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Development and validation of spectrofluorimetric method for determination of diflunisal and its impurity

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ABSTRACT

A new sensitive, simple, rapid, accurate and precise spectrofluorimetric method for determination of diflunisal and its impurity is developed. Determination of diflunisal is based on first derivative spectrofluorimetric method, while its impurity can be determined by zero order spectrofluorimetric method. Diflunisal was measured at zero-crossing wavelength 394nm (zero crossing point with its impurity) which was selected for quantification of diflunisal. The impurity was measured directly at 334 nm, using 0.05 M phosphate buffer (pH = 9) as solvent. The analytical signal resulting from first derivative and zero order spectra were measured for diflunisal and its impurity, respectively. Linearity was over the range of 0.1-0.9 µg/mL for both with detection limit of 0.02 and 0.03 µg/mL and quantitation limit of 0.07 and 0.09 µg/mL for diflunisal and its impurity, respectively. The proposed method was validated as per ICH guidelines.The accuracy was checked by applying the proposed method for the determination of the drug and its impurity, the mean percentage recoveries were found to be 99.61±0.911 and 100.41±1.373 for diflunisal and its impurity, respectively. RSD values for repeatability testing were 0.268 and 0.569 and for intermediate precision were 0.224 and 0.259 for diflunisal and its impurity, respectively. The proposed method was effectively applied to analysis of studied drug in its tablet formulation. The results obtained by it were statistically compared with the reported method revealing high accuracy and good precision.

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1. Introduction

Diflunisal (DIF) is a salicylic acid derivative with analgesic and anti-inflammatory activity designated as 2', 4'-difluoro-4-hydroxybiphenyl-3-carboxylic acid. DIF acts by inhibiting the production of prostaglandin's hormones which are involved in inflammation and pain. DIF is an official drug in the British Pharmacopoeia (BP) [1] (Figure 1a). According to BP, biphenyl-4-ol is the major impurity of DIF (Figure 1b) [1].

Different methods for determination of DIF were reported. The methods include liquid chromatography (LC) [2-6], TLC-densitometry [7], gas chromatography [8], spectrophotometry [9,10] and spectrofluorimetry [11-13]. Reviewing the literature in hand, there was no method found for the determination of DIF in presence of its impurity.

Spectrofluorimetric method proved to be more selective than normal UV-spectroscopy due to quantitation of substance at characteristic excitation and emission wavelengths. Derivative spectrofluorimetry provides a greater selectivity and spectral discrimination than common spectrofluorimetry [14,15]. It is a powerful approach for resolution of one analyte whose peak is hidden by a large overlapping peak of another analyte in multi-component analysis. The aim of the presented work was to develop simple, economic, sensitive and rapid method for the simultaneous quantitative determination of both DIF and its impurity (BPL) in bulk powder and in tablet dosage form by first derivative and zero order spectrofluorimetry, respectively, based on their native fluorescence.

2. Experimental

2.1. Apparatus

Cary Eclipse fluorescence spectrophotometric (USA) connected to IBM-PC computer and HP Laser Jet 1100 series printer. The emission of all samples was recorded against a solvent blank in 1 cm quartz cuvettes and scanning at the following parameters: Band width = 1.5 nm, Scan speed = 1200 nm/min, Data Interval = normal (1 nm), Smoothing = high.

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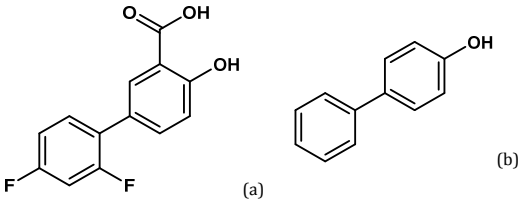

Figure 1. Chemical structure of diflunisal (a) and biphenyl-4-ol (b).

2.2. Chemicals and reagents

All chemicals and reagents used were of analytical grade. Diflunisal and BPL were kindly provided from Rameda Pharmaceutical Company, Egypt. Its purity was found to be 98.96% based on the company analysis certificate. Sodium dihydrogen orthophosphate, methanol, sodium hydroxide and acetonitrile were purchased from Sigma Aldrich (Germany). APO-Diflunisal® 500 tablets manufactured by Apotex Pharmaceutical Company (Batch number: KE8472) was purchased from Canada.

2.3. Procedure

2.3.1. Stock standard solutions of DIF and BPL (1 mg/mL)

DIF (100 mg) and BPL (100 mg) were accurately weighed and transferred into two separate 100 mL volumetric flasks and dissolved in 5mL 0.05M phosphate buffer, separately. The flasks were shaken and volume was completed with 0.05M phosphate buffer (pH = 9).

2.3.2. Working standard solutions of DIF and BPL

From the two standard stock solutions, 10 mL was transferred from each to two separate 100 mL volumetric flasks; the volume was completed to 100 mL with 0.05 M phosphate buffer (pH = 9) to obtain a standard working solution of DIF and BPL having final concentration of 100 µg/mL of each.

2.3.3. Construction of calibration curve

Aliquots from working standard solutions equivalent to 1-9 µg of DIF and BPL were transferred into two sets of 10 mL volumetric flasks. The volumes were completed with the buffer to obtain a series of concentrations of 0.1-0.9 µg/mL. The zero order spectra (D_0) of each dilution of the impurity were recorded, and then peak amplitudes were measured at emission wavelength of 334 nm after excitation at 254 nm and plotted against corresponding concentrations. First derivative spectrofluorimetry was computed for DIF and the peak amplitudes were measured at 394 nm and plotted against the corresponding concentrations. The concentrations of the drug and its impurity were calculated each from the corresponding calibration curve equation.

2.3.4. Pharmaceutical dosage form

Ten tablets of marketed formulation APO-Diflunisal®; each containing 500 mg of DIF was taken and accurately weighed. Average weight was determined and tablets were crushed into fine powder. An accurately weighed quantity of powder equivalent to 100 mg DIF was transferred to volumetric flask of 100mL capacity, 0.05 M phoshate buffer was added, the flask was sonicated for 15 min. The volume was completed with 0.05 M phosphate buffer. The solution was filtered through Whatmann filter paper (No. 41). The solution was filtered and diluted to obtain 100 µg/mL working solution. Linearity procedure was followed and the drug concentration was calculated from the corresponding regression equation. The validity of the method was assessed through applying the standard addition technique by mixing different concentrations of the standard drug to a fixed amount of its formulation. The concentrations of standard added were calculated from the corresponding regression equations.

3. Results and discussion

Derivative spectrofluorimetry offers greater selectivity than normal spectrophotometry for simultaneous determination of two or more compounds without previous chemical separation [16]. The presented spectrofluorimetric method was applied for quantitative determination of DIF and BPL in pure form and tablet dosage form. DIF exhibits native fluorescence at emission wavelength of 426 nm after excitation at 257 nm (Figure 2), while BPL exhibits fluorescence at emission wavelength of 334 nm after excitation at 254 nm in 0.05 M phosphate buffer (Figure 3). The zero order emission spectra of DIF with BPL revealed some spectral overlap as shown in Figure 4. Zero order spectra showed that the impurity could be determined by measuring the peak amplitude at 334 nm without any interference from DIF. The first derivative spectra of DIF and BPL showed that DIF could be determined by measuring the peak amplitude at 394 nm for DIF without any interference from BPL as shown in Figure 5. Linear relationships were found in the range 0.1-0.9 µg/mL for both DIF and BPL by first derivative spectroflourimetry and zero order spectrofluorimetric method, respectively.

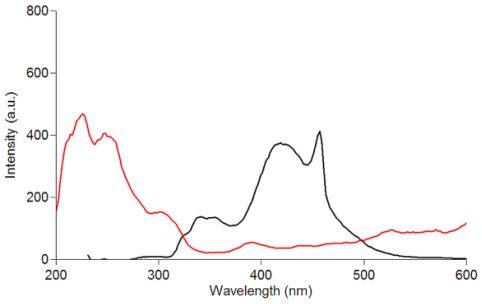

Figure 2. Excitation and emission spectra of diflunisal (0.4 µg/mL) in 0.05 M phosphate buffer.

The regression equation for DIF and BPL were computed and found to be:

At $\lambda_{394\ nm}$; y = 24.6549x +0.0364 r = 0.9997 for DIF (1)

At $\lambda_{334\ nm}$; y = 571.7890x + 96.5248 r = 0.9998 for imp. (2)

where y is the peak intensity, x is the concentration in µg/mL and r is the correlation coefficient.

Table 1. Regression and analytical parameters of the proposed spectroflourimetry method for determination of diflunisal and its impurity.

Parameters	DIF by first derivative	Impurity by zero order
Linearity		
Calibration range, µg/mL	0.1-0.9	0.1-0.9
Slope	24.6549	571.7890
Intercept	0.0364	96.5248
Correlation coefficient (r)	0.9997	0.9998
Accuracy (mean±%RSD)	99.61±0.911	100.41±1.375
Precision (%RSD)		
Repeatability [a]	0.268	0.569
Intermediate precision [b]	0.224	0.259
LOD, µg/mL	0.022	0.031
LOQ, µg/mL	0.067	0.094

[a] The intra-day (n = 9) average of three different concentrations (0.1, 0.2 and 0.5 µg/mL) repeated three times within 1 day.
[b] The inter-day (n = 9) average of three different concentrations (0.1, 0.2 and 0.5 µg/mL) repeated three times in 3 successive days.

Table 2. Statistical comparison of the results obtained by applying the proposed spectroflourimetry method and the official titrimetric method for determination of DIF in pure form.

Parameters	Spectroflourimetric method	Official method [a]
Mean	100.01	99.76
S.D.	0.762	0.761
n	10	6
Student´s *t*-test	0.301 (2.160)	-
F-test	1.433 (5.990)	-

[a] BP acid base titration method [1], The values between parentheses represent the corresponding tabulated values of *t* and *F* at *p* = 0.05.

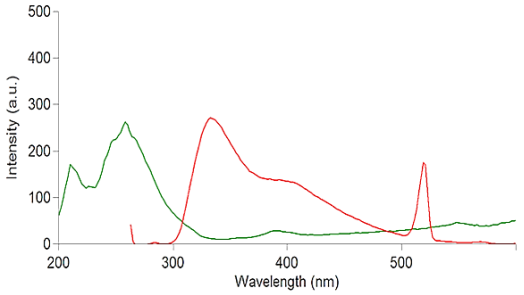

Figure 3. Excitation and emission spectra of biphenyl-4-ol (0.4 µg/mL) in 0.05 M phosphate buffer.

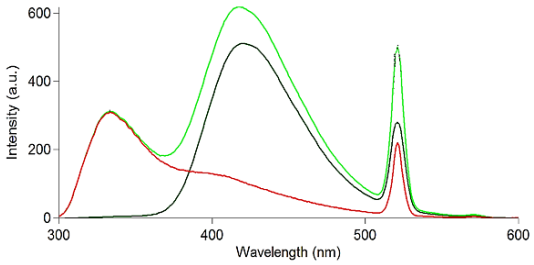

Figure 4. Zero order emission spectra of 0.4 µg/mL diflunisal (Black), 0.4 µg/mL of the impurity (Red) and mixture of 0.4 µg/mL diflunisal and 0.4 µg/mL of BPL (Green) in 0.05 M phosphate buffer (pH = 9).

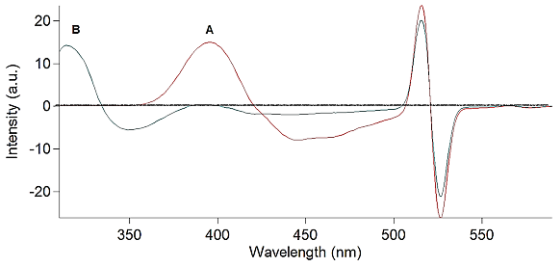

Figure 5. First derivative emission spectra of 0.9 µg/mL diflunisal (A) and 0.9 µg/mL of BPL (B) in 0.05 M potassium phosphate buffer (pH = 9).

The LOD and LOQ for DIF were found to be 0.022 and 0.067 µg/mL, respectively, and 0.031 and 0.094 µg/mL for BPL respectively, Table 1.

The proposed method was applied for the determination of pure sample of DIF and BPL over the concentration range 0.1-0.9 µg/mL in order to determine accuracy of the proposed method. The results obtained are summarized in Table 1.

The results obtained by applying the proposed first derivative spectrofluorimetric method for the analysis of the studied compound in pure drug sample and dosage form were statistically compared to the official BP method [1]. The values of the calculated *t* and *F* are less than the tabulated ones which reveal that there is no significant difference with respect to accuracy and precision as shown in Table 2. The method was used for determination of the dosage form and accuracy was further assessed by application of the standard addition technique, the results are summarized in Table 3. The specificity of the proposed first derivative spectrofluorimetric method for DIF and zero order method for the impurity were emphasized by analyzing laboratory prepared mixtures containing different percentage of each drug with BPL. The method was valid in the presence of up to 100% of BPL for DIF as shown in Table 4.

3.1. Optimization of method

Different solvents and systems were tested in order to find the best conditions like solubility, fluorescence intensity, stability and spectral discrimination (clear separation) of the drug and BPL.

3.1.1. Excitation and emission spectra

Scanning of excitation and emission spectra was done to obtain the optimum wavelengths for the resolution of the mixture. DIF can be determined at 394 nm using first derivative fluorimetric method and its impurity can be determined at 334 using zero order spectrofluorimetric method.

3.1.2. Effect of solvents

Different solvents were used to measure the maximum fluorescence intensity as acetonitrile, water, 0.1 M sodium hydroxide, methanol and 0.05 M phosphate buffer.

Table 3. Determination of diflunisal in Apo-diflunisal® tablets by the proposed spectroflourimetry method and results of standard addition.

Pharmaceutical Formulation	Taken (µg/mL)	%Found±RSD [a]	Standard addition technique	
			Pure added (µg/mL)	Recovery % [a]
Apodiflunisal® tablets	0.4	101.00±0.762	0.3	99.00
			0.4	101.25
			0.5	98.60
			Mean±%RSD	99.62%±1.429

[a] Average of 3 determinations.

Table 4. Specificity of the proposed FDF method and zero order method for the determination DIF and BPL in laboratory prepared mixtures

Mixture no.	DIF			Impurity		
	Claimed taken (µg/mL)	Found (µg/mL)	Recovery % [a]	Claimed taken (µg/mL)	Found (µg/mL)	Recovery % [a]
1	0.1	0.099	99.00	0.9	0.902	100.22
2	0.2	0.195	99.50	0.7	0.710	101.43
3	0.4	0.407	101.94	0.4	0.395	98.75
4	0.7	0.701	100.14	0.3	0.305	101.67
5	0.9	0.891	99.00	0.1	0.991	99.00
Mean±S.D.			99.72±1.38			100.41±1.61

[a] Average of 3 determinations.

The maximum fluorescence intensity was observed in 0.05 M phosphate buffer, pH = 9.

3.1.3. Effect of pH

The fluorescence intensity of diflunisal solutions was measured over a pH range from 4.0 to 13.5, by using 0.05 M phosphate buffer and adjusting the pH with hydrochloric acid and sodium hydroxide solutions. The maximum fluorescence intensity was observed at pH = 9.

3.2. Method validation

Validation was done according to International Conference on Harmonization (ICH) guidelines [17].

3.2.1. Linearity

Under optimum experimental conditions, DIF and BPL were determined in triplicates in the range of 0.1-0.9 µg/mL for both for first derivative spectrofluorimetric method and zero order spectrofluorimetric method, respectively. The linearity of the calibration graphs were validated and the regression equations were then computed, Table 1.

3.2.2. *Accuracy*

Pure samples of DIF and its impurity were analyzed by the proposed method; the mean percentage recoveries were then calculated. The accuracy was further assessed by standard addition technique. It was done by spiking the pre-analyzed DIF sample 0.4µg/mL with an extra 0.3, 0.4, and 0.5 of standard DIF. The experiment was conducted in triplicate. The percentage recovery and percentage relative standard deviation (% RSD) were calculated for each concentration, Table 3.

3.2.3. *Precision*

3.2.3.1. Repeatability (intra-assay precision)

It was evaluated by assaying freshly prepared solutions in triplicateon the same day at concentrations of (0.1, 0.2 and 0.5 µg/mL) for DIF and (0.1, 0.3 and 0.5 µg/mL) for its impurity to determine intraday variation.

3.2.3.2. Intermediate precision

The previous procedures were repeated for the same concentrations three times on three consecutive days to determine precision (inter-day). The results and relative standard deviations (%RSD) were calculated, Table 1.

3.2.4. Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components. Synthetic mixtures of drug and its impurity were prepared. Aliquots equivalent to 9-1 µg/mL of DIF were separately transferred from their working standard solutions into 10 mL volumetric flask. To the previous solutions aliquots equivalent to 1-9 µg/mL of BPL were added from their working standard solutions and the volume was completed to the mark with the buffer. The concentrations of the intact drug and its impurity were calculated from its corresponding regression equations, Table 4.

3.2.5. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is the lowest concentration of an analyte in a sample that can be detected and calculated by the following formula LOD = 3.3×(SD/S) where SD is the standard deviation and S is the slope. LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions of the method LOD = 10×(SD/S).

4. Conclusion

From the data obtained, it is noted that the proposed method is accurate, precise, and specific over defined range and could be used for purity testing, quality control and routine analysis of DIF in pure and in dosage forms. The advantages of the proposed method are low cost, rapidity, sensitivity and environmental protection. It is suitable for quality control laboratories where economy and time are essential. The spectrofluorimetric method was applicable for assay and purity testing of DIF in bulk and pharmaceutical formulations without interference of additives in the pharmaceutical preparation. It also can determine the impurity of DIF in trace concentrations, so it can be applied to detect very low extent of impurity of DIF and no previously published method approached that.

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References

[1]. The British Pharmacopoeia. Her Majesty's Stationery Office, London, **2010**, pp. 2606-2610, 2615.
[2]. Maher, H. M. *J. Liq. Chrom. Tech.* **2014**, *37*, 634-652.

[3]. Shaalan, R. A.; Belal, T. S. *Sci. Pharm*. **2013**, *81*, 713-731.
[4]. Wahbi, A.; Mabrouk, M. M.; Moneeb, M. S. *Pak. J. Pharm. Sci.* **2009**, *22*, 8-17.
[5]. El-Saharty, Y.; Refaat, M.; El-Khateeb, S. *Bull. Fac. Pharm. Cairo Univ.* **2002**, *40*, 47-64.
[6]. Streete, P. *Biomed. Sci. Appl.* **1989**, *495*, 179-193.
[7]. Bebawy, L; Elkousy, N. *Pharm. Biomed. Anal.* **1999**, *20*, 663-670.
[8]. Haj, B. M; El-Arini, A. M.; Hassan, M. H.; Khadem, R. K.; Marzouq, M. S. *Foren. Sci. Inter.* **1999**, *105*, 141-53.
[9]. Srinivas, B; Yadagiriswamy, P.; Venkateswarlu, G. *Inter. J. Pharm. Sci. Res.* **2015**, *6*, 1002-1010.
[10]. Sayin, F.; Kir, S. *FABAD J. Pharm. Sci.* **2004**, *29*, 121-126.
[11]. Murillo-Pulgarin, J. A; Alanon-Molina, A. *Appl. Spectrosc.* **2010**, *64*, 949-55.
[12]. Ioannou, P. C.; Lianidou, E. S.; Konstantianos, D. G. *Anal. Chim. Acta* **1995**, *300*, 237-241.
[13]. Pulgarin, M.; Molina, A.; Lopez, F. P. *Anal. Chim. Acta* **2007**, *583*, 55-62.
[14]. Neeli, S. A.; Anuganti, H.; Sathesh, R.; Subrahmanyam, V. S. *Dhaka Univ. J. Pharm. Sci*. **2013**, *12(1)*, 35-40.
[15]. Murillo, J.; Garcia, L. *Anal. Lett*. **1996**, *29*, 423-438.
[16]. Dress, J. C.; Wu, A. H. B. Analytical Techniques, Wolters Kluwer Vitalstream, 2008, Chapter 5, pp. 140.
[17]. ICH, Q. R., Validation of Analytical Procedures: Text and Methodology, ICH Harmonized Tripartite Guideline, 2005.