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Development, validation and comparison of UHPSFC and UHPLC methods for the determination of agomelatine and its impurities

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ABSTRACT

Agomelatine is one of the newest antidepressants. Due to a different mechanism of action it offers a completely new approach in the treatment of depressive disorders. Two chromatographic methods for determination of agomelatine and its impurities were developed. The separations on UHPSFC system were accomplished using stationary phase based on BEH 2-EP and gradient elution with CO_2 and methanol containing 20 mM ammonium formate and 5% of water. The UHPLC separations were performed on stationary phase BEH Shield RP18. The mixture of acetonitrile and methanol in ratio 1:1 and ammonium acetate buffer pH 9.5 were used as mobile phase.

Both developed methods were properly validated in terms of linearity, sensitivity (LOD, LOQ), accuracy and precision according to ICH guidelines. The UHPSFC method was linear in the range 0.25–70 μg/ml for all analytes with method accuracy ≥97.4% and ≥100.2% and method intra-day precision RSD ≤2.4 and ≤0.8 for impurities and API (active pharmaceutical ingredient), respectively. The UHPLC method was linear in the range 0.1–10 μg/ml for all analytes except three impurities for which the linear range was larger 0.1–25 μg/ml. Method accuracy ≥95.7% and ≥95.2% and method intra-day precision RSD ≤2.6 and ≤1.5 were found for impurities and API, respectively.

The measurement of tablet samples was performed and the selected parameters of the methods were compared. In conclusion, both methods were appropriate for the determination of agomelatine and its impurities in pharmaceutical quality control (QC), although the UHPSFC method was found as more convenient especially in the method development phase. The advantages of newly developed UHPSFC-PDA (photo diode array detector) method were its environmental friendliness due to the mobile phase used, a very good resolution, selectivity and high speed of analysis (total time of separation was 4.1 min).

1. Introduction

Agomelatine is the first antidepressant from a new group of melatonin agonists and selective serotonin antagonists (MASSA). Chemically, it is *N*-(2-(7-methoxynaphtalen-1-yl)ethyl)acetamide (Fig. 1). Agomelatine is a synthetic analogue of epiphysis hormone melatonin, which regulates circadian rhythms and is indicated for the treatment of depression disorders in adults. The serotonin receptor 5-HT_{2C} inhibits the release of noradrenalin and dopamine. Agomelatine as an antagonist of this receptor counteracts this inhibition which leads to increasing levels of these monoamines [1]. Agomelatine regulates circadian rhythms due to positive affecting of melatonine receptors MT_1 and MT_2. It can also correct the disorders of sleep-wake cycles [2]. The clinical trials proved that the efficiency of agomelatine is comparable with the efficiency of many antidepressants commonly used in clinical practice such as venlafaxine, paroxetine and fluoxetine [1–4]. This is important finding given the unique mechanism of action and better tolerability [5].

Six potential impurities of agomelatine have to be monitored simultaneously in pharmaceutical QC (quality control) analysis of the drug substance and/or the drug product: (2-(7-methoxynaphtalen-1-yl)ethan-1-amine hydrochloride (AgoSalt), bis[2-(7-methoxynaphtalen-1-yl)-ethyl]amine (amine), *N*,*N*-bis[2-(7-methoxynaphtalen-1-yl)-ethyl]acetamide (dimer), 2-(7-methoxynaphtalen-1-yl)acetonitrile (nitrile), 2-(7-methoxynaphtalen-1yl)acetamide (amide), 2-(7-methoxynaphtalen-1-yl)acetic acid (acid). Although these substances are structurally quite close to agomelatine, their physicochemical properties differ (Fig. 1) including acidic,

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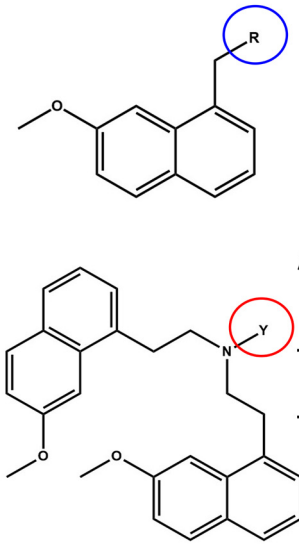

	R	Mw	logP	pKa (acid)	pKa (basic)
Acid	-COOH	216.23	2.41	4.33	-
Amide	$-CONH_2$	215.25	1.76	16.11	-0.67
Nitrile	-CN	197.23	2.69	NA	NA
Sal	$-CH_2-NH_2$. HCl	237.73	2.23	10.00	-
Agomelatine	$-CH_2-NHCO-CH_3$	243.30	2.46	16.17	-0.53

	Y	Mw	logP	pKa (acid)	pKa (basic)
Amine	-H	385.50	6.96	-	9.73
Dimer	$-COCH_3$	427.53	6.58	-	0.59

Fig. 1. The structures of agomelatin and its six potential impurities included in this study and their physicochemical properties.

basic and neutral molecules as well as a salt. Therefore, their simultaneous analysis remains challenging.

Agomelatine is relatively new drug. There are only two published methods dealing with the separation of agomelatine and its potential impurities [6,7]. Indumathi et al. [7] published a study dealing with the forced degradation of agomelatine where the degradation impurities were determined by HPLC method using Hypersil ODS C18 (150 × 4.6 mm, 5.0 μm) and gradient elution with acetonitrile and ammonium acetate. Total time of this analysis was 30 min. The second mentioned separation [6] was accomplished using RP-HPLC using Hypersil BDS C18 column (250 × 4.6 mm, 5 μm) and gradient elution with mobile phase formed by acetonitrile and potassium dihydrogen phosphate (15 mM, pH adjusted to 3.0). In spite of 50 min analysis, the optimal selectivity and resolution was not obtained [6] considering close elution of two critical pairs of impurities.

On the other hand, few methods for the determination of agomelatine itself using RP-HPLC have already been published [8–11]. RP-HPLC method with UV detection was developed for the determination of agomelatine in bulk and dosage forms [9]. Saracino et al. [10] published another HPLC method developed for the determination of agomelatine in human plasma, saliva and dried blood spots. This method took advantages of the natural fluorescence of agomelatine and used the fluorescence detection. However, using acetonitrile with phosphate buffer as a mobile phase resulted in the need of prolonged column washing cycles and incompatibility with MS detection. Similar method was developed by El-Shaheny [8] for agomelatine in tablets and human plasma. This separation was performed with the same detection and the buffer as in previous method, while the second part of the mobile phase was formed by methanol. Another approach included liquid chromatography technique with radioimmunoassay's detection (LC-RIA) [11]. Two LC–MS/MS methods [12,13] for determination of agomelatine in human plasma have also been described as well as the GC–MS method [11].

The separation and the determination of the group of structurally similar substances with different physicochemical properties, such as active pharmaceutical ingredient (API) and its impurities, are the basis of pharmaceutical QC. HPLC methods with various detection techniques are considered as gold standard in QC of drug substances. In this chromatographic system, a wide range of parameters can be optimized (stationary phase type, composition of mobile phase, pH of buffers, gradient elution, gradient slopes, temperature) [14]. However, in some cases none of the modifications may allow the separation of all substances with adequate resolution. Therefore, a number of SFC separations in pharmaceutical industry has increased recently due to its different selectivity [14–16].

SFC is a unique separation technique allowing the use of columns with both polar and non-polar stationary phases [15,16]. Even though polarity profile of SFC is basically the same as for normal phase chromatography, SFC can be used for separation of both, polar and non-polar, substances [14,17–19]. For analysis of polar compounds in pharmacy modification of the non-polar CO_2—based mobile phase is needed in order to extend the polarity range. Routinely, methanol, ethanol or acetonitrile, are used as organic modifiers to improve the solvent strength. Moreover, the peak shapes may be further improved by additives [20,19]. The term UHPSFC (ultra-high performance supercritical fluid chromatography) is used for supercritical fluid chromatography performed on stationary phase with sub-2 μm or superficially porous particles. According to Khater et al. [21] the change in particle size from 5 μm to 1.7 μm does not affect the interactions, but similarly to UHPLC, sub-2-μm particles enable ultra-fast and efficient separations [22,23].

The aim of this study was to develop and validate fast UHPSFC and UHPLC methods with UV detection for the separation and the determination of the group of structurally similar substances, agomelatine and its six impurities. Although these substances are structurally quite close, their physicochemical properties differ. Therefore, one of the main goals was to compare the retention and selectivity not only in supercritical fluid chromatography system and liquid chromatography system but also under different separation conditions. During method development different chromatographic conditions such as stationary phases, mobile phases, additives and buffers were tested. After the optimization of separation conditions complete separations were obtained in both systems. Both developed methods were properly validated according to ICH guidelines [24]. The measurement of tablet samples was performed and the methods were compared in the selected param-