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CHROMATOGRAPHIC SPECTROPHOTOMETRIC DETERMINATION USING REVERSE PHASE HPLC TECHNIQUE FOR MESALAZINE OR MESALAMINE (MESA)

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Abstract.

Aim: The aim of this paper is to estimate Mesalazine or Mesalamine (MESA) in pharmaceuticals.

Methodologies: The reversed-phase HPLC (RP-HPLC) results were used to evaluate the type of Mesalazine. Chromatographic analysis was carried out using an HPLC-UV method along with an Ion Pac column (Arcus EP-C18; 5 m, 4.6 mm, 250 mm) and a mobile phase of acetonitrile: acetic acid: water, 40:40:20 (v/v/v) + 0.5 M potassium dihydrogen orthophosphate buffer at pH 3.3, at a flow rate of 1.0 ml/min. At 260 nm, UV detection was employed in the HPLC method. Exactness, precision, particularity, linearity, and affectability were all accepted for the technique. The (MESA) had a maintenance time of (3.17) minutes. The (MESA) alignment plots were over the target ranges of 1–5 g/L, R^2 0.9998. The quantitation limit was 0.3613 g/ml, with a detection limit of 1.636 g/ml. The precision of the proposed procedure, which ranged from 98.0 percent to 100 percent, was determined through recovery experiments.

Conclusion: The modern HPLC-UV approach was used to analyze generic drug products, and the planned technique's efficiency was confirmed. The study's findings show that precision, accuracy, and efficiency are all within reasonable limits, so there is no substantial difference between the values obtained using the proposed methodology and those obtained using the traditional method.

Key words. Mesalazine (MESA) chromatographic, mesalamine degradation, mesalamine crud.

Introduction.

Mesalazine (MESA), also named mesalamine, its chemical name is 5-amino-2-hydroxy benzoic acid. The powder or crystals of MESA has a white or light grey or light pink color (Britishpharmacopia, 2013). It is soluble in oil. acidic and alkaline medium, fairly insoluble in chloroform, ether, ethyl acetate, and n-hexane [1].

Mesalamine (Figure 1) also known as Mesalazine or 5-amino salicylic acid (5-ASA), is an anti-inflammatory drug used to treat inflammatory bowel diseases, such as ulcerative colitis and mild-to-moderate Crohn's disease. Mesalamine is a bowel-specific aminosalicylate drug that acts locally in the gut and has its predominant actions, thereby having few systemic side effects. As a derivative of salicylic acid, Mesalamine is also thought to be an antioxidant that traps free radicals, which are potentially damaging byproducts of metabolism. Mesalamine is considered the active moiety of Sulfasalazine, which is metabolized to Sulfapyridine and Mesalamine. A literature survey revealed that a few analytical methods have been reported for the determination of Mesalamine in pure drug, pharmaceutical dosage forms, and biological samples using spectrophotometry, HPLC, UPLC, and LC-MS either in single or in combined forms. The aim of the present work is to develop and validate a simple, fast, and reliable isocratic RP-HPLC method with UV detection for the determination of Mesalamine in bulk and in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination of Mesalamine in bulk and tablet dosage forms [2-9].

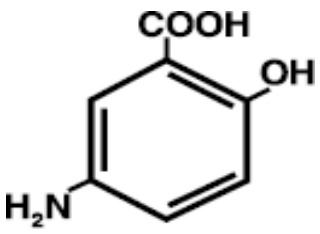

Figure 1. *Passive external rotation of the shoulder while lying on the back.*

Synthesis of Mesalamine.

The synthetic step in the synthesis disclosed therein is the reaction of a cyano group on the biphenyl ring with an azide, such as tributyl tin azide. as follows:

Synthesis of Mesalazine

The current study's aim was to establish and validate an RP-HPLC system with an ultraviolet (UV) detector for quantitative Mesalazine determination in pharmaceuticals.

Experiment.

Tools:

Completely automatic digital computer control is standard on the LC-100 series S-HPLC. Its electronic circuit design, internal mechanical construction techniques, processing technology, cinematography workstation functions, and technical requirements make it one of the most stable and reliable instruments available double-beam optical spectrometer (Angstrom Advanced Inc. USA), a sort UV-100 PC with a 1 cm light frequency quartz cell, and an IBM compatible PC make up the LC100-style HPLC-UV. The replica was made out of UPVC. PLS Toolbox for Matlab R2003b, VP pumps, and a UV indicator with variable frequency programming, as well as PLS Toolbox for Matlab R2003b, chemometric techniques, and the halfway least squares process, were all great (PLS). An Angstrom Developed Inc. LCsolution programming tool was used to coordinate peakareas. An Ion Pac segment and an ArcusEP-C18 analyticalcolumn were used to conduct the chromatographic separation and measurement at room temperature (250 mm 4.6 mm; molecule size 5 m). Before

being injected into the HPLC device, drug standard and tablet test arrangements were processed via a millipore layer channel in the portable stage [10-12].

Chemicals and Reagents.

Pure Standard: Mesalazine (MESA) with a quality assertion of 99.9 percent, granted by Industries Pharma SIGMA-ADRICH.COM CHEM Gmbh, MKCH4095, under the number A79809-5GM for medical devices and pharmaceuticals, depending on the company's factory certificate. Mesalazine - SIGMA-ADRICH®, Germany.

Market Sample: Mesalazine - Pentasa-tablets Ferring®, Switzerland, contains 500 mg per Pentasa-tablets, Match no: T13613A.

Set up the Samples for Measuring:

• Sigma-Aldrich® HPLC grade solution

• To prepare a concentration of 1 mg/ml from MESA, stock standard solutions were prepared in Methanol: chloroform (25:75) and 1 mM acetic acid at pH 3.3.

• MESA (standard solution) concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 g/ml were prepared in a mobile phase of acetonitrile: acetic acid: water, 40:40:20 (v/v/v) + 0.5 M potassium dihydrogen orthophosphate buffer at pH 3.3, at a flow rate of 1.0 ml/min. At λ_{max} 260 nm, with an Ion Pac column (Arcus EP-C18; 5 m, 4.6 mm, 250 mm).

Modernization example: To conduct sample Modernization, various examples of mesalazine - Pentasa-tablets Ferring® containing known amounts from standard MESA-500 mg tablets developed by Ferring® were added to the streamlined PLS alignment package. One known emphasis on three oblique convergences of measures, each of which was divided into different groupings, was included as a justification for performing the fundamental modification and the updated example's precognitive capacity was assessed using outer approval tests, then figure out how to conduct test refreshing for each section using the produced strategy. In the RP-HPLC, three centralizations of additional refreshing examples were used.

Results.

Procedure and Standard Drug Remedy:

In traditional setups, the mobile phase is commonly used as a solvent. Dissolving a clearly specified quantity of MESA (50 mg) in 100 ml of flexible stage in a 250 ml volumetric flagon yielded a normal stock arrangement of Mesalazine (500 g/ml). The cup was therefore made suitable using the portable stage. MESA working standard arrangements (1, 2, 3, 4, and 5 g/ml) were created after the stock arrangement was adequately undermined with the changed number.

Chromatographic Parameters:

Table 1 shows the critical parameter values acquired by utulizing reverse-phase chromatography process. (High-performance liquid chromatography, or RP-HPLC).

The proposed strategy's Calibration Curve:

Alignment bends were prepared over a focus range of 1–5 g/ml for Mesalazine. The three-fold arrangement was prepared, and 20 µL of each arrangement was injected onto the section. At 260 nm, the pinnacles were resolved. mesalazine 's adjustment bend was created by plotting the pinnacle zone versus concentration.

Table 1. *Shows the values of the basic parameters obtained using the reverse-phase chromatography system (RP-HPLC).*

Mobile phase	acetonitrile: acetic acid: water, 40:40:20 (v/v/v) + 0.5 M potassium dihydrogen orthophosphate buffer at pH 3.3
Run time	10 min
Retention time	3.17 min
Column temperature	25^{o}C
Detection wavelength	260 nm
Flow rate	1.0 ml/minute
Injection volume	20 µL

Exercising degradation Research:

Different ICH-recommended pressure conditions, such as acidic, basic, oxidative, wet, and photolytic effort, were used in the effort degradation studies [13-20].

Acid degradation:

In a 100 ml volumetric cup, 500 mg of Mesalazine tablet powder was taken. The jar being loaded with 5 mL 0.1 N HCl and held at 70-80°C in a reflux state for 2–3 hours. The arrangement was killed with 0.1 N NaOH after the strain was reached, and the flexible stage was used to finish the job. Hydrochloric acid can be used to break down Mesalazine. Hydrolysis, or water splitting, is one such reaction. ”Any acid or base stimulates amine hydrolysis (Figure 2).

Base degradation:

By using sources to suppress amine, such as NaOH or potassium hydroxide, the product is amine salt. In a 100 ml volumetric carafe, 500 mg of Mesalazine tablet powder was taken. The jar being loaded with 5 mL 0.1 N NaOH and held at 70-80°C in a reflux state for 2–3 hours. A dynamic stage was used to complete the structure after it was killed with 0.1 N HCl and after the pressure was completed (Figure 3).

Oxidative degeneration:

In a 100 ml volumetric flask, 500 mg of MESA tablet powder and 5 ml of 20% H_2O_2 were combined. For 2–3 hours, the flask was held at 70-80°C in a reflux state. The jar was finished sufficiently with the portable stage after the pressure culmination (Figure 4).

Degradation due to photolysis:

For the photolytic degradation analysis, 500 mg of Mesalazine e tablet powder is being put in a glass Petri dish and exposed to direct sunlight for 2–3 hours. The tablet powder is being moved to a 100 mL volumetric cup and formed suitable with the portable amount after applying pressure. The solution's infrared spectrum is then examined. Figure 5 shows how the HPLC-UV peaks are unstable and often overlap, this decomposition process results in partial disintegration of the mesalazine compound and uncontrolled interaction with pharmaceutical additives.

Thermal degradation:

500 mg MESA tablet powder was baked for 2–3 hours at 105°C in a glass Petri dish. In a 100 ml volumetric flask, the tablet powder was dissolved, and a solution was composed. to the stain

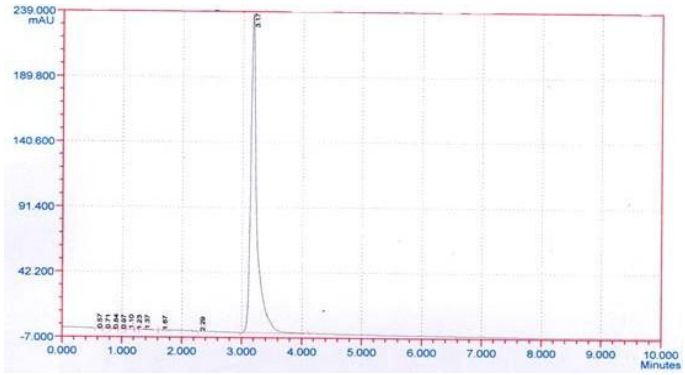

Figure 2. *Acid degradation.*

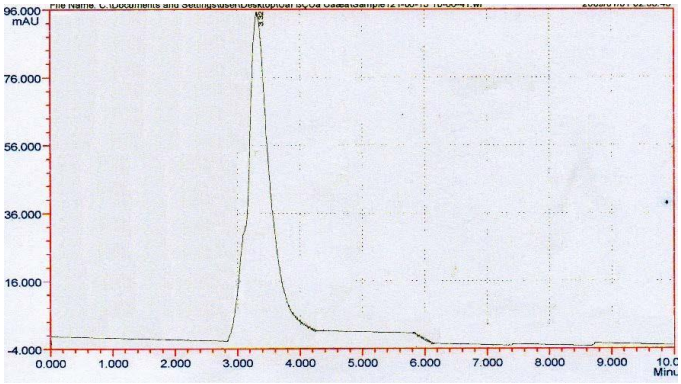

Figure 3. *Base degradation.*

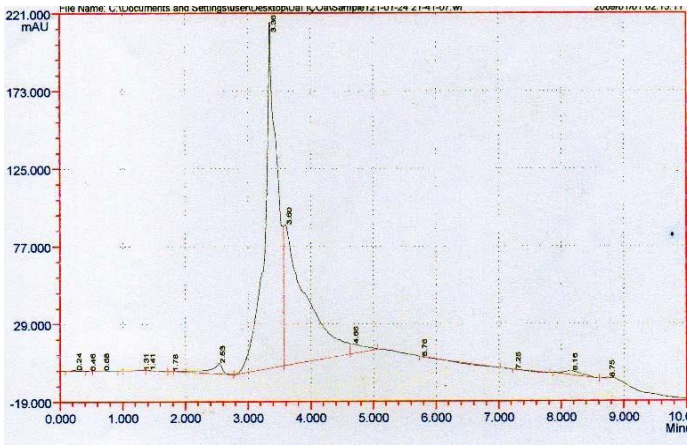

Figure 4. *Oxidative degradation.*

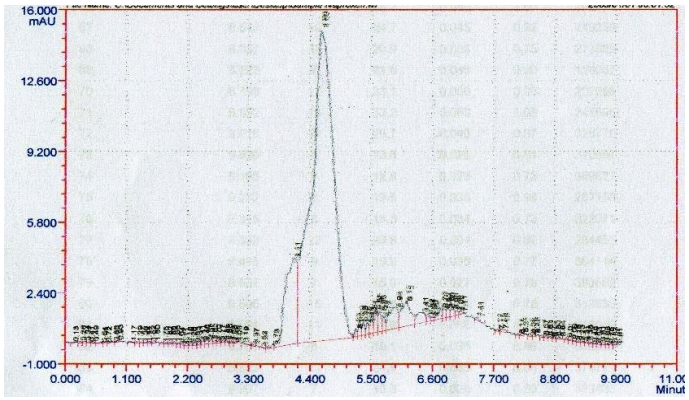

Figure 5. *Photolysis degradation.*

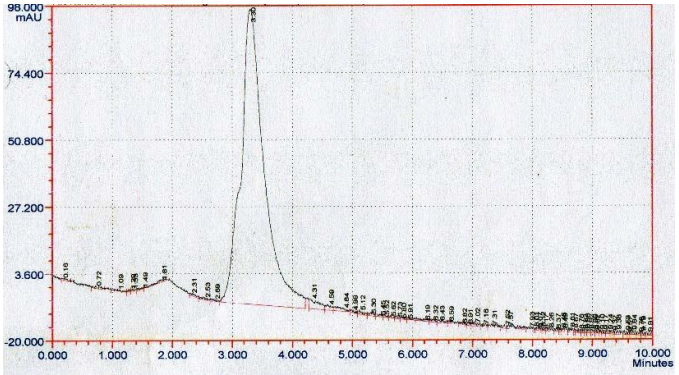

Figure 6. *Thermal degradation.*

after a given time with the handheld portion. Controlling the Mesalazine 's synthetic structure and thus achieving complete thermal dissolution of the compound becomes difficult as the temperature of the mesalazine solution rises above 100°C, as it is appeared in Figure 6.

MESA Infrared Spectrophotometer:

In the shape of a potassium bromide plate, the compounds are prepared. KBr's infrared measurements were performed at Basrah University / College of pharmacy in the area (4000500 cm^{-1}) at room temperature with a system of the type FTIR-84005-SHIMADZU, made in Germany. The active groups can be seen in the FT-IR spectrum of the compound Mesalazine (Figures 7 and 8) [21-25].

For Mesalazine -Standard:

Mesalazine -Standard's infrared spectrum, important peaks for stretching and bending vibrations can be seen in (Figure 7), which are compatible with the structure. Standard FT-IR mesalazine 's spectrum appears to be small, with peaks at 3415 cm^{-1} (M) for (OH) Carboxylic acids and 1604 cm^{-1} (S) for (C=O) Carboxylic acids, the 1730 cm^{-1}(S) for (C=O) Carboxylic acids, the 1205 cm^{-1}(M) and 759cm^{-1}(M) for (C-N), the 3415cm^{-1}(m) for (N-H), Aromatic stretching of C-H is allocated to 3062 cm^{-1}(W), while aliphatic stretching of C-H is allocated to 2962 cm^{-1}(M). Aromatic C=C peaks occur in the range 1411 cm^{-1} (M).

For mesalazine -Sample:

The Mesalazine sample's infrared spectrum (Figure 8) displays peaks that correspond to the standard model's peaks, with vibrations that lead to the structure's extension and curvature Standard-FT-IR Mesalazine 's spectrum seems to be small, with peaks at 3460 cm^{-1} (M) for (OH) Carboxylic acids and 1604 cm^{-1} (S) for (C=O) Carboxylic acids, the 1732 cm^{-1}(S) for (C=O) Keton, the 3450 cm^{-1} for OH Alcohol (S) Brod band, the 1205 cm^{-1}(m) and 759cm^{-1}(M) for (C-N), the 3419 cm^{-1}(m) for (N-H), Aromatic stretching of C-H can be assigned to 3059 cm^{-1}(W) and aliphatic stretching of C-H to 2962 cm^{-1}(M). Aromatic C=C peaks occur in the range 1409 cm^{-1} (M).

Debate on the Findings.

Improvements to HPLC conditions:

To isolate all of the degradation products from the Mesalazine peaks, chromatographic conditions were established. The Ion Pac Arcus EP-C18 has a length of 5 meters, a diameter of 4.5

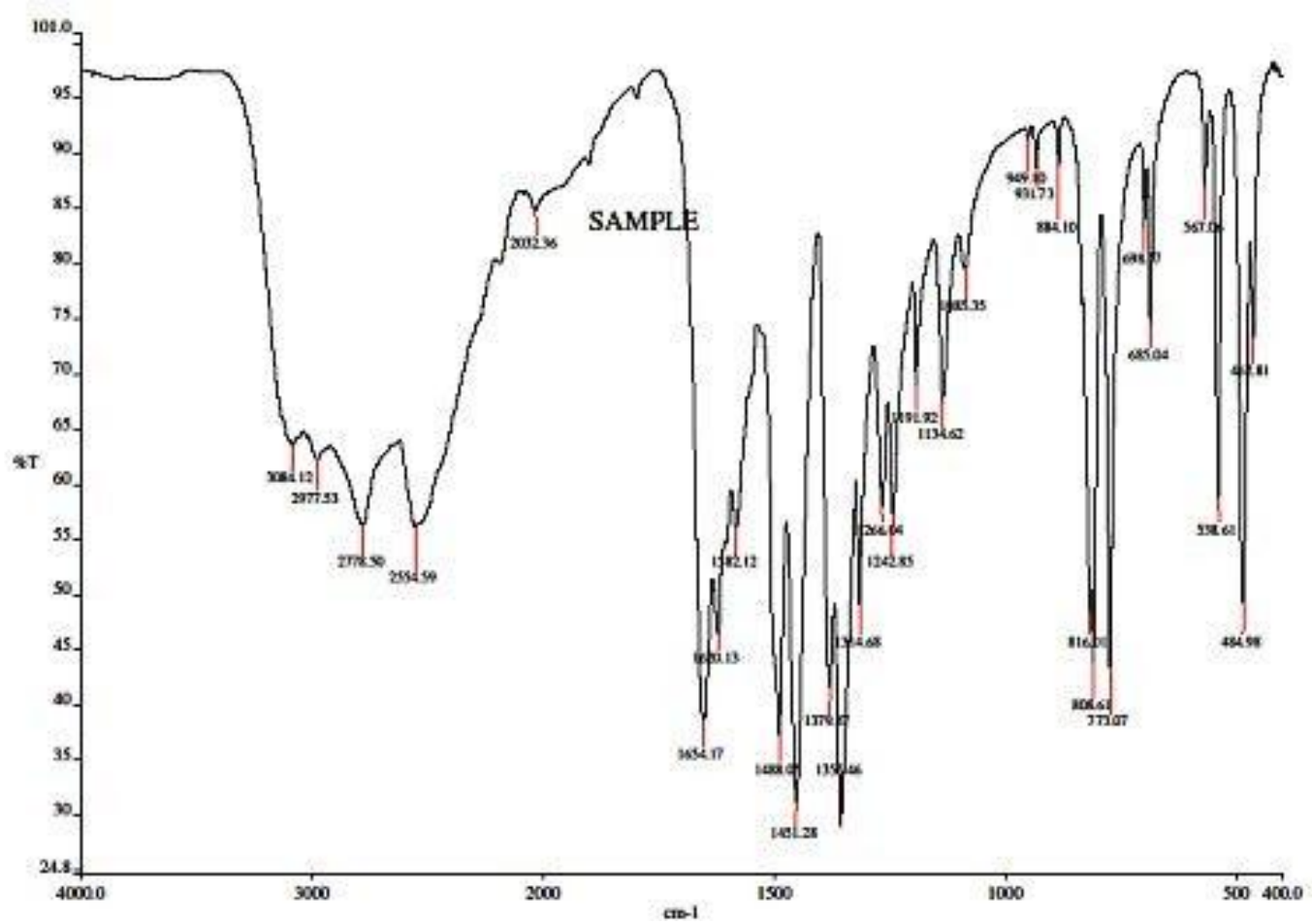

Figure 7. *FT-IR For Mesalazine Standard.*

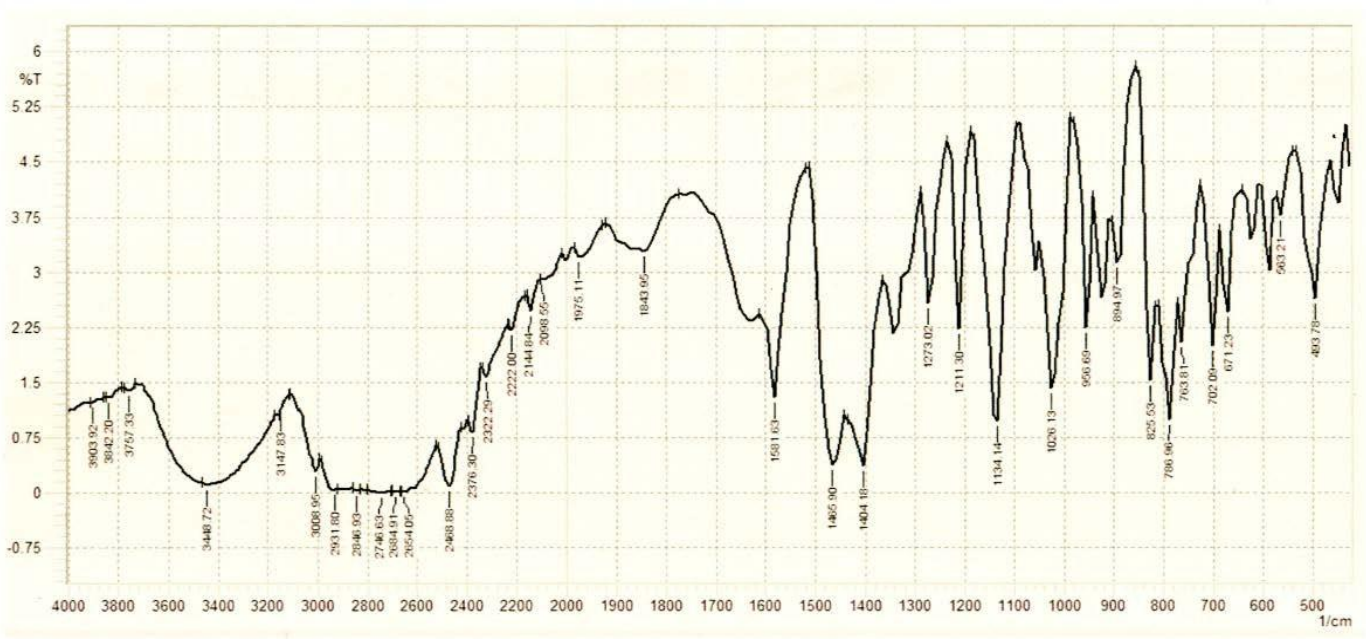

Figure 8. *FT-IR For Mesalazine sample.*

millimeters, and a diameter of 250 millimeters, as well as the requisite organic step. Methanol was used in several trials, during the process of HPLC technique optimization: acetonitrile: acetic acid: water, 40:40:20 (v/v/v) + 0.5 M potassium dihydrogen orthophosphate buffer at pH 3.3, and 1 ml/min flow rate during the process of HPLC technique, the wavelength was measured to be 260 nanometers [26]. Mesalazine had a retention time of 3.17 minutes. The new analytical method produced a good peak shape (Figure 9).

Suitability of the System:

The HPLC-UV device was subjected to research in order to adapt it. Three replicas of the same concentration were repeated using the ideal method using the normal Mesalazine (3 g/mL). The machine suitability is shown in Table 2. These findings follow the separation method's criteria as well as Mesalazine estimates in different pharmaceuticals [27].

The Validation of Methods and Assays:

Specificity, linearity range, and sensitivity, as well as regression, precision, accuracy, and rigidity, were employed specifically in order to validate the new chromatographic technique HPLC-UV in accordance with ICH: To determine process validity, the impact of experimental conditions on the peak areas of the analytes was investigated. At a Mesalazine concentration of 3 g/ml, the technique's validity was checked. Table 4 listed all of the research results. The results showed that minor changes in flow rate, mobile phase work of art, temperature, and detection wavelength had no impact on the drug peak areas, indicating that the method was valid.

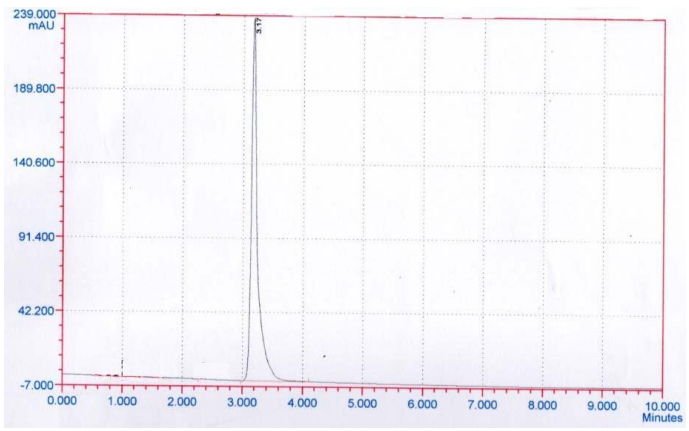

Figure 9. *Optimum conditions for Mesalazine in the HPLC-UV method.*

Table 2. *System suitability analysis of MESA.*

Injections	Drug	RT	Area	% area	USP plate count	USP tailing
1	MESA	3.10	52628.2	99.400	3906	0.40
2	MESA	3.225	108628.3	99.850	3903	0. 40
3	MESA	3.17	160228.2	99.900	3907	0. 40
4	MESA	3.20	212628.4	99.750	3908	0. 40
5	MESA	3.12	267628.5	99.525	3905	0. 40
MEAN			160348.32	RT- Retention Time 3.17 ± 0.45 min		
SD			0.41			
% RSD			0.40			

Table 3. *Linearity of MESA (n=3).*

Sl. No.	Concentration µg/ml	Area
1	1	52628.2
2	2	108628.3
3	3	160228.2
4	4	212628.4
5	5	267628.5

y = 53440x (R^2 = 0.9998) for Mesalazine

Table 4. *Regression characteristics of linearity of MESA.*

Parameters	Results
Linearity range (µg/ml)	1- 5
Regression equation (y=mx+b)	y = 53440x (R^2 = 0.9998)
Slope (m)	53409.4
Intercept (b)	53440
The correlation coefficient (R^2)	0.9998
limit of detection (LOD)	0.3613
limit of quantitation (LOQ)	1.636

Table 5. *Recovery study results of MESA.*

Sl. No.	Accuracy range	Amount of APB added (mg)	Amount recovered (mg)	% Recovery
	50 % Accuracy	50	49.5	99.0
		50	49.6	99.0
		50	49.4	98.0
	100% Accuracy	100	100	100
		100	100	100
		100	100	100
	150% Accuracy	150	150	100
		150	150	100
		150	150	100
Mean				100
SD				0.41
% RSD				0.40

The Specificity:

Forced deprivation was used to investigate the specificity of the proposed plan. The research was carried out to ensure that During the forced degradation analysis, Mesalazine could be distinguished from the potential degradation products using the proposed process. The tablet sample was tested using acid, base, oxidation, photolysis, and heat at a concentration of 3 g/ml Mesalazine. The outcomes of forced decomposition are shown in Table 5. The shapes of chromatograms are depicted in Figures 2-6, and figure 9. The drug's alkaline conditions resulted in the highest percentage of degradation [28,29]. The lowest percentage of mesalazine degradation occurred when it was exposed to heat and when it was exposed to photosynthesis. Decomposition goods showed a single peak of degradation. Other stress-related degradation products do not interfere with Mesalazine identification, so the tool can be used as a stability indicator.

The Linearity Range and Sensitivity:

A solid relationship was formed when the pinnacle regions for the drug were plotted against the medication fixation (g/ml) under ideal test conditions. The target range of Mesalazine

was discovered to be (1–5) g/ml. The following conditions were obtained from the straight relapse investigation of the information.

On the basis of the following assumptions: y = peak area, x = drug convergence (g/mL), and R^2 = regression coefficient [30]. The high relapse coefficient estimations with a small catch illustrate the adjustment bend's great linearity, as shown in Figure 10, and table 3.

The Regression:

Calculating the limit of quantitation (LLOQ) and edge of detection helped determine the proposed process's comprehension (LLOD). The following equations were used to measure the LOD and LLOQ [31].

LLOD=3.3SD/S; LLOQ=10SD/S

Where SD denotes the drug rejoinder's standard deviation and S denotes the calibration curve's slope. The LLOQ values were found to be 0.3613 g/ml, while the LLOD values were 1.636 g/ml. These figures show that the sensitivity of the predicted technique for studying the chosen drug is adequate. The regression statistics of the anticipated method are shown in Table 4 [32].

The Accuracy:

Three separate quantities of a recognized volume of standard solution were put to the pre-analysis tablet sample solutions, 10 percent, 20 percent, and 30 percent. The predicted methodology was used to re-analyze the solutions. With a percentage of RSD of 0.40 percent, the percentage recovery was between 98 and 100 percent. The results show that the procedure is very accurate. The non-interference of the excipients was determined by analyzing the analytes to determine the process' selectivity [33]. Table 5 shows a summary of the findings.

Table 6. *Method precision.*

SL. NO.	Sample weight (mg)	Area	Mean Area Counts	% Label Claim
1	100	52628.2	160228.2	100
2	100	108628.3	160228.2	100
3	100	160228.2	160228.2	100
4	100	212628.4	160228.2	100
5	100	267628.5	160228.2	100
MEAN				100
SD				0.41
% RSD				0.40

Table 7. *Intermediate precision.*

SL. NO	Sample weight (mg)	Area	Mean	% Label Claim
1	100	52628.2	160348.32	100
2	100	108628.3	160348.32	100
3	100	160228.2	160348.32	100
4	100	212628.4	160348.32	100
5	100	267628.5	160348.32	100
6	100	52628.2	160348.32	100
Mean				100
SD				0.41
% RSD				0.40

Table 8. *Assay of MESA in tablets.*

Analyte	Labeled claim (mg)	Found (mg)	Mean (mg)	%Recovery	%RSD
Standard - MESA MESA -500	500 500	500 500	500 500	100 100	±0.401 ±0.402

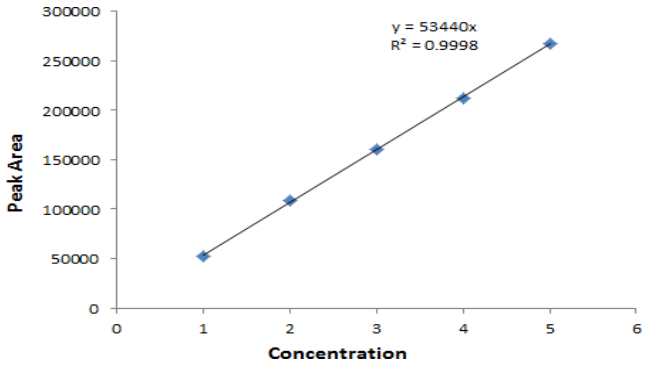

Figure 10. *Linearity of the calibration curve.*

The Precision:

Mesalazine was analyzed at a concentration of 3 g/ml to determine precision. The precision of the method was checked by using the established technique for estimating Mesalazine in pure standard mesalazine three times (n=3). The method's precision was checked by repeating the mesalazine investigation in tablet samples three times (n=3). Table 6 and 7, show a summary of the findings. System and method precision percentage RSD values were both less than 0.40 percent, suggesting that the proposed Mesalazine investigation strategy is extremely precise [34,35].

Discussion and Applications of Method:

Examining commercially available Mesalazine -500 mg tablets was used to test the analytical process (Mesalazine-Pentasa-tablets Ferring®). The proportion of Standard- mesalazine was discovered to be 100, ±0.401percent, while the ratio of Mesalazine in Mesalazine-500 (Mesalazine - Pentasa-tablets Ferring®) was discovered to be 100, ±0.402percent. This result indicates that the proposed approach was reliable and precise in analyzing mesalazine in dosage types, as shown by the percentage recovery and RSD percent values. The results of the applications were presented in Table 8.

The presence of Mesalazine in two commercial pharmaceutical products was determined using an HPLC system (LC100 Angstrom advanced) with a UV detector in this analysis. This tried-and-true approach is easy to use, low-cost, and only needs a small amount of sample. It also employs an ultraviolet detector, which, due to a single peak in the chromatogram, makes this system extremely sensitive. Since pharmaceutical drugs have such low concentrations, high sensitivity is not needed in this application. The method was validated using HPLC-UV guidelines, and the established technique meets Beer's law for drug fixation in the range of 1.0–5.0 g/mL.

Conclusion.

The study shows the critical analytical approach used to determine the existence of Mesalazine in the measurements structure in the light of the findings. Easy, accurate, exact, delicate, explicit, rough, and hearty describes the established and authorized HPLC-UV safety showing technique for Mesalazine measurement. In this way, the proposed technique can be used on a routine basis to analyze Mesalazine in the tablet dose structure.

Acknowledgments.

Assist Professor H. N.K. AL-Salman from the College of Pharmacy's Pharmaceutical Chemistry DEP/ University of Basra, Iraq. assisted the authors in the preparation of this manuscript and therefore they are thankful for such assistance.

Efforts of the Researchers.

The research was conducted in the College of Pharmacy at the University of Basrah. This research took three months to complete with significant and consistent effort, and the results were excellent in terms of evaluating a clear and sensitive method for estimating the Mesalazine.

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