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TECHNICAL REPORT: AMT_TR_PHARM_25

TITLE: **INVESTIGATING THE METHOD MODERNIZATION FOR THE OMEPRAZOLE UNITED STATES PHARMACOPEIA MONOGRAPH**

MARKET SEGMENT: PHARMACEUTICAL

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ABSTRACT

This report presents an updated method for the analysis of omeprazole and its impurities, modernizing the existing USP monograph for omeprazole delayed-release capsules. The current USP method, published in 2013, relies on older column technologies and complex mobile phases, resulting in lengthy analysis times and potential inefficiencies. By transitioning to more advanced chromatographic techniques, such as smaller column dimensions, reduced particle size, and a more sustainable mobile phase composition, the proposed method significantly improves both separation efficiency and environmental sustainability. The modified approach utilizes a high-pH mobile phase and a HALO® Elevate C18 column, achieving enhanced resolution and faster analysis times, reducing run time by up to 76% compared to the original method. Additionally, switching from acetonitrile to methanol as the organic solvent results in better separation of omeprazole related isomers and offers a more environmentally friendly solution. The method also demonstrates excellent robustness, with the HALO® Elevate C18 column exhibiting minimal changes in performance over 500 injections, ensuring reliability for quality control. Overall, this modernization offers a more efficient, environmentally conscious, and reliable alternative to the outdated USP method for omeprazole analysis, advancing both analytical precision and sustainability in pharmaceutical testing.

INTRODUCTION

The United States Pharmacopeia (USP) is responsible for setting and publishing official standards for medicines, food ingredients, and dietary supplements in the United States. This includes many medicines that we use every day, including a drug called omeprazole. Omeprazole is a proton pump inhibitor (PPI) that works by reducing the amount of acid produced in the stomach. Its use is widespread to treat conditions related to excessive stomach acid production. Following safety regulations and protocols, monitoring the impurities and concentrations of said impurities when manufacturing the drug is required.

Monitoring the quality control production of any drug is an important task and this includes the chromatographic separation of the omeprazole drug component and its impurities. The methods that are set by the USP have specific requirements that must be met when it comes to the chromatography of the drug and impurities being tested. These methods can be outdated, requiring the use of older technologies. Mobile phases, columns, and packing material are just a few variables that are set by the USP monographs that can be modernized. In this report we present an example of modernizing the USP method for omeprazole and its impurities, taking advantage of the newer column technologies that are available.

The current USP monograph for omeprazole delayed-release capsules was published in 2013 and employs the use of an L7 column. The column required for the method is a 4.6 x 150 mm, 5 µm with a C8 stationary phase. The suggested method improvements in this report fall outside of the allowable changes set by the USP, requiring validation of the new method. The suggested improvements over the current USP monograph are as

follows, reducing column dimensions from 4.6 x 150 mm to a 2.1 x 100 mm, reducing particle size from 5 µm to 2.7 µm, and changing column packing from a C8 (L7) to a C18 (L1). Along with changes in column technology, changes to gradient, mobile phase, and temperature were suggested. The gradient time has been reduced by 52%, mobile phase A preparation is less complicated becoming 0.03% ammonium hydroxide in water instead of glycine in water adjusted with to pH 9 with sodium hydroxide, mobile phase B is simplified to either acetonitrile or methanol, and temperature has been fixed at 50°C where the monograph has no requirement for column temperature. These changes to the USP monograph reduce complicated and unnecessary methods, saving time and resources.

EXPERIMENTAL

A Shimadzu Nexera UHPLC system (Columbia, MD) was used for the chromatographic separations and generation of data. Standards, solvents, and chemicals were obtained via Sigma Aldrich (St. Louis, MO). Columns used for the separations are indicated below.

Columns:

- HALO® 120 Å Elevate, 2.7µm, 2.1 x 50 mm
  Part Number: 92272-402
- HALO® 120 Å Elevate, 2.7µm, 2.1 x 100 mm
  Part Number: 92272-602
- HALO® 90Å C8, 5µm, 4.6 x 150 mm
  Part Number: 95814-708

Mobile Phase A:
0.03% Ammonium Hydroxide, pH 10.65
(Figure 3, 6, 7, 8, 10, 11)
20mM Potassium Phosphate, pH 7.1 (Figure 2)
0.1% Formic Acid, pH 2.8 (Figure 1, 4)
40 mM Glycine, pH 11 (Figure 5)

Mobile Phase B:
Acetonitrile (Figure 1, 2, 3, 4, 6)
Methanol (Figure 7, 8, 10, 11)
85:15 Acetonitrile:Methanol (Figure 5)

Flow Rate:
0.4mL/min (Figure 1, 2, 3, 4, 6, 7, 8, 10, 11)
1.2mL/min (Figure 5)

Temperature:
60 °C (Figure 1, 2, 3, 4)
40 °C (Figure 6, 7, 8, 10, 11)
Ambient (Figure 5)

Detection:
UV 305 nm

Initial Back Pressure:
167 bar (HALO® 50 mm)
260 bar (HALO® 100 mm)
233 bar (HALO® 150 mm C8)

Sample(s):
Omeprazole (62.5µg/mL) and Related Impurities/ Compounds (12.5µg/mL)
Omeprazole slow-release capsules
Weigh and mix the contents of NLT 20 Capsules. Transfer an accurately weighed portion of the Capsule content, equivalent to 20 mg of omeprazole, to a 100 mL volumetric flask, add about 50 mL of Diluent, and sonicate for 15 min. Cool, dilute with Diluent to volume, mix, and pass through a membrane filter of 0.45 µm or finer pore size.

Sample Solvent:
Following USP diluent guidelines. Dissolve 7.6 g of sodium borate decahydrate in about 800 mL of water. Add 1.0 g of edetate disodium and adjust with 50% sodium hydroxide solution to a pH of 11.0 ± 0.1. Transfer the solution to a 2000 mL volumetric flask, add 400 mL of dehydrated alcohol, and dilute with water to volume.

Data Rate: 40 Hz
Response Time: 0.05 sec.
Flow Cell: 1 µL

Peak Identities:
1. Related compound F&G
2. Related compound B
3. Related compound E
4. Related compound A
5. Impurity B
6. Omeprazole
7. Impurity H
8. N'-Methyl omeprazole
9. Impurity C

RESULTS

Omeprazole is a proton pump inhibitor (PPI) that contains a benzimidazole ring with a methoxy group and a sulfinyl group, making it sensitive to acidic conditions. Under low pH, omeprazole undergoes hydrolysis of its pyridine ring, resulting in the formation of a sulfenic acid intermediate that can further degrade. This degradation pathway can result in the loss of omeprazole's pharmacologically active structure and leads to the formation of various degradation products. These products could interfere with chromatographic analysis, causing inaccurate quantification or misleading results.

Furthermore, omeprazole exists in a pH-dependent equilibrium, where at acidic pH values, it may become protonated (forming a cation), which alters its physicochemical properties, such as polarity and solubility. This can significantly change its retention behavior in liquid chromatography and affect both the separation efficiency and detection sensitivity. Therefore, omeprazole is typically analyzed under neutral or mildly basic conditions to preserve its stability and ensure reliable chromatographic performance.

Effect of pH

To demonstrate the effect of pH on omeprazole analysis, we employed a series of mobile phases to provide an example of how the buffer system impacts the separation. The example is provided below.

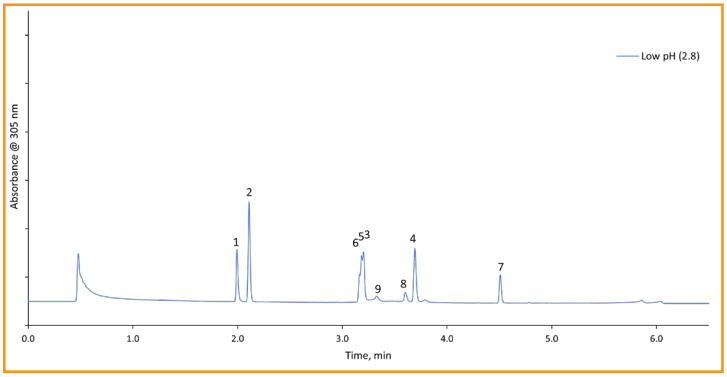

Figure 1. A separation of omeprazole and related compounds/impurities under acidic conditions using 0.1% formic acid at a pH of 2.8. Gradient of 5-55% in 6 minutes.

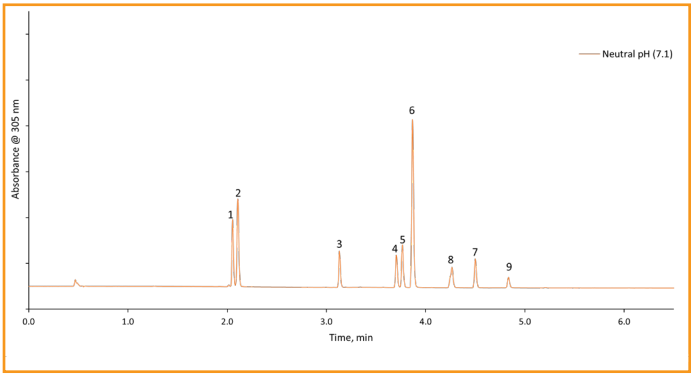

Figure 2. A separation of omeprazole and related compounds/impurities under neutral conditions using 20mM potassium phosphate at a pH of 7.1. A gradient of 5-55% in 6 minutes.

The low pH mobile separation shows a clear difference between resolution and peak shape when compared to the neutral (pH 7.1) and high pH (pH 10.6) counterparts. Under acidic conditions three compounds fully coelute, peaks 3, 5, and 6. Peak 6 being omeprazole is particularly problematic, making accurate quantitation impossible via UV. The resolution of peaks 5 and 6 increased from complete coelution to a value of 1.7, just by increasing the pH of mobile phase A to 7.1. By increasing to a pH of 10.6, the minimum resolution value increased to 4.5. This supported the USP monograph that to obtain an optimal separation of omeprazole and its related compounds/impurities, with regards to resolution and peak asymmetry, the pH of the mobile phase must be either neutral or slightly basic. Both separations show baseline resolution between each compound with a pH of 10.6 giving the best separation overall. The same gradient was employed throughout the pH analysis while mobile phase composition was changed to reach each desired pH.

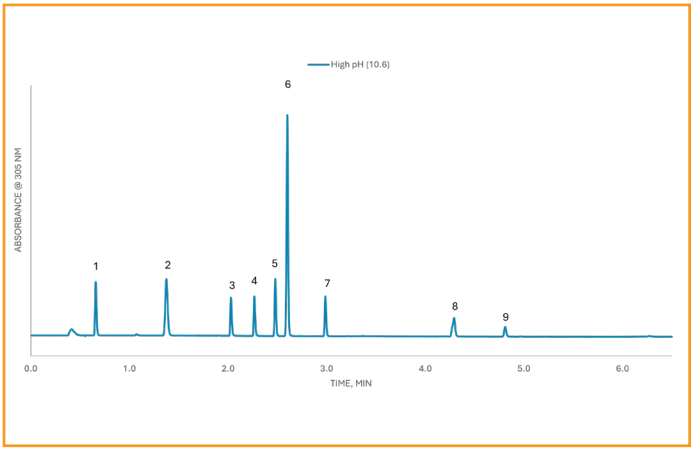

Figure 3. A separation of omeprazole and related compounds/impurities under basic conditions using 0.03% ammonium hydroxide at a pH of 10.6. A gradient of 5-55% in 6 minutes.

Degradation

Due to the chemical structure of omeprazole, degradation can readily occur under acidic conditions. To investigate the effects of degradation under acidic conditions, the pure omeprazole standard was injected without related compounds or impurities at a pH of 2.8 under the following gradient of 5-55% B in 6 minutes. The separation showed chromatographic issues, resulting in multiple peaks and poor peak shape for the omeprazole standard. When injecting a pure standard, a singular peak is expected along with symmetrical peak shape. The pure omeprazole standard, injected under acidic conditions, has 5 major peaks with the most responsive peak having a tailing value of 4.2. An example of this separation can be seen in Figure 4.

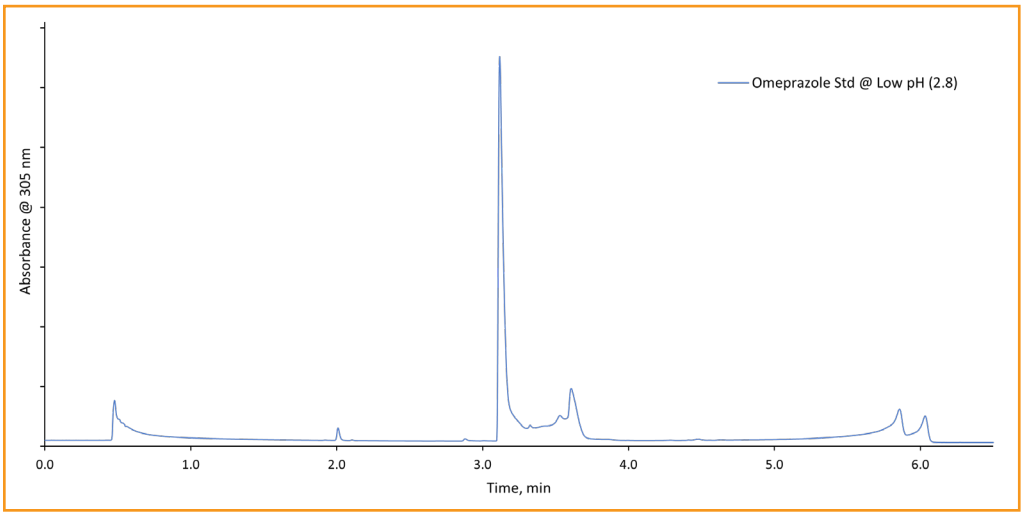

Figure 4. Omeprazole standard separated under formic acid conditions at a pH of 2.8. The separation results in poor peak shape and multiple peaks which can be potential degradants of the standard. Gradient of 5-55% in 6 minutes.

METHOD IMPROVEMENTS

With the focus on modernizing the current USP method, some variables were targeted to remove unnecessary long runtimes, complex mobile phases, and decrease reliance on toxic and expensive solvents. The current USP method, Figure 5, calls for a glycine buffered mobile phase A, at a pH of 11, along with a mixed mobile phase B containing acetonitrile and methanol. While we have previously shown that basic conditions provide optimal separation of omeprazole from its related compounds/impurities, a pH of 11 is not needed to provide symmetrical peak shapes and may only reduce column lifetime. The USP method requires the use of a 4.6 x 150 mm, 5 µm, C8 column, a 25 minute long gradient, and a complex mobile phases. With the changes discussed below, improvements to the USP method can be obtained, but the methods will need to be revalidated due to the switch in stationary phase.

By reducing the column ID, length, and particle size, the separation time can be reduced from 25 minutes to 12 minutes. By reducing the column length to 100 mm, a time savings of 52% is demonstrated. By utilizing a 50 mm column length, analysis time can be reduced by 76% compared to the original method. Examples below show the utility of smaller columns using a high pH stable stationary phase on SPP technology. The HALO® Elevate C18 column provides a high pH stable stationary phase that can withstand the requirements of modernizing the omeprazole USP method. Along with the Fused-Core® technology the HALO® Elevate C18 column can reliably separate omeprazole and the numerous related compounds/impurities with baseline resolution in a gradient time as short as 6 minutes. Fused-Core® particles have better mass transfer effects than FPP technology, improving separations by reducing run time and increasing resolution.

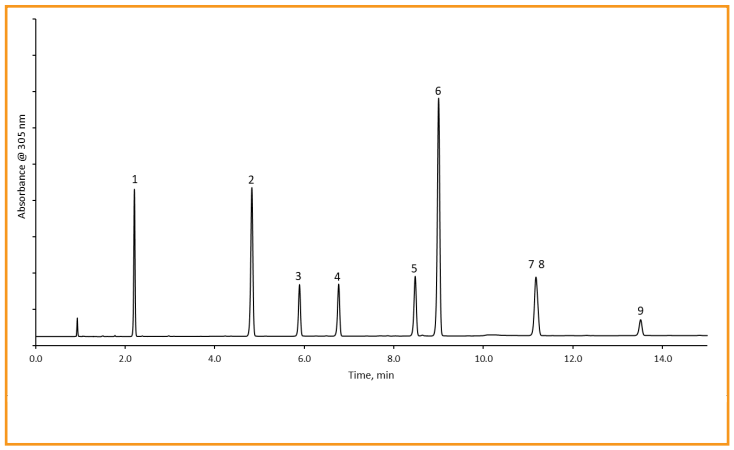

Figure 5. A separation of omeprazole and related compounds/impurities under the USP chromatography impurity conditions. On a 4.6 x 150 mm, 5 µm, C8 column the separation is completed in under 15 minutes with minor fronting on all peaks. A full coelution between peaks 7 (Impurity H) and 8 (N'-Methyl omeprazole) is the outcome of the USP method.

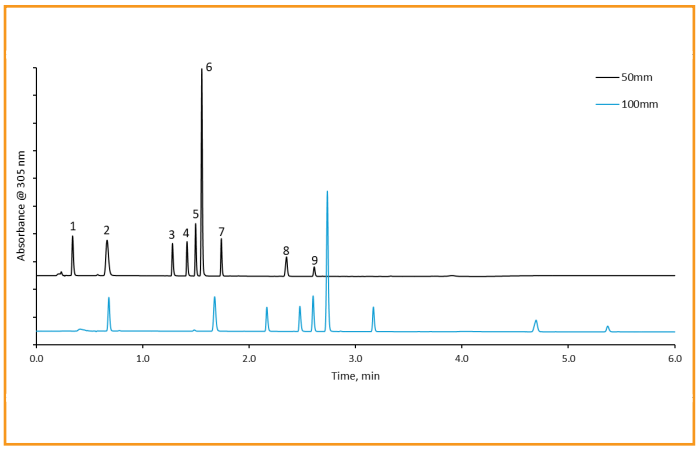

Figure 6. Comparison of two different HALO® Elevate C18 columns. A 50 mm column (167 bar) completes the omeprazole separation in under 3 minutes while the 100 mm (260 bar) completes the separation in under 6 minutes. Gradient of 5-55% in 3 minutes for the 50 mm column and a gradient of 5-50% in 6 minutes for the 100 mm column.

SUSTAINABILITY

Modernizing a method can also include other variables, such as making a method more environmentally friendly. While reducing column ID and length, one may reduce solvent consumption. By switching organic modifiers, chromatographers can decrease the use of harmful mobile phases. An example of this is the conversion from a mixed mobile phase B, acetonitrile:methanol (85:15), as a standard organic modifier to exclusively methanol. Methanol, being a more sustainable and environmentally friendly organic solvent, should be investigated as a suitable replacement for acetonitrile. In this report, methanol improved the separation by maintaining the same elution order and separating isomers of N'-Methyl omeprazole which were not resolved with acetonitrile or the 85:15 ratio from the monograph. The impact of these sustainability changes can be calculated by the analytical method greenness score (AMGS), developed by the American Chemical Society (ACS), and used to compare which method is more environmentally friendly. The lower the AMGS score, the more environmentally friendly the method. When coupled with the modernized method, using a HALO® 2.1 x 100 mm Elevate C18 column with methanol can reduce the AMGS, from 73.93 (current monograph) to 17.93 (modernized monograph).

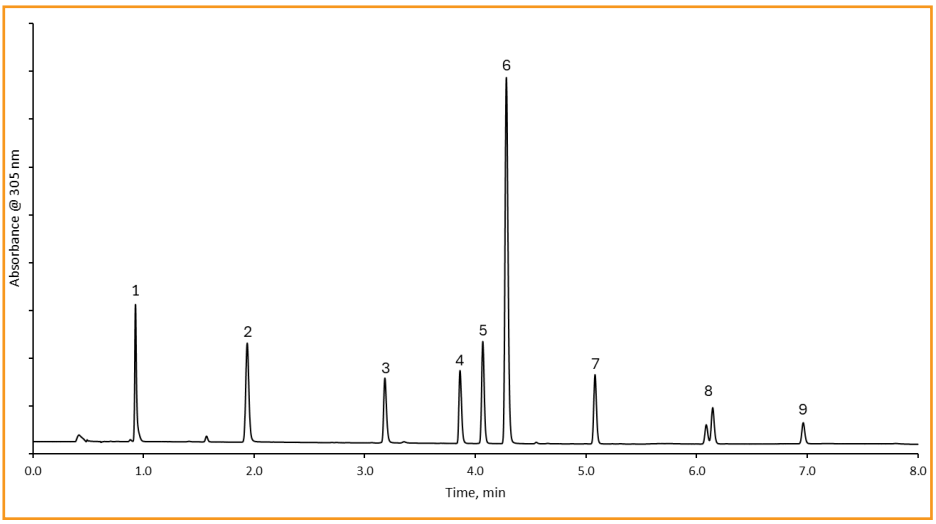

Figure 7. A separation of omeprazole and its related compounds/impurities separated under high pH conditions with methanol as the organic solvent. The incorporation of methanol improves the overall separation, by increasing resolution for the early eluting peaks and maintaining baseline resolution of critical pairs. Methanol also promoted more separation between the isomers of N'-Methyl omeprazole (peak 8). Gradient of 10-60% in 6 minutes.

ROBUST METHODOLOGY

Modernizing a USP method requires robust methods. Labs that perform quality control need stable methods that can be run consistently with little change in the chromatography to comply with method requirements. The HALO® Elevate C18 column by Advanced Materials Technology has demonstrated lifetime by thoroughly completing a lengthy stability test of omeprazole injections. The chromatography resulted in little to no changes, with a max of 2% RSD in retention for the omeprazole standard, from injection 1 to injection 500 under method conditions for our modernized method. In validating new methods, stability of materials such as the column, need to be verified. The HALO® Elevate C18 column displays stability of 500 injections or (34,000 column volumes) at minimum and proves to be a great choice for USP modernization.

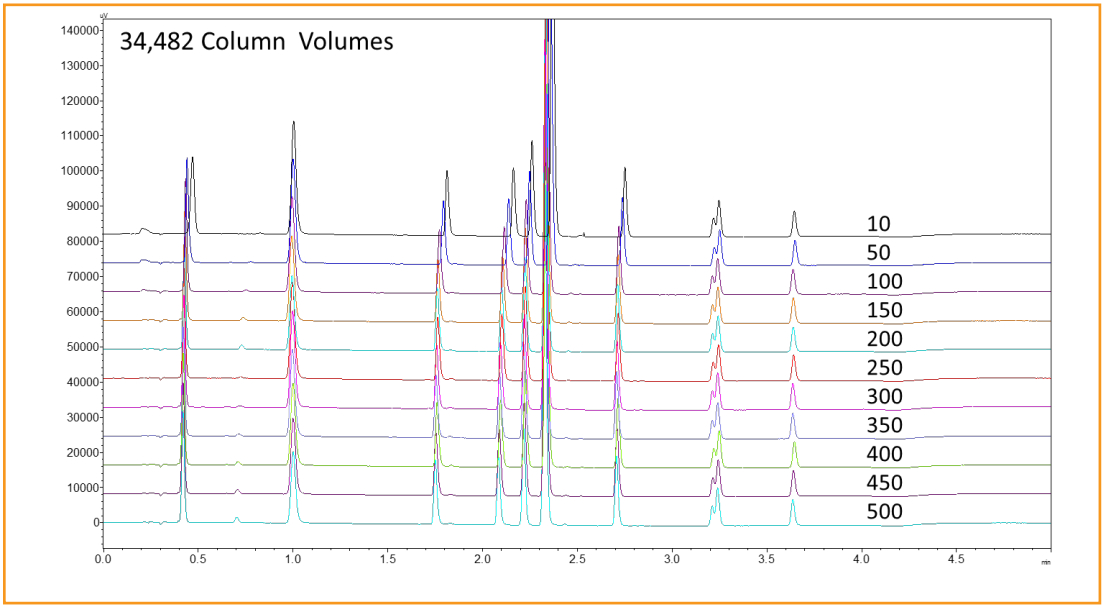

Figure 8. A stability test of a modernized USP omeprazole method conducted on a 2.1 x 50 mm HALO® Elevate C18 column. 500 injections, 34,000 columns volumes. Gradient of 10-60% in 3 minutes. Initial backpressure at 156 bar and final backpressure at 165 bar.

Another way to confirm that a method is robust is to perform a calibration curve. By maintaining a linear calibration curve, chromatographers can be sure that when testing different concentrations of drug, their system and methods are operating adequately. A calibration curve of omeprazole was performed to demonstrate the accuracy of the method, instrumentation, and column.

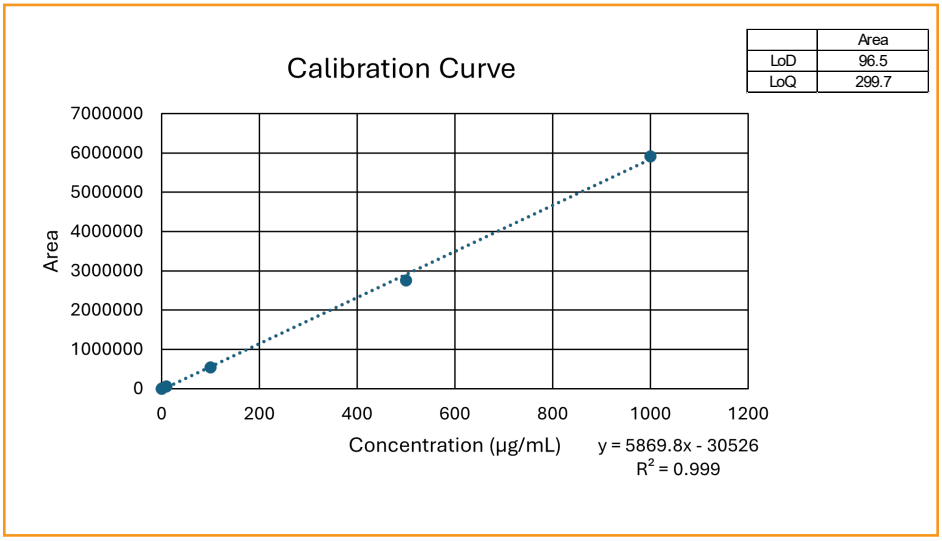

Figure 9. A calibration curve of the omeprazole standard. Also calculated in the upper corner are the LoD and LoQ values for the omeprazole standard.

As an added proof of concept, a 20 mg capsule of delayed-release omeprazole was dissolved, following the USP method sample preparation instructions, and injected on a 2.1 x 100 mm HALO® Elevate C18 column using the same method shown earlier in this report. As expected, there were fewer impurity peaks than the standard impurity mix. One peak (peak 1) could be matched to the Related compounds F&G. These related compounds were 0.05% of the omeprazole capsule; just over the LoD and LoQ values. With this value, the delayed release capsule would pass the quality requirements set by the USP.

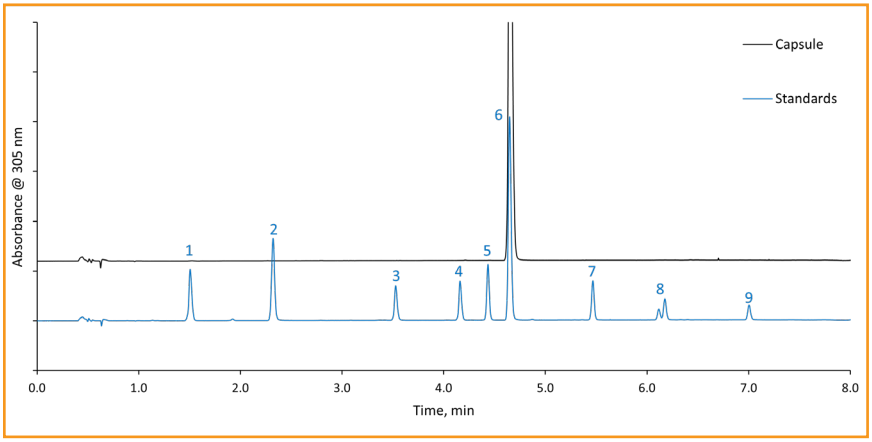

Figure 10. A comparison of a dissolved omeprazole capsule and the standard impurity panel that was used throughout the report. The omeprazole peak elutes at the same time for each sample, 4.65 minutes, illuminating the consistency with the chosen method in 6 minutes.

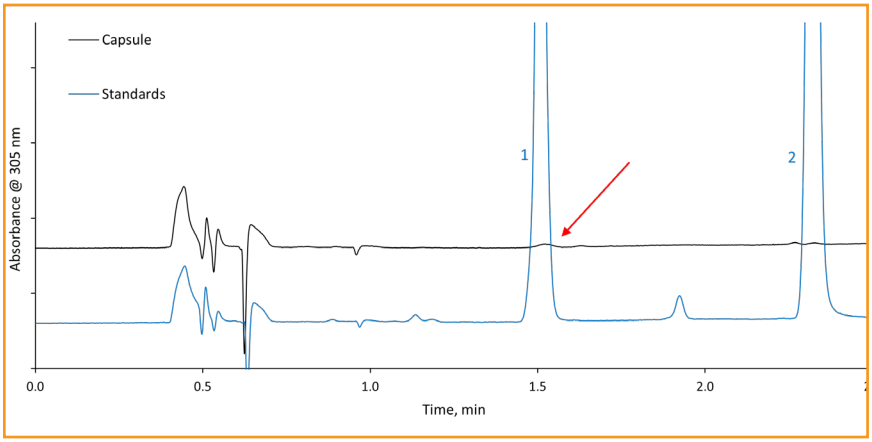

Figure 11. A comparison of a dissolved omeprazole capsule and the standard impurity panel. A zoomed-in view of a potential impurity from the commercial capsule of omeprazole. The potential impurity peak aligns with peak 1, Related compound F&G, from the impurity panel. The peak passes the LoQ of 299.7, with an area of 685, making it a 0.05% composition of the omeprazole peak.

CONCLUSION:

In conclusion, this report demonstrates a successful improvement to the current USP method for analyzing omeprazole and its impurities by utilizing more efficient and environmentally friendly chromatographic techniques. By optimizing factors such as mobile phase pH, column specifications, and solvent choice, improved separation efficiency, reduced analysis time, and minimized solvent consumption were achieved. Specifically, the use of a high pH mobile phase and smaller column dimensions significantly enhanced resolution while maintaining MS compatibility. Moreover, replacing acetonitrile with methanol proved to be not only environmentally beneficial, but also more effective in separating omeprazole and its related impurities as demonstrated with the isomer separation.

Additionally, the modernized method was investigated for improvement through stability tests, with the HALO® Elevate C18 column showing exceptional durability over 500 injections, ensuring consistency and reliability in quality control processes. Ultimately, the approach presented in this report offers a more efficient, sustainable, and potentially more reliable alternative to the outdated USP method, benefiting both analytical performance and environmental impact in the pharmaceutical industry.

REFERENCES:

1. *Monograph: USP. Omeprazole Delayed-Release Capsules. In: USP–NF. Rockville, MD: USP; July 5, 2023. DOI: https://doi.org/10.31003/USPNF_M58645_01_01*
2. *Low, M., Singh, S., Venkataya, B., Pearson, J., Ibrahim, M., Jarouche, M., Khoo, C., Rowe, J. and Li, C.G. (2022), Stability of omeprazole in a commercial calcium carbonate based oral suspension at 2, 5 and 10 mg/mL stored under refrigeration (4°C) for 70 days. J Pharm Pract Res, 52: 34-41. https://doi.org/10.1002/jppr.1782*
3. *Srinivasarao Koppala, V. Ranga reddy, Jaya Shree Anireddy, Development and Validation of a Novel Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Related Substances of Ketoprofen and Omeprazole in Combined Capsule Dosage Form, Journal of Chromatographic Science, Volume 54, Issue 5, May 2016, Pages 765–775, https://doi.org/10.1093/chromsci/bmw008*
4. *Iuga, C. A., & Bojita, M. (2010). Stability study of omeprazole. Farmacia, 58, 203–210.*
5. *Seshadri, R. K., Raghavaraju, T. V., & Chakravarthy, I. E. (2013). A Single Gradient Stability-Indicating Reversed-Phase LC Method for the Estimation of Impurities in Omeprazole and Domperidone Capsules. Scientia pharmaceutica, 81(2), 437–438. https://doi.org/10.3797/scipharm.1209-12*
6. *Murakami, F. S., Cruz, A. P., Pereira, R. N., Valente, B. R., & Silva, M. A. S. (2007). Development and Validation of a RP‐HPLC Method to Quantify Omeprazole in Delayed Release Tablets. Journal of Liquid Chromatography & Related Technologies, 30(1), 113–121. https://doi.org/10.1080/10826070601034485*
7. *Ferretti, R., Zanitti, L., Casulli, A., & Cirilli, R. (2018). Unusual retention behavior of omeprazole and its chiral impurities B and E on the amylose tris (3-chloro-5-methylphenylcarbamate) chiral stationary phase in polar organic mode. Journal of pharmaceutical analysis, 8(4), 234–239. https://doi.org/10.1016/j.jpha.2018.04.001*

KEY WORDS:

Omeprazole, USP monograph, chromatographic separation, proton pump inhibitor (PPI), impurities, high-performance liquid chromatography (HPLC), UHPLC, Fused-Core®, modernization, C18 stationary phase, mobile phase optimization, pH effect, gradient optimization, column dimensions, particle size reduction, sustainability, methanol as solvent, acetonitrile replacement, chromatographic method validation, analytical method greenness score (AMGS), degradation products, related compounds, impurity quantification, environmental impact, column stability, resolution, separation efficiency, retention time, calibration curve, omeprazole slow-release capsules.