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# Analysis of potential genotoxic impurities in rabeprazole active pharmaceutical ingredient via Liquid Chromatography-tandem Mass Spectrometry, following quality-by-design principles for method development

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ABSTRACT

A novel Liquid Chromatography-tandem mass spectrometry (LC–MS/MS) method is presented for the quantitative determination of two potential genotoxic impurities (PGIs) in rabeprazole active pharmaceutical ingredient (API). In order to overcome the analytical challenges in the trace analysis of PGIs, a development procedure supported by Quality-by-Design (QbD) principles was evaluated. The efficient separation between rabeprazole and the two PGIs in the shortest analysis time was set as the defined analytical target profile (ATP) and to this purpose utilization of a switching valve allowed the flow to be sent to waste when rabeprazole was eluted. The selected critical quality attributes (CQAs) were the separation criterion *s* between the critical peak pair and the capacity factor *k* of the last eluted compound. The effect of the following critical process parameters (CPPs) on the CQAs was studied: %ACN content, the pH and the concentration of the buffer salt in the mobile phase, as well as the stationary phase of the analytical column. D-Optimal design was implemented to set the plan of experiments with UV detector. In order to define the design space, Monte Carlo simulations with 5000 iterations were performed. Acceptance criteria were met for $C_8$ column (50 × 4 mm, 5 μm), and the region having probability $\pi \geq 95\%$ to achieve satisfactory values of all defined CQAs was computed. The working point was selected with the mobile phase consisting of ACN, ammonium formate 11 mM at a ratio 31/69 v/v with pH = 6,8 for the water phase. The LC protocol was transferred to LC–MS/MS and validated according to ICH guidelines.


## 1. Introduction

Potential genotoxic impurities (PGIs) are impurities that have a structural alert related to genotoxicity, whereas genotoxic impurities (GTIs) are PGIs that were proved to be genotoxic during toxicological assessment [1]. Both pharmaceutical industries and regulatory agencies have recognized the importance of GTIs in human health and regulatory issues related to the presence of GTIs in new drug formulations have been released in the last decade by the European Medicines Agency (EMEA) [2,3] and U.S Food and Drug Administration (USFDA) [4]. Current regulatory issues are also included in the Pharmaceutical Research and Manufacturing Association (PhRMA) task force white paper [5], and the more recent ICH M7 guideline [6]. Among others, a Threshold of Toxicological Concern (TTC) was established and refers to a threshold exposure level of 1.5 μg/day, which is considered to be associated with an acceptable risk.

Rabeprazole is a member of the Proton pump inhibitors (PPIs) class, which has emerged as the drug class of choice for treating patients with acid-related diseases, including peptic and duodenal ulcer, gastroesophageal reflux disease (GERD), as well as Barrett's esophagus and Zollinger-Ellison syndrome [7]. PPIs have proven to be well tolerated, with few clinically evident side effects [8]. However, the safety of a drug product is dependent not only on the toxicological effects of the active pharmaceutical ingredient (API), but also on the impurities it contains. Among others, two PGIs have been identified [5], originating from the route of synthesis of rabeprazole, labeled as CPAR (chloropropoxy analogue of rabeprazole) and FBCI (free base of chloro intermediate) (Fig. 1). Based on the maximum daily dosage of rabeprazole (120 mg), the

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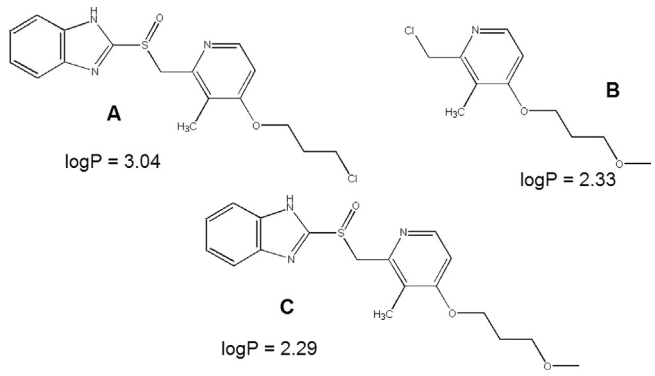


**Fig 1.** Structures of A) CPAR, B) FBCI and C) rabeprazole along with their logP values.

estimated permitted level of these impurities in rabeprazole API is 12.5 ppm/day.

Regarding analysis of PGIs, separate methods are usually developed and validated. Conventional techniques such as HPLC with UV or PDA detector or GC with flame ionization detection (FID) are usually inadequate for their accurate determination in low concentrations. In challenging cases, like the current one, when low ppm levels have to be reached, sophisticated techniques such as LC–MS or GC–MS have become very popular in the field of GTIs trace analysis due to their superior sensitivity and specificity [9–13]. Therefore, a robust method should be developed and validated for similar cases and to this purpose chemometrics constitutes a valuable tool [14].

Modern trends in pharmaceutical industry and regulatory documents strongly suggest the implementation of Quality by Design (QbD) concept in pharmaceutical product development [15–18]. Since analytical methods are also processes with a great impact on the quality of the final product, QbD should be applied to the analytical method development. QbD leads to the establishment of the design space (DS), defined as the *multidimensional combination and interaction of input variables that have been demonstrated to provide assurance of quality*. For this purpose, the method development starts with the establishment of the analytical target profile (ATP), which is the set of objectives of methods and quality requirements. The critical quality attributes (CQAs) are those properties or characteristics that should be within an appropriate limit or range to ensure the desired product quality, while the critical process parameters (CPPs) are the parameters whose variability has an impact on a CQA. The most efficient way to establish a relationship between the CQAs and the CPPs with the minimum number of experiments is the design of experiments (DoE). The DS is then considered as a "safe zone" where no considerable changes in the CQAs are observed as a consequence of small, deliberate changes of CPPs. Various papers pointed out that the model uncertainty should be taken into account when defining the DS, by estimating the errors in the criteria, therefore the optimal region obtained by response surface methodology (RSM) should not be considered a DS. Monte-Carlo simulations have been used in order to investigate the propagation of the predictive errors of the model, providing assured quality of analytical data [19–23].

In the current study, the aim was to develop a LC-UV method for the effective separation of investigated solutes in order to split the run in 2 parts: one containing the rabeprazole and the other one containing its PGIs (CPAR and FBCI). Such approach enables designation of exact moment for eluent redirection to waste in order to protect ESI source from unacceptably high concentrations of rabeprazole that is to be used in this particular analysis. Further on, this method will be transferred to a Liquid Chromatography tandem mass spectrometry (LC–MS/MS) system for validation and subsequent quantitation of the PGIs. Method development was supported by the QbD approach and in order to define the CPPs some preliminary experiments were conducted. D-optimal experimental design was chosen in order to simultaneously examine both categorical and numerical factors with a minimal number of experiments. Monte Carlo simulations propagating the error that originates from the model coefficients' calculation helped us define the DS. This way quality in DS was not only predicted but also ensured.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Standards of rabeprazole and the potential genotoxic impurities CPAR and FBCI were obtained from TLC Pharmaceutical Standards (Ontario, Canada). Acetonitrile and methanol (HPLC grade) were purchased from Fisher Chemical (Tiniakos, Athens, Greece), while ammonium acetate and ammonium formate 98% (analytical reagent grade) were obtained from Sigma-Aldrich (Athens, Greece). The pH of the mobile phase was adjusted using ammonium hydroxide solution 25% (Fluka, Athens, Greece) and formic acid 98% (Panreac, Athens, Greece). De-ionized and further purified water (Resistivity 18.2 MΩ × cm) was obtained via Synergy UV water purification system (Merck Millipore, Athens, Greece).

### 2.2. Instrument analysis

Method development including the preliminary experiments for the definition of the CPPs and optimization of the chromatographic parameters by D-optimal experimental design were carried out using a GBC LC 1120 isocratic pump (Darmstadt, Germany) system with a GBC LC 1210 UV–vis detector. Samples were introduced through a Rheodyne injector valve with a 20 μL sample loop. Data acquisition and analysis were performed using Empower software (Waters, Milford, MA, USA). Chromatographic separations were performed on YMC ODS and $C_8$, 50 mm × 4 mm, 5 μm particle size columns under isocratic elution at 25 ± 2 °C and the flow rate was set to 0.5 mL/min. Rabeprazole, as well as its impurities (CPAR, FBCI) were detected at 263 nm. Mobile phase composition, column selection, pH and salt concentration of the mobile phase were modified according to the plan of experiments defined by the D-optimal design (Table 1). All mobile phases were filtered through a 0.45 μm nylon-membrane filter, obtained from Gelman Sciences (Northampton, UK), and degassed under vacuum prior to use.

The LC–MS/MS system included one Agilent 1100 series binary pump, a degasser and a column oven/cooler (Hellamco, Athens, Greece). The CTC PAL autosampler (Hellamco) included two individually controlled valve drives, a 6-port injection valve and a 10-port switching valve. Mass spectral data were acquired on a Sciex API 4000 Qtrap mass spectrometer (Antisel, Athens, Greece) interfaced with the LC via an Electrospray Ionization source (ESI), operating in positive mode. Data were processed using the Analyst 1.5.2 software.

Parameters for MS/MS analysis were optimized by infusing a solution containing both impurities (200 ng/mL) *via* a syringe pump (Harvard Apparatus, Holliston, MA, USA). The turbo ionspray source temperature and the turbo ionspray voltage were set at 550 °C and 5000 V, respectively. High purity nitrogen (99.9%) was used for all gases in the mass spectrometer. Curtain gas was set at 10.0 a.u. (arbitrary units), collisionally activated dissociation (CAD) gas setting was medium, the nebulizer gas ($GS_1$) was set at 10 a.u., while the turbo ionspray gas ($GS_2$) at 700 L/h. Declustering potential (DP) was adjusted at 56 V and 66 V for CPAR and FBCI respectively. The collision cell exit potential (CXP) for both compounds was set at 12 V. Collision energies were set at 19 eV for CPAR and 25 eV for