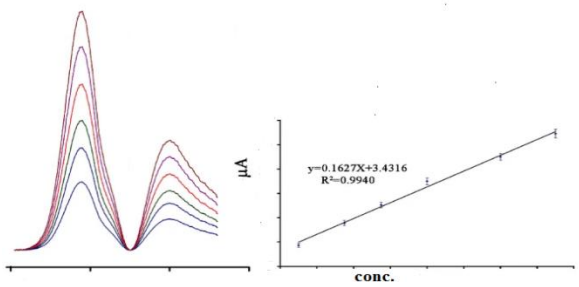
Theophylline Determination in Pharmaceuticals Using a Novel High-performance Liquid Chromatographic Process

H.N.K. AL-Salman[1*], Ekhlas Qanber Jasim[2], Hussein H. Hussein[3], Falah Hassan Shari[4]

Highlights

- A novel technique for predicting theophylline in pharmaceuticals.
- Theophylline is estimated using HPLC-UV technology for LC100.
- Investigate the structural combination of theophylline in neutral, base, and acidic, conditions.
- Investigating the comparative stability of theophylline throughout the experimental evaluation method.
- Execute a series of requests to test the chromatographic process for estimating theophylline.

Graphical Abstract



Abstract

Objective: The current study aims to find a suitable, accurate, and faster RP-HPLC technique for the determination of theophylline, which could then be validated in accordance with the International Conference on Harmonization (ICH) guidelines. **The Aim of this Study:** The aim of this study was to develop an efficient, accurate, and faster RP-HPLC method for determining theophylline, which was then validated using the International Conference on Harmonization (ICH) guidelines. **Methods:** In the HPLC analysis, the Waters 2695 was used. The drug was isolated better using an Ion Pac zorbax 300-SCX Agilent Column, 5 m, 4.6 250 mm, with a liquid phase of Orthophosphoric acid (0.1 percent Orthophosphoric acid in HPLC acetonitrile and Methanol in the ratio of 50:50 v/v at a flow rate of 1ml/min, with discovery at 280 nm using a PDA detector. **Results:** Theophylline's preservation time was discovered to be 3.747 0.127 min. In the 5-25 mg/l range, the procedure was found to be linear, with a parallel coefficient (R^2) of 0.9998. The LOD and LOQ of the system were determined to be (0.99 and 3) g/ml, respectively. The technique and system precisions were predicted using, and the outcomes were determined as percent RSD principles, which were noticed to be within the strict limitations. Theophylline recovery was detected to be in the 99-100 percent range, confirming the method's precision. **Conclusion:** Using basic ICH guidelines, the suggested RP-HPLC process was validated. The following methodology can be used successfully and easily for routine diagnostic analysis.

Key Words: Chromatographic Method for Theophylline, Exertion Degeneration, FT-IR for Theophylline.

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Introduction

Behind a wide range of premium brands, theophylline, also identified as 1,3-dimethylxanthine, is a phosphodiesterase inhibitor utilized in the treatment of COPD and asthma are connected to respiratory illness such as emphysema and bronchitis.

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It is a representative of the xanthine family and shares systemic and biochemical resemblances with caffeine and theobromine. It is abundant in natural surroundings and can be found in tea [AL-Salman H N K et al, 2020; Kanakal M M et al, 2014].

One of the byproducts of caffeine oxidative metabolism is a trace amount of theophylline in the liver.

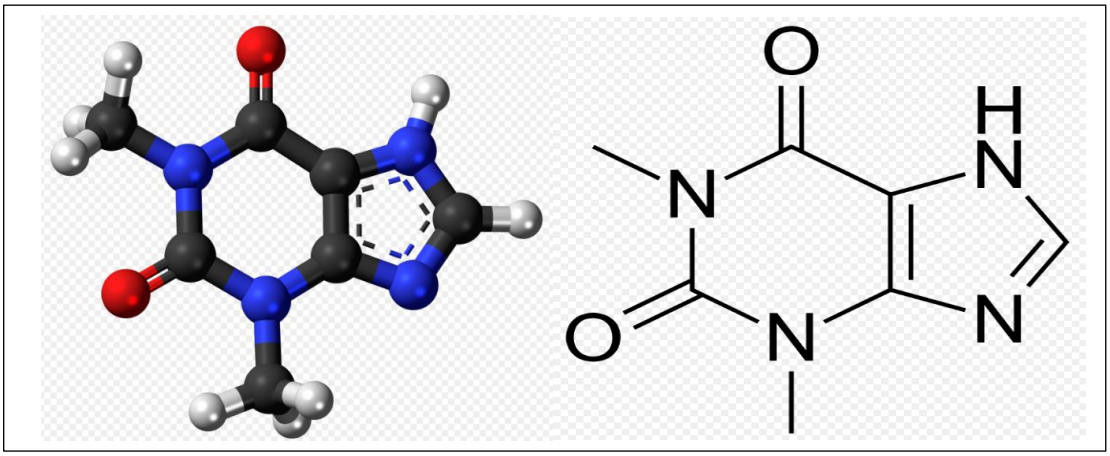


Figure 1. Chemical structure of Theophylline

IUPAC Theophylline (also known as dimethylxanthine, a member of the class of pharmaceutical drugs) It is commonly used to treat COPD and asthma. Pharmacopoeia has traditionally used buffer as an acid solution, as well as an aqueous solution for phycochemical analysis [Shan Y L et al, 2012; - Doijad R C et al, 2007].

These approaches produced good outcomes, but they are insufficient due to exorbitant costs and considerable time constraints required to prepare the sample for simultaneous estimation in industrial sector.

The life expectancy of the column, which is influenced either by acid and salts used during stock solution, accounts for a larger portion of routine HPLC analysis costs. Salts used during dilutions could initiate in the form of organic diluents, raising the cost of HPLC pump maintenance. The total cost of routine analysis is also affected by the number of organic compounds used [Alassadi E A S et al, 2019; AL-Salman H N et al, 2020].

For the determination of Theophylline, an isocratic liquid chromatographic technique with ultraviolet (UV) sensing at 280 nm is characterised. The drug was separated chromatographically on the reversed-phase column Agilent Zorbax-SCX-C18 (5 m, 250 mm 4.6 mm). A symmetric peak shape is provided by the developed liquid chromatographic method [Al-Salman H N K et al, 2020].

Numerous analytical methods of analysis Theophylline have also been observed in the academic literature. These techniques rely on spectrophotometric HPLC. Because HPLC-UV spectrophotometry requires trace amounts of the analyses, it is suitable for the analysis of pharmaceuticals and biological fluids. This method is quite costly because it necessitates a lengthy and laborious pre - treatment of the specimens as well as laborious cleaning up protocols prior to analysis. As a result, it is important to develop a straightforward and appropriate analytical procedure for measuring Theophylline. Because of its low cost and inherent simplicity, UV-visible spectrophotometry is widely used in research labs, hospitals, and pharmaceutical corporations [Alassadi EAS et al, 2020; Abd-Alrassol KS et al, 2020].

Synthesis of Theophylline [Daly J W et al, 1987]

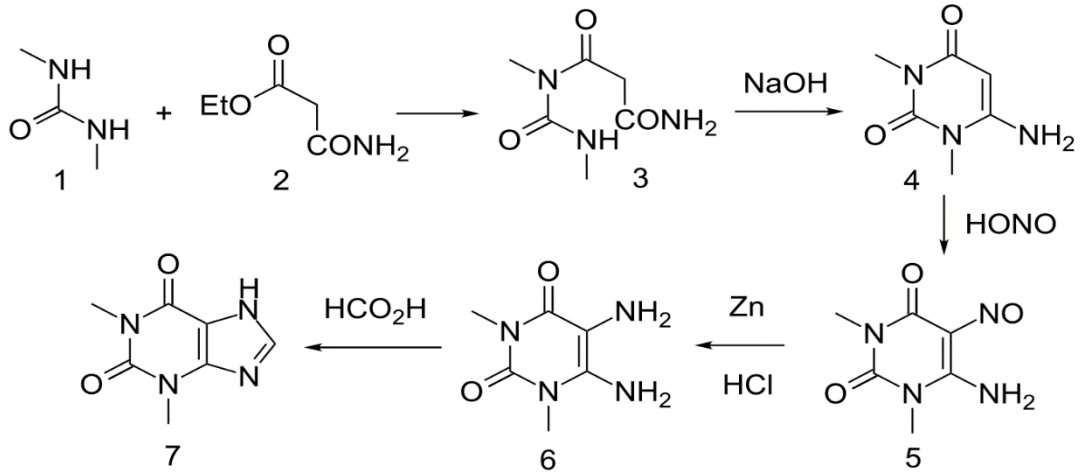
The proposed design suggests an artificial technique Scheme 1 for a type of theophylline, which includes the following steps:

S1, amino-1, preparation 3-FU dimethyl In the first reaction vessel, 6-: methyl-sulfate, 6-Urea, amino-pyrimidine, sodium hydroxide solution, and water are provided; after 4-5 hours of stirring in the crystal tub, water treatment, cleaning, and evaporation yield 6-amino-1,3-FU dimethyl.

S2, preparation 5-nitroso-group-6-amino-1,3-FU dimethyl: by S1 obtained 6-amino-1, after 3-FU dimethyl, acetic acid and water assign and blend in the 2nd test tube, be heating up to 78-82 DEG C, after which liquid sodium nitrite in aqueous phase,

shielding 28-32min, don't really pause in the insulating procedure to agitate, then be allowed to cool to do after 10-14his mixed in 0-2 DEG C of perpetuation and screen, filter cake mopping with ice water, and soaking are accessed amino-1, the 3-FU dimethyl of 5-nitroso-group-6.

S3, prepare theophylline: in a hydrogen autoclave, insert S2 managed to gain 5-nitroso-group-6-amino-1,3-FU dimethyl, palladium-carbon catalyst, and methyl alcohol, going to pass into hydrogen causes the friction in the hydrogen autoclave to be 2.5-3.5MPa, after keeping pressure for 3-4 hours, diatomite purification is used to acquire permeate, distillate flywheel vaporisation is acquired brown oil, the temperature of rotary.



Scheme 1. synthesis of Theophylline

The Objective of the Study

The aim of the study was to establish and validate an RP-HPLC technique for quantifying Theophylline in pharmaceuticals using an ultraviolet (UV) detector.

Experimenting

Tools

The LC-100 series S-HPLC has computer processor power that is super easy. Its electronic signal layout, internal electrical structural system, process optimization, cinematic workspace operations, and specifications distinguish it as a leading instrument with exceptional stability and reliability. The LC100-type HPLC-UV is made up of a double-beam optical spectrometer (Angstrom Advanced Inc.USA), a category UV-100 PC with a 1 cm beam wavelength quartz cell, and it is linked to an IBM compatible PC. The product was a UPVC Matlab rendition, R2003b was used for chemometric schemes, the midway least squares (PLS) have been incredible with PLS Toolbox that can be used with Matlab R2003b, VP pumps, and a UV indicator with variable frequency programmability. summit areas were embedded. using Angstrom Advanced Inc.'s LC solution programming programme. The chromatographic partition and estimation were conducted on an Ion Pac section; a 5 m, 4.6 250 mm Ion Pac zorbax 300-SCX Agilent Column. The analytical column remained stable at room temperature. Before injection into the HPLC framework, drug conventional strategies and tablet test agreements were processed through a millipore layer stream in the portable stage [Shao Y et al, 2004; Nirogi R V et al, 2007; Al-Salman H N K et al, 2018].

Reagents and Chemical

Standard theophylline with a transparency assertion of 99.9% based on the manufacturing plant certificate, and approved for medical tools and pharmaceuticals by Industries Pharma Samarra Alfayhaa®Iraq under NO. DH02821114 [ICH Q2B, 1996].

Market Sample

Theophylline - ASMASAM®, SDI-IRAQ -tablets, includes 120 mg per tablet of ASMASAM®, tablets ®.

Set up the Samples for Measuring

- Solution of HPLC grade (Sigma-Aldrich®).
- To formulate a concentration of 1 mg/ml of Theophylline, stock content was determined in Methanol: Acetonitrail (50:50) and 1 mM acetic acid at pH 4.5.

- Theophylline (standard solution) was formulated in a 50:50 methanolic solution and acetonitrail to produce concentration levels of 5, 10, 15, 20, and 25 g/ml.

The Sample Modernization

The streamlined PLS configuration set was augmented with different concepts of Theophylline -Samarra® tablets comprising recognised from basic Theophylline -200mg tablets generated by Samarra® to conduct sample Modernization. One recognised attention to three unknown divergences of tests covering multiple subgroups of all was introduced reason for complete the fundamental modification and the predictive ability of the revamped instance was inspected incorporating outer authorization test results, at that juncture start figuring the attend test invigorating for every section leveraging the generated tactic RP-HPLC with three concentration of the extra free refreshing [Al-Salman H N K et al, 2018].

Preparation of Solution

Preparation of buffer (0.1% of Orthophosphoric acid)

1 ml of orthophosphoric acid was considered accurate and provided to 1000 ml of volumetric flask; the capacity was disintegrated and dissolved to 1000 ml with HPLC grade liquid (Milli-Q water). The solution PH was maintained to 4.00.05 utilising tri ethyl amine HPLC grade, and the buffer was centrifuged in an ultrasonic water bath and space filtered through a 0.45m filtration [Baboota S et al, 2007].

Getting ready for the mobile process

The mobile step was made by mixing methanol and acetonitrile in a 50:50 v/v ratio. The liquid phase was vacuum filtered through a 0.45m filter after being heated in an ultrasonic boiling water bath for 10 minutes [Al-Salman H N K, 2017].

Preparation of Eiluent

As a dilution, the mobile phase was also used.

Preparation Theophylline Standard Solution

200 mg of Theophylline having to work requirement were measured into a 100 ml volumetric flask and diluted to quantity with solvent (Mobile phase). A diluent has been used to dilute 15ml of the above solution to 50ml, generating an ultimate concentration of 100 g/ml [Chaibva FA et al, 2007].

Preparation of Theophylline Sample Solution

Twenty heophylline tablets (each containing 200 mg of Theophylline) have been shattered into a particle in a concrete. A 10 mg specimen of theophylline was transported to a 100ml volumetric flask and complete up with dilution. To dissolve the materials, it was incubated for thirty min. A solvent has been used to dilute 15ml of above solvent to 50ml, generating a very last concentration of 100 g/ml [Mohammadi A et al, 2007].

Selection of Wavelength for Method Development

A standard solutions of 1000 mg/ml Theophylline was ready, and different concentrations were performed to achieve a concentration of 100g/ml with a methanol: acetonitril mixture. Inspecting the above-mentioned standard drug alternative between 190 and 300 nm yielded the wavelength. The scanned outcomes show that the highest absorption was at 280 nm. As a direct consequence, 280 nm was chosen as the recognition wavelength for the RP-HPLC study (Figure 2).

Method Development

The chromatographic procedure was introduced by conducting trials with the responding as the United States Pharmacopoeia (USP) sure that the amount and tailing component in mind. For the means of performing laboratory experiment, the dependent variables were column type and solvent system concentration. All through the trials, the spectrum was maintained consistent at 280 nm, the stream rate was kept consistent at 1.0 ml/min, and the flow rate was maintained fixed at 20°C. Table 1 shows this same innovative chromatographic condition for process validation [Hayun et al, 2017].

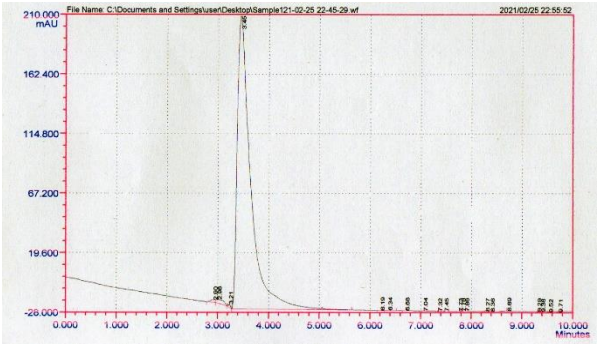


Figure 2. UV spectra of Theophylline

Table 1. Experimental conditions of method development

Trial No	Composition of mobile phase	Buffer	Column dimensions and pore size	Flow rate	Injection volume	Observations
1	ACN: Methanol (20:80)	--	Ion Pac column; Arcus EP-C18; 5 µm, 4.6 mm× 250 mm	1.00 ml/min	10 µl	Plate count was not within the limit
2	ACN: Methanol (30:70)	-	Ion Pac column; Arcus EP-C18; 5 µm, 4.6 mm× 250 mm	1.00 ml/min	20 µl	Broad peak was obtained
3	CAN: Methanol (40:60)	-	Ion Pac column; Arcus EP-C18; 5 µm, 4.6 mm× 250 mm	1.00 ml/min	20 µl	Plate count was not within the limit
4	CAN: Methanol (50:50)	-	Ion Pac zorbax 300-SCX Agilent Column, 5 µm, 4.6 × 250 mm.	1.00 ml/min	20 µl	USP Tailing was not within the limit
5	ACN: Buffer (20:80)	0.1% TFA	Ion Pac column; Arcus EP-C18; 5 µm, 4.6 mm× 250 mm	1.00 ml/min	20 µl	Less retention time
6	ACN: Buffer (30:70)	0.1% TFA	Ion Pac column; Arcus EP-C18; 5 µm, 4.6 mm× 250 mm	1.00 ml/min	20 µl	Plate count and tailing was not within the limit
7	ACN: Buffer (20:80)	0.1% TFA	Ion Pac zorbax 300-SCX Agilent Column, 5 µm, 4.6 × 250 mm.	1.00 ml/min	20 µl	Peak tailing was not within the limit
8	ACN: Buffer (30:70)	0.1% TFA	Ion Pac zorbax 300-SCX Agilent Column, 5 µm, 4.6 × 250 mm.	1.00 ml/min	20 µl	Two peaks were observed
9	ACN: Buffer (20:80)	0.1% OPA	Ion Pac zorbax 300-SCX Agilent Column, 5 µm, 4.6 × 250 mm.	1.00 ml/min	20 µl	Peak shape was not proper
10	ACN: Buffer (30:70)	0.1% OPA	Ion Pac zorbax 300-SCX Agilent Column, 5 µm, 4.6 × 250 mm.	1.00 ml/min	20 µl	Plate count and tailing were within the limit.

ACN – Acetonitrile, TFA- Tri fluoro acetic acid, OPA- Ortho Phosphoric acid

Construction of Calibration Curve

Aliquots of different doses of known absorption were designed utilising diluent (Mobile phase) to achieve concentrations of (5, 10, 15, 20, and 25) g/ml. The chromatograms were managed to capture under ideal experimental parameters. The hromatograms were used to determine the total peak areas at different concentrations levels. The linear plot was then created by combining the average standard solution of their corresponding times.

The Exertion Degeneration Studies

The effort degradation studies were carried out under various ICH suggested conditions i.e., such as acidified, basic, oxidative, hot, and photochemical effort [Yano Y et al, 2006; Panda S S et al, 2013; Kanakal M M et al, 2014; Thumma S et al, 2008].

1. Acid Degeneration

Theophylline received 60 milligrammes in a 100 millilitres of powder was administered to the patient. The jar was filled with 5 ml of 0.1 N HCl and kept at 70–80 oC reflux for 2–3 hours. Following the accomplishment of the influence, the configuration was killed with 0.1 N NaOH and concluded sufficiently with the portable stage. Hcl may be produced during the electrolysis of water of theophylline. Hydrolysis, or dividing with liquid, is one such reaction. ”Any acid or base stimulates amine hydrogenation [Figure 3].

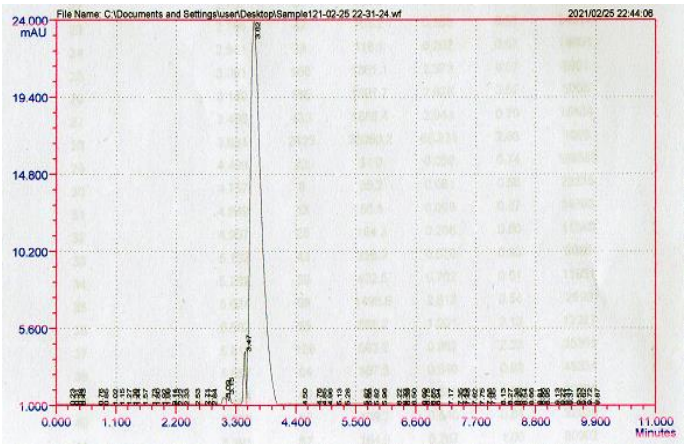


Figure 3. Acid degeneration

2. Base Degeneration

When use foundations such as sodium hydroxide to handle amine salts 60 milligrammes of theophylline was mixed with 100 millilitres of volume. approximately a 2 litres of the 0.1 N hydrochloric acid was added to the container at a maximal

intensity for 2 to 3 hours, or 2 to 3 litres of reflux for a 2 to 3 hours After dissolution of the salt, the solution was bled to obtain 0.1 N Hydrochloric acid and completed with phase water. Figure 4.

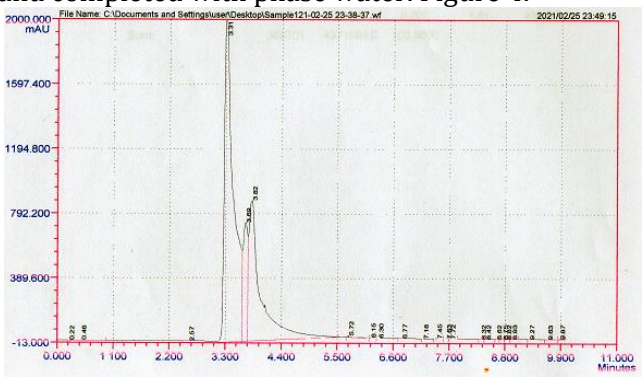


Figure 4. Base degeneration

3. Oxidative Degeneration

In a 100 ml flask, 60 mg of tablet Valsan and 5 ml of the 20% H2 formulation were added to the powder. the container was held at a reflux temperature for two to three hours. After putting pressure, the container was sufficiently compacted and was ready for transport in Figure 5.

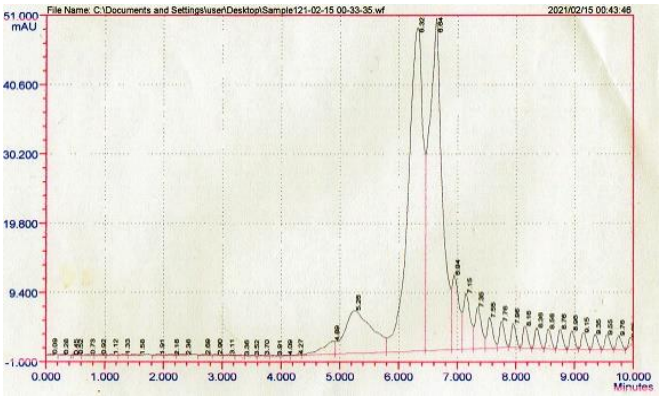


Figure 5. Oxidative degeneration

4. Photolytic Degeneration

as in this experiment, 60 mg of Valsanadoseptinate was placed in a Petri plate and left in direct sunlight for two to three hours for photo-degradation. Once the pressure was finished cooking, the tablet was removed to a ml volumetric flask and diluted and stirred well. The frequency band of the solvent is scanned in the infra-red portion of the electromagnetic spectrum. In this manner, the Theophylline breaks down to some degree and interferesse with pharmacological preservatives can be seen in Figure 6.

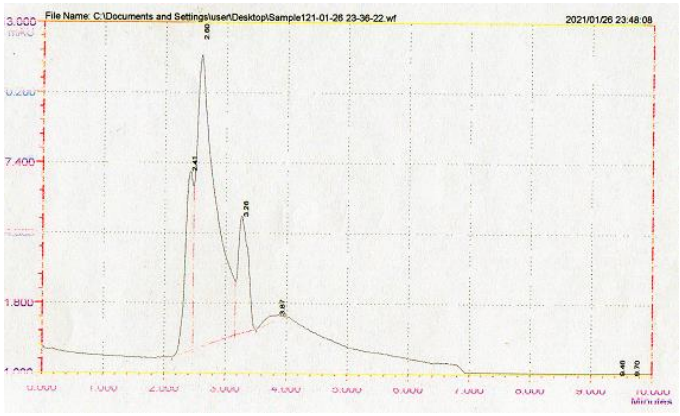


Figure 6. Photolytic degeneration

5. Thermal Degeneration

Valsanazolite was added to a glass plate and set into a hot oven for 2–3 hours at 105°C. A 100 ml graduated cylinder was fitted with the particles and placed in the sink. An increase of temperature of the solvent to >100 C means that Theophylline has been greatly completed with and thermal disintegration of the molecule is demonstrated in Figure 7.

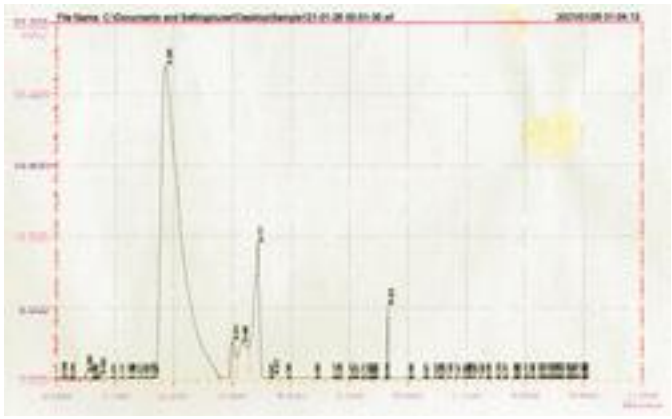


Figure 7. Thermal degeneration

Infrared Spectrophotometer of Theophylline [BABU A R S et al, 2011; KASAWAR G B et al, 2010]

IR was recorded on KBr discs planned at Basra University of Science and Technology and Education at ambient temperature, and a handset of the type FT-IR-84005582-SHIMZU was used.

For Theophylline -Standard

In standard [Figure 8, Table 2] the ultraviolet light provides performance absorption and oscillation frequencies that consent with the framework of theophylline. + 4060506093 cm-1 is weak and 1668 and 17 cm-1 appear two strong. This discovery can be credited to (the idea of) bending of the curve (frequency), where the sample

exhibited a maximum value of 1487 cm-1 The stretching and bending wavelengths of NH3 are 3122 and 1566 cm-1. The spectra in Figure 8.

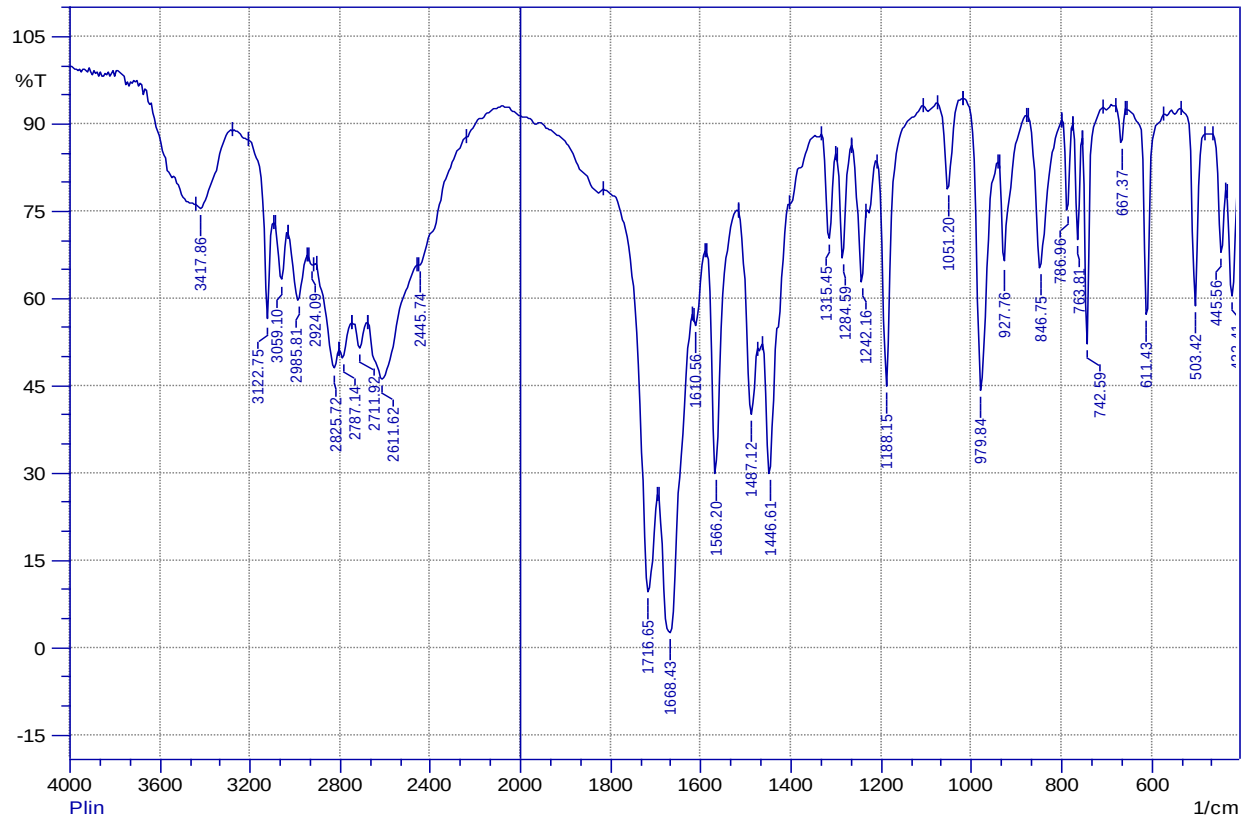


Figure 8. FT-IR spectrum of Theophylline standard

For Theophylline - Sample

Some essential peaks in the infrared spectrum of the infrared spectrum of Theophylline [shown in figure 9, table 2] follows suit] and C has a strong series of absorbances between about 3160 and 1662 cm, while the Infrared spectra reveals weak (or single) prominent absorbance at C The FT-IR spectrum of standard Ketonophylline has a series of weak and prominent peaks at K This discovery can be credited to (the idea of) bending of the curve (frequency), where the sample exhibited a maximum value of 1487 cm-1 The stretching and bending wavelengths of NH3 are 3122 and 1566 cm-1. Spectral analysis of V of FT.

Table 2. Characteristic absorption bands in the infrared spectra of Theophylline Standard and sample

	Functional groups	Theophylline Standard (cm^{-1})	Theophylline sample(cm^{-1})
1	(C=O) Keton-1	1716 S	1716 S
2	(C=O) Keton-2	1668 S	1668 S
3	(N -H) Stretch bend	3122 m 1566 S	3122 m 1566 S
4	(C=N)	1446 m	1446 m
5	(C-N)	1242 m	1242 m
7	(C=C)	1487 m	1487 m
8	(C-H) alkene o.o.p	3059 w 979 S	3062 w 979 S

Notes :- S = strong, M = middle, W = weak

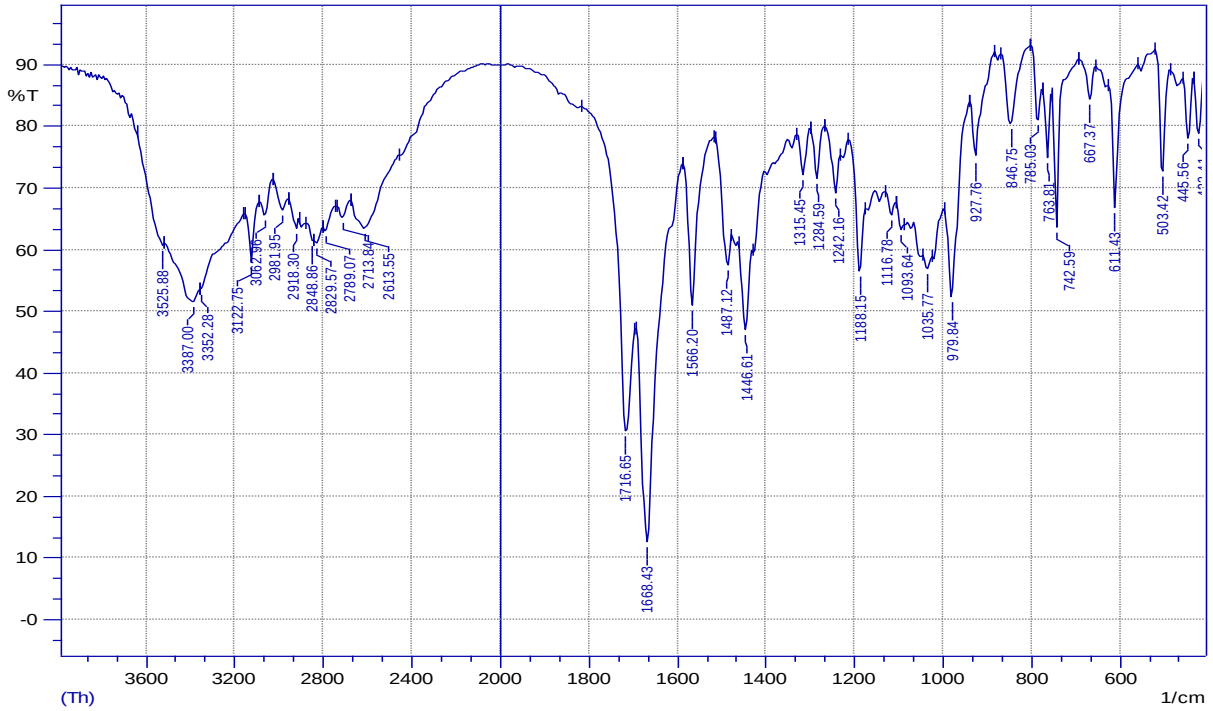


Figure 9. FT-IR spectrum of Theophylline –Sample

Method Validation

The technique was found to be effective for high specificity, scheme, predictability, accuracy, and finesse, as well as the ICU guidelines limits for limit of quantification, with a robustness, among an ICH [KUMAR V et al, 2011].

Specificity

The integrity of the mixture was proven by raising a defined mixture of a specific concentration of the suitable contaminants. Theinine was measured in 6 recreate at a concentration of 100 µg/mL, and precision was found to be within the specified limits.

System Suitability

System compliance is delineated as ensuring the operational acceptability of a scheme, prior to analysis of uncertainties Six regurgitates of Theophylline at a dosage of 100ng/ml [MAITHANI M et al, 2011] To be within 2 percent of the mean plus or minus 2 percent from the average peak absolute standard deviation (means) for maximum absorption and refractive indices.

Linearity and Range

a clear link between test results and the amount of the analyte present The detection of eight different doses of theophylline and a direct relationship to the test results of data study After three different absorptions of Theophylline (5, 10, and 15 µg/ml) and up to a dose of twenty-five µg/mL of Theophylline. The salt concentration was recorded as well as a function of the characteristic peaks. Linear methods were used to approximate the linearity of the tangent. A model that has an R^2 equal to or above 0.999 has good enough fit to meet your standards. The concentrations of various salts were plotted against the average peak areas. The least-squares method was used to measure the linearly. When the coefficient of correlation (R^2) is greater than or equal to 0.999, the model appears to fit the data adequately [MALLU U R et al, 2011; Alsaad A A A et al, 2019].

Accuracy

Accuracy is defined as how close the expected return is to the true worth. The percentage of the analyte retrieved may be calculated, and displayed as R Since there are three concentrations of the Theophylline solution (50 µg/ml, 100 µg/ml, and 150 µg/ml), successive analysis (n=3) was used to test the developed calibration curve for each of the developed method. the experiment's data were analysed using the [% Recovery = (Recovered concentration /Injected concentration) times 100] to see if the proposed technique was accurate. It must not deviate from this goal by more than

one-tenth of a percentage point to be considered acceptable [MARTIS E et al, 2011; PRASANNA B L et al, 2012].

Precision

For a concentration of Theophylline at 100 mg/mL, six measurements were taken to use the proposed model, with the percentage of RSD calculated. The RSD accuracy was less than two percent, and was rated insufficient by the United States Pharmacopeial Convention [PRASANNA B L et al, 2012].

Robustness

You are talking about being flexible in method system parameters; robustness is an attribute of the method. Analytical conditions must be carefully monitored and incorporated in the methodology when needed. Injection amounting a robustness was evaluated in the present research by giving a single obvious change in the natural buffer concentration (7 RSD and 3% vs. the control value of 7%), and organic buffer velocity (approximately 2 ml/min) was assessed [WU J et al, 2011].

Limit of Detection and Limit of Quantification

under the asserted lab conditions, the level of LOD may be known, but there is no assurance that this level of LOD will be present in the samples. Precise and accurate measurements are available at the lowest possible LOQ. Calculated using the equation.

Results and Discussion

Development of HPLC method

In the chromatographic A, the mobile phase contains zero to three percent of theophylline via a mean initial concentration of 3.45 ± 0.011 minutes. In the equation 3, the mobile phase contains 0-3% of theophylline with such a median detection limit of 3.45 ± 0.01. This indicates the highly precise method development of the developed HPLC method.

System Suitability

In getting the idea analyses, the providing guidance and support was confirmed with the Theophylline accumulation of 100mg/mL. As specified in Table 3, the audit experience was ±2% for the RSD for the peak height area and residence time are given as has been shown.

Table 3. System suitability analysis of Theophylline

Injections	Drug	RT	Area	% area	USP plate count	USP tailing
1	Theophylline	3.45	38000	98.733	706	2.10
2	Theophylline	3.45	74245	98.812	5623	2.10
3	Theophylline	3.45	114330	98.324	5580	2.10
4	Theophylline	3.45	152375	98.621	5600	2.10
5	Theophylline	3.45	190600	98.755	5558	2.10
MEAN			114333	RT- Retention Time 3.45 ± 0.011 min		
SD			0.33			
% RSD			0.33			

Linearity

Various concentrations of stock solutions (ranging from 5 to 25 µg/mL) were prepared and their spectra were made. The documented chromatograms gave the chromatographic peaks their respective mean quantities, and the linearity plot was formulated by averaging the calculated values. The correlation was found to be almost perfect, at 0.9995. The linearity of Theobromine has been established in the Tables 4 as well as 5, and Fig.4.

Table 4. Linearity of Theophylline

Sl. No.	Concentration µg/ml	Area
1	5	38000
2	10	74245
3	15	114330
4	20	152375
5	25	190600

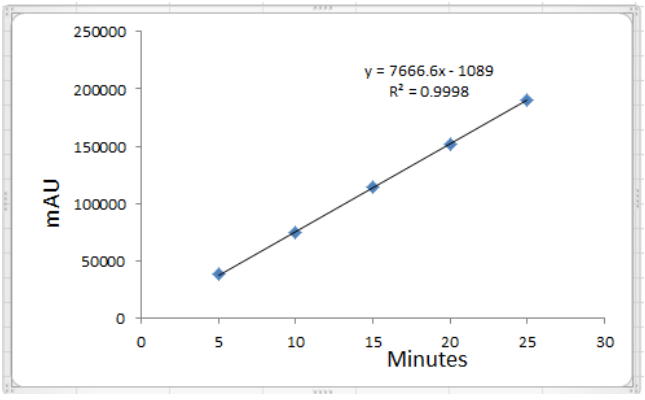


Figure 10. Calibration plot of Theophylline

Table 5. Regression characteristics of linearity of Theophylline

Parameters	**Results**
Linearity range (µg/ml)	5- 25
Regression equation (y=mx+b)	y=7666.6x-1089
Slope (m)	17543.45
Intercept (b)	1089
The correlation coefficient (R^2)	0.9998

Accuracy

50%, 100%, and 150% In order to get 3 average recovery rates for each stage, the experiments were undertaken 3 phase and the SD of the percentage of the consequences was computed. 100.1% was discovered to be the "the developments and improvements rate" (Table 6).

Table 6. Recovery study results of Theophylline

Sl. No.	Accuracy range	Amount of API added (mg)	Amount recovered (mg)	% Recovery
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
SD				0.311
% RSD				0.311

Precision

Tables 7 & 8 show the means and standard deviations, or calculs, respectively, of the various studies. The system's accuracy was discovered to be the less than 2%.

Table 7. Method precision

SL. NO.	Sample weight (mg)	Area	Mean Area Counts	% Label Claim
1	100	114330	114330	100
2	100	114330	114330	100
3	100	114330	114330	100
4	100	114330	114330	100
5	100	114330	114330	100
6	100	114330	114330	100
MEAN				100
SD				0.32
% RSD				0.32

Table 8. Intermediate precision

SI NO	Sample weight (mg)	Area	Mean	% Label Claim
1	100	114330	114330	100
2	100	114330	114330	100
3	100	114330	114330	100
4	100	114330	114330	100
5	100	114330	114330	100
6	100	114330	114330	100
Mean				100
SD				0.30
% RSD				0.30

Robustness

The calibration accuracy was validated by making minor changes in fluid velocity (±0.2ml/min) and buffer/solution concentration (±7%) and can be seen in Table 9. Ease of handling is a measurement of observational procedure's capability and self- robustness when put under typical operation. Slight flow velocity and molarity change was discovered to be less than 2% of the starting solution.

Table 9. Robustness of the developed method for Theophylline

SL NO	Parameters	Sample weight	Area	% Label claim	Mean	SD	%RSD
1	Flow plus (1.0ml/min)	100	114330	100	100	0.306	0.31
		100	114330	100			
		100	114330	100			
2	Flow minus (0.5ml/min)	100	114330	98	98	0.30	0.3
		100	114330	97.5			
		100	114330	98.5			
3	Organic (Methanol: ACN) plus (75: 25)	100	114330	100	100	0.04	0.04
		100	114330	100			
		100	114888	100			
4	Organic (Methanol: ACN) minus (65:35)	100	114867	100	100	0.04	0.04
		100	114956	100			
		100	114901	100			

Limit of Detection and Limit of Quantification

They are being discovered at the concentrations of 0.1 and 1 microgram and 1 microliter, including both. The lower bounds (LOD) and the upper limits (LOQ) show the extreme precision of the developed method.

The Applications of Method

We used commonly produced tablets as a dosage for the the scientific method of Theophylline-40 mg was measured (TAJ pharma-tablets, INDIA, claiming to contain 40 mg of Theophylline). Results have found that Standard- the percentage of theophylline was found where measurements were 100% ± 2.2%, while the the Limited TAJ version measured 99% ± 1% This result proving the precision and accuracy of the suggested formulations for the outcomes for the Theophylline. Outcomes were displayed in Table 10.

Table 10. Assay of Theophylline in tablets

Analyte	Labeled claim (mg)	Found (mg)	Mean (mg)	%Recovery	%RSD
Standard - Theophylline	40	40	40	100	±0.216
Theophylline -40	40	39.5	39.75	99	±0.212

Conclusion

The advanced RP-HPLC method's verification criteria was excellent. Accuracy is less than two percent with respect to estimates of SDS. the T The theoretical elimination half-life of theophylline was determined to be 3.747 minutes. The precision of the mean extraction technique for Theophylline was discovered to be 98 percent.6.6 percent The low value of LOD and LOQ confirms the method's ability to discriminate between the signal and noise. These characteristics indicate that the established RP-HPLC technique may be simple and quick for routine checks.

Competition for Disclaimers of Interest

Several writers declared that they had no conflict of interest. Nobody wants to use that as an excuse to file lawsuits. The aim of the study is to improve the societal response to medicines. Moreover, the costs of the research study were provided by the authors only.

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