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DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF INDACATEROL MALEATE AND GLYCOPYRRONIUM BROMIDE IN PHARMACEUTICAL DOSAGE FORM AND THEIR COMPARISION USING STUDENT t-TEST

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ABSTRACT

Indacterol maleate and Glycopyrronium bromide combination is used for chronic obstructive pulmonary disease and newly introduced in market. It is necessary to develop suitable quality control methods for rapid and accurate determination of these drugs. High-performance liquid chromatographic (HPLC) and UV spectrophotometric methods were developed and validated for the quantitative determination of Indacaterol maleate (IND) and Glycopyronium bromide (GLY). Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on reversed-phase Enable C18 column by using acetonitrile: phosphate buffer pH 3.5 (40:60 v/v) as a mobile phase. The retention time for IND and GLY was 3.71 min and 6.79 min respectively. The absorption correction UV spectrophotometric method was performed at 305 nm for IND and at 240 nm for GLY by using Methanol as a solvent. The linearity of the calibration curves for each analyte in the desired concentration range was good ($r^2 > 0.999$) by both the HPLC and UV methods. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. The two methods were compared using student t-test and t-calculated value was found to be less than t_{tab} value indicating that there is no significant difference in the assay results by the two methods. All methods were found to be rapid, specific, precise and accurate and these methods require no preliminary separation and found no interferences from the capsule excipients so it can be used for routine analysis of both drugs in quality control laboratories.

INTRODUCTION:

Indacaterol Maleate (IND) is chemically known as 2-[(5,6-Diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxyquinolin-2(1H)-one (Figure 1). IND stimulate adrenergic β_2 receptors in the smooth muscle of the airways. IND prevents airway spasms caused by chronic obstructive pulmonary disease (COPD). This drug is indicated for the treatment of COPD. This causes relaxation of the muscle, thereby increasing the diameter of the airways, which becomes constricted in asthma and COPD [1,2,3]. Glycopyrronium bromide (GLY) is a chemically 1, 1 -

dimethylpyrrolidin-1-ium-3-yl 2-cyclopentyl-2-hydroxy phenyl acetate bromide (Figure 2). GLY is a synthetic anticholinergic agent with a quaternary ammonium structure. It reduces secretions in the mouth, throat, airways, and stomach before surgery [4,5]. It used along with other medicines to treat peptic ulcers. The combination of Indacaterol Maleate and Glycopyrronium Bromide mainly used as β_2 adrenoreceptor -agonist and anticholinergic agent with a quaternary ammonium structure and widwly used in COPD [6]. The deep literature review revealed that various analytical methods like spectrophotometric, HPLC, HPTLC, stability indicating HPLC, LC-MS and other methods are reported for estimation of IND and GLY individually and in combined with other dosage form and in biological fluids but none of the analytical method is reported for simultaneous estimation of both the drugs in combined pharmaceutical dosage form [7-13]. Therefore, there is a challenge to develop RP-HPLC and UV spectrophotometric method for the simultaneous estimation of Indacaterol maleate and Glycopyrronium bromide. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical, and precise RP-HPLC and Absorption correction UV spectrophotometric method for the simultaneous estimation of two drugs in pharmaceutical dosage form.

MATERIALS AND METHODS

2.1 Reagent and chemicals

IND (Cipla pvt. Ltds., Mumbai) and GLY (Vav Life Sciences Pvt Ltd., Mumbai.) were received as gift sample. Marketed formulation containing 110 mcg of IND and 50 mcg of GLY was purchased from Local Market. HPLC grade acetonitrile and purified grade potassium di-hydrogen phosphate were purchased from Merck Specialities Pvt Ltd., Mumbai, India. All other reagents employed were of high purity analytical grade. All weighing was done on a calibrated digital balance (Shimadzu ATX 224, Japan). Calibrated glass wares were used throughout the work. Double distilled water and Mili-Q water were used in the UV method and RP-HPLC method respectively.

2.2 RP-HPLC method

2.2.1. Instrumentation

The analysis was carried out on a HPLC system (Shimadzu-LC 20AT) equipped with UV detector, pressure controlled by prominence pump and operated by LC solution. Enable C18 column (250 mm × 4.6 mm i.d., particle size 5 µm) was used for separation. Mobile phase used for separation of mixture containing acetonitrile: phosphate buffer pH 3.5 (40:60 v/v). The flow rate was kept at 1.0 mL/min, column temperature was ambient (25°C), eluents were detected by UV detector at 230 nm, and the injection volume was 25 µL.

2.2.2. Chromatographic Condition

Optimal composition of the mobile phase was determined to be acetonitrile: phosphate buffer pH 3.5 (40:60 v/v). The mobile phase was filtered through nylon 0.46 µ membrane filter and was degassed before use (30 min). Stock solution was prepared by dissolving IND and GLY (10 mg each) that were weighed accurately and separately transferred into 100 ml volumetric flasks. Both drugs were dissolved in 25 ml of mobile phase to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with mobile phase.

These standard stock solutions were observed to contain 100 µg/ml of IND and 100 µg/ml GLY. Appropriate volume from this solution was further diluted to get appropriate concentration levels according to the requirement. From the above stock solutions, dilutions were made in the concentration range of 2.2-13.2 µg/ml of IND and 1.0-6.0 µg/ml of GLY, respectively. A volume of 25 µL of each sample was injected into column.

2.2.3. Preparation of buffer

Dissolve 3.6 gm of potassium dihydrogen orthophosphate and 2 ml of triethylamine in 800 ml water adjust the pH to 3.5 with orthophosphoric acid and add sufficient water to produce 1000 ml with distill water. The prepared buffer was passed through 0.46 µ membrane filter (Milipore, USA) and the same was used for mobile phase preparation.

2.2.4. Preparation of mobile phase:

Mobile phase was prepared by mixing HPLC grade acetonitrile and 15mM potassium dihydrogen phosphate buffer (pH 3.5) in 40:60 (v/v) proportions. Mixture was shaken vigorously and sonicated for 30 min prior to use.

2.2.5. Preparation of stock solutions (IND, GLY and binary mixture)

Aqueous solution (100 µg/ml) of IND, GLY and its binary mixture was prepared by adding accurately weighed 10 mg of IND and GLY and binary mixture of both drugs in 50 ml of mobile phase, then sonicated for 10 min and diluted up to 100 ml. Series of test solutions were prepared in the concentration range of 2.2-13.2 µg/ml and 1.0-6.0 µg/ml of IND and GLY respectively, by diluting appropriate volume of the stock solution (100 µg/ml) with mobile phase. The dilutions were first vortexed and then used for further analysis.

2.2.6. Preparation of calibration curve

The calibration curve was prepared by injecting concentration 2.2-13.2 µg/ml of IND and 1.0-6.0 µg/ml of GLY and binary mixture solutions manually to the HPLC system at detection wavelength of 230.0 nm. Mean of n = 6 determinations was plotted as the standard curve. The calibration curve was tested by validating it with inter-day and intra-day measurements.

2.3 UV spectrophotometric method

2.3.1. Instrumentation

The UV method was performed on SHIMADZU double beam spectrophotometer (Model: UV-1800) with 2 nm spectral band width; wavelength accuracy of 0.5 nm using 10 mm matched quartz cuvettes. Data acquisition was done by using UV-probe software version 2.33. The absorption spectra of reference and test solution were carried out over the range of 200–400 nm.

2.3.2. Selection of common solvent

Methanol of analytical grade reagent was selected as a common solvent for developing spectral characteristics of both drugs. The selection was made after assessing the solubility of both drugs in different solvents like water, methanol, chloroform etc.

2.3.3. Determination of wavelength of maximum absorbance (λ_{max}) of IND and GLY

Wavelength of maximum absorption was determined by scanning 22 µg /ml solution of IND and 10 µg /ml GLY using UV–visible double beam spectrophotometer from 200 to 400 nm using Methanol as blank.

2.3.4. Preparation of standard stock solutions (IND, GLY and Binary mixture)

Aqueous solutions (100 µg/ml) of IND, GLY and its binary mixture were prepared by adding accurately weighed 10 mg of IND and GLY and binary mixture of both drugs in 50 ml of methanol, then sonicated for 10 min and diluted up to 100 ml.

2.3.5 Absorption correction method

UV spectra of IND and GLY in methanol, it was observed that GLY has zero absorbance at 305 nm, where as IND has substantial absorbance (Figure 6). Therefore, IND was estimated at 305 nm with no interference from GLY. To estimate GLY, absorbance of IND was measured at 240 nm using standard solution of IND (10µg/mL).

The contribution of IND was deducted from the total absorbance of sample mixture at 240 nm. The calculated absorbance for GLY was called as 'Corrected Absorbance' for GLY. The concentration of GLY was determined from calibration curve at 240 nm using corrected absorbance.

Corrected Absorbance = Total Absorbance - Interfering Absorbance

To construct Beer's plot for IND and GLY, stock solutions of both the drugs were prepared in methanol [100 µg/ml]. Also Beer's plot was constructed for IND and GLY in solution mixture at different concentration. Both the drugs followed linearity individually in IND (4.4, 8.8, 13.2, 17.6, 22, 26.4 µg/ml) and GLY (2, 4, 6, 8, 10, 12 µg/ml) and in mixture with the concentration range IND:GLY are (4.4:2, 8.8:4, 13.2:6, 17.6:8, 22:10, 26.4:12 µg/ml). The concentration of two drugs in the mixture can be calculated using following equations

$A = abc$
$Cx = A_1 / ab$
$Cx = A_1 / ax_1 * b$…… (1)
$A_2 = A_{IND} + A_{GLY}$
$A_2 = (ay_2 * Cy * b) + (ax_2 * Cx * b)$
$A_2 = (ay_2 * Cy) + (ax_2 * Cx)$
$Cy = [A_2 - (ax_2 * Cx)] / ay_2$…….(2)

where A_1 , A_2 are absorbance of mixture at 240 nm (λ1) and 305 nm (λ2), respectively, ax_1 and ax_2 are absorptivities of IND at λ1 and λ2 , respectively, ay_1 and ay_2 are absorptivities of GLY at λ1 and λ2 , respectively, Cx and Cy are concentrations of IND and GLY, respectively

2.4 Method Validation

Validation was carried out according to ICH guideline [14].

2.4.1. Linearity: The linearity of measurement by RP-HPLC was evaluated by analyzing standard solutions of IND and GLY in the range of 2.2-13.2 µg/ml and 1-6 µg/ml for both drugs respectively. Calibration curve were constructed by plotting average peak area versus concentration for both drugs and shown in figure 4 and 5. While in Absorption correction method, calibration curves were plotted over a concentration range of 4.4-26.4 µg/ml for IND and 2-12 µg/ml GLY. The absorbances of solution were then measured at 305 nm and 240 nm for IND and GLY respectively. The calibration curves were constructed by plotting absorbances versus concentration for both drugs and shown in figure 9 and 10.

2.4.2. Accuracy

For studying the accuracy of the proposed methods, and for checking the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method at three level(.e. spiking 50%, 100%, 150% of IND and GLY). This study was performed by addition of known amounts of IND and GLY to a known concentration of sample solution. The amounts of standard recovered were calculated in terms of mean recovery with the upper and lower limits of % RSD.

2.4.3. Precision

2.4.3.1. Repeatability: The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for IND and GLY without changing the parameter of the proposed spectrophotometry methods.

2.4.3.2. Intermediate Precision: Intra-day precision and inter-day precision for the developed methods were measured in terms of % RSD. The experiments were repeated three times a day for intra-day precision and on 3 different days for inter-day precision. The concentration values for both intra-day precision and inter-day precision were calculated three times separately and % RSD were calculated.

2.4.4. Limit of detection (LOD) and limit of quantitation (LOQ): ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise

ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 σ/S and 10 σ/S criterions, respectively; Where; σ is the standard deviation of y-intercepts of regression lines

S is the slope of the calibration curve

2.4.5. Specificity:

The method specificity was assessed by comparing the chromatograms (HPLC) and scans (UV) obtained from the drug and the most commonly used excipient mixture with those obtained from blank (excipient solution in methanol without drug).

2.4.6. Robustness

Influence of small changes in chromatographic conditions such as change in flow rate, that is, ± 0.2 ml/min, mobile phase composition ±2 ml and wavelength ± 2 nm was studied to determine the robustness of the method for the development of RP-HPLC method for the simultaneous estimation of IND and GLY and their %RSD was determined.

2.4.7. System Suitability

The stock solution containing 3.6 µg/ml of IND and 3 µg/ml of GLY was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit.

2.5. Analysis of marketed formulation:

Commercially available marketed formulation (Capsule) containing both IND and GLY (Ultibro breezhaler) were used for the study. Twenty Capsules (each containing 110 mcg IND and 50 mcg GLY) were accurately weighed and finely powdered. A quantity of powder equivalent to 11 mg of IND and 5 mg of GLY was weighed and transferred to 100 ml volumetric flask. This stock solution was prepared in Methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. Then solution was filtered through whatman filter paper No. 41. This stock solution contains IND 110 µg/ml and GLY 50 µg/ml which used for further dilution for determination of both drugs by UV and RP-HPLC method.

2.5.1. For RP-HPLC

From the above stock solution, 0.4 ml of solution was taken and diluted up to 10 ml with methanol which contains 4.4 µg/ml of IND and 2 µg/ml of GLY. A volume of 25 µL of sample was injected into column. The amount of IND and GLY in sample solution of capsule was calculated. This procedure was repeated for six times. The amount of the drug found in dosage form was shown in Table 8.

2.5.2. For absorption correction method

From the above stock solution, 0.8 ml of solution was taken and diluted up to 10 ml with methanol which contains 8.8 µg/ml of IND and 4 µg/ml of GLY. The absorbance of sample solution was measured at all selected wavelengths. The content of IND and GLY in sample solution of capsule was calculated. This procedure was repeated for six times. The amount of the drug found in dosage form was shown in Table 8.

RESULT AND DISCUSSION

3.1. RP-HPLC method and UV-method validation

RP-HPLC and Absorption correction UV-Spectrophotometric methods were developed for IND and GLY which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations.

The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (Rt) of IND and GLY were 3.71 min and 6.79 min, respectively. The system suitability parameters were shown in table 1.

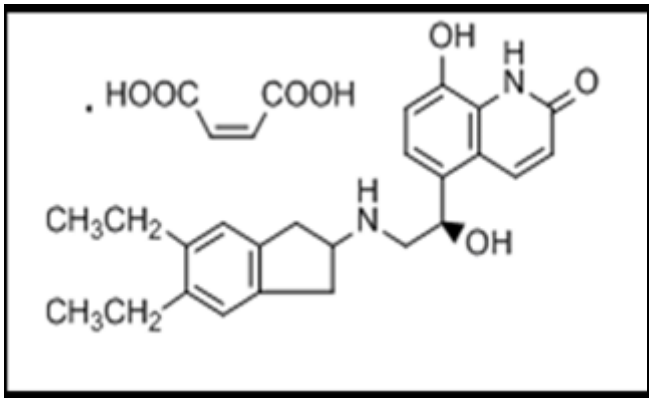

Figure 1: Chemical Structure of Indacaterol maleate

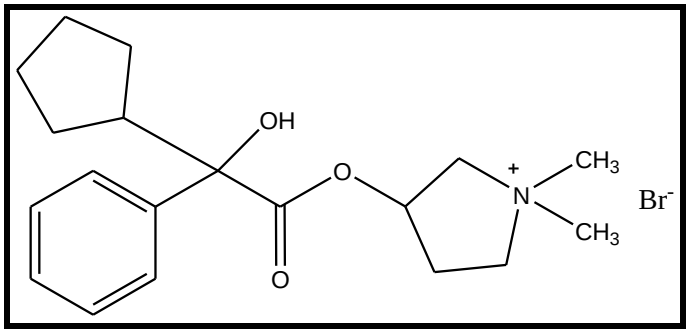

Figure 2: Chemical Structure of Glycopyrronium bromide

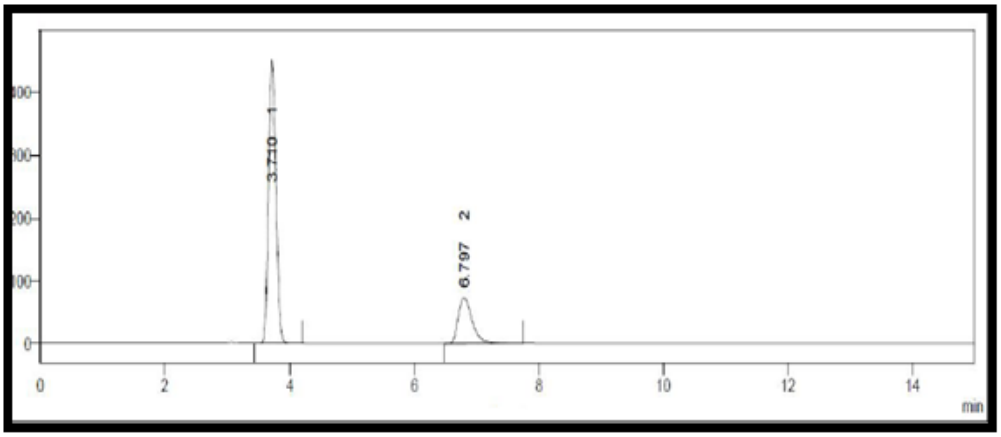

Figure 3: Chromatogram of IND (4.4 µg/ml) and GLY (2 µg/ml) in binary mixture

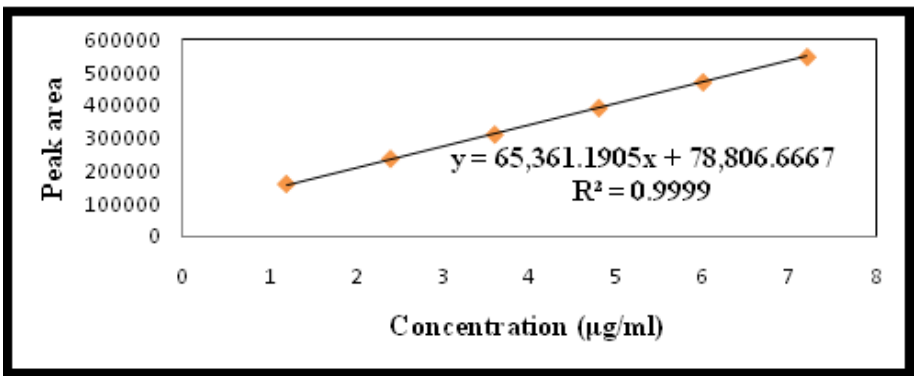

Figure 4: Calibration curve of IND (2.2-13.2 µg/ml) (RP-HPLC method)

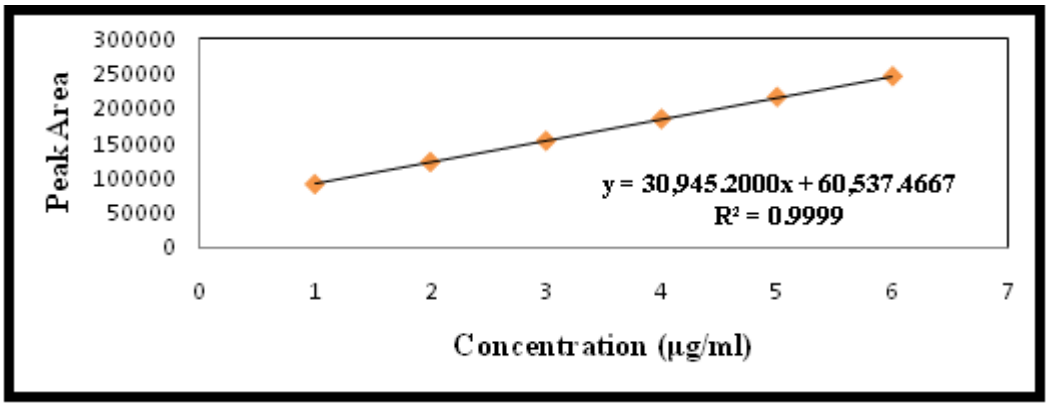

Figure 5: Calibration curve of GLY (1-6 µg/ml) (RP-HPLC method)

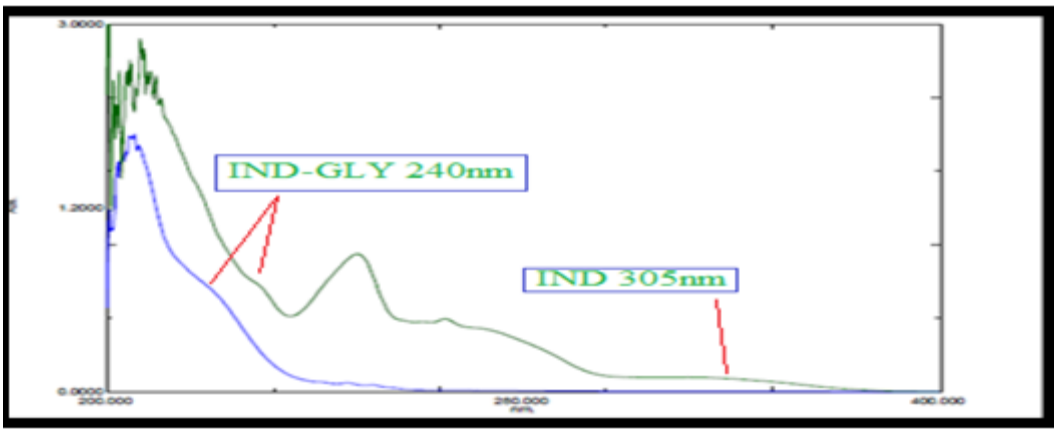

Figure 6: Overlain Absorption correction spectra of IND (22µg/ml) and GLY for (10µg/ml)

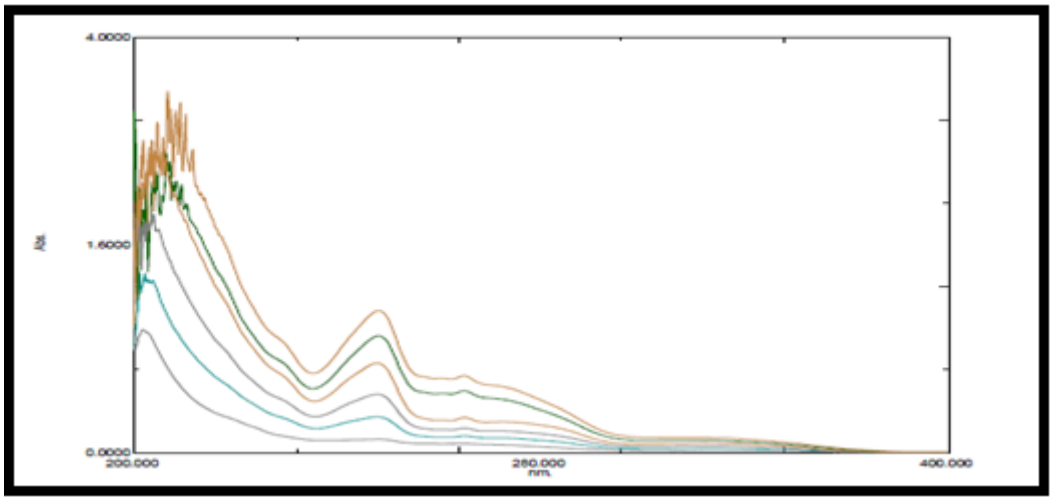

Figure 7: Overlain spectra of IND (4.4-26.4 µg\ml) (UV spectrophotometry)

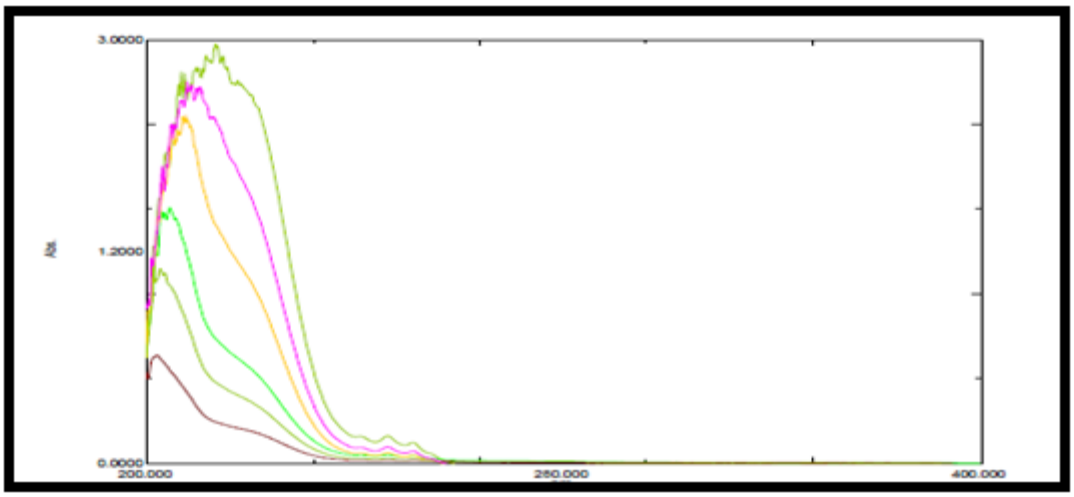

Figure 8: Overlain spectra of GLY (2-12 µg\ml) (UV spectrophotometry)

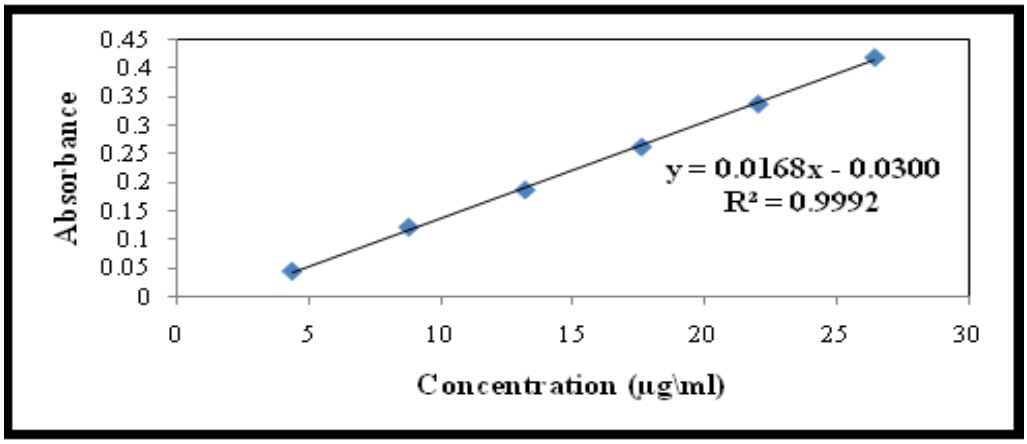

Figure 9: Calibration curve of IND (4.4-26.4 µg\ml) at 305nm (UV spectrophotometry)

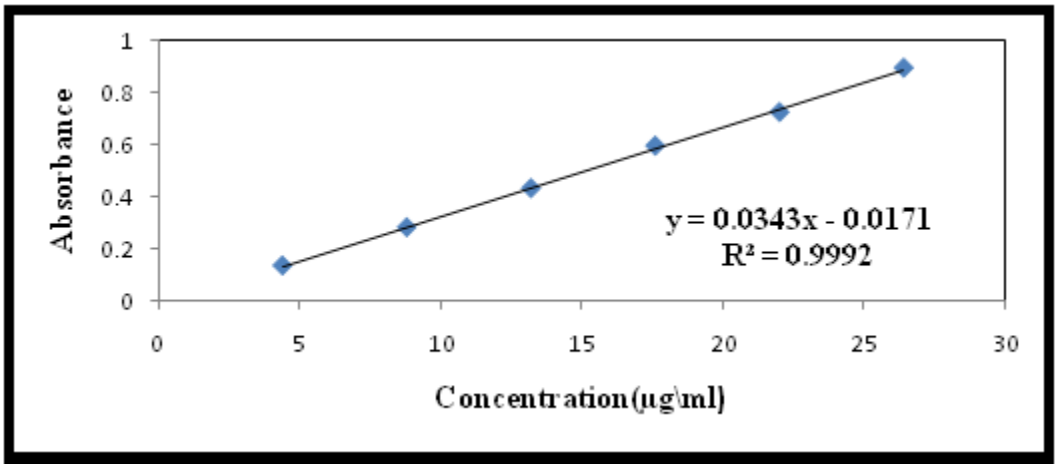

Figure 10: Calibration curve of GLY (2-12 µg\ml) at 240nm (UV spectrophotometry)

Table 1: System suitability parameters

Parameters	Data obtained		Acceptance criteria
	IND	GLY	
Retention time (Rt)	3.727	6.797	% RSD < 2
Theoretical plates per column (N)	4512	4443	> 2000
Symmetry factor/ tailing factor	1.3	1.6	< 1.5
Resolution (Rs)	9.81		> 2

Table 2: Linearity of IND and GLY (RP-HPLC method)

Sr no.	Concentration (µg/ml)		Peak area ± SD (n=6)		%RSD	
	IND	GLY	IND	GLY	IND	GLY
1	2.2	1	158865 ± 5340.61	91110 ± 474.48	0.33	0.52
2	4.4	2	234566 ± 2929.85	122058 ± 2307.64	1.23	1.87
3	6.6	3	312562 ± 1579.57	152463 ±1479.85	0.50	0.96
4	8.8	4	398563 ± 5132.69	182784 ± 2984.61	1.30	1.61
5	11	5	478563 ± 5669.55	215478 ± 867.18	1.20	0.40
6	13.2	6	552364 ± 2700.05	245123 ± 392.27	0.49	0.15

Table 3: Linearity data for IND and GLY in binary mixture (Absorption correction Method)

Conc. (µg/ml)	Absorbance ± SD (n=6)		% RSD	
	240 nm	305 nm	240 nm	305 nm
4.4:2	0.136 ± 0.001528	0.045 ± 0.00057	1.11	1.27
8.8:4	0.283 ± 0.001528	0.122 ± 0.00015	0.53	1.24
13.2:6	0.433 ± 0.00200	0.187 ± 0.00115	0.46	0.61
17.6:8	0.596 ± 0.00115	0.262 ± 0.00152	0.19	0.58
22:10	0.725 ± 0.00152	0.337 ± 0.00152	0.21	0.45
26.4:12	0.895 ± 0.00100	0.418 ± 0.00100	0.11	0.23

Table 4: Intraday and Interday precision data of IND and GLY(RP-HPLC and UV method)

Drug	RP-HPLC					UV Spectrophotometry				
	Conc. (µg/ml)	Intraday Variation (n=3)		Interday Variation (n=3)		Conc. (µg/ml)	Intraday Variation (n=3)		Interday Variation (n=3)	
		Mean Peak Area ± SD	%RSD	Mean Peak Area ± SD	%RSD		Mean Abs ± SD	%RSD	Mean Abs ± SD	%RSD
IND	4.4	232993 ± 2165.41	0.92	233277 ± 2745.85	1.17	8.8	0.099 ± 0.00100	1.01	0.102 ± 0.00100	0.99
	6.6	311661 ± 1563.46	0.50	312277 ± 2296.76	0.73	13.2	0.155 ± 0.00057	0.37	0.155 ± 0.00057	0.37
	8.8	394013 ± 3720.90	0.94	393838 ± 3830.14	0.97	17.6	0.229 ± 0.00057	0.25	0.230 ± 0.00057	0.25
GLY	2	121717 ± 489.81	0.40	121437 ± 536.09	0.44	4	0.098 ± 0.00057	0.58	0.100 ± 0.00100	1
	3	153347 ± 1259.40	0.82	153104 ± 1147.19	0.74	6	0.136 ± 0.00100	0.73	0.137 ± 0.00057	0.41
	4	181658 ± 1299.76	0.71	182626± 1820.61	0.99	8	0.181 ± 0.00057	0.31	0.182 ± 0.00057	0.31

Table 5: Summary of Validation Parameters (RP-HPLC and UV method)

Parameters	RP-HPLC		UV Spectrophotometry	
	IND	**GLY**	**IND**	**GLY**
Working wavelength (nm)	230	230	305	240
Concentration range (µg/ml)	22 - 13.2	1 - 6	4.4 – 26.4	2 - 12
Sandell's sensitivity (µg/cm^2/0.001A.U)	NA	NA	0.660	0.038
Regression Equation	Y=65361.1905x + 78806.6667	Y= 30945.20 + 60537.4667	Y= 0.016x - 0.030	Y = 0.0343x - 0.0171
Correlation coefficient (r^2)	0.9996	0.9999	0.9992	0.9992
SD of slope	65361.21	30945.15	0.016	0.020
SD of intercept	1296.40	1971.95	0.029	0.016
Data Point	6	6	6	6
Retention Time (minute)	3.71	6.79	NA	NA
LOD (µg/ml)	0.06	0.21	0.180	0.024
LOQ (µg/ml)	0.19	0.63	0.547	0.074
Precision				
Repatability (n=6) %RSD	1.177 %	0.767 %	0.52 %	1.17 %
Intraday (n=3) %RSD	0.50-0.94 %	0.40-0.82 %	0.25 - 0.99 %	0.31 – 1.00 %
Interday (n=3) %RSD	0.73-1.17 %	0.44-0.99 %	0.25 - 1.01 %	0.31 – 0.73 %

Table 6: Recovery study of IND and GLY (RP-HPLC and UV method)

Method	Drug	Amt. Present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recover (µg/ml)	% Recovery ± SD	%RSD
RP-HPLC	IND	4.4	2.2	6.6	6.6	100.90 ± 0.77	0.78
			4.4	8.8	6.5	98.48 ± 1.86	1.87
			6.6	11	6.5	98.48 ± 1.48	1.49
	GLY	2	1.	3	5.9	100.01 ± 0.97	1.00
			2	4	6	100.05 ± 0.98	1.00
			3	5	5.89	101.60 ± 1.05	1.07
UV Spectrophotometry	IND	8.8	4.4	13.2	13.1	99.99 ± 1.50	1.51
			8.8	17.6	17.4	99.23 ± 1.18	1.18
			13.2	22	21.9	99.87 ± 1.57	1.59
	GLY	4	2	6	5.9	99.84 ± 1.62	1.63
			4	8	8.1	100.15 ± 1.43	1.43
			6	10	9.8	98.88 ± 0.96	0.97

Table 8: Assay of marketed formulation (RP-HPLC and UV)

Assay Method	Drug	Label Claim (µg/ml)	Amount found (µg/ml)	% Label Claim ± SD (n=6)
RP-HPLC	IND	110	111.01	100.89 ± 0.396
	GLY	50	50.49	100.98 ± 0.407
UV Spectrophotometry	IND	110	110.70	100.63 ± 1.46
	GLY	50	50.15	100.30 ± 0.637

Table 7: Robustness study of IND and GLY (RP-HPLC method)

S.no	Parameters	Variation	%Assay		± SD (n=3)		% RSD	
			IND	GLY	IND	GLY	IND	GLY
1	Flow rate (1± 0.2ml/min)	0.8ml/min	98.33	99.71	0.161	0.206	0.164	0.207
		1.0ml/min	98.33	99.66				
		1.2ml/min	98.61	99.33				
2	Wavelength (nm)	218	98.31	99.68	0.205	0.223	0.208	0.223
		220	98.49	99.76				
		222	98.72	99.34				
3	Mobile phase (%v/v)	38:62	98.19	99.71	0.262	0.258	0.265	0.259
		40:60	98.39	99.76				
		42:58	98.71	99.29				

Table 9: Data for statistical comparison result of UV and HPLC method

Drug	% Assay result				t-test
	HPLC		UV Spectrophotometry		
IND	101.31	101.33	103.23	99.56	0.15
	100.43	100.44	100.46	99.38	
	100.91	100.92	101.61	101.62	
GLY	101.23	101.34	100.25	100.89	0.77
	100.23	101.16	100.75	100.82	
	101.12	100.82	101.85	100.01	
Limit of 95% confidence interval, t_{table} = 2.015					

The chromatograms have been shown in Figure 3. A six point calibration curve was constructed with working standards and was found linear (r^2 = 0.999) for each of the analytes over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram (Figure 4 and 5). The developed RP-HPLC method was accurate, precise, reproducible and very sensitive. Figure 7 and 8 shows Overlain UV spectra of IND and GLY. The regression coefficient of the correlation equation curve was greater than 0.999 (figure 9 and 10) and the method was validated by using binary mixture of both drugs with less than 2% RSD. Table 2 and 3 shows Linearity data of RP-HPLC method and UV method respectively. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 4). All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines as shown in Table 5. The percent recovery (content uniformity) of both drugs in the commercial formulations by RP-HPLC and UV methods is shown in Table 6. Moreover the %R.S.D. (less variation) shows good precision of both developed methods. Table 7 shows the robustness study of IND and GLY and % RSD value for all changed parameter was found to be within limit. Hence, it can be considered that the proposed method is robust. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. Hence, the methods were suitably employed for assaying both the drugs in commercial marketed formulation (Table 8).

Statistical comparison of HPLC and UV methods

Statistical comparison was done on assay results obtained from UV and HPLC methods for marketed formulation (Ultibro breezhaler) by using student's t-test as shown in table 9. Calculated values for t-test were 0.15 and 0.77 for IND and GLY respectively which is less than t_{table} value

(2.015) indicating that there was no significant difference between the HPLC method and UV method.

CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of IND and GLY in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of IND and GLY in multi-component formulations without interference of each other. The results of UV method showed no significant difference from the HPLC method.The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of capsule dosage forms. The developed method can also be conveniently adopted for dissolution testing of IND and GLY in commercial formulation.

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