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Research Article

Validated Simultaneous Derivative Spectrophotometric Estimation of Diflunisal and Lignocaine in Bulk and Pharmaceutical Formulation

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Abstract

Two simple, sensitive, accurate and precise spectrophotometric methods were developed and validated for the quantitative determination of Diflunisal (DIF) and Lignocaine (LIG) in bulk and pharmaceutical dosage form. Method A is based on the first-order derivative (D1), and Method B is based on the second-order derivative (D2) spectrophotometric method. In Method A, absorbance was measured at 224nm and 264nm being the zero crossing points for DIF and LIG, respectively. In Method B, absorbance was measured at 232nm and 273nm being the zero crossing points for DIF and LIG, respectively. For Method A, the two drugs obeyed Beer's law in the concentration range of 3-39µg/ml for DIF and 4-40 µg/ml for LIG with correlation coefficients 0.999 and 0.995, respectively. For Method B, the two drugs obeyed Beer's law in the concentration range of 3-30µg/ml for DIF and 4-48µg/ml for LIG with correlation coefficients 0.998 and 0.996, respectively. Both methods can be used for routine analysis of these two drugs in their pharmaceutical dosage form. Results for analysis of both methods were tested and validated for various parameters according to ICH guidelines.

Keywords: Derivative, Diflunisal, Lignocaine, first order, second order

INTRODUCTION

Diflunisal (DIF) chemically is 2, 4 -Difluoro-4hydroxybiphenyl-3-carboxylic acid. Diflunisal is a salicylic acid derivative nonsteroidal drug with analgesic, anti-inflammatory and antipyretic properties, and it is a peripherally-acting non-narcotic analgesic drug. It is used for symptomatic treatment of mild to moderate pain accompanied by inflammation (e.g. musculoskeletal trauma, post-dental extraction, post-episiotomy), osteoarthritis, and rheumatoid arthritis. It is official in British Pharmacopoeia[1]. The most commonly used techniques for the determination of DIF in pharmaceutical dosage form are derivative spectrophotometry[2], HPLC-UV detection by chemometric spectrophotometry[3], synchronous fluorescence spectrometry[4-5], HPLC-DAD[6], HPLC-fluorescence detection[7], TLC-densitometry[8], LC-DAD[9], LC-DAD-MS[10], differential-pulse polarography[11], differential-pulse and square-wave stripping voltametry[12], capillary electrophoresis with luminescence detection[13].

Lignocaine chemically is 2-(Diethylamino)-N-(2, 6-dimethylphenyl), 2-(Diethylamino)-2, 6-acetoxylidide. Lignocaine is an amide-type local anaesthetic commonly used in injectable dosage forms or designed for local application in mucous membranes. It is official in Indian Pharmacopoeia[14]. The most commonly used techniques for the determination of LIG in pharmaceutical dosage form are UV spectrophotometry[15], RP-HPLC[16], HPTLC[17], UPLC[18], LC-MS/MS[19], GC-FID[20], capillary electrophoresis with electrochemiluminescence detection[21], partial least squares multivariate calibration[22].

As per our knowledge, no derivative UV spectrophotometric method has been reported for simultaneous estimation of Diflunisal and Lignocaine from their formulation. Hence we have developed two derivative spectrophotometric methods for simultaneous estimation of these drugs from bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals and Reagents

Lignocaine was purchased from Balaji Drugs, Surat (Gujarat). Diflunisal was purchased from Dolphin Pharmacy Instruments Pvt. Ltd (Mumbai). The ointment for combination of Diflunisal and Lignocaine was prepared in the laboratory by using

chemicals such as liquid paraffin, carboxymethyl cellulose sodium and white petroleum jelly. AR grade methanol was used throughout the analysis.

Instrument

A double-beam UV-Visible Spectrophotometer (Jasco, Model V-630) was employed with a pair of 1cm quartz cells for all analytical work.

Selection of Common Solvent

For both drugs, methanol was used as a common solvent for developing spectral characteristics by assessing the solubility in various solvents.

Preparation of standard stock solution:

The standard stock solutions of Diflunisal and Lignocaine were prepared by dissolving 10 mg of each drug in 40 ml of methanol. The final volume was adjusted with methanol to get a 100µg/ml solution of each drug. To select the analytical wavelength, a standard solution of 20 µg/ml of each Diflunisal and Lignocaine was prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the UV range of 200-400 nm. The spectral data were processed to obtain each drug's first-order derivative spectrum, and the above process was repeated for the second-order derivative method.

Derivative Spectrophotometric Methods

Method A: First Order Derivative Method

Each pure drug's first-order derivative (D1) overlain spectra showed zero crossing points (ZCP). They assisted in their simultaneous estimation, as shown in **Figure 1.** The first order derivative wavelength considered for DIF was 264 nm, at which LIG shows zero absorbance. Similarly, the analysis of LIG was carried out at 224nm, at which the DIF showed zero absorbance. Calibration curves were plotted between absorbance observed at D1 for two drugs at selected wavelengths against the concentration range 3-39µg/ml and 4-40µg/ml for DIF and LIG, respectively.

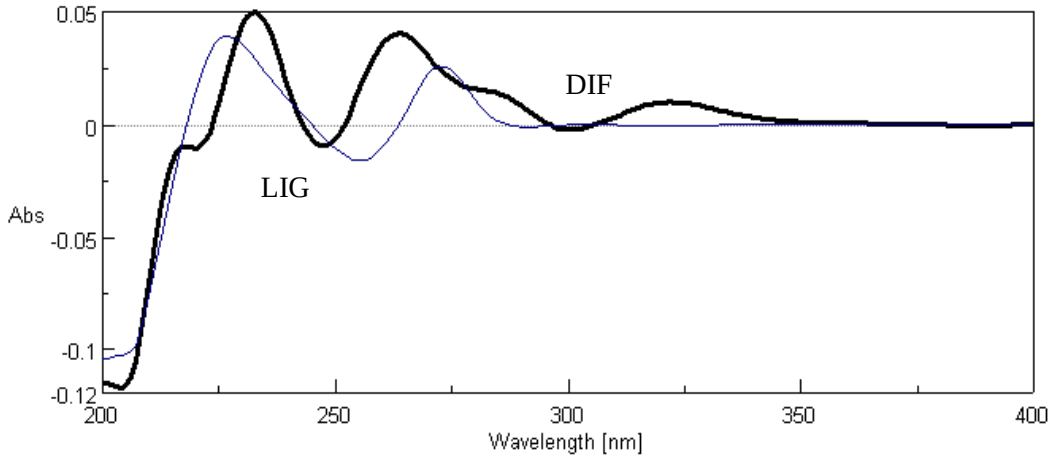

Figure1. First Order Derivative Overlain Spectra of Diflunisal and Lignocaine

Method 2: Second Order Derivative Method

The second order derivative (D2) overlain spectra of each pure drug were found to show zero crossing point (ZCP) and assisted in their simultaneous estimation, as shown in **Figure 2.** The second order derivative wavelength considered for DIF was 273nm, at which LIG shows zero absorbance. Similarly, the analysis of LIG was carried out at 232nm, at which DIF show zero absorbance. Calibration curves were plotted between absorbance observed at D2 for two drugs at selected wavelengths against the concentration range 3-30 µg/ml and 4-48 µg/ml for DIF and LIG, respectively.

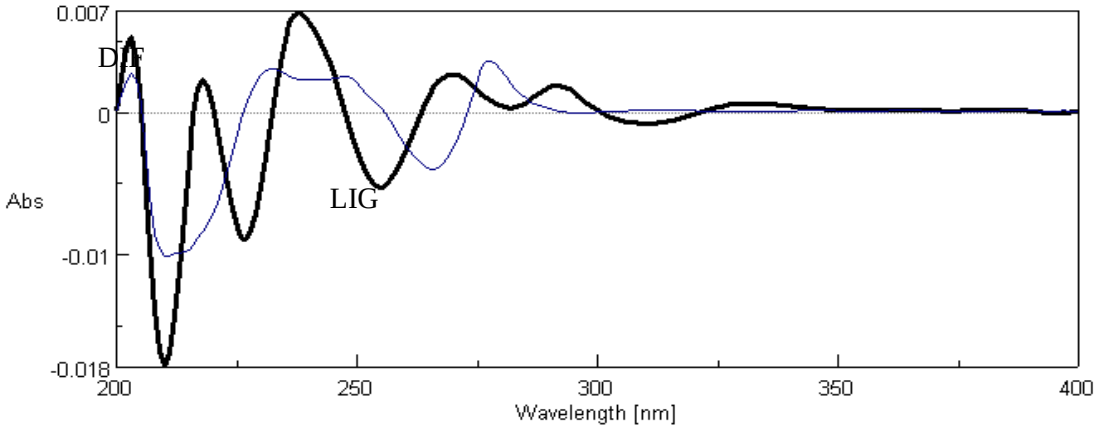

Figure2. Second Order Derivative Overlain Spectra of Diflunisal and Lignocaine

Analysis of Formulation

The oral ointment containing DIF and LIG was prepared and analysed as follows[23-27]:

0.5 gm of each DIF and LIG pure drugs were ground in a mortar, and then 0.5 ml of liquid paraffin was added and mixed well to make a suspension. Then about 18 gm of white petroleum jelly was gradually mixed in 5 gm of carboxymethyl cellulose sodium (CMC-Na). After that above suspension of drugs was added into base and mixed well, subsequently more base was added to make 25 gm of ointment. Afterwards 1 gm of this ointment was dissolved in 40 ml of methanol, sonicated for 10 minutes and diluted up to 200 ml with methanol. Then sample solution was filtered through Whatman filter paper (no. 41). After appropriate dilutions, the absorbance of sample solutions was recorded at corresponding wavelengths, and the results were recorded as shown in **Table 1.**

Table 1: Result of Formulation Analysis

Parameters	Method A		Method B	
	DIF	LIG	DIF	LIG
%Drug Content	99.05	100.37	99.55	96.90
SD*	0.013	0.02	0.041	0.015
%RSD	0.014	0.023	0.046	0.016

*Mean of three determinations

Validation

The methods were validated according to International Conference on Harmonization (ICH) Q2B guidelines for validation of analytical procedures to determine the linearity, precision and accuracy of each analyte[24]. Both precision and accuracy were determined with standard samples prepared in triplicates at different concentration levels covering the entire linearity range.

RESULTS AND DISCUSSION

Linearity

The linearity was determined at concentration range 3-39µg/ml and 4-40µg/ml for DIF and LIG, respectively for the first order derivative method. For the second order derivative method, linearity was determined at concentration range 3-30µg/ml and 4-48µg/ml for DIF and LIG, respectively.

Precision

Precision was determined by studying repeatability and intermediate precision. The experiment was repeated three times a day for intra-day and on three different days for inter-day precision. The results of the precision study are presented in **Table 2**. S.D. in intra-day and inter-day precision study for both methods was found to be not more than 2.0%, which indicates excellent repeatability and intermediate precision.

Table 2: Optical Characteristics and Validation Parameters

Parameters	DIF		LIG	
	Method A	Method B	Method A	Method B
Working Wavelength (nm)	264	273	224	232
Beer-Lambert's Law range (µg/ml)	3-39	3-30	4-40	4-48
Precision* i) Intraday precision (SD)	0.59	0.06	1.5	0.08
ii) Interday precision (SD)	0.15	0.064	0.17	0.10
LOD(µg/ml)*	0.3	0.4	0.5	0.4
LOQ(µg/ml)*	0.95	1.3	1.7	1.2
Regression Values				
Slope	0.045	0.062	0.045	0.036
Intercept	0.008	0.007	0.001	0.001
Regression Coefficient(R^2)	0.999	0.998	0.995	0.996

*Mean of Three determinations

Accuracy

Recovery studies by standard addition method assessed the validity and reliability of the proposed methods. The results are shown in **Table 3**. The SD for the mean of % recovery values was <2.0 for both methods.

Table 3: Result of Recovery Studies

Drug	Recovery Level	%Recovery ± SD*	
		Method A	Method B
DIF	50%	100.13±0.06	100.37±0.073
LIG		99.44±0.05	99.05±0.04
DIF	100%	99.62±0.03	99.55±0.04
LIG		101.07±0.02	99.90±0.05
DIF	150%	99.04±0.04	101.2±0.03
LIG		100.85±0.05	100.3±0.07

*Mean of three determinations

CONCLUSION

The proposed UV spectrophotometric derivative methods for simultaneous estimation of DIF and LIG are found to be accurate and precise. The results obtained were found to be within the acceptable limit. The proposed methods are simple, rapid and easy to perform, and these methods are applicable for simultaneous estimation of DIF and LIG in pure and pharmaceutical dosage form. The good validation criteria of the proposed methods allow their use in quality control laboratories.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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