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Research Article

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UV Spectrophotometric Method for Simultaneous Estimation of Glycopyrrolate and Formoterol Fumarate in their Synthetic Mixture by Absorbance Correction Method

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ABSTRACT

A simple, precise, accurate and validated UV-spectroscopic method absorbance correction menthod for simultaneous estimation of Glycopyrrolate and Formoterol Fumarate in their synthetic mixture was developed. The Estimation was done based on measurement of absorbance at two wavelengths 295 nm (λ1) and 209 nm (λ2). Were λ1 was the point at which Formoterol Fumrate showed significant absorbance and Glycopyrrolate showed zero absorbance and λ2wavelength at which both drugs showed significant absorbance. Linearity was obtained over a range of 2-10 µg/ml for glycopyrrolate and 1-3 µg/ml for Formoterol Fumarate respectively. The results of proposed method were validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. This method can be successfully be applied for simultaneous estimation of drugs in all commercial products.

Keywords: Glycopyrrolate; Formoterol Fumarate; Absorbance; Correction method, UV spectroscopic method

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INTRODUCTION

Glycopyrrolate chemically is 3-Hydroxy-1,1-dimetylpyrrolidinium bromide α-cyclopentylmandelate. It is in a class of medications called anticholinergics. It decreases stomach acid production by blocking the activity of a certain natural substance in the body. It decreases acid secretion in the stomach and so may be used for treating stomach ulcers, in combination with other medications. It can also be used in treating asthma and COPD. The chemical structure of Glycopyrrolate is shown in Figure 1 [1-3].

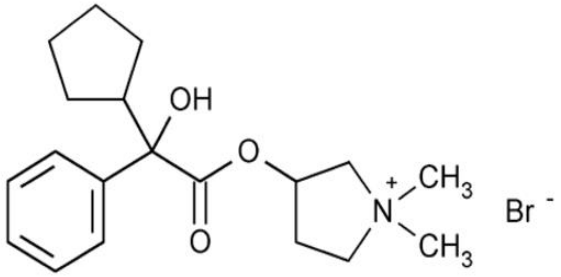

Figure 1: Structure of Glycopyrrolate

Formoterol Fumarate chemically is 2'-Hydroxy-5'-[1-hydroxy-2-[[p-methoxy-a methylphenethyl]amino]ethyl]formanilide-2-Butenedioate. It is a long-acting (12 hours) beta2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD). Inhaled formoterol works like other beta2-agonists, causing bronchodilatation through relaxation of the smooth muscle in the airway to treat the exacerbation of asthma. The chemical structure of Formoterol Fumarate is shown in Figure 2 [4].

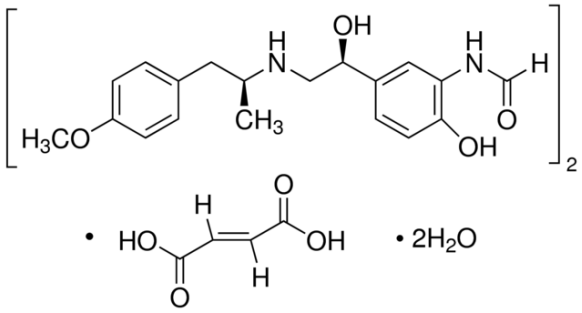

Figure 2: Structure of Formoterol Fumarate

BEVESPI AEROSPHERE is indicated for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema[1].BEVESPI AEROSPHERE is not indicated for the relief of acute bronchospasm or for the treatment of asthma, and is contraindicated in patients with hypersensitivity to glycopyrrolate, formoterol fumarate, or to any component of the product[1].BEVESPI AEROSPHERE is a combination of glycopyrrolate, a long-acting muscarinic antagonist (LAMA), and formoterol Fumarate, a long-acting beta2-adrenergic agonist (LABA). [5,6].

Literature Survey reveals that there is no any single method for simultaneous estimation of Glycopyrrolate and Formoterol Fumarate has been reported. However, UV Spectroscopic method for glycopyrrolate [7] and RP-HPLC method [8-13] have been noted. No UV Spectroscopic methods for Formoterol Fumarate alone has been reported and RP-HPLC [14-24] have been noted. Henceforth the following experiment was performed.

MATERIALS AND METHODS

Reference standard of Glycopyrrolate and Formoterol Fumarate were received as gift sample from Glenmark Pharmaceuticals (Mumbai). Methanol AR grade was purchased from Ahmedabad, India. UV Visible Spectrophotometer (Double beam) Make: Labtronics, Model: LT-2900, capable of multicomponent analysis, was used for quantitation.

Method of Analysis [25]
Diluent: Methanol
Wavelength: 209 nm and 295 nm.

Experimental Work

Preparation of standard solutions
The standard stock solution of both drugs was prepared by accurately weighing 10 mg of Glycopyrrolate and 10 mg Formoterol Fumarate in 10 mL volumetric flask respectively and making volume up to mark with diluent. Then 1 ml of standard stock solution was diluted to 10 mL with diluent to make final standard concentration of Glycopyrrolate (100 µg/mL) and Formoterol Fumarate (100 µg/mL) respectively (Figure 3 and Figure 4).

Preparation of synthetic mixture
18 mg of pure glycopyrrolate and 9 mg of Formoterol Fumarate were weighed and transferred to a porcelain dish. The ingredients like lactose (45 mg), HPMC (40 mg), PVP (40 mg) and starch powder (48 mg) were added and the mixture was triturated properly for uniform mixing. The mixture obtained was used for the further analysis.

Preparation of test solution
Sample powder of about 111.11 mg (synthetic powder equivalent to 10 mg Glycopyrrolate and 5 mg of Formoterol Fumarate) was weighed accurately and transferred into a 100-mL volumetric flask. Then add about 50 mL diluent and sonicate for 40 minutes with intermittent shaking. Then volume was made up to the mark with diluent to make final standard solution of Glycopyrrolate (100 µg/mL) and Formoterol Fumarate (50 µg/mL) respectively. The test solution was filtered through 0.45 µm Millipore filter paper. Then 4 mL of sample stock solution was diluted to 100 mL with diluent to make final standard concentration of Glycopyrrolate (4

µg/mL) and Formoterol Fumarate (2 µg/mL), respectively. The sample solution was analysed by using UV Spectrophotometer.

UV spectra for Glycopyrrolate (4 µg/mL) and Formoterol Fumarate (2 µg/mL) for wavelength maxima selection is shown in Figures 3-6. Absorbance at both wavelength 209 nm and 295 nm for both drugs are shown in Tables 1 and 2. And the wavelength selection is shown in Figure 7 and Figure 8.

Method Validation [25]

Validation was carried out with respect to various parameters, as required under ICH guideline Q2 (R1). The developed method validated with respect to parameters such as linearity, precision, accuracy, ruggedness, robustness, LOD, LOQ and solution stability.

Linearity

To achieve linearity and range, stock solution containing Glycopyrrolate (100 µg/mL) and Formoterol Fumarate (100 µg/mL) were separately prepared. Glycopyrrolate and Formoterol Fumarate stock solutions were diluted with diluent to yield solutions in the concentration range of 2 - 10 µg/ml and 1 - 3 µg/ml respectively. The solutions were analysed by using UV Spectrophotometer. Overlay linearity spectra for Glycopyrrolate and Formoterol Fumarate are shown in Figure 5 and 6. Calibration curve for both drugs are shown in Figures 9-11. The results of linearity are presented in Table 3.

Precision

Intraday precision

The method precision was done by preparing solution containing the mixture of 4 µg/ml and 2 µg/ml of Glycopyrrolate and Formoterol Fumarate respectively. Analysis was replicated for 6 different times within same day. The results are presented in Table 4 and Table 5. The results obtained were within 2% RSD.

Interday precision

The method precision was done by preparing solution containing the mixture of 4 and 2 µg/ml of Glycopyrrolate and Formoterol Fumarate respectively. Analysis was replicated for 6 different days (Figure 10 and Figure 11). The results are presented in Tables 6 and 7. The results obtained were within 2% RSD.

Robustness

Robustness test was determined by obtaining results by varying parameters like change of scanning speed and change in manufacturer of methanol. The value of percentage RSD was below 2.0%, this showed robustness of developed method. The results are presented in Tables 8-11.

Ruggedness

Ruggedness test was determined by obtaining a solution containing mixture of 4 µg/ml Glycopyrrolate and 2 µg/ml Formoterol Fumarate prepared from their respective stock solutions and then analysis done by two different analysts. The value of percentage RSD was below 2.0%, this showed ruggedness of developed method. The results are presented in Tables 8 and 9.

Accuracy

The difference between theoretical added sample amount to the Synthetic Mixture and practically achieved sample amount from Synthetic Mixture (after UV analysis) is called accuracy of analytical method. Accuracy was determined at three different level 80%, 100% and 120% of the target concentration in triplicate. The results are presented in Table 2 and Table 3.

Solution stability

The standard and sample solutions were found to be stable for 24 hours at room temperature. The results are presented in Table 12.

Limit of detection (lod) and limit of quantitation (loq)

The results of LOD and LOQ are mentioned in Table 13.

Summary

All parameters of validation are summarized in Table 14.

RESULTS AND DISCUSSION

Proper wavelength selection for estimation of both drugs depends on nature of drugs and their solubility. Method employs solving of equations based on measurement of absorbance at 209 nm and 295 nm which were selected as λ_2 and λ_1 respectively. Calculation for both drugs are done as per below formula:

$$C_X = A_1/ax_1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$
$$C_Y = [A_2 - (ax_2 \times C_X)]/ay_2 \ldots\ldots (2)$$

Where,

- A_1 and A_2 are absorbance of mixture at 295 nm (λ_1) and 209 nm (λ_2),
- ax_1 and ax_2 are absorptivities of Formoterol fumarate at λ_1 and λ_2, respectively,
- ay_1 and ay_2 are absorptivities of Glycopyrrolate at λ_1 and λ_2, respectively,
- C_X and C_Y are concentrations of Formoterol Fumarate and Glycopyrrolate, respectively.

Table 1: Calibration Table for glycopyrrolate and formoterol fumarate

Sr. No	For Glycopyrrolate		For Formoterol Fumarate		
	Concentration (µg/ml)	Absorbance at 209.0 nm (λ_2)	Concentration (µg/ml)	Absorbance at 209.0 nm (λ_2)	Absorbance at 295.0 nm (λ_1)
1	0	0.000	0	0.000	0.000
2	2	0.156	1	0.078	0.015
3	4	0.310	1.5	0.153	0.019
4	6	0.379	2	0.197	0.028
5	8	0.479	2.5	0.227	0.034
6	10	0.627	3	0.389	0.041

Table 2: Accuracy Data for Glycopyrrolate (GLY) and Formoterol Fumarate (FF)

Level of % Recovery	Amount Present (mg)		Amount of Standard Added (mg)		Total Amount Recovered (mg)		% Recovery	
	GLY	FF	GLY	FF	GLY	FF	GLY	FF
80	9	4.5	7.2	3.6	16.30	8.02	100.66	99.12
80	9	4.5	7.2	3.6	16.06	8.22	99.14	101.54
80	9	4.5	7.2	3.6	16.30	8.02	100.66	99.12
100	9	4.5	9	4.5	18.28	9.13	101.58	101.54
100	9	4.5	9	4.5	18.38	9.07	102.11	100.82
100	9	4.5	9	4.5	18.28	9.13	101.58	101.54
120	9	4.5	10.8	5.4	20.10	9.79	101.51	98.90
120	9	4.5	10.8	5.4	20.19	9.72	101.99	98.25
120	9	4.5	10.8	5.4	19.90	9.92	100.55	100.22

Table 3: Statistical Validation for Accuracy

Level of % Recovery	% Mean Recovery*		Standard Deviation*		Co-Efficient of Variation* (% R.S.D.)		Standard Error*	
	GLY	FF	GLY	FF	GLY	FF	GLY	FF
80	100.16	99.93	0.879	1.396	0.878	1.396	0.507	0.805
100	101.76	101.30	0.306	0.418	0.300	0.412	0.176	0.241
120	101.35	99.12	0.736	1.007	0.726	1.015	0.425	0.581

Table 4: Data for inter day precision

Sr. No.	Concentration (µg/mL)		% of Label Claim	
	GLY	FF	GLY	FF
1.	4	2	99.20	99.12
2.	4	2	99.44	99.12
3.	4	2	98.41	101.54
4.	4	2	99.20	99.12
5.	4	2	98.16	101.54
6.	4	2	98.41	101.54

Table 5: Statistical Validation of interday precision

Drug	Mean* (%)	Standard Deviation*	Co-Efficient of Variation* (% R.S.D.)	Standard Error*
GLY	98.80	0.537	0.543	0.220
FF	100.33	1.324	1.319	0.540

Table 6: Data for intraday precision

Sr. No.	Concentration (µg/mL)		% of Label Claim	
	GLY	FF	GLY	FF
1.	4	2	99.44	99.12
2.	4	2	98.41	101.54
3.	4	2	99.44	99.12
4.	4	2	99.69	99.12
5.	4	2	98.16	101.54
6.	4	2	99.20	99.12

Table 7: Statistical Validation of intraday precision

Drug	Mean* (%)	Standard Deviation*	Co-efficient of Variation* (% R.S.D.)	Standard Error*
GLY	99.06	0.622	0.627	0.253
FF	99.93	1.249	1.249	0.509

Table 8: Result of Ruggedness study

Variation and Level		Concentration (µg/ml)		% Labelled Claim	
		GLY	FF	GLY	FF
Different analyst	Analyst 1	4	2	99.75	101.54
	Analyst 2	4	2	99.24	99.73

Table 9: Statistical validation for ruggedness study

Drug	Mean* (%)	Standard Deviation*	Co-efficient of Variation* (% R.S.D.)	Standard Error*
GLY	99.50	0.357	0.356	0.253
FF	100.64	1.282	1.273	0.906

Table 10: Result of Robustness study

Variation and Level		Concentration (µg/ml)		% labelled Claim	
		GLY	FF	GLY	FF
Change in scanning speed	Fast	4	2	100.94	99.91
	Medium	4	2	99.23	101.00
	Slow	4	2	100.26	100.09
Change in Methanol Manufacturer	1	4	2	100.65	100.82
	2	4	2	100.81	100.09

Table 11: Statistical Validation for Robustness study

Variation and Level	Mean* (%)		Standard Deviation*		Co-efficient of Variation* (% R.S.D.)		Standard Error*	
	GLY	FF	GLY	FF	GLY	FF	GLY	FF
Change in scanning speed	100.15	100.33	0.861	0.583	0.860	0.584	0.497	0.336
Change in Methanol Manufacturer	100.73	100.45	0.116	0.5134	0.115	0.515	0.082	0.363

Table 12: Solution stability data for standard solution and sample solution

Standard Solution				
Time (hr)	Absorbance		% Difference	
	209 nm	295 nm	Glycopyrrolate	Formoterol Fumarate
0	0.372		==	==
8	0.373			
24				
Sample Solution				
Time (hr)	Absorbance		% Difference	
	209 nm	295 nm	Glycopyrrolate	Formoterol Fumarate
0	0.262		==	==
8				
24				

Table 13: LOD and LOQ data for sample solution

Parameter	Glycopyrrolate	Formoterol Fumarate
LOD	0.154 µg/ml	0.241 µg/ml
LOQ	0.468 µg/ml	0.730 µg/ml

Table 14: Summary of validation parameters for simultaneous estimation of Glycopyrrolate and Formoterol Fumarate

Parameters of Validation		Acceptance Criteria	Glycopyrrolate	Formoterol Fumarate
Linearity (µg/mL)		Follows Lambert's Beer law	2 – 10 µg/mL	1-3 µg/mL
Correlation Coefficient (R^2)		R^2>0.9	0.9876	0.9931
Accuracy (%)	**80%**	Recovery 98 – 102%	100.16	99.93
	100%		101.76	101.30
	120%		101.35	99.12
LOD (µg/mL)		-	0.154	0.241
LOQ (µg/mL)		-	0.468	0.730
Intraday Precision (% RSD) n=6		RSD<2%	0.627	1.249
Interday Precision (% RSD) n=6			0.543	1.319
Robustness (% label claim)	**Scanning Speed**	RSD<2%	100.14	100.33
	Methanol manufacturer	RSD<2%	100.73	100.45
Ruggedness (% label claim)		RSD<2%	99.75	101.54
Solution Stability		>12 hour	Stable for 24 hr	Stable for 24 hr

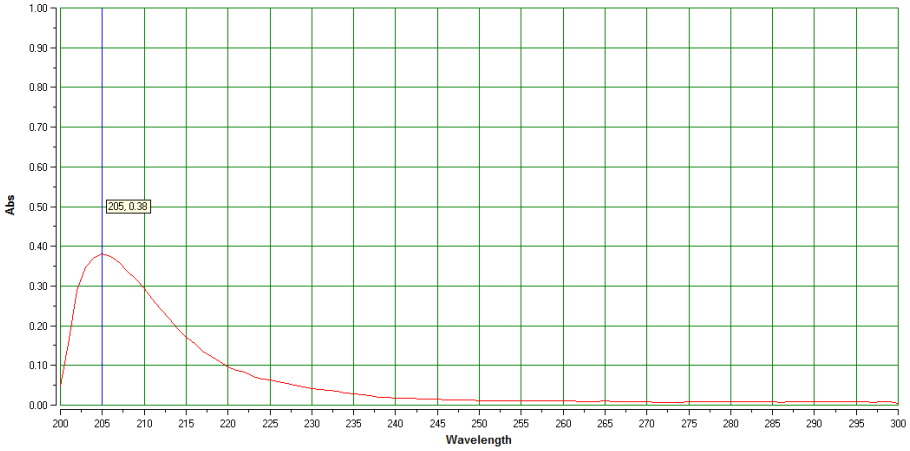

Figure 3: Spectra of Glycopyrrolate (4 µg/mL)

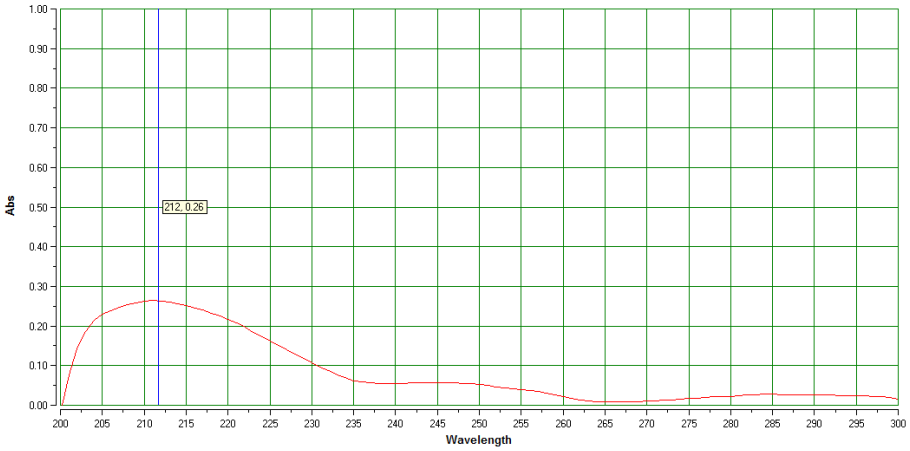

Figure 4: Spectra of Formoterol Fumarate (2 µg/mL)

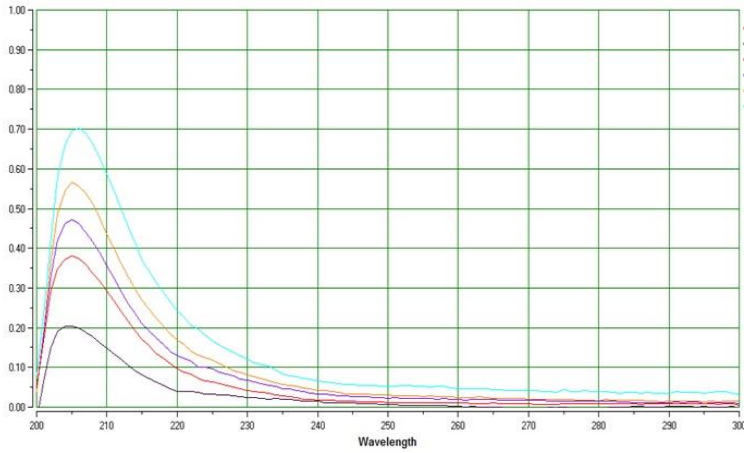

Figure 5: Overlay spectra of linearity of Glycopyrrolate

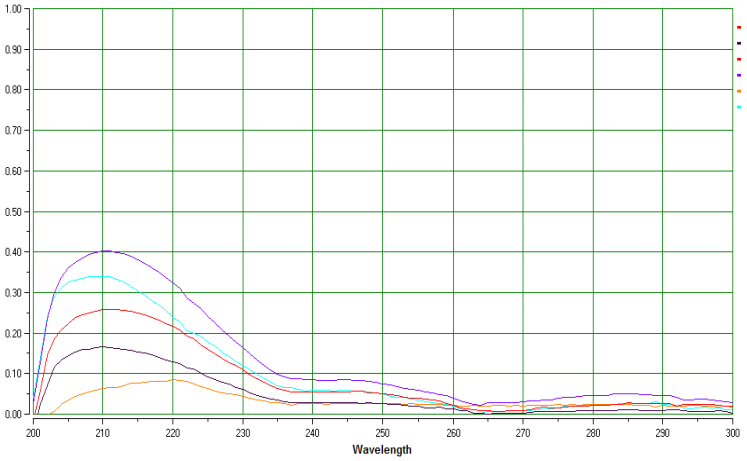

Figure 6: Overlay spectra of linearity of Formoterol Fumarate

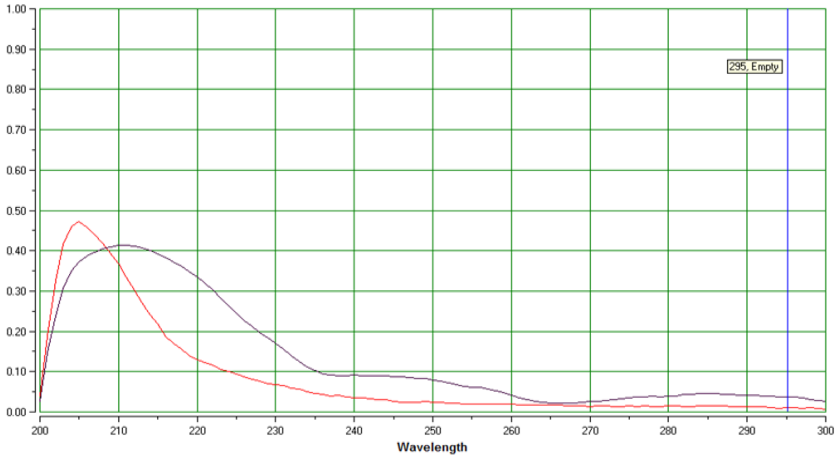

Figure 7: Selection of λ1 for the method

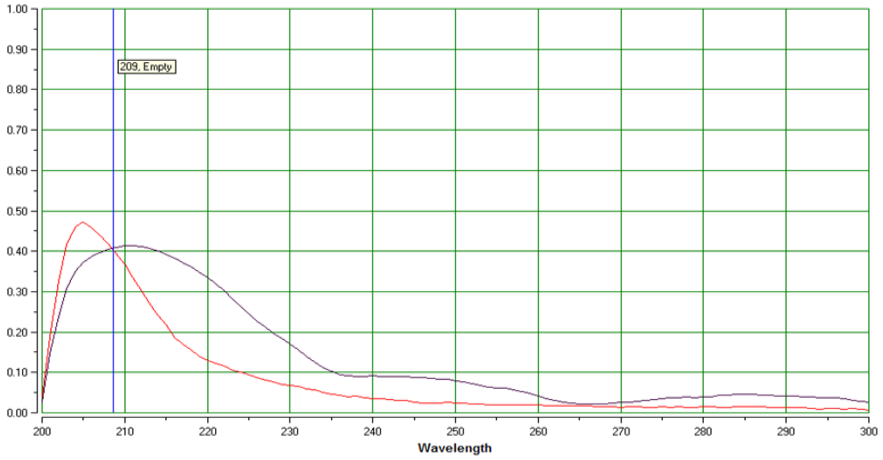

Figure 8: Selection of λ2 for the method

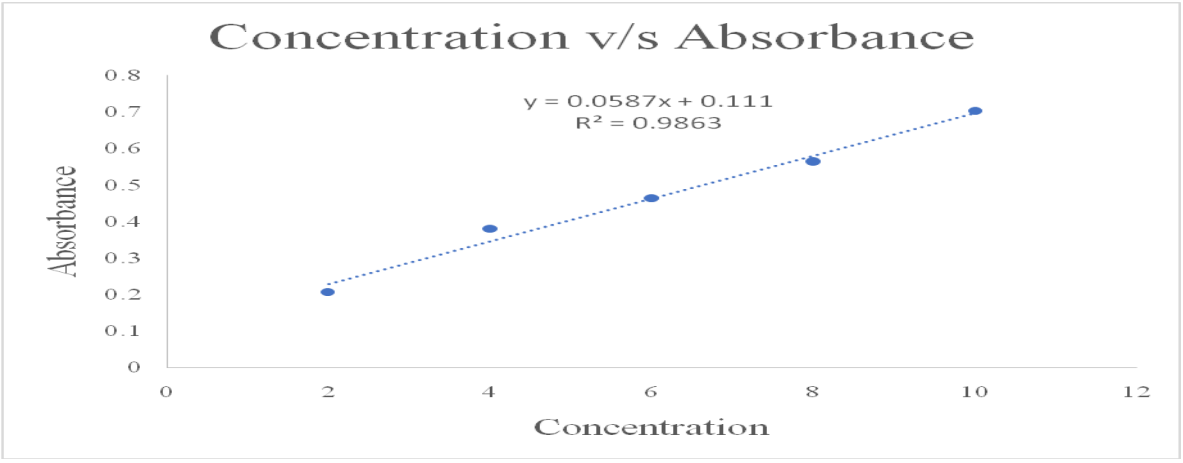

Figure 9: Calibration curve for Glycopyrrolate at 209 nm

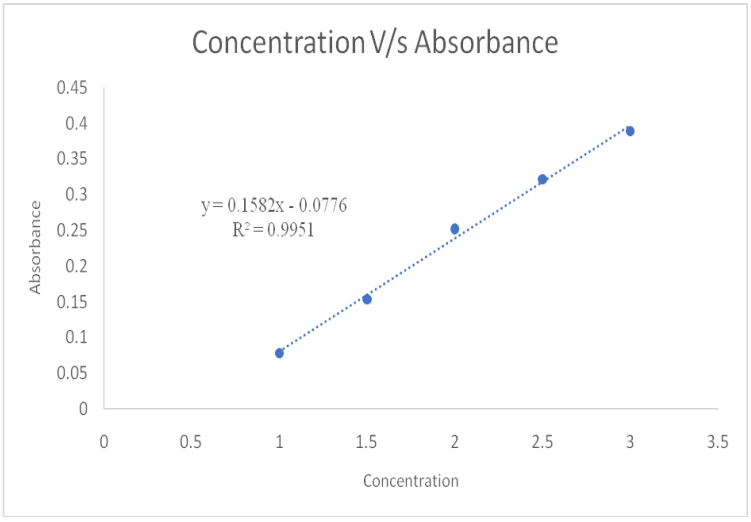

Figure 10: Calibration curve for Formoterol Fumarate at 209nm

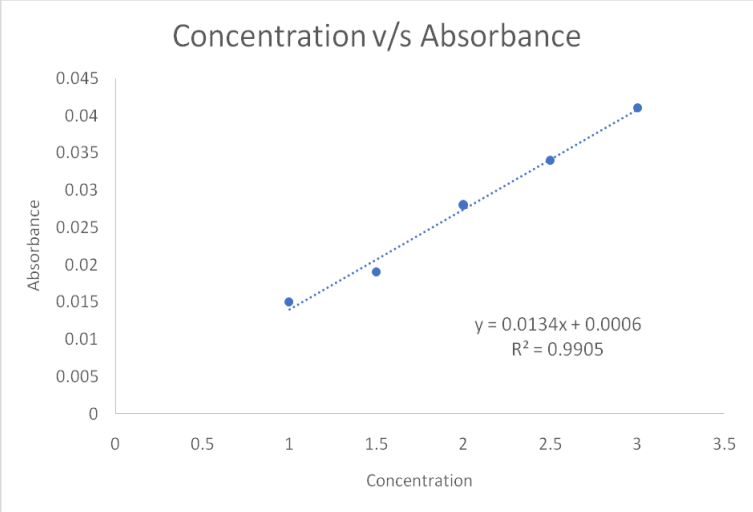

Figure 11: Calibration curve forFormoterol Fumarate at 295nm

CONCLUSION

The proposed method is found to be simple, accurate and precise method for simultaneous estimation of Glycopyrrolate and Formoterol Fumarate in commercial dosage form in routine and quality control laboratories.

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