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Development and Validation of a Stability Indicating Spectrofluorimetric Method for the Determination of Lanzoprazole via its Degradation Product

Ghaleb Oriquat[1], Afaf Osman[2], Mohammad Abdul-Azim [3] and Sawsan Abuhamdah[4*]

[1]Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Umm Al- Qura University Makkah- Saudi Arabia, [3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt, [4]Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, University of Jordan, Amman, Jordan.

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ABSTRACT

A simple and a stability indicating spectrofluorometric method was developed and validated for the analysis of lanzoprazole via its degradation product in formulation. The proposed method was based on measuring the fluorescence intensity of the degradation products at 410 nm for the emission wavelength and at 322 nm for the excitation wavelength. The method was validated in accordance with the ICH requirements, which involved accuracy, precision, linearity, selectivity and both limit of detection and limit of quantification. Linearity was obtained in concentration range 1– 10 µg/ml. The mean percentage recoveries were 99.39 ± 0.11%. The degradation product was obtained in acidic stress condition. The proposed procedure was successfully applied for the determination of lanzoprazole in pure form, laboratory-prepared mixtures, tablet and expired batch. Statistical comparison between the results obtained by the suggested method and that obtained by the official method for the determination of the drug was done and it was found that there were no significant differences between them. For ease and convince of such method, where analysis can be done within a short period of time in comparison with the chromatographic methods. The method was validated according to United States Pharmacopeia Guidelines.

INTRODUCTION

Lanzoprazole is a new, highly potent proton pump (H^+, K^+ ATPase) inhibitor with potent anti-secretory effects. It was demonstrated to be effective in the treatment of duodenal and gastric ulcers, reflux esophagitis and Zollinger-Ellison syndrome (Hardman & Limbird, 2005). Lanzoprazole, (2-[3-Methyl-4-(2,2,2,-trifluoroethoxy)-2-pyridinyl] methyl] sulfinyl]-1-H-benzimidazole (Fig. 1). Molecular formula: $C_{16}H_{14}F_3N_3O_2S$, Molecular weight (369.363) is a white to brownish-white odorless crystalline powder that is practically insoluble in water[2]. It is freely soluble in dimethylformamide (DMF), soluble in methanol, slightly soluble in ethylacetate, acetonitrile or methylene chloride, very slightly soluble in ether. It degrades in aqueous solution and rate of degradation increases with decreasing pH at 25°C (Delgado & William 1998; Gennaro, 2000). Lanzoprazole is a substituted benzimidazole that can be easily degraded. It is unstable at a low pH, so the oral dosage forms are supplied as enteric-coated granules. The granules dissolve only in alkaline pH, thus preventing degradation of the drug by acidity of the esophagus and stomach (Gennaro, 2000; Martindale and Sweetman, 2002). Several methods have been reported for its determination in biological fluids and pharmaceutical formulations, including quantification of Lanzoprazole in oral suspension by ultra-high-performance liquid chromatography hybrid ion-trap time-of-flight mass spectrometry (Stacy *et al.*, 2011; Yardimci, and Ozaltin 2001). Electrochemical studies and differential pulse polarographic analysis of Lanzoprazole in pharmaceuticals (Dogrukol-Ak, 2001). The determination of Lanzoprazole in pharmaceutical preparation by capillary electrophoresis (El-Sherif, 2005), Stability-indicating methods for the determination Lanzoprazole (El-Sherif,2009), Reversed phase high performance liquid chromatographic method for determination of Lanzoprazole, omeprazole and pantoprazole

* *Corresponding Author*
Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, University of Jordan, Amman, Jordan. Tel.: +926-6-5355000 Ext 23358; Fax: +962-6-5300250; P.O.Box: 13380, Amman 11942 Jordan. E-mail address: s.abuhamdah@ ju.edu.jo or smaabuhamdah@gmail.com

sodium sesqui-hydrate in presence of their acid-induced degradation products (Ermer & Miller, 2005) in presence of related substance in Lanzoprazole for injection (Moustafa, 2000) Spectrophotometric methods for the determination of Lanzoprazole and pantoprazole sodium sesquihydrate (Khaled *et al.,* 2012). Fluorometric determination of drugs containing α-methylene sulfoxide functional groups using N-methylnicotin amide chloride as a fluorogenic agent (ICH, 1993).

The International Conference on Harmonization (ICH) guidelines titled "Stability Testing of New Drug Substances and Products" stated that stress testing helps to determine the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products and to validate the stability-indicating power of the analytical procedures used (Schulman, 1985). No fluorometric method was reported for the detection and determination of the degradation products of the drug. The aim of this work was to develop and establish new, validated, simple, cheap, selective, sensitive and reproducible stability-indicating spectrofluorometric method for determination of the drug via its degradation products and for detection of impurities that may present.

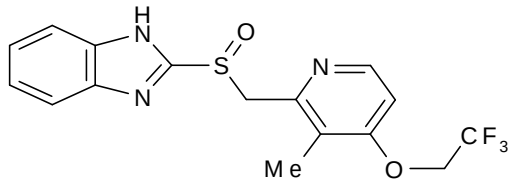

Fig. 1: Structure of lansoprazole.

EXPERIMENTAL

Reference Substance

Due to the lack of Lanzoprazole reference substance, the active pharmaceutical ingredient was extracted from commercial tablets (**Sedico** Pharmaceutical Company), 6th October City, Egypt. Its purity was certified 100.98%. Pharmaceutical formulation **Lopral capsules** supplied by T3A Co. Assuit, Egypt. B.N. 010639. Labeled to contain (30 mg) of Lanzoprazole per capsule. All chemicals and solvents were analytical grade. Methanol (Lab-Scan), acetonitrile (LC grade, Mumbai) Chloroform (BDH), acetone (Lab-Scan), ethanol (Lab-Scan), 1% sulfuric acid, acetic acid 0.1M, 0.1 M hydrochloric acid aqueous (Riedel-de Haen, Germany) and 0.2 M sodium hydroxide , EL-Nasr pharmaceutical Co., Egypt. Spectrofluorimetric Method was performed on a Spectrofluorometer (Shimadzu Scientific Instruments Inc., Columbia, MD) Model RF-1501, equipped with a xenon lamp and 1 cm quartz cells. Sensitivity, low; excitation and emission band width, 20. PH meter (Hanna Instruments, Ann Arbor, MI) Model 8417.

Method

Degradation under Forced Conditions

100 mg Lanzoprazole were accurately weighed and crushed to a fine powder. An appropriated amount was transferred into an individual 50 ml volumetric flask, after addition of 0.1 M hydrochloric in a conical flask, and the solution was left at room temperature (25 ± 2°C) for not <2 h. Subsequently, the solution was neutralized with 0.2 M sodium hydroxide, evaporated over a boiling water bath nearly to dryness, dissolved in 80 mL methanol, and filtered, quantitatively transferred into a 100 mL volumetric flask, and completed to volume with methanol.

Preparation of Standard Lanzoprazole stock and blank (Unhydrolysed drug) solutions

The stock reference solution was prepared by weighing accurately 100 mg of Lanzoprazole transferred, to 100 ml volumetric flask and diluted to volume 100 ml with methanol obtaining a concentration of 200μgml^{-1} then dilute (0.05 – 0.5) ml of the solution with methanol in a series of 10 ml volumetric flasks, and complete to volume with the same solvent to obtain working solution from 1-10 μgml^{-1}.

Lanzoprazole degradation stock solutions (Test experiment; hydrolysed drug)

To prepare the sample solutions, were prepared at a concentration of 200 μgml^{-1}, then diluted (0.05 – 0.5 ml) of this solution with methanol in a series of 10 ml volumetric flasks and make up to volume with the same solvents to obtain 1 – 10 μgml^{-1}.

Laboratory prepared mixtures

Mixtures containing different ratios of Lanzoprazole degradation stock solution (Test experiment) (90-10 μgml^{-1}) and a complementary amounts of Lanzoprazole standard stock solution (Blank) (10-90 μgml^{-1}) were prepared in 10-ml volumetric flask. Volume was completed with methanol.

Scanning of the Fluorescence Spectra

0.5 ml of Lanzoprazole degradation stock solution (10 μgml^{-1}) was transferred into a 10-ml volumetric flask. Volume completed with methanol and fluorescence intensity was measured at λ_{em} 410 nm with λ_{ex} 322 nm against a blank (10 μgml^{-1} of Lanzoprazole standard stock solution).

Construction of the calibration graph for method

Aliquots (0.05, 0.1.....,0.5 ml) of Lanzoprazole degradation [test experiment] (0. 2 mg/ml) were transferred into a series of 10-ml volumetric flasks. The volume was then completed with methanol. The fluorescence intensity was measured using excitation and emission wavelengths at 410 and 322 nm respectively, against Lanzoprazole standard stock solutions (0.05, 0.1.....,0.5 ml) (blank) The difference in the fluorescence intensity between the test experiment and blank is measured at λ_{em} 410 nm with λ_{ex} 322 nm, and plotted versus the corresponding concentrations. A linear calibration curve was constructed and the regression equation was computed.

Application of the proposed method for the analysis of pharmaceutical formulation

Powdered content of ten capsules were mixed well and quantity of the powder equivalent to 20 mg Lanzoprazole transferred to a conical flask and the procedure in the degradation under Forced Condition was followed. 2.5 ml to 100 ml were diluted with methanol. Fluorescence intensity were measured at λ_{em} 410 nm with λ_{ex} 322 nm against blank similarly treated without addition of 0.1M hydrochloric acid. The concentration of the drug was determined from the corresponding regression equation.

Detection of degradation products in degraded market samples (capsules)

The proposed procedure was applied to an expired batch. Content of ten capsules were accurately weighed and crushed to a fine powder, 30 mg of Lanzoprazole was transferred into a 100 ml volumetric flask. After addition of 25 ml of methanol, the flasks were vortex mixed for 1 hr. Filtered into 50-ml volumetric flask and completed the volume with methanol. Dilute a portion of the result solution to obtain a solution contain 4.5µg/ml with the same solvent. Measure the fluorescence intensity against a blank.

Validation of the method

Analytical method development and validation play a major role in the discovery, development, and manufacture of pharmaceutical (Shabir *et al.*, 2007). The International Conference on Harmonization (ICH) 2005 requires the stress testing to be carried out to elucidate the inherent stability characteristic of the active substance. A stability-indicating method is the one that quantifies the drug and also resolves its degradation products (Rockville, 2005; Shabir *et al.*, 2007; Shabir, 2003). Intraday and interday precision, linearity range, accuracy, selectivity, LOQ, LOD, robustness, and ruggedness were evaluated to validate the methods.

RESULTS AND DISCUSSION

Analytical method development and validation play a major role in the discovery, development and manufacture of pharmaceutical (Shabir et al., 2007). The International Conference on Harmonization (ICH) 2005 requires the stress testing to be carried out to elucidate the inherent stability characteristic of the active substance. A stability-indicating method is the one that quantifies the drug and also resolves its degradation products (Rockville, 2005; Shabir *et al.*, 2007; Shabir, 2003).

The present method was validated using samples of tablet dosage forms with the label claim of 30 mg by determination of the following parameters: Specificity, linearity range , Intraday and interday precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) , robustness and system suitability test to validate the methods.

While Lanzoprazole is non-fluorescent, it was observed that the acidic degradation products have an intense fluorescence, Figure (2). This observation was used to develop a sensitive, indirect, stability-indicating fluorometric method via its degradation products and also for the direct detection of the purity of the drug. The measurements for the acidic degradation products were done at 410 nm emission and 322 excitation as shown in (Figure 2).

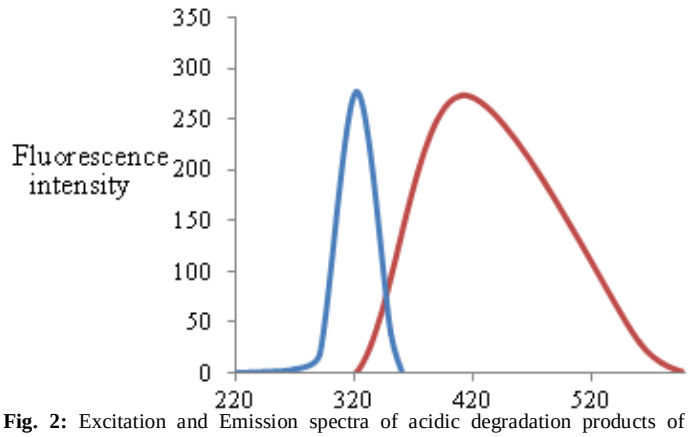

Fig. 2: Excitation and Emission spectra of acidic degradation products of lansoprazole (10µg/ml) at 410 nm (emission) and 322 nm (excitation).

The effect of different solvents, such as methanol, ethanol, acetonitrile, 0.1M HCl, 0.1M NaOH, acetic acid, acetone, 50% sulfuric acid, and distilled water was also investigated. Methanol was found to be the best solvent. Spectrofluorimetric methods have an advantage over spectrophotometric ones, because they offer much greater selectivity and sensitivity. Thus, procedures based upon fluorescence should be considered when measuring techniques are being advised for small quantities of materials, for example, in the analysis of trace impurities in drug substance or in unit dose assays of certain drugs such as alkaloids and steroids, which are administered at very low dose (Rockville, 2005; Shabir *et al.*, 2007).

The method suggested in the present investigation for the spectrofluorimetric determination of Lanzoprazole via its degradate depends upon the native fluorescence of the methanolic solution of the degradate solution, where the drug solution does not exhibit any fluorescence intensity.

Figure (2) shows the excitation and emission maxima of Lanzoprazole degradate in methanol, where Lanzoprazole degrade shows a maximum emission at 410 nm when excited at 322 nm, while a similar concentration of Lanzoprazole has no emission nor excitation , using the same excitation wavelength, with very much lower fluorescence intensity. This would permit the determination of Lanzoprazole degraded in presence of Lanzoprazole intact molecule.

The fluorescence intensity of different concentrations of Lanzoprazole degraded in methanol were recorded against the same concentration of Lanzoprazole as a blank, using 322 nm and 410 nm as excitation and emission wavelengths, respectively.

The fluorescence intensity is found to be a linear function of concentration of Lanzoprazole degraded in the range of 1.0-10.0 µg/ml, and the regression equation was computed and found to be:

$I_f = 27.049\ C + 4.3443$ $r = 0.9988$

Where I_f is the fluorescence intensity, C is the concentration of Lanzoprazole degraded in µg/ml and r is the correlation coefficient. Several factors affecting the fluorescence intensity were also tested ; and it was found that upon using methanol as solvent, satisfactory results were obtained.

The proposed spectrofluorimetric method was successfully applied for the determination of the drug via its degradate with mean percentages recovery of 99.39± 0.11%, (Table I) and comparison of the results obtained by the proposed fluorimetric method and official method (Shabir *et al.*, 2007). for determination of Lanzoprazole (Table II) shows. and no significant difference was observed.

Table. 1: Results of analysis of Lanzoprazole in pure form by the proposed fluorometric method.

Item	Taken µg/ml	Found µg/ml	Recovery%
	2	1.99	99.50
	4	3.96	99.00
	6	5.96	99.33
	8	7.94	99.25
	9	8.99	99.89
Mean			99.39
S.D			0.33
S.E			0.15

S.D: Standard Deviation , S.E: Standard Error

Table. 2: Statistical analysis of the results obtained by the proposed Fluorometric method and the official method for the determination of Lanzoprazole in pure powder form.

Values	Fluormetric method	Official method*
Mean	99.39	100.98
SD	0.33	0.27
Variance	0.11	0.07
n	5	3
F*		1.57 (19.300)*
Student's t*		1.04 (2.447)*

The figures in the parenthesis are the corresponding tabulated values at P = 0.05.
* HPLC method.
S.D: Standard Deviation,

The specificity of the method was proved by the analysis of a laboratory prepared mixture containing different percentages of the degradation product. The specificity of the method was achieved in presence of up to 90% of it's degraded, (Table III).

The concentration of the drug from an expired batch stored at ambient temperature under normal conditions was determined by direct measurement of its fluorescence at the specified wavelengths (Table IV).

The LOD and LOQ were calculated using the following equations:
LOD = 3.3 (SD/S) and LOQ = 10 (SD/S) where SD is the standard deviation of response and S is the slope of the graph (Table V).

The stability of Lanzoprazole degradate in methanol has been determined by keeping one sample in refrigerator and other in a tightly capped volumetric flask placed at ambient temperature under normal lighting condition .The sample were checked for assay in three successive days of storage and compared with freshly prepared degraded sample by the proposed method. The RSD% values of assay were found to be below 2.0% in both cases. This indicates that Lanzoprazole degrade is stable in the solution.

Validation of the proposed methods was made by measuring range, accuracy, precision, linearity, specificity, LOD and LOQ. Results obtained are in (Table V). These data render the applicability of the proposed method for the quality control of the drug formulations.

Table. 3: Determination of synthetic mixtures of lanzoprazole standard stock solution (1) and Lanzoprazole degradation stock solution (2) by the proposed fluorometric method .

Mix No.	Concentration µgml^{-1} (1)	(2)	Degradation (%)	Recovery (%)
1	1.0	9.0	10	99.60
2	3.0	7.0	30	99.82
3	5.0	5.0	50	98.93
4	7.0	3.0	70	99.84
5	9.0	1.0	90	99.63
Mean			99.56	
S.D.			0.37	
RSD.			0.37	

S.D: Standard Deviation.
RSD: Relative Standard Deviation

Table. 4: Analysis of expired pharmaceutical formulation by the proposed fluorometric method.

Expired Preparation	Claimed amount µgml^{-1}	µg of degradation found	Recovery (%) of degradation
Lopral capsules		0.122	2.72
	4.5	0.111	2.47
		0.111	2.47

Table. 5: Results of validation parameters of the responses and the regression equation obtained by the proposed method.

Parameters	Fluorometric method
Slope	27.049
Intercept	4.3443
Correlation coefficients	0.9988
Concentration range	1-10 µg/ml
Accuracy (%) mean± RSD	99.39± 0.33
Specificity mean± RSD	99.56± 0.37
LOD (µg/ml)	0.0007
LOQ (µg/ml)	0.002

(LOD): Limit of Detection, (LOQ): Limit of Quantification, RSD: relative standard deviation

Accuracy

The accuracy was assessed from three replicate determinations of three different solutions containing different concentrations for Lanzoprzole. The absolute means obtained are shown in (Table I). With a mean value of 99.39% and SD = 0.33 demonstrating that the method is accurate within the desired range.

LOD and LOQ for calculating of the LOD and LOQ, a calibration equation, was generated by using the mean values of the three independent analytical curves. The LOD and LOQ were obtained by using the mean of the slope, and the standard deviation of the intercept of the independent curves, determined by a linear regression line (Table V). The LOD and LOQ calculated were 0.0007 and 0.002 ug/ml respectively.

Precision The precision evaluated as the repeatability resulted in a relative standard deviation (RSD) value (n=5, 3) the

intermediate precision was assessed by analyzing two samples of pharmaceutical formulation on three different days (inter-day).

Robustness Robustness was examined by evaluating the influence of small variation of method variables, including solvent supply and excitation wavelength. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation of method variables did not significantly affect the procedures. This provided an indication for the reliability of the proposed method during its routine application for the analysis of the drug. Ruggedness was also tested by applying the proposed method to the assay of drug using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were found to be reproducible.

System suitability test The RSD values calculated in the system suitability test for the parameters tested were within the acceptable range as shown in (Table V), indicating that the system is suitable for the analysis intended.

CONCLUSIONS

A stability indicating assay method was successfully developed for determination of Lanzoprazole. The proposed fluorometric method is simple, accurate, rapid and reproducible for analysis of Lanzoprazole in raw material and enteric coated granules, without interference from excipients and in the presence of its acidic degradation products and for the detection of its impurity.

The advantages of the fluorometric technique are very well established for the quality control of most pharmaceutical due to its effectiveness, significant precision and accuracy. Therefore, the proposed method was successfully applied and suggested for the quantitative analysis of Lanzoprazole in tablet dosage forms, contributing to improve the quality control and to assure the therapeutic efficacy.

Competing interests

Authors have declared that no competing interests exist.

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