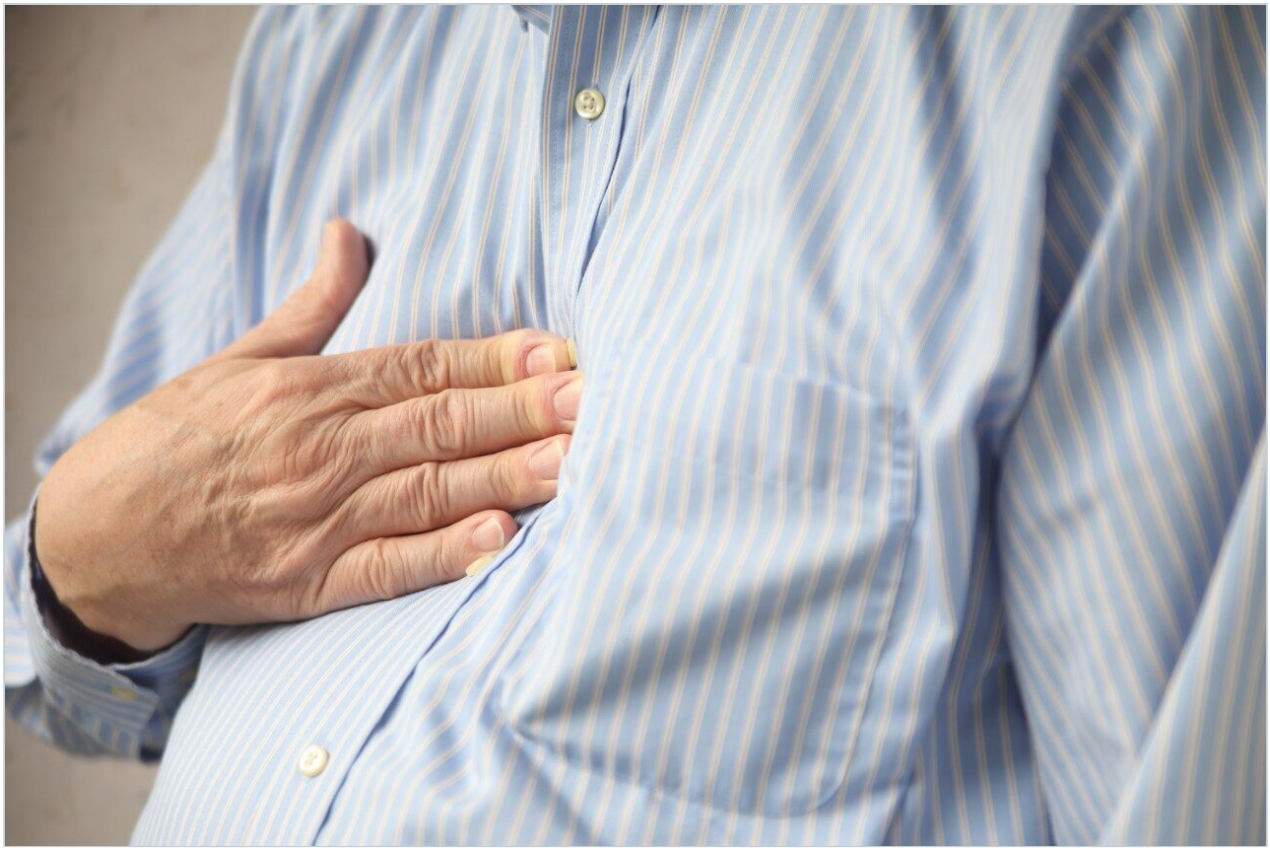
Waters™

응용 자료

Forced Degradation Analysis of Omeprazole Using CORTECS 2.7 µm Columns

Kenneth D. Berthelette, Thomas Swann, Kenneth J. Fountain

Waters Corporation



Abstract

A forced degradation study of Omeprazole was performed using a CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column on an Alliance HPLC System equipped with both a PDA and QDa Detector. This column exhibited high resolution as shown for two critical pairs of isobaric degradants, as well as backpressures compatible with traditional HPLC (<5,000 psi). The use of the CORTECS C_{18}+ stationary phase gave sharp and symmetrical peak shape for Omeprazole (a weak base) and its degradants, even when using low pH, low ionic strength pH modifiers (***e.g.*** formic acid). The combination of CORTECS 2.7 µm Columns, as well as PDA and mass detection using the QDa Detector allows for the rapid separation and identification of closely eluting compounds in complex sample mixtures. On an HPLC system, 150 mm length CORTECS 2.7 µm Columns can be used at an appropriate flow rate without exceeding the pressure limits, thus maximizing the potential for increased resolution in HPLC.

Benefits

- CORTECS C_{18}+ Columns provide superior peak shape for bases using low pH, low ionic strength pH modifiers (e.g. formic acid).
- CORTECS 2.7 µm, 4.6 x 150 mm Columns provide high resolution of complex mixtures while operating within the pressure limits of an HPLC system, enabling separation of structurally similar compounds.
- Combining UV detection with the ACQUITY QDa Detector provides easy and reliable mass detection for simple peak identification.

Introduction

Chemical stability testing is important when manufacturing pharmaceutical compounds. This is especially true with pharmaceuticals which are administered orally. The digestion process can alter the active pharmaceutical ingredient (API), producing potentially harmful by-products. In the development process, it is important to be able to detect these by-products and characterize them. In order to perform characterization of a compound, all degradation products and the main compound should be resolved from each other. CORTECS 2.7 µm Columns offer superior peak shape and resolution for the analysis of complex samples. The columns have 2.7 µm solid-core particles which allow for higher resolution, and lower backpressures than fully porous columns. Traditionally, the use of 150 mm sub-3-µm columns on HPLC systems is limited due to

the backpressure generated. However, CORTECS 2.7 µm Columns allow an analyst to use 150 mm columns on their HPLC system, offering the highest resolution possible while operating within the pressure limits of the system (<5,000 psi). The forced degradation of Omeprazole will be shown as an example and analyzed using a CORTECS C_{18}+, 2.7 µm, 150 mm Column on an Alliance HPLC System with both UV and mass detectors.

Omeprazole is a basic compound which acts as a proton pump inhibitor used in the treatment of acid reflux and heartburn. This API is also unstable at low pH.[1] Forced degradation under acidic conditions is needed to identify/characterize by-products formed under such conditions. CORTECS 2.7 µm Columns allow for high resolution between peaks in complex mixtures such as forced degradation samples. The use of the Waters ACQUITY QDa Detector allows for quick identification of peaks by mass. Due to the simplicity of the instrument, the ACQUITY QDa requires minimal training in order to use it effectively and can provide quick and reliable mass data. Unlike traditional mass spectrometers, the ACQUITY QDa Detector does not require regular tuning or calibration. This maximizes the ease of use for inexperienced analysts. By using the newest particle technologies and detection techniques, an analyst can quickly and reliably separate complex mixtures and easily obtain mass spectral data of the peaks. This can lead to faster decisions in method development, potentially reducing total development time.

Experimental

LC conditions

System:	Alliance HPLC
Column:	CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm (p/n 186007408)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	10–78% B in 16.5 min, return to 10% B in 0.1 min, hold for 3.4 min

Flow rate:	1.2 mL/min
Column temp.:	30 °C
Detection (UV):	280 nm
QDa setting:	ESI+ full scan from 120–420 *m/z*
QDa cone voltage:	15V
QDa capillary voltage:	0.8 kV
Injection volume:	9.6 µL
Data management:	Empower 3 CDS

Sample Preparation

Two Omeprazole tablets (20 mg Omeprazole) were separately crushed with a mortar and pestle and transferred to two 100 mL volumetric flasks. To one flask (A), 25 mL 0.1 N HCl was added and the solution was left at room temperature for 1.5 hours. 25 mL 0.1 N NaOH was added to neutralize the solution. Methanol was added to the flask to bring the sample up to 100 mL. The other solution (B) was diluted to 100 mL with 50:50 methanol:water. Both solutions were then filtered through a 0.2 µm filter. To create the sample for injection 0.66 mL of solution A and 0.34 mL of solution B were combined in an LCMS Certified Max Recovery Vial (p/n 600000749CV).

Results and Discussion

The sample was injected onto an Alliance e2695 HPLC System equipped with a 2998 PDA detector and an ACQUITY QDa Detector. In the case of Omeprazole, there are at least 8 known degradation compounds found in literature searches and references to USP standards.[2,3,4] Table 1 lists the compounds and their associated masses. Two sets of isobaric compounds exist in this separation. Related Compounds F and G have the same mass (312.36 *m/z*), and Omeprazole-n-Oxide and Omeprazole Sulphone have the same mass

(362.42). Figure 1 shows the separation of the acid degradation sample on a CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column.

Peak ID Number	Compound	Mass (M+H)
1	Omeprazole	346.4
2	5-methoxy-2-benzimidazole-2-thiol	181.2
3	Omeprazole Sulphide	330.4
4	Omeprazole Desmethoxy	316.4
5/6	Omeprazole Related Compound F/G	312.4
7/8	Omeprazole Sulphone	362.4
7/8	Omeprazole-n-oxide	362.4

Table 1. Known degradation products and structurally related compounds of Omeprazole and the associated masses of each compound.

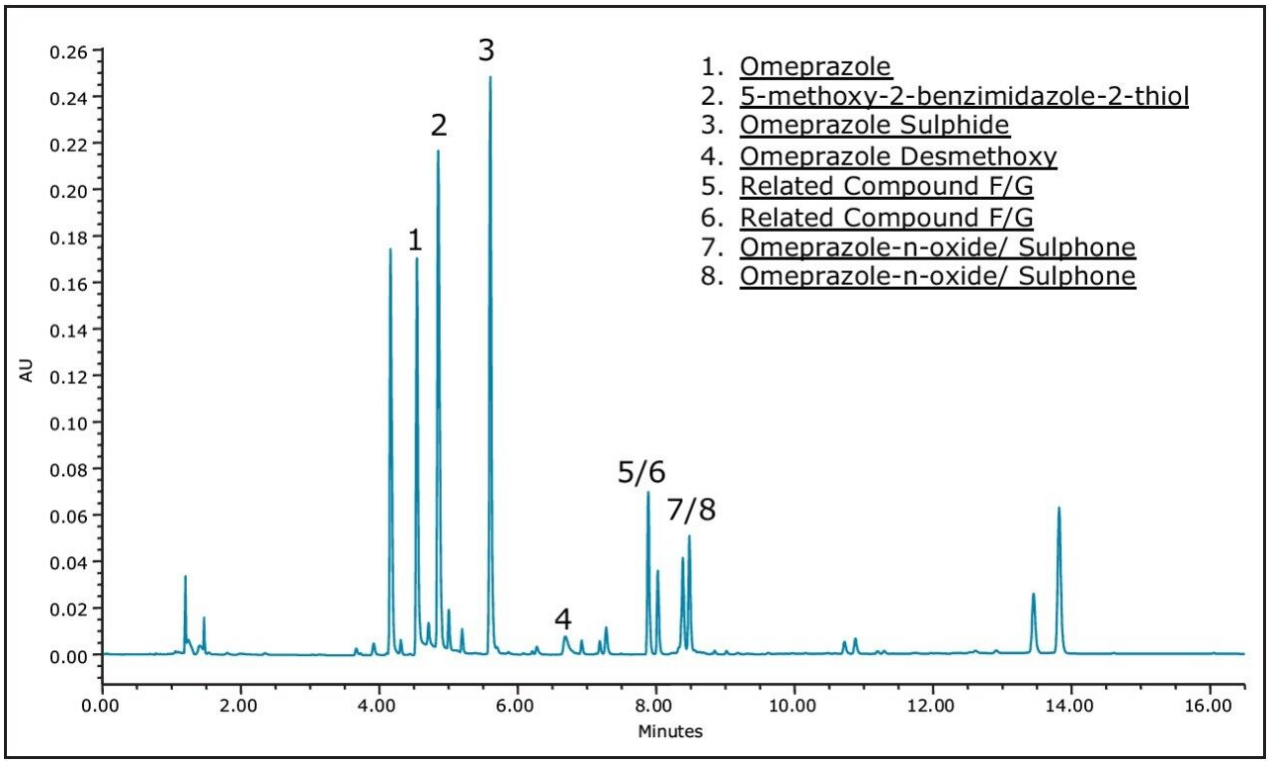


Figure 1. Separation of the forced degradation sample of Omeprazole tablets.

As seen in the above chromatography, all of the eluting peaks were sharp and well resolved. Since the

backpressure on the CORTECS 2.7 µm Columns is low compared to fully porous columns with the same particle size, a 150 mm length column can be used for maximum resolution, which is critical for the two isobaric peak pairs in this separation. In addition, use of the ACQUITY QDa Mass Detector allowed matching the UV peaks to the components in Table 1, and even though the unit mass resolution does not permit isobaric pairs 5/6 and 7/8 to be distinguished, injection of pure standards for these compounds would confirm their elution order.

Extracted ion chromatograms (EICs) were used to confirm peak identification. Figure 2 shows the EICs of Omeprazole and 5-methoxy-2 benzimidazole-2-thiol as examples. The ACQUTIY QDa Detector can also be used to generate a combined mass spectrum for a given peak. Figure 3 shows the combined spectrum for Omeprazole and Omeprazole Sulphide. Combined spectrum analysis is typically used to confirm peak identity and detect co-elution of additional compounds.

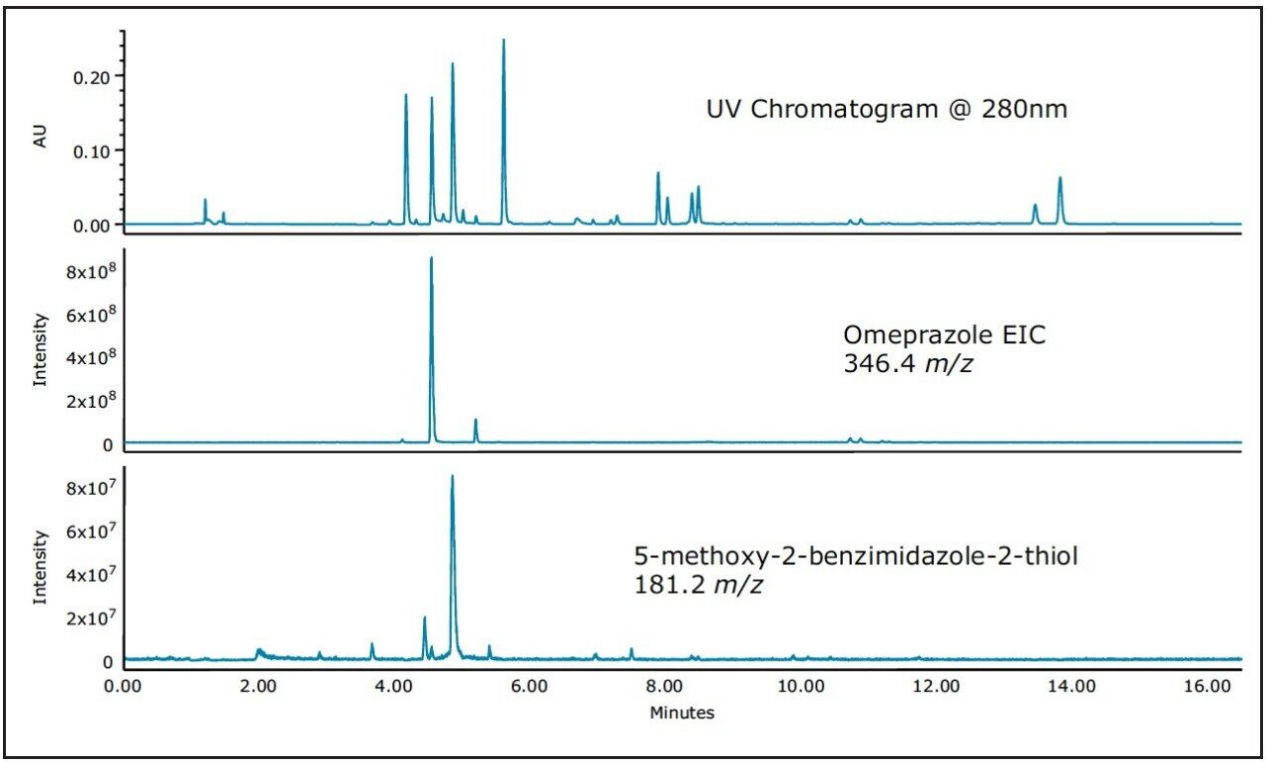


Figure 2. Extracted ion chromatograms of Omeprazole (346.4 m/z) and 5-methoxy-2- benzimidazole-2-thiol (181.2 m/z) showing positive identification of these two peaks.

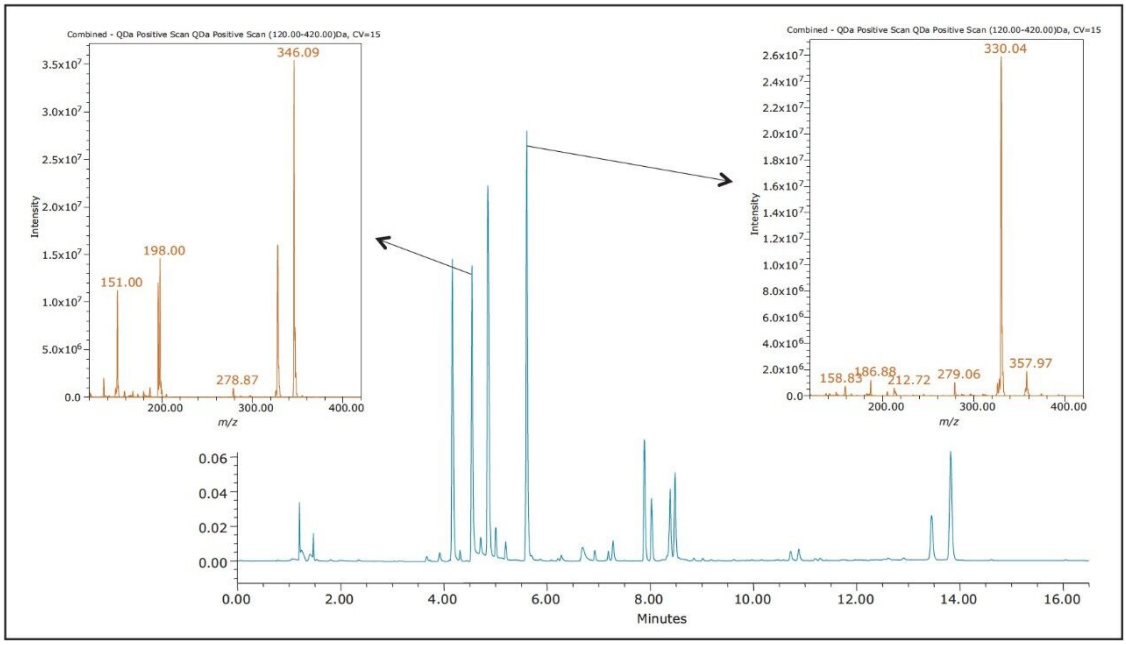


Figure 3. Combined spectrum of all masses present during the elution of Omeprazole (346.09 m/z) and Omeprazole Sulphide (330.04 m/z).

Identification of the degradants present was possible due to the high resolution separation obtained on a CORTECS 2.7 µm, 150 mm Column, and the mass data obtained on the ACQUITY QDa Detector. The use of a CORTECS 2.7 µm Column allowed for the separation of the eight separate compounds in the forced degradation sample. The high efficiency and low backpressure of these columns allows the highest possible resolution on an HPLC system.

Conclusion

A forced degradation study of Omeprazole was performed using a CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column on an Alliance HPLC System equipped with both a PDA and QDa Detector. This column exhibited high resolution as shown for two critical pairs of isobaric degradants, as well as backpressures compatible with traditional HPLC (<5,000 psi). The use of the CORTECS C_{18}+ stationary phase gave sharp and symmetrical peak shape for Omeprazole (a weak base) and its degradants, even when using low pH, low ionic strength pH modifiers (e.g. formic acid). The combination of CORTECS 2.7 µm Columns, as well as PDA and mass detection using the QDa Detector allows for the rapid separation and identification of closely

eluting compounds in complex sample mixtures. On an HPLC system, 150 mm length CORTECS 2.7 µm Columns can be used at an appropriate flow rate without exceeding the pressure limits, thus maximizing the potential for increased resolution in HPLC.

References

1. Bozdag, S.; Calis, S.; Sumnu, M. Formulation and stability evaluation of enteric-coated Omeprazole formulations. S.T.P. *Pharma Sciences* 1999, 9, 321–327.
2. Seshadri, R.K.; Raghavaraju, T.V., Chakravarthy, I.E. A Single Gradient Stability-Indicating Reversed-Phase LC Method for the Estimation of Impurities in Omeprazole and Domperidone Capsules. *Scientia Pharmaceutica* 2012, 437–458.
3. Rajab, A; Touma, M.; Rudler, H.; Afonso, C.; Seuleman, M. Slow, spontaneous degradation of lansoprazole, omeprazole and pantoprazole tablets: isolation and structural characterization of the toxic antioxidants 3H-benzimidazole-2-thiones. *Pharmazie* 2013, 749–754.
4. Search Results USP Reference Standards, http://store.usp.org/OA_HTML/usp_ibeCSrdSrchResults.jsp?kw=Omeprazole.

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

720005103, July 2014