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Development of RP UPLC-TOF/MS, stability indicating method for omeprazole and its related substances by applying two level factorial design; and identification and synthesis of non-pharmacopoeial impurities

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ABSTRACT

A new UPLC-TOF/MS compatible, reverse phase-stability indicating method was developed for determination of Omeprazole (OMP) and its related substances in pharmaceutical dosage forms by implementing Design of Experiment (DoE) i.e. two level full factorial Design (2^3 + 3 center points = 11 experiments) to understand the Critical Method Parameters (CMP) and its relation with Critical Method Attribute (CMA); to ensure robustness of the method. The separation of eleven specified impurities including conversion product of OMP related compound F (**13**) and G (**14**) i.e. Impurity-I (**1**), OMP related compound-I (**11**) and OMP 4-chloro analog (**12**) was achieved in a single method on Acquity BEH shield RP18 100 × 2.1 mm, 1.7 μm column, with inlet filter (0.2 μm) using gradient elution and detector wavelength at 305 nm and validated in accordance with ICH guidelines and found to be accurate, precise, reproducible, robust and specific. The drug was found to degrade extensively in heat, humidity and acidic conditions and forms unknown degradation products during stability studies. The same method was used for LC-MS analysis to identify *m*/*z* and fragmentation of maximum unknown impurities (Non-Pharmacopoeial) i.e. Impurity-I (**1**), Impurity-III (**3**), Impurity-V (**5**) and Impurity-VIII (**9**) formed during stability studies. Based on the results, degradation pathway for the drug has been proposed and synthesis of identified impurities i.e. impurities (Impurity-I (**1**), Impurity-III (**3**), Impurity-V (**5**) and Impurity-VIII (**9**)) are discussed in detail to ensure in-depth understanding of OMP and its related impurities and optimum performance during lifetime of the product.

1. Introduction

OMP—the "substituted benzimidazole"; is a covalent class of PPIs (Proton Pump Inhibitors); inhibits the pump by irreversibly binding to cysteines in the pump. The irreversibility of the covalent bond results in inhibition of acid secretion until more enzymes are synthesized i.e. inhibition of enzymes H^+/K^+-ATPase [Hydrogen–Potassium Adenosine Triphosphates] at the secretory surface of the gastric parietal cell. This effect leads to inhibition of both basal and stimulated acid secretion, irrespective of the stimulus, for more than 24 h [1–3]. OMP acts as "prodrug", (they require modification of their structure to become active) and rapidly metabolized by hepatic "cytochrome P-450 isoenzyme CYP2C19", and almost completely absorbed, with peak plasma levels occurring 1–3 h after ingestion. It is highly (95%) protein-bound and rapidly distributed in plasma resulting in a very short plasma half-life of 40–60 min. Despite the usual short half-life of OMP, the covalent bond with the pump provides sustained inhibition of acid. Therefore, OMP is used for the treatment of diseases caused due to excess acid secretion such as "ulcers", "dyspepsia", "Gastroesophageal Reflux Disease" (GERD) "Laryngopharyngeal Reflux" (LPR) and the "Zollinger–Ellison syndrome" etc. [4,5].

Most of the oral OMP preparations are enteric-coated, to prevent rapid degradation of the drug in the acidic conditions of the stomach. This is achieved by formulating tablets [6], granules within capsules and the multiple-unit pellet system (MUPS) etc. [7] these

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preparations are pH dependant and releases medicine in intestine i.e. above pH 5.5 and not in stomach; to avoid degradation in acidic conditions.

DoE has statistics based approach and has several advantages, to achieve predictive knowledge of the method with little number of experiments and helps in optimization and development of robust and rugged method [8,9]. As a result of DoE study, the method performance can be understood and improved due to knowledge of CMPs, CMAs, overlay plots Design Space; and hence Normal Operating Range (NOR), Proven Acceptable Range (PAR) [10] and control strategy, which will ensure optimal performance and reliability of the method and the data generated.

The official monographs; Indian Pharmacopoeia (IP), [11] British Pharmacopoeia (BP) [12], European Pharmacopoeia (EP), [13] and United States Pharmacopoeia (USP) [14] have listed maximum eight specifiedimpurities of OMP and has separate UV–vis spectroscopy method for related compound F (**13**) and G (**14**). Although, several methods on OMP have been recently reported. Refs. [15–18] however, published reports have not dealt with synthesis and characterization of four non-pharmacopoeial impurities as well as separation of eleven impurities in a single UPLC/TOF-MS compatible method. Present work describes plausible degradation pathway and application of DoE for optimization of chromatographic conditions.

2. Experimental

2.1. Reagents and chemicals

The active pharmaceutical ingredient (API), placebo/matrix, tablets and its impurities were received from "Dr.Reddy's Laboratories Ltd", IPDO, Hyderabad, India. Emsure grade ammonium acetate and ammonia were purchased from Merck, Darmstadt, Germany. Di-sodium tetra borate i.e. borax was purchased from Merck, Mumbai, India. HPLC-grade acetonitrile (ACN) and methanol (MeOH) was purchased from Rankem, Thane, India. High-purity water obtained from Milli-Q water purification system, Bedford, USA) was used for study. Test solutions were filtered through Whatman Uniflo 0.2 μm PVDF filter and purchased from GE Healthcare, Buckinghamshire, UK. NMR solvent CDCl3, DMSO-d6, D2O and CD3OD were purchased from Cambridge Isotope Laboratories Inc., MA, USA.

2.2. Instrumentation and chromatographic conditions

LC analysis was performed on a Waters Acquity UPLC (Waters Corporation, Milford, MA, USA) equipped with a photodiode array detector (PDA) at 305 nm using "Empower-2" software (Waters Corporation, Milford, MA, USA). Separation was accomplished on an Acquity BEH shield RP18 column (100 × 2.1 mm, 1.7 μ) attached with inlet filter (Waters Corporation, NJ, USA). With flow rate 0.3 mL min^{-1} using following gradient program (Time in min)/(%mobile phase-B): 0/0, 4/0, 12/8, 26/25, 34/40, 42/70, 45/70, 46/0 and 48/0. The following solutions were prepared: Buffer solution, ammonium acetate (0.06 M) pH 8.9 with ammonia-; mobile phase-A, buffer: ACN (950:50, v/v) and mobile phase-B, ACN: MeOH (65:35, v/v). The column temperature was maintained at 40 °C, and the injection volume was 6 μL with sample cooler at 5 °C. DoE and statistical analysis were performed using "Design-Expert®" software, version 9.0.1.0 (Stat-Ease, Inc., Minneapolis, MN, USA).

2.3. UPLC-TOF/MS and NMR conditions

MS analysis was performed on Waters Acquity TOF/MS (Waters Corporation, Manchester, UK) which was connected to UPLC via an "Electrospray Ionization Interface" (ESI). The ESI source was operated in positive ion mode with and a capillary voltage at 2.4 KV, source and desolvation temperature were set at 120 and 350 °C respectively. The concentration and desolvation gas flows were 50 and 600 L h^{-1} respectively. "Leucine-encephalin" was used as the lock mass generating an $[M+H]^+$ ion (m/z 556.2771) at a concentration of 200 pg mL^{-1} and flow rate 50 μL min^{-1} to ensure accuracy during analysis. The lock spray interval was set at 10 s. All data collected in centroid mode using "Mass Lynx" software (Waters Corporation, Milford, MA, USA). The ^{1}H NMR spectra were recorded on Varian Mercury plus 400 MHz FT-NMR spectrometer. The chemical shifts were reported in δ (ppm) relative to TMS as internal standard.

2.4. Preparation of standard and sample

OMP standard stock solution was prepared by dissolving 80 mg (800 μg mL^{-1}) in 10 mL of methanol & diluted to 100 mL with diluent-I. (Diluent-I: 0.01 N disodium tetra borate solutions: MeOH, 750:250 v/v) Similarly, 0.4 μg mL^{-1} standard solution was prepared by appropriate dilution of stock solution with diluent-I.

OMP DR tablets were (equivalent to 100 mg – 20 mg × 5Tablets) transferred to 250 mL volumetric flask containing 100 mL of 0.01 N disodium tetra borate solution and kept for shaking on rotary shaker at 200 RPM for about 30 min; added 100 mL of methanol and kept for sonication (sonicator bath temperature maintained between 15–20 °C) for about 30 min with intermittent shaking. Subsequently, the solution was diluted to 250 mL with diluent-II (Diluent-II: 0.01 N disodium tetra borate solution: MeOH, 500:500 v/v) and mixed well and centrifuged at 4000 RPM for about 10 min and diluted the centrifuged solution with 0.01 N disodium tetra borate solution to made the final concentration 200 μg mL^{-1} [(5 mL of centrifuged solution was transferred to 10 mL volumetric flask and diluted with 0.01 N disodium tetra borate solution] and filtered the final solution with Whatman Uniflo 0.2 μm PVDF filter by discarding about 5–7 mL of filtrate.

3. Results and discussion

3.1. Method development and optimization

We aimed to develop LCMS/TOF compatible method to separate all process related and degradation impurities from each other and from OMP; by selecting volatile ammonium acetate buffer (pKa 9.2), borax buffer as a diluent (9.2 pH in water) with sample cooler (5 °C) to avoid degradation. For selectivity MeOH and ACN were used in mobile phase. Column inlet filter (0.2 μm) was attached to column to increase the column life, as solution was hazy and viscous even after centrifugation. 305 nm wavelength was selected as per USP monograph.

Gradient program, (Time in min)/% mobile phase-B): 0/6, 0.5/6, 1/10, 9/10, 13/25, 17/25, 22/30, 29/60, 33/70, 34/6, 37/6 was considered during optimisation and it was determined that best resolution between all impurities was obtained with (Time in min)/% mobile phase-B): 0/0, 4/0, 12/8, 26/25, 34/40, 42/70, 45/70, 46/0, 48/0; except Impurity-V (5) and Impurity-VI (6) (**R2**). Similarly, it was observed that, by increasing column oven temperature from 30 °C to 40 °C resolution of R2 was increased to 2.5. Decreasing pH of mobile phase-A buffer from 9.1 to 8.9, resolution further increased to 3.0. While at pH 9.3 both impurities were merged with each other and reversed the retention of both the impurities. Hence, fixed 40 °C as column oven temperature and pH of mobile phase buffer 8.9 resolution of **R2** increased to 4.6; therefore to check the relation between CMA and CMP. DoE experiments were carried out using above finalized chromatographic conditions.