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Determination of lornoxicam in pharmaceutical preparations by zero and first order derivative UV spectrophotometric methods

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Zero and first order derivative UV spectrophotometric methods were developed for the analysis of lornoxicam (LOR). The solutions of the standards and pharmaceutical samples were prepared in 0.05 N NaOH. Absorbances of LOR were measured at 376 nm for the zero order by measuring height of peak from zero and at 281 and 302 nm for the first order derivative spectrophotometric method by measuring peak to peak height. The linearity ranges were found to be 0.5–35 μg/mL for the zero order and 0.2–75 μg/mL for the first order derivative UV spectrophotometric method. The methods were validated and applied to the determination of LOR in pharmaceutical preparations (tablet and injectable, both containing 8 mg LOR). It was concluded that the methods developed were accurate, sensitive, precise, robust, rugged and useful for the quality control of LOR in pharmaceutical preparations.

1. Introduction

Lornoxicam (6-chloro-4-hydroxy-2-methyl-*N*-2-pyridinyl-2H-thieno[2,3-*e*]-1,2-thiazine-3-carboxamide 1,1-dioxide) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory and antipyretic properties. It is distinguished from established oxicams by a relatively short elimination half-life, which may be advantageous from a tolerability standpoint (Balfour et al. 1996; Olkkola et al. 1994; Skjodt and Davies 1998).
Voltammetric (Ghoneim 2002) and high performance liquid chromatographic (HPLC) methods have been reported for the analysis of LOR in plasma (Radhofer-Welte and Dittrich 1998; Suwa et al. 1993; Kohl et al. 2000; Dittrich et al. 1990; Bareggi et al. 1997). Joseph-Charles et al. developed an HPLC method for the analysis of oxicams in pharmaceutical preparations (Joseph-Charles and Bertucat 1999). No spectrophotometric method for the determination of LOR in pharmaceutical formulations has been reported in the literature.

The main purpose of the studies presented was to develop simple, rapid, accurate, precise, linear, sensitive, robust and rugged spectrophotometric methods for the determination of LOR in pharmaceutical formulations which can be considered a useful alternative to the HPLC method.

2. Investigations, results and discussions

The zero order derivative UV spectra of LOR, obtained from different solutions (0.05 N NaOH, MeOH, MeOH containing 0.05 N NaOH, MeOH containing 0.05 N HCl and 20 mM borate buffer pH:11.06), are given in Fig. 1 except for 20 mM borate buffer pH:11.06 because of its complete fit with 0.05 N NaOH. The maximum of the main band was found to be dependent on the pH of the media much more than on their solvent content. Increasing pH caused a bathochromic shift and decreased abundance of the absorption band. Given this result we considered whether an acid medium could be chosen as a

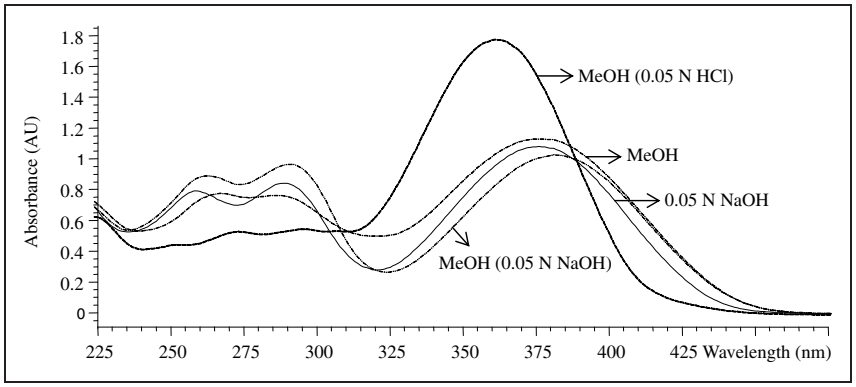

Fig. 1:
Zero order derivative UV spectra of LOR (25 μg/mL) in different solutions

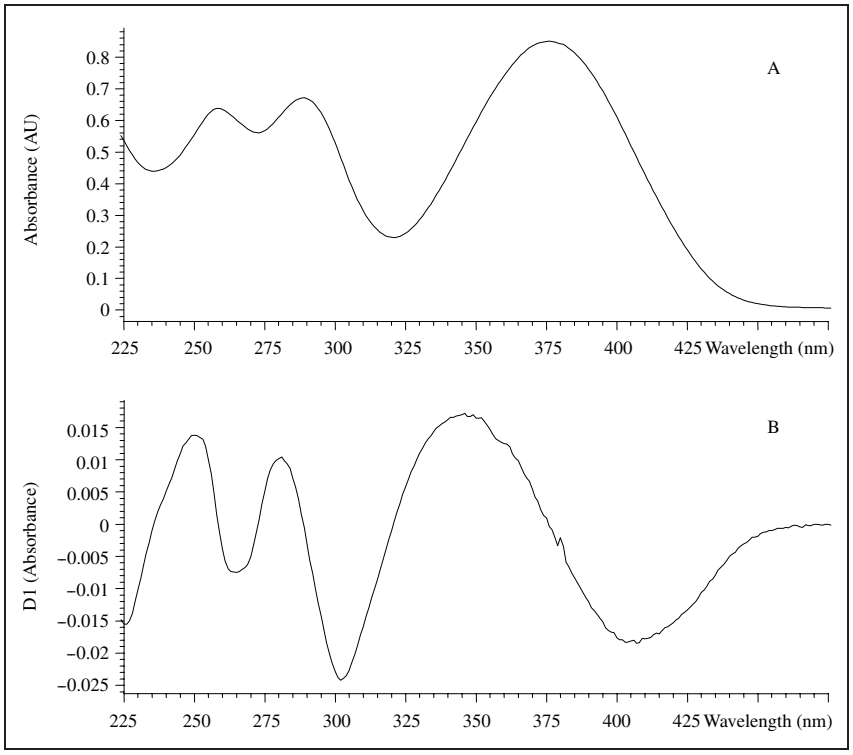

Fig. 2:
UV spectra of LOR (20 μg/mL) A) zero and B) first order derivative UV spectrum

working solution to increase sensitivity. But LOR is very poorly soluble in acidic media, and its solubility increases with increasing pH (Tsai et al. 1993). Therefore, a basic medium was chosen for the working solution to increase solubility and to obtain better resolution than with acidic media at the three absorption bands (258, 288 and 376 nm).

The principle advantage of derivative spectrophotometry is the improvement of resolution of overlapping absorption bands, so the accuracy and precision of UV absorption methods are considerably improved (Bebewy 1998; Wang and Asgharnejad 2000; Karljikovic-Rajic et al. 2003). In this study, a derivative technique is used to resolve overlapping bands at 258 nm and 288 nm (Fig. 2A) and to increase the linearity range of the calibration curve. 20 μg/mL of LOR in 0.05 N NaOH was measured with first, second, third and fourth order derivative spectrophotometric techniques to determine the degree of the derivative spectrophotometric method. A good resolution and sensitivity for LOR was observed with first order derivative spectrophotometry.

The effect of pH on the spectrum for zero and first order derivative UV spectrophotometry was investigated at pH 7.24, 8.00, 9.00, 9.96, 11.06 (using 20 mM borate buffer) and 12.70 (using 0.05 N NaOH). At these pH values, absorption values and maximum wavelength did not differ. Therefore it was concluded that the spectrum of LOR was not affected above pH 7.24.

At the end of these studies, 0.05 N NaOH was chosen, because of the time gain while preparing solutions and cost saving by eliminating the purchase and disposal of organic solvents. The zero and first order derivative UV spectra of LOR under these conditions are illustrated in Fig. 2.

The assay of LOR was validated with respect to linearity, precision, accuracy, selectivity/sensitivity, robustness and ruggedness (ICH 1995; Sabry et al. 1999; Castro et al. 1999).

The standard stock solutions of LOR, which were protected from daylight, were stored in two different conditions, i.e. +4 °C and room temperature for 2 weeks. During this period, the solutions were analyzed and the spectrum was compared with the spectrum of a standard solution prepared freshly each day, and no difference was found between them. It was concluded that LOR is stable under the conditions mentioned for at least 2 weeks.

The calibration plots were constructed after analysis of ten different concentrations with each concentration was measured three times. The regression equations and correlation coefficients of the mean of six consecutive calibration curves are given in Table 1.

The *limit of detection* (LOD) ($k = 3.3$) and *limit of quantification* (LOQ) ($k = 10$) of the method were established according to the ICH definitions ($C_l = k\ S_0/s$ where C_l is LOD or LOQ, S_0 is the standard error of blank determination s is the slope of the standard curve and k is the constant related to the confidence interval. The standard errors of absorbance measurement for blank solution in zero and first order derivative UV spectrophotometric methods were 1.92×10^{-3} and 2.56×10^{-5} ($n = 15$), respectively. The LOD and LOQ values of the methods are given in Table 1.

Table 1: Data for calibration graphs ($n = 6$) for LOR using zero and first order derivative UV spectrophotometric methods

	Zero order derivative UV spectroscopy	First order derivative UV spectroscopy
Slope	0.0438 ± 0.0007	0.0172 ± 0.0008
Intercept	-0.0076 ± 0.0014	0.0021 ± 0.0011
Correlation coefficient	0.9992	0.9998
Linearity range (μg/mL)	0.5–35	0.2–75
LOD (μg/mL)	0.13	0.06
LOQ (μg/mL)	0.5	0.2

Mean ± Standard error

Table 2: Precision and accuracy of spectrophotometric methods developed for analysis of LOR (n = 6)

	Added (μg/mL)	Intra-day			Inter-day		
		Found $\bar{x}$ (μg/mL) ± SE	Accuracy Bias (%)	Precision RSD (%)	Found $\bar{x}$ (μg/mL) ± SE	Accuracy Bias (%)	Precision RSD (%)
Zero order	2.5	2.51 ± 0.04	0.03	2.13	2.48 ± 0.03	−0.80	2.35
derivative UV	10	10.07 ± 0.08	0.70	2.03	10.02 ± 0.03	0.18	0.62
spectrophotometry	20	20.06 ± 0.10	0.29	1.27	20.01 ± 0.03	0.05	0.38
First order	2.5	2.54 ± 0.03	1.60	2.01	2.47 ± 0.02	−1.07	2.12
derivative UV	20	19.95 ± 0.11	−0.25	1.35	20.05 ± 0.07	0.23	0.80
spectrophotometry	50	50.16 ± 0.13	0.32	0.63	50.21 ± 0.16	0.42	0.78

$\bar{x}$: Mean, SE: Standard error, RSD: Relative standard deviation, Bias: [100 × (found − added)/added]

Table 3: Recovery of methods for analysis of LOR (n = 6)

Added (μg/mL)	Zero order derivative UV spectrophotometry			First order derivative UV spectrophotometry		
	$\bar{x}$ (μg/mL) ± SE	RSD (%)	Recovery %	$\bar{x}$ (μg/mL) ± SE	RSD (%)	Recovery %
15	15.09 ± 0.04	0.67	100.60	15.01 ± 0.04	0.58	100.07
20	20.19 ± 0.04	0.53	100.95	20.01 ± 0.05	0.57	100.05
25	25.23 ± 0.05	0.53	100.92	24.99 ± 0.05	0.49	100.04

$\bar{x}$: Mean, SE: Standard error, RSD: Relative standard deviation

Accuracy was investigated by analyzing three concentrations of LOR in the linear range in six independent replicates on the same day and on six consecutive days. Accuracy was expressed as bias (%). The bias values were close to zero (Table 2).

The recovery studies were carried out by spiking placebo (starch, lactose and magnesium stearate, which are common constituents of solid pharmaceutical formulations) with LOR at 75% (15 μg/mL), 100% (20 μg/mL) and 125% (25 μg/mL) of the standard solution concentration. The percentage recoveries of the three concentrations were found to be close to 100% (Table 3). The high percentage recoveries indicate no interferences from ingredients and excipients that might be found in different formulations.

The low bias values and high recovery indicated that the methods have a high accuracy.

Repeatability is based on the results of the method operating over a short time interval under the same conditions. The low RSD values of intra-day precision (Table 2), recovery (Table 3) and pharmaceutical preparations (Table 4) showed that the methods give a high repeatability.

Table 4: Results of pharmaceutical formulations analysed by spectrophotometric methods (n = 6)

Tablets (8 mg LOR)		Injectable (8 mg LOR)	
Zero-order UV	First-order UV	Zero-order UV	First-order UV
8.02 ± 0.01	7.97 ± 0.01	8.14 ± 0.01	8.27 ± 0.01
7.99 ± 0.01	7.99 ± 0.01	8.09 ± 0.01	8.23 ± 0.01
7.99 ± 0.01	7.93 ± 0.01	8.27 ± 0.01	8.39 ± 0.01
8.03 ± 0.01	7.99 ± 0.01	7.98 ± 0.01	8.08 ± 0.01
7.94 ± 0.01	7.91 ± 0.02	8.01 ± 0.01	8.12 ± 0.01
7.93 ± 0.01	7.90 ± 0.01	8.05 ± 0.01	8.19 ± 0.01
$\bar{x}$: 7.98 ± 0.017	$\bar{x}$: 7.95 ± 0.02	$\bar{x}$: 8.09 ± 0.04	$\bar{x}$: 8.22 ± 0.05
SD: 0.04	SD: 0.04	SD: 0.10	SD: 0.11
RSD: 0.53%	RSD: 0.52%	RSD: 1.31%	RSD: 1.34%
$p = 0.207 > p = 0.05$		$p = 0.116 > p = 0.05$	

$\bar{x}$: (Mean) ± Standard error, SD: Standard deviation, RSD: Relative standard deviation

Three different concentrations of LOR in the linear range were analyzed in six independent series on the same day (*intra-day precision*) and six consecutive days (*inter-day precision*) with three measurements of every sample in each series. The data evaluated using calibration plots are summarized in Table 2. The RSD values varied from 0.38 to 2.35 for zero order derivative UV spectrophotometry and from 0.63 to 2.12 for first order derivative UV spectrophotometry. The low intra-day and inter-day RSD values and also the low RSD values obtained from the analyses of pharmaceutical formulations (Table 4) indicated that the intermediate precision of the methods was good.

The spectra obtained from pharmaceutical formulations and placebo solution (Fig. 3) were identical with those obtained from standard solutions containing an equivalent concentration of LOR (20 μg/mL) (Fig. 2). In addition the standard addition technique was applied to the same preparations which were analysed by calibration curve methods. The regression equations of standard addition curves of methods for tablet analysis were found to be $y = 0.0429x + 0.822$ ($r^2 = 0.9999$) for zero and $y = 0.0171x + 0.3482$ ($r^2 = 0.9991$) for first order derivative UV spectrophotometry. There was no difference between the slopes with standard and standard addition techniques. These results show that there was no interference from matrix components. Therefore it could be said that the methods are highly *selective*.

The *robustness* of the methods was tested by making deliberate small changes in wavelength and NaOH concentration (Table 5). For *ruggedness*, LOR analyses were performed by a different analyst and in a different laboratory (interdisciplinary laboratory) with a different device (Agilent 8453 UV spectrophotometer). A tablet sample containing 20 μg/mL was analysed six times. These results were close to those obtained under standard conditions and also when a statistical comparison was done with Friedman analysis there was no difference between the results ($p = 0.068 > p = 0.050$ for zero and $p = 0.072 > p = 0.050$ for first order derivative UV spectrophotometry). Therefore the methods are rugged and robust under small changes in experimental conditions.

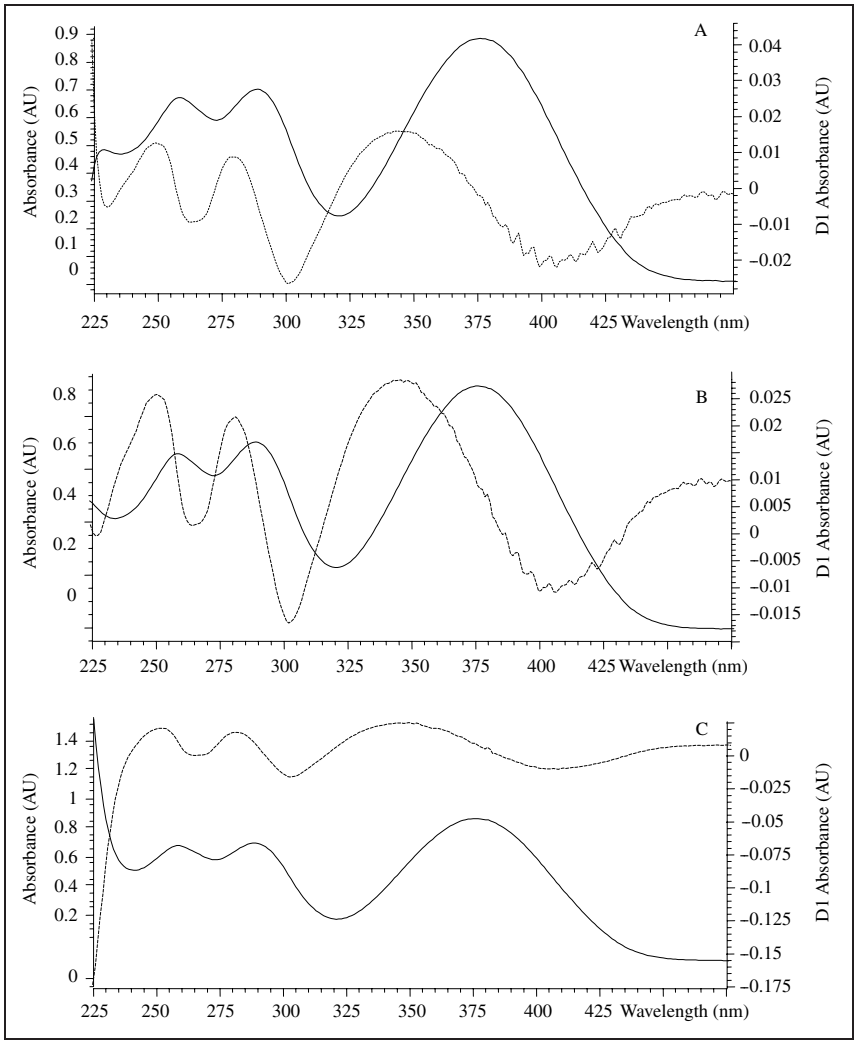

Fig. 3:
Zero (——) and first order (------) derivative UV spectra of LOR in 0.05 N NaOH; A) Tablet solution B) Injectable solution and C) Spiked placebo solution. All solutions contained 20 μg/mL LOR

Quantitative analyses of LOR in *tablets and injectables* were performed using the two methods developed. Pharmaceutical formulations were analysed in six independent series and samples from each series were measured three times. The statistical comparison of methods was performed with the Wilcoxon paired test. There was no statistically significant difference between the results (Table 4).

Table 5: Robustness and ruggedness[a] data for zero and first order derivative UV spectrophotometric methods (LOR 20 μg/mL)

Method	Conditions	$\bar{x} \pm SE$	RSD %	Bias %
Zero order derivative UV spectrophotometry	Standard	19.83 ± 0.01	0.09	−0.83
	0.04 N NaOH	19.78 ± 0.03	0.39	−1.09
	0.06 N NaOH	19.72 ± 0.01	0.07	−1.42
	Wavelength 375 nm	19.83 ± 0.01	0.09	−0.87
	Wavelength 377 nm	19.81 ± 0.01	0.10	−0.94
	Different analyst[a]	19.84 ± 0.01	0.15	−0.81
	Different device[a]	19.73 ± 0.05	0.56	−1.33
First order derivative UV spectrophotometry	Standard	19.91 ± 0.04	0.46	−0.47
	0.04 N NaOH	19.83 ± 0.002	0.28	−0.85
	0.06 N NaOH	19.91 ± 0.02	0.23	−0.45
	Wavelength 301 nm	19.67 ± 0.03	0.33	−1.66
	Wavelength 303 nm	19.66 ± 0.03	0.36	−1.69
	Different analyst[a]	19.94 ± 0.02	0.19	−0.32
	Different device[a]	19.92 ± 0.03	0.32	−0.38

3. Experimental

3.1. Apparatus

Spectrophotometric measurements were carried out using an Agilent 8453 model UV-VIS spectrophotometer with a diode array detector (190–1100 nm). A 10 mm quartz cuvette was used. pH of solutions was measured with a pH meter (Mettler Toledo MA235).

3.2. Chemicals and reagents

LOR (a/a 99.8% purity) was kindly supplied by Abdi Ibrahim Drug Company (Turkey) and was used without further purification. Methanol, sodium hydroxide and boric acid were purchased from Merck. Milli-Q water was used for the preparation of buffers and other aqueous solutions. Pharmaceutical preparations of LOR were obtained from local pharmacies.

3.3. Standard and sample solutions

3.3.1. Standard solutions

For the LOR standard stock solution (250 μg/mL) in deionised water, LOR (2.5 mg) is weighed into a 10 mL volumetric flask, dissolved in 5 mL of deionised water and 0.5 mL of 0.05 N NaOH and then diluted with deionised water to a final volume of 10 ml.
For the LOR standard stock solution (150 μg/mL) in MeOH, LOR (1.5 mg) is weighed into a 10 mL volumetric flask and dissolved in 7.5 mL of MeOH. It is then diluted with MeOH to a final volume of 10 mL.
Standard stock solutions of LOR in MeOH and deionised water were kept in the dark at +4 °C.
Appropriate volumes of the standard stock solution were taken and diluted to 10 mL with 0.05 N NaOH to give the desired final analyte concentrations.

3.3.2. Tablet solutions

Ten tablets (8 mg LOR per tablet) were weighed and powdered. An amount of powder equivalent to one tablet was weighed and transferred to

a 50 mL volumetric flask. 30 mL of 0.05 N NaOH was added, and the flask was sonicated for 15 min and diluted to the mark with 0.05 N NaOH. After centrifugation for 5 min at 3000 rpm, 1.25 mL supernatant was taken and diluted to 10 mL with 0.05 N NaOH. Then zero and first order derivative UV absorbance of tablet sample solution was measured for quantitative analysis.

3.3.3. Injectable solutions

Each of the injectable flacons (8 mg LOR per flacon) was dissolved with 2 mL deionised water and transferred to a 50 mL volumetric flask and than diluted to the mark with 0.05 N NaOH. The solutions were analysed as for the tablet solutions.

3.4. Procedures

Before the analysis of solutions containing LOR, the spectrophotometer was adjusted with the matrix solution as the blank. The spectra were recorded from 225 nm to 475 nm. The quantitative analysis of appropriate dilutions of stock, tablet and injectable solutions was performed at 376 nm for the zero order derivative UV spectrophotometric method by measuring the height of the peak from zero, and at 302 and 281 nm for the first order derivative UV spectrophotometric method by measuring the peak to peak height.

All statistical analysis was performed with SPSS software (Version 10.7).

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