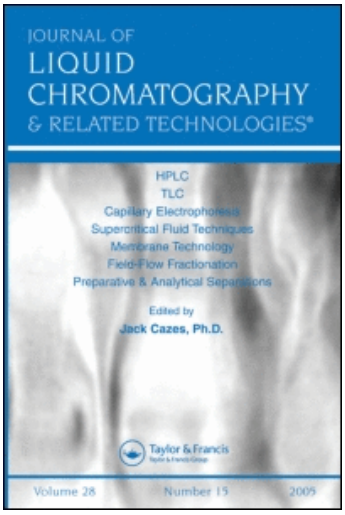

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FAST AND SENSITIVE NEW HPLC-UV METHOD FOR DETERMINATION OF OMEPRAZOLE AND MAJOR RELATED SUBSTANCES IN PHARMACEUTICAL FORMULATION

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FAST AND SENSITIVE NEW HPLC-UV METHOD FOR DETERMINATION OF OMEPRAZOLE AND MAJOR RELATED SUBSTANCES IN PHARMACEUTICAL FORMULATION

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□ *A simple, fast, and sensitive HPLC method with UV detection has been developed for the quantitation of omeprazole (OMZ) and major related substances in raw material and pharmaceutical formulation (paste) using a column of reduced length (50 mm) and diameter (2.1 mm) packed with hybrid particles. Chromatographic conditions were: 25° C, 1 μl injection volume, and UV detection at WV of 280 nm. The flow rate was 0.3 mL/min using methanol-phosphate buffer (pH 7.6) (40:60) as the mobile phase. Chromatographic purity was also determined with the same chromatographic conditions. The method was validated according to international guidelines (ICH guidelines) for specificity, linearity, LOD, LOQ, precision, accuracy, and robustness. The HPLC-UV method was found to be suitable for the quality control and stability studies of OMZ in a pharmaceutical formulation.*

Keywords fast and microbore column, omeprazole, pharmaceutical formulation, related substances

INTRODUCTION

Omeprazole (OMZ), a substituted benzimidazole sulfoxide (Figure 1) is a proton pump inhibitor, used in the treatment of acid-related diseases. OMZ inhibits selectively and irreversibly the gastric H^+/K^+ATPase, which is the proton pump involved in the final step in the acid secretion. Hence, OMZ is administered in the treatment of peptic ulcer, *Helicobacter*

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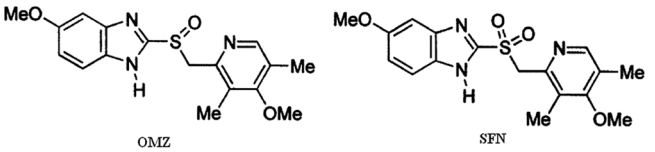

FIGURE 1 Chemical structures of omeprazole (OMZ) and its sulfone derivative (SFN).

pylori infection, gastrooesophageal reflux disease, and Zollinger-Ellison syndrome.[1]

OMZ is a hydrophobic compound with weak basic proprieties that can be degraded unless it may be protected against acidic conditions.[2]

Several analytical methods have been reported for the quantitation of OMZ in pharmaceutical and biological samples such as spectrophotometric,[3] voltammetric,[4] thing layer chromatographic (TLC),[5] and capillary electrophoretic methods.[6] Several HPLC methods coupled to UV, electrochemical (ECD), or mass spectrometric detector (MS)[7–10] have been reported for the determination of OMZ and its degradation products in biological fluids and in stability studies. Both ECD and MS HPLC methods provide higher selectivity and sensitivity compared to other analytical techniques.[8] However, these procedures require much longer time or involve the completion of several steps for operation of the instruments, making them unsuitable for routine analysis.

The USP codified a method applied both to OMZ assay and chromatographic purity by HPLC[11] using a conventional C8 column and phosphate buffer-acetonitrile as mobile phase.

OMZ solutions are unstable, specially at pH values below 7,[2] therefore, rapid analysis is convenient. Also, it is necessary to develop a highly sensitive method for the analysis of OMZ related compounds.

Miniaturization of the instrumentation that includes reduction of column (diameter and length) and particulate size is one of the major current trends in the improvement of a separation method. This modification of the column design allowed reduced analysis time, less solvent consumption with high resolution, sensitivity, and robustness. These conditions are very useful in the pharmaceutical and biopharmaceutical analytical field.[12]

HPLC with UV detection is generally the most common instrumentation employed in the analytical laboratory due to its advantages like low cost of operation, versatility, easy requirement for assembly, and simple operation.[13]

The use of HPLC coupled to UV detector and equipped with columns of reduced diameter and length could be an appropriate alternative that allows high resolution, low limits of detection, and quantitation values and less run time.

To our knowledge, there is no report in literature available regarding the use of a HPLC-UV method for the analysis of OMZ and the major related substances in active pharmaceutical ingredient (API) and paste formulation.

The aim of this work is to develop a simple, fast, and highly sensitive method for the analysis of omeprazole as an API and paste formulation using fast and reduced diameter column (microbore) packed with hybrid particles. In addition, this method could also be applied to the determination of OMZ and its major related substances in a single run.

EXPERIMENTAL

Chemical and Reagent

Omeprazole, 5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinil] bencimidazol (OMZ) and related sulfone (SFN) were obtained from Sigma (St. Louis, MO, USA). Methanol (HPLC grade), acetonitrile (HPLC grade), sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, sodium borate 10-hydrate, and phosphoric acid were supplied by E. Merck (Darmstadt, Germany). Ultrapure water was obtained by an EASY pure^{TM} RF equipment (Barnstead, Dubuque, IA, USA). All solutions were filtered through a 0.45 μm nylon membrane (Micron Separations Inc., Westboro, MA, USA) and degassed before use.

Equipment

The HPLC system consisted of a Waters 1525 Binary HPLC pump, 717 plus autosampler, and 2487 Dual λ Absorbance detector (Waters, MI, USA). Chromatograms were processed using Breeze Software (Waters, MI, USA).

Chromatographic Conditions

Separation was carried out using Xterra analytical microcolumn (50 mm × 2.1 mm i.d., 3.5 μm particle size). The column temperature was set at 25°C. The mobile phase consisted of methanol:0.036 M phosphate buffer (pH = 7.6) (40:60) and the flow rate was set at 0.3 mL/min. The UV detection was carried out at 280 nm. The injection volume was 1 μL. A run time of 4.5 min was employed for determination of OMZ in API and paste formulation. Only 14 min was the run time necessary to completely separate OMZ from its major related substances in the sample analyzed.

Preparation of Standard Solutions

Standard Solution of OMZ

A stock solution of OMZ containing 1 mg/mL was prepared in methanol and the standard solution of 50 μg/mL of OMZ was obtained by appropriate dilution in diluent (75% 0.01 M sodium borate: 25% acetonitrile).

Standard Solution of OMZ and OMZ Sulfone Derivative

A stock solution of OMZ and OMZ sulfone derivative (SFN) containing 1 mg/mL of each one was prepared in methanol and the standard solution of 50 μg/mL for OMZ and 40 μg/mL of SFN was obtained by appropriate dilution in diluent.

Sample Preparation

The content of omeprazole in API and the paste formulation were assayed. The content of 5 syringes of paste was mixed with a spatula. An amount of 0.3 g was accurately weighed into a 100 mL volumetric flask and 80 mL of diluent was added. The mixture was sonicated for 30 min and the solution was diluted to the mark with diluent. An adequate portion was centrifuged at 4000 rpm for 10 min. An aliquot of 1.0 mL of this solution was diluted to 25 mL with diluent.

The API sample was prepared by weighting 25 mg of OMZ into a 25 mL volumetric flask and then dissolving with diluent to obtain a 1 mg/mL solution of OMZ.

Stress Conditions

Oxidation: 25 mL of a 3% v/v hydrogen peroxide solution was added to 25 mg of OMZ accurately weighted. Acidic: 20 mg of OMZ with 100 mL of 0.5 M hydrochloric acid solution were refluxed during 1 hr. Alkaline: similar condition to the method described under "acidic," using 0.5 M sodium hydroxide solution. Light: 1 mg/mL of OMZ solution was exposed to white light during one week. All samples were diluted with diluent to obtain a final concentration of 50 μg/mL of OMZ.

RESULTS AND DISCUSSION

HPLC-UV Method Development

Most of the HPLC methods applied to quantification of OMZ in pharmaceutical and biological samples use C8 or C18 conventional column, phosphate buffer (pH 7 to 7.6) acetonitrile as mobile phase and UV

detection. Other HPLC methods also use ED or MS as the detector, achieving better selectivity and sensitivity. However, a long time is necessary to obtain a stabilized baseline before analysis in the case of EC detector; and in the case of the MS detector, it is expensive equipment that requires special installation and qualified operators.

Rapid analysis with high resolution and sensitivity were achieved with the use of a fast (50 mm, lenght) microbore column (2.1 mm diameter) with reduced particles size (3.5 μm), allowing the quantitation of OMZ in short times with less consumption of solvents and sample using a simple UV-detector (Figure 2). Moreover, it is important to remark that time of analysis must be reduced due to the rapid decomposition of OMZ specially in solutions at pHs below 7.[2]

On the other hand, the official USP method and most of the reported methods, employ acetonitrile as solvent in the mobile phase at a high flow. The use of a column with hybrid particles allowed the replacement of acetonitrile with methanol even at a very low flow (0.3 ml/min). This fact is convenient especially due to the acetonitrile shortage taking place since 2008.[14] Moreover, the use of this column with hybrid particles allowed the resolution of OMZ and its major related substances using methanol instead of acetonitrile in a short time with adequate peak shape (Figure 3).

The development of the chromatographic method was found to be suitable and provided good results in terms of chromatographic parameters.

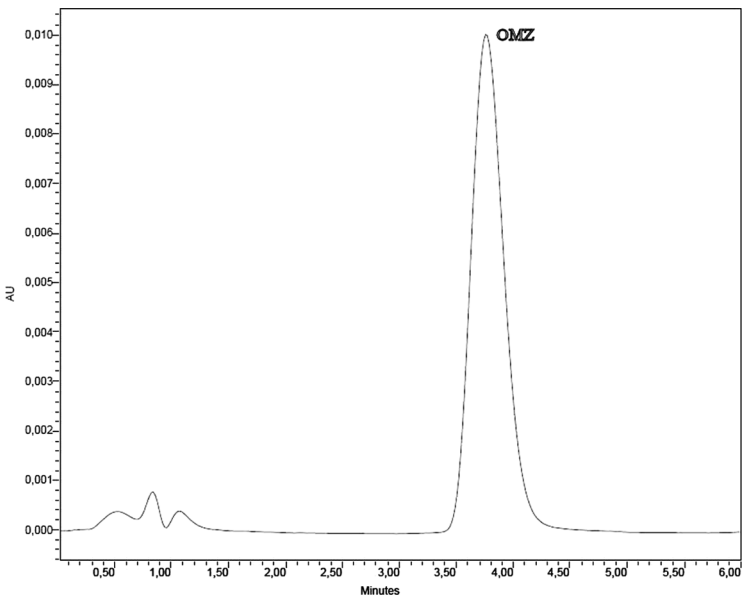

FIGURE 2 Representative chromatogram of OMZ (50 μg/mL). Experimental conditions are given in the text.

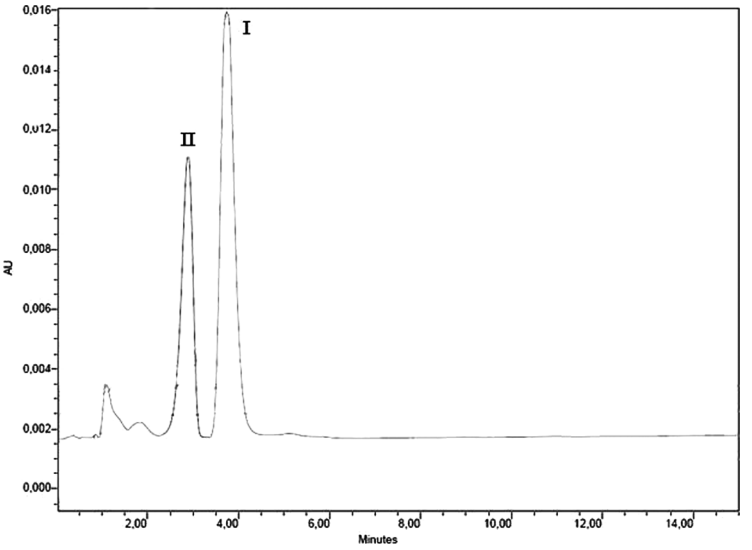

FIGURE 3 Chromatogram of OMZ 50 μg/mL (I) and its sulfone derivative 40 μg/mL (II). Experimental conditions are given in the text.

Efficiency (N), retention factor (k′), and tailing factor (T) are shown in Table 1 according to USP 32 pharmacopeia.[15]

Related Substances

Impurities in the drugs often possess unwanted pharmacological and/or toxicological effects. For these reason, quality and safety of the drugs are generally assured by monitoring and controlling the impurities of the drugs which are used as active pharmaceutical ingredient (API) in pharmaceutical formulation.

HPLC coupled to UV-detector is the most useful tool for quantitation of these drugs and their related substances.

One of the OMZ related substance is the sulfone OMZ derivative (SFN) (Figure 1). HPLC method with the use of a column with hybrid particles

TABLE 1 Chromatographic Parameters of OMZ and its Sulfone Derivative (SFN)

Parameter	OMZ	SFN
k′	3.5	2.2
N	911	630
T	1.06	1.09
Rs[a]	3.10	

[a]Between OMZ and SFN.

allows the complete separation not only of OMZ and its sulfone derivative but also of the other related substances. The analytical column assayed contains hybrid particles in which one out of every silanols is replaced with a methyl group. As a result of this process the column packed with this type of hybrid particles is able to operate at high speed and a wide pH range. In addition, it is possible to obtain symmetrical peaks (special for basic compounds) and high efficiency separation of compounds with different hydrophobicity.[13]

Figure 3 shows a chromatogram of OMZ and its sulfone derivate. Chromatographic parameters are given in Table 1.

Validation

The validation of the developed HPLC method was accomplished following the International Conference on Harmonization (ICH) guidelines. The evaluated parameters were specificity, linearity, LOD, LOQ, precision, accuracy, and robustness.[16]

Specificity

The stability indicating capability of the method was studied by accelerated stress conditions (acid, alkaline, oxidation, and light) and specificity was also examined by comparing chromatograms of excipient blanks of the pharmaceutical formulation tested (paste). Degradation was observed when OMZ was exposed to acidic, alkaline, and oxidation and the presence of OMZ sulfone derivative and unknown peaks were detected in the chromatograms. Figure 4 shows the complete degradation of OMZ in acidic medium together with the disappearance of the OMZ peak. As a result of acidic degradation, OMZ sulfone derivative was observed along with unknown peaks. Exposure to light stress resulted in minimal degradation of OMZ.

These experiments indicated that the proposed HPLC method is suitable in screening test of OMZ related substances in API (Figure 5) and in stability studies.

Linearity, LOD, and LOQ

Calibration curves at six concentration levels (5.0, 10.0, 25.0, 50.0, 75.0, and 100.0 μg/mL) of OMZ were assayed by duplicate in three separate runs. LOD (S/R = 3) and LOQ (S/R = 10) were 0.4 and 1.3 μg/mL of OMZ, respectively, equivalent to 0.4 ng and 1.3 ng injected on column (Table 2).

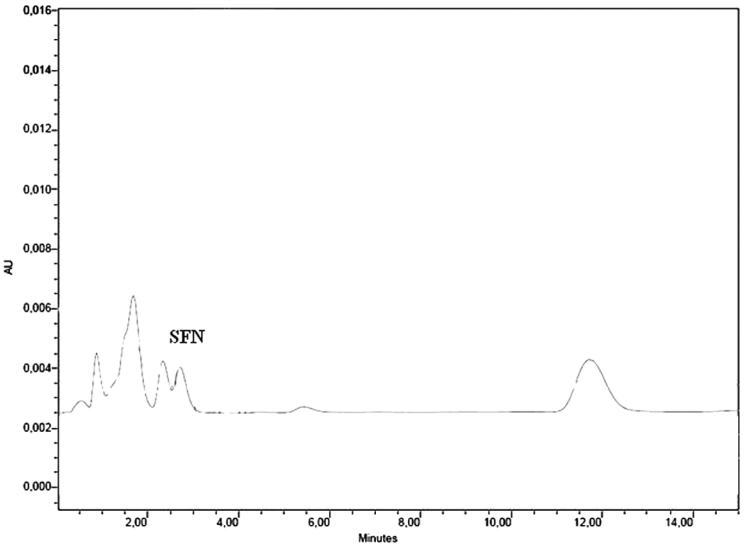

FIGURE 4 Chromatogram of OMZ under acidic hydrolysis. Experimental conditions are given in the text.

Precision and Accuracy

Precision was evaluated for intra-day (n = 6) and inter-day assays (n = 18), and it was expressed as % RSD for retention times and areas (Table 2).

Accuracy was calculated from recovery studies. Placebo samples prepared with all excipients contained in the paste formulation were spiked with OMZ at 80, 100, and 120% concentration levels. Preparations of each level were assayed by triplicate. Percentages of recovery values were obtained in the range of 99.9 and 100.6% (Table 2).

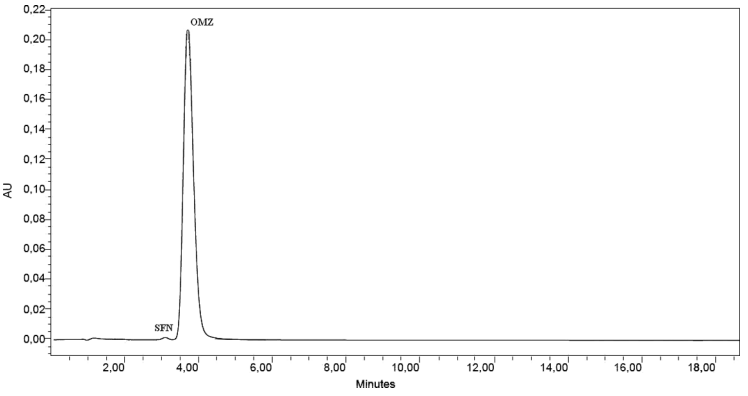

FIGURE 5 Chromatogram of OMZ (1 mg/mL) with the presence of SFN and other related substances.

TABLE 2 Figures of Merit Obtained for the Analysis of OMZ

Parameter	OMZ		
Linear range [μg/mL]	5–100		
r^2	0.9992		
LOD [μg/mL]	0.4		
LOQ [μg/mL]	1.3		
Precision % RSD			
Intra-day (n = 6)			
Migration time	1.2		
Peak area	0.9		
Inter-day (n = 18)			
Migration time	1.5		
Peak area	1.5		
Accuracy			
Spiked levels	80%	100%	120%
Paste	100.6 (1.8)	100.5 (1.9)	99.9 (1.8)

Robustness

Robustness was studied following a Plackett-Burman design. Seven parameters were selected: percentage of organic solvent, buffer pH, column temperature, flow rate, injection volume, detector wavelength, and time of use of the column. These parameters were selected because they reflected the potential changes that could affect the analytical method. The effect of each variable was studied at two levels as indicated in Table 3. In that model, the seven parameters selected were investigated using eight experimental conditions following Youden and Steiner's experimental design[17] (Table 4). Each series in the design consisted of six replicate injections of OMZ standard solution and three replicate injections of pharmaceutical formulation samples. The effects of variations in the chromatographic parameters were evaluated using retention factor (k′), mean theoretical plates (N), tailing factors (T), and the content of OMZ in the real sample. Each variable was compared to critical E statistic,

TABLE 3 Variables and their Levels for Robustness Test

Selected variables[a]	Units	Abbreviation	High level	Low level
Organic solvent	%	A.a	42	38
Buffer pH	–	B.b	7.7	7.5
Column temperature	°C	C.c	27	23
Flow	mL/min	D.d	0.35	0.25
Injection volume	μL	E.e	1.2	0.8
Wavelength	nm	F.f	285	275
Time of use of the column	–	G.g	New	Old

[a]Upper and lower case letter represent high and low level of the variable respectively.

TABLE 4 Robustness Test Design

	Experimental Number							
Variables	1	2	3	4	5	6	7	8
	A	A	A	A	a	a	a	a
	B	B	b	b	B	B	b	b
	C	c	C	c	C	c	C	c
	D	D	d	d	d	d	D	D
	E	e	E	e	e	E	e	E
	F	f	f	F	F	f	f	F
	G	g	g	G	g	G	G	g
Observed result	s	t	u	v	w	x	y	z

Experimental design according to reference[17].

which is calculated as a product between a tabulated t critical value and the standard deviation of each variable (Table 5). Data and analyses obtained confirmed the robustness of the analytical method.

Quantitative Analysis of OMZ in API and Paste

Determination of content of OMZ in API and paste formulation was performed under the experimental conditions described previously (Figure 6). The results are in good agreement with the labeled values of the commercial products (Table 6).

Abbreviations

API, active pharmaceutical ingredient; ECD, electrochemical detector; HPLC, high performance liquid chromatography; ICH, International Conference on Harmonization; LOD, limits of detection; LOQ, limits of

TABLE 5 Deviations for Each Result Obtained Using Younder and Steiner's Statistical Method

	N	k′	T	Content of OMZ
DA	−35.44	−2.08	−0.01	1.58
DB	50.74	0.33	0.05	−0.93
DC	47.06	−1.28	−0.03	2.58
DD	1.11	0.20	−0.10	0.38
DE	29.15	−0.05	−0.08	2.97
DF	17.54	0.20	−0.07	1.78
DG	34.31	0.33	−0.09	−0.12
Ec[a]	163.66	4.46	0.32	8.41

[a]Critical statistic (Ec = tc (p = 0.05) $\sqrt{2}$ SD).

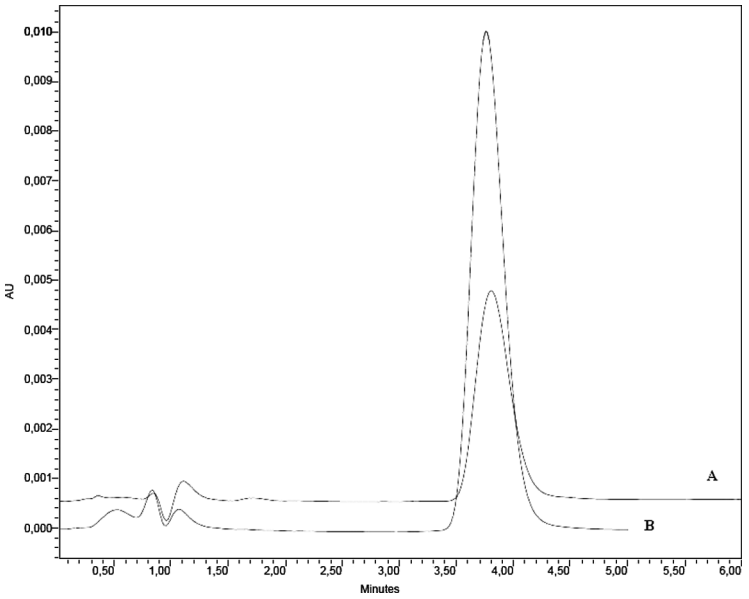

FIGURE 6 (A) Chromatogram of OMZ (50 μg/mL); (B) Chromatogram of OMZ in paste formulation. Experimental conditions are given in the text.

TABLE 6 Analysis of OMZ in API and Pharmaceutical Formulation

	Label	Found[a]
API (%)	100.0	99.9 (1.0)
Paste (37 g/100 g)	37	36.8 (1.8)

[a]Results are expressed as mean values (n = 3), RSD values in parenthesis.

quantitation; MS, mass spectrometry; OMZ, omeprazole; SFN, omeprazol related sulfone; TLC, thin layer chromatography; USP, United States Pharmacopeia; UV, ultraviolet; WV, wavelength.

CONCLUSIONS

A simple, fast, highly sensitive, and resolute HPLC-UV method has been developed for the quantitation of OMZ in pharmaceutical formulation (paste) as well as in active pharmaceutical ingredients. Moreover, the present method allowed the complete separation of major OMZ related substances. The advantages of the proposed method with those previously reported are shorter analysis time, less consumption of solvent (methanol)

and sample, and a complete resolution of known and unknown related substances in a single run, as well as make the use of acetonitrile unnecessary. In conclusion, all these features make this method suitable to be used in a routine laboratory, for quality control of OMZ in pharmaceutical formulation as well as the stability studies.

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REFERENCES

1. Shi, S.; Klotz, U. Proton Pump Inhibitors: An Update of Their Clinical Use and Pharmacokinetics. *Eur. J. Clin. Pharmacol.* **2008**, *64*, 935–951.
2. Mathew, M.; Das Gupta, V.; Bailey, R. Stability of Omeprazole Solutions at Various pH Values as Determined by High-Performance Liquid Chromatography. *Drug Develop. Ind. Pharm.* **1995**, *21* (8), 965–971.
3. Wahbi, A.; Abdel-Razak, O.; Gazy, A.; Mahgoub, H.; Moneeb, M. Spectrophotometric Determination of Omeprazole, Lansoprazole and Pantoprazole in Pharmaceutical Formulations. *J. Pharm. Biomed. Anal.* **2002**, *30* (4), 1133–1142.
4. Pinzauti, S.; Gratteri, P.; Furlanetto, S.; Mura, P.; Dreassi, E.; Phan-Tan-Luu, R. Experimental Design in the Development of Voltammetric Method for the Assay of Omeprazole. *J. Pharm. Biomed. Anal.* **1996**, *14* (8–10), 881–889.
5. Agbaba, D.; Novovic, D.; Karljikovié-Rajie, K.; Marinkovié, V. Densitometric Determination of Omeprazole, Pantoprazole, and their impurities in Pharmaceuticals. *J. Planar Chromatogr. Mod. TLC.* **2004**, *17*, 169–172.
6. Pérez-Ruiz, T.; Martínez-Lozano, C.; Sanz, A.; Bravo, E.; Galera, R. Determination of Omeprazole, Hydroxyomeprazole and Omeprazole Sulfone Using Automated Solid Phase Extraction and Micellar Electrokinetic Capillary Chromatography. *J. Pharm. Biomed. Anal.* **2006**, *42* (1), 100–106.
7. Yuen, K.; Choy, W.; Tan, H.; Wong, J.; Yap, S. Improved High Performance Liquid Chromatographic Analysis of Omeprazole in Human Plasma. *J. Pharm. Biomed. Anal.* **2001**, *24* (4), 715–719.
8. Espinosa Bosch, M.; Ruiz Sánchez, A. J.; Sánchez Rojas, F.; Bosch Ojeda, C. Analytical Methodologies for the Determination of Omeprazole: An Overview. *J. Pharm. Biomed. Anal.* **2007**, *44*, 831–844.
9. Sluggett, G. W.; Stong, J. D.; Adams, J. H.; Zhao, Z. Omeprazole Determination using HPLC with Coulometric Detection. *J. Pharm. Biomed. Anal.* **2001**, *25*, 357–361.
10. Hofmann, U.; Schwab, M.; Treiber, G.; Klotz, U. Sensitive Quantification of Omeprazole and its Metabolites in Human Plasma by Liquid Chromatography–Mass Spectrometry. *J. Chromatogr. B* **2006**, *831* (1–2), 85–90.
11. United States Pharmacopeia 32 Revision; United States Pharmacopeial Convention: Rockville, MD, USA, 2009; p. 3132.
12. Rozing, G.; Nägele, E.; Hörth, P.; Vollmer, M.; Moritz, R.; Glatz, B.; Gratzfeld-Hüsgen, A. Instrumentation for Advanced Microseparations in Pharmaceutical Analysis and Proteomics. *J. Biochem. Biophys. Methods.* **2004**, *60*, 233–263.
13. Tripodi, V.; Flor, S.; Contin, M.; Lucangioli, S. Simple, Highly Sensitive Micro HPLC Method for the Determination of Coenzyma Q10 and its Major Related Substances. *J. Liq. Chromatogr. & Rel. Technol.* **2009**, *32* (6), 860–873.
14. Food and Drug Administration. "Acetonitrile Shortages: Recommendations for Reporting Changes in Analytical Procedures." www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM171776.pdf. September 14, 2009.

15. United States Pharmacopeia 32 Revision; United States Pharmacopeial Convention: Rockville, MD, USA, 2009, p. 227.
16. Guidance Q2 (R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization. Final version **2005**, http://www.ich.org
17. Vander Heyden, Y.; Nijhuis, A.; Smeyers-Verbeke, J.; Vandeginste, B.; Massart, D. Guidance for robustness/ruggedness test in method validation. *J. Pharm. Biomed. Anal.* **2001**, *24*, 723–753.