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Precision improvement for omeprazole determination through stability evaluation

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A new spectrofluorimetric method for the determination of omeprazole (OMP) based on its degradation reaction catalyzed by ultraviolet (UV) light is proposed. OMP in aqueous solution is very unstable, which renders a serious difficulty for controlling its quality. It does not show native fluorescence, but when exposed to UV radiation, it generates a highly fluorescent degradation product with adequate stability for indirect OMP quantification. Under the studied optimal experimental conditions (pH, temperature, exposure time to UV radiation), a specific rate constant of 2.851 min^{-1}-described by zero-order kinetic – was obtained for the degradation reaction. Using λ_{exc} 293 nm and λ_{em} 317 nm, a linear relationship was obtained (r^2 0.9998) in the concentration range of 0.1 to 1.3 $\mu g\ mL^{-1}$, with a detection limit of 1.07 10^{-3} $\mu g\ mL^{-1}$ ($S/N = 3$). The methodology developed was successfully applied to OMP quality control in pure drugs and tablet dosage forms without previous treatment, with good tolerance to common excipient, and a high level of concordance between the nominal and experimental values. This work constitutes an important contribution to knowledge of the degradation mechanism of OMP. It has been shown to be appropriate for OMP quality control, to have an adequate sampling rate, low cost instrument, and to be a less polluting procedure. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: omeprazole; quality control; UV degradation; sensitized fluorescence.

Introduction

Omeprazole (R,S)-(5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulphinyl]-1H-benzimidazole) is the first class of drug known as proton-pump inhibitors. It acts on the inhibition of gastric acid secretion in the stomach, and it is used to treat various acid-related gastrointestinal disorders. OMP is, in fact, a prodrug, which is converted into a reactive intermediate sulfenamide at low pHs, combining with thiol groups of the H^+/K^+ ATPase in gastric parietal cells. OMP is used in the treatment of peptic ulcers, reflux oesophagitis, Zollinger–Ellison syndrome, and the infection caused by *Helicobacter pylori*.[1–3] Although its elimination half-life from plasma is short, it has been reported to be about 0.5–3 h. Its action is much longer compared to inhibition of acid secretion, allowing it to be used in single daily doses.[4]

OMP degrades rapidly in aqueous solutions at low pH values. Preformulation studies have shown that moisture, solvents, and acidic compounds have important effects on the stability of OMP.[5] UV radiation, various salts[6] and some metal ions[7] also accelerate its degradation. It is a lipophilic, weak base with $pK_{a1} = 4.2$ and $pK_{a2} = 9$ and will be degraded unless it is protected against acid conditions.[8]

OMP has been determined in formulations through a variety of methods, such as spectrophotometry,[9–11] chromatography,[12,13] micellar electrokinetic capillary chromatography,[14] and non-aqueous capillary electrophoresis.[15] Shaghaghi *et al*. described a first attempt to indirectly determine OMP in capsules by spectrofluorimetry. The method is based on fluorescence quenching of Tb^{3+}-1, 10-phenanthroline complex produced by OMP. They obtained a poor sensitivity and narrow linearity range.[16] British[17] and European Pharmacopoeias[18] describe a non-selective titrimetric method with a standard NaOH and potentiometric end point as assay for OMP.

The determination of OMP by conventional UV methods suffers from interferences due to UV absorbing compounds. Many of the reported UV-visible spectrophotometric methods involve the formation of charge transfer complexes with different electron acceptors, which render a similar reaction with all basic compounds. Different separative methods have been proposed for the analysis of the drug in mixtures, the determination of degradation products, as well as in stability studies.[4] Although in most studies UV detection systems are used, the severe effect of OMP degradation in solution catalyzed by UV light has never been mentioned in the literature.

The aim of the present work is to develop a methodology for OMP quality control based on the effect of UV radiation on degradation reaction and the consequent modification of fluorescent properties in a solution. Accordingly, the OMP degradation process is investigated in order to suggest a reaction mechanism, establish a reaction order and optimize the reaction conditions.

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Experimental

Apparatus

A Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp and 1-cm quartz cells was used for the fluorescent measurements.

A UV lamp Sankyo Denky (Japan) 6 W and 15 cm of length was used for the degradation procedure.

A Beckman DU 520 UV–visible spectrometer with quartz cells of 10-mm path length for absorptiometric measurements was used.

Reagents

OMP was kindly provided by Bagó Lab (Argentina). System pH levels were adjusted by adding 1 mol L^{-1} of sodium hydroxide solutions (Mallinckrodt Chemical Works, New York, NY; Los Angeles, CA; St Louis, MO, USA), 0.02 mol L^{-1} of sodium tetraborate (Mallinckrodt Chemical Works, New York, NY; Los Angeles, CA; St Louis, MO, USA), and 0.01 or 1 mol L^{-1} of acetic acid (Mallinckrodt Chemical Works, New York, NY; Los Angeles, CA; St Louis, MO, USA), depending on the desired pH.

Stock standard solutions

OMP standard solution containing 0.345 mg mL^{-1} was prepared by dissolving 34.54 mg of the drug (MW 345.417) in absolute methanol (Cicarelli, Rosario, Santa Fe, Argentina). Under these conditions, OMP solution was found to be stable for several days when kept in the dark.

The standard working solution of 0.345 μg mL^{-1} was prepared daily by diluting stock standard solution with methanol and stored in a dark bottle.

Sample solutions

An accurately weighted OMP anti-acid capsule containing an equivalent of about 1 mg of OMP was finely powdered and dissolved in 20 mL absolute methanol. The liquid was filtered to remove insoluble material; then it was transferred to a 25-mL volumetric flask and taken to volume with methanol. A solution of 0.4 μg mL^{-1} was prepared by dilution of the sample solution with methanol.

All reagents used were of analytical and/or spectroscopic grade.

General procedure

Sample and standard solutions were adjusted at pH 4 by the addition of acetic acid 1 mol L^{-1}. All systems were directly exposed to a UV light at the same time in order to degrade OMP for 60 min. This procedure was performed at room temperature, in 10-mL graduated polyethylene centrifuge tubes. The samples were placed horizontally to provide the maximum exposure area to the light source.

Sample and standards solutions were then introduced into the spectrofluorometer, and the fluorescent emissions were measured at λ_{em} 317 nm, using a λ_{exc} 293 nm.

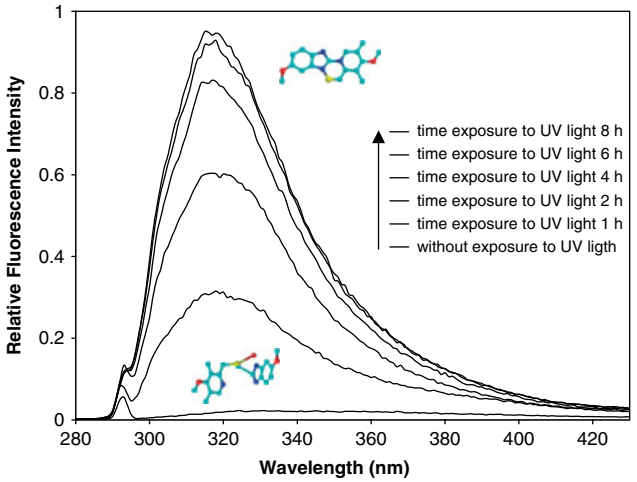

Figure 1. Fluorescent spectra of omeprazol degradation product produced by exposing the drug to direct ultraviolet light during different periods of time.

Validation procedure

In order to demonstrate the validity of this methodology, 34.5 mg of commercial capsules powder from two different batches containing 3 mg OMP were dissolved and transferred to a 50-mL volumetric flask and taken to volume with absolute methanol. 100 μL portions of this sample solution were transferred to ten volumetric flasks of 10 mL. This methodology was applied to six portions, and the average quantity of OMP obtained with the proposed method was taken as a base value. Then, increasing quantities of OMP were added to other four aliquots of sample and total OMP was determined by applying the standard addition method. All samples were taken to volume with absolute methanol.

Results and Discussions

Spectral characteristics of OMP and its degradation product

Figure 1 shows the emission spectra obtained for a 0.345 μg mL^{-1} of OMP methanolic solution at pH 4 with and without exposing it to UV light. Before exposure, the drug does not show a fluorescent emission, but after exposure, a maximum emission at 317 nm appears when it is excited at 293 nm. This fluorescence intensity increases with exposure time due to the degradation product formation. These wavelengths were selected to measure the fluorescence intensity for the following assays. Some researchers[19–23] have proposed the scheme of OMP degradation in acid medium.

The subsequent formation of sulfenamide from OMP decomposition is in accordance with known reactions between sulfenic acids and amines. Both OMP and the sulfenamide are significantly more stable in methanol than in aqueous solutions. However, the degradation process is highly catalyzed by UV light in acidic solutions of methanol, reducing the whole process duration from days to a few hours. These two facts constitute the basis of the highly sensitive and precise spectrofluorimetric method for OMP determination proposed in this work. Results obtained through calculations from molecular modelling (Figure 2) show that sulfenamide formation leads to an increase in rigidness and coplanarity of all double bonds in the molecule, which is the cause of the high fluorescence intensity. Simultaneously, the highest stability

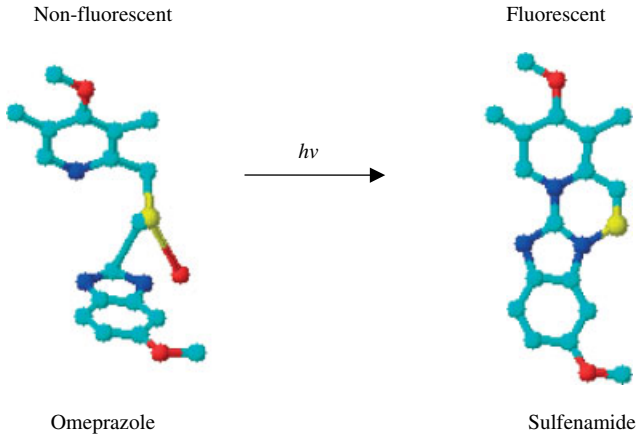

Figure 2. Omeprazole and sulfenamide stereochemical modeling.

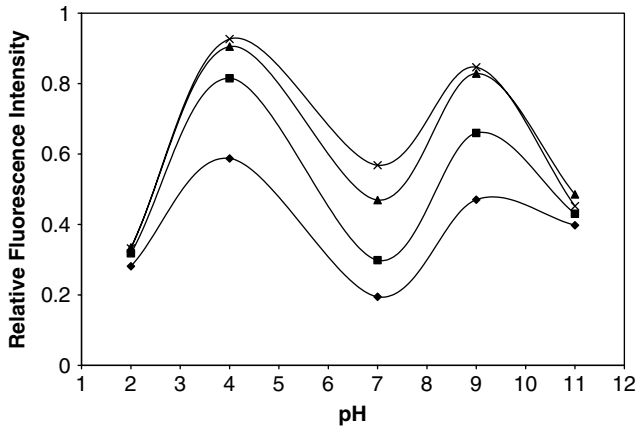

Figure 3. Effect of pH on fluorescent intensity of omeprazole degraded in methanolic solutions (0.345 μg mL^{-1}). Exposure time to UV light 2 h (♦); 4 h (■); 6 h (▲); 8 h (×).

of OMP and the sulfenamide formed in methanol produces the immediate decrease in the degradation reaction speed, when the OMP solutions are taken out from UV light exposure. Under this condition, a negligible growth in the intensity of fluorescence signal is produced, allowing a precise spectrofluorimetric measurement.

Analysis of spectrofluorimetric data for OMP degraded in methanolic solutions shows that the fluorescent intensity increases at pH 4–5, decreases between 6 and 8, and increases again at pH 9–10. The pH selected for successive measurement was 4 due to the highest fluorescence intensity obtained. The effect of pH in the fluorescent signal is shown in Figure 3.

Degradation kinetic of OMP in solution

The medium acidity plays an important role in the degradation kinetics of OMP. Different acids were proved to adjust the pH in 1 to 5 ranges. The highest fluorescent intensity and the degradation kinetics were achieved when acetic acid was used. OMP degradation was evaluated in methanol at pH 4. The spectrofluorimetric measurements were carried out every 10 min, during a period of 480 min at room temperature.

The absorption UV and fluorescent spectra of OMP do not vary when the solutions undergo different temperatures, showing that temperature has no effect over the degradation process.

OMP does not show native fluorescence, but when it is degraded in an acid medium, its degradation product gives an intensive fluorescent signal. The fluorescence peak intensity of this product increases with the exposure time to UV light. The degradation kinetics is described by zero-order kinetics in the experimental conditions of this study. The correlation coefficient is 0.9963.

The velocity constant (k) of degradation reaction catalyzed by UV light was obtained according to Eqn 1.

$$F = kt \quad (1)$$

where F is fluorescent signal at 317 nm ($\lambda_{exc} = 293$ nm) at time t.

The specific constant k was 2.851 min^{-1}. This value shows that the decomposition rate is high and OMP can be totally degraded in approximately 6 h at room temperature. Therefore, the exposure time of OMP to UV light has an important effect on all quantification measurements, especially on those in which UV light is used as the detection system.

Data obtained by UV–Vis spectrophotometry showed that there are differences between the absorbance values before and after exposure of the drug to UV light when measured every 10 min. This decomposition is shown by a shift of the absorption maximum to shorter wavelengths and, simultaneously, a decrease of absorption signal intensity.[23] However, it can be observed that this change in absorbances is small compared to the high increase in fluorescent emission signal for the same samples and exposure times. For instance, for 1 h of exposure time, the fluorescence emission is approximately ten-fold that of non-degraded OMP. The difference between UV–Vis spectrophotometry and spectrofluorimetry as regards sensitivity for monitoring the degradation process of OMP is evident.

Method validation

Linearity range and sensitivity

The calibration curve was constructed covering a concentration range from 0.1 to 1.5 μg mL^{-1}. Equation for calibration graph was obtained by least-square linear regression analysis of the fluorescent signals versus analyte concentrations:

$$F = 71.939 + 12.856\,C \quad (2)$$

where F is the relative fluorescence intensity and C the concentration of OMP. Correlation coefficient was 0.9998. The figures of merit obtained demonstrate the good performance of the calibration.

Using $\lambda_{exc} = 293$ nm and $\lambda_{em} = 317$ nm, a good linear relationship was obtained in the range 0.1–1.3 μg mL^{-1} of OMP with a detection limit of 1.07×10^{-3} μg mL^{-1} ($S/N = 3$).

Precision and accuracy

The intra-day and inter-day precision of the method based on repeatability was performed, by replicating the method ($n = 6$) on five sample solutions of the same batch number of commercial capsules of OMP using the standard addition method, which gave a relative standard deviation lower than 4.9% in all cases. The intra-day and inter-day recovery ranged from 94.57 to 109.66%. The validation method results are shown in Table 1.

Table 1. Validation of the method for the determination of OMP in commercial pharmaceutical formulae. ($n = 6$)

Sample Ulcozol	Base Value (mg)	OMP Added (mg)	OMP Found (mg)	Recovery[a] ± % RSD
Intraday	$5.96\ 10^{-3}$	–	$5.91\ 10^{-3}$	99.16 ± 2.5
	$5.96\ 10^{-3}$	$1.38\ 10^{-3}$	$7.29\ 10^{-3}$	96.38 ± 3.1
	$5.96\ 10^{-3}$	$2.76\ 10^{-3}$	$8.98\ 10^{-3}$	109.42 ± 2.9
	$5.96\ 10^{-3}$	$4.14\ 10^{-3}$	$1.02\ 10^{-2}$	102.41 ± 3.0
	$5.96\ 10^{-3}$	$5.52\ 10^{-3}$	$1.15\ 10^{-2}$	100.36 ± 2.3
Interday	$5.96\ 10^{-3}$	–	$5.97\ 10^{-3}$	100.16 ± 4.9
	$5.96\ 10^{-3}$	$1.38\ 10^{-3}$	$7.32\ 10^{-3}$	98.55 ± 3.8
	$5.96\ 10^{-3}$	$2.76\ 10^{-3}$	$8.57\ 10^{-3}$	94.57 ± 3.0
	$5.96\ 10^{-3}$	$4.14\ 10^{-3}$	$1.05\ 10^{-2}$	109.66 ± 2.8
	$5.96\ 10^{-3}$	$5.52\ 10^{-3}$	$1.12\ 10^{-2}$	94.92 ± 2.5

[a] [(found − base)/added] × 100

Table 2. Analysis of OMP in pharmaceutical samples by the developed spectrofluorimetric method. ($n = 6$)

Sample	OMP Nominal quantity (mg)	OMP Found (mg)	%E[a]
1	20	20.59 ± 2.5[c]	2.95
2	20	19.72 ± 2.8[c]	1.38

1) Ulcozol (Bagó, Bs. As., Argentina).
2) Aziatop (Elea, Bs. As., Argentina).
[a] Percentual relative error (calculated considering that the preparations contain the amount reported by the manufacturing laboratories).

Selectivity

The use of the degradation reaction for producing high fluorescence emission has an important advantage: the exposure to UV light gives an OMP degradation product with high fluorescence emission, without spectral interference from other common components (excipients) of the pharmaceutical formulae. This was proved by measuring the fluorescent emission of the OMP samples with and without exposing them to UV light. The results are the same as those observed in Figure 2, without variation respect to the pure drug solution spectrum.

Applications

Using the previously selected parameters, the proposed method was applied to the analysis of OMP in two commercial pharmaceutical samples. The results obtained were in good agreement with the labelled content of OMP, and they are listed in Table 2.

Some reported analyses for OMP solutions,[9–18] including the USP 26 official method,[13] mainly use UV detection systems and involve previous separation methods like HPLC or liquid-liquid extraction, which take between 15 and 20 min for each injection or extraction step for each sample and standard. The rapid decomposition of the drug in solution, even when only exposed to natural light, suggests that the UV absorbance measurements in these methods may not be accurate and precise, since there is a gap of several minutes between the sample/standard preparation and the instrumental detection. The instability in solution should also be taken into account when OMP is quantified in solution by other methods, such as titration.

In this work, samples and standards were simultaneously treated for 60 min under UV light and the fluorescence signals corresponding to a more stable compound were immediately measured. The whole process takes approximately 80 min, competing, from this point of view, with the majority of the methods described in the literature. This method does not need a previous separation step since the degradation conditions and fluorescence emission parameters are almost exclusive to OMP, providing high analytical selectivity and sensitivity. In addition, the method is simple, requires the use of few reagents and inexpensive equipment.

Conclusion

The method reported here demonstrates that OMP concentration can be accurately determined with a simple, fast, economical, precise, sensitive, selective, and low-pollutant methodology, solving the difficulty of OMP instability in aqueous solutions and taking advantage of the UV catalyzed decomposition of the drug to enhance the fluorescence signal. The high selectivity and sensitivity of this methodology permits its application to stability studies, where detection of small changes in concentration without interferences is critical. The method can also be satisfactorily applied to the quality control of commercially available pharmaceuticals without separating the analyte from common excipients of the formula. It constitutes a worthy alternative to other costly, time-consuming and expensive quality control methods. Due to the small amount and nature of the reagents used and the simplicity of the analytical procedure, this method contributes to a clean or environmentally friendly analytical chemistry without sacrificing important parameters, such as precision, sensitivity, and speed of analysis.

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