult to treat; it affects between 1% and 2% of the general population but is present in up to 50% of patients seen in headache centers [12]. It is believed that only withdrawing medications and beginning an adequate prophylaxis can lead to an improvement of the disorder [13]. However, relapses are frequent even after a detoxification treatment; therefore the monitoring and follow-up of the patients are crucial to the success of the treatment [12,13]. For this purpose, indirect methods such as self-reports and headache diaries are usually used [14], since measurements of triptan levels in plasma and urine are not adequate, reflecting only a recent ingestion (short-term therapeutic monitoring).

Hair analysis is increasingly used to monitor adherence to pharmacological treatments [15] and to document objectively the progress of detoxification and rehabilitation programs of drug addicts [16]. Hair analysis has a very wide time-window of detection that depends on the hair length; this is based on the assumption that the drugs present in blood diffuse and are incorporated into the hair matrix, which grows at a fairly constant rate (about 1 cm/month), where drugs and metabolites can stay unaltered for long time, protected from metabolism and degradation. Therefore hair analysis allows monitoring a long period of time and provides valuable information on the patient's compliance to the prescribed therapy; in addition, hair analysis allows the clinician to distinguish between occasional and chronic use/overuse [17,18].

Until now, no analytical procedures were reported in the literature for the quantitative determination of triptans in hair, while several papers focused on LC–MS analyses of triptans in various human biological fluids, such as plasma or serum [19–32] and urine [32]. These drugs, except naratriptan, are available in Italy in oral formulations. The recommended initial doses for oral triptans in adults are: 12.5 mg for AL; 40 mg for EP, 5 or 10 mg for RIZ; 50 or 100 mg for SUM and 2.5 or 5 mg for ZP; the maximum daily dose for oral triptans are: 25, 80, 20, 300 and 10 mg for AL, EP, RIZ, SUM and ZP, respectively.

The main purpose of the present study was to develop and validate a suitable and sensitive method for the quantification of five triptans in human hair: AL, EP, RIZ, SUM and ZP, which are the triptans most commonly prescribed in the Headache Centre of Modena University Hospital. Fig. 1 shows the chemical structures of these antimigraine compounds.

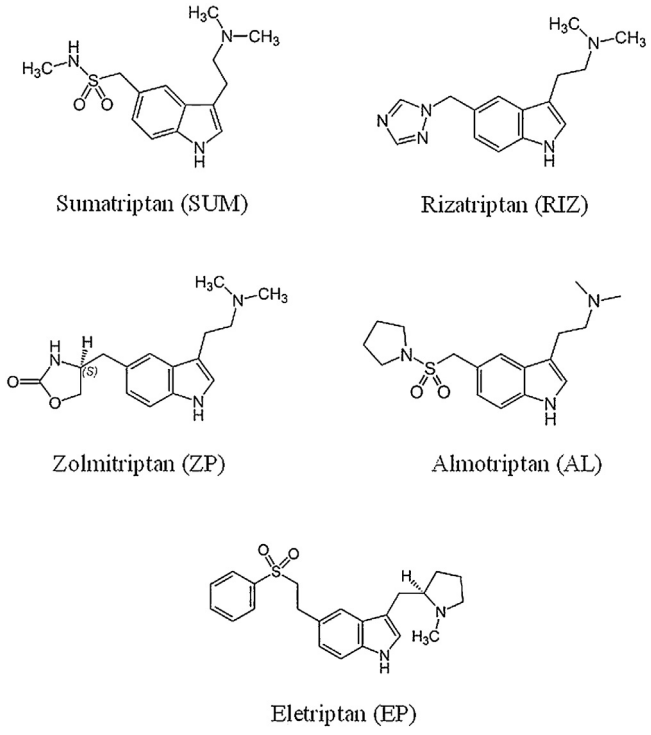

Fig. 1. Chemical structures of the target triptans.

2. Material and methods

2.1. Chemicals

AL, EP, RIZ and ZP were supplied from Clearsynth INC. (San Diego, CA, USA). SUM was purchased from LGC Standards (Bury, Lancashire, UK). Zolmitriptan-D_6 (ZP-D_6) was used as internal standard (IS) and was from Alsachim (Illkirch Graffenstaden, France).

All solvents and chemicals for HPLC–MS/MS were of LC–MS purity grade (Baker-VWR, Milano, Italy), while other chemicals used for sample preparation were of analytical grade (Carlo Erba, Milano, Italy).

Strata^{TM}-X-C 33 μm Polymeric Strong Cation SPE Cartridges (100 mg/6 mL) were supplied from Phenomenex (Bologna, Italy).

2.2. Hair samples

Drug-free hair specimens for the preparation of quality control samples were collected from known volunteers abstinent from any drugs. Authentic hair specimens for toxicological investigations were collected from headache patients afferent to the Headache Centre of the Modena University Hospital. For each patient a detailed pharmacological history relative to the previous three months was collected. All patients provided written informed consent and the Ethics Committee of the Province of Modena approved the study.

Of a total of 300 consecutive headache patients we selected the 147 migraine sufferers who declared the intake of at least one triptan and the corresponding dose over the three months before the hair sampling. Some of these 147 patients fulfilled the diagnostic criteria of the ICHD-3 beta for medication-overuse headache (MOH) [1].

Hair samples (at least 4 cm in length) were cut from the scalp of the posterior vertex of the head as close as possible to the scalp and stored at room temperature until processing. The hair samples were most brown/dark brown in color; in some cases, evidence of cosmetic treatments was present.

2.3. Preparation of standard solutions

A stock solution of the analytes (AL, EP, RIZ, SUM and ZP) was prepared in methanol at a concentration of 1 μg/μL; this solution was diluted with methanol to obtain working solutions at ten