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Quantification and structural elucidation of potential impurities in agomelatine active pharmaceutical ingredient

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ABSTRACT

Seven impurities in agomelatine drug substance were determined by a newly developed RP-HPLC method. Structures of potential impurities were confirmed by NMR and IR analysis. Efficient chromatographic separation was achieved on Hypersil BDS C_{18} column (250 mm × 4.6 mm, 5 μm) in gradient mode by using a binary mixture of potassium dihydrogen phosphate (15 mM, pH adjusted to 3.0) and acetonitrile at a flow rate of 1.0 ml/min. A photodiode array detector set at 230 nm was used for detection. Forced degradation studies showed that the proposed method was specific, and agomelatine was found to be susceptible to acidic and alkaline conditions. The method was validated according to ICH guidelines with respect to specificity, sensitivity, precision, linearity, accuracy, robustness and system suitability. Detection limit of impurities was in the range of 0.0008–0.0047%. Regression analysis showed correlation coefficient value greater than 0.999 for agomelatine and its seven impurities. Accuracy of the method was established based on the recovery obtained between 94.4% and 106.7% for all impurities. The validation results demonstrated that the developed method was suitable for the quantitative determination of potential impurities in agomelatine. A possible mechanism for the formation of impurities was proposed.

1. Introduction

Agomelatine (N-[2-(7-methoxy-1-naphthyl)ethyl]acetamide), a melatonin analog, represents a new class of antidepressants. It is a potent melatonergic agonist (MT_1 and MT_2) and also has 5-hydrotryptamine 2C (5-HT_{2c}) antagonist properties [1]. In particular, a great deal of pre-clinical and clinical data indicated that agomelatine has a comparable antidepressant efficacy to other well-established antidepressants [2–5]. Furthermore, there are no increases in specific side effects such as sexual dysfunction and body-weight gains, which are common with some classes of antidepressants [6–8]. Agomelatine as the first known antidepressant that works via a non-monoaminergic mechanism of action was approved for the treatment of major depression in Europe in 2009, and was available in Europe under the brand name Valdoxan [1].

For active pharmaceutical ingredient (API) produced by chemical synthesis, impurities include the un-reacted starting materials, intermediates, by-products and degradation products. The control of impurities originating in the manufacturing process has become an important element of drug development [9–12].

After a comprehensive and systematic investigation on manufacturing process of agomelatine, two unreported potential impurities (Imp-3 and Imp-4) were designed and synthesized. Besides, there were three impurities (Imp-2, Imp-6 and Imp-7) mentioned previously in the patent about the synthesis of agomelatine [13]. Agomelatine was manufactured by the catalytic hydrogenation of (7-methoxy-1-naphthyl)acetonitrile (starting material, Imp-5) in lab. Therefore, starting material (Imp-5), hydrogenated products (intermediate, Imp-1) and the other five impurities were considered as potential impurities in agomelatine. The structures of agomelatine and its seven impurities are shown in Fig. 1.

There are several methods for determination of agomelatine and its impurities in bulk drugs and pharmaceutical dosage forms reported in literature [14–16]. However, structural elucidation and quantitative determination of impurities have not been found. In this study, structures of impurities were confirmed by characterization using impurity reference standards and with the help of NMR and IR techniques. During HPLC analysis of agomelatine samples, none of the currently available analytical methods can efficiently separate and quantify all impurities. Thus, a specific, accurate and stability indicating HPLC method was developed. The newly developed method was validated according to ICH guidelines [17]. Stress

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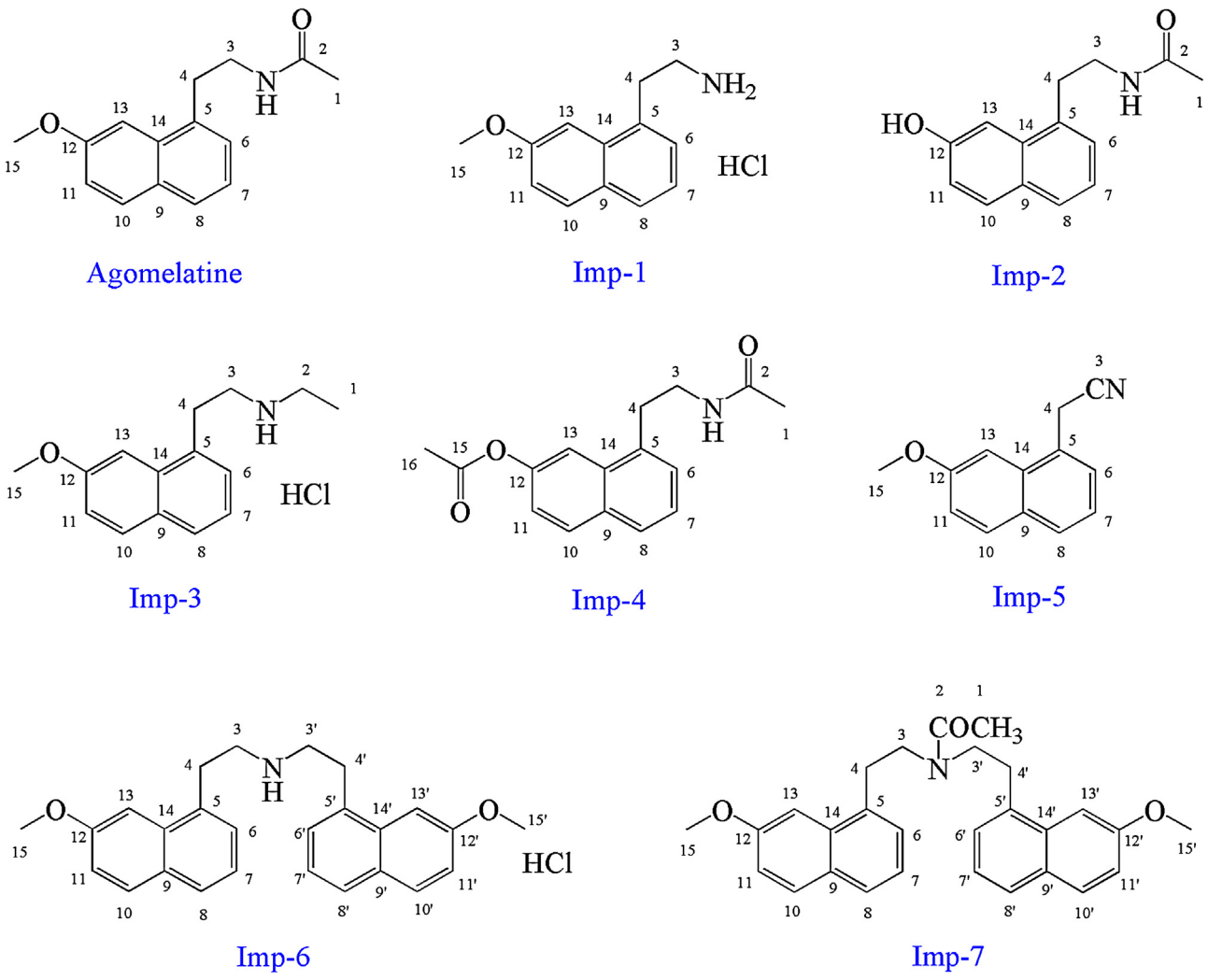

Fig. 1. Chemical structures of agomelatine and impurities with numbering assigned for NMR characterization.

studies were carried out on agomelatine drug substance according to ICH drug stability test guidelines [18] in order to elucidate its inherent stability characteristics and identify potential degradation products. Possible mechanism for the formation of potential impurities was proposed.

2. Experimental

2.1. Materials and reagents

Agomelatine and standards of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6 and Imp-7 were supplied by the Department of Medicinal Chemistry of China Pharmaceutical University. HPLC grade acetonitrile was purchased from Tedia Company Inc. (USA). Purified water (Robust, Guangdong, China) was used in the preparation of solutions. Other chemicals were of analytical grade.

2.2. RP-HPLC analysis

Chromatographic studies were performed on a Shimadzu HPLC system equipped with LC-20AT liquid chromatography pump, SPD-M20A diode-array UV detector, SIL-20AHT auto sampler, CTO-20A column oven and CBM-20A communications bus module (Shimadzu Corporation, Kyoto, Japan). The chromatograms were recorded and analyzed employing LC-solution software. A Hypersil BDS C_{18} column (250 mm × 4.6 mm, 5 μm particle size, Thermo Electron Corporation, UK) was employed. Column oven temperature was maintained at 30 °C. Mobile phase A was potassium dihydrogen orthophosphate buffer (15 mM, pH adjusted to 3.0 with phosphoric acid) and phase B was acetonitrile. The HPLC gradient program was time (min)/%B (v/v): 0/31, 20/31, 40/85, 45/85, 47/31 and 55/31, flow rate was 1.0 ml/min, UV detector wavelength was 230 nm. The agomelatine sample was prepared in diluent (mobile phase A and acetonitrile, 69:31, v/v) at 100 μg/ml concentration and 20 μl of sample solution was injected into HPLC system.

2.3. LC–MS analysis

The LC–MS analysis was performed on LCMS-2020 mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The analysis was performed in SCAN mode with electro-spray ionization source and triple quadrupole mass analyzer. The samples were analyzed in positive and negative ion electrospray mode. Curved desolvation line and heat block temperatures were 250 °C and 400 °C respectively. Desolvation gas flow was fixed at 300 l/h. Detector voltage was 1.10 kV. The HPLC system was equipped with LC-20AD liquid chromatography pump, SPD-M20A diode-array UV detector, SIL-20A auto sampler, CTO-20A column oven and CBM-20A communications bus module (Shimadzu Corporation, Kyoto, Japan). The HPLC conditions for LC–MS analysis matched those described