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Development of UV-Visible and HPIC Analytical Methods for Quantitative Analysis of Antiulcer Drugs in Dosage Forms

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Abstract

Precise measurement of active pharmaceutical ingredients (APIs) in dosage forms is crucial in the pharmaceutical business to guarantee the quality, effectiveness, and safety of the product. This research is focused on creating and confirming analytical techniques for accurately measuring the amount of antiulcer medicines in different types of medication. The measurement of antiulcer medicines in their respective formulations was carried out using two analytical techniques: UV-Visible spectroscopy and High-Performance Liquid Chromatography (HPLC). UV-Visible spectroscopy is a straightforward and cost-effective method that allows for quick examination. It is particularly useful for regular quality control applications. HPLC, in contrast, offers exceptional sensitivity, specificity, and precision, facilitating the isolation and measurement of many constituents in complex matrices. The technique development procedure included optimizing experimental parameters, including solvent systems, detection wavelengths, column types, mobile phase composition, and flow rates. The reliability and robustness of the suggested procedures were ensured by validation studies done in accordance with ICH recommendations. The adequacy of the procedures for their intended purpose was assessed by evaluating parameters such as linearity, precision, accuracy, specificity, and robustness. The UV-Visible and HPLC techniques that were developed showed exceptional linearity over the examined concentration range, with correlation values (R^2) above 0.99. The precision experiments showed low relative standard deviations (RSDs), suggesting excellent repeatability and moderate accuracy. The accuracy checks demonstrated that the recovery values were within acceptable ranges, therefore verifying the dependability of the methodologies used for quantitative analysis.

In summary, the new analytical approaches provide effective instruments for precisely determining antiulcer medicines in pharmaceutical dosage forms. Their usefulness in regular quality control labs enables prompt and dependable evaluation of product quality, assuring adherence to regulatory requirements and enhancing patient safety.

INTRODUCTION

In the realm of modern pharmaceuticals, quality control serves as the cornerstone in ensuring the safety and effectiveness of medicinal products. Rigorous standards are set to ascertain the quality, safety, and efficacy of medicines intended for human use. The evaluation and maintenance of these aspects heavily rely on robust methods for quality control [1].

Medicinal formulations today are intricate compositions comprising active ingredients alongside various inert materials such as diluents, disintegrants, colors, and flavors. To guarantee the quality and stability of the final product, it becomes imperative to segregate and quantify these mixtures. Modern analytical techniques, including spectroscopic methods, chromatographic methods, electrometric methods, and thermometric methods, stand out for their high specificity and sensitivity. They furnish precise and comprehensive data even from small material samples. Furthermore, these techniques are characterized by rapid application and are readily adaptable to automation, thus facilitating their widespread utilization [2].

Material and Method:

Experimental Conditions

INSTRUMENTS

- Shimadzu- Isocratic High performance liquid chromatographic system comprising Shimadzu LC-10AT (VP series) pump
- Variable wavelength programmable UV/VIS detector SPD-10AVP Rheodyne injector (7725i) with 20 pL fixed loop
- Phenomenex Gemini Cig / Luna Cig column having i.d. 250 x 4.6 mm and 5 pm particle size.
- Shimadzu (1650PC) UV-visible double beam spectrophotometer with matched quartz cell (lcm).
- Shimadzu (AX 200) electronic balance
- Ultrasonic bath (PCI instruments Ltd.)
- Digital pH meter (Eutech Instruments, Model: pH tester-1)
- Perkin Elmer (GX FT-IR) - Fourier transform infra-red spectrophotometer

Scan range: 4000 - 400 cm'1
Resolution: 0.15 cm'1

CHEMICALS AND MATERIALS

We received analytically pure ATV and HCTZ samples as gifts from the Mumbai, India-based organization M/s Blue Cross Laboratory Ltd.

An Ahmedabad, India-based firm called M/s Torrent Pharmaceuticals Ltd. graciously provided us with analytically clean samples of AML, EZT, SMV, NIC, NEB, and ITC.

As a gift, we received analytically pure ASP from M/s Mercury Laboratories Ltd. of Vadodara, India. As a gift, we received an analytically pure sample of RAB from M/s Mepro Pharmaceutical Ltd., India.

Analytical grade methanol was sourced from E. Merck in Mumbai, India, for the UV method. S.D. Fine Chemicals of Mumbai supplied the acetic acid, ammonium acetate, o-phosphoric acid, and potassium dihydrogen phosphate used in the liquid chromatography procedure. These came all the way from India and were of the highest analytical quality.

Methanol, acetonitrile, and deionized water suitable for high-performance liquid chromatography (E. Merck, Mumbai, India) were used in the procedure. Hydrochloric acid, sodium hydroxide, and hydrogen peroxide of analytical reagent quality were procured from Qualigen Fine Chemicals of India for use in liquid chromatography.

I purchased the STARCAD and AVAS-AM tablet formulations from the local market. Lupin Labs. Ltd. of India and Micro Laboratories Ltd. of India, respectively, produce these medications. Each tablet contains 10 mg of atorvastatin and 5 mg of amlodipine.

Both the ECOSTAT (Skymax Lab. Ltd., India) and ASOVAS (J. B. Chemicals Ltd., India) tablet formulations, including 10 mg of atorvastatin and ezetimibe, respectively, were surveyed on the local market.

The STARSTAT-EZ tablet formulation by Lupin Labs. Ltd. and the Simvas EZ tablet formulation by Micro Labs. Ltd. both include 10 mg of ezetimibe and 10 mg of simvastatin, respectively.

Everything was sourced from neighborhood markets.

The local market was scoured for the capsule formulations Ato Plus (10 mg atorvastatin and 75 mg aspirin) and Atchol ASP (Aristo Pharma. Ltd., India) that include these active ingredients.

The active ingredient in the TONACT PLUS 1/table formulation is AVAS PLUS, manufactured in India by Micro Labs Ltd.

A specimen containing 375 mg of nicotinic acid and 10 mg of atorvastatin was obtained from the adjacent market. The specimen was prepared by Lupin Lab. in India. The Indian pharmaceutical companies Torrent Pharmaceuticals Ltd. and Cadila Pharmaceutical Ltd. produce the tablet formulations Nebicard-H and Nodon-H, respectively. The amount of nebivolol in Nebicard-H is 5 mg, but in Nodon-H it is 12.5 mg.

Hydrochlorothiazide was purchased at a local supermarket. Zorite, made in India by Indoco Remedies Ltd., had 150 mg of itopride hydrochloride and Rabium Plus, 20 mg of rabeprazole sodium, was the two pill formulations that were available. We got these recipes from the grocery store down the street.

Identification of Drug Samples

Determination of melting point

The melting points of the medicines were determined using a melting point instrument.

Table 1: List of medicine and their melting point

Name of the drug	Melting point found
Atorvastatin calcium	167-169°C (168-170 °C)
Amlodipine besylate	199-202 °C (199 - 201 °C)
Ezetimibe	165-166 °C (164-166 °C)
Simvastatin	136 - 138°C (135 - 138 UC)
Aspirin	134-137^{0}C
Nicotinic acid	235 - 237 UC (236.6 °C)
Rabeprazole sodium	140-143 °C (140 -141UC)
Itopride hydrochloride	190-193 °C (191 -193 °C)
Nebivolol hydrochloride	139- 142°C (140.7 °C)
Hydrochlorothiazide	273 – 276 UC (273 - 275 °C)

In order to perform an infrared spectroscopic study, the pharmaceutical component and KBr were crushed into pellets using a hydraulic pellet press under pressure ranging from 7 to 10 tonnes. The range of 4000-400 cm^1 was scanned using a Fourier Transform Infrared (FT-IR) instrument. The IR spectra were contrasted with the IR standard mentioned in the published works.

EXPERIMENTAL

FIRST ORDER DERIVATIVE SPECTROPHOTOMETERIC METHOD

Preparation of standard stock solutions

Accurately measuring 25 mg of ATV and AML, we transferred them to separate 25 ml volumetric flasks that contained a little quantity of methanol in order to produce stock solutions. Through the use of agitation, the solids were dissolved in the containers. Both medications were concentrated to 1000 pg/ml by adjusting the volumes with methanol. Working standards with a concentration of 100 pg/ml for each medicine were created by diluting parts of the original solutions with methanol.

Selection of wavelengths for estimation of ATV and AML

Both the ATV and AML working standard solutions were properly diluted with methanol to produce solutions with a concentration of 10 pg/ml. using a 2 nm bandwidth and a wavelength range of 400 to 200 nanometers, the spectra of the solutions were examined in spectrum mode. Utilizing a scaling factor of 50 and an interpoint spacing of 2 nanometers, we generated first-order derivative spectra from the zero-order spectra of ATV and AML.

Through the use of memory channels, the spectra of first-order derivatives were superimposed. The ATV's zero crossing point (ZCP) values were recorded, which stood for the AML's derivative response. Since 337.4 nm did not produce any response for ATV, it was chosen as the measurement wavelength for AML. When the derivative response for AML hit zero, a measurement of 289.4 nanometers was also utilised to quantify ATV. Drug concentration manipulation allowed us to determine ATV and AML specific wavelengths (ZCPs).

Calibration curves for ATV and AML

Appropriate portions of the working standard solutions for ATV and AML were put into individual 10 ml volumetric flasks. The aliquots were diluted with methanol to the mark, resulting in final concentrations of ATV at 5, 10, 15, 20, and 30 pg/ml, respectively.

The amount of AML is measured in milliliters. In order to get spectra of corresponding first-order derivatives, solution spectra were scanned and processed throughout the 400–200 nanometer range. For ATV, the concentrations were determined by constructing calibration curves at 337.4 nm, and for AML, at 289.4 nm. After that, AML and all-terrain vehicles (ATVs) were both included into regression models [95].

Validation

The accuracy, precision, specificity, and robustness of the approach were validated by following the suggested protocol.

Precision

This study tested the reliability of the first derivative spectrophotometric method over many days as well as within a single day. We were able to do this by measuring the derivative responses over three days at three different concentrations of AML (10, 20, 30 pg/ml) and ATV (5, 15, 25 pg/ml). A relative standard deviation (RSD) was used to display the findings.

Six estimations of responses to three different dosages of AML (10, 20, 25 pg/ml) and ATV (5, 15, 20 pg/ml) were performed to test the instrument's accuracy. Values representing relative standard deviation (RSD) were used to display the outcomes of these computations.

Accuracy

The procedure's correctness was checked by calculating the ATV and AML recoveries using the standard add approach. By analyzing the derivative response at the corresponding wavelengths, the concentrations of ATV and AML were obtained. A preset sample solution was enriched with ATV at 0, 4, 8, and 12 pg/ml, and AML at 0, 3, and 6 pg/ml. measuring the same medications in two different formulations at the same concentrations allowed us to confirm the recovery

Specificity

Mixed with a predetermined quantity of medicine were many tableting formulations including commonly used excipients. By measuring the absorbance, the concentrations of the medications were ascertained. Allied Chemical Corporation of India supplied the talc, microcrystalline cellulose, starch, and carboxymethyl cellulose utilized as excipients in this research.

Robustness

To test how well the approach held up, we kept an eye on the drug solutions' stability for 24 hours at a temperature of 25 ± 2°C.

Mixed standard solutions

Appropriate 10-milliliter portions of AML working standard solutions were obtained using a variety of volumetric containers. The volume of the ATV working standard solutions was diluted with methanol before being added to the flask in appropriate portions. The concentrations of AML and ATV were adjusted using iterative methods until they reached 5, 15, 20, and 25 pg/ml, respectively.

Analysis of marketed formulations

Twenty tablets were precisely measured and ground into a powder. Powdered ATV tablets of 10 milligrams (6.40 milligrams of AML) were added to a 50 milliliter volumetric flask. The vial was sonicated for five minutes after 20 ml of methanol was added to it. A 50 ml volumetric flask was used to filter the solution using Whatman filter paper (No. 1). Afterwards, the same solvent was used to get the volume up to the mark.

Methanol filled the volumetric flask to the top. Next, an appropriate portion of the liquid was mixed with a solution containing 16.24 pg/mL of AML and 16.50 pg/mL of ATV. We calculated the derivative

responses at 337.4 and 289.4 nanometers after analyzing the solution spectra over the 400 to 200 nanometer range. The ATV and AML concentrations were calculated by plugging the derivative responses into the linear equation that mapped out the compounds' calibration curves [96].

RESULTS AND DISSCUSSION

FIRST ORDER DERIVATIVE SPECTROPHOTOMETERIC METHOD

For the simultaneous equation to be used for the precise determination of medications, their respective wavelength maxima must be dissimilar. Due to the fact that AML contributes considerably to the absorbance of ATV at its maximal absorbance value, the derivative method was attempted to estimate the presence of AML and ATV. Figure 5.1.2.1.1 illustrates the overlapping zero order spectra of ATV (10 pg/ml) and AML (10 pg/ml) in methanol.

In contrast to AML, which exhibits a zero-crossing point (ZCP) at 337.4 nm and a large derivative response, ATV does not absorb at this wavelength, according to their first-order derivative spectra (Dl) (fig. 5.1.2.1.2). There was no change to the Zero Crossing Points (ZCPs) of the two medications. As a result, ATV was estimated at 337.4 nanometers and AML at 289.4 nanometers due to these considerations. The derivative response at 337.4 nanometers rose in direct proportion to the concentration of AML. Between the 5–30 pg/ml concentration range and the AML responses, there was a very high correlation value of 0.9984. The results showed that the responses were linearly related. In a similar vein, at concentrations ranging from 5 to 25 pg/ml, the derivative responses for ATV at 289.4 nm demonstrated a linear connection with a correlation value of 0.9995. You may find the results of the regression analysis for the calibration curves in tables 5.1.2.1.1 and 5.1.2.1.2.

Exact research was carried out to examine the inter- and intra-day variability in responses. Table 5.1.2.1.3-5 shows that the approach's accuracy is reflected by a low relative standard deviation (RSD) value. The proposed method was tested by recovery experiments to determine its accuracy. Table 5.1.2.1.6 shows that the ATV recovery was 98.54% to 99.75% and the AML recovery was 100.04%; both values are excellent.

At the analytical wavelengths utilised in the specificity experiments, neither drug's derivative response was affected by the excipients. The investigation into robustness found no significant changes in the derivative response of any therapy after 24 hours. Between 98.46% and 99.72% of AML and ATV samples showed some degree of recovery.

The percentages are as follows: 97.56%, 99.26%, and higher. This component makes the method robust and accurate in determining AML and ATV. You may see a summary of the validation settings in Table 5.1.2.1.7.

In Table 5.1.2.1.8, we can see the average percentages of AML and ATV found in various laboratory-prepared combinations utilising the approach. The accuracy and precision of the approach may be evaluated by looking at the average recoveries. In order to find out if their combination pill formulations included AML and ATV, the suggested method was used. The acquired values were in agreement with those reported in the corresponding tables (5.1.2.1.9).

The proposed research outlines a spectrophotometric method utilizing first order derivatives to estimate the mixture concentration of ATV and AML. It was determined that the validated method was straightforward, accurate, and precise. The recovery percentage indicates that the method is unaffected by the excipients incorporated into the formulation. Consequently, the proposed method may be implemented routinely to analyses combined dosage forms of ATV and AML.

METHOD DEVELOPMENT FOR ANTIULCER DRUG COMBINATION

EXPERIMENTAL

STABILITY INDICATING RP-HPLC METHOD

Preparation of mobile phase

To make the mobile phase, 450 millilitres of water was mixed with 0.693 grammes of ammonium acetate. The solution's pH was adjusted to 6.0 ± 0.2 by adding 0.1 M of acetic acid. Mixing 200 millilitres of acetonitrile with 350 millilitres of methanol resulted in a solution. A 10-minute sonication treatment followed by filtering the solution with Whatman filter paper (No. 1) degassed it before use.

Preparation of standard stock solutions

To make the mobile phase, 450 millilitres of water was mixed with 0.693 grammes of ammonium acetate. The solution's pH was adjusted to 6.0 ± 0.2 by adding 0.1 M of acetic acid. Mixing 200 millilitres of acetonitrile with 350 millilitres of methanol resulted in a solution. A 10-minute sonication treatment followed by filtering the solution with Whatman filter paper (No. 1) degassed it before use.

Chromatographic conditions

The reverse phase C-18 column (Phenomenex, Gemini) was equilibrated using a mobile phase that consisted of 0.02M ammonium acetate (pH 6), acetonitrile, and methanol in a 45:20:35 (v/v/v) ratio. The mobile phase flow rate was maintained at 1 ml/min, and effluents were seen at a 284 nm wavelength. The sample was injected using a fixed loop with a volume of 20 picoliters and duration of 15 minutes.

Calibration curves for RAB and ITC

10 ml vials were used to separate portions of the RAB working standard solution. Containers for vaporisation. The ITC working standard solution was carefully measured and added to the identical containers. The mobile phase was used to adjust the volumes to the necessary level, resulting in RAB concentrations of 0.8, 4, 8, 40, and 80 jag/ml.

JLntiufcer (Drugs)

One hundred fifty micrograms per millilitre is the concentration of ITC. A fixed loop system was used to inject solutions with a volume of 20 pL, and the chromatograms of those solutions were recorded. We also created calibration curves and calculated regression equations for RAB and ITC.

Method Validation

Evaluating the method's robustness, detection limit, specificity, precision, and accuracy were all part of the validation procedure.

Precision

The results are shown with respect to the RSD, or Relative Standard Deviation. In order to determine how precise RAB and ITC are, researchers computed their reactions in three separate solutions with different concentrations. On each of the three days, we tested the answers three times. Relative standard deviation (RSD) is used to express the results. Six separate mixed solutions including varying concentrations of RAB (1, 5, 15 pg/ml) and ITC (4, 40, 120 pg/ml) were injected to assess the instrumental accuracy.

Accuracy

By computing the recoveries of ITC and RAB using the standard adds technique, we were able to assess the correctness of the methodology. Additions of ITC (0, 0.8, 40, 80 pg/ml) and RAB (0, 1, 5, 15 pg/ml) were made to the pre-quantified sample solutions. In order to find the RAB and ITC concentrations, the peak areas were measured and then fitted to the linear equation of the calibration curve.

Specificity

Common additives like as starch, microcrystalline cellulose, and magnesium stearate were mixed with a pre-measured quantity of drugs. By using suitable dilutions, the chromatogram was acquired, and the concentrations of the compounds were ascertained.

Detection limit and Quantitation limit

In accordance with the ICH standards, the following equation was used to compute the LOQ and LOD. The slope of the calibration curve, denoted as S, determines the limits of quantification and detection, respectively, which are 10 xg/S and 3.3 xo/S, respectively. a represents the dispersion of the regression lines' y-intercepts.

Robustness

A stability test of the sample solution was conducted at a temperature of 25 ± 2°C for 24 hours in order to assess the method's resilience.Common additives like as starch, microcrystalline cellulose, and magnesium stearate were mixed with a pre-measured quantity of drugs. By using suitable dilutions, the chromatogram was acquired, and the concentrations of the compounds were ascertained.

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Robustness

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STABILITY INDICATING RP-HPLC METHOD

Optimization of mobile phase

Among other things, the acetonitrile: water (70:30, v/v) mobile phase resulted in low peak resolution and a 3.36-minute peak for RAB and a 2.29-minute peak for ITC. In a 70:30, v/v ratio of methanol to 0.02M ammonium acetate (pH 6), the experiment produced a broad peak for RAB and ITC. At 7.2 minutes, we found a peak for RAB, and at 3.58 minutes, for ITC, with enhanced asymmetry, using a mixture of acetonitrile, methanol, and 0.02M ammonium acetate in a volume/volume/volume ratio of 40:20:40 (pH 6). A peak for RAB was seen after 7.0 minutes and for ITC after 3.2 minutes when a mixture of acetonitrile, methanol, and 0.02M ammonium acetate (pH 5) was used in a volume/volume/volume ratio of 40:20:40. A mobile phase consisting of 0.02M ammonium acetate, acetonitrile, and methanol in the ratio of 45:20:35 (v/v/v) was used to generate clear and clearly identifiable peaks for RAB and ITC. Using 0.1 M acetic acid, the mixture's pH was brought down to 6. We kept the flow rate at 1 ml/min. A resolution of 17.8 was observed in Figure 4.1, which reveals that RAB had retention times of 8.8 minutes and ITC of 3.8 minutes.

Selection of analytical wavelength

There was a noticeable absorption at 284 nm in the UV overlain spectra of both RAB and ITC. So, for chromatographic tests, this was the selected detection wavelength (fig. 4.2).

Method validation

To create the calibration curves, we plotted the peak area vs concentration for RAB (0.4–20 pg/ml) and ITC (0.8–150 pg/ml). Table 4.1 contains the results of the regression analysis used to create the calibration curves. Through the execution of a repeatability test, the RSD values for RAB and ITC were ascertained,

and the accuracy of the instruments was ascertained. The precision investigations conducted both within and between days findings are shown in table 4.2 using RSD as the unit of analysis. It is clear that the approach is accurate because of the little RSD values. The technique's accuracy was assessed by determining RAB and ITC recoveries using the method of standard addition. According to table 4.2, the recoveries for RAB were determined to be 98.28 - 99.37%, while for ITC they were 98.23 - 100.70%. The results show that the approach is correct. Both RAB and ITC had quantitation limits of 0.4 pg/ml and 0.8 pg/ml, respectively, whereas their detection limits were 0.1 and 0.3 pg/ml, respectively. According to the data shown above, it is possible to calculate the exact concentration of both medicines to the nanogram level. In order to ensure that the formulations' excipients did not interfere with the results of the specificity investigation, a synthetic combination combining the medications and excipients was prepared. Both RAB and ITC exhibited peaks in the chromatogram independently of one another, with recoveries of more than 97% for each medication. Table 4.2 summarizes the parameters obtained from system suitability experiments conducted on newly generated standard stock solutions of ITC and RAB. The stability examination of the solutions showed that RAB was unstable and hydrolyzed with a recovery percentage of 45.23 percent, but ITC remained stable for 24 hours without showing any signs of deterioration. Figure 4.3 shows that the percentage recovery of ITC was 98.72%.

Analysis of marketed formulations

Combination pharmaceutical formulations of RAB and ITC were evaluated for their concentrations using the proposed method (Capsules 1 and 2). Table 6.1.2.1.8 shows that the values of RAB and ITC were close to the indicated levels.

Forced degradation study

At retention durations (RT) of 3.137, 3.867, and 5.093 minutes for RAB, the chromatograms of the material that was subjected to base degradation showed peaks of degradation products. On the other hand, ITC did not change. The results are shown in figures 4.4 and 4.5. Because RAB was so easily broken down by acids, the treated samples became brown. Under room temperature circumstances, the acid-deteriorated samples showed maxima for the degradation product at 3.347, 5.89, and 7.383 minutes for RAB. The results of the acid hydrolysis tests at room temperature and 80 °C demonstrate that ITC is resistant, as seen in figures 4.6 and 4.7.

The medication was completely degraded at retention durations (RT) of 3.33, 3.547, 4.967, and 8.18 minutes for RAB in the samples that were damaged by hydrogen peroxide, suggesting that the degradation product peaks occurred at those periods. Figure 6.1.2.1.8 also shows that ITC deterioration peaked at 3.673 minutes. There was no degradation peak seen, and both medications showed stability when subjected to dry heat. Due to dry heat deterioration, the RAB powder changed its physical appearance to a brownish hue (see to figures 4.8 and 4.10 for reference). During the light degradation experiment, the chromatogram showed that RAB deteriorated at 3.433, 3.940, 4.167, and 8.593 minutes. However, it was shown that ITC remained steady. Figures 4.11 and 4.12 show that the RAB powder changed its physical appearance, becoming brown. According to the degradation research, RAB showed stability against dry heat degradation but was susceptible to hydrolysis by acids and bases, degradation by oxidative stress, and degradation by ultraviolet light. Oxidative stress may lead to the breakdown of ITC, as seen in table 4.2.

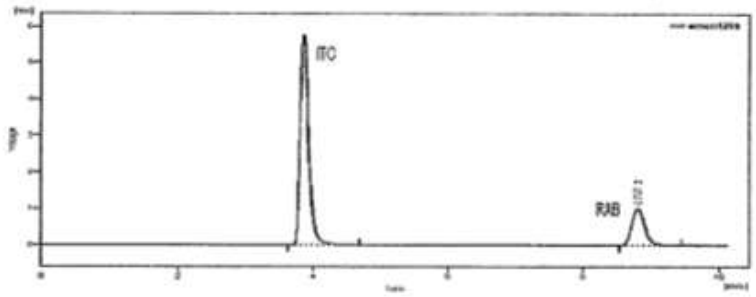


Figure: Liquid Chromatogram of ITC (40µm, 3.8 min) RAB (5µg/ml, 8.8 min)

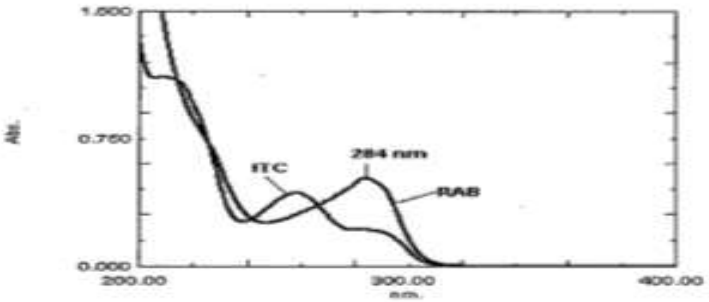


Figure: UV overlain spectra of ITC (10 µg/ml) and RAB (10 µG/ML)

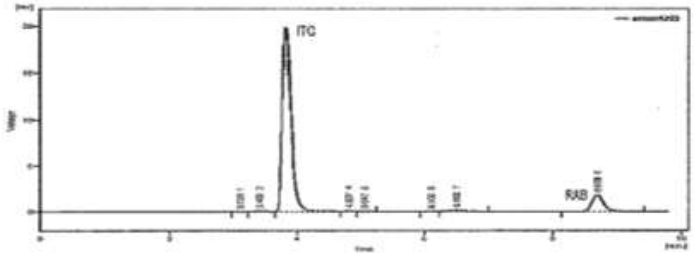


Figure: Chromatogram of solution stability study for RAB (20 µg/ml) and ITC (150 µg/ml)

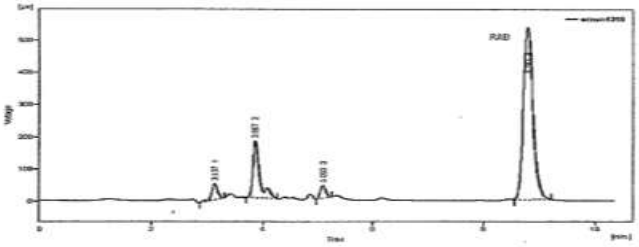
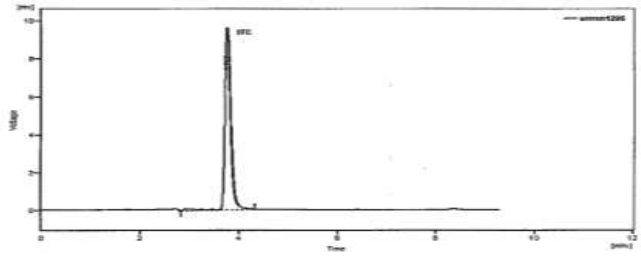


Figure: Chromatogram of base treated RAB (6 µg/ml) at room temperature for 24 h



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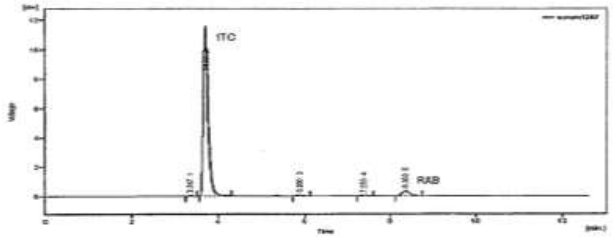

Figure: Chromatogram of base treated RAB (10 µg/ml) and ITC (75 µg/ml) at room temperature for 70 min.

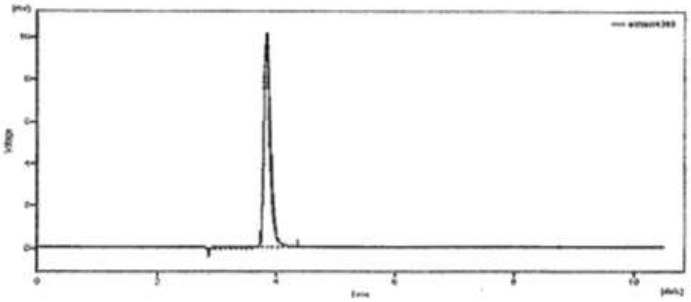

Figure: Chromatogram of dry heat degradation study of ITC (6 µg/ml) at 80^{0C} for 1 hr.

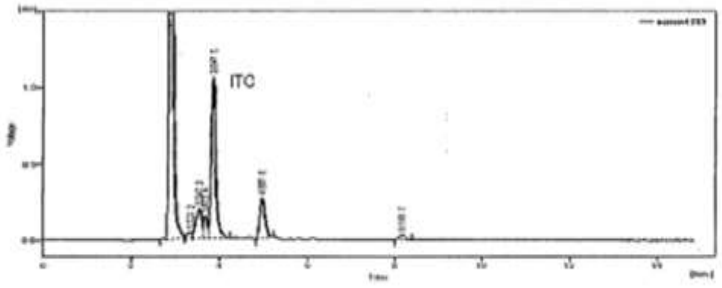

Figure: Chromatogram of hydrogen proxide (3%) treated RAB (6 µg/ml) ITC (6 µg/ml) at 80^{0}C for 1 h.

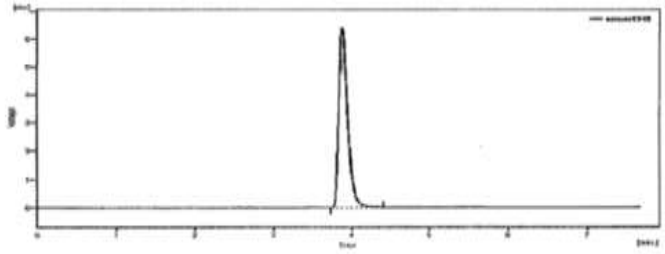

Figure: Chromatogram of dry heat degradation study of ITC (40 µg/ml) at 80^{0C} for 1 hr.

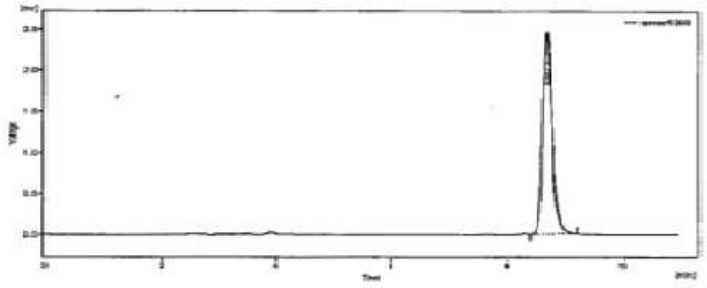

Figure: Chromatogram of dry heat degradation study of RAB (10 µg/ml) at 80^0C for 1 hr

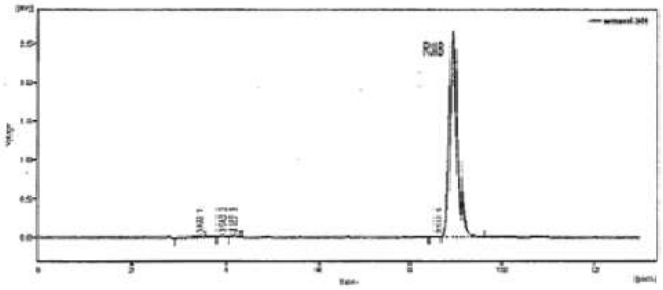

Figure: Chromatogram of dry photo degradation study of RAB (10 µg/ml) at 80^0C for 1 hr

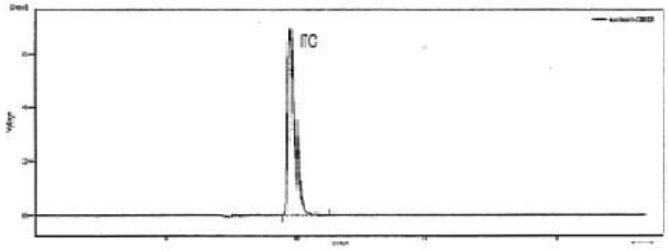

Figure: Chromatogram of dry photo degradation study of ITC (40 µg/ml)

Table: Regression analysis of calibration curve for RAB

Concncentration (µg/ml)	Mean (Peak area)± SD (n=6)	CV
0.4	9.25± 0.50	5.41
1	23.14±0.74	3.20
5	124.14±2.58	2.07
10	251.49±4.10	1.63
15	379.71±4.34	1.14
20	507.27±6.43	1.27
Slope Interrecept	25.44±0.19 -1.97±1.53 0.9995	0.75 - -

$Y=25.44x-1.97, R^2=0.9995$

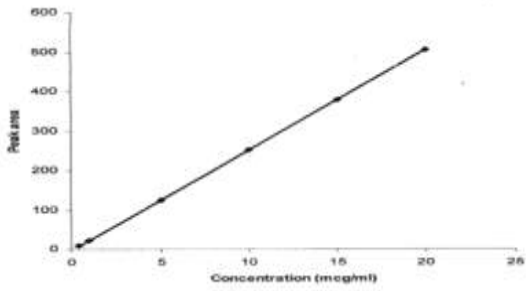

Figure: Regression analysis of calibration curve for RAB

Table: Regression analysis of calibration curve

Concncentration (µg/ml)	Mean (Peak area)± SD (n=6)	CV
0.8	9.05± 0.55	6.08
4	51.14±1.83	3.58
8	101.13±2.92	2.89
40	503.12±7.09	1.41
80	1012.79±4.34	0.78
150	1917.89±18.68	0.97
Slope Interrecept Con.Coef.	12.78±0.13 -2.98±1.24 0.9999	1.02 - -

$Y=12.78x-2.98, R^2=0.9999$

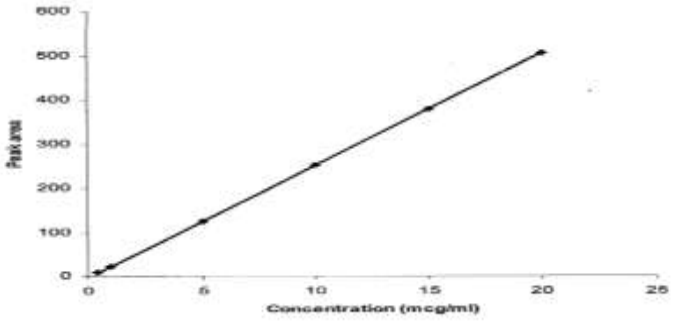

Figure: Regression analysis of calibration curve for RAB

CONCLUSION

The combination of RAB and ITC medicines has been tested using a stability-indicating liquid chromatographic method. Estimation was carried out using a Cis column serving as the stationary phase and pH 6 ammonium acetate solutions at a concentration of 0.02 M. Acetonitrile and methanol make up the combination.

There is a 45:20:35 ratio of volume to volume in the mobile phase. A steady flow rate of one milliliter per minute was maintained. There was an 8.8-minute retention time for RAB and a 3.3-minute retention time for ITC. For both ITC and RAB, the method showed linearity between 0.8 and 150 femtograms/mL and 0.4 and 20 femtograms/mL, respectively. It was found that the method was very sensitive, exact, and accurate after verification.

The peaks of degradation products were effectively separated from the medicine peaks during forced degradation experiments.

Spectrophotometry, high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC) have all been used in publications to determine RAB and ITC together. The found technique uses a simple mobile phase and is stability indicating, in contrast to the published RP-HPLC strategy. Medicines in combination dosage forms were successfully estimated using the proposed method.

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