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A Promising Analytical Method has been Crafted, Validated, and Quantified for Estimating Theophylline Utilizing UV Spectroscopic and RP-HPLC Techniques in Conjunction with Stress Degradation Studies to Pinpoint Deterioration

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The current study aimed to develop and assess UV-spectrophotometric (zero order, first order, second order, area under the curve) and RP-HPLC methods for estimating theophylline in its pharmaceutical dosage form.

Methods: A less toxic solvent composition of acetonitrile and 0.1% orthophosphoric acid (25:75 v/v) is used as a mobile phase and diluent for developing both UV-spectroscopic and RP-HPLC techniques. Shimadzu Prominence LC-20A Modular HPLC system with a C_{18} column (250 × 4.6 mm; 5 µm) was used to develop the RP-HPLC method.

Results: Method A is a zero-order spectrophotometric method for determining theophylline at 271 nm, and the correlation coefficient in the linearity study was found to be 0.9958, LOD, and LOQ are 0.52 and 1.71 µg/mL. Method B is a first-order spectrophotometric method for determining theophylline at 258 nm, and the correlation coefficient in the linearity study was found to be 0.9983, LOD, and LOQ are 0.46 and 1.51 µg/mL. Method C is a second-order spectrophotometric method for determining theophylline at 218 nm, and the correlation coefficient in the linearity study was found to be 0.9941, LOD, and LOQ are 0.38 and 1.25 µg/mL. Method D is an area under the curve spectrophotometric method for determining theophylline at 260 to 282 nm, and the correlation coefficient in the linearity study was found to be 0.9978, LOD, and LOQ are 0.57 and 1.88 µg/mL. Method E is the RP-HPLC method for the determination of theophylline at the retention time of 2.814 min, and the correlation coefficient in the linearity study was found to be 0.9923, LOD, and LOQ are 0.78 and 2.57 µg/mL. Studies on stress degradation show that oxidation and acid degradation mostly impact theophylline solutions.

Conclusion: Theophylline can be determined using the proposed approach, which is convenient, precise, affordable, and reproducible.

Keywords: Theophylline; Reverse phase high-performance liquid chromatography; Degradation study; UV-spectrophotometric.

1. INTRODUCTION

An analysis is essential in any product or service, and it is also necessary for the drug because it involves life [1]. Analytical chemistry studies the separation, measurement, and identification of chemical additives in synthetic and herbal materials comprising one or more chemicals or elements [2]. The two main categories of analytical chemistry are qualitative evaluation and quantitative evaluation. Qualitative evaluation refers to identifying the chemical additives present in the sample. Quantitative evaluation determines the amount of positive detail or compound in the substance [3]. A validated quantitative stability-indicating analytical method can identify the changes in the chemical, physical, or microbiological properties of the drug substance and products with time [4]. COPD is a significant public health issue and the world's leading cause of illness and death. Global Initiative for Obstructive Lung Disease estimates suggests that COPD will rise from the sixth to the third most common cause of death worldwide by 2020. COPD is presently the fourth most prevalent cause of death in the United States, affecting more than 16 million people [5]. While it has been used clinically for over 80 years, theophylline is among the most often prescribed medications for treating asthma and chronic obstructive pulmonary disease (COPD) globally [6]. Theophylline (1, 3 Dimethyl Xanthine) is an orally administered xanthine derivative that induces smooth muscle relaxation in the bronchial tree, causing bronchodilation [7]. It is also used as an adjunct in treating congestive heart failure and acute pulmonary edema. Still, it has no established efficacy in chronic irreversible airway obstruction patients [8]. “The mechanism of action of theophylline suggested by the non-selective inhibition of phosphodiesterase’s (PDE), inhibition of phosphoinositide 3-kinase-δ (PI3K-δ), adenosine receptor antagonism, and increased activity of certain histone deacetylases (HDACs) that deacetylate lysine residues in chromatin, thereby silencing gene transcription” [9]. “Theophylline is also an antagonist of adenosine receptors with affinities against the human-cloned adenosine receptors in the mM range (A1 receptor, 10–30 µM; A2A receptor, 2–10 µM; A2B receptor, 10–30 µM; A3 receptor, 20–100 µM), levels that can be achieved clinically” [10]. Theophylline's adverse effects include stimulation of the central nervous system (CNS), suppression of adenosine A2A receptor signalling may

paradoxically increase inflammation, cardiac arrhythmias (through blocking of A1receptors), gastric hypersecretion, gastroesophageal reflux, and diuresis [11].

Various analytical techniques have been reported for quantifying theophylline by UV-spectrophotometric and RP-HPLC methods. Reported crucial approaches are the determination of theophylline in pharmaceutical dosage forms by buffer-free high-performance liquid chromatography method [12], validation and quantification of theophylline and salbutamol using ion-pair liquid chromatography [13], HPLC and UV method for the estimating of theophylline in rabbit plasma [14], RP-HPLC method for simultaneous estimation of salbutamol sulphate and theophylline in pharmaceutical syrup dosage form [15], development and validation of UV spectrophotometric methods for simultaneous estimation of amlodipine besylate and theophylline [16].

According to the previously reported analytical methods, our main aim is to develop robust, rapid, sensitive, selective, linear, and precise different types of UV-spectrophotometric and RP-HPLC methods for determining theophylline. The method was validated as per the United States Pharmacopeia [17] and ICH guidelines [18]. The proposed analytical method developed by the appropriate selection of less toxic solvents is an enormous challenge because better separation and quantification analysis is much more important. As per ICH Q2(R1) guidelines [19], linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ) are performed and utilized in determining the drug content of the theophylline in a pharmaceutical product.

2. MATERIALS AND METHODS

2.1 Materials

Mylan Laboratories Ltd., Hyderabad, India, provided theophylline bulk powder as a kind gift. Theophylline commercial formulation Unicontin-E (400 mg) was purchased from the local pharmacy. The investigation used only chemical reagents of analytical quality. From GlaxoSmithKline Pharmaceuticals Limited in Mumbai, India, HPLC-grade acetonitrile and orthophosphoric acid were procured. From Gujarat, India's Ideal Chemicals Pvt. Ltd., we received sodium hydroxide, hydrogen peroxide, and hydrochloric acid.

2.2 Instrumentation

Shimadzu 1800 UV spectrophotometer was used for this analysis, with 1 cm matched quartz cells for all measurements and UV probe 4.2 series software. The quantitative determination was conducted with a Shimadzu Prominence LC-20A Modular HPLC system (Shimadzu, Kyoto, Japan) consisting of a solvent delivery unit, sample injector, and PDA detector (SPD-M20A). Data were acquired and processed using LC-solution software (version 1.24, Shimadzu). A C_{18} column (250 × 4.6 mm; particle size 5 µm) maintained at 40°C was used to separate the compound. The investigation employed a digital analytical balance (Mettler Toledo, India), an ultrasonic sonicator (Spectra Lab, India), and validated borosilicate glass pipettes, volumetric flasks, and beakers.

2.3 Selection of Solvents Based on the Solubility and Stability Studies

Several solvents were used to assess the drug's solubility and stability at 25°C. Each volumetric flask had 10 mL of the drug dissolved in various solvents and buffers, including water, ethanol, methanol, acetonitrile, phosphate buffer saline pH 7.2, phosphate buffer pH 5.5, 5.6, 7.2, and 7.4. Comparatively, the drug was detected throughout the experiment, and the observed drug was entirely soluble in water, ethanol, methanol, and acetonitrile but not in buffers. Prepare 10 µg/mL solutions using the abovementioned solvents to perform more research on solution stability. At 2, 4, 6, 8, 10, and 12 hours, continually analyze the samples using UV-visible spectroscopy. Most organic phase prepared solutions in the stability study are stable at room temperature for more than 24 hours.

2.4 Different Methods of Development

A novel analytical technique was created to identify the drug's identification, purity, physical attributes, potency of the drug's bioavailability, and stability. The UV and HPLC approaches have the benefit of not requiring the complex processing and processes often connected with specific traits. It takes less time and is more cost-effective. The HPLC technique is superior to the UV approach in terms of accuracy and precision, according to a statistical comparison of the quantitative determination of drugs. The findings show that theophylline may be accurately measured using HPLC and UV spectroscopic techniques for pure and dosage forms.

2.4.1 Method A (Zero order spectrophotometric method)

Using the UV-spectroscopy concept is the simplest technique to conduct numerous investigations. A blank solution for the diluent was maintained [20]. From 200 to 400 nm, samples were recorded. After the optimization study, the λ_{max} was confirmed to be 271 nm. Fig. 1 represents the optimized spectrum by zero-order spectrophotometric method at 10 µg/mL concentration.

2.4.2 Method B (First-order spectrophotometric method)

The approach can recover unresolved band spectra with qualitative and quantitative data [21]. The diluent was kept as a blank solution. Spectra between 200 and 400 nm were measured. The zero-order spectra were transformed into first-order derivative spectra (delta lambda 8, scaling factor 1) using the inbuilt software of the instrument. After interpreting optimization study data, the λ_{max} was found to be 258 nm. Fig. 2 represents the optimized spectrum by first-order spectrophotometric method at 10 µg/mL concentration.

2.4.3 Method C (Second-order spectrophotometric method)

Developed primary data may be used by recovering unresolved band spectra [22]. A blank solution was preserved for the diluent. We measured the spectra between 200 and 400 nm. The instrument's built-in software converted zero-order to second-order derivative spectra (delta lambda 2, scaling factor 1). The standard drug was examined, and the lambda max was determined to be 218 nm. Fig. 3 represents the optimized spectrum by second-order spectrophotometric method at a 10 µg/mL concentration.

2.4.4 Method D (Area under the curve spectrophotometric method)

Effectively solves the broad spectrum with the methodology is two effective points on the mixed spectrum are directly proportional to the concentration of the spectral component of interest [23]. A reference solution was preserved for the diluent. Samples were captured between 200 and 400 nm. Using UV probe software-2.42, the spectra between 260 and 282 nm were recorded. The area versus concentration data was used to conduct the linearity assessment. Fig. 4 represents the optimized spectrum by the area under the curve spectrophotometric method at 10 µg/mL concentration.

2.4.5 Method E (RP-HPLC method)

In the reverse phase, HPLC is used to determine and separate most classes of chemical compounds by using polar solvents for the mobile phase and no-polar components like $C_{18}H_{37}$ or C_8H_{17} for the stationary phase [24].

2.4.6 Optimization of chromatographic detection for RP-HPLC method

Chromatographic conditions were optimized to establish a routine analysis of theophylline, with excellent technique reproducibility and analytical throughput. Numerous columns were used for this analysis, like the Inertsil ODS, C_{18}, C_8, Kromasil nonpolar, and cyano columns. In contrast, the Shimadzu Prominence LC-20A Modular HPLC system with a C_{18} column (250 × 4.6 mm; particle size 5 µm), with acetonitrile and 0.1% orthophosphoric acid (25:75 v/v), was used to achieve the best resolution, appropriate run duration, short retention time, nice peak shape, and enhanced responsiveness. Robustness studies revealed that using C_{18} columns from various vendors and a slight modification in the mobile phase composition had no impact on the analysis. As shown in Fig. 5, the chromatogram of the theophylline reference standard had a retention time of 2.814 min, a constant flow rate of 1.00 mL/min, at a column temperature of 25°C, at 271 nm, isocratic mode, and a 10 min run-time.

2.5 Preparation of Mobile Phase

HPLC-grade acetonitrile and 0.1% orthophosphoric acid (25:75 v/v) prepare the mobile phase. To prepare 0.1% orthophosphoric acid, 0.1 mL of liquid orthophosphoric acid dissolved in the HPLC grade water and volume up to 100 mL. The selected mobile phase was mixed well and degassed in an ultrasonic water bath for 20 min, and then the resulting solution was filtered through a 0.45 µm filter under vacuum filtration.

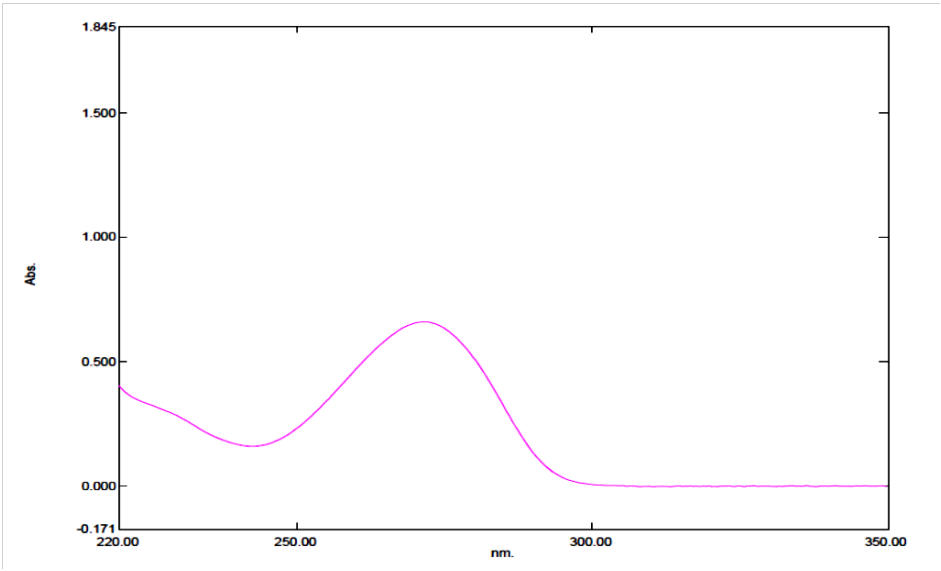

Fig. 1. Optimised spectrum of theophylline at 10 µg/mL concentration by zero-order spectroscopic method

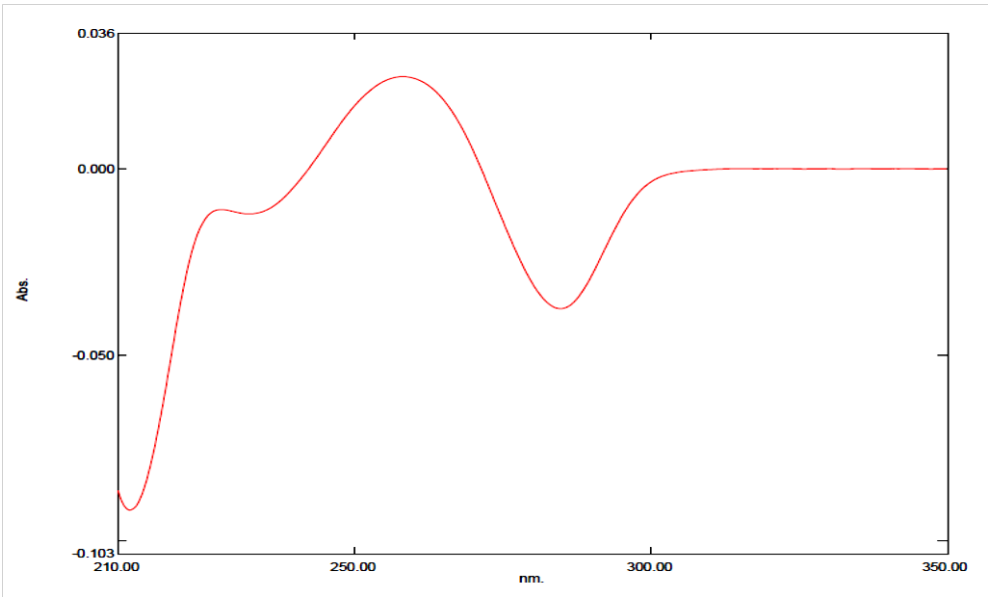

Fig. 2. Optimised spectrum of theophylline at 10 µg/mL concentration by first-order spectroscopic method

2.6 Sample Preparations UV-spectroscopic and HPLC methods

2.6.1 Preparation of solution for standard drug

Accurately weigh and transfer 10 mg of theophylline into a 10 mL clean, dry volumetric flask, add the mobile phase, and sonicate to dissolve it completely and make volume up to the mark with the mobile phase. Based on the requirement, samples are prepared for methods A, B, C, D, and E by dilution with the mobile phase made from the working standard.

2.6.2 Preparation of solution for commercial formulation

Ten commercial tablets (Unicontin-E, 400 mg) were carefully weighed for the assay investigation, and the average weight was determined. The tablets were crushed uniformly

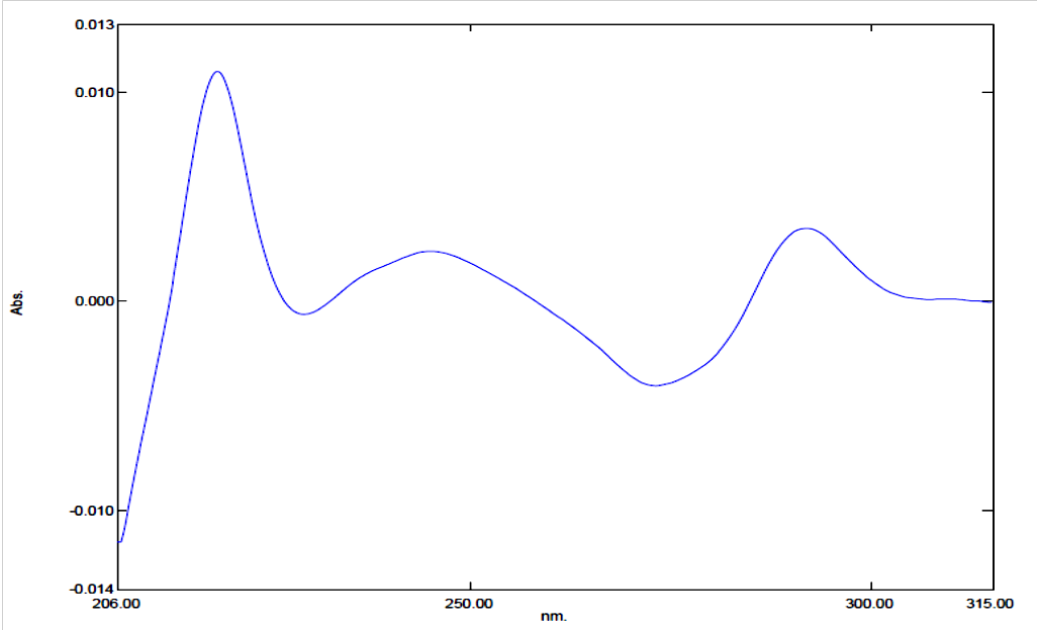

Fig. 3. Optimised spectrum of theophylline at 10 µg/mL concentration by second-order spectroscopic method

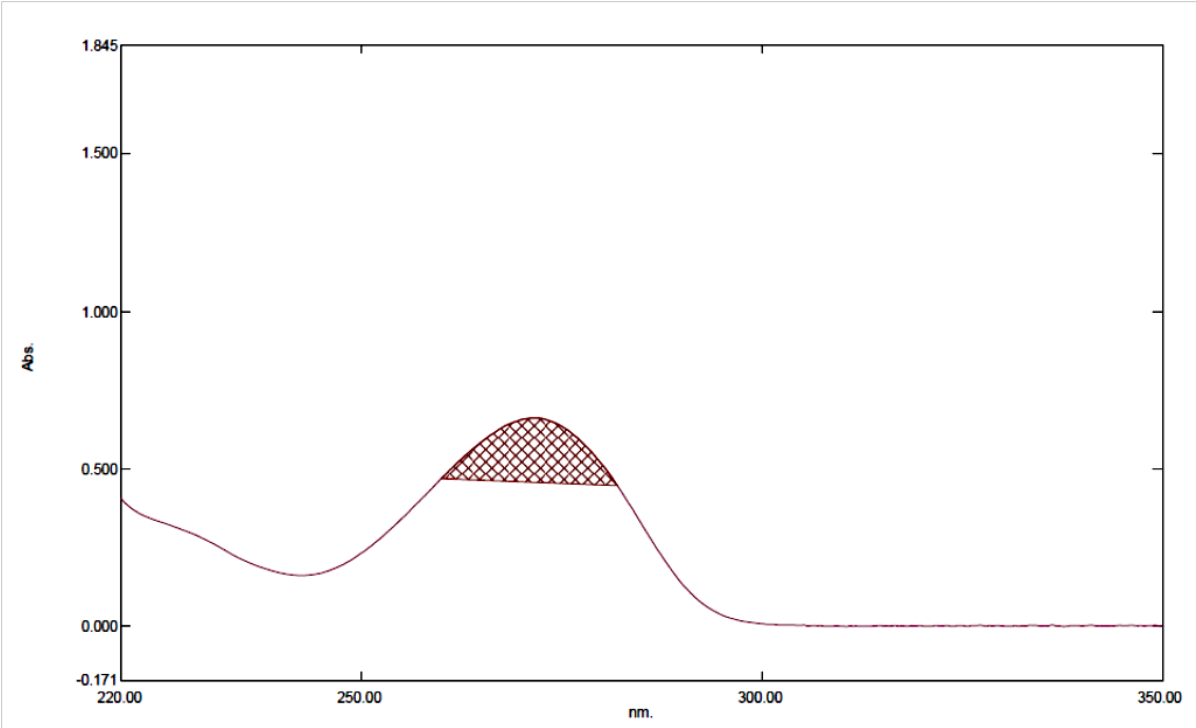

Fig. 4. Optimised spectrum of theophylline at 10 µg/mL concentration by the area under the curve spectroscopic method

to obtain a fine powder. The powder equivalent to 25 mg of theophylline was transferred into the volumetric flask of 25 mL volume and sonicated for 15 minutes with sufficient mobile phase to dissolve the drug; the volume was regulated up to the mark with the mobile phase. The obtained solution was filtered using the Whatman filter paper. After the filtration solution was diluted with the mobile phase to produce the sample solutions for methods A, B, C, D, and E, the

percentage estimation of the drug was calculated using the assay formula.

2.7 Method Validation

A crucial task in the pharmaceutical sector is method validation. Validation data are used to verify that the analytical method utilised for a particular test is appropriate for its goals. These data demonstrate the analytical method's efficacy, dependability, and consistency. Following ICH Q2 (R1) recommendations, the analytical methodology was carried out to validate analytical methods for system suitability, linearity, detection limit, quantification, accuracy, precision, and robustness for HPLC and UV-spectroscopic methods [25].

2.8 Stress Degradation Studies by HPLC and UV-spectroscopic Method

Degradation studies provide a tool for analysing the stability of medication samples in the pharmaceutical industry. The molecule's chemical stability influences the safety and efficacy of pharmaceutical products. The information required to select the ideal formulation, container, storage condition,

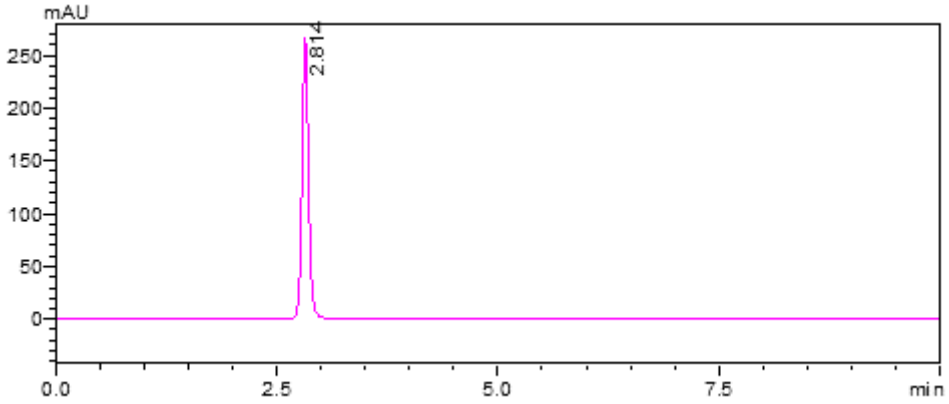

Fig. 5. Optimised chromatogram of theophylline at 18 µg/mL concentration by RP-HPLC method

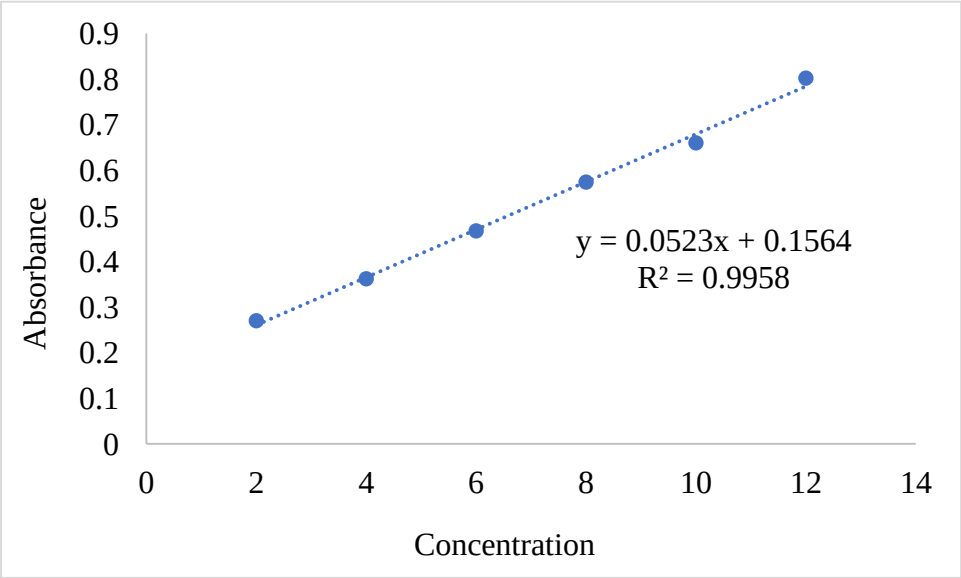

Fig. 6. Calibration curve of theophylline for method A (Zero-order spectrophotometric method)

Table 1. Intraday precision for methods A, B, C, and D of theophylline

Sl.No.	Conc. (µg/mL)	Method A Absorbance	Method B	Method C	Method D Area	%RSD Method			
						A	B	C	D
1	10	0.661	0.025	0.012	2.847	0.24%	0%	0%	0.84%
2	10	0.665	0.025	0.012	2.818				
3	10	0.661	0.025	0.012	2.873				
4	10	0.662	0.025	0.012	2.812				
5	10	0.661	0.025	0.012	2.825				
6	10	0.661	0.025	0.012	2.856				

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Second-order spectrophotometric method) Method D (Area under the curve spectrophotometric method).

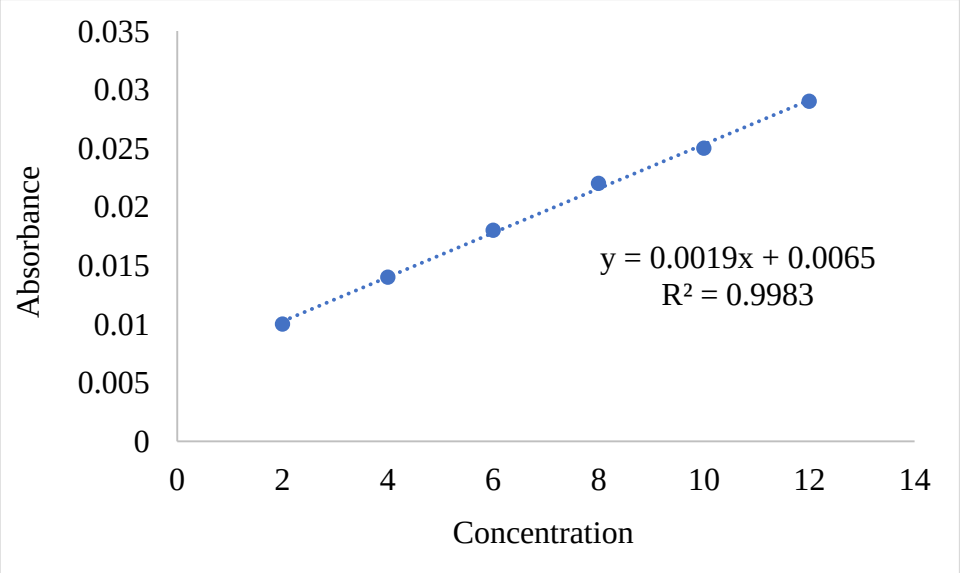

Fig. 7. Calibration curve of theophylline for method B (First-order spectrophotometric method)

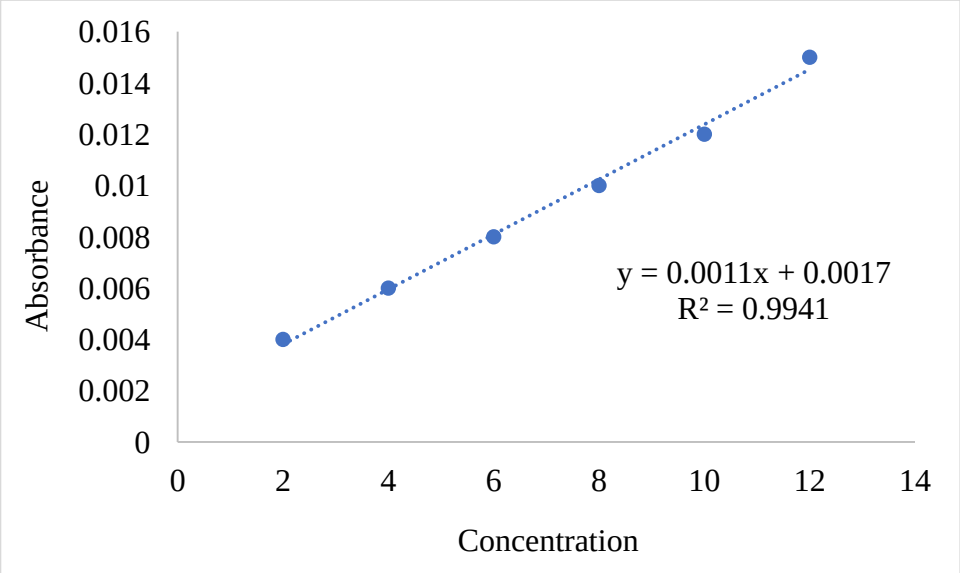

Fig. 8. Calibration curve of theophylline for method C (Second-order spectrophotometric method)

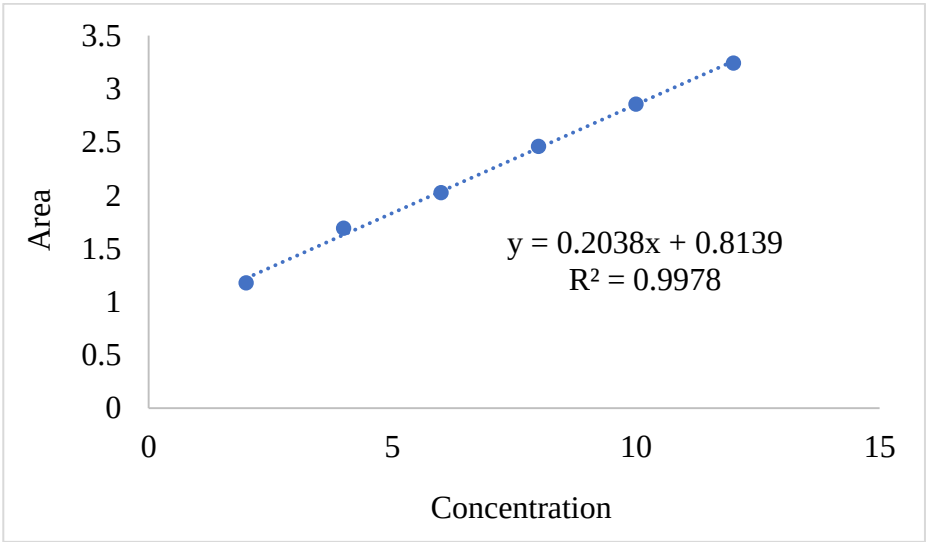

Fig. 9. Calibration curve of theophylline for method D (Area under the curve spectrophotometric method)

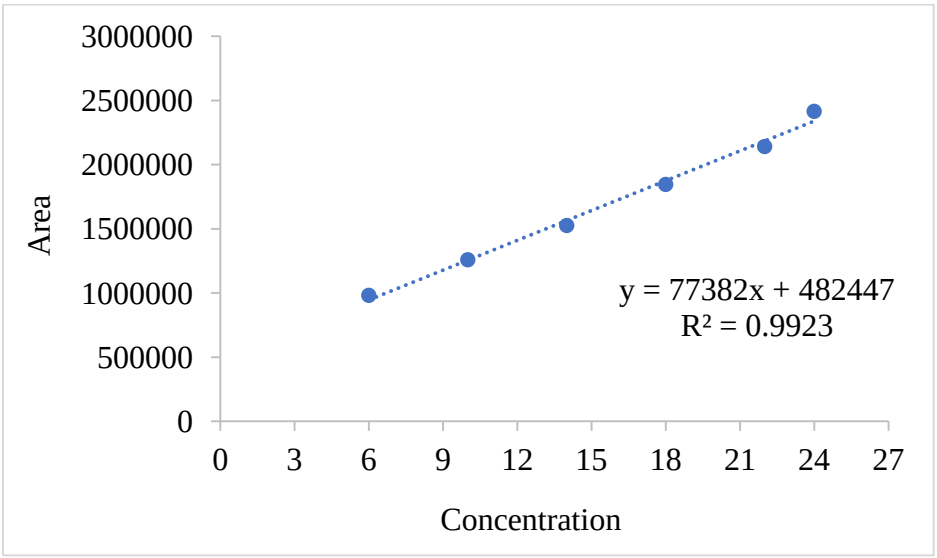

Fig. 10. Calibration curve of theophylline for method E (RP-HPLC method)

Table 2. Interday precision for methods A, B, C, and D of theophylline

Sl.No.	Conc. (µg/mL)	Method A	Method B	Method C	Method D	%RSD			
		Absorbance			Area	Method			
						A	B	C	D
1	10	0.645	0.025	0.012	2.852				
2	10	0.652	0.025	0.012	2.756				
3	10	0.661	0.025	0.012	2.789	1.41%	0%	0%	1.89%
4	10	0.668	0.025	0.012	2.875				
5	10	0.665	0.025	0.012	2.884				
6	10	0.649	0.025	0.012	2.879				

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Second-order spectrophotometric method), Method D (Area under the curve spectrophotometric method)

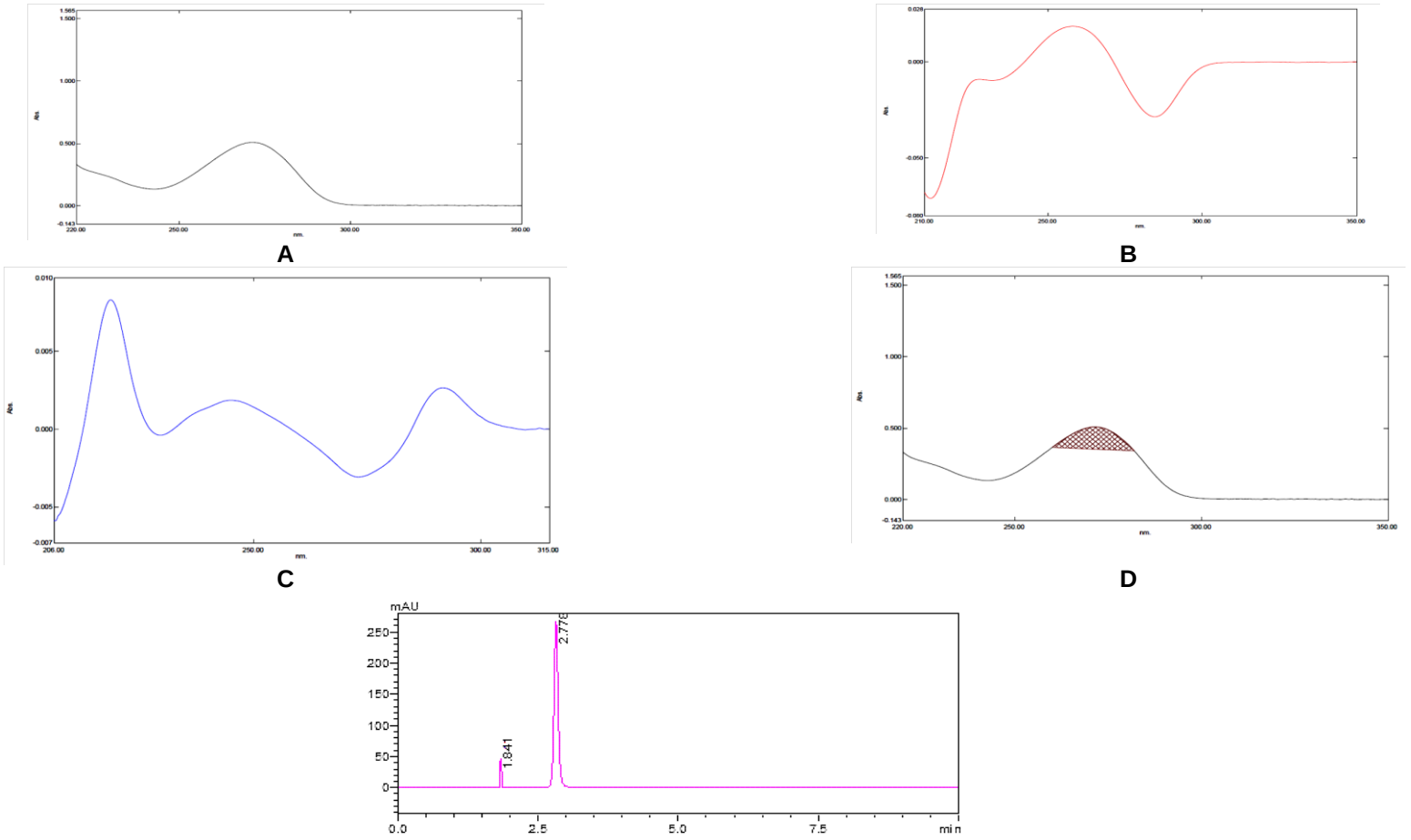

Fig. 11. The oxidative stress degradation studies spectrum and chromatograms for methods A, B, C, D, and E

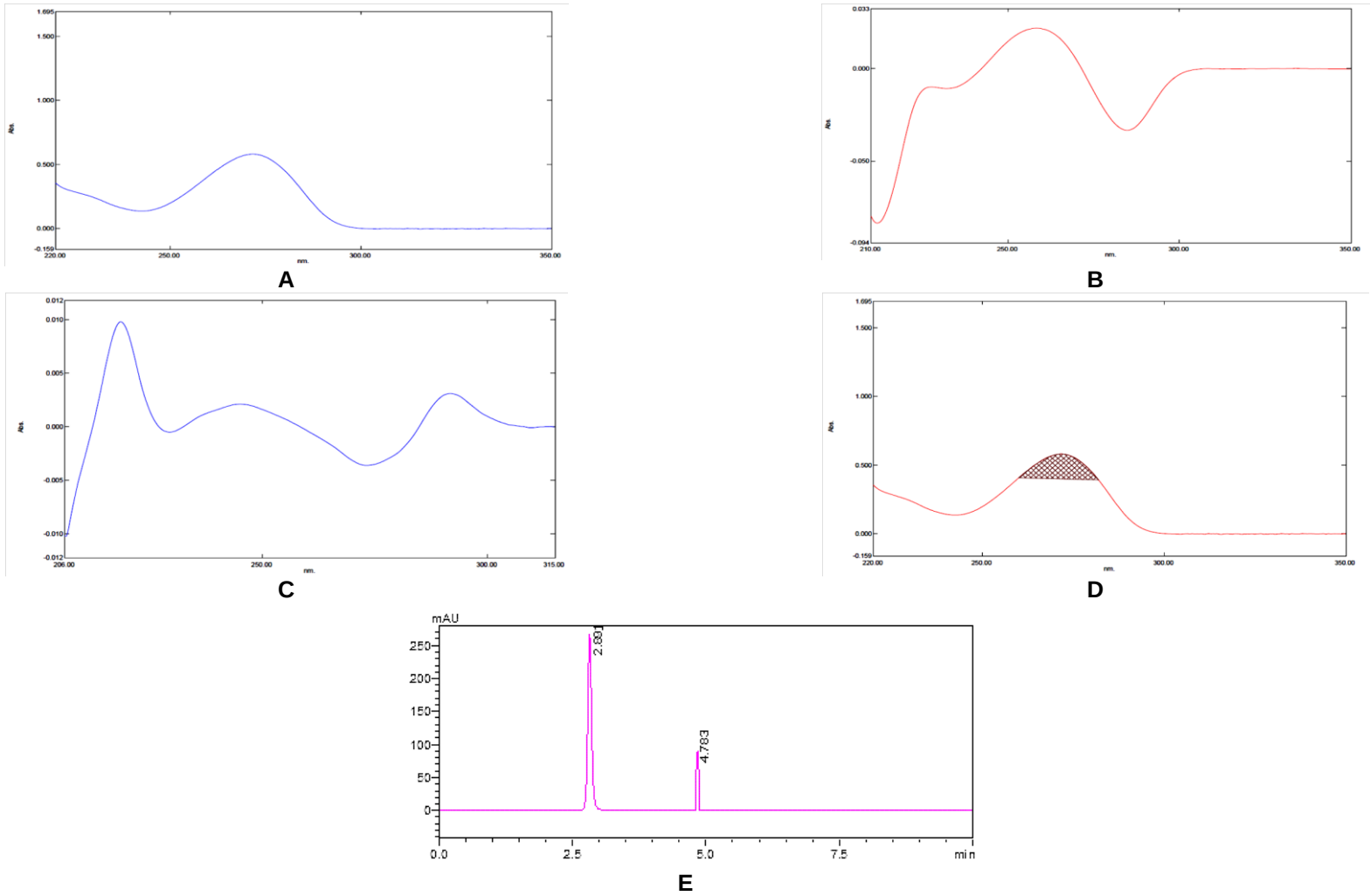

Fig. 12. The acid stress degradation studies spectrum and chromatograms for methods A, B, C, D, and E

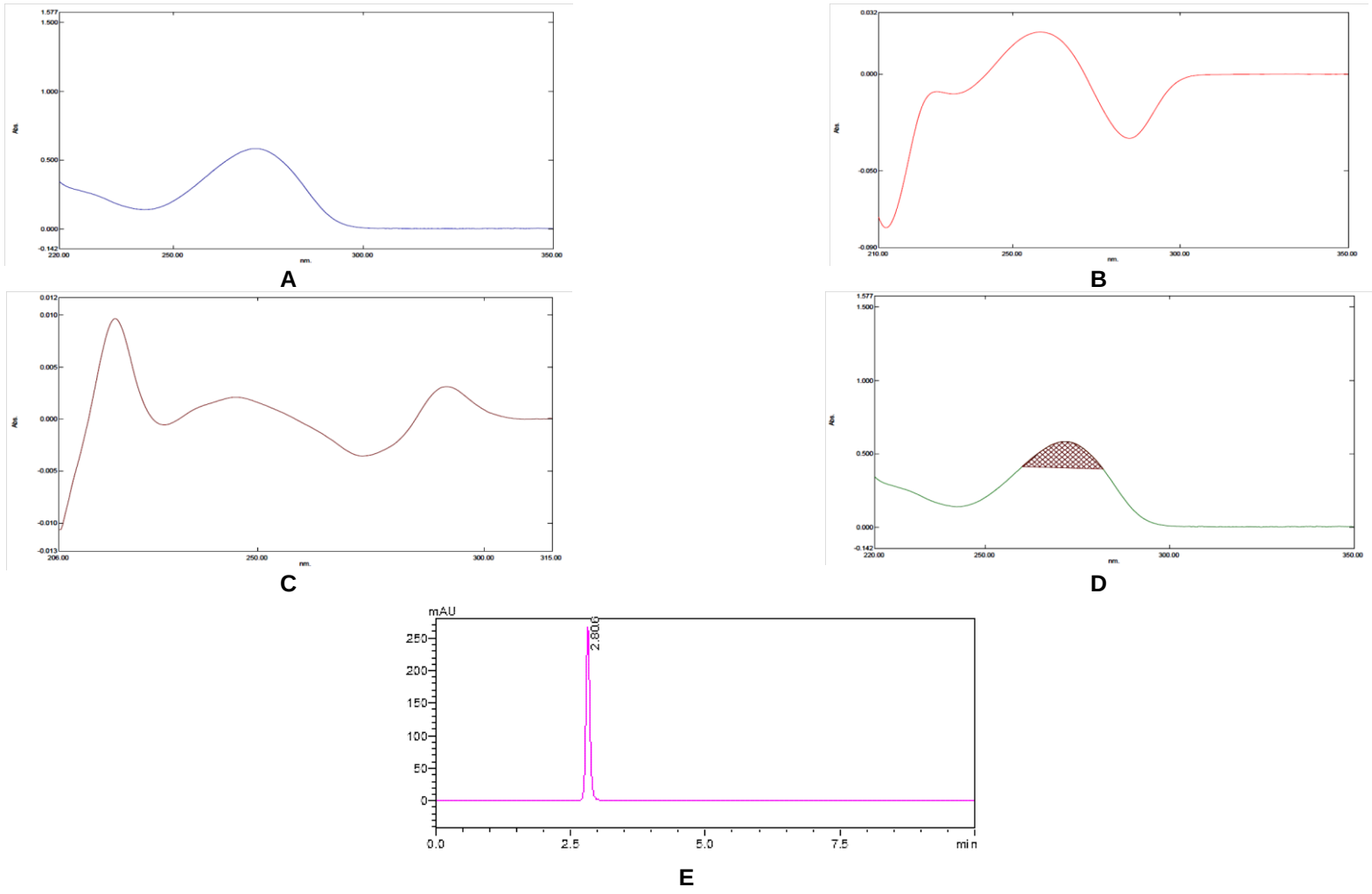

Fig. 13. The alkali stress degradation studies spectrum and chromatograms for methods A, B, C, D, and E

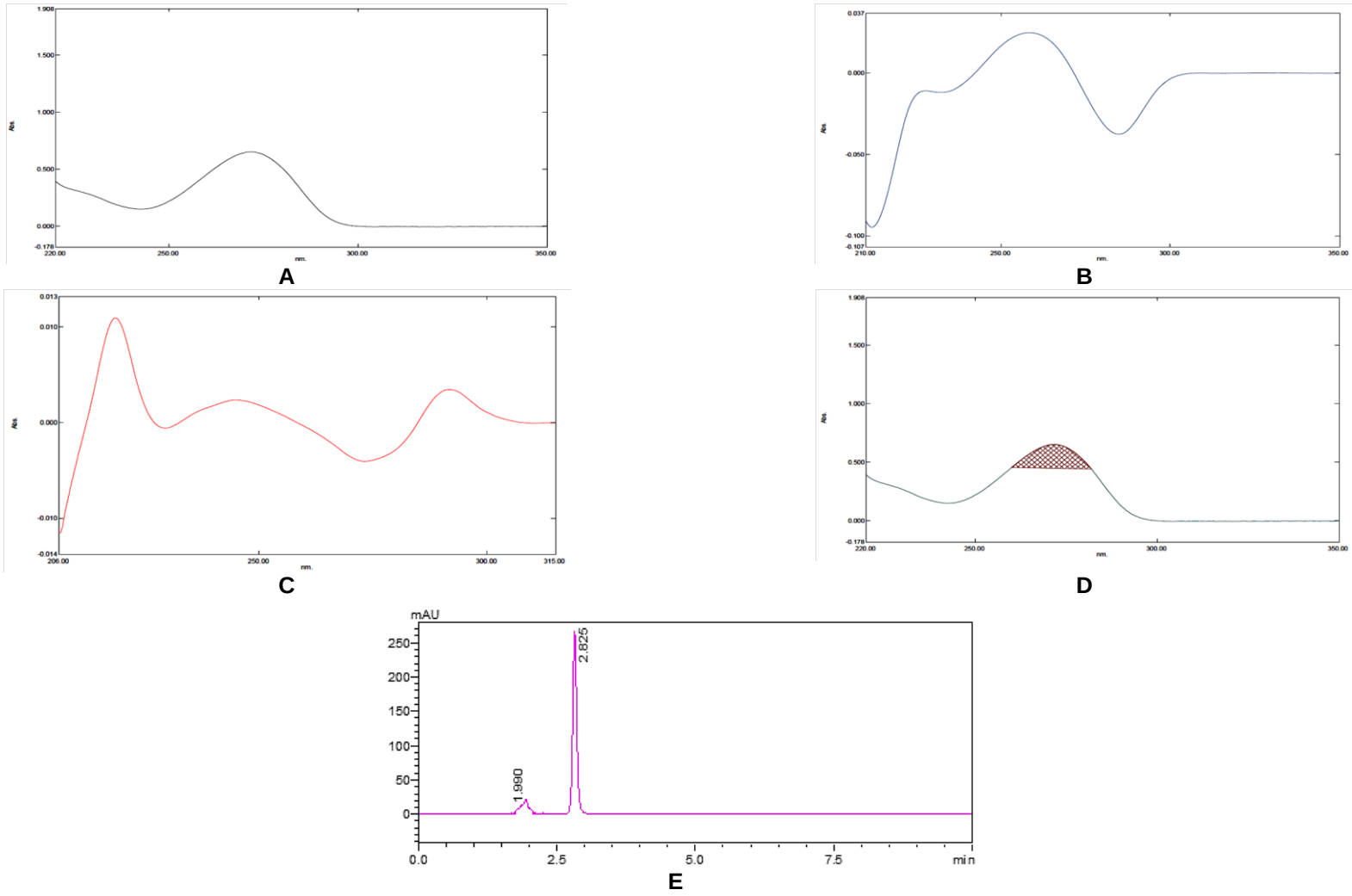

Fig. 14. The thermal stress degradation studies spectrum and chromatograms for methods A, B, C, D, and E

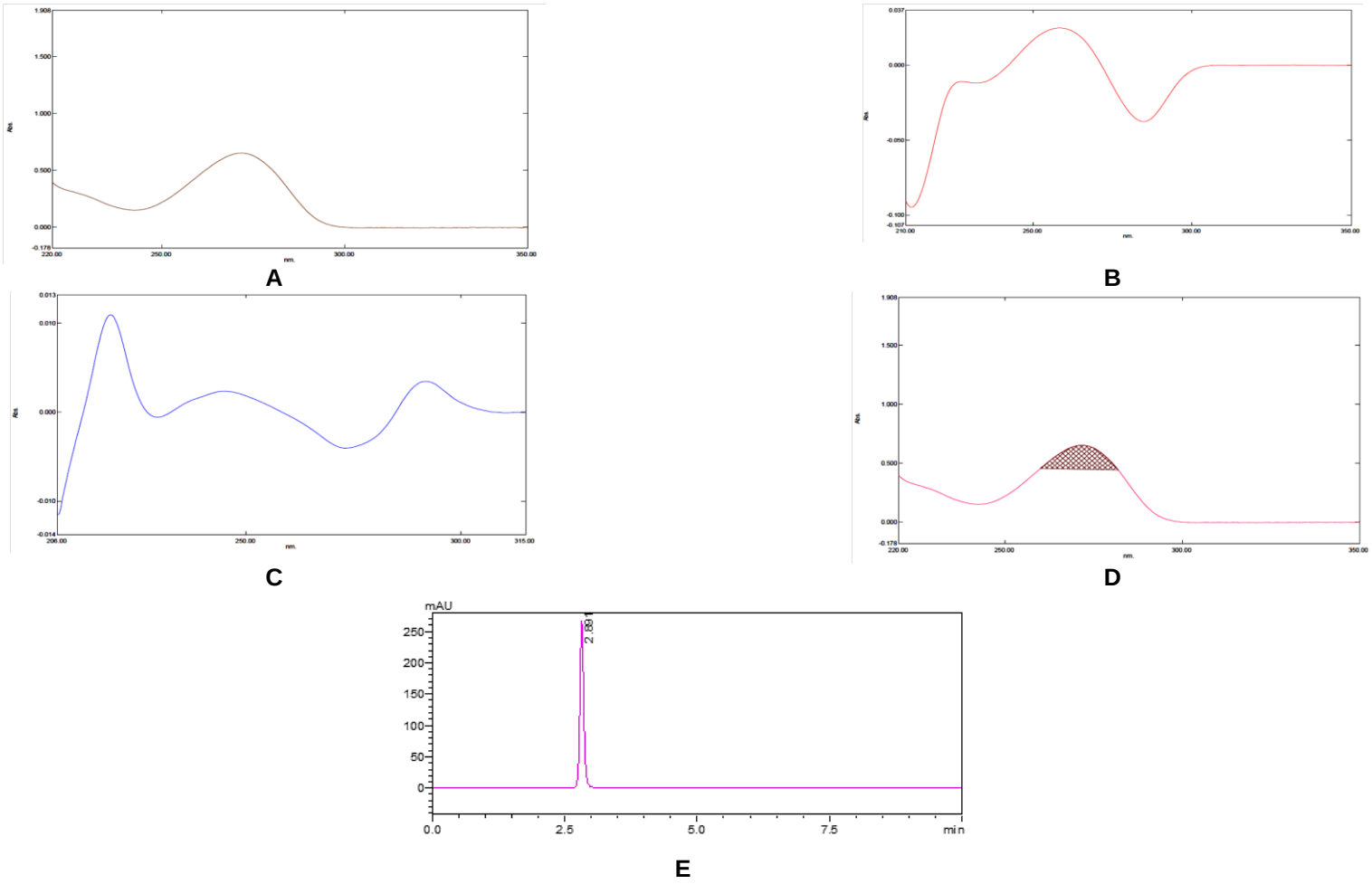

Fig. 15. The photolytic stress degradation studies spectrum and chromatograms for methods A, B, C, D, and E

Table 3. Intraday and Interday precision for method E (RP-HPLC method)

Sl.No.	Conc. (µg/mL)	Intraday precision area	Interday precision area
1	12	1843258	1825874
2	12	1842514	1832514
3	12	1839587	1834145
4	12	1841258	1836258
5	12	1844125	1832147
6	12	1845258	1826895
%RSD		0.11%	0.22%

and shelf life is provided by knowledge of molecular stability. These facts are significant and play a big role in regulatory documentation. Novel therapeutic compounds must undergo stability tests before completing the registration dossier. Stability studies must be conducted per International Conference on Harmonization (ICH) recommendations (Q1A) to suggest a shelf life for novel drug ingredients and products. To assess the suggested method's stability indicating characteristics and specificity, stress degradation studies by HPLC and UV-spectroscopic methods of theophylline were also carried out, followed by ICH recommendations [26,27] and Mondal et al. (2016) [28]. All solutions used in stress studies were prepared at an initial concentration of 1mg/mL of theophylline and further diluted in the mobile phase to give a final concentration of 10 µg/mL for methods A, B, C, D, and 18 µg/mL for method E and filtered the solutions before injection. Acid degradation was conducted in 0.5M hydrochloric acid, alkaline degradation was conducted using 0.5N sodium hydroxide, and solutions for oxidative stress studies were prepared using 10 % hydrogen peroxide refluxed for 90 min at 60°C. The drug solution was heated in a thermostat for thermal stress degradation testing, and the sample solution was cooled and used. Photostability was exposed to UV light for 6 hours under a UV chamber (365 nm) and analyzed.

3. RESULTS

3.1 Method Validation

The developed spectroscopy and chromatographic techniques are reliable, precise, accurate, and specific for estimating theophylline. The International Conference on Harmonization (ICH) recommendations provided the framework for the results. This guideline relates to new or updated analytical techniques for assessing the release and stability of chemical, biological, and biotechnological pharmacological ingredients and products.

3.1.1 Linearity

Linearity is an analytical method that produces test results directly proportional to the concentration of analyte present in the test sample. In Linearity studies, calibration curves were graphed in a 2-12 µg/mL concentration range for methods A, B, C, D and 6-16 µg/mL for method E. The linear regression equation of method A is y = 0.0523x + 0.1564 with a correlation coefficient of 0.9958 (Fig. 6), Method B is y = 0.0019x + 0.0065 with a correlation coefficient of 0.9983 (Fig. 7), Method C is y = 0.0011x + 0.0017 with a correlation coefficient of 0.9941 (Fig. 8), Method D is y = 0.2038x + 0.8139 with a correlation coefficient of 0.9978 (Fig. 9), Method E is y = 77382x + 482447 with a correlation coefficient of 0.9923 (Fig. 10).

3.1.2 Precision

When RSD in precision studies was less than 2%, the suggested procedure had acceptable reproducibility. The performance of intraday and interday precision and the percent RSD for the response of six replicate measurements in each developed method A, B, C, D, and E were within the acceptable ranges. Results from the intraday and interday precision studies are summarized in Tables 1, 2, and 3.

3.1.3 Accuracy

The percentage of recovery values in the accuracy studies demonstrates that the proposed method is accurate, and that interference response exists. By adding an adequate amount of theophylline standard stock solution to the sample solution, accuracy was evaluated at three different concentration levels (50%, 100%, and 150%). Three replicate measurements are performed for methods A, B, C, D, and E, showing that the percent recovery was within the allowed ranges (Tables 4 and 5).

Table 4. Theophylline accuracy observations for methods A, B, C, and D

Level	Conc. (µg/mL)	Amount of drug added (µg/mL)		Amount recovered (µg/mL)				% Recovery			
		Pure	Formulation	Method				Method			
				A	B	C	D	A	B	C	D
	3	2	1	2.96	2.89	2.98	2.95	1.61%	1.88%	1.71%	0.70%
50%	3	2	1	2.89	2.98	2.88	2.92				
	3	2	1	2.87	2.88	2.92	2.96				
	6	2	4	5.96	5.92	5.97	5.98	1.33%	0.35%	0.17%	0.44%
100%	6	2	4	5.84	5.89	5.96	5.97				
	6	2	4	5.99	5.93	5.98	5.93				
	9	2	7	8.96	8.95	8.99	8.98	0.49%	0.42%	0.29%	0.42%
150%	9	2	7	8.88	8.89	8.98	8.91				
	9	2	7	8.95	8.96	8.94	8.97				

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Second-order spectrophotometric method), and Method D (Area under the curve spectrophotometric method)

Table 5. Theophylline accuracy observations for methods E (RP-HPLC method)

Level	Conc. (µg/mL)	Amount of drug added (µg/mL)		Amount recovered (µg/mL)	% Recovery
		Pure	Formulation		
50%	6	4	2	5.97	
	6	4	2	5.95	0.42%
	6	4	2	5.92	
100%	12	4	8	11.95	
	12	4	8	11.96	0.08%
	12	4	8	11.94	
150%	18	4	14	17.92	
	18	4	14	17.88	0.12%
	18	4	14	17.91	

Table 6. The theophylline robustness data for several approach techniques using UV and HPLC techniques

Method	Condition	%RSD
A	Wavelength 269 nm	0.23
	Wavelength 273 nm	0.47
B	Wavelength 256 nm	0.58
	Wavelength 260 nm	0.69
C	Wavelength 216 nm	0.89
	Wavelength 220 nm	0.41
D	Wavelength 258 nm to 280nm	0.78
	Wavelength 262 nm to 284nm	0.35
E	Flow rate 0.8 mL/min	0.52
	Flow rate 1.2 mL/min	1.74
	Mobile phase acetonitrile: 0.1% orthophosphoric acid (20:80 v/v)	0.52
	Mobile phase acetonitrile: 0.1% orthophosphoric acid (30:70 v/v)	1.24
	Temperature 27°C	0.56
	Temperature 33°C	0.87

**Mean of six observations.*

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Second-order spectrophotometric method), Method D (Area under the curve spectrophotometric method), Method E (RP-HPLC method)

Table 7. Employing UV and HPLC techniques, the theophylline sensitivity assessments (LOD and LOQ)

Method	LOD (µg/mL)	LOQ (µg/mL)
Method A	0.52	1.71
Method B	0.46	1.51
Method C	0.38	1.25
Method D	0.57	1.88
Method E	0.78	2.57

**Mean of three observations.*

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Second-order spectrophotometric method), Method D (Area under the curve spectrophotometric method), Method E (RP-HPLC method)

3.1.4 Robustness

The robustness study was performed by altering the wavelength (±2 nm) in method A and the flow rate (±0.1mL/min), mobile phase ratio, and temperature (±3°C) in method B. All the parameters were passed with no notable changes. The percent RSD was within the acceptable range (Table 6).

3.1.5 Limits of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ parameters were determined from the regression equation of theophylline; LOD = 3.3 σ/S, LOQ = 10 σ/S, where the standard deviation of the response (σ) and S is the slope of the corresponding calibration curve. In the LOD analysis, the detection limits for methods A, B, C, D, and E were 0.52, 0.46, 0.38, 0.57, and 0.78 µg/mL, while the LOQ was 1.71, 1.51, 1.25, 1.88 and 2.57 µg/mL, respectively. Table 7 displays the relevant LOD and LOQ values for theophylline.

3.2 Analysis of Marketed Formulations

The commercially available Unicontin-E, 400 mg formulations of theophylline assay were carried out, and the purity percentage was assessed by methods A, B, C, D, and E. Neither substantial variation was found during the percentage purity analysis. The interpretation findings for the marketed theophylline tablet are depicted in Table 8.

3.3 Stress Degradation Studies

Studies on stress degradation were carried out under various stressful conditions, but no significant degradation was observed. The highest degradation percentage was observed in oxidation stress tribunals, where methods A, B, C, and D observed 22.87%, 24%, 25%, and 23.57% of degradation, respectively. However, method E showed 8.15% degradation at 2.778 min retention time (Fig. 11).

Studies on acid stress degradation indicated that methods A, B, C, and D exhibited 11.66%, 12%, 16.66%, and 11.83% degradation, respectively. In contrast, method E showed a degradation percentage of 5.65% at 2.891 min of retention time (Fig. 12).

In investigations on alkali stress degradation, it was revealed that methods A, B, C, and D exhibited degradation rates of 11.51%, 12%, 16.66%, and 11.06%, respectively. In contrast, method E had a degradation rate of 5.31% at 2.806 min of retention time (Fig. 13).

Thermal stress degradation studies observed less degradation, with methods A, C, and D finding 0.15%, 8.33%, and 0.03%, and method E finding 0.73% at 2.856 min retention time. However, no degradation was seen for method B throughout the analysis period (Fig. 14).

Regarding photolytic stress degradation, method A showed degradation percentages of 1.06%; however, methods B, C, and D showed no degradation at all, and method D showed a degradation percentage of 0.08% at 2.891 min retention time (Fig. 15).

The summary of the validation parameters is illustrated in Table 9, while Table 10 summarises the desired outcome of stress degradation studies.

4. DISCUSSION

The findings of the current investigation indicate that efficient separation and selectivity were achieved in a shorter runtime using the developed and validated method. The spectrophotometric method linear response was obtained in the 2-12 µg/mL concentration range, with a correlation coefficient of 0.99. Using a gradient technique, the chromatogram was monitored with the mobile phase flow rate. The system suitability parameters were evaluated. The developed and validated method was found to be linear, accurate, precise, and robust against the wide concentration of theophylline, which might help qualitative and quantitative validation. The significant objective of this study was to pinpoint the spectroscopic and chromatographic techniques that were reliable enough to produce an appropriate separation of the components with a good spectrum for the UV-spectroscopic methods and chromatogram within a reasonable run time for the reverse phase high-performance liquid chromatographic method. The target analytical profile was created to identify critical method attributes influencing critical quality attributes, and a systematic risk analysis was conducted. The most important quality variables were specificity, tailing factor, resolution, separation factor, and retention time. Mobile phase characteristics were discovered to be the most crucial for the specified analysis based on risk priority number. Therefore, three parameters were selected as crucial technique features: the acetonitrile ratio, the 0.1% orthophosphoric acid ratio, and the flow rate in the mobile phase. This experimental effort discovered the most significant separated peak observed at 271 nm after obtaining the UV spectrum in the PDA detector. The mobile phase of acetonitrile and 0.1% orthophosphoric acid (25:75 v/v) at a 1 mL/min flow rate characterizes the recognized point by combining the unique crucial method features. The design space presents the operable method region where the changes will not affect the quality of the analysis. The methods were validated as per International Council of Harmonization (ICH) guidelines [18-19], Validation of Analytical Procedures: Text and Methodology Q2 (R1). According to studies on stress degradation, UV-spectroscopic and HPLC analysis of theophylline solutions demonstrated no indications of insignificance degradation [14-15].

Table 8. Assay data for the commercially available theophylline formulations (Unicontin-E) using UV and HPLC techniques

Drug and label claim	Amount estimated (mg/tab)					Purity (% w/w) ± S.D, (%RSD)				
	Method					Method				
	A	B	C	D	E	A	B	C	D	E
Unicontin-E (400 mg)	399 ± 0.85	399 ± 0.78	399 ± 0.34	399 ± 0.17	399 ± 0.61	99.71 ± 0.41 (0.61%)	99.63± 0.34 (0.57%)	99.31 ± 0.26 (0.44%)	99.23 ± 0.36 (0.11%)	99.59 ± 0.26 (0.31%)

**Mean of three observations.*

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Second-order spectrophotometric method), Method D (Area under the curve spectrophotometric method), Method E (RP-HPLC method)

Table 9. Overview of theophylline UV-spectrophotometric and RP-HPLC validation parameters

Parameters	Method A	Method B	Method C	Method D	Method E
λmax	271 nm	258 nm	218 nm	260-282 nm	271 nm
Linearity (µg/mL)	2-12 µg/mL	2-12 µg/mL	2-12 µg/mL	2-12 µg/mL	6-16µg/mL
Regression coefficient	R^2 = 0.9958	R^2 = 0.9983	R^2 = 0.9941	R^2 = 0.9978	R^2 = 0.9923
Regression equation (y=mx+c)	y = 0.0523x + 0.1564	y = 0.0019x + 0.0065	y = 0.0011x + 0.0017	y = 0.2038x + 0.8139	y = 77382x + 82447
Intra-day precision (% RSD)	0.24%	0%	0%	0.84%	0.11%
Inter-day precision (% RSD)	1.41%	0%	0%	1.89%	0.22%
Robustness (% RSD)	0.23-0.47	0.58-0.69	0.41-0.89	0.35-0.78	0.52-1.74
LOD (µg/mL)	0.52	0.46	0.38	0.57	0.78
LOQ (µg/mL)	1.71	1.51	1.25	1.88	2.57

Table 10. The desired outcome of theophylline stress degradation studies employing UV-spectrophotometric and RP-HPLC approaches

Degradation condition	Method A	Method B	Method C	Method D	Method E	% Degradation				
	Absorbance			Area		Method				
						A	B	C	D	E
Oxidation	0.509	0.019	0.008	2.182	1695286	22.87%	24%	25%	23.57%	8.15%
Acid	0.583	0.022	0.010	2.517	1741256	11.66%	12%	16.66%	11.83%	5.65%
Alkali	0.584	0.022	0.010	2.539	1747536	11.51%	12%	16.66%	11.06%	5.31%
Thermal	0.659	0.025	0.011	2.854	1832142	0.15%	0%	8.33%	0.03%	0.73%
Photolytic	0.653	0.025	0.012	2.855	1844128	1.06%	0%	0%	0%	0.08%

5. CONCLUSION

The current research proposes an accurate, efficient, and specific UV-spectroscopic and HPLC approach for routine theophylline analysis. We have explored the specific maximum wavelength regions in the zero, first, and second-order derivative spectra and area under curve techniques for the estimation of theophylline, and this has yet to be reported in previous studies. It may identify related substances or contaminants during storage conditions and estimate the analyte of interest without interferences. Using a Shimadzu Prominence LC-20A Modular HPLC system, with a C_{18} column (250 × 4.6 mm; particle size 5 µm) column and composition of acetonitrile and 0.1% orthophosphoric acid (25:75 v/v) as a mobile phase in this study resulted in superior analyte elution with high resolution, increased plate count, increased capacity factor, and reduced tailing. The reported development methods were validated as per ICH Q2 (R1) guidelines. UV-spectroscopic and HPLC methods can analyze the theophylline analyte in bulk and dosage forms.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

For this work, the authors report no conflicts of interest.

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