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# APPLICATION OF TLC DENSITOMETRIC AND UV SPECTROPHOTOMETRIC TECHNIQUES FOR SIMULTANEOUS DETERMINATION OF INDACATEROL AND GLYCOPYRRONIUM IN INHALATION CAPSULES USED FOR TREATMENT OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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## ABSTRACT
Indacaterol and glycopyrronium are co-formulated as a maintenance bronchodilator treatment to relieve symptoms in adult patients with chronic obstructive pulmonary disease (COPD). In the present work; first reported, accurate, precise TLC densitometric method and UV spectrophotometric methods developed for simultaneous determination of indacaterol and glycopyrronium in their bulk and dosage form. The proposed TLC densitometric method based on determination of the UV-visualized bands after TLC separation of indacaterol and glycopyrronium using 60 F254 silica gel plates and mobile phase consists of (60% Methanol:30%ethylacetate:10%water by volume) with UV detection at 260 nm. The relationship between the concentration of standard solutions and the peak response is linear within the concentration range of 1-6 µg/spot for indacaterol and 0.5-3 µg/spot for glycopyrronium. Alternatively the UV spectrophotometric methods based on determination of indacaterol in presence of glycopyrronium using zero order spectrophotometric method also determination of glycopyrronium in presence of indacaterol through manipulation of the ratio spectra by three methods namely; ratio difference, ratio derivative and mean centering. The proposed techniques have been validated according to ICH guidelines and show high sensitivity, accuracy and precision and the results were statistically compared to reported method.

**KEYWORDS:** Indacaterol, Glycopyrronium, TLC-Densitometry, UV-spectrophotometric methods, Ultibro®.

## INTRODUCTION
Indacaterol maleate is 5-{(1*R*)-2-[(5,6-diethyl-2,3-dihydro-1*H*-inden-2-yl)amino]-1- hydroxyethyl}-8-hydroxy-2(1*H*)-quinolinone maleate **(Fig.1).** It's white to very slightly grayish or very slightly yellowish not hygroscopic powder. It is soluble in methanol, very slightly soluble in water, insoluble in 0.9% NaCl; it's molecular weight; 508.57 and molecular formula; $C_{28}H_{32}N_2O_7$.[1] Glycopyrronium bromide is 3-(2-cyclopentyl-2-hydroxy-2-phenylacetoxy)-1,1-dimethylpyrrolidinium bromide **(Fig.2).** It's a white, non-hygroscopic powder, freely soluble in water, soluble in methanol (96%), very slightly soluble in methylene chloride it's molecular weight; 398.34 and molecular formula; $C_{19}H_{28}BrNO_3$.[1] Ultibro® Breezhaler is a medicine that contains two active substances, indacaterol (143 micrograms) and glycopyrronium (63 micrograms). It is used as maintenance treatment to relieve symptoms of chronic obstructive pulmonary disease (COPD) in adults. COPD is a long-term disease in which the airways and air sacs inside the lungs become damaged or blocked, leading to difficulty breathing air in and out of the lungs.[2] There is a reported spectrophotometric method for simultaneous determination of indacaterol and glycopyrronium.[3] Few LC-MS methods were reported for determination of indacaterol in plasma and urine.[4-6] Spectrophotometric method were also reported for determination of indacaterol in pharmaceutical formulation,[7] Literature survey revealed that different methods for determination of glycopyrronium in biological fluids using LC-MS,[8,9] HPLC,[10] GC,[11] visible spectrophotometric method.[12]

To date there is no reported TLC-densitometric method for simultaneous estimation of indacaterol and glycopyrronium in combined dosage forms also the proposed spectrophotometric methods were firstly applied for analysis of both drugs simultaneously.

Hence, the objective of this work is to develop an accurate, selective and precise TLC-densitometric and UV spectrophotometric techniques for simultaneous

determination of indacaterol and glycopyrronium in bulk and inhalation capsules.

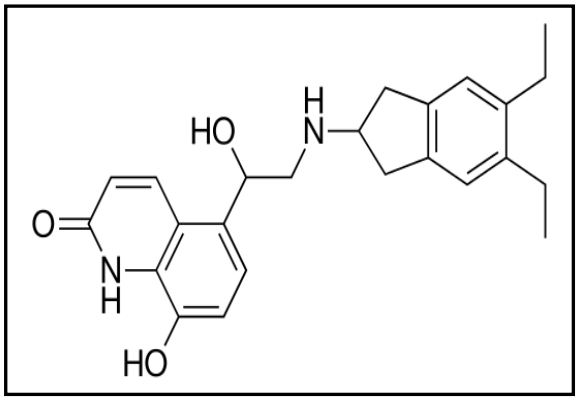


**Figure 1: Structural formula of indacaterol maleate.**

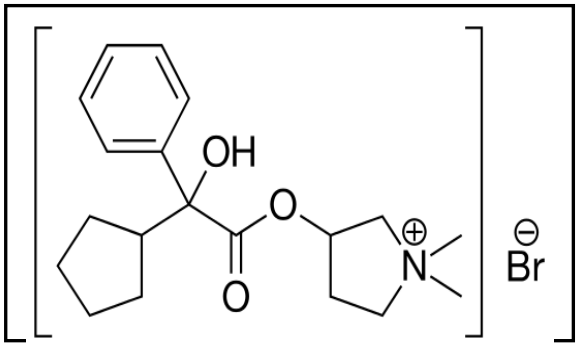


**Figure 2: Structural formula of glycopyrronium bromide.**

## EXPERIMENTAL

***Instruments***

- Camag-Linomat 5 auto sampler (Switzerland) was used for data acquisition. The system is equipped with a deuterium and halogen tungsten lamp as a radiation, while a 100 µL syringe(Hamilton, Bonaduz, Switzerland) was used for sample application, the scanning mode is absorbance mode, the slit dimension is 3 mm × 0.45 mm, the scanning speed is 20 mm s–1.
- Pre-coated TLC plates, silica gel 60 GF254 (20 × 20 cm), (Fluka chemie, Switzerland).
- SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and a HP1020 LaserJet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 0.2 nm interval.

- ***Software***

Matlab 8.2.0.701 (R2013b), all calculations were performed using Intel(R) Core(TM) i3 CPU, 2.13 GHz, and 4.00 GB of RAM under Microsoft Windows 7 Home Premium™.

## MATERIALS

**Pure samples**

- Pure indacaterol maleate (99.7 %) was kindly supplied by Novartis Company, Egypt.
- Pure glycopyrronium bromide (99.5 %) was kindly supplied by Novartis Company, Egypt.

**Pharmaceutical preparation**

- Ultibro® Breezhaler: each capsule claimed to contain 143µg indacaterol maleate and 63 µg glycopyrronium bromide (B.No.S0308, manufactured by Novartis pharma stein, Switzerland), purchased from local market.

**Chemicals and solvents**

All chemicals and reagents used throughout this work were of analytical grade. Methanol, ethyl acetate purchased from (El-Nasr Pharmaceutical Chemicals Co. Abu- Zabaal, Cairo, Egypt).Water is freshly distilled.

**Standard solutions**

A standard solution of indacaterol (1mg/ml) was prepared by dissolving 100 mg of the drug powder in 50 ml of methanol and the volume was completed to 100 ml with the methanol. Working solution of (100 µg/ml) was prepared from the stock solution by suitable dilution with methanol.

A standard solution of glycopyrronium (1mg/ml) was prepared by dissolving 100 mg of the drug powder in 50 ml of methanol and the volume was completed to 100 ml with the methanol. Working solution of (100 µg/ml) was prepared from the stock solution by suitable dilution with methanol.

**Pharmaceutical sample solution**

Contents of 25 capsules of **Ultibro®Breezhaler** were mixed and weighed. All amount equivalent to 25 capsules content (3.575 mg of indacaterol maleate and 1.575 mg of glycopyrronium bromide) was extracted by shaking with 20 ml of methanol for 15 minutes, then filtered into 25 ml volumetric flask and the volume was adjusted with the methanol to obtain a solution labeled to contain (143 µg/ml of indacaterol maleate and 63 µg/ml glycopyrronium bromide).Apply the general procedure using aliquots covering the working concentration range. Determine the content of the Breezhaler® capsules from the corresponding regression equations.

**Procedures**

- **Chromatographic condition for TLC-densitometric method**

Analysis was performed on pre-coated 20 × 10 cm TLC aluminum silica gel 60 GF254 plates. Samples were applied to the plates using Hamilton micro syringe (50-µL). Plates were spotted 1 cm apart from each other and 1 cm apart from the bottom edge. The chromatographic tank was pre-saturated with the mobile phase for 20 min, then developed by ascending chromatography using (60% Methanol: 30%ethylacetate: 10%water by volume)

as a mobile phase. The plates were air dried and the spots were scanned at 260 nm with CAMAG TLC scanner. The produced bands were scanned under deuterium lamp at 260 nm. The software used for data measurement, collection and analysis is Wincats software. Concentrations of indacaterol and glycopyrronium in the produced bands were appears as peak areas in the TLC-densitometric chromatograms. The produced peak areas were plotted versus analyte concentrations and the regression equation of the linear relation was computed.

**Linearity and construction of calibration graphs**

Into two separate series of 10 mL volumetric flasks, aliquots of standard solutions equivalent to (1-6 mg) and (0.5-3 mg) of indacaterol and glycopyrronium respectively, were transferred separately and diluted to volume with methanol. So the series of flasks contain (100-600) µg/mL and (50-300 µg/mL), 10µL of each solution were applied to a TLC plate following the above mentioned specific chromatographic conditions and scanned at 260 nm.

**Spectral characteristics of indacaterol and glycopyrronium**

**Construction of Calibration Curves**

Aliquots equivalent to 12–48 µg/ml indacaterol and 6–20 µg/ml glycopyrronium are accurately transferred from their standard working solutions (100µg/ml) into two separate series of 10-ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 to 400 nm and stored in the computer.

**Zero order method; for determination of indacaterol**

The measured absorbance values at 293 nm (zero crossing for glycopyrronium) versus the final drug concentrations in µg/ml were plotted to get the calibration graph and the regression equation was derived.

**Manipulation of ratio spectra; for determination of glycopyrronium**

- **Ratio difference method:** the absorption spectra of glycopyrronium divided by the spectrum of indacaterol solution (14µg/ml). The difference in the peak amplitudes (ΔP) at the ratio spectra was measured at 227 and 269 nm (ΔP 227-269 nm).

- **Ratio derivative method ($^1$DD)**

The stored spectra of glycopyrronium are divided by the spectrum of 14µg/ml indacaterol, then the first derivative of the ratio spectra ($^1$DD) with Δ=4 nm is obtained. A calibration graph relating the peak amplitude at 234 nm to the corresponding concentrations in µg/ml of glycopyrronium is constructed.

- **Mean centering method**

The scanned spectra of glycopyrronium are divided by the spectrum of 14µg/ml indacaterol and the obtained ratio spectra are mean centered. The calibration curve of glycopyrronium is constructed by plotting the mean centered values at 227 nm versus the corresponding concentration.

**Assay of laboratory prepared mixtures**

Aliquots of indacaterol and glycopyrronium were mixed to prepare different mixtures containing different ratios of both. The procedures mentioned under construction of calibration curves for both techniques were followed and the concentrations of both drugs were calculated.

**Assay of pharmaceutical formulation**

**For TLC-densitometric method:** Aliquots from pharmaceutical sample solution (143µ/mL of indacaterol: 63µg/mL glycopyrronium); were 10, 15,20,25,30 µL applied to a TLC plate following the above mentioned specific chromatographic conditions and scanned at 260 nm.

**For spectrophotometric methods:** By using aliquots covering the working concentration ranges from pharmaceutical sample solution (143µ/mL of indacaterol: 63µg/mL glycopyrronium). The content of the capsules were calculated using the mentioned general procedure for each method.

## RESULTS AND DISCUSSION

In the present study, a simple, accurate and precise TLC-densitometric and UV-spectrophotometric techniques were applied as first reported methods for simultaneous analysis of indacaterol and glycopyrronium in bulk and pharmaceutical formulation:

- **TLC-densitometric method**

**Method optimization:** To obtain the most appropriate mobile phase, selection of suitable mobile phase is carried out by controlled trials and errors. Different developing systems of different compositions and different percentages of each component were tried, such as water - methanol - chloroform (40:40:20, by volume), methanol - acetonitrile - tri ethylamine (50:20:30, by volume), methanol- ethyl acetate- toluene (50: 40: 10, by volume); which gave poor resolutions, band broadening, tailing and an asymmetric peaks. Upon using a mixture of methanol: ethyl acetate - water in different ratios, resolution was obtained with tailed bands to some extent until using the ratio (60: 30: 10, by volume) which gave the optimum resolution. The Rf values were 0.27 ± 0.005 and 0.69 ± 0.01 for indacaterol and glycopyrronium, respectively (**Fig.3**). Quantitative determination of indacaterol and glycopyrronium was performed by scanning the bands at 260 nm.

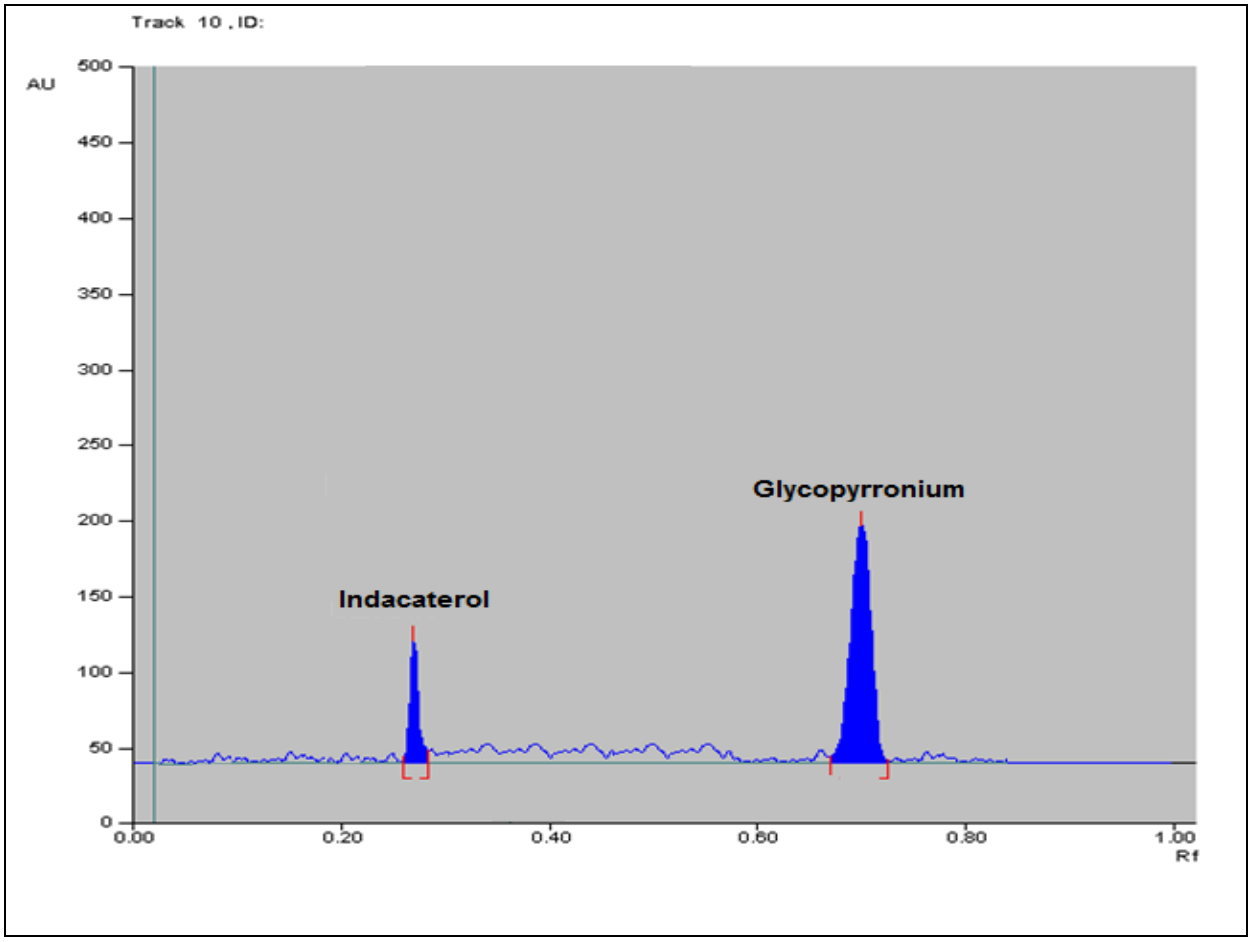


**Fig. 3: Two-dimensional TLC densitogram of indacaterol and glycopyrronium at 3 and 1.5 µg/band, respectively.**

**Spectrophotometric methods**

**Zero order method; for determination of indacaterol**

The zero-order absorption spectra of indacaterol and glycopyrronium show zero crossing of glycopyrronium in the range from 290-400 nm, which allows direct determination of indacaterol at 293 nm without interference from glycopyrronium, as shown in **(Fig 4).** The absorbance of the zero-order spectra of indacaterol was measured at 293 nm due to increased sensitivity.

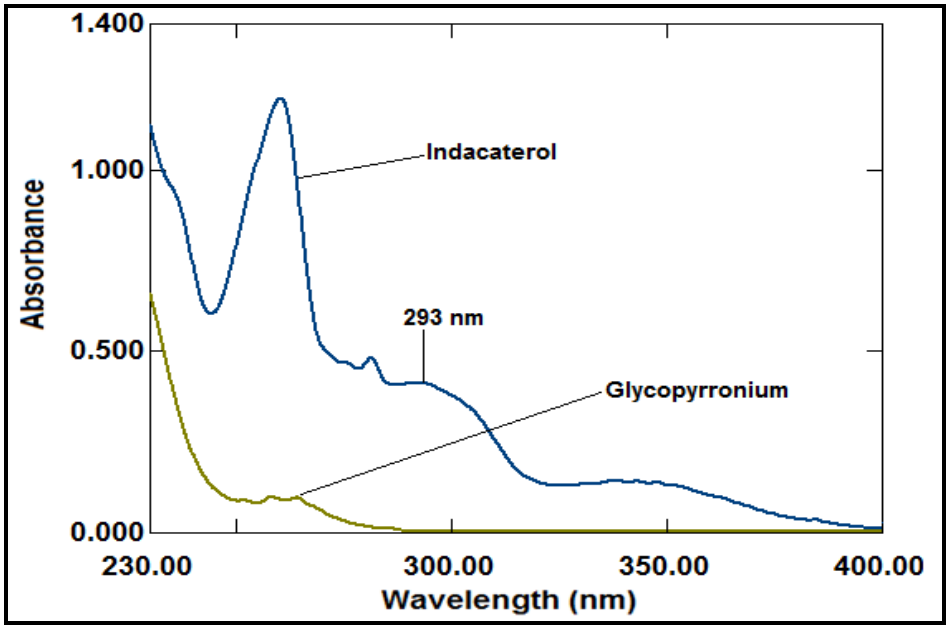


**Figure 4: Zero-order absorption spectra of indacaterol (24 µg/ ml) and glycopyrronium (20 µg/ ml) in methanol.**

**Manipulation of ratio spectra; for determination of glycopyrronium**

- **Ratio difference method**

The absorption spectra of glycopyrronium were divided by the absorption spectrum of indacaterol (14µg/ml) as a divisor to get the ratio spectra, as shown in (**Fig.5**). The difference in peak amplitudes between 227 and 269 nm in the ratio spectra is proportional to the concentration of glycopyrronium without interference from indacaterol.

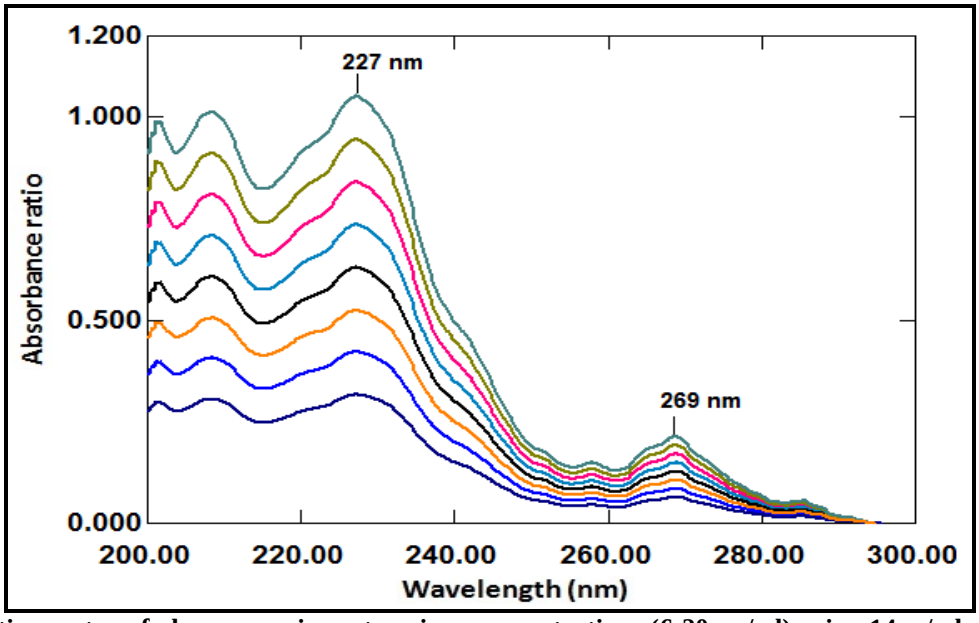


**Figure 5: Ratio spectra of glycopyrronium at various concentrations (6-20 µg/ml) using 14µg/ml of indacaterol as a divisor.**

**Ratio derivative method: ($^1$DD)**

The absorption spectra of glycopyrronium were divided by the absorption spectrum of indacaterol (14µg/ml) as a divisor to get the ratio spectra, as shown in (**Fig.5**). The amplitude of the first derivative of the ratio spectra at 234 nm are proportional to the concentrations of glycopyrronium without interference from indacaterol, as shown in (**Fig.6,7**).

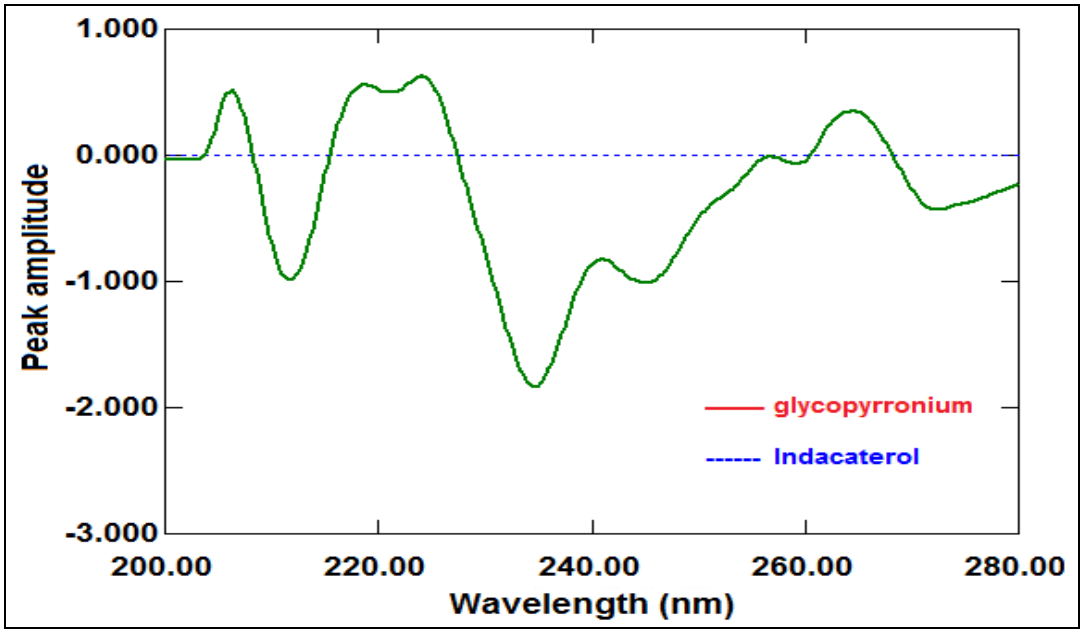


**Figure 6: First derivative of the ratio spectra of glycopyrronium (14µg/ml) and indacaterol (14µg/ml) using 14 µg/ml of indacaterol as a divisor.**

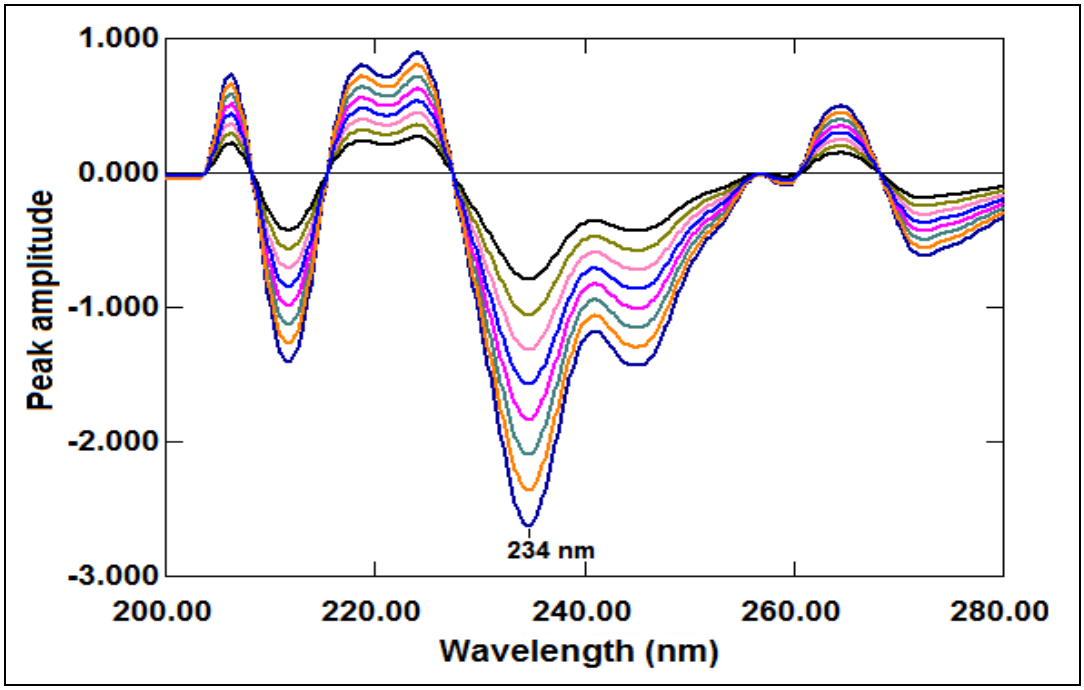


**Figure 7: First derivative of the ratio spectra of glycopyrronium at various concentrations (6-20 µg/ml) using 14 µg/ml of indacaterol as a divisor.**

**Mean centering method**

For further improvement of the selectivity to analyze glycopyrronium in presence of indacaterol, a simple method is applied; this method is based on the mean centering of ratio spectra. It eliminates the derivative step and therefore the signal-to-noise ratio is enhanced. In this method, the absorption spectra of glycopyrronium were divided by the absorption spectrum of indacaterol (14 µg /ml) as a divisor to get the ratio spectra as shown in (**Fig.5**). The obtained ratio spectra (200-300 nm) were mean centered. The mean centered values at 227 nm are proportional to the concentrations of glycopyrronium without interference from indacaterol, as shown in (**Fig.8**).

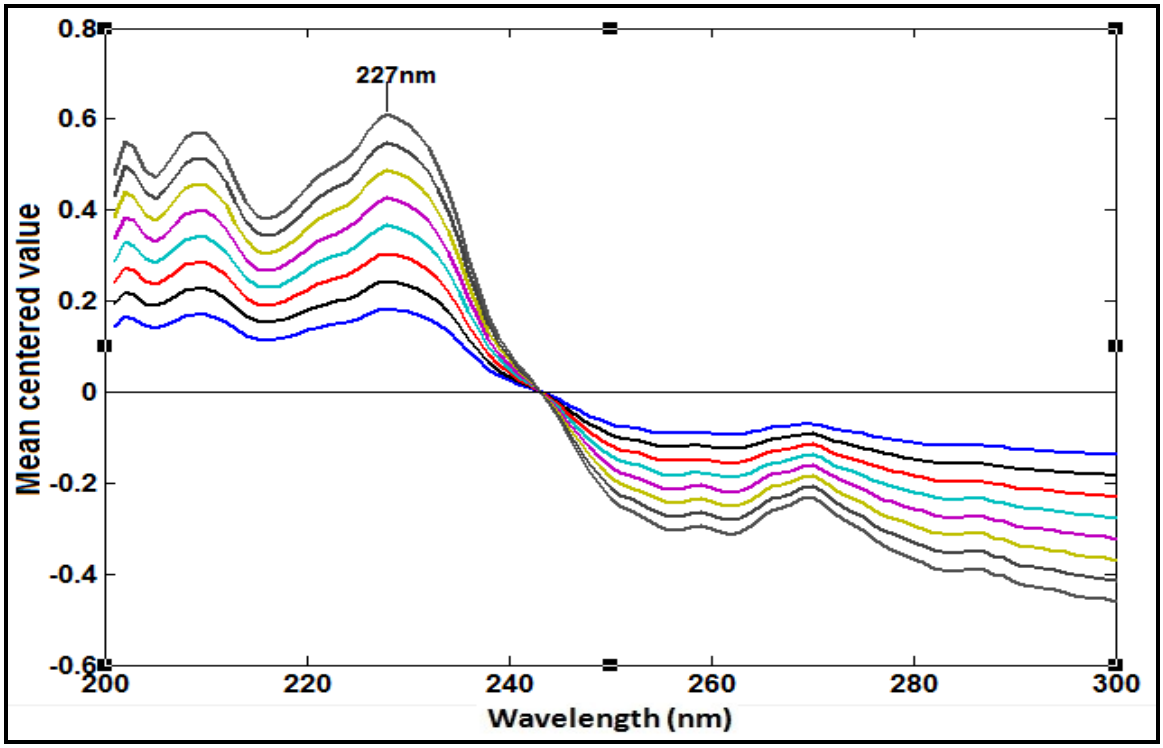


**Figure 8: Mean centering of the ratio spectra of glycopyrronium at various concentrations (6-20 µg/ml) using 14 µg/ml of indacaterol as a divisor.**

**Validation of the methods**[14]

Method validation was performed according to International Conference on Harmonization (ICH) guidelines.

**(a) Linearity and range**

**- For TLC-densitometric method**

Six concentration levels of indacaterol and glycopyrronium were selected to construct the calibration curves. Linearity of the proposed method was evaluated and found to be in the concentration range of (1-6) and (0.5-3) µg/band for indacaterol and glycopyrronium respectively. (**Fig.9).**The regression plots were found to be linear over the mentioned ranges, the linear regression equations were:

Y1= 701.5286 C1 + 274.0667 ($r^2$ = 0.9997).
Y2 = 1255.4280C2 + 594.8460 ($r^2$ = 0.9998).

Where Y1 and Y2are the integrated peak areas for indacaterol and glycopyrronium respectively, C1 and C2 are the corresponding concentrations in µg/band for indacaterol and glycopyrronium respectively and $r^2$ is the determination coefficient.

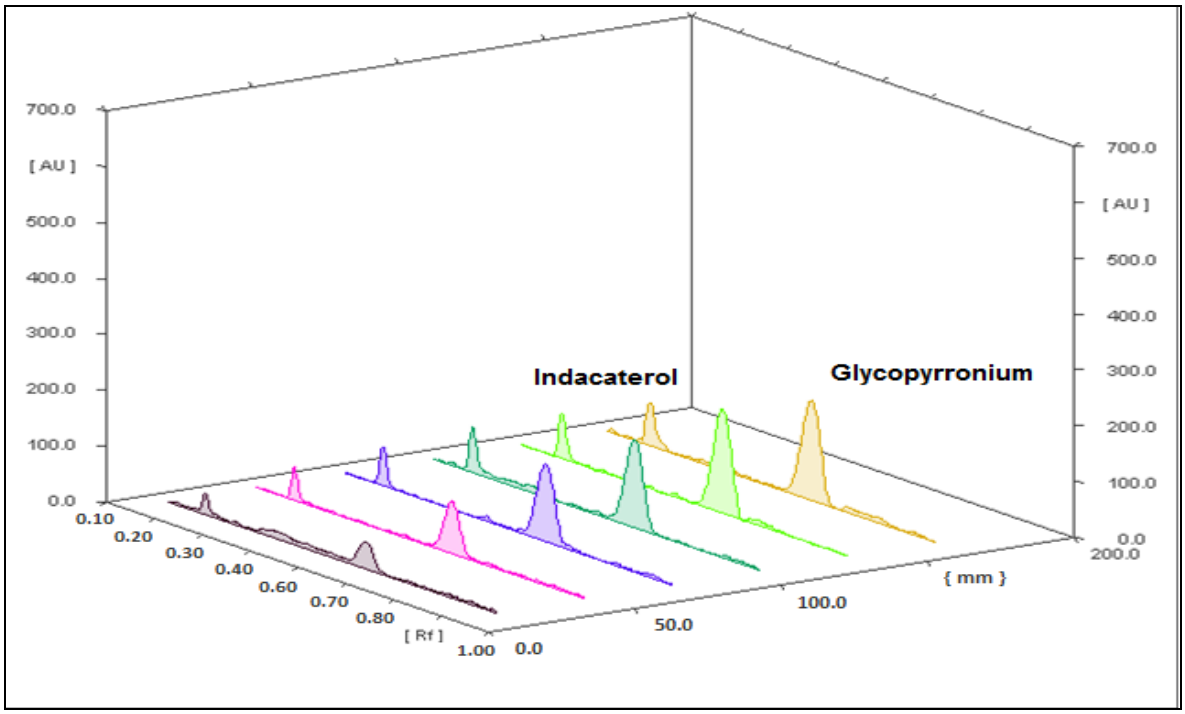


**Figure 9: Three-dimensional TLC densitogram of indacaterol and glycopyrronium mixtures ranging from (1-6) µg/band and from (0.5-3) µg/band, respectively.**

The high values of the determination coefficients and the low intercept value indicate the excellent linearity of the proposed method. Linearity range, regression equation, intercept, slope and the determination coefficient for the calibration data were presented in **table 1.**

**-For Spectrophotometric methods:** Linearity range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in **table 2.**

**(b) Sensitivity - Limit of detection (LOD) and Limit of quantitation (LOQ)**

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

LOD = 3.3 Sa / slope LOQ = 10 Sa / slope

Where Sa is the standard deviation of the intercept of regression line.

**(c) Accuracy**

Three replicate determinations of three different concentrations of indacaterol and glycopyrronium in pure forms within linearity range were performed. Accuracy as percent recovery (%R) was calculated, the calculated values of (%R) confirms excellent accuracy as shown in **table 1, 2.**

**(d) Precision**

Three replicate determinations of three different concentrations of indacaterol and glycopyrronium in pure forms within linearity range were performed in the same day (repeatability) and on three successive days (intermediate precision) for the analysis of the three chosen concentrations using the proposed method. Acceptable % RSD was obtained, confirming the precision of the methods as shown in **table 1, 2.**

**(e) Specificity**

The specificity of the proposed procedures was assured by applying it to laboratory prepared mixtures of

indacaterol and glycopyrronium using the standard addition technique. Laboratory prepared mixtures of indacaterol and glycopyrronium are subjected to analysis by the proposed method in presence of the same concentration of pharmaceutical formulation. The obtained results were satisfactory as shown in **table 3, 4**. The methods were suitable for the determination of the indacaterol and glycopyrronium in raw materials and pharmaceutical formulation; therefore, it is considered to be specific and selective.

**Application to pharmaceutical formulation**

The proposed procedures were applied for simultaneous determination of indacaterol and glycopyrronium in Ultibro® inhalation capsules. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [3]. No significant differences were found by applying t-test and F-test at 95% confidence level [15], indicating good accuracy and precision of the proposed method for the analysis of the studied drug in their pharmaceutical dosage form, as shown in **table 4, 5.**

**Table 1: Calibration data for simultaneous determination of indacaterol and glycopyrronium by the proposed TLC-densitometric method:**

| Calibration parameters | | TLC-densitometric method | |
|---|---|---|---|
| | | Indacaterol | Glycopyrronium |
| Wavelength (nm) | | 260 | |
| Linearity range (µg/band) | | 1-6 | 0.5-3 |
| - Regression Equation | | *y= b x+ a* | *y= b x+ a* |
| -Slope (b) | | 701.528 | 1225.428 |
| -Intercept (a) | | 274.066 | 594.846 |
| Coefficient of determination ($r^2$) | | 0.9997 | 0.9998 |
| Accuracy (%R)* | | 99.67 | 100.02 |
| Precision (%RSD)* | Repeatability | 0.869 | 0.773 |
| | Intermediate precision | 0.963 | 0.865 |
| LOD(µg/band) | | 0.108 | 0.047 |
| LOQ(µg/band) | | 0.328 | 0.143 |

**Average of three replicates determinations of three concentrations (1, 2, and 4) and (0.5, 1 and 2) µg/band of indacaterol and glycopyrronium respectively.*

**Table 2: Regression and validation parameters for determination of indacaterol and glycopyrronium by the proposed spectrophotometric methods:**

| | | Indacaterol | Glycopyrronium | | |
|---|---|---|---|---|---|
| **Parameters** | | **Zero order** | **Ratio difference** | **Ratio derivative** | **Mean centering** |
| Wavelength (nm) | | 293 | 227 and 269 | 234 | 227 |
| Linearity range (µg/mL) | | 12-48 | 6-20 | 6-20 | 6-20 |
| - Regression equation | | $y^* = b^{**} x + a$ | $y^* = b^{**} x + a$ | $y^* = b^{**} x + a$ | $y^* = b^{**} x + a$ |
| - Slope (b) | | 0.0168 | 0.0407 | 0.1279 | 0.0305 |
| - Intercept (a) | | 0.0073 | 0.0154 | 0.0178 | 0.0030 |
| Coefficient of determination ($r^2$) | | 0.9997 | 0.9997 | 0.9996 | 0.9996 |
| LOD (µg/mL) | | 0.845 | 0.279 | 0.364 | 0.357 |
| LOQ (µg/mL) | | 2.562 | 0.847 | 1.102 | 1.080 |
| Accuracy (%R)*** | | 100.19 | 99.49 | 100.15 | 100.04 |
| Precision (%RSD)*** | Repeatability | 0.520 | 0.661 | 0.812 | 0.720 |
| | Intermediate precision | 0.949 | 1.110 | 1.153 | 0.949 |

*y* is the specific response of each method*
*x** is the concentration in µg/mL*
**** Values for 3 determinations of 3 different concentrations*

**Table 3: Recovery study by applying standard addition technique for determination of indacaterol and glycopyrronium by the proposed TLC-densitometric method**

| Pharmaceutical taken (µg/band) | | Pharmaceutical found (µg/band) | | Pure added µg/band | | Pure found µg/band | | % Recovery of pure found | |
|---|---|---|---|---|---|---|---|---|---|
| Indacaterol | Glycopyrronium | Indacaterol | Glycopyrronium | Indacaterol | Glycopyrronium | Indacaterol | Glycopyrronium | Indacaterol | Glycopyrronium |
| 1.43 | 0.63 | 1.43 | 0.63 | 1 | 0.5 | 0.98 | 0.50 | 97.96 | 99.25 |
| | | | | 2 | 1 | 1.96 | 0.99 | 97.87 | 99.24 |
| | | | | 3 | 1.5 | 2.99 | 1.50 | 99.51 | 99.84 |
| | | | | Mean | | | | 98.45 | 99.44 |
| | | | | % RSD | | | | 0.935 | 0.342 |

*[a]Average of three determinations of the same pharmaceutical sample containing 1.43 and 0.63µg/band of indacaterol and glycopyrronium respectively.*

| Table 4: Recovery study of indacaterol and glycopyrronium by applying standard addition technique. | | | | | | |
|---|---|---|---|---|---|---|
| **Method** | | **Pharmaceutical taken ( µg/mL)** | **Pharmaceutical found ( µg/mL)** | **Pure added (µg/mL)** | **Pure found (µg/mL)** | **% Recovery** |
| **Indacaterol** | **Zero order** | 14.30 | 14.21* | 12 | 11.92 | 99.29 |
| | | | | 24 | 24.19 | 100.81 |
| | | | | 30 | 30.14 | 100.47 |
| | | Mean ± %RSD | | | | 100.19±0.796 |
| **Glycopyrronium** | **Ratio difference** | 6.30 | 6.23* | 6 | 6.02 | 100.34 |
| | | | | 10 | 9.90 | 99.03 |
| | | | | 12 | 11.82 | 98.49 |
| | | Mean ± %RSD | | | | 99.29±0.959 |
| | **Ratio derivative** | 6.30 | 6.23* | 6 | 6.03 | 100.52 |
| | | | | 10 | 9.99 | 99.87 |
| | | | | 12 | 12.10 | 100.82 |
| | | Mean ± % RSD | | | | 100.40± 0.482 |
| | **Mean centering** | 6.30 | 6.23* | 6 | 6.00 | 99.99 |
| | | | | 10 | 9.80 | 98.03 |
| | | | | 12 | 12.01 | 100.11 |
| | | Mean ± %RSD | | | | 99.37± 1.175 |

| Table 4: Statistical comparison of the results obtained by applying the proposed TLC-densitometric and reported method for simultaneous determination of indacaterol and glycopyrronium in Ultibro® capsules. | | | | |
|---|---|---|---|---|
| | **TLC-densitometric method** | | **Reported method[3]*** ** | |
| | **Indacaterol** | **Glycopyrronium** | **Indacaterol** | **Glycopyrronium** |
| **Mean** | 99.72 | 99.87 | 99.23 | 99.92 |
| **N*** | 5 | 5 | 5 | 5 |
| **Variance** | 0.276 | 0.986 | 0.440 | 1.141 |
| **%RSD** | 0.527 | 0.994 | 0.668 | 1.069 |
| ***t*****| 1.289 (2.306) | 0.071 (2.306) | —— | —— |
| **F**** | 1.591 (6.388) | 1.158 (6.388) | —— | —— |

** No. of samples.*

*** The values in the parenthesis are tabulated values of t and F at (p= 0.05).*

**** Spectrophotometric determination using savitzky golay filter, the peak amplitude at 264 and 236 nm corresponding to the concentrations of indacaterol and glycopyrronium[3]*

| Table 5: Statistical comparison of the results obtained by applying the proposed spectrophotometric and reported method for determination of indacaterol and glycopyrronium in Ultibro® capsules. | | | | | | |
|---|---|---|---|---|---|---|
| | **Indacaterol** | **Glycopyrronium** | | | **Reported method[3]*** ** | |
| | **Zero order** | **Ratio difference** | **Ratio derivative** | **Mean centering** | **Indacaterol** | **Glycopyrronium** |
| **Mean** | 99.61 | 99.75 | 100.41 | 100.04 | 99.23 | 99.92 |
| **N*** | 5 | 5 | 5 | 5 | 5 | 5 |
| **Variance** | 0.353 | 0.215 | 0.761 | 0.345 | 0.440 | 1.141 |
| **%RSD** | 0.596 | 0.465 | 0.869 | 0.587 | 0.668 | 1.069 |
| ***t*****| 0.945 (2.306) | 0.323 (2.306) | 0.794 (2.306) | 0.227 (2.306) | —— | —— |
| **F**** | 1.247 (6.388) | 5.307 (6.388) | 1.501 (6.388) | 3.312 (6.388) | —— | —— |

** No. of samples.*

*** The values in the parenthesis are tabulated values of t and F at (p= 0.05).*

**** Spectrophotometric determination using savitzky golay filter, the peak amplitude at 264 and 236 nm corresponding to the concentrations of indacaterol and glycopyrronium[3]*

## CONCLUSION

In this study; sensitive and selective TLC-densitometric and UV-spectrophotometric procedures has been developed and validated for the simultaneous determination of indacaterol and glycopyrronium in their pure form and in their pharmaceutical preparation.

The developed TLC-densitometric procedure if compared to the reported methods, it has the advantage of being more sensitive and selective. Furthermore this TLC-densitometric procedure can replace the reported HPLC method when HPLC requirements are unavailable.

The developed method is time saving where many bands can be run at the same time. This method is also economic since a small quantity of mobile phase as a developing system was used unlike HPLC procedures.

Finally we can conclude that the described TLC-densitometric and UV spectrophotometric procedures can be used in routine analysis of indacaterol and glycopyrronium in their pure forms and pharmaceutical dosage form without previous separation.

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