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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF OMEPRAZOLE, DOMPERIDONE AND THEIR RELATED SUBSTANCES.

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ABSTRACT

The present study describes the development of a comprehensive science and risk based UPLC method and subsequent validation for the simultaneous analysis of related substances of omeprazole and domperidone active pharmaceutical ingredient (API)
using a quality by design approach. An efficient experimental design based on systemat ic scuti ng of all three components of the UPLC method (column temperature, mobile phase A and B) is presented. The optimized UPLC conditions (column temperature of 50°C, mobile phase A organic modifier (buffer: methanol, 900: 50), mobile phase B organic modifier (acetonitrile: methanol, 830: 170)) resulted in fully resolved peaks. The QbD based method development helped in generating a design space and operating space with the knowledge of all method performance characteristics and limitations and successful method robustness within the operating space.

KEYWORDS: Quality by design, UPLC, Omeprazole, Domperidone, Design space

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INTRODUCTION

Quality by design (QbD) is a key principle that has gained much discussion since its initiation as part of the U.S. Food and Drug Administration's vision for the 21st century cGMPs and the International Conference on Harmonisation (ICH) guidance on pharmaceutical development [1,2]. Quality by Design (QbD) has become an important concept for the pharmaceutical industry that is further defined in the International Confe rence on Harmonisation
(ICH) guidance on pharmaceutical developmen t as "a systematic approach to development That
begins with predefined objectives and emphasi zes product and process understanding and process control, based on sound science and q uality risk management".The quality of LC methods has become increasingly important in a QbD environment. For the purpose of QbD of LC methods, robustness and ruggedness should be verified early in the method development stage to ensure method performance over the lifetime of the product. Otherwise, if a non-robust or no n-rugged method is adopted, significant time a nd resource may be required to redevelop, rev alidate and retransfer analytical methods.The p rimary objective of this study was to implement Qbd approach to develop and validate an UPLC method that could separate omeprazole and domperidone from its potential related substances and to establish an in-depth understanding of the method and build in
the quality during the method development to e nsure optimum method performance over thelif etime of the product. The combination of Omeprazole and Domperidone is used duodenal ulcers, gastric ulcers, reflux or ulcerative oesophagitis, etc [3-4]. So far various reported RP-LC (HPLC) methods [5-7] include estimation of impurities in Omeprazole and Domperidone individually both in formulation and active pharmaceutical ingredient. But none of the methods described the single method for estimation of impurities in combination of Omeprazole and Domperidone in active pharmaceutical ingredient. Rapid, accurate and economical analytical methods has been already reported for the determination of Paracetamol and Diclofenac sodium in the combined tablet dosage form [8]. The present paper describes the quantitative estimation of impurities in combination product.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

HPLC gradient grade ACN and methanol from Merck (Mumbai, India) has been used. Di sodium hydrogen phosphate (AR grade), ortho phosphoric acid and triethylamine Solution from Merck have been used. Demineralized water was further purified in the laboratory by filtering through an ultrapure Milli-Q (Millipore, Milford, MA, USA). The working standard (USP) and active pharmaceutical ingredients of Omeprazole and Domperidone were obtained from Interlabs India Pvt limited Hyderabad, India, as gratis sample.

2.2 Instrumentation and liquid chromatographic conditions

Chromatographic separation was carried out on a Waters Aquity UPLC with photodiode array detector. The output signal was monitored and processed using Empower 2 software. The mobile phase buffer consisted of 0.01M of Di sodium hydrogen phosphate, added 1mL of triethylamine and pH adjusted to 7.5 with diluted ortho phosphoric acid as mobile phase buffer and mobile phase A (buffer: methanol (900:50)) and mobile phase B (acetonitrile: methanol (830:170)). The chromatographic separation was performed in gradient mode (min/%B, 0/20, 0.5/25, 5/50, 5.5/72, 6.2/85, 6.8/85, 7.0/20, 8.0/20) The chromatographic separation was carried out in used Zorbax XDB C 18 (100 mm X 4.6 mm, and 1.8 µm particle size) at a flow rate of volume 1.5 ml/min with 6.0 µl injection volume.

The column temperature was at 50°C. UV detection was performed at λmax 285 nm. The standard and sample preparation was made with methanol: water: diethylamine in the ratio of 800:200:1 as diluents.

2.3 Standard preparations for assay for active pharmaceutical ingredient

Accurately weighed and transferred Omeprazole and Domperidone working standard in 50ml volumetric flask, dissolved in 30 ml of diluents and sonicated for 5 minutes and made up to volume with diluents and further dilutions are made to get the final concentration of 3µg/mL and filtered through 0.22 µm filter.

2.4 Sample preparation for assay for active pharmaceutical ingredient

Accurately weighed and transferred Omeprazole and Domperidone active pharmaceutical ingredient in 100ml volumetric flask, dissolved in 60 ml of diluents and sonicated for 5 minutes made up to volume with diluents to get the final concentration of 1000µg/ mL and filtered through 0.22 µm filter.

3. RESULTS AND DISCUSSION

3.1 Method Development

A new UPLC method for simultaneous determination of related substances Omeprazole, Domperidone and its main impurities has been developed and evaluated. The UPLC method was tested for selectivity, linearity, sensitivity, accuracy and precision. The initially developed method is described in Table 1, and the obtained chromatogram for the analyses of active pharmaceutical sample is presented in (Figure 1). As could be seen from the chromatograms, resolution of impurity B of Domperidone and sulphone impurity of Omeprazole, desmethoxy impurity of omeprazole and Omeprazole, sulphide impurity of Omeprazole and Domperidone, as well as that of impurity C and impurity B of Domperidone represented the main problem because of close eluting pattern. In the optimization experiments, the effects of three study parameters; column temperature (40, 50, and 55°C), mobile phase A organic modifier (methanol), mobile phase B organic modifier, (acetonitrile: methanol) were simultaneously evaluated to assess the effects of these parameters on each of the four response variables

Table 1
A Comparison of Initial UPLC Method and Optimized UPLC Method

Parameter	Initial method	Optimized method
Column	C 18	C 18
Flow rate	1.5 ml/ min	
Column temperature	40°C	50°
Injection volume	4µL	6µL
Detection	290 nm	
Mobile phase A	Buffer : methanol (900mL:100mL)	Buffer : methanol (900mL:50mL)
Mobile phase B	Acetonitrile : Methanol (850:150)	Acetonitrile : Methanol (830:170)

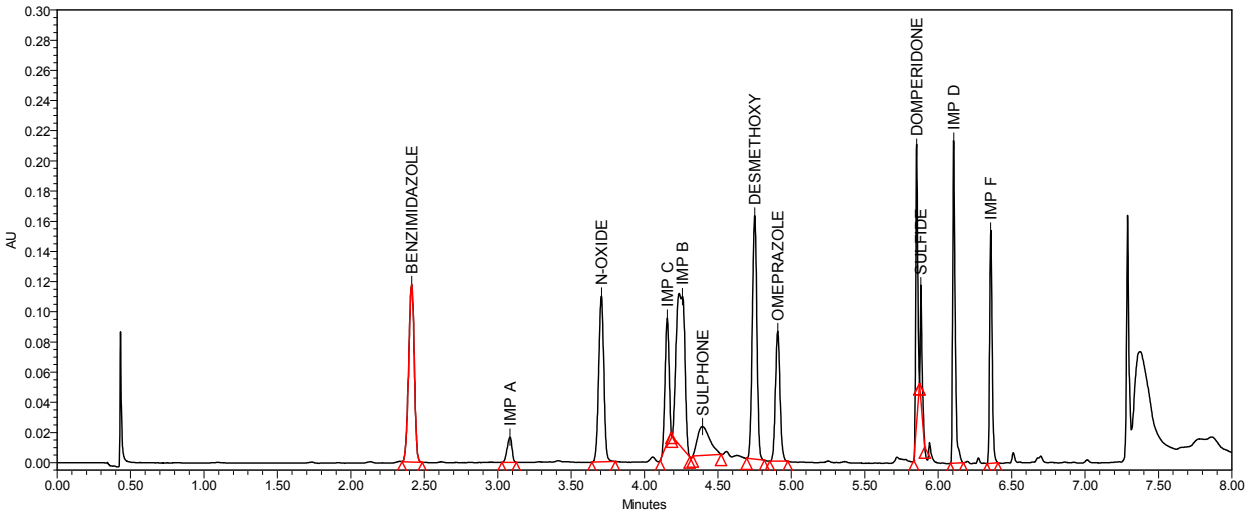

Figure 1
Chromatogram obtained for Omeprazole, Domperidone and its main impurities.

Preliminary analyses revealed the following: 1. Lower column temperature 40°C led to a poor separation of sulphide impurity of Omeprazole and Domperidone, as well as that of impurity C and impurity B of Domperidone (Figure 1). At higher column temperature 55°C, reduction of methanol content in the mobile phase A from 100ml to 50ml resulted in a better separation of impurity C and impurity B of Domperidone. Further decrease of acetonitrile concentration to 0% provided the complete coelution of impurity B of Domperidone and sulphone impurity of Omeprazole . At higher column temperature 55°C, mobile phase A (Buffer: methanol, 900 mL: 50 mL), addition of methanol to mobile phase B, resulted in a satisfactory separation of the impurities. But, at the column temperature of 50°C, mobile phase A (Buffer: methanol, 900 mL: 50 mL), mobile phase B (acetonitrile: methanol, 830 mL: 170 mL) at which better separation of the impurities was achieved.

3.2 Method Validation

Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results. Once the chromatographic conditions had been selected, the method was validated, whereby attention was paid to the selectivity, linearity, limit of detection, limit of quantification, precision and accuracy.

3.2.2 Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The specificity of the method was checked by injecting standard solution, sample solution, diluents as blank, and all impurities individually. To check the performance of the optimized LC method for the separation of degradation products, the drug was subjected to various stress conditions (Table 1). The corresponding chromatograms are shown in Figure 2. As can be seen from the figures, the method is capable of separating all the degradation products formed under the various stress conditions.

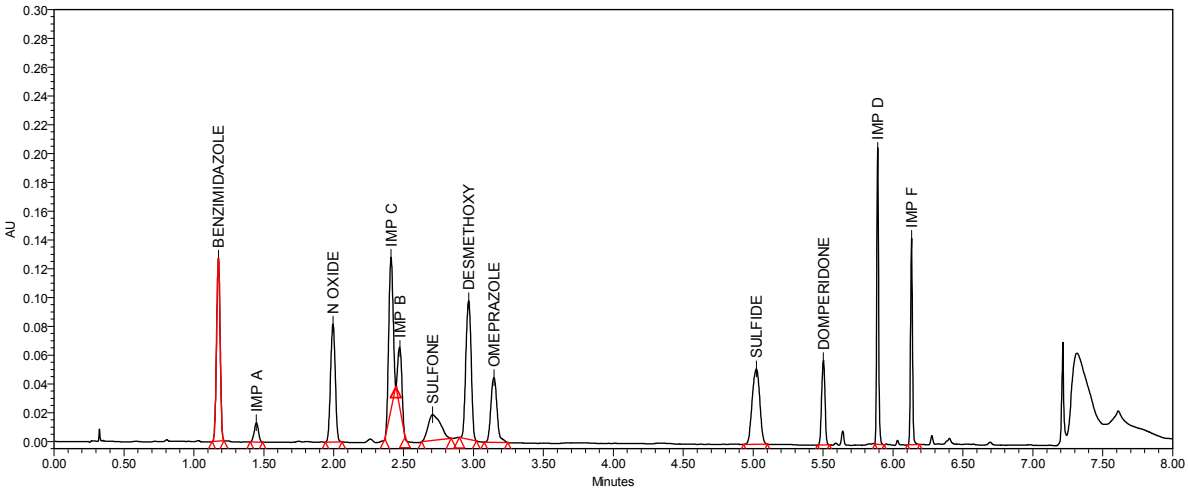

Figure 2
Chromatogram obtained after subjecting the sample to stress conditions.

3.2.3 Precision

The precision of the method was evaluated by performing six independent related substances of formulation sample the % RSD was calculated. The %RSD values for all the six impurities found to be less than 10. The intermediate precision of the method was investigated by repeating the precision studies on other days by different analysts on different system using reagents from different lots. The intermediate precision, expressed as the %RSD was found to be less than 10. The data obtained suggest that the method exhibited an excellent precision and intermediate precision.

3.2.4 Limit of Detection and Quantification

Prepared a series of dilutions of impurities and analyte in different concentrations and injected them into the chromatographic system till the signal to noise ratio is between 2 and 3.4 for limit of detection and signal to noise ration ratio is between 9.0-11.4 for a limit of quantification. Prepared six individual solutions containing impurities concentration at the limit of quantification level Injected each solution once and calculated the % RSD for the area of impurities.

3.2.5 Accuracy

The accuracy of the method was studied by recovery studies. The sample solution was prepared at six different concentration levels i.e. 25%, 50%, 100%, 150%, 200% & 300%, specified amounts of impurities had been added to sample solutions and recovery of these solutions was performed. The added amounts were calculated in terms of recovery, which were found to be between 90 – 110%.

3.2.6 Linearity

Linearity was demonstrated by injecting impurities at limit of quantification level, 25%, 50%, 75%, 100%, 125% and 150% with respect to the specification level. Plotted the calibration curve by taking concentration on X-axis and peak area on Y-axis, calculated the correlation coefficient and % y-intercept at 100% specification level. The linearity study reveals that the method is linear from LOQ to 150%.

CONCLUSION

This present paper describes the quantitative estimation of impurities in a combination product. The above developed method was found to be novel, selective, linear and accurate for the estimation of Omeprazole, Domperidone and its main impurities.

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