AAPS PharmSciTech, Vol. 16, No. 1, February 2015 (© 2014)
DOI: 10.1208/s12249-014-0207-7

Research Article

Development and Validation of a UPLC Method for Rapid and Simultaneous Analysis of Proton Pump Inhibitors

Richard T. Addo,[1,2] Kenneth Davis,[1] Ruhi Ubale,[1] Joel S. Owen,[1] and E. Blake Watkins[1]

Received 10 April 2014; accepted 14 August 2014; published online 27 August 2014

Abstract. Proton pump inhibitors (PPIs) are used extensively for the relief of gastroesophageal reflux, peptic ulcers, and other hypersecretory conditions. Some of the commonly used PPIs—omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole—were used in this study with the aim of developing a rapid ultra performance liquid chromatography (UPLC) method for detecting each and allowing separation and quantification of a mixture of PPIs. An analysis of samples was performed on a UPLC system equipped with a quaternary solvent delivery system, a refrigerated sample manager, a column heater, a photo diode array detector scanning from 210 to 400 nm, and a C_{18} analytical column (50 mm×3.0 mm, 1.7-μm particle size). The chromatographic analysis of the PPI samples and standards was performed using gradient elution with acetonitrile and water. The calibration curve range varied for each of the PPIs ranging from a lower limit of 0.75–1.78 μg/mL to a maximum concentration of 200 μg/mL with a regression coefficient (r^2) of ≥0.98. The accuracy and precision were calculated, and the %RSD was determined to be ≤0.21% (intraday) and ≤5% (interday). The LOD was 0.23–0.59 μg/mL and the LOQ was 0.71–1.78 μg/mL for each of the drugs analyzed. The method was capable of detecting and quantifying each drug in a mixture with good resolution and a total run time of less than 5 min. Herein, we report an efficient and rapid analytical method for the simultaneous detection of multiple PPIs in a mixture.

KEY WORDS: HPLC; method development; proton pump inhibitors; simultaneous analysis; UPLC.

INTRODUCTION

Proton pump inhibitors are a class of widely prescribed drugs that cause a long lasting reduction of gastric acid secretion and are useful for the treatment of gastroesophageal reflux disease (GERD), peptic ulcer disease (PUD), and gastritis. Proton pump inhibitors (PPIs) are prodrugs and, therefore, undergo activation through an acid-catalyzed rearrangement leading to covalent binding to the H^+/K^+-ATPase enzyme (proton pump), thereby inhibiting gastric acid secretion (1–3). All currently FDA-approved PPIs (omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole) contain a 2-pyridylmethylsulfinylbenzimidazole pharmacophore (Fig. 1) and differ only in the nature of substituents on the pyridine and benzimidazole rings (4).

As part of a program to evaluate stability of finished drug products, we desired a method capable of detecting individual PPIs as well as mixtures of various PPIs (5). The method described, herein, provides broad flexibility in PPI analysis. This procedure can be used for the determination of the aforementioned PPIs in final dosage forms to monitor for content uniformity. Stability studies of various dosage forms can be performed rapidly as well, facilitating the analysis of a large number of samples in a short period of time. To our knowledge, this is the first account of an ultra performance liquid chromatography (UPLC) method capable of the simultaneous detection and quantification of multiple PPIs in a single run.

Existing methods utilize HPLC for the analysis of multiple PPIs in a mixture (4,6). UPLC provides the benefit of small injection volumes, shortened run times (<5 min), and reduced solvent usage, making it the more economical method for quantitation. Additionally, UPLC provides greater peak resolution, owing to the use of 1.7-μm-particle size columns and higher operating pressures (≤15,000 psi) (7–9). In the current study, we have developed a convenient, cost-effective, validated UPLC method (10,11) that enables simultaneous detection and quantitation of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole.

MATERIALS AND METHODS

Chemicals

USP analytical samples were purchased from Sigma-Aldrich (St. Louis, MO, USA). The structures of these agents are shown in Fig. 1. HPLC grade acetonitrile (ACN) and water were purchased from VWR Scientific (Radnor, PA, USA).

[1] Department of Pharmaceutical Sciences, School of Pharmacy, Union University, Jackson, Tennessee 38305, USA.
[2] To whom correspondence should be addressed. (e-mail: raddo@uu.edu)

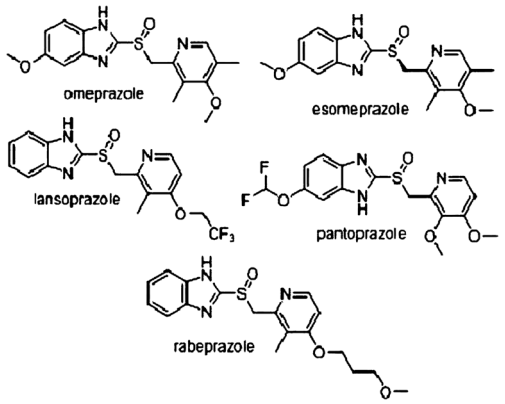

Fig. 1. FDA-approved proton pump inhibitors

Equipment

The analysis of samples was performed on a Waters (Milford, MA, USA) Acquity H-class UPLC system equipped with a quaternary solvent delivery system, a refrigerated sample manager, a column heater, and a photo diode array detector.

Preparation of Standards

Omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole were obtained from Sigma-Aldrich. HPLC grade acetonitrile and water were obtained from VWR Scientific. Each of the USP standards (10 mg) was weighed on a USP-certified, calibrated, analytical balance and dissolved in 1:1 acetonitrile/water (50 mL) to give a 200-μg/mL stock solution. This stock solution was diluted appropriately to prepare standards of the following concentrations: 150, 100, 75, 50, 25, 10, 1, and 0.75 μg/mL. Prior to UPLC analysis, samples were filtered through a 0.2-μm filter.

Preparation of Samples

Stock solutions (200 μg/mL) of each of the drugs were prepared in 1:1 acetonitrile/water. A sample containing all five drugs was prepared by mixing equal volumes of each stock solution such that the final concentration of each PPI was 40 μg/mL. The samples were filtered through a 0.2-μm filter prior to UPLC analysis.

Chromatographic Conditions

The analysis of samples was performed on a Waters Acquity H-class UPLC system equipped with a quaternary solvent delivery system, a refrigerated sample manager, a column heater, a photo diode array detector scanning from 210 to 400 nm with a Waters BEH C_{18} (50 mm× 3.0 mm, 1.7-μm particle size) analytical column. The chromatographic analysis of the PPI samples and standards was performed using a linear gradient (80:20 to 50:50 water/ACN over 4.5 min). The sample storage compartment was maintained at 10.0°C, while the column was maintained at 35.0°C throughout each run with a mobile phase flow rate of 0.45 mL/min. The UPLC injection volume was 1.0 μL. Omeprazole exhibits a λ_{max} at 302 nm (12), lansoprazole λ_{max} at 286 nm (13), rabeprazole λ_{max} at 284 nm (14), pantoprazole λ_{max} at 262 nm (15), and esomeprazole λ_{max} at 302 nm (16). A standard curve was developed with standards ranging from 0.75 to 200 μg/mL in 1:1 ACN/water. Analytical data was captured electronically using Masslynx 4.1 software (Waters, Milford, MA). Statistics were computed using Microsoft Excel 2010 (Redmond, WA, USA).

Method Validation

Method validation was performed by evaluating various parameters according to well-established guidelines (11). The method was validated for linearity, precision, accuracy, limit of quantification, and limit of detection.

The linearity of the calibration curve for each PPI was determined over the range of 0.75–200 μg/mL by diluting appropriate amounts of stock solutions (200 μg/mL) with 1:1 acetonitrile/water. Three calibration curves were prepared on the same day with the following concentrations (0.75, 1, 10, 25, 50, 75, 100, 150, and 200 μg/mL). For validation, each point on the calibration curve was run in triplicate. The curves were calculated by determining average peak areas and plotting these against the sample concentration. A standard curve equation was derived using linear least-squares regression analysis.

The specificity of the method for the pure drug was determined using forced acid-catalyzed degradation by exposing the pure drug solution to 1 N hydrochloric acid. The degraded drug solution was then analyzed for presence of pure drug as well as interference from degradation products. The specificity of the method for the drug is proven when the degradation products do not show significant interference.

Accuracy of the method was determined by comparing the average concentration of standard solutions to a reference standard (50 μg/mL) measured in ten replicates.

Table I. Linearity of the UPLC Method as Calculated from the Respective Calibration Curves

	Omeprazole	Esomeprazole	Lansoprazole	Pantoprazole	Rabeprazole
Mean of slopes (min)[a]	14,318±14.6	15,549±22.7	10,697.67±35.1	14,128.67±83.8	13,654±22.5
Mean of intercepts (μg min^{-1} mL^{-1})[a]	17,751±74.7	11,250.67±299.3	27,977.67±760.8	9,594.87±1522.5	73,847±399.0
Mean of correlation coefficient	0.9993	0.9981	0.9981	0.9992	0.9824

[a] The units used for slope and intercept were based on the calibration curve which is a plot of peak area (μg min^{-1} mL^{-1}) *vs* concentration (μg/mL)

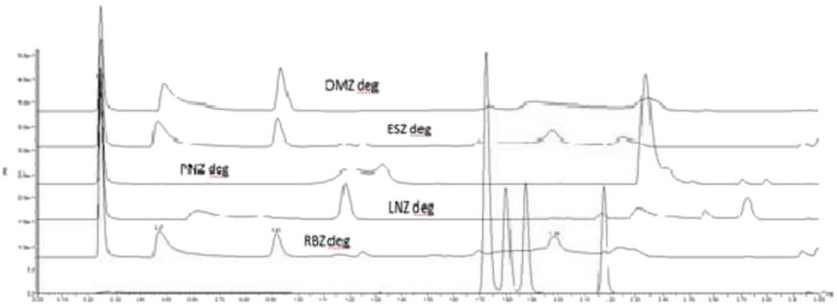

Fig. 2. Chromatogram of the degradation products of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole

Precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of standard solutions. Precision was determined in ten replicates of drug standard solution on the same day (intraday precision) and daily for six times over a period of 1 week (interday precision). The results were expressed as %RSD (relative standard deviation) of the measurements.

Limit of detection (LOD) and limit of quantitation (LOQ) were determined using the calibration curve method according to International Conference on Harmonization Validation of Analytical Procedures Guideline [ICH Q2(R1)] recommendations (11). The LOD (k=3.3) and LOQ (k=10) of the proposed method were calculated using the following equation:

$$A = k\sigma/S$$

where A is LOD or LOQ, k is a constant, σ is the standard deviation of the response, and S is the slope of the calibration curve.

RESULTS

Linearity

The UPLC method was used to construct calibration curves for each of the five drugs—omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole. The calibration curve was constructed using standard solutions at concentrations of 200, 150, 100, 75, 50, 25, 10, 1, and 0.75 μg/mL. These standards were run in triplicate each day for a total of 6 days. For each analyte, the method produced a calibration that ranged from a lower limit of 0.75–1.78 μg/mL (depending on the specific PPI) to 200 μg/mL. The regression coefficient of the standard curves of all analytes was calculated to be ≥0.98 (Table I). The mean±SD of three standard curve slopes along with the correlation coefficients (r^2) were also determined. The injection volume used in this method (1.0 μL) was about 20 times smaller than that used in a typical HPLC run (20 μL). Hence, the amount of drug that was actually being detected with an injection volume of 1.0 μL was as low as 750 pg per injection.

Selectivity

The selectivity of the UPLC assay was determined by exposing a known concentration of the individual drug solutions to degrading conditions of 1 N HCl. This allowed us to determine if the degradation products were also detected by the UPLC method and whether their retention times were distinctly different from the parent drug. The analysis of each degraded sample and comparison with the original chromatogram (Fig. 2) demonstrate the selectivity of this method as each degradation product has a distinct retention time from its parent drug.

Accuracy

The accuracy of the method determines the degree of agreement between the result of a measurement and its true value (i.e., how close the detected concentration is to the actual concentration of the drug). The accuracy of the method with respect to each of the five drugs was calculated as a

Table II. Validation of UPLC Method: The UPLC Method was Evaluated for Linearity, Accuracy, Precision, and Sensitivity

	Omeprazole	Esomeprazole	Lansoprazole	Pantoprazole	Rabeprazole
Linearity (r^2)	0.99	0.99	0.99	0.99	0.98
Linearity range[a]	1.78–200.00	1.42–200.00	0.75–200.00	1.08–200.00	1.00–200.00
Accuracy (%)	99.08	101.70	101.58	98.68	101.87
Precision (%RSD)					
Intraday (n=10)	0.17	0.14	0.13	0.21	0.11
Interday (n=6)	3.51	3.90	4.77	2.67	4.61
Sensitivity					
LOD[a]	0.59	0.47	0.23	0.36	0.33
LOQ[a]	1.78	1.42	0.71	1.08	1.00

LOD limit of detection, *LOQ* limit of quantitation, *RSD* relative standard deviation
[a] μg/mL

Table III. Analysis of a Mixture of PPIs

	Actual[a]	Measured[a]
Omeprazole/esomeprazole	80.0	79.2
Lansoprazole	40.0	40.8
Pantoprazole	40.0	39.5
Rabeprazole	40.0	40.7

[a] μg/mL

percentage of the actual concentration of a reference standard (Table II).

Precision

The precision of a method determines the closeness of agreement between a series of measurements of the same sample. Both the intraday and interday precision were examined at 50 μg/mL to give a measure of the repeatability and magnitude of variation of the assay. Precision is expressed as %RSD and was found to be ≤0.21% for intraday variation and ≤4.77% for interday variation (Table II).

Sensitivity

LOD and LOQ are measures of the sensitivity of an assay. The LOD is a value that is defined as the lowest amount of an analyte that can be detected by the method but not necessarily quantitated accurately and precisely. Conversely, the LOQ is a value that is the lowest amount of an analyte that can be quantified with a reasonable level of accuracy and precision. The LODs for the five drugs ranged from 0.23 to 0.59 μg/mL, while the LOQs ranged from 0.71 to 1.78 μg/mL (Table II).

Analysis of Samples

We examined the utility of the UPLC method by analyzing a mixture containing the five drugs—omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole. The ability of the method to detect and accurately quantify the analytes was evaluated. A correlation of 0.98 was seen between the actual and measured value of the analytes (Table III). The chromatogram in Fig. 3 shows the effective detection of the five PPIs with good resolution. The total run time required for the analysis was less than 5 min which demonstrates the efficiency of the method. The retention times of the drugs were 1.73 min for omeprazole/esomeprazole, 1.79 min for rabeprazole, 1.87 min for pantoprazole, and 2.17 min for lansoprazole. Esomeprazole is the single optical isomer of racemic omeprazole and, therefore, displays an identical retention time on an achiral column.

DISCUSSION

The primary objective of this study was the simultaneous detection of multiple PPIs in a mixture in order to facilitate easy detection and quantification. Since UPLC offers numerous advantages over HPLC techniques, it has become increasingly popular and is favored over HPLC by analytical scientists (17–21). The simultaneous detection of drugs using UPLC techniques has been gaining importance over the past few years as it enables rapid and effective analysis as compared to traditional HPLC (22–25). The UPLC method described herein can considerably simplify the analysis of PPIs. Such a method can facilitate the simultaneous assaying of dosage forms containing PPIs for content uniformity and stability. This method has previously been used for the analysis of omeprazole in marketed dosage forms (5). Therefore, this method could likely be used for the analysis of the other PPIs in marketed dosage forms following a similar extraction process. The current method would likely allow for the detection of the five PPIs in plasma samples and other biological matrices. This method provides for rapid analysis of a large number of samples as well by reducing the time required to analyze each PPI sample individually. In this study, we have presented a simple UPLC method with UV detection for simultaneous determination of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole with complete validation parameters and its application to the simultaneous detection of five PPIs in a mixture.

The developed method has an optimum retention time of analytes that facilitates rapid analysis and prudent, cost-effective use of resources. Previous research in this area has reported retention times that were three to four times longer than those reported in this study, which demonstrates the efficiency of this method (4,6). The clean separation of peaks also proves that the method is capable of differentiation between the different PPIs and for individual quantification.

The method was validated according to the ICH guidelines Q2(R1). Linearity was established by constructing a calibration curve, and the regression coefficient for the curve was ≥0.98, demonstrating that this method could be used effectively in the

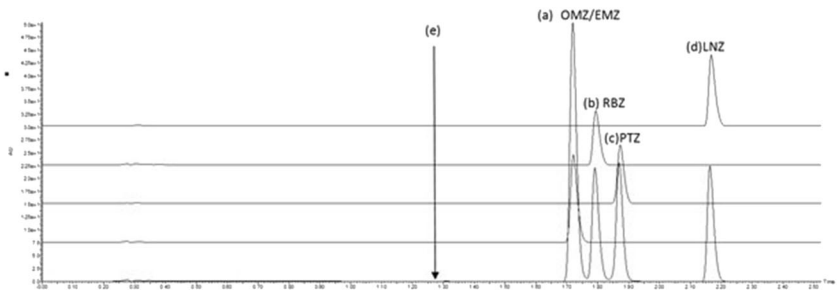

Fig. 3. Chromatograms of (**a**) omeprazole/esomeprazole, (**b**) rabeprazole, (**c**) pantoprazole, (**d**) lansoprazole, and (**e**) mixture of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole

concentration range mentioned earlier for all five PPIs. The specificity of the method was established as the degradation products of the PPIs were not found to interfere with the peaks of the pure drug. The method was also shown to be highly accurate with accuracy ranging from 98.68% for pantoprazole to 101.87% for rabeprazole. The intraday variation of the method was ≤0.21%, while the interday variation was ≤4.77%. This method is both accurate and precise, irrespective of the timeframe of analysis. Lastly, the sensitivity of the method was measured by calculating the limit of detection and limit of quantification for each of the five drugs. It was observed that the LOD ranged from 0.23 to 0.59 μg/mL and LOQ ranged from 0.71 to 1.78 μg/mL. It is particularly important to note that the injection volume used in this method is only 1.0 μL. Therefore, the minimum amount of analyte that can be quantified in a single injection is as low as 750 pg. This proves the sensitivity of the method and its effectiveness, which exceed those of other published methods in the detection of multiple PPIs.

The developed method was also used successfully for the detection of the five PPIs in a mixture. The quantification was accurate with a correlation coefficient of 0.98 (Table III) and with substantial separation of peaks, proving the reliability of the method for the simultaneous assay of multiple PPI drugs. The good sensitivity and separation of analytes suggest that with some refinement this method might additionally be useful for the analysis of PK samples in biological matrices, provided adequate extraction methods are developed.

CONCLUSIONS

A UPLC method coupled with photodiode array detector was developed, that was validated and shown to be linear, accurate, precise, sensitive, and specific for the simultaneous detection of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole. This method is also valid for the rapid detection of PPIs in a mixture and can be used for the detection of PPIs in dosage forms for evaluation of content uniformity and stability.

ACKNOWLEDGMENTS

The authors wish to thank Ronald Bingham and EMG Clinics for providing a grant that partly funded this project.

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