Alprazolam benzoate (APB) determination by the improvement of a micro-high-performance liquid chromatography technology in their dose pharmaceuticals and standard powder

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HIGHLIGHTS

• A novel technique for calculating Alprazolam Benzoate (APB) levels in medicines.

• For LC100 in Alprazolam benzoate (APB) estimation, HPLC-UV technology is used.

• Research the neutral, acidic, and basic structural synthesis of alprazolam benzoate (APB).

• Analyzing Alprazolam benzoate's relative stability during the experimental estimation procedure.

• Use various programs to verify the chromatographic method for calculating Alprazolam Benzoate (APB) levels.

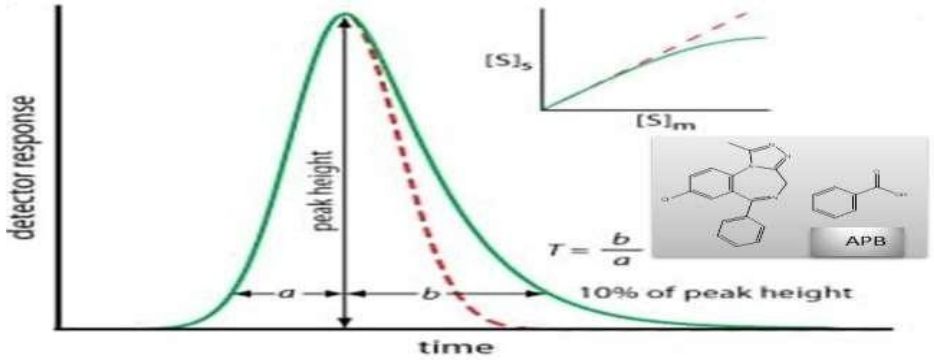
Abstract

Context: This paper aims to explain and develop a high-performance liquid chromatography (HPLC) technique to measure Alprazolam Benzoate (APB) in pharmaceuticals. Method: The form of (APB) was ascertained using the Reversed- Phase HPLC (RP-HPLC) technique, the findings of which were obtained. An Ion Pac column and HPLC-UV system with an Arcus EP-C18 sizing 5 m, 250 mm, and
4.6 mm were used to conduct the chromatographic analysis. Acetonitrile was usedas the mobile phase with a 0.5 M potassium dihydrogen orthophosphate
+ Triethylamine 30:70 (v/v) buffering at pH 4.5 and an average flow rate of 1.0 ml/min. UV detection was performed by the HPLC equipment at 310 nm. Results: The accuracy, linearity, precision, sensitivity, and specificity of the approach were all confirmed. The (APB)'s perseverance lasted for about 1.10 minutes. The Alprazolam benzoate calibration plots were linear for the concentration ranges of 1 to 5 g/L. The Limit of Detection (LOD) was 0.0316 g/ml, and the Limit of Quantitation (LOQ) was 0.0143 g/ml respectively. Studies on recovery were used to evaluate the suggested method's accuracy, and they found it to be 100% accurate. Conclusion: Commercial tablet manufacturing has been effectively analyzed utilizing the devised technique of High-Performance Liquid Chromatography-Ultra Violet. The specificity of this technique, its accuracy, as

well as the precision of it, have been verified. They were determined to be within reasonable boundaries. Additionally, there was no discernible difference between the results obtained using the proposed approaches and those acquired using the recommended method.

Keywords: (APB) drug, the limit of detection, the limit of quantification, micro- high-performance liquid chromatography, statistical analysis

Abstract in Graphic



INTRODUCTION

Alprazolam Benzoate (APB) is utilized as an antiprotozoal with AUPIC as the chemical name: $C_{17}H_{13}CIN_4$, Mol. Wt. 308,765 (8-chloro-1-methyl-6-phenyl-4H-benzo[f] [1]) is an APB compound that is a white powder constructed with crystal or a faint yellow odour. It is soluble in dichloromethane, chloroform, and acetone in ethanol but almost insoluble in water.It is the derivation of benzoic acid with a melting point between 99 and 102 and has antiamebic, anti-bacteria and anti-protozoa properties. [Figure 1], [2].

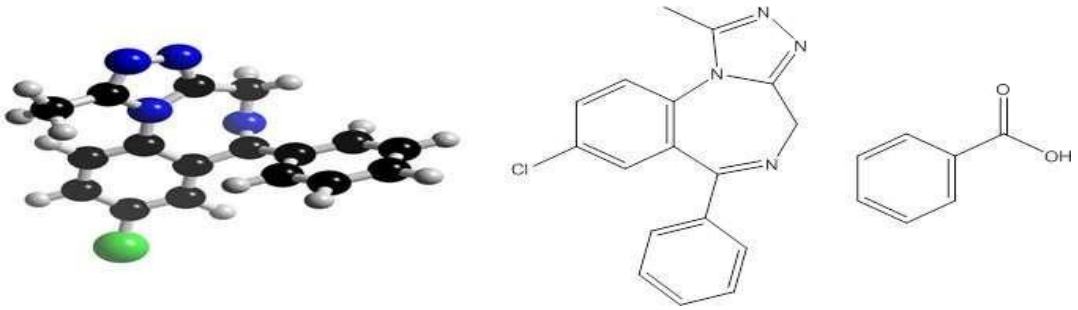


Figure 1: Chemical Structure of Alprazolam benzoate (APB)

APB has antibacterial and antimicrobial compounds as its medicinal attributes. Infections of anaerobic bacteria are also treated using derivatives of nitroimidazole. By mixing pyroxene and verdoxine oxidase enzymes, the drug is converted into anaerobic microorganisms. The linked ferredoxin group or ferredoxin metabolism degrades the nitro group in APB mechanically. As a result, the novel substance has

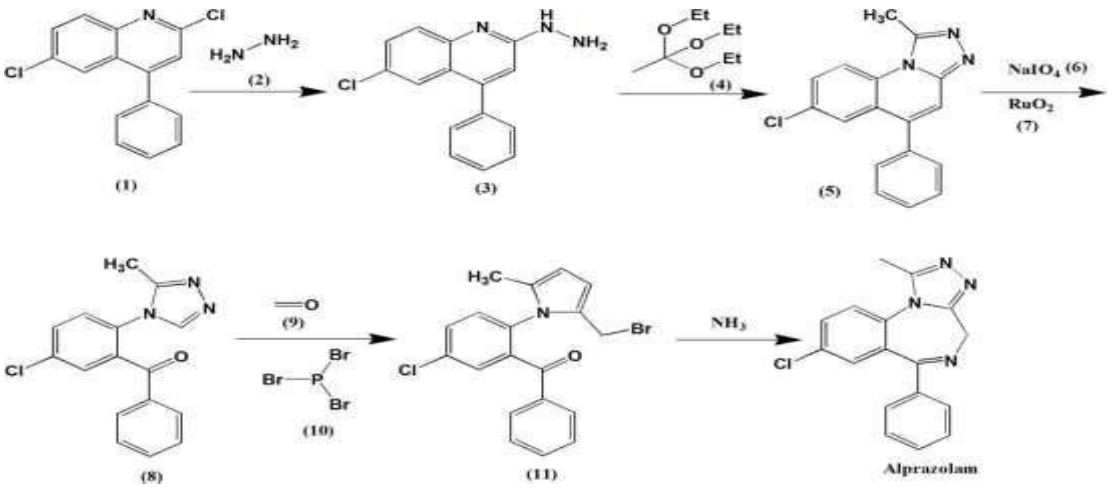
been held accountable for shattering the DNA structure of spiral chains, which prevents bacteria and other microscopic organisms from synthesizing DNA. [3-5].

APB in pharmaceuticals can be checked and detected using a wide range of analytical techniques, particularly the high-performance liquid chromatography approach, considered cutting-edge and contemporary. Pharmaceuticals are quantified for APB using high-performance liquid chromatography (HPLC). [6, 7].

Reversed-phase HPLC (RP-HPLC), a straightforward, quick, and highly accurate technique for calculating APB in medicines, was created in this study employing an ultraviolet sensor. The samples' stability has been evaluated in various laboratory settings. It is crucial to create a suitable analytical approach to gauge the amount of APB in its pharmacological formulations. Acetonitrile, methanol, and potassium hydrogen phosphate are a few solvents used in the eluent solution for the HPLC process. This drug is measured quantitatively and qualitatively using a chromatographic separation column (Ion Pac Arcus EP-C18; 5 m, 4.5 mm 250 mm), and appropriate separation conditions are utilized. This approach was validated in accordance with the Food and Drug Administration's "Dynamic Verification Method" Guidance Document from May 2001. [8, 9]. The International Council for Chemical Harmonization (IHC) criteria also approved the RP-HPLC technique.

The Synthesis of Alprazolam

As stated in the following equation's steps (Scheme 1), APB synthesizes imidazoles or ethylenediamine and acetic acid and then treats the mixture with lime [10].



Scheme 1: Alprazolam Synthesis

The Study Objective

The aim of this research was to develop and validate an RP-HPLC technology with an UV sensor for quantitatively measuring APB in pharmaceuticals.

EXPERIMENTAL ASPECTS

Instrumentation

Digital control via the computer is autonomous in the LC-100 series S-HPLC. It is a pioneering device with exceptional dependability and reliability due to its digital

circuit design, inner mechanical frame conception, processing technological advances, functionalities of the cinematography workspace, and technical specifications. The UV-100 PC model with a quartz cell with a 1 cm path length is the basis of the LC100-type HPLC-UV, which is hooked to a computer compatible with IBM via a optical spectrometer which is double-beam (Angstrom Advanced Inc., USA). These suggested chemometric approaches utilized PLS_Toolbox to operate with Matlab R2003b, VP motors, and varying spectrum configurable Ultraviolet sensors. R2003b, Matlab, the UVPC personal spectroscopic software, was employed. LC solution program from Angstrom Advanced Inc. was used to incorporate peak zones. For chromatographic separation and measurement, an Arcus EP-C18 (250 mm 4.6 mm; sized 5 cm particle) experimental column stored at room temperature was implemented. Before inserting the mobile phase, conventional pharmaceutical solutions, and pill sample solutions into the HPLC procedure, they were purified using a millipore tissue filter. [11-14]

MATERIALS AND CHEMICAL SUBSTANCES

Pure Standard

APB benchmark with a purported purity of 99.8%, approved for use in pharmaceuticals and healthcare equipment by PubCem Drug Industries, USA, Cas number: 28981-97-7, EINECS: 249-349-2, BRN: 1223125, and MDL number: MFCD00078881.

Market Sample

Alprazolam LPH- Serie® pills batch No. 486346, made by Labormed for Pharmaceuticals and Medical Appliances LPH- Serie®, had been identified as containing 0, 25 mg APB per tablet.

Measurement Sample Configuration

• Solutions provided by Germany's Sigma-Aldrich® Chemie GmbH Grade HPLC

• APB standard stock solutions were produced in acetonitrile with a 30:70 (v/v) triethylamine ratio and a pH 4.5 potassium dihydrogen orthophosphate buffer.[15,16]

• Working standard solutions of APB with concentrations of 1.0 up to 5.0 g/ml were prepared in acetonitrile using triethylamine and a 0.5 M potassium dihydrogen orthophosphate buffer at pH 4.5.

Sample Updating

To update the simulation, multiple specimens of Labormed's Alprazolam LPH-Serie® tablets with known levels of standard APB were added to the optimized PLS calibration set. Three unknown samples with different quantities of each were combined with one known quantity for the initial calibration. The updated sample's ability to predict outcomes was evaluated using external validation samples. The estimated sample updating was then performed for each component using the devised method RP-HPLC with each of the three concentrations of the extra updating samples. [17-20]

PROCEDURES

The Solution of Standard Drug

Conventional solutions have been developed using the mobile stage as a solvent. A properly weighed quantity of APB (25 mg) was dissolved in a 100 mL volumetric flask in a 50 mL mobile phase to create an APB conventional stock solution (250 g/mL). The cylindrical container was then filled with the mobile phase to the appropriate level. The standard working solutions of APB (1 up to 5 g/mL) were formed by properly diluting the olution of stock with the mobile phase.

RESULTS

The Calibration Curve

The recommended technique's calibration curves were generated over APB's 1–5 g/ml concentration range. 20 l of every single solution, which was created in triplicate, was inserted into the column. At 310 nm, the peaks were determined (Table 1).

Plotting the peak area versus concentration allowed for the creation of the APB curve for calibration.

Table 1: The fundamental parameter values determined with the RP-HPLC/ reverse-phase chromatography technique

Mobile Phase	At pH 4.5, acetonitrile: triethylamine 30:70 (v/v) plus 0.5 M potassium dihydrogen orthophosphate buffer
Column temperature	25 ^{o}C
Run time	10 min
Detection wavelength	310 nm
The Volume of Injection	20 µL
Flow rate	1.0 ml/minute

Studies on Stress-Related Damage

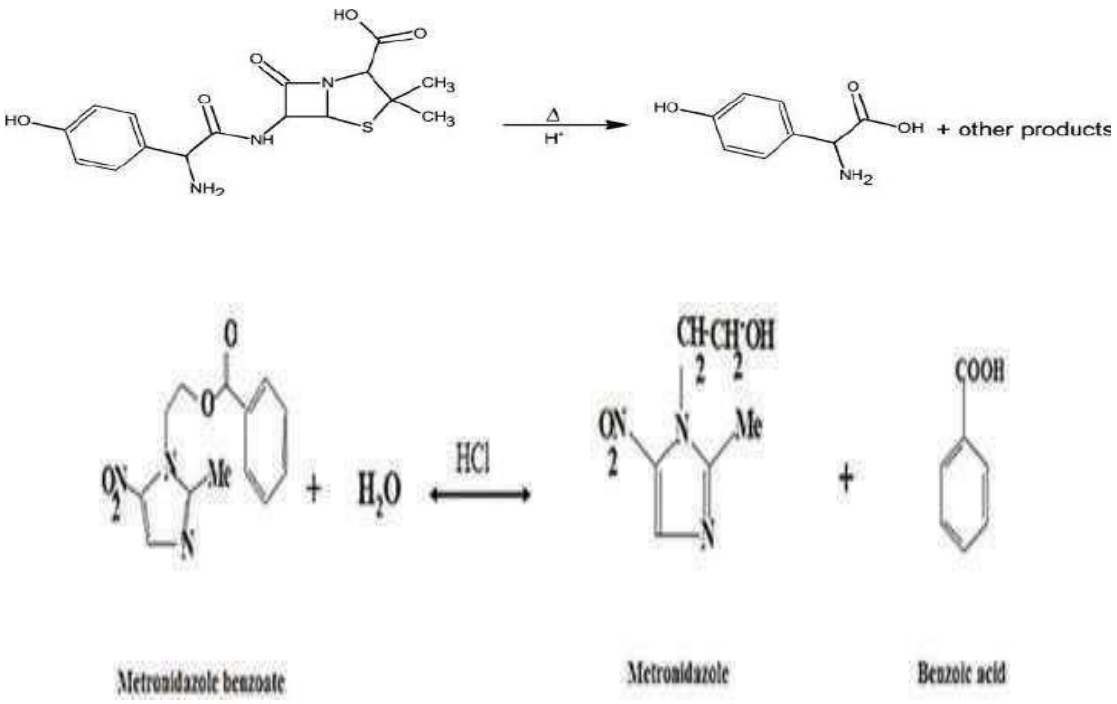
Different ICH-recommended stress conditions were used in stress degradation investigations, including acidic stressor, basic stressor, oxidative stressor, thermal stressor, and photolytic stressor. [21-23]

Degradation by Acid

APB tablet powder containing 60 mg was dissolved in a 100 ml volumetric flask. The flask received 5 ml of 0.1 N HCl for two to three hours and was kept at 70–80 °C in reflux condition.

After the process was done, the neutralization of the solution with 0.1 N NaOH before being brought reached the required level with the mobile phase. Hydrochloric acid may hydrolyse APB.

Hydrolysis—"splitting with water"—is a particular process. Every acid or base can cause esters to hydrolyze. (Scheme 2 and Figure 2).



Scheme 2: APB benzoate Structure

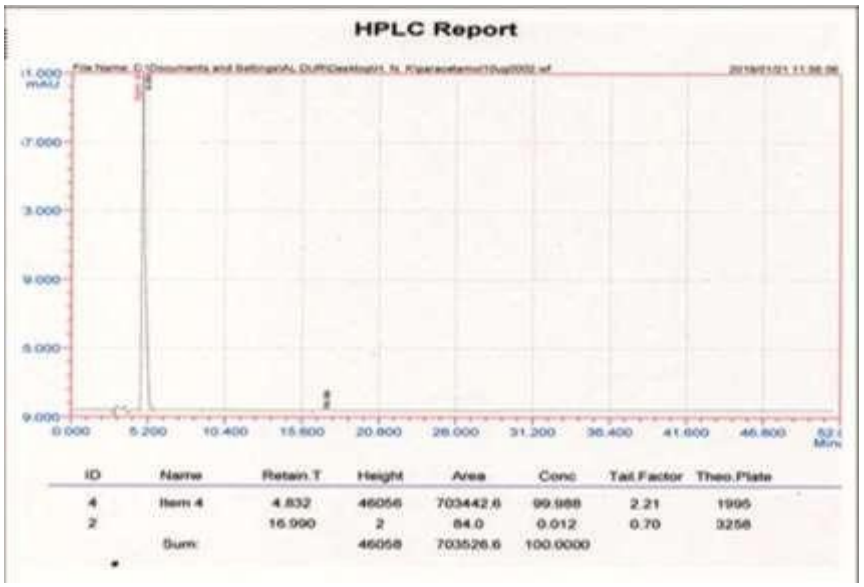
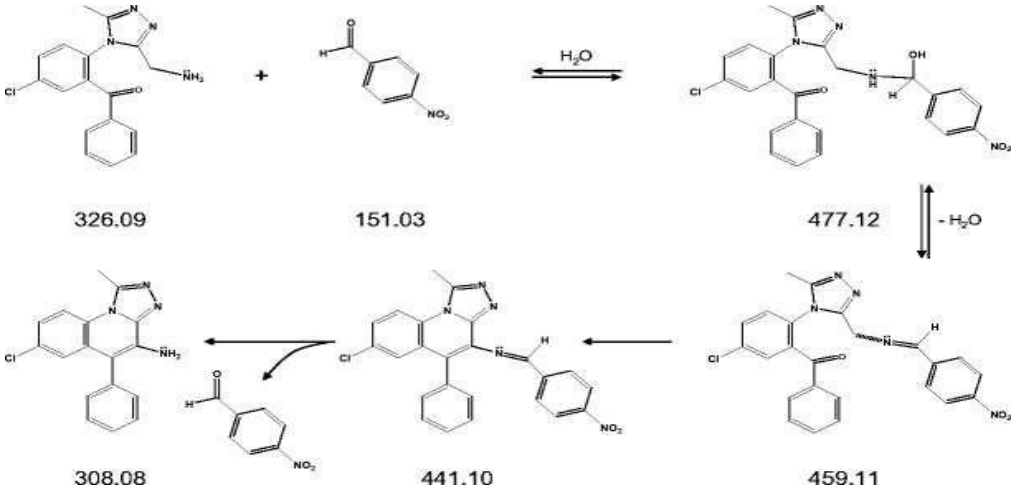


Figure 2: Acid Degradation Chromatogram

The Degradation Basis

When suppressing alcohol and carboxylic salt compounds called ester with bases like potassium hydroxide or NaOH. APB capsule powders containing 60 mg were ingested in a volumetric flask of 100 cc. With 5 ml of 0.1 N NaOH, the container

was filled up and, for two to three hours, kept in reflux at 70–80°C. After the stress, with 0.1 N HCl, the solution neutralization was conducted before being brought to the desired mobile phase level [Scheme 3 and Figure 2].



Scheme 3: Base Degradation Mechanism

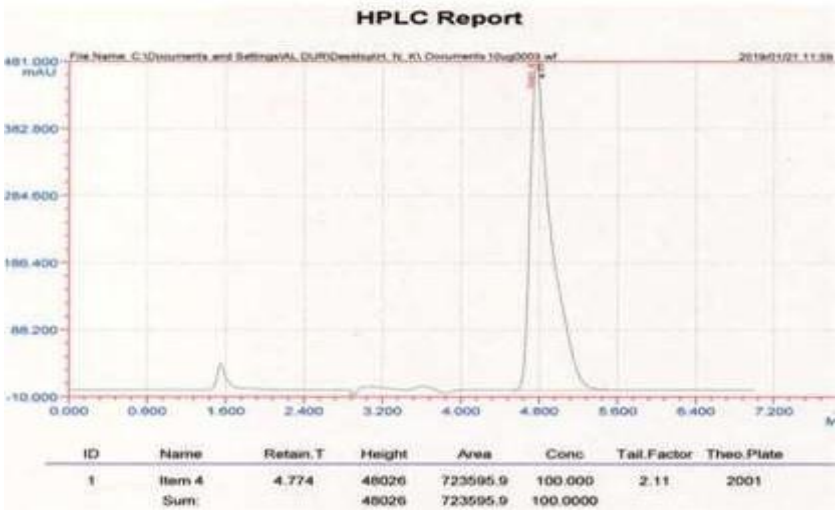


Figure 3: Base Degradation Chromatogram

Degradation by Oxidation

A 100 ml volumetric flask was filled with 5 ml of 20% H2O2 and 60 mg of APB tablet powder. For two to three hours, the flask was held at 70-80°C in a refluxing condition. The flask became full with the mobile phase after the stress (Figure 4).

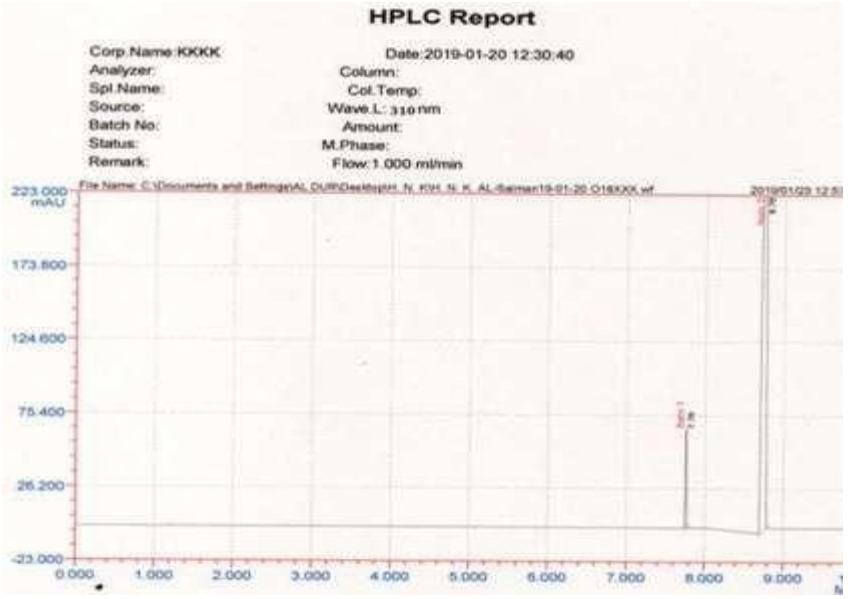


Figure 4: Degradation by Oxidation Chromatogram

Photolytic Degradation

A clear glass Petri dish containing 60 mg of APB benzoate tablet powder was exposed to sunlight for two to three hours as part of the photolytic degradation investigation. In a volumetric flask, the tablet powder contained 100 millilitres when the stressing process was finished and brought up to the required concentration by applying the mobile phase. The solution's infrared spectrum is next examined. This kind of breakdown results in the partial the APB chemical disintegration and the interactions that were uncontrolled with medicinal additives, where the HPLC-UV peak appearance is erratic and occasionally coincides, shown in Figure 5.

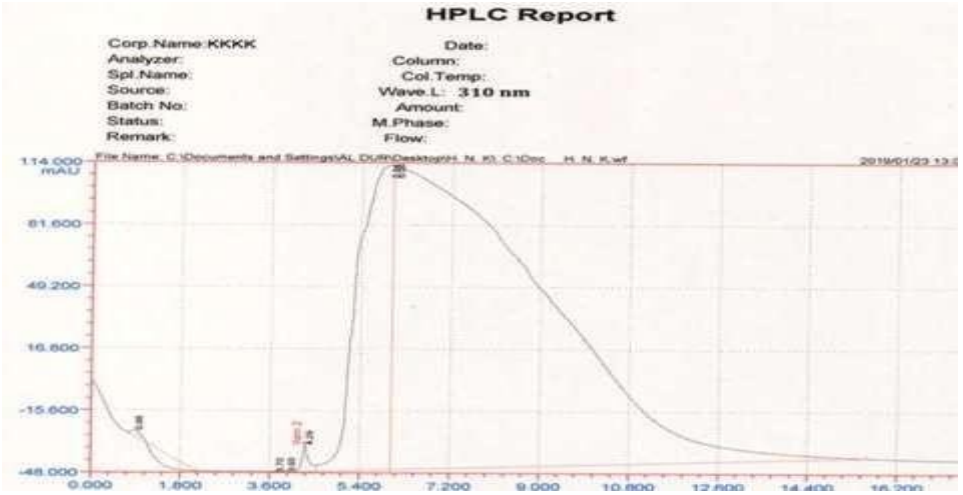


Figure 5: Photolytic Degradation Chromatogram

Thermal degradation

A glass Petri dish containing APB tablet powder as much as 60 mg was used and heated to 105°C for two to three hours.

After the timer ran out, the tablet containing the powder was transferred to a 100 ml volumetric flask and stuffed with the mobile phase. The inability to manage APB's synthetic structure and achieve a thorough thermal dissolution of the compound is indicated by an increase in the APB solution temperature above 100°C. In Figure 6, it is depicted.

Figure 6: Thermal Degradation Chromatogram

Alprazolam Infrared Spectrum

The active groups of the standard substance APB and the model's FT-IR values show a clear convergence in the FT-IR observations, which proves that the

chromatographic method used in this study to separate the active ingredient from the drug form was successful in doing so.

For Pure APB Powder

In the IR of standard APB [Figure 7], The characteristic absorption bands at -C-H (Aromatic, Med) 3300 cm^{-1}, -C-H (strach, strong) 2850-3000 cm^{-1}, -C-H (Bending, Variable) 135-1450 cm^{-1}, =C-H (strach, Med) 3010-3100 cm^{-1}, =C-H (Bending, strong) 675-1000 cm^{-1}, -C=C- (strach, Med) 1620-1650 cm^{-1}, -C-Cl (strach, strong) 600 cm^{-1}, -C-N (strach, Med) 1080-1360 cm^{-1}, -C-N (strach, Med) 2210-2260 cm^{-1}-C=N (strach, Med) 1500-1700 cm^{-1}, in the structure of APB, are stored in the test standard APB [24,25].

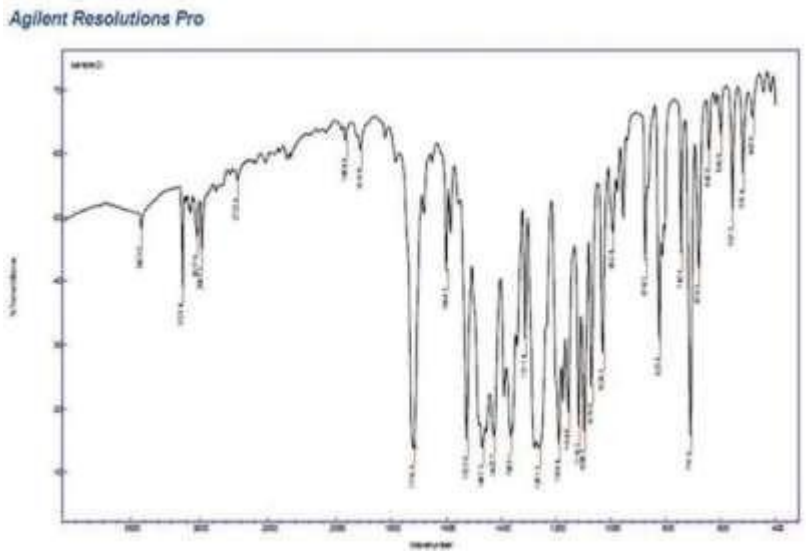


Figure 7: FT-IR spectrum of standard APB

APB and APB Crud

The bands of characteristic absorption at -C-H (Aromatic, Med) 3150 cm^{-1}, -C-H (strach, strong) 3000 cm^{-1}, -C-H (Bending, Variable) 1404 cm^{-1}, =C-H (Bending, strong) 726 cm^{-1}, -C-Cl (strach, strong) 586 cm^{-1}, =C-H (strach, Med) 3008 cm^{-1}, -C=C- (strach, Med) 169 cm^{-1}, -C-N (strach, Med) 1026 cm^{-1}, -C-N (strach, Med) 2222 cm^{-1}, -C=N (strach, Med) 1581 cm^{-1}, in the structure of APB, have been manifested in the samples of the test (Figure 8).

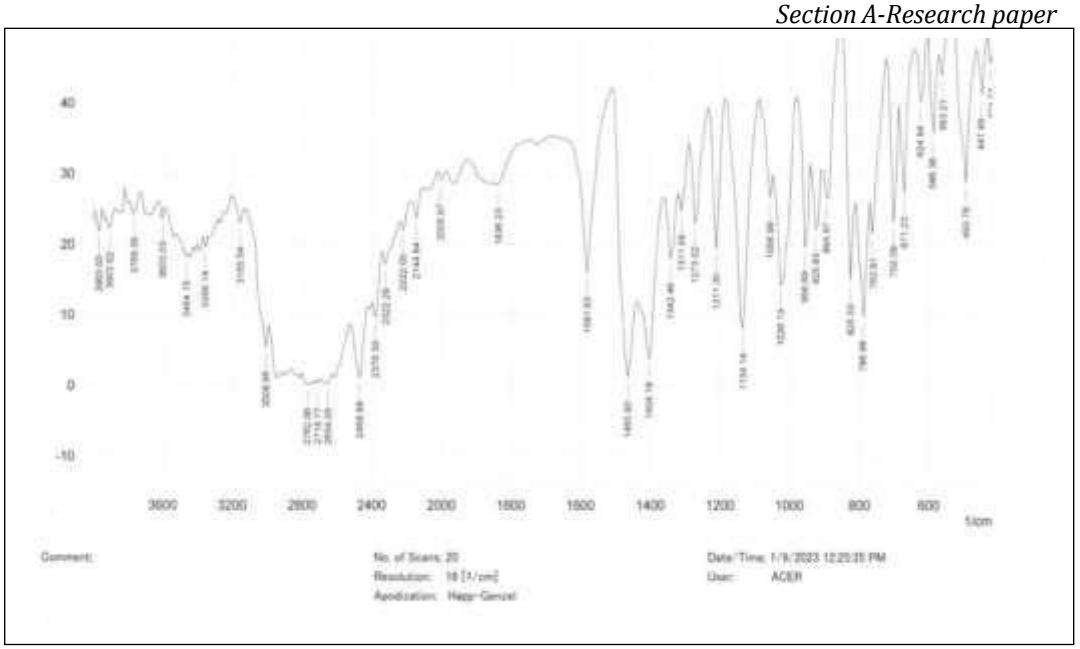


Figure 8: APB Crud FT-IR Spectrum

RESULT DISCUSSION

The HPLC condition Optimisation

The conditions of chromatography were created in order to extract all of the degradation products from the APB peaks.

In multiple trials to optimize the HPLC process, Ion Pac Arcus EP-C18, 5 m, 4.5 mm, and 250 mm, with the appropriate organic phase, acetonitrile: triethylamine 30:70 (v/v) + 0.5 M potassium dihydrogen orthophosphate buffer at pH 4.5 and 1 ml/min flow rate, were utilized. At 310 nm, the wavelength was tracked. APB had a retention period of 1.10 minutes. An excellent peak shape was seen using the new analytical technique, as it is shown in Figure 2.

The Suitability System

Studies were done to modify the HPLC-UV system. The best method was used to create three identical samples of the same concentration using the reference APB (3 g/mL). Table 2 displays the applicability of the system. These outcomes follow Alprazolam estimates in different medications and the separation method'sspecifications.

Table 2: APB Suitability Analysis System

Drug	Injections	Retention Time	USP Tailing	USP Plate Count	Area	Area in %
APB	1	1.10	1.10	3326	25000	99.400
APB	2	1.105	1.10	3543	61245	99.540
APB	3	1.14	1.10	3541	11330	99.000
APB	4	1.14	1.10	3678	22375	99.318
APB	5	1.15	1.10	3125	60600	99.425
MEAN			36110	RT- Retention Time 1.10 ± 0.041 min		
SD			0.38			
% RSD			0.30			

Method and Assay Validation

The new chromatographic method HPLC-UV was validated using the ICH guidelines [26] and metrics including accuracy, linearity range and sensitivity, precision, rigidity, regression and specificity. The effect of experimental conditions on the peak regions of the analytes was examined to assess the method's validity. The method's validity was tested at a concentration of 3 g/mL APB. All research findings were provided in Tables 3-7. The results verified the method's major validity by demonstrating that minor adjustments in flow rate, mobile phase composition, temperature, or detecting wavelength had no effect on the drug peak regions. [27,28].

The Particularity [29,30]

The research of forced deterioration was implemented to examine the method's particularity. The examination was done to ensure the proposed method could distinguish APB from any potential degradation products created while the investigation of degradation by force was conducted.

At a concentration of 3 g/ml APB, the tablet sample was subjected to acid, base, heat, oxidation, and photolysis tests. Figures 2–6 show chromatograms in their various shapes. The drug's rate of breakdown was at its fastest under alkaline

conditions. Thermal and photosynthesis-related APB degradation accounted for the smallest fraction of the total deterioration. In the byproducts of breakdown, one peak degradation was discovered. The approach can be used as a stability indicator because other stress-related degradation products do not obstruct the detection of APB.

The Sensitivity and Range of Linearity [31, 32]

The linear connection that would exist under the ideal experimental circumstances was constructed by graphing the drug's peak regions against its total concentration (g/mL). The concentration range for APB was discovered to be 1-5 g/mL. Thelinear regression analysis of the data produced the following formulas: For APB, y = 120130x + 933.5 (R2 = 0.9998). Assume that R2 is the regression coefficient, y is the peak area, and x is the drug

concentration (in g/mL). Figure 8 and Table 3 show that the calibration curve has great linearity, with high regression coefficient values and a moderate intercept. point.

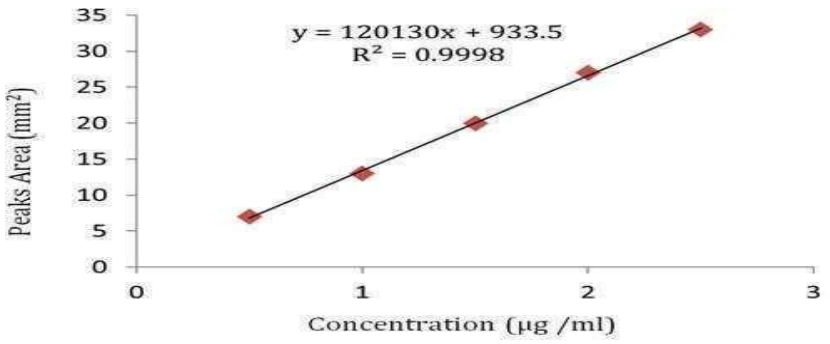


Figure 8: The Calibration Curve Linearity

Table 3: APB Linearity

Sl. Number	Concentration (µg/ml)	Peaks Area
1	1	25000
2	2	61245
3	3	11330
4	4	22375
5	5	60600

The Regression [33,34]

The estimates for the limit of quantitation (LOQ) and limit of detection (LOD) allowed for a sensitivity analysis of the proposed approach. The following are the LOD and LOQ values: SD is the standard deviation of the drug response, and S defines the slope of the calibration curve. LLOQ=10 SD/S; LLOD=3.3 SD/S SD denotes the standard deviation of the drug reaction, and S denotes the slope of the calibration curve. The LLOQ value was 0.0316 g/ml, and the LLOD value was 0.0143 g/ml, according to the results. These statistics demonstrate that the sensitivity of the proposed method is enough for the drug analysis. Table 6 (see Table 4) displays the regression data for the proposed technique.

Table 4: APB Linierity Regression Characteristics

Parameters	Results
Range of Linearity (µg/ml)	1- 5
Slope (m)	120130
Intercept (b)	933.5
Regression equation (y=mx+b)	y = 120130x + 933.5
Limit of Quantitation (LOQ)	0.0316
Limit of Detection (LOD)	0.0143
The correlation coefficient (R^2)	0.9998

The Accuracy [35,36]

Three different levels of standard solution were applied to the pre-analysis tablet sample solutions: 10%, 20%, and 30%. The recommended methodology was used to review the solutions. The recovery percentage ranged from 100% to 150%, with an RSD of less than 1%. The process is reasonably accurate, according to the results. The selectivity of the method's design prevents excipients from interfering with the analysis of the analyses. Table 5 provides a summary of the findings.

Table 5. APB Recovery Study Results

Sl. Number	Range of Accuracy	APB Added amount (mg)	Recovered amount (mg)	Recovery Percentage (%)
1.	50 % Accuracy	50	49.5	99.0
2.		50	49.6	99.2
3.		50	49.4	98.8
4.	100% Accuracy	100	100	100
5.		100	100	100
6.		100	100	100
7.	150% Accuracy	150	150	100
8.		150	150	100
9.		150	150	100
Mean				100
Standard Deviation				0.38
% Recovery Standard Deviation				0.30

The Precision [37,38]

To ascertain the degree of precision, 3 g/ml of APB was analyzed. To evaluate the system's correctness, the developed method for calculating APB in the pure standard APB was applied three times in a row (n = 3). Three consecutive APB assays on tablet samples (n = 3) were used to test the method's precision. The results are summarized in Tables 6 and 7. The percent of the RSD values for the method and system precision were both 0.01%, indicating that the proposed process is accurate enough for interpreting APB results.

Table 6. Method Precision

SL Number	Peak Area	Weight of Sample (mg)	Mean Area Counts	Label Claim Percentage (%)
1	11330	100	36110	100
2	11330	100	36110	100

3	11330	100	36110	100
4	11330	100	36110	100
5	11330	100	36110	100

Mean	100
Standard Deviation	0.38
% Recovery Standard Deviation	0.30

Table 7. Intermediate Precision

SL Number	Weight of Sample (mg)	Area	Mean of Area	Label Claim Percentage (%)
1	100	114330	36110	100
2	100	114330	36110	100
3	100	114330	36110	100
4	100	114330	36110	100
5	100	114330	36110	100
6	100	114330	114330	100
Mean				100
Standard Deviation				0.38
% Recovery Standard Deviation				0.30

The Method Applications [39-41]

By inspecting commercially accessible tablets advertised to contain 0.25 mg of APB (Alprazolam comprimate, LPH, 0.25 mg, Labormed Pharmaceutical Industries Limited, Syria), the analytical method of APB was evaluated. APB was determined as a percentage where the values were 100 ± 0.300% and as a ratio where the values were 100 ± 0.01%. Based on the percentage recovery and RSD% values, this result demonstrates that the suggested procedure was precise and accurate in APB analysis in dosage forms. The application's findings are summarized in Table 8.

Table 8: APB in Tablet Assay

Analysis	**Labelled claim (mg)**	**Found (mg)**	**Mean (mg)**	**Recovery Percentage (%)**	**RSD Percentage (%)**
Standard - APB APB -0.25	0.25 0.25	0.25 0.25	0.25 0.25	100 100	±0.300 ±0.302

CONCLUSION

The amount of APB in two marketed drugs was determined in this study using an HPLC system (LC100 Angstrom advanced) outfitted with a UV detector. The established approach is straightforward, affordable, and only requires a very small sample volume. This technique is made extremely particular by one peak in the chromatogram, which is also employed as an ultraviolet detector. Due to the extremely low concentration of medicinal medications, this application does not require high sensitivity. The procedure was approved in accordance with the HPLC-UV recommendations, and it was designed to adhere to Beer's rule for drug concentrations between 1.0 and 5.0 g/mL.

In light of the findings, this study discloses the crucial analytical technique utilized to find APB in the dose form. The HPLC-UV method for quantifying APB created and validated is straightforward, exact, sensitive, focused, robust, and robust. Therefore, the suggested method may be used for routine analysis of APB in tablet dose formulation.

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DISCLAIMER OF COMPETITIVE INTERESTS

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