



## **New Simple UV Spectrophotometric Method for Determination of Certain NSAIDs present in a Physical Mixture with Pantoprazole in Different pH Values**

Esmat Zien El-Deen\*, Mamdouh Ghorab, Shadeed Gad, Heba Yassin

Pharm. Technology Dept., Faculty of Pharmacy, Tanta University\*, Tanta, Egypt, and Pharmaceutics Dept., Faculty of Pharmacy Suez Canal University, Ismailia, Egypt

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### **ABSTRACT**

Non-steroidal anti-inflammatory drugs are among the most commonly prescribed agents for rheumatic disorders such as osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. We review the co-prescription of proton pump inhibitors for the prevention of NSAID-induced gastropathy. Ketorolac/ pantoprazole formula and diclofenac/ pantoprazole formula were been studied. An accurate simple and précised method was adopted for simultaneous determination of ketorolac and pantoprazole in a physical mixture form. The method is based on measuring the first derivative amplitudes at 285.2nm and 270.9nm for ketorolac and pantoprazole respectively in 0.1NHCl using 0.1NHCl as a blank. The first derivative values of absorbance at 340nm and 227nm were measured for ketorolac and pantoprazole respectively in phosphate buffer (pH7.4) using phosphate buffer (pH7.4) as a blank. The first derivative values of absorbance at 285nm and 272.6nm for diclofenac and pantoprazole respectively in 0.1NHCl using 0.1NHCl as a blank. The first derivative values of absorbance at 288.9nm and 275.6nm were measured for diclofenac and pantoprazole respectively in phosphate buffer (pH7.4) using phosphate buffer (pH7.4) as a blank. The obtained results were validated for accuracy, precision, LOD, LOQ and were found to be satisfactory. The proposed method is sample, rapid and suitable for the assay of such combinations.

Keywords: Ketorolac, Pantoprazole, Physical Mixture, First Derivative

### **INTRODUCTION**

Ketorolac, [(±) - 5-benzoyl -2, 3-dihydro - 1H-pyrrolizine - 1- carboxylic acid], is a non-steroidal anti-inflammatory drug (NSAID) which has a strong analgesic activity (1). The drug can be administered intravenously, intramuscularly or orally as the water-soluble tromethamine salt. Several studies suggest that ketorolac is comparable to opioids when used to treat acute pain (2, 3). Diclofenac sodium or sodium-2-[(2, 6-dichlorophenyl) amino] phenyl] acetate, is widely used as non-steroidal anti-inflammatory agent in therapeutics, it inhibits the cyclooxygenase enzyme1. Diclofenac sodium is used as analgesic, antipyretic, anti-inflammatory and approved in the United States for the long term symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (4). The anti-nociceptive action on NSAIDs is primarily due to the inhibition of prostaglandin biosynthesis through the inhibition of cyclooxygenase enzymes: COX-1(constitutive) and COX-2 (inducible in inflammatory processes)

(5, 6). Pantoprazole is 6-(difluoromethoxy)-2- [(3, 4-dimethoxypyridin-2-yl methane] sulfinyl-1H-1, 3-benzodiazole. Pantoprazole is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastro esophageal reflux disease (7). Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H<sup>+</sup>,K<sup>+</sup>) - ATPase enzyme system at the secretory surface of the gastric parietal cell (8). Fixed NSAID/PPI combinations will likely help to solve the gastrointestinal compliance problem. The first representative of this group of drugs for treating the signs and symptoms of OA, RA, and ankylosing spondylitis, and for decreasing the risk of developing gastric ulcers in patients at risk has just been approved by the FDA (9). An additional advantage of PPI combination is the lower incidence of heartburn, acid regurgitation, and sleep disturbance. Future guidelines will probably recommend combination of NSAIDs, as well as coxibs with a PPI, as first-line medication for all

\*Corresponding Author Address: Esmat Zien El-Deen, Pharm. Technology Dept., Faculty of Pharmacy, Tanta University\*, Tanta, Egypt,

risk patients (10). The purpose of the present study was to prepare a validated method in order to estimate both NSAIDs and pantoprazole present in the same formula.

## MATERIALS

Ketorolac tromethamine (Sigma- Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Amriya pharmaceuticals industries, Alexandria, Egypt, Diclofenac sodium (Sigma- Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Pharco Pharmaceuticals industries, Ismailia, Egypt, Pantoprazole (Sigma- Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Sigma pharmaceuticals industries, Quweisna, Egypt. All other reagents and chemicals were analytical grades and were used as received.

### Method:

**Determinations of NSAIDs and pantoprazole in the prepared blend:** A derivative spectrophotometric method was developed. Since the zero-order spectra of the two drugs are overlapping, the determination of those ingredients using the conventional UV spectrophotometry has become invalid. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands. The derivative absorbance at certain chosen wavelengths allowed the concurrent determination of the two components without preliminary separation or extraction of any of them. The zero-crossing method is the most common procedure for conducting analytical calibration in derivative spectrophotometry (11-14).

**Instrumentation:** UV and derivative spectra of the solutions were recorded on double beam UV-Vis spectrophotometer (Shimadzu 1800) using 10 mm path length quartz cells, scan range of 200–400 nm, delta wavelength 5nm and scaling factor 1.

### Preparation of standard solutions and construction of calibration curves for ketorolac/pantoprazole formula:

**For ketorolac:** Stock standard solution of ketorolac was prepared in distilled water to give a final concentration of 1mg.ml<sup>-1</sup>. Different aliquots from this stock solution were taken and diluted with 0.1N HCl to obtain solutions of ketorolac in the concentration range of 5-30 µg.ml<sup>-1</sup>. The zero order absorption spectra were recorded against 0.1N HCl as a blank. Calibration curves were constructed by plotting the values of the first derivative absorbance (<sup>1</sup>D) at 285.2 nm against the corresponding concentrations of the standard solutions. Stock standard solution of ketorolac

was prepared similarly in phosphate buffer (pH 7.4) to obtain a final concentration of 1mg.ml<sup>-1</sup>. Different aliquots from this stock solution were taken and diluted with the same buffer to obtain solutions of ketorolac in the concentration range of 5-30 µg.ml<sup>-1</sup>. The zero order absorption spectra were recorded against phosphate buffer (pH 7.4) as a blank. Calibration curves were constructed by plotting the values of the first derivative absorbance (<sup>1</sup>D) at 340 nm against the corresponding concentrations of the standard solutions.

**For pantoprazole:** Stock standard solution of pantoprazole was prepared in 0.1N HCl to give a final concentration of 1.0 mg.ml<sup>-1</sup>. Different aliquots from this stock solution were taken and diluted with 0.1N HCl to obtain solutions of pantoprazole in the concentration range of 5-30 µg.ml<sup>-1</sup>. The zero order absorption spectra were recorded against 0.1N HCl as a blank. The absolute values of the first order derivatives were obtained by zero-crossing technique. Calibration curves were constructed by plotting the values of the first derivative absorbance (<sup>1</sup>D) at zero-crossing point for ketorolac 270.9 nm against the corresponding concentrations of the standard solutions. Stock standard solution of pantoprazole was prepared similarly in phosphate buffer (pH 7.4) to obtain a final concentration of 1mg.ml<sup>-1</sup>. Different aliquots from this stock solution were taken and diluted with the same buffer to obtain solutions of pantoprazole in the concentration range of 5-30 µg.ml<sup>-1</sup>. The zero-order absorption spectra were recorded against phosphate buffer (pH 7.4) as blank. Calibration curves were constructed by plotting the values of the first derivative absorbance (<sup>1</sup>D) at 227nm against corresponding concentrations of standard solutions.

### Preparation of standard solutions and construction of calibration curves for diclofenac/pantoprazole formula:

**For diclofenac:** Stock standard solution of diclofenac was prepared in distilled water to give a final concentration of 1mg.ml<sup>-1</sup>. Different aliquots from this stock solution were taken and diluted with 0.1N HCl to obtain solutions of diclofenac in the concentration range of 5-30 µg.ml<sup>-1</sup>. The zero order absorption spectra were recorded against 0.1N HCl as a blank. Calibration curves were constructed by plotting the values of the first derivative absorbance (<sup>1</sup>D) at 285.2 nm against the corresponding concentrations of the standard solutions. Stock standard solution of diclofenac was prepared similarly in phosphate buffer (pH 7.4) to obtain a final concentration of 1mg.ml<sup>-1</sup>. Different aliquots from this stock solution were taken and diluted with the same buffer to obtain solutions of diclofenac in the concentration range

of 5-30  $\mu\text{g}\cdot\text{ml}^{-1}$ . The zero order absorption spectra were recorded against phosphate buffer (pH 7.4) as blank. Calibration curves were constructed by plotting the values of the first derivative absorbance ( $^1\text{D}$ ) at 288.9 nm against the corresponding concentrations of the standard solutions.

**For pantoprazole:** Stock standard solution of pantoprazole was prepared in 0.1N HCl to give a final concentration of 1.0  $\text{mg}\cdot\text{ml}^{-1}$ . Different aliquots from this stock solution were taken and diluted with 0.1N HCl to obtain solutions of pantoprazole in the concentration range of 5-30  $\mu\text{g}\cdot\text{ml}^{-1}$ . The zero order absorption spectra were recorded against 0.1N HCl as a blank. The absolute values of the first order derivatives were obtained by zero-crossing technique. Calibration curves were constructed by plotting the values of the first derivative absorbance ( $^1\text{D}$ ) at zero-crossing point for ketorolac 272.6 nm against the corresponding concentrations of standard solutions. Stock standard solution of pantoprazole was prepared similarly in phosphate buffer pH 7.4 to give a final concentration of 1 $\text{mg}\cdot\text{ml}^{-1}$ . Different aliquots from this stock solution were taken and diluted with the buffer to obtain solutions of pantoprazole in the concentration range of 5-30  $\mu\text{g}\cdot\text{ml}^{-1}$ . The zero-order absorption spectra were recorded against phosphate buffer (pH 7.4) as blank. Calibration curves were constructed by plotting the values of the first derivative absorbance ( $^1\text{D}$ ) at 275.6 nm against corresponding concentrations of standard solutions.

#### Assay of the prepared blend:

**Simultaneous determination of ketorolac and pantoprazole:** The zero order spectrum of this aliquot of dissolution medium was recorded against 0.1 N HCl (dissolution medium 1) or phosphate buffer (pH 7.4) (dissolution medium 2) as blank. For dissolution medium (1): the  $^1\text{D}$  value was recorded at 285.2 and at 270.9 for determination of ketorolac and pantoprazole respectively, then the concentration of each drug was calculated from the

corresponding regression equation of its calibration curve. For dissolution medium (2): the  $^1\text{D}$  value was recorded at 340 and at 227 for determination of ketorolac and pantoprazole respectively, then the concentration of each drug was calculated from the corresponding regression equation of its calibration curve.

**Simultaneous determination of diclofenac and pantoprazole:** The zero order spectrum of this aliquot of dissolution medium was recorded against 0.1 N HCl (dissolution medium 1) or phosphate buffer (pH 7.4) (dissolution medium 2) as blank. For dissolution medium (1): the  $^1\text{D}$  value was recorded at 285.2 nm and at 272.6 nm for determination of diclofenac and pantoprazole respectively, then the concentration of each drug was calculated from the corresponding regression equation of its calibration curve. dissolution medium (2): the  $^1\text{D}$  value was recorded at 288.9 nm and at 275.6 nm for determination of diclofenac and pantoprazole respectively, then the concentration of each drug was calculated from the corresponding regression equation of its calibration curve.

## RESULTS AND DISCUSSION

**For the first formula (ketorolac and pantoprazole):** Since the zero-order spectra of ketorolac and pantoprazole in 0.1 N HCL (pH 1.0) and in phosphate buffer (pH 7.4) are overlapping as shown in Fig.1(A) and Fig.2 (A) respectively, the determination of both ingredients utilizing the conventional UV spectrophotometry has become invalid. A first derivative spectrophotometric method was adopted for their simultaneous determination where the first derivative spectra revealed zero-crossing point for pantoprazole allowing the measurement of ketorolac and the contrary zero-crosses points for ketorolac allowing the measurement of pantoprazole Fig. 1(B) and Fig. 2 (B).

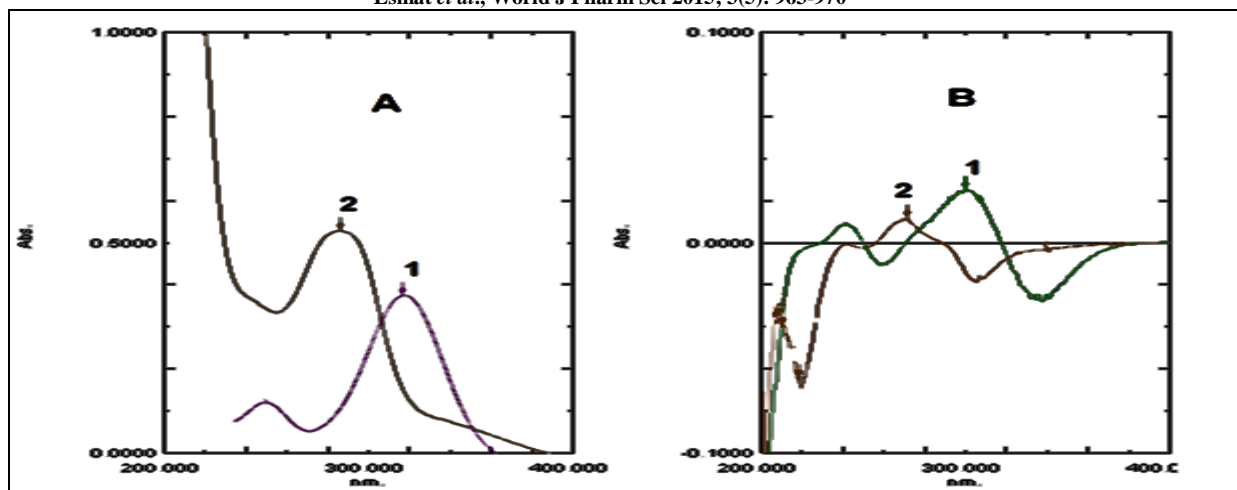


Fig (1) Overlain of zero-order spectra (A) for ketorolac (1) & pantoprazole (2) and 1<sup>st</sup> order spectra (B) for ketorolac (1) & pantoprazole (2) in phosphate buffer (pH 1.0)

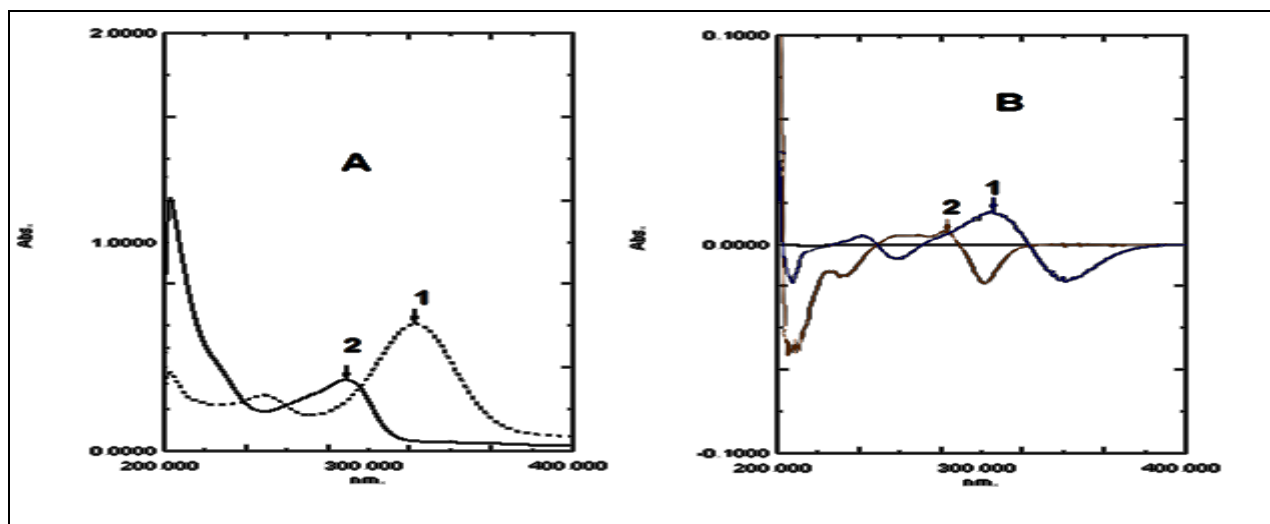


Fig (2) Overlain of zero-order spectra (A) for ketorolac (1) & pantoprazole (2) and 1<sup>st</sup> order spectra (B) for ketorolac(1) & pantoprazole (2) in phosphate buffer (pH 7.4)

**Validation of the proposed first derivative spectrophotometric method for first formula:**

The Validity of the method was tested regarding linearity, specificity, accuracy, and precision according to ICH guide lines (ICH-Q2B, 2005) (15).

**Linearity and range:** The calibration graphs for the determination of ketorolac and pantoprazole by the proposed method were constructed by plotting the derivative amplitudes versus the concentrations. The graphs were found to be rectilinear over the concentration ranges cited in Table (1).

Table 1: Statistical data of calibration curves of ketorolac and pantoprazole

Parameter	In pH 1.0		In pH 7.4	
	ketorolac	pantoprazole	ketorolac	pantoprazole
Linearity Range (µg.ml <sup>-1</sup> )	5- 30	5- 30	5- 30	5-30
Regression equation	${}^1D_{285.2}=0.0013x-0.0004$	${}^1D_{270.9}=0.0007x+0.0006$	${}^1D_{340}=0.0015x-0.0012$	${}^1D_{227}=0.0022x+0.0002$
Correlation coefficient	0.999	0.999	0.9999	1
SD about slope	0.000002	0.00017	0.0016	0.0004

SD about intercept	0.00040	0.001300	0.0003	0.00200
LOD ( $\mu\text{g.ml}^{-1}$ )	0.41000	0.34000	0.4000	0.3000
LOQ ( $\mu\text{g.ml}^{-1}$ )	1.2300	1.0600	1.1300	0.900

Statistical analysis of the data showed high values of correlation coefficients of the regression equations, small values of the standard deviations of intercept (Sa), and of slope (Sb). These data proved the linearity of the calibration graphs and the agreement of the result with Beer's law.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ) for first formula:** The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected, while the limit of quantitation (LOQ) was determined by establishing the lowest concentration that can be measured above which the calibration graph is nonlinear. The results are

shown in Table (1). LOQ and LOD were calculated according to the following equations (15):

$$\text{LOQ} = 10 S_a / b, \text{LOD} = 3.3 S_s / b$$

Where Sa is the standard deviation of the intercept of regression line, and b is the slope of the calibration curve.

**Accuracy and precision for first formula:** To prove the accuracy of the proposed methods several synthetic mixtures of ketorolac and pantoprazole in the ratio 1:1 were analyzed. Statistical analysis of the obtained results involving the mean percent recoveries of both drugs in the proposed mixtures are summarized in Tables 2 and 3.

**Table (2) Recovery of synthetic mixtures of ketorolac and pantoprazole**

drug	Concentration ( $\mu\text{g.ml}^{-1}$ )	Mean* % recovery	
		In pH 1.0	In pH 7.4
ketorolac	10	99.30 $\pm$ 0.06	102.00 $\pm$ 0.02
	20	99.00 $\pm$ 0.19	99.57 $\pm$ 0.02
	30	100.30 $\pm$ 0.03	99.80 $\pm$ 0.14
pantoprazole	10	100.10 $\pm$ 0.09	99.70 $\pm$ 0.02
	20	99.95 $\pm$ 0.07	100.22 $\pm$ 0.06
	30	99.73 $\pm$ 0.01	99.68 $\pm$ 0.04

\*Average of three determinations  $\pm$  S.D

**Table (3): Precision data for the determination of ketorolac and pantoprazole**

drug	Concentration ( $\mu\text{g.ml}^{-1}$ )	Intra-day *		Inter-day *	
		Concentration found ( $\mu\text{g.ml}^{-1}$ )		Concentration found ( $\mu\text{g.ml}^{-1}$ )	
		In pH 1.0	In pH 7.4	In pH 1.0	In pH 7.4
ketorolac	10	9.98 $\pm$ 0.02	9.97 $\pm$ 0.04	9.98 $\pm$ 0.06	9.98 $\pm$ 0.01
	20	20.01 $\pm$ 0.12	20.01 $\pm$ 0.02	19.99 $\pm$ 0.12	19.97 $\pm$ 0.05
	30	29.98 $\pm$ 0.03	29.99 $\pm$ 0.01	30.01 $\pm$ 0.14	30.03 $\pm$ 0.03
pantoprazole	10	9.99 $\pm$ 0.04	10.20 $\pm$ 0.02	9.99 $\pm$ 0.02	9.99 $\pm$ 0.07
	20	19.97 $\pm$ 0.01	19.99 $\pm$ 0.02	19.98 $\pm$ 0.09	19.96 $\pm$ 0.01
	30	30.03 $\pm$ 0.05	29.99 $\pm$ 0.06	29.99 $\pm$ 0.01	29.98 $\pm$ 0.08

\*Average of three determinations  $\pm$  S.D

Intraday (repeatability) and inter-day (intermediate) precisions were assessed using three concentrations. The standard deviations were found to be very small indicating good repeatability over the entire concentration range, which revealed the precision of the proposed method as shown in Table 3.

**For the second formula (diclofenac and pantoprazole):** Since the zero-order spectra of diclofenac and pantoprazole in 0.1 N HCL (pH 1.0) and in phosphate buffer (pH 7.4) are overlapping as

shown in Fig.3 (A) and Fig.4 (A) respectively, the determination of both ingredients utilizing the conventional UV spectrophotometry has become invalid. A first derivative spectrophotometric method was adopted for their simultaneous determination where the first derivative spectra revealed zero-crossing point for pantoprazole allowing the measurement of diclofenac and the contrary zero-crosses points for diclofenac allowing the measurement of pantoprazole Fig. 3 (B) and Fig. 4 (B).

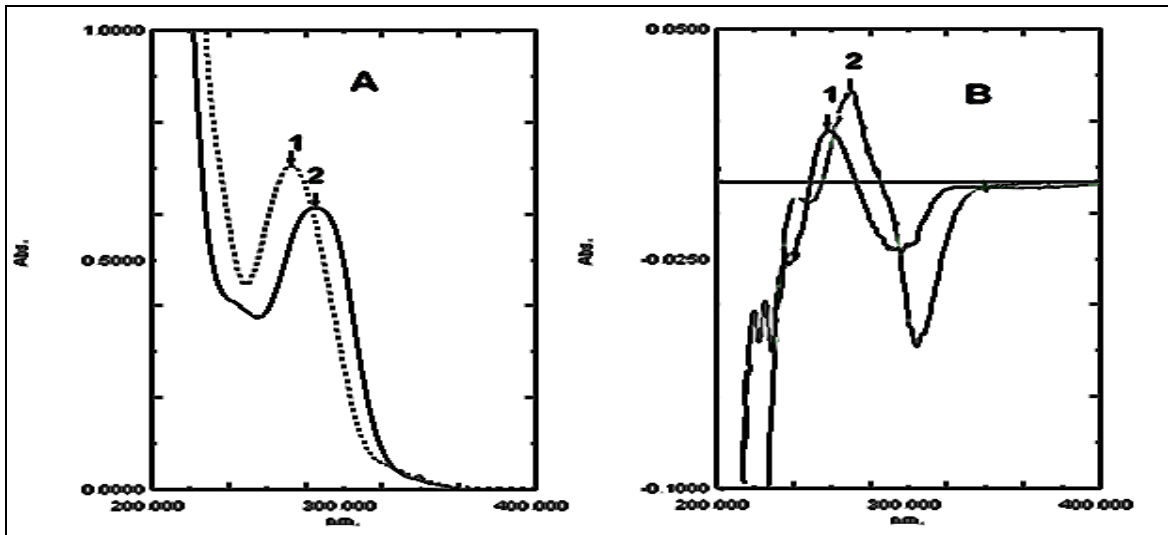


Fig (3) Overlain of zero-order spectra (A) for diclofenac (1) & pantoprazole (2) and 1<sup>st</sup> order spectra (B) for diclofenac (1) & pantoprazole (2) in phosphate buffer (pH 1.0)

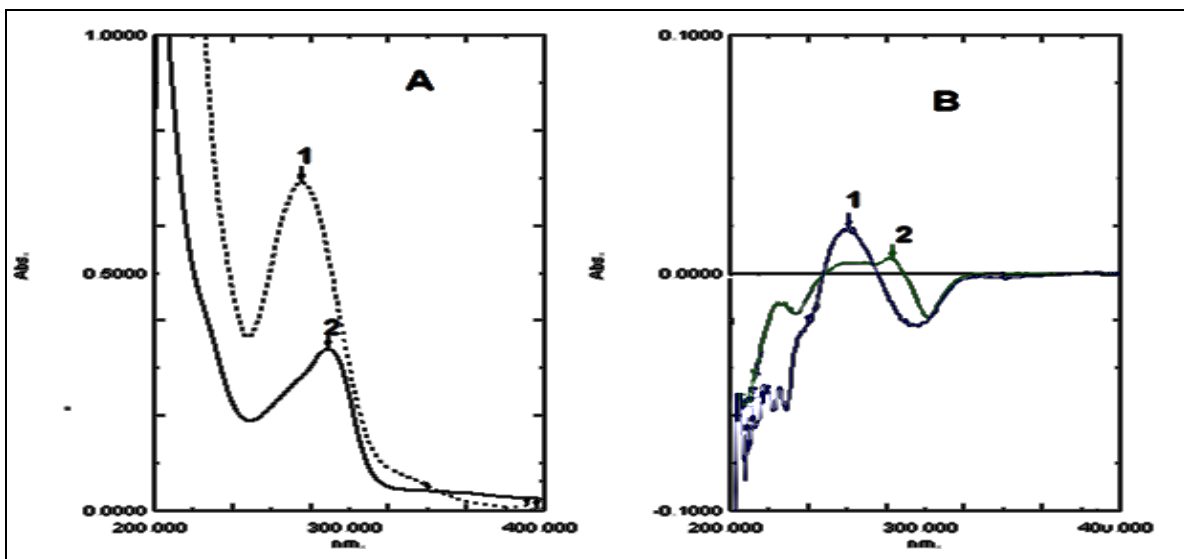


Fig (4) Overlain of zero-order spectra (A) for diclofenac (1) & pantoprazole (2) and 1<sup>st</sup> order spectra (B) for diclofenac (1) & pantoprazole (2) in phosphate buffer (pH 7.4)

**Validation of the proposed first derivative spectrophotometric method for second formula:**

The Validity of the method was tested regarding linearity, specificity, accuracy, and precision according to ICH guide lines (ICH-Q2B, 2005) (15).

**Linearity and range:** The calibration graphs for the determination of ketorolac and pantoprazole by the proposed method were constructed by plotting the derivative amplitudes versus the concentrations. The graphs were found to be rectilinear over the concentration ranges cited in Table (4).

**Table 4: Statistical data of calibration curves of diclofenac and pantoprazole**

Parameter	In pH 1.0		In pH 7.4	
	diclofenac	pantoprazole	diclofenac	pantoprazole
Linearity Range ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	5- 30	5- 30	5- 30	5-30
Regression equation	${}^1\text{D}_{285.2}=0.0007x-0.0001$	${}^1\text{D}_{272.6}=0.0008x-0.002$	${}^1\text{D}_{288.9}=0.0007x-0.003$	${}^1\text{D}_{275.6}=0.005x+0.001$
Correlation coefficient	0.999	0.999	0.9999	0.9999
SD about slope	0.000001	0.00013	0.0012	0.0002
SD about intercept	0.00001	0.00003	0.0002	0.00400
LOD ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	0.43000	0.41000	0.4000	0.33000
LOQ ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	1.400	1.2300	1.3200	1.100

Statistical analysis of the data showed high values of correlation coefficients of the regression equations, small values of the standard deviations of intercept (Sa), and of slope (Sb). These data proved the linearity of the calibration graphs and the agreement of the result with Beer's law.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ) for the second formula:** The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected, while the limit of quantitation (LOQ) was determined by establishing the lowest concentration that can be measured above which the calibration graph is nonlinear. The

results are shown in Table (4). LOQ and LOD were calculated according to the following equations (15):  $\text{LOQ} = 10 \text{ Sa} / b$ ,  $\text{LOD} = 3.3 \text{ Ss} / b$

Where Sa is the standard deviation of the intercept of regression line, and b is the slope of the calibration curve.

**Accuracy and precision for second formula:** To prove the accuracy of the proposed methods several synthetic mixtures of ketorolac and pantoprazole in the ratio 1:1 were analyzed. Statistical analysis of the obtained results involving the mean percent recoveries of both drugs in these mixtures are summarized in Tables 5 and 6.

**Table (5) Recovery of synthetic mixtures of diclofenac and pantoprazole**

drug	Concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	Mean* % recovery	
		In pH 1.0	In pH 7.4
diclofenac	10	99.30 $\pm$ 0.06	102.00 $\pm$ 0.02
	20	99.00 $\pm$ 0.19	99.57 $\pm$ 0.02
	30	100.30 $\pm$ 0.03	99.80 $\pm$ 0.14
pantoprazole	10	100.10 $\pm$ 0.09	99.70 $\pm$ 0.02
	20	99.95 $\pm$ 0.07	100.22 $\pm$ 0.06
	30	99.73 $\pm$ 0.01	99.68 $\pm$ 0.04

\*Average of three determinations  $\pm$  S.D

**Table (6): Precision data for the determination of diclofenac and pantoprazole**

drug	Concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	Intra-day *		Inter-day *	
		Concentration found ( $\mu\text{g}\cdot\text{ml}^{-1}$ )		Concentration found ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	
		In pH 1.0	In pH 7.4	In pH 1.0	In pH 7.4
diclofenac	10	9.97 $\pm$ 0.01	9.99 $\pm$ 0.01	9.98 $\pm$ 0.02	10.00 $\pm$ 0.03
	20	20.04 $\pm$ 0.02	20.06 $\pm$ 0.05	19.99 $\pm$ 0.14	19.98 $\pm$ 0.01
	30	29.99 $\pm$ 0.22	29.98 $\pm$ 0.02	30.04 $\pm$ 0.04	29.99 $\pm$ 0.02
pantoprazole	10	10.00 $\pm$ 0.03	10.10 $\pm$ 0.09	9.99 $\pm$ 0.01	9.98 $\pm$ 0.06
	20	19.98 $\pm$ 0.05	20.21 $\pm$ 0.11	19.99 $\pm$ 0.07	19.97 $\pm$ 0.04
	30	30.01 $\pm$ 0.13	29.97 $\pm$ 0.08	29.99 $\pm$ 0.05	30.05 $\pm$ 0.13

\*Average of three determinations  $\pm$  S.D

Intraday (repeatability) and inter-day (intermediate) precisions were assessed using three concentrations. The standard deviations were found to be very small indicating good repeatability over the entire concentration range, which revealed the precision of the proposed method as shown in Table 6.

#### Conclusion:

The derivative spectrophotometric technique provides a simple and sensitive means of

determining more than one drug in the presence of each other e.g. ketorolac & pantoprazole and diclofenac & pantoprazole as a physical mixture. It has also the advantages of acceptable accuracy and precision. This method is also easier and cheaper to perform than HPLC separations and do not require expensive reagents or organic solvents. These advantages coupled with acceptable precision make the method suitable for routine quality control.

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