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Development and Validation of Novel UV-Spectrophotometric Method for the Estimation of Flurbiprofen and Glipizide Using Hydrotropic Solubilization Technique in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Effective and advantageous Hydrotropic Solubilization technique has been developed for the estimation of two drugs i.e, Flurbiprofen and Glipizide in bulk and its pharmaceutical formulations. Hydrotropic Solubilization technique is one of the aqueous solubility enhancing methods employed for the poorly water soluble drugs and found to be simple, precise and cost effective. Solvents like Piperazine, Urea, Sodium Salicylate, Sodium benzoate etc are the commonly used as hydrotropic solventsin different concentrations. The use of these hydrotropic solvents are of proper choice since the use of organic solvents can be reduced as it is hazardous, costlier and causes environmental pollution. In this context, 1M piperazine has been used as a solubilizing agent to enhance solubility of both the drugs, Flurbiprofen and Glipizide. The absorption maximum of Flurbiprofen and Glipizide was found to be at 246nm and276nm in Zero order derivative spectrum (Method A), calculation of Area Under Curve (AUC)(Method B) was done in the wavelength range of 236-256nm for Flurbiprofen and 266-286nm for Glipizide. The Beer-Lambert's law has been followed in the concentration range of 2-10µg/ml for Flurbiprofen and 5-35µg/ml for Glipizide for both the methods. The methods were validated as per ICH guidelines and all the validation parameters were found to be within the acceptable range. The developed methods were successfully practiced to estimate the amount of Flurbiprofen and Glipizide in bulk and pharmaceutical dosage forms in routine analysis.

Keywords: Flurbiprofen, Glipizide, Piperazine, Validation, Zero order derivative spectroscopy, Area under Curve.

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INTRODUCTION

Flurbiprofen (FLB) chemically, [1,1'-biphenyl]-4-acetic acid, 2-fluoro-alphamethyl-(±). It is a racemic mixture of (+)S and (-)R-enantiomers. It belongs to the family of Non-Steroid Inflammatory Drugs (NSAIDS) and it is phenyl alkanoic acid derivative used to treat the inflammation and pain of arthritis. Flurbiprofen acts by blocking of prostaglandins synthesis which inhibits cyclooxygenase, which is responsible for converting arachidonic acid to cyclic endoperoxide and these moieties are the precursors of prostaglandins[1]. The molecular weight is 224.26 and molecular formula is $C_{15}H_{13}FO_2$[3].It is soluble in methanol, DMSO, DMF, ethanol, and buffers of ionic strength ≥0.1 M and pH ≥7.4. It has very poor aqueous solubility. From the literature survey, it was found that flurbiprofen can be estimated by spectrophotometry[5-7], HPLC methods[8-10] individually or in combination with other drugs. Glipizide (GLZ) is an oral blood-glucose lowering drug belonging to the class of sulfonyl ureas. The IUPAC name of Glipizide is 1-cyclo hexyl-3-[[p [2-(5-methylpyrazine-carboxamido) ethyl] phenyl] sulfonyl] urea. The molecular formula of Glipizide is $C_{21}H_{27}N_5O_4S$ and its molecular weight is 445.55[2].The mechanism of action of Glipizide follows by partially blocking K channels among beta cells of pancreatic islets of Langerhans. The cell depolarizes resulting in the opening of voltage-gated calcium channels which encourages insulin release from beta cells. Glipizide is a white powder which is odorless and having a pKa value of 5.9. It is insoluble in water and alcohols, but soluble in 0.1 N NaOH and freely soluble in dimethylformamide[4].From the literature survey, it was found that Glipizide can be estimated by spectrophotometry[11-14], HPLC methods[15,16] individually or in combination with other drugs. The aim of the present work is to develop and validate new hydrotropic spectrophotometric methods for the estimation of Flurbiprofen and Glipizide in bulk and pharmaceutical formulations.

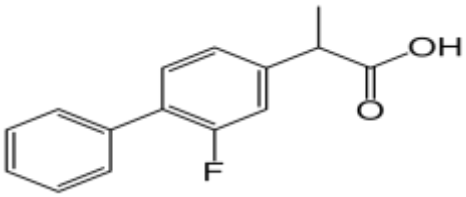

Figure 1: Structure of Flurbiprofen

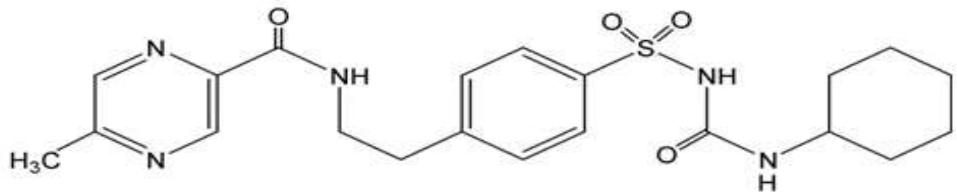

Figure 2: Structure of Glipizide

MATERIALS AND METHOD

Chemicals and Reagents

Flurbiprofen (FLB) and Glipizide (GLZ)working standards were kindly provided by Mylan Laboratories, Hyderabad and were used for the present study. A commercial tablet formulations (Froben 100mg) and (G Trol SR 10mg) were purchased from the local pharmacy and used for study which contains Flurbiprofen - 100mg and Glipizide - 10mg. 1M Piperazine is used as hydrotropic agent and double distilled water were used for the present study.

Instrument

A double beam UV-VIS spectrophotometer (Evolution 220, Thermo Scientific, Japan) connected to computer loaded with spectra manager software "Thermo Insight" was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu, Japan).

Preparation of standard stock solutions

The standard solutions of FLB and GLZ were prepared by dissolving accurately weighed 10 mg of each drug in 1ml of1M Piperazine to enhance solubility of the poorly aqueous soluble drugs and then the volume was made upto 10 ml with double distilled water to obtain a final concentration of 1mg/mL. These stock solutions were used to prepare further dilutions of working standard solutions.

Preparation of working standard solutions

From the above standard stock solutions, 1mL of each were pipetted out into a 10mL cleaned and dried volumetric flasks and the volume was made up to the mark with double distilled water to get a concentration of 100µg/mL (working standard solutions).

Method A: Zero order derivative spectroscopy

Series of dilutions of FLB and GLZ, stock solutions were made by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0mL of FLB and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5mL of GLZ into separate 10mL cleaned and dried volumetric flasks and diluting to volume of 10 ml with double distilled water to produce the concentrations ranging from 2-10 µg/mL of FLB and 5-35 µg/mL of GLZ. The above solutions were scanned over the range of 400 nm to 200 nm against blank. The λ_{max} of FLB and GLZ were found to be at 246.0 nm and 276.0 nm. The present study was carried out at 246.0 nm and 276nm for FLB and GLZ, where the Beer- Lambert's law had been followed. The calibration curves were constructed by plotting concentration on X-axis versus absorbance on Y-axis at 246.0 nm and 276nm for FLB and GLZ.

Method B: Area under curve

The AUC (area under curve) method is one of the UV methods for the determination of drugs. This method of AUC is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. The calibration curves were constructed by plotting concentration on X-axis versus AUC on Y-axis for both the drugs.

Estimation of Flurbiprofen and Glipizide in bulk and in formulations

For the analysis of drugs in bulk, accurately weighed 10 mg samples of FLB and GLZ were dissolved in 1ml of 1M piperazine (hydrotropic agent) initially and then volume was made up to 10 mL with distilled water in a suitable volumetric flasks. After suitable dilution with double distilled water, the spectrums of the final samples prepared, were recorded against blank. For the analysis of the pharmaceutical dosage forms, ten tablets each of Flurbiprofen and Glipizide were weighed and were finely powdered. A quantity of powder equivalent to 50 mg of each of the drugs were transferred to a 50 mL cleaned and dried volumetric flasks and dissolved in 5ml of1M piperazine and then with double distilled water up to the mark by keeping on an ultrasonicaton bath for 5 to 10 minutes. The solutions were filtered through Whatman filter paper (No. 41). From that stock solutions, further dilutions were made with double distilled water to get required concentration. The concentration of Flurbiprofen and Glipizide were determined by measuring the absorbances of sample solutions at 246.0nm and 276.0nm. The assay procedure was repeated six times (n=6).

Method Validation

Linearity

For both the drugs, 6-point (2.0–10.0 µg/ mL) for FLB and 7-point (5.0–35.0 µg/ mL) for GLZ, calibration curves were prepared on 3 different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression analysis method.

Precision

The intra-day and inter-day precisions of the proposed spectrophotometric methods for both the drugs Flurbiprofen and Glipizide were determined by estimating the corresponding response 3

times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of FLB (6.0, 8.0, and 10.0 µg/mL) and GLZ (15.0, 20.0, and 25.0 µg/mL) and the results were reported in terms relative standard deviation (RSD) and the value should be ≤ 2% for the method to be precise.

Accuracy

This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100 and 120% which consisted of adding known amounts of FLB and GLZ reference materials to a pre quantified sample solution. The recovery was verified by estimation of both the drugs in triplicate preparations at each specified concentration level. The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery to state whether the method is accurate.

RESULTS AND DISCUSSION

The methods discussed in the present work provided a convenient and accurate way for the analysis of Flurbiprofen and Glipizide in bulk and in pharmaceutical dosage forms. The absorbance maxima of Flurbiprofen and Glipizide was found to be 246nm and 276nmfor the method A of both the drugs and for method B the area under curve in the range of 236-256nm for Flurbiprofen and 266-286nm for Glipizide was selected for the analysis. The statistical data of Zero derivative Spectroscopy of FLB and GLZ was given in tables 1,2 and the calibration curves of Zero Order Spectroscopy of FLB and GLZ were given in figures 7 and 8. The statistical data of Area Under Curve for FLB and GLZ were given in tables 3,4 and the calibration curves of Area Under Curve method for FLB and GLZ were given in figures 11 and 12.Linearity for the two drugs were observed in the concentration range of 2-10 µg/mL and5-35 µg/mL as shown in the tables 7,8 and figures5&6.The assay of the two methods for FLB and GLZ was found to be within the range of 98-102% as shown in the tables 5&6. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. In both the intra-day and inter-day precision study for two methods the %RSD was found to be less than 2.0 indicating the good precision of the method as shown in the tables 9,10(FLB) and 11,12(GLZ) respectively. The validation of proposed methods was further confirmed by recovery studies, the %recovery values vary from 98- 102% as shown in the tables 13&14. Based on results obtained, it was found that the proposed methods were found to be accurate, precise and reproducible and can be employed for routine quality control analysis of Flurbiprofen and Glipizide in tablet dosage forms.

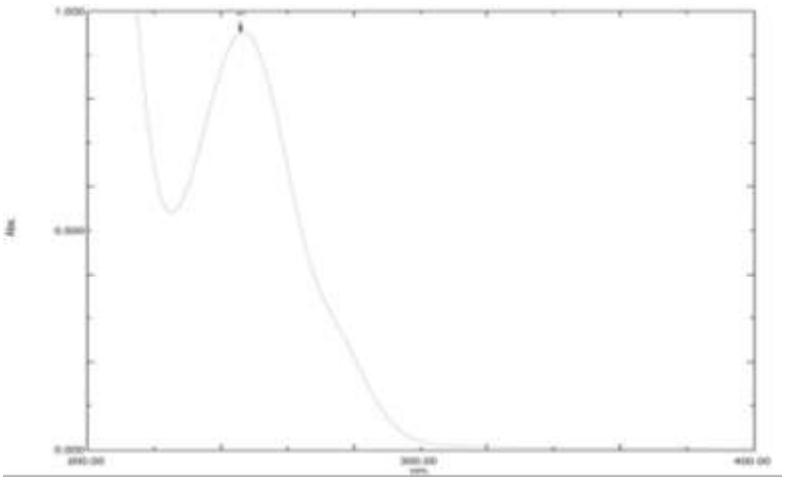

Figure 3: λ_{max} determination for Flurbiprofen

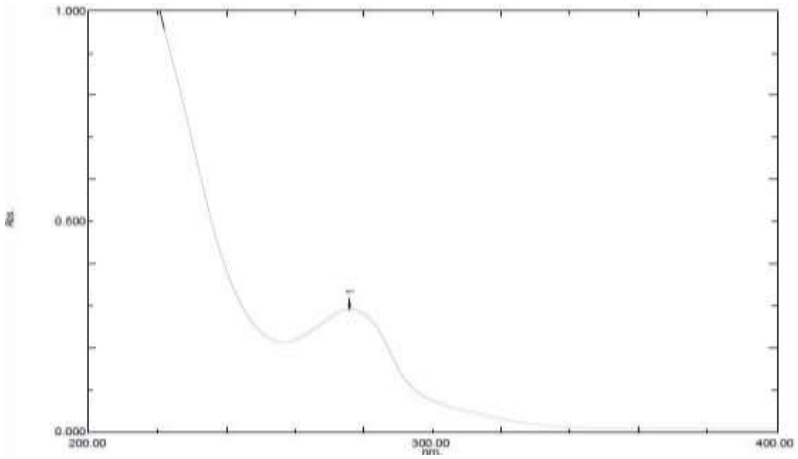

Figure 4: λ_{max} determination for Glipizide

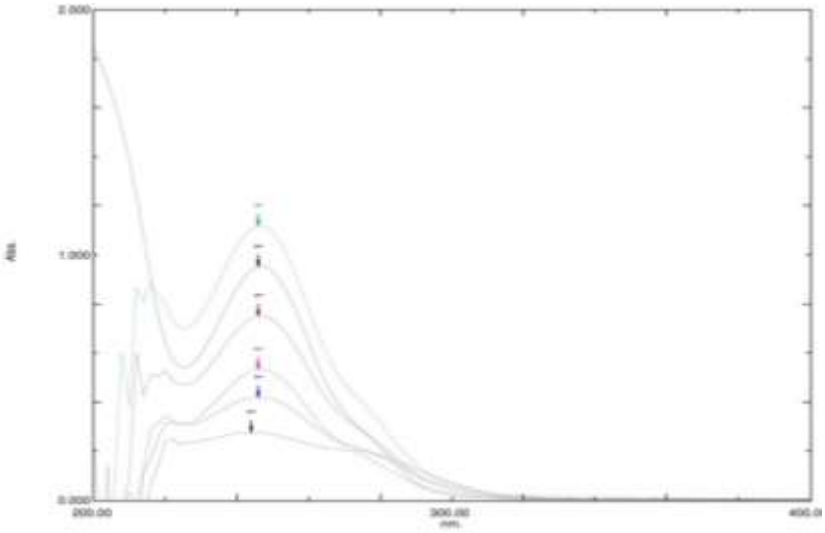

Figure 5: Linearity for Zero- derivative absorption spectrum of FLB

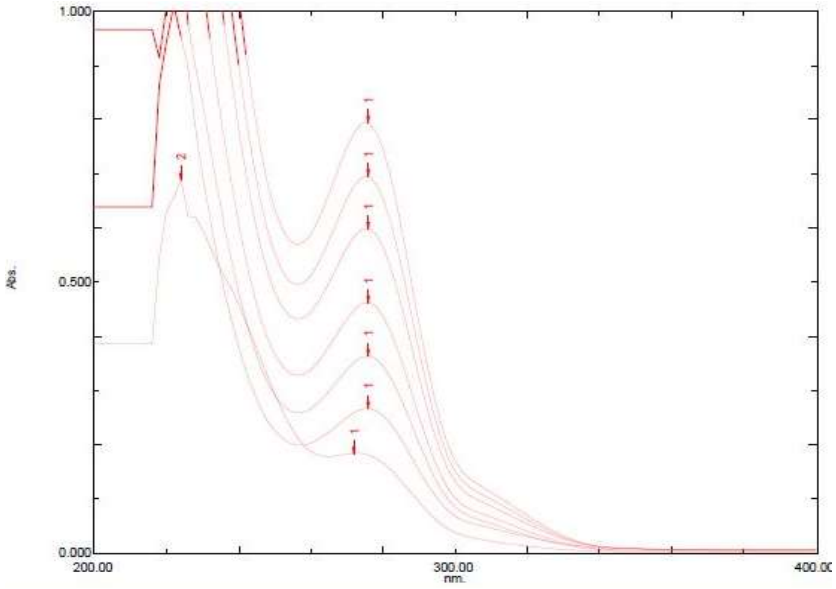

Figure 6: Linearity for Zero- derivative absorption spectrum of GLZ

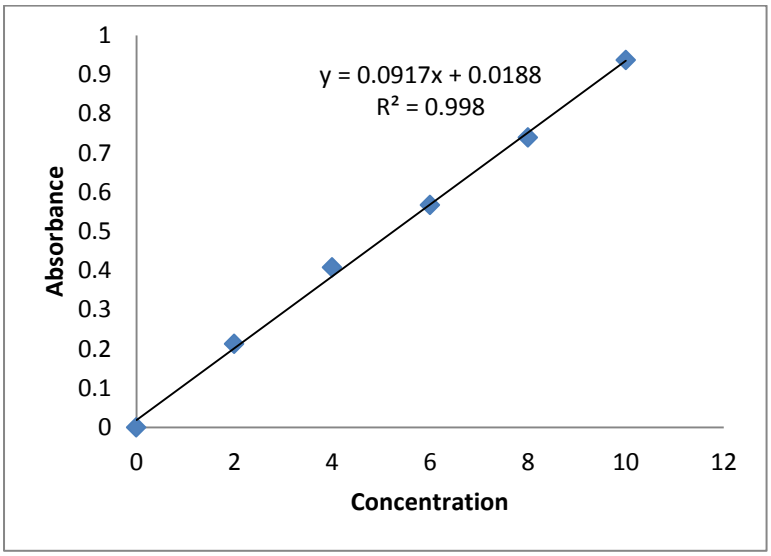

Figure 7: Calibration curve for Zero-order derivative spectrum of FLB

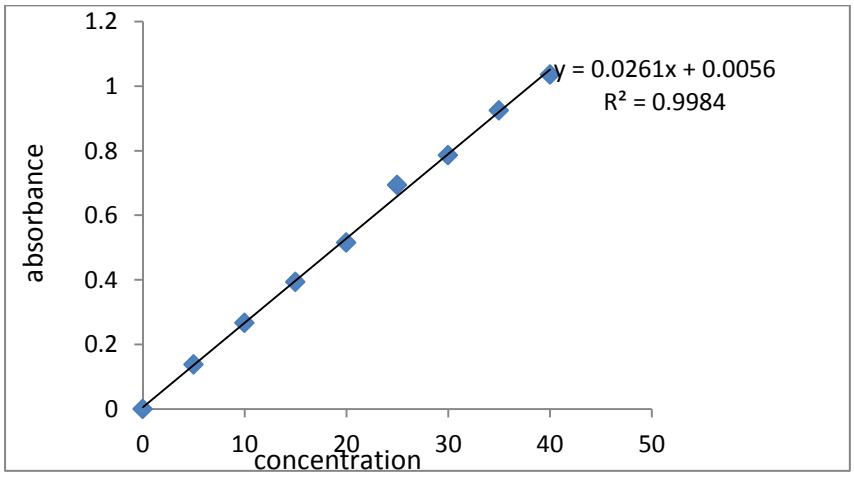

Figure 8: Calibration curve for Zero-order derivative spectrum of GLZ

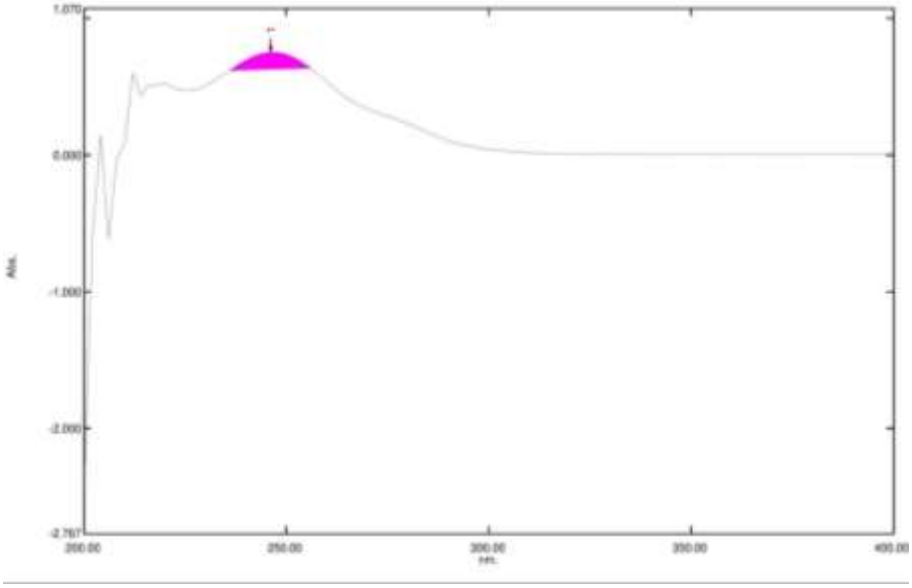

Figure 9: Area Under Curve for Flurbiprofen

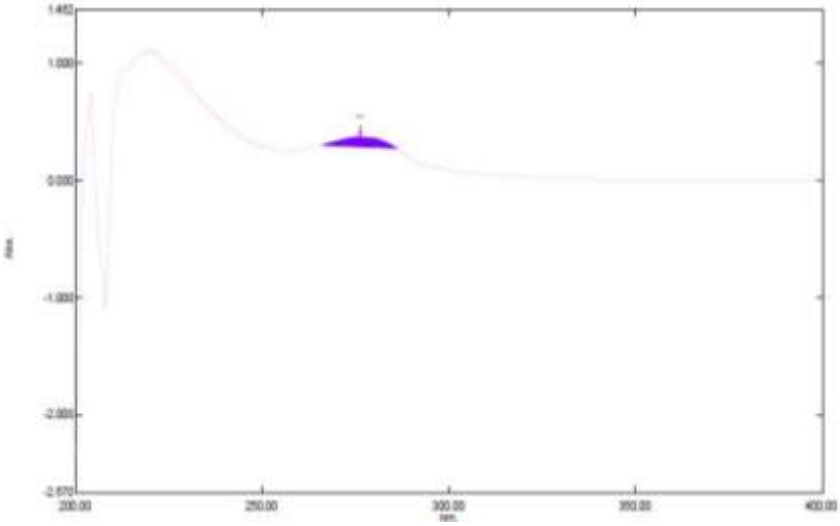

Figure 10: Area Under Curve for Glipizide

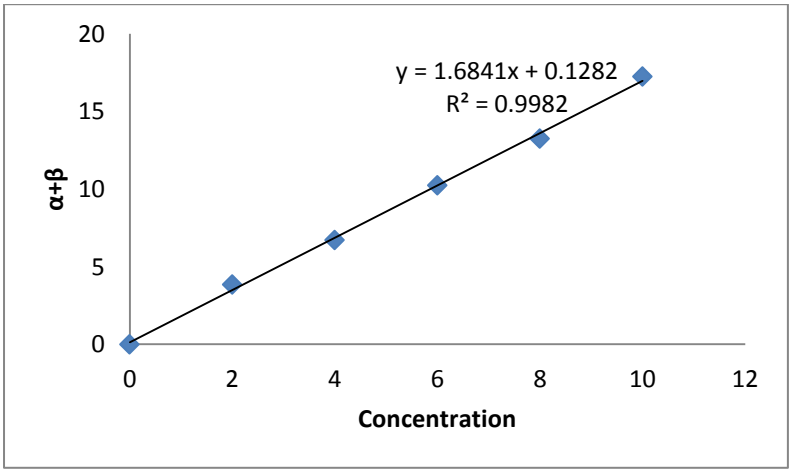

Figure 11: Calibration curve for Area under curve of FLB.

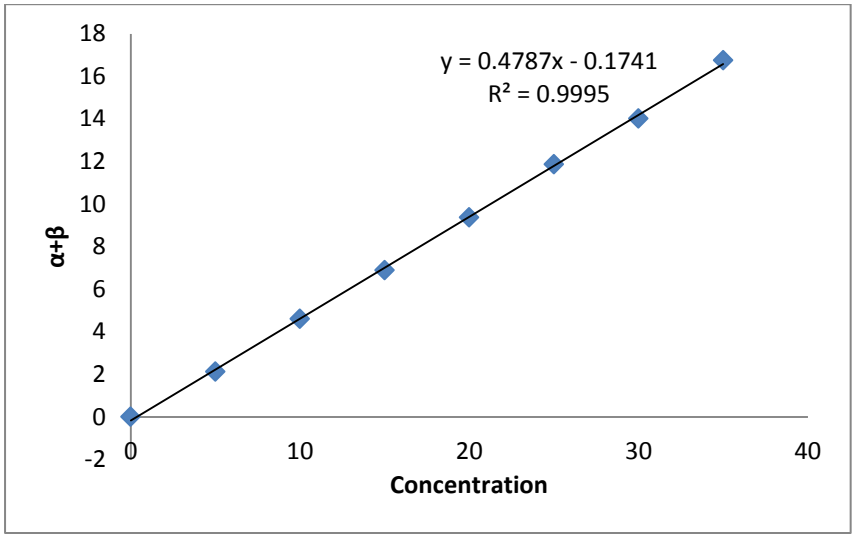

Figure 12: Calibration curve for Area under curve of GLZ.

Table 1: Statistical data for zero order derivative spectroscopy of FLB

S. No.	Concentration (µg/mL)	Absorbance	Standard deviation*	Slope	Intercept	R^2
1	2	0.213	0.004	0.091	0.018	0.998
2	4	0.408	0.003			
3	6	0.567	0.003			
4	8	0.739	0.002			
5	10	0.936	0.003			

* n=6

Table 2: Statistical data for zero order derivative spectroscopy of GLZ

S. No.	Concentration (µg/mL)	Absorbance	Standard deviation*	Slope	Intercept	R^2
1	5	0.138	0.002	0.0261	0.0056	0.9984
2	10	0.267	0.003			
3	15	0.394	0.002			
4	20	0.516	0.002			
5	25	0.694	0.003			
6	30	0.786	0.003			
7	35	0.925	0.002			

*n=6

Table 3: Statistical data for Area Under Curve of FLB

S. No	Concentration (µg/mL)	α+β	Standard deviation*	Slope	Intercept	R^2
1	2	3.853	0.0119	1.684	0.128	0.998
2	4	6.718	0.0139			
3	6	10.236	0.0548			
4	8	13.249	0.0466			
5	10	17.236	0.0283			

* n=6

Table 4: Statistical data for Area Under Curve of GLZ

S. No	Concentration (µg/mL)	α+β	Standard deviation*	Slope	Intercept	R^2
1	5	2.125	0.0169	0.4689	0.059	0.9986
2	10	4.598	0.0130			
3	15	6.896	0.0248			
4	20	9.364	0.0598			
5	25	11.869	0.0743			
6	30	14.021	0.0910			
7	35	16.759	0.0337			

* n=6

Table 5: Assay of the Marketed Formulation of FLB

Analysis method	Label claim (mg)	Amount found(mg)	% Recovery
A	100 mg	99.65	99.65
B	100mg	98.9	98.9
Bulk drug	100mg	99.8	99.8

Table 6: Assay of the Marketed Formulation of GLZ

Analysis method	Label claim (mg)	Amount found(mg)	% Recovery
A	10 mg	9.92	99.2
B	10mg	10.02	100.2
Bulk drug	100mg	99.87	99.87

Table 7: Linearity studies of Flurbiprofen by proposed methods

S. No.	Parameter	Method A	Method B
1	Linearity(µg/mL)	2-10	2-10
2	Slope	0.091	1.684
3	Intercept	0.018	0.128
4	Correlation coefficient	0.998	0.998

Table 8: Linearity studies of Glipizide by proposed methods

S. No.	Parameter	Method A	Method B
1	Linearity(µg/mL)	5-35	5-35
2	Slope	0.0261	0.4689
3	Intercept	0.0056	0.059
4	Correlation coefficient	0.9984	0.9986

Table 9: Intra-day and Inter-day Precision data of Flurbiprofen (zero order)

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Mean ± SD*	% RSD	Mean ± SD*	% RSD
6	0.556±0.0006	0.107	0.561±0.003	0.534
8	0.739±0.0023	0.311	0.730±0.0046	0.630
10	0.927±0.0014	0.151	0.934±0.0015	0.160

* n=3

Table 10: Intra-day and Inter-day Precision data of Flurbiprofen (AUC)

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Mean ± SD*	% RSD	Mean ± SD*	% RSD
6	10.233±0.0015	0.014	10.239±0.0019	0.018
8	13.245±0.0012	0.009	13.245±0.0024	0.018
10	17.237±0.0011	0.0063	17.243±0.0028	0.016

* n=3

Table 11: Intra-day and Inter-day Precision data of Glipizide (zero order)

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Mean ± SD*	% RSD	Mean ± SD*	% RSD
15	0.385±0.00042	0.109	0.395±0.00053	0.135
20	0.505±0.00088	0.174	0.515±0.00086	0.166
25	0.682±0.00055	0.08	0.695±0.00063	0.09

* n=3

Table 12: Intra-day and Inter-day Precision data of Glipizide (AUC)

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Mean ± SD*	% RSD	Mean ± SD*	% RSD
15	6.873±0.0044	0.064	6.792±0.026	0.38
20	9.318±0.0066	0.07	9.356±0.007	0.074
25	11.80±0.00902	0.076	11.850±0.0068	0.056

* n=3

Table 13: Recovery studies of Flurbiprofen by proposed methods

Concentration taken (µg/mL)	Spiked level (%)	Amount added (mg)	%Recovery			
			A	B	A	B
4	80	3.2	7.11	7.06	98.83	98.16
4	100	4	7.94	7.86	99.37	98.25
4	120	4.8	8.81	8.69	100.1	98.80

Table 14: Recovery studies of Glipizide by proposed methods

Concentration taken (µg/mL)	Spiked level (%)	Amount added (mg)	%Recovery			
			A	B	A	B
15	80	12	26.8	27.1	99.2	100.3
15	100	15	29.6	30.2	98.6	100.6
15	120	18	28.3	27.7	101.07	98.9

CONCLUSION

The proposed methods were found to be simple, sensitive, accurate and precise and showed no interference from the common additives and excipients. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. The proposed methods were also more economical because, here we used 1M Piperazine and double distilled water for the standard stock solution and further dilutions made with double distilled water. Hence

the proposed methods can be routinely used for the estimation of Flurbiprofen and Glipizide in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

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