



## Sensitive, Direct and Rapid Methods for Determination of Flurbiprofen through Ion Associate Complexes and First Derivative Spectrophotometry

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### ABSTRACT

Two simple, accurate and sensitive methods for determination of flurbiprofen (FLUR) in pure and dosage forms had been developed and validated. The first method is based on the formation of ion-associate complexes between the drug and arsenazo I (ARS I) or Bromophenol blue (BPB) to give colored products maximally absorbed at 556 and 635 nm with the two reagents, respectively. The optimum conditions that favor the formation of the ion pair complexes were extensively studied and optimized. Beer's law was obeyed up to 90.20 and 100.85  $\mu\text{g mL}^{-1}$  while Ringboom concentration ranges are 1.89 - 89.20 and 2.00 - 96.60  $\mu\text{g mL}^{-1}$  for the two reagents, respectively. In the second method, first derivative (<sup>1</sup>DD) spectrophotometry was used for the determination where it was found that FLUR exhibits maximum amplitude at 259 nm. At this wavelength, linear relation between concentration and absorbance was obtained with high correlation coefficients. The methods were validated, in terms of accuracy and precision and the results are satisfactory. The proposed methods were successfully applied for determination of FLUR in pure and pharmaceutical dosage form without interference from the common excipients. The results obtained by the proposed methods were comparable with those obtained by reference methods.

**Keywords:** Flurbiprofen; Spectrophotometric determination; Ion pair complexes; First derivative spectroscopy

### INTRODUCTION

Flurbiprofen is a propionic acid derivative of non-steroidal anti-inflammatory drugs (NSAIDs). It is used in muscle skeletal and joint disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis, in soft-tissue disorders such as sprains and strains. It is also used as lozenges in the symptomatic relief of sore throat. [1]. Throughout the literature survey, a variety of techniques was found to be used for micro determination of flurbiprofen either in pure form or in pharmaceutical formulation. The most famous of these techniques are spectrophotometry [2-6], HPLC [7-14] and gas chromatography [15]. These techniques require sophisticated instruments and expensive reagents, besides being time consuming. On the other hand, the formation of ion-associate complexes between organic dyes and drugs is one of the techniques available for determination of pharmaceutical compounds [16-20]. Also derivative spectrophotometry offers greater selectivity than do normal spectrophotometry in the simultaneous determination of two or more compounds without previous chemical separation. The principles and advantages of this technique had been described by O'Haver and Green [21].

The work described herein developed two spectrophotometric methods that can be used in laboratories without modern and expensive instrumentation such as that required for gas chromatography or HPLC. The first method involved the formation of ion associate complexes of flurbiprofen with arsenazo I (ARSI) or Bromophenol blue (BPB) as chromophoric reagents, while the second method used the first derivative spectroscopy (<sup>1</sup>D) at 259 nm for determination of the drug. The proposed procedures were applied successfully for determination of flurbiprofen in pure and in pharmaceutical preparation with good accuracy and precision.

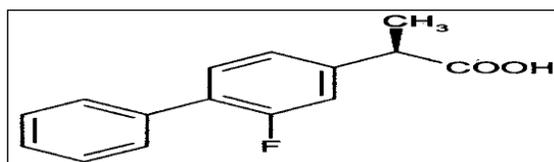
## EXPERIMENTAL SECTION

### Instrumentation

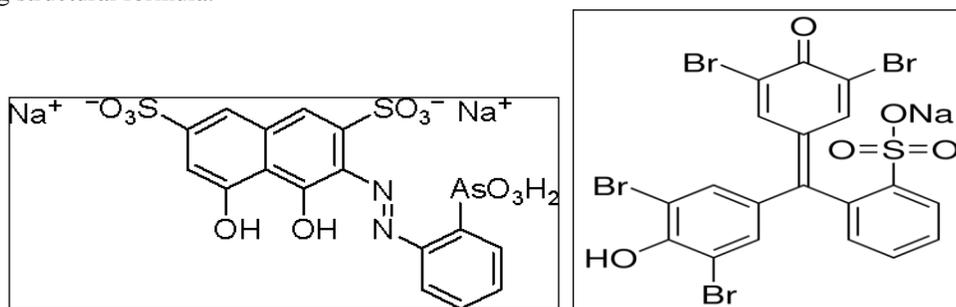
All absorption measurements were made by using Shimadzu UV1601 double beam UV-VIS spectrophotometer loaded with Shimadzu UV-Prob Version 1.10 software and interfaced to Pentium-4 computer and Canon-810 laser printer to record the spectra and perform subsequent calculations of their derivatives (NODCR). The spectrophotometric measurements were made at wavelength range 200-600 nm using 10 mm quartz matched cells. The first derivative spectra were derived in the wavelength range (220-350) nm using  $\Delta\lambda = 8$  nm and scaling factor = 10. An Orion research model 601A/digital Ionalyzer pH meter was used to check the pH of the universal buffer solution.

### Materials and reagents

All the chemicals used were of analytical reagent grade and the solvents were of spectroscopic grade. Flurbiprofen was obtained from El-Kahira Company, Shoupra Elkhyma City, Egypt. The purity of the samples was found to be 99.8% on the dried bases according to the United States Pharmacopoeia (USP) method [22] and was used as received. Its IUPAC name is 2-Fluoro- $\alpha$ -methyl[1,1-biphenyl]-4-acetic acid ;2-(2-fluoro-4-bi- phenyl) propionic acid;3-fluoro-4-phenylhydatropic acid; ( $C_{15}H_{13}FO_2$ , Molecular Weight: 244.261 g/mol). It has the following structural formula:



The analytical reagents used in the present work are arsenazo I (ARZ I) and Bromophenol blue (BPB). They have the following structural formula:



Arsenazo I (ARZ I) Bromophenol blue (BPB)

Stock solutions of  $1.0 \times 10^{-3}$  M of the reagents were prepared by dissolving the accurate weight of each reagent initially in 25 mL ethanol followed by dilution in 100 mL measuring flask by ethanol to the mark. Universal buffer solutions of different pH values (2.04-12.06) were prepared as recommended earlier [23].

### Pharmaceutical dosage forms

Froben gel (Kahira pharma – Egypt; under license of Abbott international lab –Pakistan) labeled to contain 5gm flurbiprofen in 100gm gel.

### Drug standard solutions

A stock solutions containing (0.1 mg/mL) of flurbiprofen was prepared by dissolving 10 mg of the pure sample in the least amount of ethanol and completed to 100 mL with the same solvent. Working standard solutions (10.0  $\mu$ g/mL) were prepared by subsequent dilution. For molar ratio and continuous variation methods  $10^{-3}$  M solution of the drug was prepared by dissolving 0.0244 g in least amount of ethanol then complete to 100 mL in measuring flask.

### Construction of the calibration graphs

#### Method (I)

In a 10 mL volumetric flask, a series of solutions containing 2.0 mL of  $1 \times 10^{-3}$  M reagent solution followed by 3.0 mL universal buffer solution at the optimum pH and different concentrations of the drug (in  $\mu\text{g/mL}$ ) were mixed well. The mixture was diluted to volume with ethanol and the solution was allowed to stand for 5.0 min at room temperature ( $25 \pm 2^\circ\text{C}$ ). The absorbance was measured at the recommended wavelength using a reagent blank similarly prepared without drug. By plotting the concentration of the drug (in  $\mu\text{g/mL}$ ) against the absorbance, straight line is obtained (up to certain limit) after which deviation from Beer's law was observed.

#### Method (II)

Different aliquots from FLUR standard stock solution (0.1- 5.0 mL) was transferred into series of 10 ml volumetric flasks to obtain solutions over concentration rang of (1-50  $\mu\text{g/mL}$ ). The first derivative spectra were recorded and the amplitude measured at 259 nm. The derivative amplitudes were then plotted against the final concentrations to get the calibration graphs. Alternatively, the corresponding regression equation was derived.

### Preparation of pharmaceutical dosage form samples

An accurately weighed quantity of gel equivalent to 5 mg of FLUR was transferred into 100 mL calibrated flask, 60 mL of ethanol was added and shaken for 10 minutes. The volume was then made to the mark, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and the filtrate was diluted appropriately to get 50  $\mu\text{g/mL}$  of FLUR for assay by the recommended method

### Stoichiometric Ratio

The stoichiometry of the ion-associate complexes was studied using the mole ratio and continuous variation methods [24, 25].

## RESULTS AND DISCUSSION

### Method (I): Determination of flurbiprofen through ion associate complex

Preliminary investigations revealed that flurbiprofen reacts directly with each of the reagents (ARS I) and (BPB) to produce soluble ion-associate complexes exhibiting absorption maxima at 556 and 635 nm, respectively. The presence of the ion-associate complexes was supported by the bathochromic shift observed from 545 and 605 nm for the reagents to 556 and 635 nm for the complexes, respectively. Representative example for FLUR-ARZ I ion pair is shown in Figure 1.

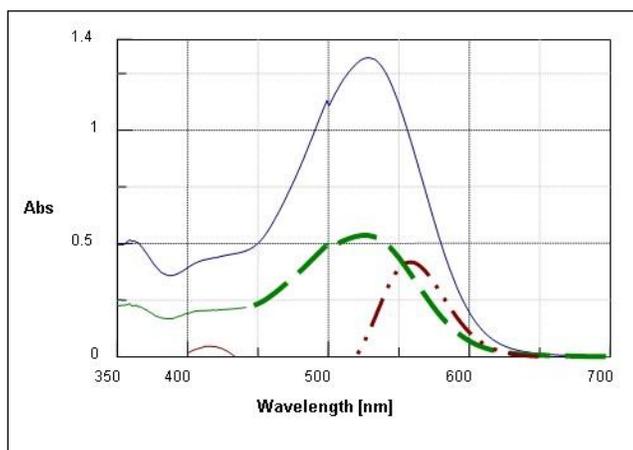


Figure 1: Difference curves of FLUR – ARZ I complex, A; (—): ARZ I vs buffer, B; (---) ARZ I + FLUR vs buffer and C; (-.-) Mixture B vs A

The optimum conditions favoring the formation of colored complexes were attained by studying the different variables affecting the reaction as follows.

**Effect of pH**

Universal buffer solutions of pH range 2.04 -12.06 was found to give the best results rather than other types of buffer solutions (acetate or Theil buffer). The optimum pH values giving maximum absorbance for FLUR with ARZ I and BPB are 10.50 and 9.23, respectively. Moreover, the optimum volume of buffer solution was examined and found to be 3.0 ml in a total volume of 10 ml.

**Effect of time and temperature**

Sample solutions containing flurbiprofen and the blank were treated identically with the reagent and buffer for different times and at different temperatures. The results obtained indicated that the ion-associate complexes were formed instantaneously at room temperature ( $25 \pm 2^\circ\text{C}$ ). The absorption spectra and color intensities were not altered by varying the temperature up to  $40^\circ\text{C}$ , above which the absorbance decreased by 10 % for every increase of  $5.0^\circ\text{C}$ . The absorbance remained stable for about 15 hrs then it began to decrease slowly.

**Effect of reagent concentration**

When various concentrations of (ARSI) and (BPB) were added to a fixed concentration of flurbiprofen at  $10\mu\text{g/mL}$ , 2.0 mL of  $10^{-3}$  M reagent solution was found to be enough to develop the color to full intensity.

**Stoichiometric ratio**

The stoichiometry of the ion-associate complexes was investigated at the optimum pH values by applying the mole ratio and continuous variation methods. The results indicated the formation of a 1:1 ion-associate complex. The logarithmic stability constants were calculated from the spectral data of both methods [26, 27] and are shown in Table (1).

**Method (II): Determination of flurbiprofen using first derivative spectrophotometric method:**

The first derivative studies of FLUR showed that it can be determined by measuring the amplitude at 259 nm (Figure 2).

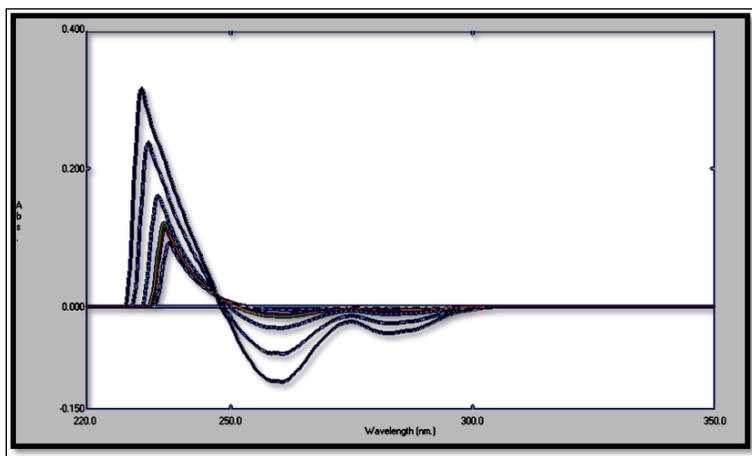


Figure 2: The first derivative absorption spectra of FLUR at different concentrations ( $1\text{DD}_{259}$ ).

**METHOD VALIDATION****Linearity and Range**

Under the optimum experimental conditions described, standard calibration curves for flurbiprofen with ARS I and BPB were constructed by plotting absorbance versus concentration. Conformity to Beer's law was evident in the concentration range of the final dilution. The linear regression equation for the method is also shown in the table. For more accurate results, Ringboom [28] optimum concentration ranges were obtained by plotting transmittance percent versus the logarithmic value of the concentration in  $[\mu\text{g/mL}]$ . Values for mean molar absorptivity and Sandell sensitivity [29] were also calculated and are collected in Table 1.

The calibration graphs for determination of (FLUR) by the first derivative spectrophotometric method was constructed by plotting the derivative amplitudes *versus* the concentrations as shown in Figures 2. The graphs were found to be rectilinear over the concentration ranges cited in Table 1.

The methods were validated by the high close to one correlation coefficient in each case for FLUR and the value of intercept on ordinate which is close to zero.

### Accuracy

Different concentrations within the linearity range of (FLUR) were analyzed in bulk powder also by adding a previously analyzed test solution with additional drug standard solution to check the accuracy of the results. The recovered concentrations  $\pm$  SD were calculated using the regression equation.

### Precision

The intraday (repeatability) and inter-day (intermediate) precisions were assessed using three concentrations and three replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed methods. The data are cited in table (2).

**Table 1: Spectrophotometric cumulative data for (FLUR) using (ARS I), (BPB) and (<sup>1</sup>DD<sub>259</sub>) methods**

Parameter	ARS I	BPB	( <sup>1</sup> DD <sub>259</sub> )
pH	10.5	9.23	-----
Wavelength ( $\lambda_{\max}$ ) (nm)	556	635	259
Beer's limits ( $\mu\text{g/mL}$ )	1.05 - 90.20	1.20 - 100.85	2.0 - 30.0
Ringbom range ( $\mu\text{g/mL}$ )	1.89 - 89.0	2.0 - 96.60	
Molar absorptivity ( $\times 10^4$ ) ( $\text{l mol}^{-1} \text{cm}^{-1}$ )	3.44	5.25	
Sandell sensitivity ( $\mu\text{g cm}^{-2}$ )	11.44	9.25	
Intercept (b)*	-0.014	-0.004	0.017
Slope (m)*	0.106	0.265	0.032
Correlation coefficient (r)	0.9992	0.9989	0.999
RSD%	0.233	0.308	0.77
LOD	0.28	0.113	0.937
LOQ	1.91	1.38	3.13
Stability constant**	5.33	5.85	
RE %	0.411	0.308	

\* Linear regression equation:  $Y = mX + b$

\*\* Mean value of Molar ratio and continuous variation methods.

### Specificity

The specificity of each of the proposed method was investigated by observing any interference encountered from common dosage form excipients. It was shown that these compounds did not interfere with the results of the proposed methods.

### LOD & LOQ

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) for the two proposed methods were calculated as  $3\sigma/S$  and  $10\sigma/S$ , respectively, where S is the slope of the calibration curves and  $\sigma$  is the standard deviation of the response and displayed in Table (1).

### Application

The content of FLUR in pharmaceutical formulate (froben® gel) was determined by applying the first derivative method using the standard addition technique. The mean percentage recoveries and standard deviation of the labeled and added amount for each drug are shown in Table (3). The data cited in Table (3) show that the mean percentage recovery of labeled drug is  $100.85 \pm 0.76$ , while that for the added authentic is  $100.22 \pm 1.4$  for FLUR at 259 nm. The obtained results show low standard deviation values which indicate that the methods is applicable for determination of drug in its pharmaceutical dosage forms without interference from additives.

**Table 2: Intra- and inter-day validation for the determination of FLUR using the proposed methods**

	Parameters	ARS I	BPB	( <sup>1</sup> DD <sub>259</sub> )
Intra-day	% Recovery	99.55	99.68	100.09
		99.96	101.36	101.21
		100.34	99.56	99.23
	Mean	99.95	100.2	100.18
	S.D.	0.39	1	0.99
	%RSD	0.39	1	0.99
	%Error	0.16	0.41	0.4
Inter-day	1 <sup>st</sup> day	100.1	102.06	100.31
	2 <sup>nd</sup> day	100.9	101.59	98.99
	3 <sup>rd</sup> day	101.98	100.66	100.11
	Mean	100.99	101.43	99.8
	S.D.	0.94	0.71	0.71
	%RSD	0.94	0.7	0.71
	%Error	0.38	0.29	0.29

The results of the proposed methods were compared with those obtained by the reference method [22] using point hypothesis test. The student t- and F- values (Table 4) at 95% confidence level did not exceed the tabulated t- and F-value, confirming no significant difference between the performance of the proposed methods and the reference methods.

**Table 3: Determination of FLUR in Froben gel using the first derivative spectrophotometric method (<sup>1</sup>D<sub>259</sub>)**

Recovery%		Found; (ug/mL)		<sup>1</sup> D <sub>259</sub>		Taken; (µg/mL)	
Added	GEL	Added	GEL	Total	GEL	Added	GEL
98.41	100.69	4.92	10.07	0.499	0.341	5	10
101.58	101.93	10.16	10.19	0.671	0.345	10	10
100.46	101	20.09	10.1	0.987	0.342	20	10
101.56	99.81	15.23	4.99	0.667	0.178	15	5
99.1	100.81	4.96	20.16	0.824	0.665	5	20
100.22	100.85						Mean
1.4	0.76						S.D

**Table 4: Statistical analysis for determination of FLUR using ARS I, BPB and (<sup>1</sup>D<sub>259</sub>) methods compared with the reference method [22]**

Formulation	Proposed methods					Reference method	
		Recovery %	RSD %	t-value	F-value	Recovery %	RSD %
Froben gel	ARS I	99.94	0.36	1.11	1.31	100.21	0.36
	BPB	99.95	0.55	0.9	2.34		
	( <sup>1</sup> DD <sub>259</sub> )	100.85	0.76	3.01	4.57		

Theoretical t-value ( $v = 8$ ) and F-value ( $v = 4.4$ ) at 95% confidence level are 2.306 and 6.39, respectively.

## CONCLUSIONS

Two spectrophotometric methods for the determination of flurbiprofen were proposed. The first method depends on the formation of highly colored ion pair complexes with two chromophoric reagents; arsinazo I and Bromophenol blue which absorb maximally at 556 and 635 nm, respectively. The second method utilizes the first derivative spectroscopy for the determination. The proposed methods are simple, less time consuming, and sensitive. They were advantageous over other reported visible spectrophotometric methods with respect to their higher sensitivity and selectivity. No interference from associated excipients, additives, and degradation products was observed. The proposed methods can be used for routine analysis and quality control laboratories for the determination of flurbiprofen in raw materials and in pharmaceutical formulation.

## CONCLUSION

In the present research, synthesized some of tetrazole derivatives may be the derivatives have biological activity towards the bacteria (positive or negative).

## ACKNOWLEDGEMENT

The authors are grateful to Prof. Dr. M. E. Moustafa, Chemistry Department, Faculty of Science, Benha University, Benha Egypt for his continuous support in completing this work.

## REFERENCES

- [1] J Reynolds, Martindale. The Complete Drug Reference, 38th Edition, volume A., The pharmaceutical press, London, UK, **2011**; 65,66 and 83.
- [2] C Sajeev; PR Jadhav; D RaviShankar; RN Saha, *Analytica Chimica Acta*, **2009**, 463(2), 207–217.
- [3] V Mandava; B Rao; B C K Reddy; T SrinivasaRao; K M Ch AppaRao, *Rasayan J.Chem*, **2009**, 2(2), 418-420.
- [4] V Adepu; KE Vnagoji; V Girijasastry. *Int. J. Pharm Pharm. Sci.*, **2014**, 6(9), 31-38.
- [5] PK Pradhan; PN Rajput; N Kumar; B Joshi; U M Upadhyay. *Int. J. Pharm. Sci. Rev. Res.*, **2014**, 27(2), 96-99.
- [6] G Patel; P Chauhan; S Shah. *J. Chem. Pharm. Res.*, **2014**, 6(7), 96-101.
- [7] SC Chi; H Kim; SC Lee, *Anal Lett*, **1944**, 27(2), 377-389.
- [8] SA Korhammer; A Bernreuther; J Fresenius. *Anal Chem*, **1996**, 354(2), 131-135.
- [9] D Loudon; A Handley; S Taylor; E Lenz; S Miller; ID Wilson; A Sage. *Anal Chem*, **2000**, 72(16), 3922-3926.
- [10] B K Patel; J Valentova; A J Hutt. *Chromatographia*, **2002**, 55(3), 135-142.
- [11] Y Sun; K Takaba; H Kido; MN Nakashima; K Nakashima, *J. Pharm Biomed Anal*, **2003**, 30(5), 1611–1619.
- [12] HI Lee; CI Choi; JY Byeon; JE Lee; SY Park; YH Kim; SH Kim; YJ Lee; CG Jang; SY Lee. *J. Chromatogr.*, **2014**, 971(1), 58-63.
- [13] MD Bubba; L Checchini; L Ciofi, S Furlanetto; L Lepri. *Biomed Chromatogr*, **2014**, 28(1), 127-134.
- [14] B Yilmaz; AF Erdem. *J Chromatogr Sci*, **2015**, 53 (9): 1443-1448.
- [15] B Yilmaz, H Sahin; V Akba; AF Erdem. *JAOAC Int*, **2014**, 97(4): 1061-1066.
- [16] H Kanazawa; A Tsubayashi; Y Nagata; Y Matsushima; C Mori; J Kizu; M Higaki. *J Chromatogr*, **2002**, 948(1-2), 303-308.
- [17] HI Shin; BH Kim; HS Chang; CH Park; CI Kim, *Korean J Urol*, **2011**, 52(4), 265-268.
- [18] L Wang; Y Tang; Y Liu. *J. Pharm. Anal*, **2011**, 1(1):51–56.
- [19] P Venkatesan; VS Janardhanan; C Muralidharan; K Valliappan. *Acta Chim Slov*, **2012**, 59(2), 242-248.
- [20] M Hirozawa; T Sambe; N Uchida Xiao; P Lee; K Sato; S Kobayashi. *Jap J. Ophthalmol*, **2015**, 59 (5), 364-371.
- [21] TC O'Haver; GL Green. *Anal Chem*. **1976**, 48, 312-318.
- [22] "The United States Pharmacopoeia", 38th Edition, the national formulary United States pharmacopoeia, **2015**, 2, 3572, 3573.
- [23] HTS Britton. Hydrogen ions 4th Edition, Chapman and Hal (London), **1952**, 364.
- [24] JH Yoe, AL Jones. *Ind. Eng. Chem. J. Anal.*, 16th Edition; **1944**, 111.
- [25] P Job; C Rend. *Anal. Chem.*, **1935**, 6, 97.
- [26] W C Vosburgh; C R Cooper. *J. Amer. Chem. Soc.*, **1941**, 63, 437-444.
- [27] R M Issa; M M El-Essawy. *Z. Phys. Chem. (Leipzig)*, **1973**, 96, 253-261.

[28] A Ringbom. *Anal. Chem.*, **1993**, 115,332-339.

[29] EB Sandell. *Colourimetric determination of trace metals*, 3rd Edition, Interscience New York, **1959**.