

RESEARCH ARTICLE



Development and Validation of a Reversed-Phase Stability-Indicating HPLC Assay Method for the Simultaneous Determination of Esomeprazole Magnesium Trihydrate and Itopride Hydrochloride in Solid Dosage Forms

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Abstract: An isocratic reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of esomeprazole magnesium trihydrate and itopride hydrochloride in bulk drug and solid dosage forms. Separation was achieved on a HiQ Sil C18HS column (250 mm×4.6 mm, 5 µm) using a mobile phase consisting of acetonitrile and 2 mM phosphate buffer (pH 7.0) containing 0.06% w/v tetrabutylammonium hydrogen sulphate (15:85 v/v) at a flow rate of 1.0 mL/min. Photodiode array detection was performed at the isobestic wavelength of 290 nm, yielding retention times of 15.09 min for itopride and 17.67 min for esomeprazole. The method was validated according to International Council for Harmonisation guidelines, showing linear responses over 50 to 750 µg/mL for esomeprazole ($r^2=0.999$) and 100 to 500 µg/mL for itopride ($r^2=0.998$). Method precision showed relative standard deviations below 1.0%, and mean recoveries from commercial capsules ranged from 98.78% to 99.49% for esomeprazole and 99.98% to 99.99% for itopride. The validated procedure was successfully applied to forced degradation studies conducted under hydrolytic (acidic, alkaline, neutral), oxidative, photolytic, and thermal stress conditions. Esomeprazole magnesium trihydrate exhibited substantial degradation under hydrolytic, oxidative, and photolytic conditions, producing up to eleven resolved degradation products, whereas itopride hydrochloride degraded selectively under concentrated acidic and alkaline reflux while remaining stable under neutral, oxidative, photolytic, and thermal stress. Photodiode array peak purity analyses confirmed that both drug peaks remained spectrally homogeneous without co-eluting degradants, establishing the method as stability-indicating for routine quality control and stability evaluations.

Keywords: Esomeprazole magnesium trihydrate; Itopride hydrochloride; Stability-indicating assay method; Forced degradation; Ion-pair liquid chromatography.

1. Introduction

Pharmaceutical quality control relies on analytical methods capable of discerning active pharmaceutical ingredients from degradation products formed during manufacture, storage, and handling [1]. Regulatory frameworks articulated by the International Council for Harmonisation (ICH Q1A (R2), Q2 (R1)) and the United States Food and Drug Administration (FDA) mandate the establishment of stability-indicating assay methods (SIAM) [1, 2]. A stability-indicating method is defined as a validated quantitative analytical procedure that can detect changes over time in the pertinent properties of the drug substance and drug product, accurately measuring the active ingredients without interference from degradation products, process impurities, or formulation excipients [1, 3]. Stress testing exposes chemical entities to elevated thermal conditions, humidity, acidic and basic media, neutral hydrolysis, light irradiation, and oxidative agents [2, 4]. These studies show that the intrinsic degradation pathways of drug substances, identify putative degradation structures, and verify the selectivity of chromatographic separations [3, 5].

Fixed-dose combination therapies targeting gastrointestinal disorders have gained clinical prominence. Gastroesophageal reflux disease is characterized by the retrograde flow of acidic gastric contents across the lower esophageal sphincter, resulting in mucosal inflammation, erosive esophagitis, persistent heartburn, and upper abdominal pain [6, 7]. The pathogenesis involves transient lower esophageal sphincter relaxations, impaired acid clearance, delayed gastric emptying, and hypersensitivity to refluxate [7, 8]. The therapeutic combination of a proton pump inhibitor and a prokinetic agent provides complementary pharmacological intervention [8, 9].

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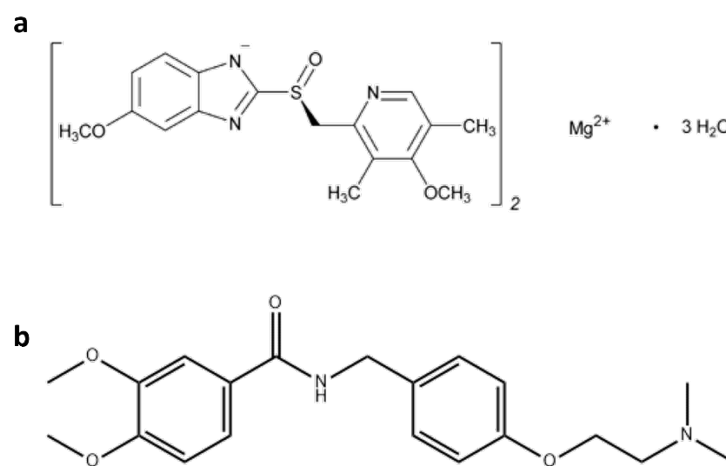


Figure 1. Chemical Structure of a. Esomeprazole magnesium trihydrate b. Itopride Hydrochloride

Esomeprazole magnesium trihydrate, the (S)-enantiomer of omeprazole, inhibits gastric acid secretion by covalently binding to the H⁺/K⁺-ATPase enzyme system located at the secretory surface of the gastric parietal cell [9, 10]. It provides sustained suppression of basal and stimulated gastric acid output superior to racemic proton pump inhibitors by blocking the terminal step of hydrogen ion transport [10, 11]. Itopride hydrochloride, a prokinetic benzamide derivative, stimulates gastrointestinal motor tone via dopamine D₂-receptor antagonism and concurrent inhibition of the enzyme acetylcholinesterase [12, 13]. It eliminates dopamine-mediated inhibitory tone on acetylcholine release by blocking dopamine D₂ receptors on enteric neurons [13, 14]. Acetylcholinesterase inhibition retards enzymatic breakdown of synaptic acetylcholine, thereby amplifying peristaltic activity, raising lower esophageal sphincter tone, accelerating gastric clearance, and exerting centrally mediated anti-emetic actions [14, 15].

Despite their widespread co-administration in fixed-dose capsules, simultaneous chromatographic determination of esomeprazole magnesium and itopride hydrochloride in the presence of their degradants presents significant chromatographic challenges [16]. Esomeprazole is a hydrophobic, acid-labile substituted benzimidazole with limited aqueous solubility and an octanol-water partition coefficient (log P) of 0.6 [11, 16]. In contrast, itopride hydrochloride is a hydrophilic benzamide derivative (log P = 2.3) with elevated water solubility and basic aliphatic amine functionality prone to secondary silanol interactions on alkyl-bonded silica phases [13, 17].

A survey of the published literature shows analytical methods reported for single-analyte determinations or simple combinations without forced degradation studies [16–20]. Existing techniques for esomeprazole encompass spectrophotometric assays and reversed-phase liquid chromatography for single-drug tablets [18, 19]. Itopride has been measured using liquid chromatography coupled to fluorescence or UV detection in biological fluids, and thin-layer or spectrophotometric assays in two-component mixtures with rabeprazole [17, 20]. However, no validated stability-indicating method exists for the simultaneous separation and quantitative analysis of esomeprazole magnesium and itopride hydrochloride in the presence of degradation products generated across ICH stress conditions.

The present work describes the development and validation of an isocratic ion-pair reversed-phase high-performance liquid chromatographic method with photodiode array detection for the simultaneous determination of esomeprazole magnesium and itopride hydrochloride in bulk and capsule dosage forms. The method was rigorously validated in accordance with ICH Q2 (R1) guidelines and subsequently applied to evaluate the stress degradation kinetics, stability profiles, and degradation pathways of both drug entities under hydrolytic, oxidative, photolytic, and thermal conditions.

2. Materials and Methods

2.1. Reagents and Chemicals

Reference standard samples of esomeprazole magnesium trihydrate and itopride hydrochloride were obtained as gift samples from Cipla Ltd. (Mumbai, India) and Ami Life Sciences Pvt. Ltd. (Baroda, India), respectively. Commercial hard gelatin capsules containing esomeprazole magnesium enteric-coated pellets (40 mg) and itopride hydrochloride sustained-release pellets (150 mg) (Raciper IT, Ranbaxy Laboratories Ltd.) were procured from a local retail pharmacy. HPLC-grade acetonitrile and methanol were obtained from Merck Specialties Pvt. Ltd. Analytical reagent-grade disodium hydrogen phosphate, potassium dihydrogen phosphate, tetrabutylammonium hydrogen sulphate (TBAHS), hydrochloric acid, sodium hydroxide, and hydrogen peroxide solution

(30% w/v) were purchased from Qualigens Fine Chemicals. Purified HPLC-grade water was generated using a Milli-Q water purification assembly.

2.2. Instrumentation and Chromatographic Conditions

Chromatographic method development, optimization, and validation were conducted on a Jasco HPLC system equipped with a Jasco PU-2080 Plus intelligent HPLC pump, a Rheodyne manual sample injector fitted with a 20 μ L sample loop, a Varian Star 800 module interface, Borwin chromatography software, and a Jasco UV-2075 Plus intelligent UV/Vis detector operated at a sensitivity of 0.01 AUFS. Peak purity determinations, degradation tracking, and spectral deconvolutions were performed on a Dionex HPLC system comprising a P680 HPLC pump, an ASI-100 automated sample injector, a UVD 340U photodiode array (PDA) detector, and Chromeleon Client software (version 6.80 SP2, Build 2284). Chromatographic separation was achieved on a HiQ Sil C18HS analytical column (250 mm length $\times$ 4.6 mm internal diameter, 5 μ m particle size).

The aqueous buffer component was prepared by dissolving 0.50 g of anhydrous disodium hydrogen phosphate and 0.301 g of potassium dihydrogen phosphate in 1000 mL of distilled water, yielding a 2 mM phosphate buffer adjusted to pH 7.0 $\pm$ 0.05. The ion-pairing reagent tetrabutylammonium hydrogen sulphate was dissolved in the buffer at a final concentration of 0.06% w/v (1.77 mM). The mobile phase was prepared by mixing acetonitrile and the buffered ion-pairing solution in a 15:85 v/v ratio. The mixture was filtered through a 0.45 μ m nylon membrane filter and degassed using an ultrasonic bath for 15 min. The mobile phase was delivered under isocratic conditions at a flow rate of 1.0 mL/min. Spectroscopic monitoring was conducted at 290 nm, corresponding to the experimentally determined isobestic point of esomeprazole and itopride.

2.3. Preparation of Standard Solutions

Primary standard stock solutions of esomeprazole magnesium (1000 μ g/mL) and itopride hydrochloride (1000 μ g/mL) were prepared independently by dissolving 10.0 mg of each reference standard in methanol in separate 10 mL volumetric flasks. Working standard solutions were prepared by serial dilution of standard stocks with the mobile phase. A combined working standard solution containing 100 μ g/mL of esomeprazole and 500 μ g/mL of itopride was utilized for routine system suitability and precision assessments.

2.4. Analysis of Marketed Formulation

To assess formulation applicability, twenty commercial capsules (Raciper IT) were accurately weighed and evacuated of their pellet contents. The composite contents were finely ground using an agate mortar and pestle. A portion of homogenized powder equivalent to the average capsule weight was accurately weighed and transferred into a 100 mL volumetric flask containing approximately 70 mL of methanol. The suspension was mechanically shaken for 10 min, sonicated for 15 min to ensure complete extraction of active drugs, diluted to volume with methanol, and filtered through Whatman No. 41 filter paper. Aliquots of the filtrate were diluted with mobile phase to yield target working concentrations of 80 μ g/mL for esomeprazole and 300 μ g/mL for itopride hydrochloride. Peak areas of standard and formulation solutions were recorded (n=5), and active drug contents were determined relative to reference calibration responses.

Recovery studies were executed using the standard addition technique across three concentration increments (50%, 100%, and 150%). Standard solutions of esomeprazole (40, 80, and 120 μ g/mL) and itopride (150, 300, and 450 μ g/mL) were added to pre-analyzed capsule composite solutions containing 80 μ g/mL esomeprazole and 300 μ g/mL itopride. Each spiked concentration level was injected in triplicate, and recovery percentages along with relative standard deviations were calculated.

2.5. Method Validation

Validation was conducted in accordance with ICH Q2 (R1) specifications. Linearity was investigated across concentration ranges of 50 to 750 μ g/mL for esomeprazole (50, 150, 250, 350, 450, 550, 650, and 750 μ g/mL) and 100 to 500 μ g/mL for itopride (100, 200, 300, 400, and 500 μ g/mL). Linear regression curves were constructed by plotting peak area against nominal analyte concentration using ordinary least-squares analysis.

Instrument precision (repeatability) was determined by calculating relative standard deviations across five consecutive injections of standard drug solutions. Intra-day precision was established by evaluating six replicate injections at three separate intervals within a single day. Intermediate precision was verified by repeating the six-replicate injection sequence across three consecutive days using freshly prepared solutions and mobile phases.

Limits of detection (LOD) and quantitation (LOQ) were established based on signal-to-noise ratios of 3:1 and 10:1, respectively, by analyzing standard samples prepared at progressively lower concentrations. System suitability parameters, including theoretical plate counts (N), peak asymmetry/tailing factor (T), and chromatographic resolution (R_s), were calculated throughout validation sequences.

2.6. Forced Degradation (Stress Testing)

Forced degradation was conducted on individual drug substances and combined solutions to verify the resolution of parent molecules from hydrolytic, oxidative, photolytic, and thermal degradants.

Table 1. Forced Degradation Conditions Employed for ES and ITP

Stress Category	Stress Condition / Reagent	Degradation Parameters for Esomeprazole Magnesium Trihydrate (ES)	Degradation Parameters for Itopride Hydrochloride (ITP)
Hydrolytic	Acid hydrolysis	1 N HCl at room temperature, and under reflux at 80°C for 1 h	Screening with 0.1 to 5 N HCl for 30 min to 3 h at room temperature and under reflux at 70°C (3 N HCl reflux at 70°C for 30 min optimized)
	Alkaline hydrolysis	1 N , 2 N , and 3 N NaOH at room temperature, and under reflux with 1 N NaOH at 80°C for 1 h	Screening with 0.1 to 5 N NaOH for 30 min to 3 h at room temperature and under reflux at 70°C (3 N NaOH reflux at 70°C for 30 min optimized)
	Neutral hydrolysis	Distilled water under reflux at 80°C for 1 h	Distilled water under reflux at 80°C for 1 h
Oxidative	Hydrogen peroxide (6% v/v)	Exposure to 6% v/v H ₂ O ₂ in dark at room temperature for 1 h	Exposure to 6% v/v H ₂ O ₂ in dark at room temperature for 1 h
	Hydrogen peroxide (30% v/v)	Exposure to 30% v/v H ₂ O ₂ in dark at room temperature for 1 h	Exposure to 30% v/v H ₂ O ₂ in dark at room temperature for 1 h
Photolytic	Direct solar radiation	Methanolic solution exposed to direct sunlight for 8 h/day over 16 days (128 h total)	Methanolic solution exposed to direct sunlight in open air for 360 h
Thermal	Dry heat	Bulk solid substance exposed in a hot air oven at 105°C for 5 h	Bulk solid substance exposed in an oven at 80°C for 6 h
Climatic / Humidity	Accelerated stability testing	Bulk drug stored at 40°C±2°C and 75%±5% RH for 3 months (sampled at 1 week, 2 weeks, 1 month, 2 months, and 3 months)	Bulk drug stored at 40°C±2°C and 75%±5% RH for 3 months (sampled at 1 week, 2 weeks, 1 month, 2 months, and 3 months)

2.6.1. Hydrolytic Degradation (Acid, Alkaline, Neutral)

For acidic degradation, 10.0 mL of drug stock solution in methanol (1 mg/mL) was mixed with 10.0 mL of 1 N hydrochloric acid and refluxed at 80°C for 1 h for esomeprazole. For itopride, solutions containing acid strengths from 0.1 to 5 N HCl were screened across ambient and heated states, with optimal stressing established using 3 N HCl refluxed at 70°C for 30 min.

Alkaline degradation was conducted by combining 10.0 mL of stock solution with 10.0 mL of 1 N sodium hydroxide refluxed at 80°C for 1 h for esomeprazole. For itopride, base concentrations from 0.1 to 5 N NaOH were evaluated, with significant hydrolysis attained under 3 N NaOH refluxed at 70°C for 30 min.

Neutral hydrolysis was performed by refluxing 10.0 mL of drug stock solutions with 10.0 mL of distilled water at 80°C for 1 h. Following exposure, all stressed solutions were allowed to cool to room temperature, neutralized with equivalent stoichiometric volumes of acid or base where applicable, and diluted with mobile phase prior to chromatographic injection.

2.6.2. Oxidative Stress

Oxidative degradation was executed by reacting 10.0 mL of methanolic stock solution with 10.0 mL of 6% v/v and 30% v/v hydrogen peroxide solutions. Reaction mixtures were stored at ambient room temperature ($25 \pm 2^\circ\text{C}$) in the dark for 1 h to mitigate thermal homolysis of peroxides. Solutions were subsequently transferred, brought to volume with mobile phase, and analyzed.

2.6.3. Photolytic Stress

Photostability testing was evaluated by exposing stock solutions of esomeprazole and itopride in methanol to direct natural sunlight. Esomeprazole was exposed for 8 h daily over 16 consecutive days (128 h total daylight exposure). Itopride solutions were subjected to continuous open sunlight for an aggregate duration of 360 h. Corresponding control samples were wrapped completely in aluminum foil and stored in parallel under identical thermal conditions.

2.6.4. Thermal and Solid-State Humidity Stress

Solid-state bulk drug samples (500 mg) were distributed in thin layers onto clean glass Petri dishes. Dry heat exposure was conducted inside a precision-controlled hot air oven maintained at 105°C for 5 h for esomeprazole and 80°C for 6 h for itopride hydrochloride. Accelerated solid-state stability evaluation was conducted by placing bulk drugs within a climatic humidity chamber maintained at $40^\circ\text{C} \pm 2^\circ\text{C}$ and $75\% \pm 5\%$ relative humidity for 3 months, with sampling at intervals of 1 week, 2 weeks, 1 month, 2 months, and 3 months.

3. Results and Discussion

3.1. Method Development and Optimization of Chromatographic Conditions

3.1.1. Selection of Detection Wavelength

Individual ultraviolet absorption spectra of esomeprazole magnesium trihydrate and itopride hydrochloride in methanol indicated absorbance maxima at 302 nm and 258 nm, respectively. Overlaid UV spectra revealed an isobestic absorption point at 290 nm. Selection of 290 nm as the monitoring wavelength provided balanced molar absorptivity for both active compounds and their respective degradation products, avoiding disproportionate detector response during simultaneous quantitation.

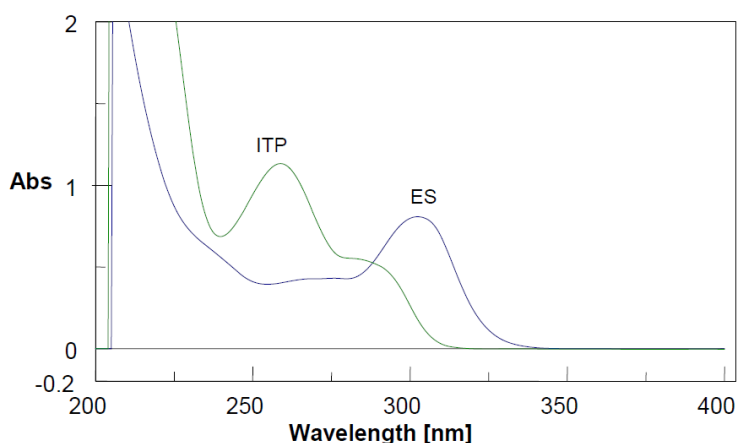


Figure 2. Overlaid UV spectra showing isobestic absorption point at 290 nm.

3.1.2. Optimization of Stationary and Mobile Phases

Initial chromatographic screening aimed to achieve symmetrical peak shapes and adequate retention for both compounds. Preliminary trials using binary mixtures of methanol-water and acetonitrile-water across diverse volume ratios failed to deliver acceptable separations. On conventional reversed-phase alkyl silica packings, itopride exhibited pronounced peak tailing, severe band broadening, and retention variability. This behavior was attributed to mixed-mode retention governed by secondary

silanophilic interactions between the protonated tertiary dimethylamino group of itopride and ionized residual silanols (Si-O^-) on the silica surface. Esomeprazole displayed insufficient retention control and tended to merge with degradation peaks.

Introduction of volatile acidic or neutral buffers, such as ammonium acetate (pH 4.5), led to peak fronting and degradant co-elution. While a neutral 2 mM phosphate buffer (pH 7.0) improved peak symmetry, it failed to separate esomeprazole from degradants EA3, EN2, and EP3. Multiple linear and step gradient elution programs combining acetonitrile and phosphate buffer (pH 7.0) over 20 to 60 min run times resolved selected degradation fractions, but were accompanied by baseline drift that hindered quantitation of trace degradants, while critical degradants continued to co-elute with the parent esomeprazole peak.

To eliminate secondary silanol interactions and enhance selectivity for both ionizable moieties simultaneously, tetrabutylammonium hydrogen sulphate (TBAHS) was incorporated into the aqueous mobile phase. At pH 7.0, TBAHS acts dual-functionally: lipophilic tetrabutylammonium cations (TBA^+) dynamically adsorb onto the hydrophobic stationary phase surface, masking ionized silanolate sites and suppressing cation-exchange interactions responsible for itopride peak tailing. Simultaneously, pairing of counter-ions modulates the retention of the substituted benzimidazole core of esomeprazole. Concentration screening identified 0.06% w/v (1.77 mM) TBAHS as optimal, producing sharp, symmetrical peaks (tailing factors <1.9) and efficiency exceeding 3800 theoretical plates for both analytes.

An isocratic mobile phase composed of acetonitrile and 2 mM phosphate buffer containing 0.06% w/v TBAHS (pH 7.0) in a 15:85 v/v ratio achieved complete resolution of itopride ($t_R=15.09$ min) and esomeprazole ($t_R=17.67$ min) from each other and from all degradation products within a 60 min run time at a flow rate of 1.0 mL/min.

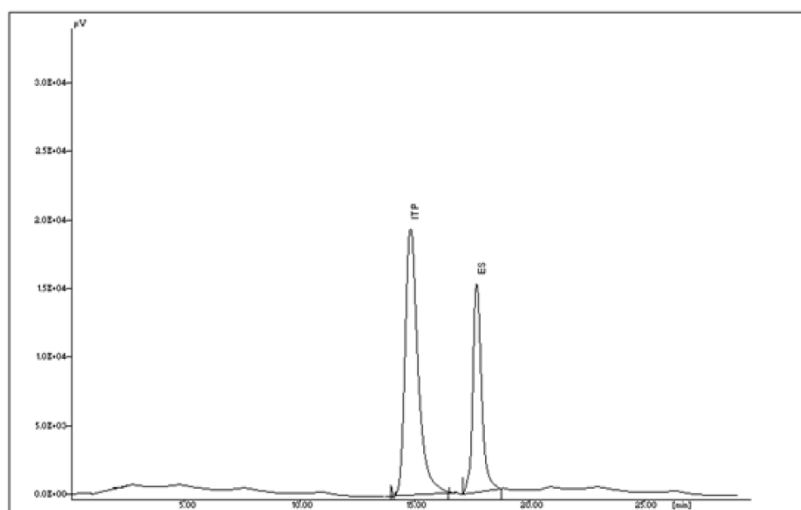


Figure 3. HPLC Chromatogram of Standards of ES and ITP

3.2. Method Validation

3.2.1. Linearity and Calibration Range

Linearity was established over the range of 50 to 750 $\mu\text{g/mL}$ for esomeprazole (50, 150, 250, 350, 450, 550, 650, and 750 $\mu\text{g/mL}$) and 100 to 500 $\mu\text{g/mL}$ for itopride hydrochloride (100, 200, 300, 400, and 500 $\mu\text{g/mL}$). Regression lines constructed by ordinary least-squares analysis showed proportionality between peak area responses and analyte concentrations.

For esomeprazole, the linear regression equation was:

$$y=25.67x+2076$$

with a coefficient of determination (r^2) of 0.999, where y represents chromatographic peak area and x represents analyte concentration in $\mu\text{g/mL}$. For itopride hydrochloride, calibration over 100 to 500 $\mu\text{g/mL}$ yielded the regression equation:

$$y=8594x$$

with a coefficient of determination (r^2) of 0.998. The working calibration parameters for both active pharmaceutical ingredients are summarized in Table 2.

Table 2. Calibration and Linearity for Esomeprazole Magnesium Trihydrate and Itopride Hydrochloride ($n = 3$)

Analyte	Nominal Concentration ($\mu\text{g/mL}$)	Mean Chromatographic Peak Area	Relative Standard Deviation (% RSD)
Esomeprazole Magnesium Trihydrate	0	0	—
	50	3,427	0.37
	150	5,734	0.32
	250	8,561	0.34
	350	10,998	0.39
	450	14,001	0.34
	550	16,327	0.37
	650	18,503	0.39
	750	21,304	0.33
Itopride HCl	0	0	—
	100	702,314	0.51
	200	1,589,743	0.83
	300	2,369,200	0.64
	400	3,141,600	0.73
	500	3,999,350	0.96

3.2.2. Sensitivity (LOD and LOQ)

Limits of detection and quantitation were determined experimentally based on signal-to-noise ratios of 3:1 and 10:1, respectively. For esomeprazole magnesium, LOD and LOQ values were established at 25.0 $\mu\text{g/mL}$ and 50.0 $\mu\text{g/mL}$. For itopride hydrochloride, enhanced molar absorptivity at 290 nm produced an LOD of 0.5 $\mu\text{g/mL}$ and an LOQ of 1.0 $\mu\text{g/mL}$.

3.2.3. Precision and Repeatability

Method precision was verified across repeatability, intra-day precision, and intermediate precision. Instrument repeatability assessed across five replicate injections of standard drug solutions showed relative standard deviations of 0.35% for peak area and 0.39% for retention time for esomeprazole (100 $\mu\text{g/mL}$), and 0.002% for peak area and 0.002% for retention time for itopride (100 $\mu\text{g/mL}$).

Intra-day precision was verified by injecting reference solutions at three separate intervals within a single day ($n=6$ per trial). Intermediate precision was established by repeating the six-replicate injection sequence across three consecutive days with freshly prepared solutions and mobile phases. For esomeprazole, intra-day precision showed a mean RSD of 0.85%, while inter-day precision across three days ranged from 0.76% to 0.99%. For itopride hydrochloride, intra-day precision gave a mean RSD of 0.054%, with inter-day values ranging from 0.027% to 0.091%. All observed RSD values remained well below the 2.0% threshold prescribed by regulatory guidelines. Precision evaluations are presented in Table 3.

Table 3. Intra-Day and Intermediate Precision Data for Esomeprazole Magnesium Trihydrate and Itopride Hydrochloride

Injection Number	ES Area (100 $\mu\text{g/mL}$)			ITP Area (50 $\mu\text{g/mL}$)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	6784.56	6865.53	6832.83	476,150.27	477,715.21	477,755.83
2	6795.31	6788.05	6865.21	476,789.35	477,795.63	477,648.52
3	6774.89	6834.18	6837.13	476,585.86	477,840.26	477,379.90
4	6876.10	6859.25	6841.59	476,634.51	476,673.25	477,662.65
5	6741.38	6884.49	6854.43	476,636.26	477,693.54	477,675.23
6	6857.34	6734.91	6683.87	476,543.51	477,573.26	477,699.52
Mean	6804.93	6827.73	6819.17	476,556.60	477,548.50	477,636.90
Standard Deviation ($\pm$)	51.49	56.34	67.35	215.74	438.57	131.44
Relative Standard Deviation (%)	0.76	0.83	0.99	0.045	0.091	0.027

3.2.4. Accuracy and Recovery

Method accuracy was validated through standard addition recovery experiments using commercial combination capsule contents (Raciper IT, Ranbaxy Laboratories Ltd., label claim: 40 mg esomeprazole magnesium, 150 mg itopride hydrochloride). Analytical reference standards were spiked into pre-analyzed capsule composite solutions containing nominal concentrations of 80 µg/mL esomeprazole and 300 µg/mL itopride at three concentration levels corresponding to 50%, 100%, and 150% of target assay levels. Each concentration was analyzed in triplicate.

Table 4. Quantitative Assay of Esomeprazole Magnesium Trihydrate and Itopride Hydrochloride in Marketed Combination Capsules (Raciper IT)

Determination Number	Esomeprazole (µg/mL)	Found	Itopride Hydrochloride (ITP)	Found
1	79.61		299.54	
2	79.12		299.75	
3	79.58		299.96	
4	79.67		299.84	
5	79.89		299.78	
Mean Concentration Found	79.57 µg/mL		299.77 µg/mL	
Standard Deviation (±SD)	0.281		0.154	
Relative Standard Deviation (% RSD)	0.353%		0.051%	
Active Amount Found per Capsule	39.78 mg		149.88 mg	
Stated Label Claim per Capsule	40.00 mg		150.00 mg	
Mean Content Found (% Label Claim)	99.46%		99.92%	

Mean recoveries for esomeprazole across the spiked concentrations (40, 80, and 120 µg/mL added) ranged from 98.78% to 99.49% with an overall recovery of 99.15% and an RSD of 1.13%. Mean recoveries for itopride across spiked levels (150, 300, and 450 µg/mL added) ranged from 99.98% to 99.99% with an overall RSD of 0.40%. Direct assay of commercial capsules gave 99.46±0.35% (39.78 mg/capsule) for esomeprazole and 99.92±0.05% (149.88 mg/capsule) for itopride hydrochloride. Standard addition recovery data are summarized in Table 5.

Table 5. Recovery for Commercial Combination Capsules (n=3)

Target Formulation Analyte	Baseline Extracted (µg/mL)	Pure Standard Added (µg/mL)	Total Expected (µg/mL)	Total Recovered (µg/mL)	Mean Recovery (%)	Relative Standard Deviation (% RSD)
Esomeprazole	80.0	0.0 (0%)	80.0	79.57	99.46	0.35
	80.0	40.0 (50%)	120.0	119.02	99.19	1.37
	80.0	80.0 (100%)	160.0	158.06	98.78	0.44
	80.0	120.0 (150%)	200.0	198.98	99.49	0.52
Mean Overall ES Recovery	—	—	—	—	99.15	1.13
Itopride HCl	300.0	0.0 (0%)	300.0	299.77	99.92	0.05
	300.0	150.0 (50%)	450.0	449.96	99.99	0.56
	300.0	300.0 (100%)	600.0	599.88	99.98	0.21
	300.0	450.0 (150%)	750.0	749.93	99.99	0.44
Mean Overall ITP Recovery	—	—	—	—	99.98	0.40

3.2.5. System Suitability Testing

System suitability was verified before each analytical run by evaluating theoretical plate counts (N), peak asymmetry/tailing factor (T), chromatographic resolution (R_s), and capacity factors (k'). Column efficiency exceeded 4000 theoretical plates for esomeprazole (N=4033) and 4800 plates for itopride (N=4821). Peak asymmetry factors remained within acceptance criteria (T=1.83 for esomeprazole and 1.64 for itopride). Chromatographic resolution between adjacent degradation peaks was maintained above 2.5 throughout validation trials. Consolidated validation parameters are compiled in Table 6.

Table 6. Consolidated System Suitability and Validation Parameters for the Developed Stability-Indicating Assay Method

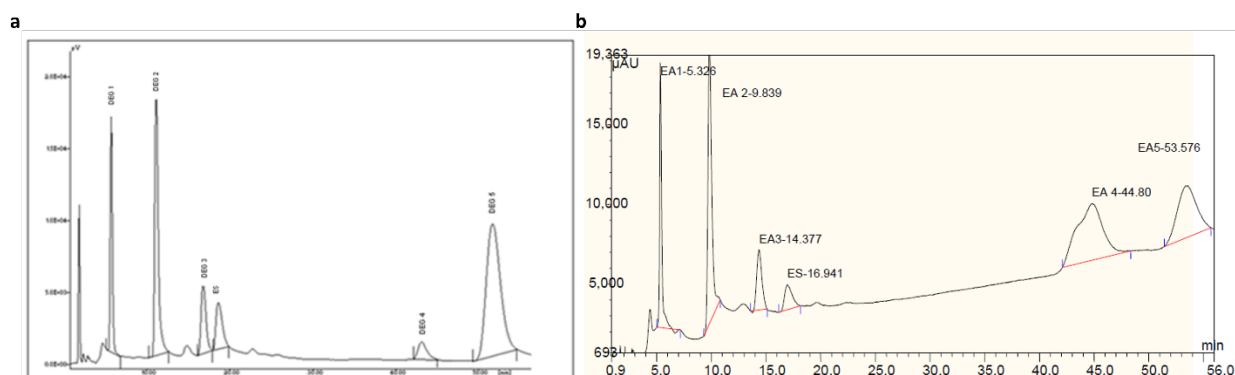
Parameter	Esomeprazole Trihydrate	Magnesium	Itopride Hydrochloride	Acceptance Limits	Threshold
Linear Dynamic Range	50 to 750 $\mu\text{g/mL}$		100 to 500 $\mu\text{g/mL}$	$r^2 \geq 0.998$	
Working Linearity Range	50 to 250 $\mu\text{g/mL}$		100 to 500 $\mu\text{g/mL}$	Operational assay range	
Regression Equation	$y = 25.67x + 2076$		$y = 8594x$	Visual linearity verification	
Coefficient of Determination (r^2)	0.999		0.998	$r^2 \geq 0.998$	
Limit of Detection (LOD)	25.0 $\mu\text{g/mL}$		0.5 $\mu\text{g/mL}$	Signal-to-noise ratio $\geq 3:1$	
Limit of Quantitation (LOQ)	50.0 $\mu\text{g/mL}$		1.0 $\mu\text{g/mL}$	Signal-to-noise ratio $\geq 10:1$	
Repeatability (Peak Area % RSD)	0.35%		0.002%	%RSD $\leq 1.0\%$	
Repeatability (t_R % RSD)	0.39%		0.002%	%RSD $\leq 1.0\%$	
Intra-Day Precision (% RSD)	0.85%		0.054%	%RSD $\leq 2.0\%$	
Inter-Day Precision (% RSD)	0.76% to 0.99%		0.027% to 0.091%	%RSD $\leq 2.0\%$	
Mean Formulation Recovery	99.15%		99.98%	Mean recovery 98.0% to 102.0%	
Dosage Form Content Assay	99.46 $\pm$ 0.35%		99.92 $\pm$ 0.05%	90.0% to 110.0% label claim	
Theoretical Plate Count (N)	4033.37		4821.50	$N > 3000$	
Peak Asymmetry / Tailing (T)	1.83		1.64	$T < 2.0$	
Resolution (R_s , Right-hand side)	10.25		3.20	$R_s \geq 1.5$	
Resolution (R_s , Left-hand side)	2.61		3.87	$R_s \geq 1.5$	

3.3. Forced Degradation Studies

3.3.1. Stress Degradation Behavior of Esomeprazole Magnesium

Esomeprazole magnesium degraded extensively across hydrolytic, oxidative, and photolytic conditions, while remaining stable against solid-state dry heat and climatic humidity.

Acid-induced stress (1 N HCl, 80°C, 1 h) produced 76.59% substrate decomposition, resolving into five degradation peaks designated EA1 through EA5. Peaks EA1 (t_R =5.33 min), EA2 (t_R =9.84 min), EA4 (t_R =44.80 min), and EA5 (t_R =53.58 min) constituted major degradation products, while EA3 (t_R =14.38 min) appeared as a minor degradant eluting immediately ahead of parent esomeprazole (t_R =16.94 min).

**Figure 4. Chromatograms of acid hydrolysis of ES a. HPLC and B. PDA**

Under alkaline conditions (1 N NaOH, 80°C, 1 h), esomeprazole underwent 70.78% degradation, generating two major peaks, EB1 (t_R = 9.79 min) and EB2 (t_R =44.74 min), alongside a minor peak EB3 (t_R =53.12 min). Neutral hydrolysis under reflux at 80°C for 1 h resulted in 73.07% loss of parent drug, yielding four degradants: EN1 (t_R =9.61 min, major), EN2 (t_R =13.97 min, minor), EN3 (t_R =19.34 min, minor), and EN4 (t_R =42.85 min, major).

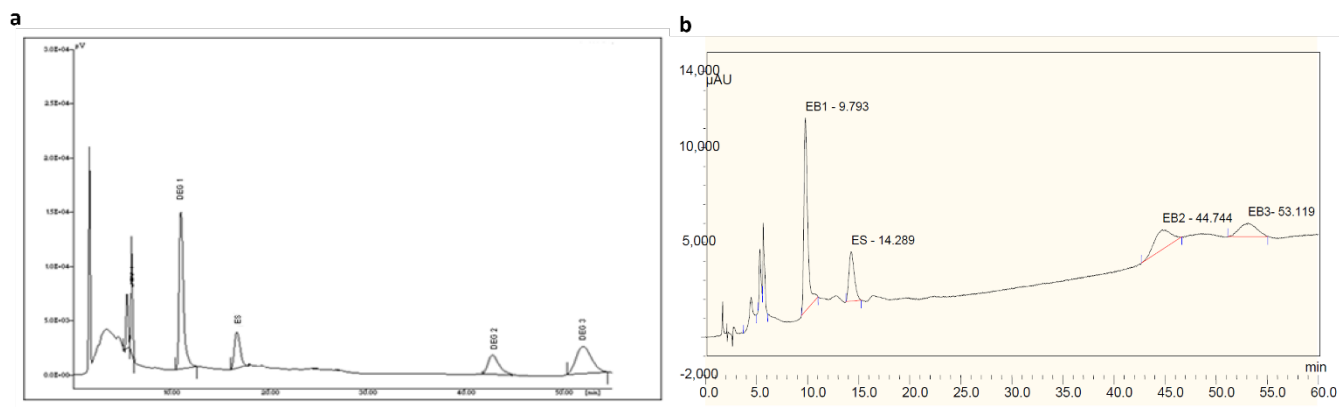


Figure 5. Chromatograms of Alkaline Hydrolysis of ES a. HPLC and PDA

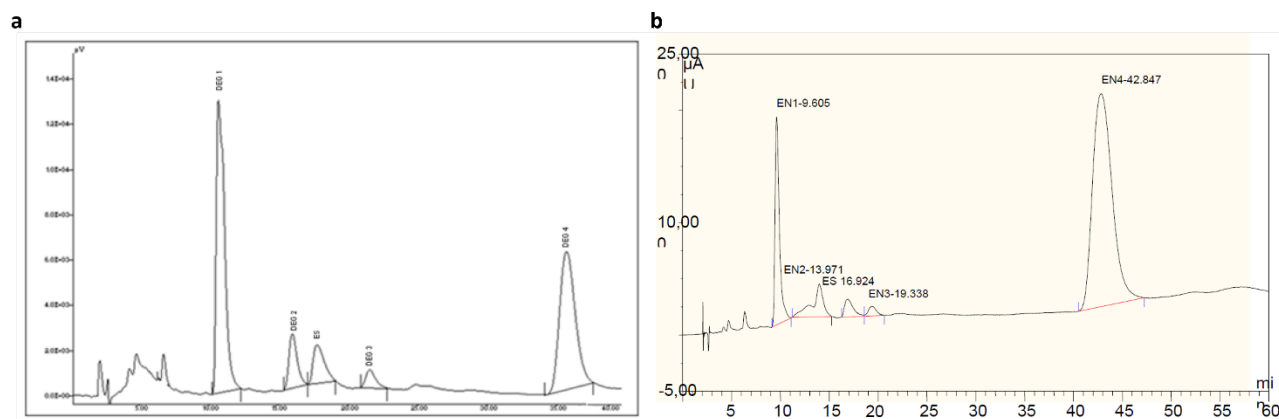


Figure 6. Chromatograms of Neutral Hydrolysis of ES a. HPLC and PDA

Oxidative challenge with 6% v/v H_2O_2 resulted in 79.84% degradation, forming three major degradation products: EOX1 ($t_R = 4.63$ min), EOX2 ($t_R = 9.60$ min), and EOX3 ($t_R = 52.19$ min). Escalating peroxide strength to 30% v/v H_2O_2 caused 65.26% degradation, yielding two major products (EOX1 at 9.60 min, EOX3 at 44.96 min) and two minor products (EOX2 at 12.92 min, EOX4 at 52.42 min).

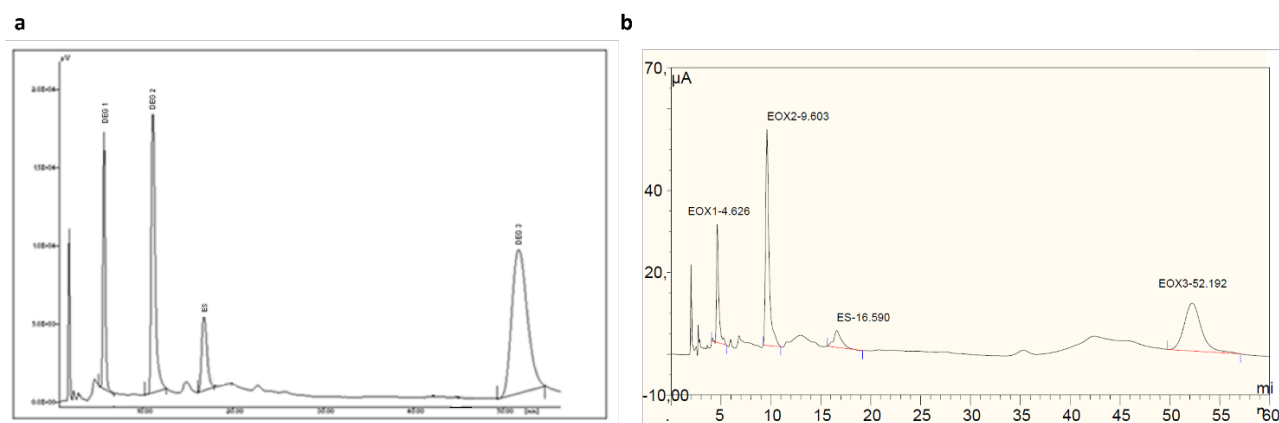


Figure 7. Chromatograms of Peroxide Oxidation of ES

Solution photolysis under natural sunlight for 128 h generated 71.74% degradation with six distinct products: EP1 ($t_R = 9.52$ min), EP2 ($t_R = 11.45$ min), EP3 ($t_R = 13.93$ min), EP4 ($t_R = 25.76$ min), EP5 ($t_R = 34.38$ min), and EP6 ($t_R = 42.26$ min). In contrast, exposure of crystalline esomeprazole to dry heat at 105°C for 5 h and exposure to $40^\circ\text{C}/75\%$ RH over 3 months yielded no detectable chromatographic decomposition.

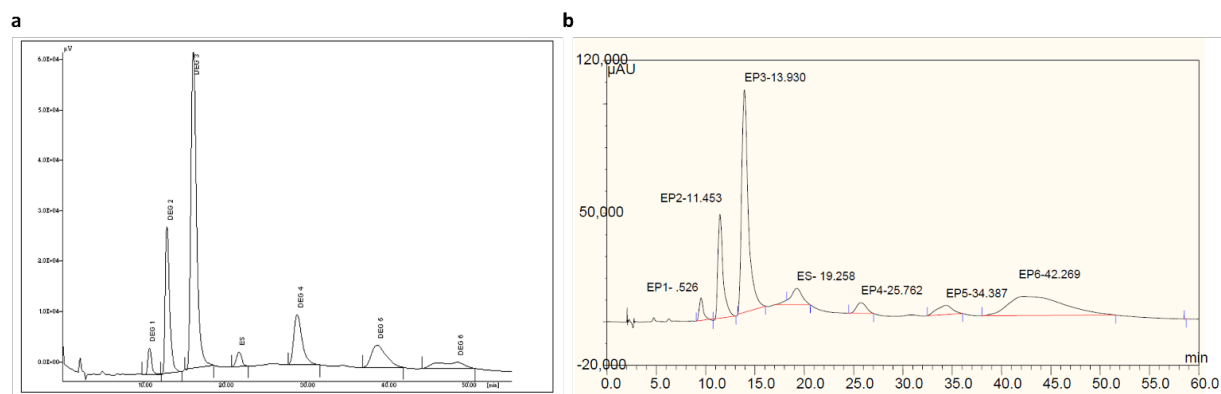


Figure 8. Chromatograms of Photolytic Degradation of ES a. HPLC and b. PDA

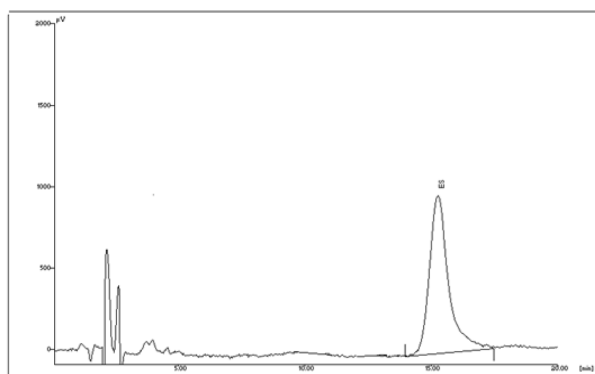


Figure 9. HPLC Chromatogram of Thermal Degradation of ES

3.3.2. Characterization of Degradation Products of Esomeprazole

Photodiode array spectral matching revealed that several degradants eluting in close proximity share identical ultraviolet absorption profiles. Specifically, degradants EA2, EN1, EOX2, EP1, and EB1 ($t_R \approx 9.6$ to 9.8 min) displayed superimposable UV spectra ($\lambda_{max} \approx 202,290.5$ nm), indicating that this peak represents a common primary solvolytic core degradant.

Table 7. Summary of Forced Degradation Behavior and Products for Esomeprazole Magnesium Trihydrate

Stress	Degradation Condition	Number of Degradants (Major)	Number of Degradants (Minor)	Total Degradants Observed	Substrate Degradation (%)
Acid Hydrolysis	1 N HCl, reflux at 80°C for 1 h	04	01	05	76.59%
Alkaline Hydrolysis	1 N NaOH, reflux at 80°C for 1 h	01	02	03	70.78%
Neutral Hydrolysis	Distilled water, reflux at 80°C for 1 h	02	02	04	73.07%
Oxidative Stress	6% v/v H ₂ O ₂ , dark, room temperature for 1 h	03	00	03	79.84%
Oxidative Stress	30% v/v H ₂ O ₂ , dark, room temperature for 1 h	02	02	04	65.26%
Photolysis	Natural sunlight, 8 h/day for 16 days (128 h total)	04	02	06	71.74%
Thermal (Dry Heat)	Hot air oven, 105°C for 5 h	00	00	00	Stable degradation (No degradation)
Accelerated Stability	40°C ± 2°C/75% ± 5% RH for 3 months	00	00	00	Stable degradation (No degradation)

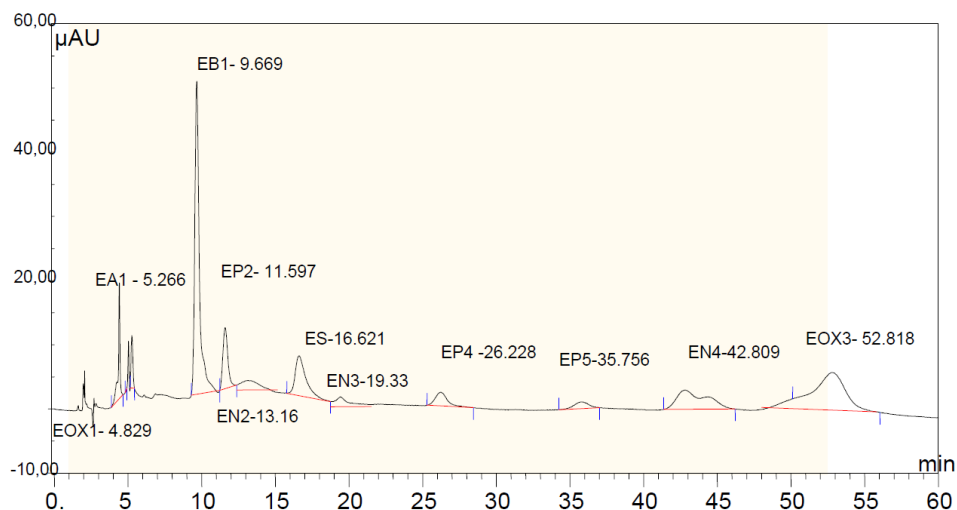


Figure 10. PDA chromatogram of combined degradants of ES

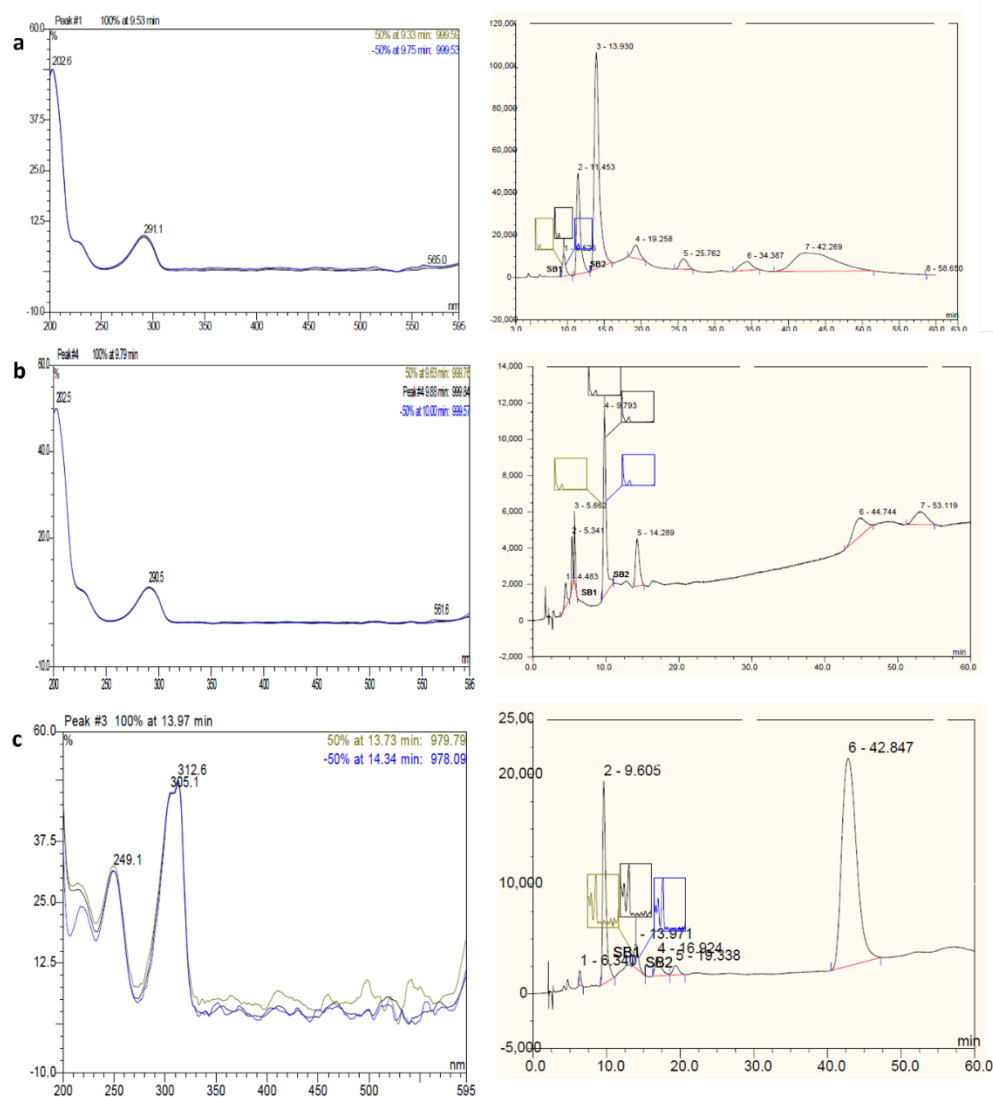


Figure 11. UV Spectra (left) and PDA (right) Chromatograms of ES Degradants a. EP1, b. EB1, and c. EN2

Similarly, the minor products EN2 (13.97 min) and EP3 (13.93 min) displayed shared spectral characteristics ($\lambda_{\text{max}} \approx 249, 305, 312 \text{ nm}$). Degradants EA5, EB3, and EO3 eluting past 52 min also showed identical absorbance spectra ($\lambda_{\text{max}} \approx 203, 291 \text{ nm}$), confirming formation of late-eluting hydrophobic polymeric or dimeric degradants across these distinct chemical environments.

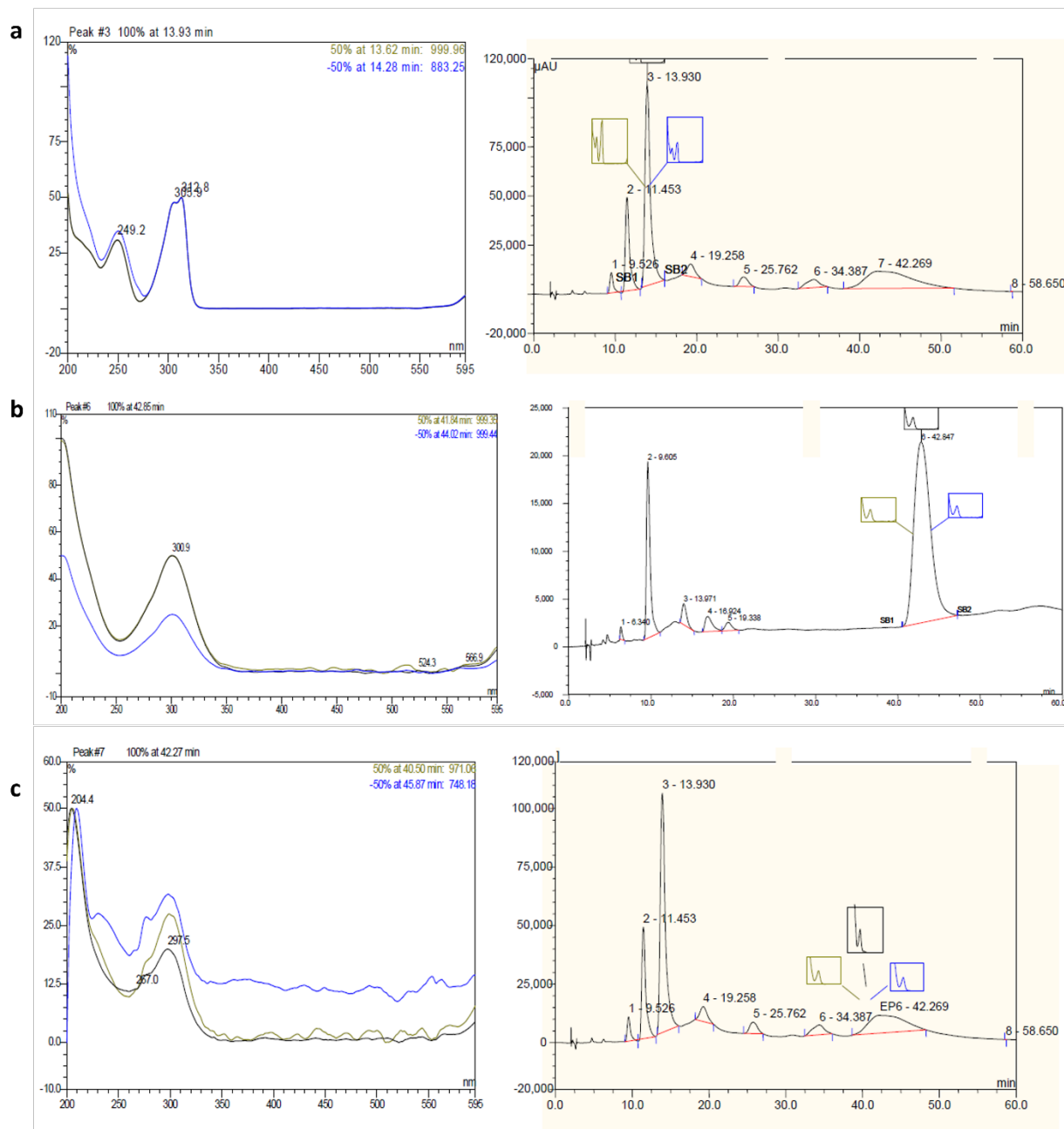


Figure 12. UV Spectra (left) and PDA (right) Chromatograms of ES Degradants a. EP3, b. EN4, and c. EP6

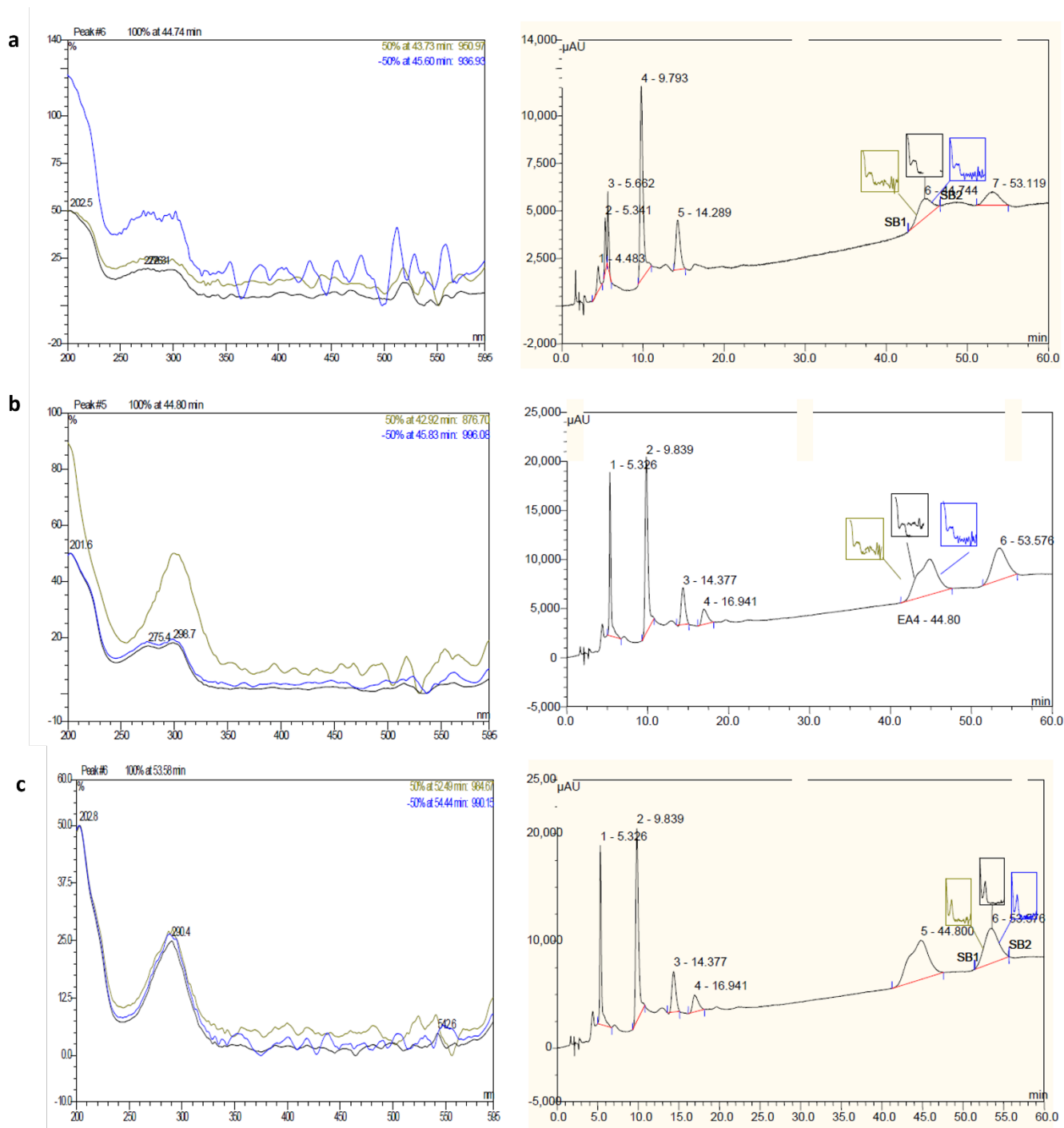


Figure 13. UV Spectra (left) and PDA (right) Chromatograms of ES Degradants a. EB2, b. EA4, and c. EA5

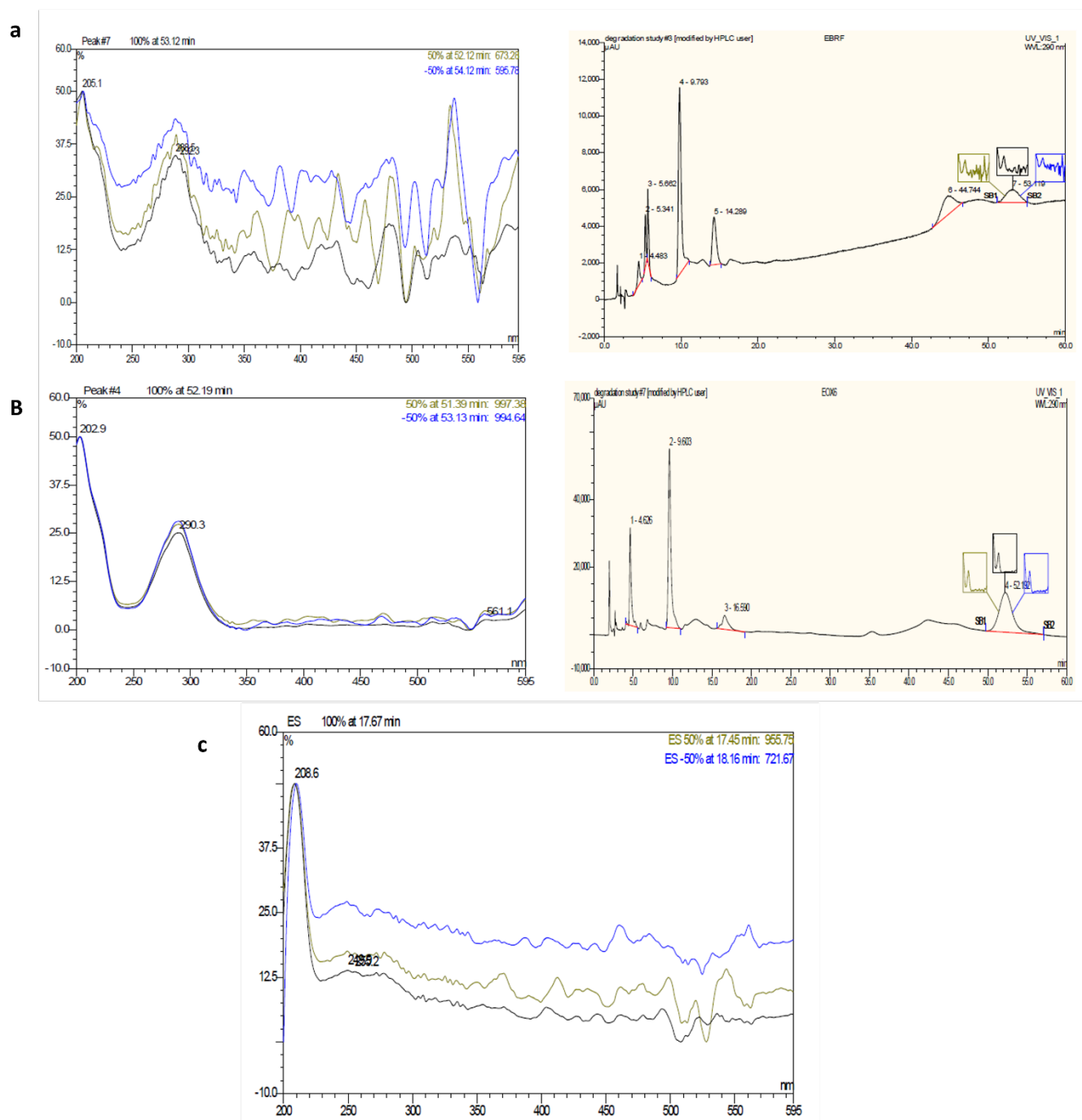


Figure 14. UV Spectra (left) and PDA (right) Chromatograms of ES Degradants a. EB3, b. EOX3, and c. UVspectra for ES

3.3.3. Stress Degradation Behavior of Itopride Hydrochloride

Itopride hydrochloride exhibited stability against neutral hydrolysis, peroxide oxidation, direct sunlight photolysis, dry heat, and climatic storage. Exposure to 6% and 30% v/v H_2O_2 for 1 h yielded no discernible degradation peaks, with the itopride peak area remaining constant (99.98% recovery). Prolonged photolytic exposure to natural sunlight for 360 h in methanolic solution induced no photolytic cleavage (99.97% recovery). Solid-state exposure to 80°C for 6 h and 40°C/75% RH over 3 months confirmed full chemical stability.

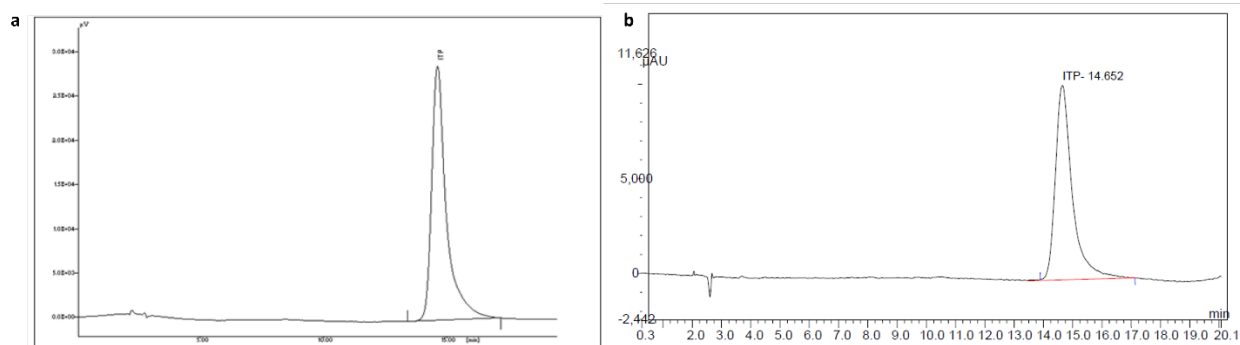


Figure 15. HPLC and PDA Standard Chromatograms of ITP

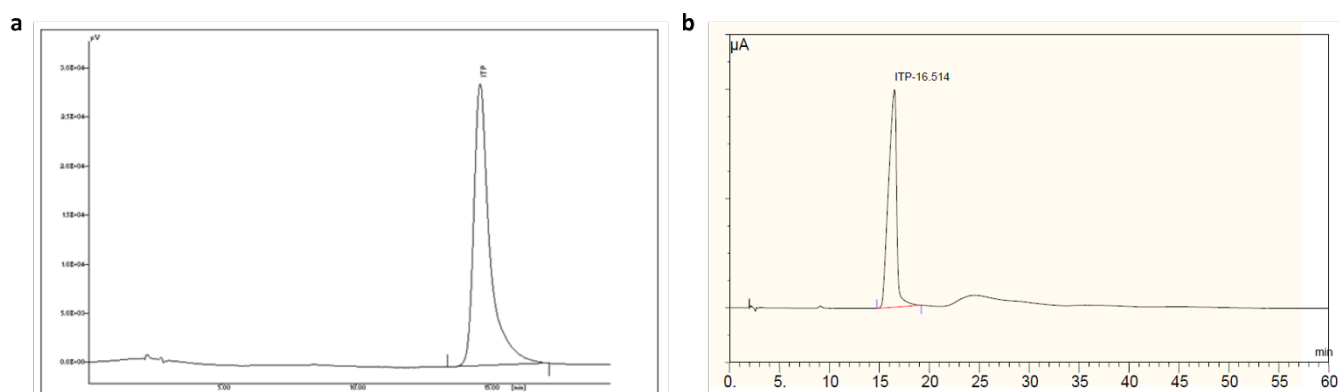


Figure 16. Chromatograms of Photolytic Degradation Studies of ITP a. HPLC and b. PDA

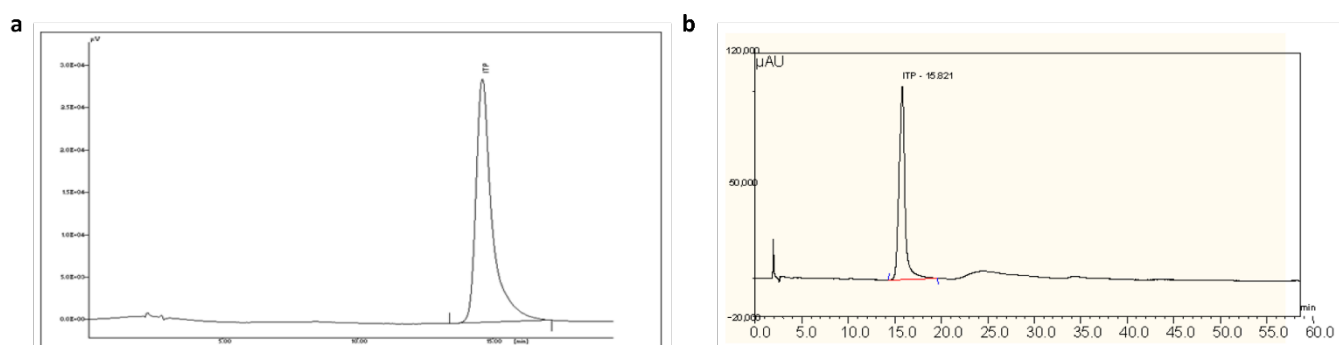


Figure 17. Chromatograms of Oxidative Degradation Studies of ITP a. HPLC and b. PDA

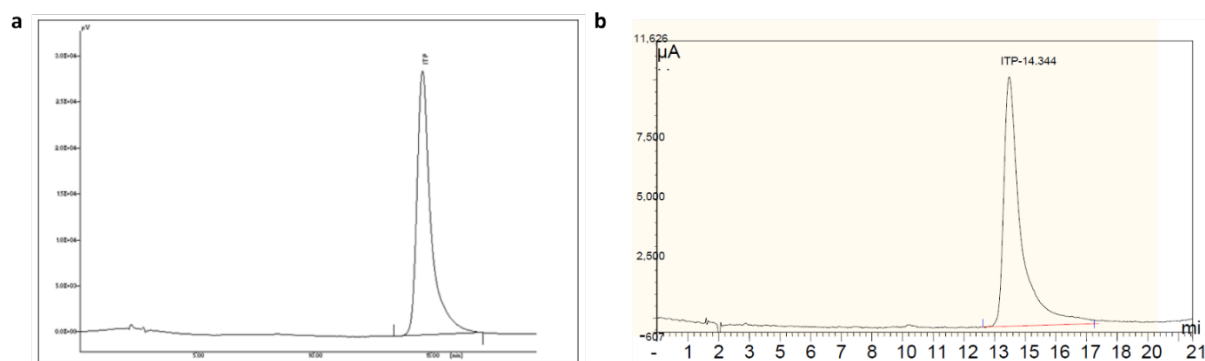


Figure 18. Chromatograms of Thermal Degradation Studies of ITP a. HPLC and b. PDA

Decomposition of itopride hydrochloride occurred under aggressive hydrolytic conditions. Digestion with 3 N HCl at 70°C for 30 min resulted in 88.0% degradation, generating a single major degradation peak designated IA ($t_R = 5.73$ min). Refluxing with 3 N NaOH at 70°C for 30 min resulted in 94.0% degradation, forming a major basic degradant designated IB ($t_R = 5.88$ min).

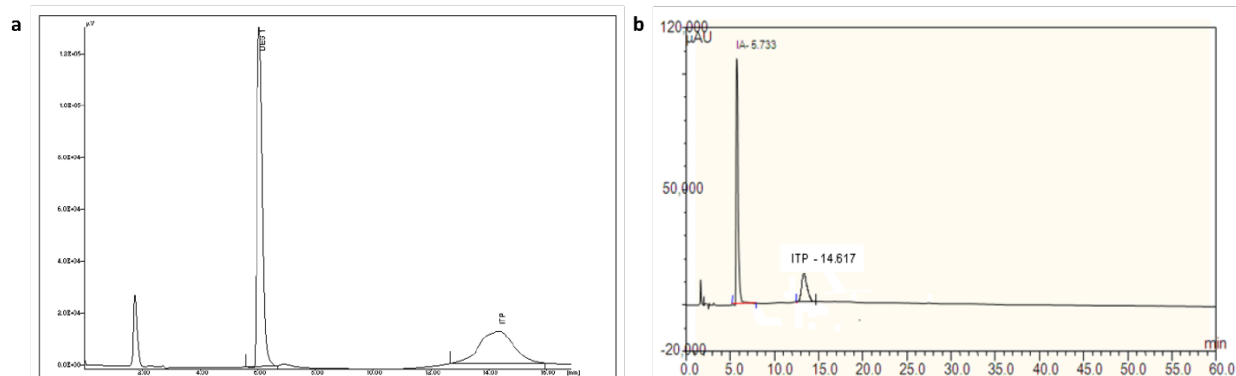


Figure 19. Chromatograms of Acid Degradation Studies of ITP a. HPLC and b. PDA

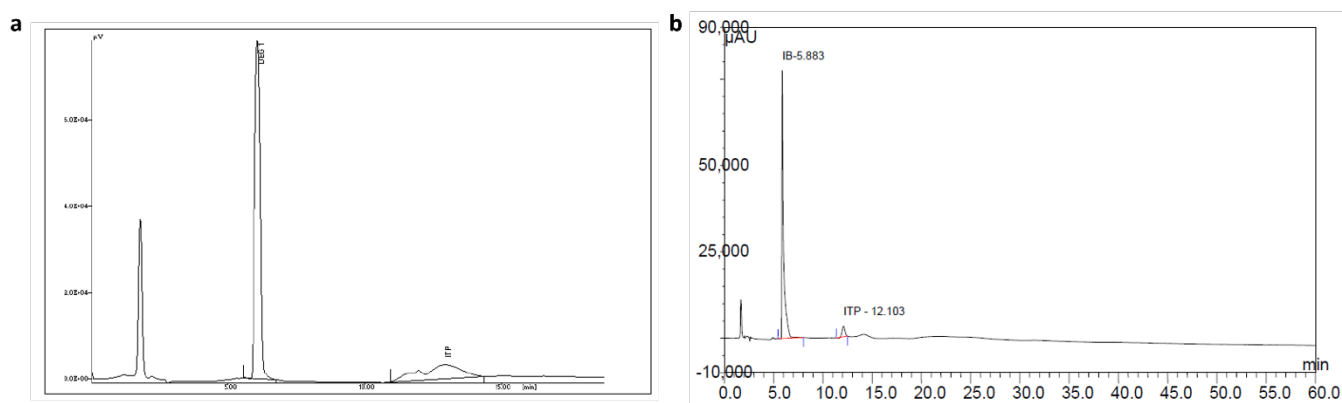


Figure 20. Chromatograms of Alkaline Degradation Studies of ITP a. HPLC and b. PDA

Table 8. Forced Degradation Behavior and Products for Itopride Hydrochloride

Stress Category	Degradation Condition	Number of Degradants (Major)	Substrate Degradation (%)
Acid Hydrolysis	Reflux with 3 N HCl at 70°C for 30 min	01	88%
Alkaline Hydrolysis	Reflux with 3 N NaOH at 70°C for 30 min	01	94%
Neutral Hydrolysis	Distilled water, reflux at 80°C for 1 h	00	Stable (No degradation)
Oxidative Stress	6% v/v H ₂ O ₂ , dark, room temperature for 1 h	00	Stable (No degradation)
Oxidative Stress	30% v/v H ₂ O ₂ , dark, room temperature for 1 h	00	Stable (No degradation)
Photolysis	Methanolic solution exposed to sunlight for 360 h	00	Stable (No degradation)
Thermal (Dry Heat)	Standard drug placed in an oven at 80°C for 6 h	00	Stable (No degradation)
Accelerated Stability	40°C ± 2°C/75% ± 5% RH for 3 months	00	Stable (No degradation)

When solutions of IA and IB were co-injected, their retention times overlapped into a single chromatographic zone eluting between 5.6 and 5.9 min. However, second-derivative UV spectral analysis obtained by photodiode array detection resolved subtle spectral divergences between the amide-cleavage products generated under respective hydronium and hydroxide catalysis.

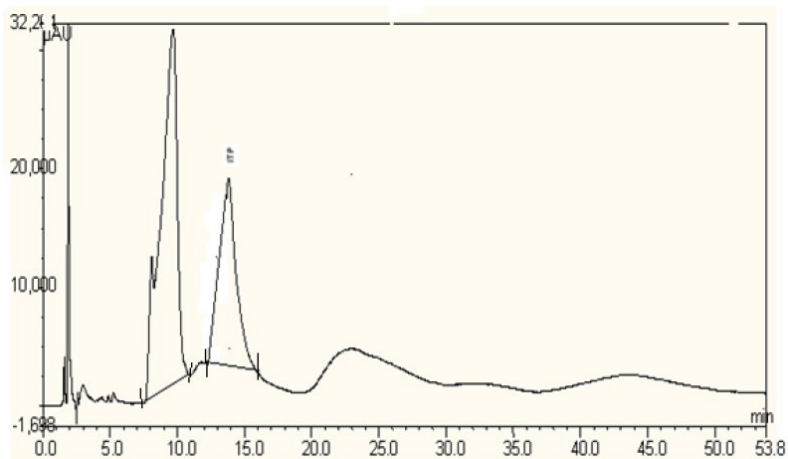


Figure 21. HPLC Chromatogram of ITP with Co-injected with Acid and Alkaline Degradant

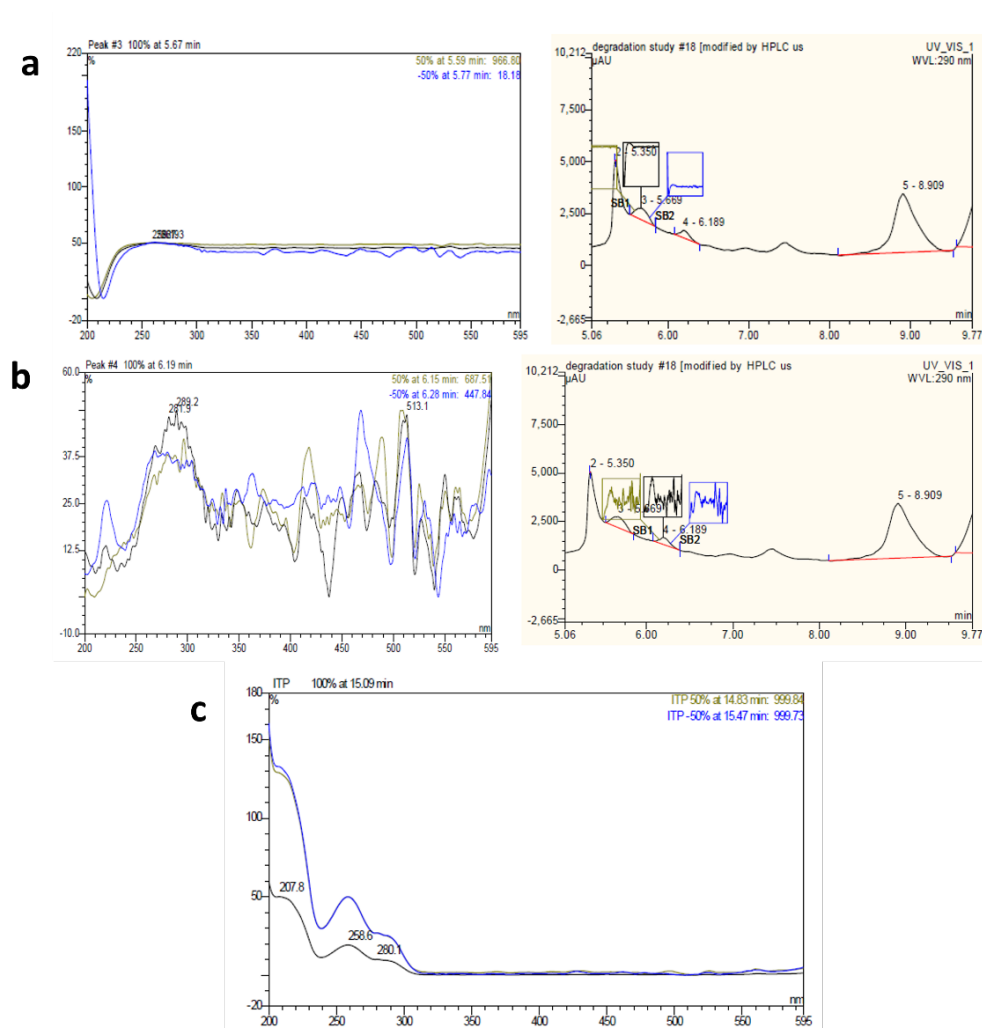


Figure 22. UV spectra and PDA chromatograms of Itopride Degradants IA, IB, and Parent

3.3.4. Specificity in Stressed Combined Mixtures

Method specificity in the presence of degradants was validated by spiking neutralized degradation mixtures of both drugs and analyzing the composite mixture. The optimized isocratic mobile phase resolved all eleven degradation products, itopride hydrochloride, and esomeprazole magnesium trihydrate. Baseline resolution was maintained between itopride and its adjacent peaks ($R_s=3.20$ on the leading edge and $R_s=3.87$ on the trailing edge), as well as for esomeprazole relative to its adjacent peaks ($R_s=2.61$ on the leading edge and $R_s=10.25$ on the trailing edge).

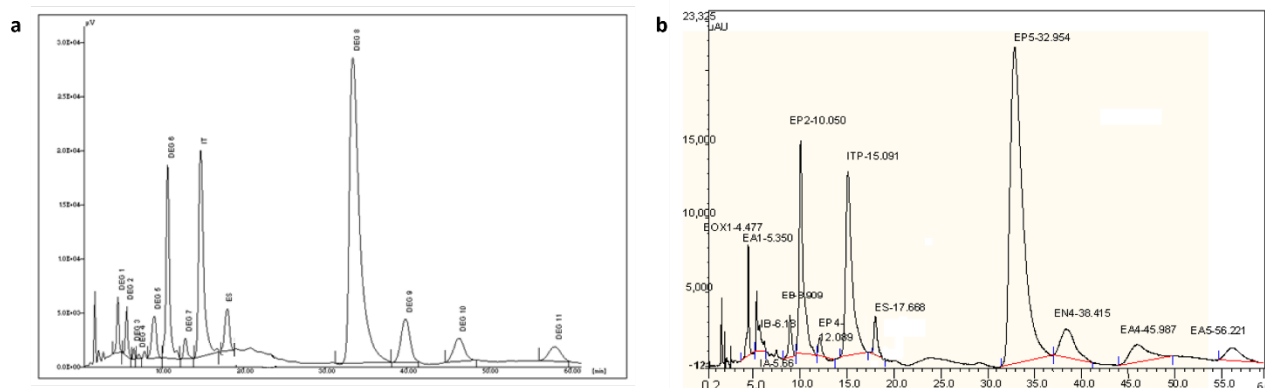


Figure 23. Resolved Combined Degradants of ES and ITP a. HPLC and b. PDA

Photodiode array spectral deconvolution across the upslope, apex, and downslope inflection points yielded peak purity match factors of 100.0% for parent esomeprazole and 99.70% for parent itopride, confirming peak homogeneity without co-eluting degradant interference. The chromatographic retention properties and spectral purity indices across the combined stress mixtures are consolidated in Table 9.

Table 9. Retention times, Peak Identification, and Spectral Purity for Combined Degradation Products of Esomeprazole Magnesium Trihydrate and Itopride Hydrochloride

Peak Designation	Retention Time (tR, min)	Source Degradation Condition	Chemical Classification	Peak Purity Index (%)
Deg 1	4.47	Oxidative Stress (6% and 30% H ₂ O ₂)	EOX1 (Major)	95.20
Deg 2	5.35	Acid Hydrolysis (1 N HCl)	EA1 (Major)	69.50
Deg 3	5.66	Acidic Hydrolysis of ITP (3 N HCl)	IA (Major)	97.10
Deg 4	6.18	Alkaline Hydrolysis of ITP (3 N NaOH)	IB (Major)	19.60
Deg 5	8.90	Photolytic Stress / Alkaline ES	EP1 / EB1 (Minor/Major)	96.00
Deg 6	10.05	Photolytic / Peroxide / Hydrolytic ES	EP2 / EA2 / EN1 / EOX2	95.20
Deg 7	12.08	Photolytic Degradation of ES	EP4 (Major)	88.20
Itopride HCl (ITP)	15.09	Intact Active Drug	Parent Prokinetic API	99.70
Esomeprazole Magnesium Trihydrate (ES)	17.67	Intact Active Drug	Parent PPI API	100.00
Deg 8	32.95	Photolytic Stress of ES	EP5 (Minor)	93.60
Deg 9	38.41	Neutral Hydrolysis of ES	EN4 (Major)	91.60
Deg 10	45.98	Acid / Base / Photolytic ES	EA4 / EB2 / EP6	83.60
Deg 11	56.22	Acid / Base / Peroxide ES	EA5 / EB3 / EOX3	79.10

4. Conclusion

A stability-indicating reversed-phase high-performance liquid chromatographic method was established for the simultaneous determination of esomeprazole magnesium trihydrate and itopride hydrochloride in the presence of their stress-induced degradation products. Forced degradation profiling clarified the chemical stability characteristics of both active entities: esomeprazole magnesium trihydrate was susceptible to hydrolytic, oxidative, and photolytic breakdown, whereas itopride hydrochloride was stable across all conditions except concentrated mineral acid and alkali digestion under elevated temperatures. Both compounds proved stable under solid-state dry heat and climatic humidity stress. Incorporation of tetrabutylammonium hydrogen sulphate as an ion-pairing and silanol-masking agent resolved chromatographic challenges arising from the disparate polarities and ionization profiles of the two analytes. Under isocratic conditions on a C18 stationary phase, the parent drugs were baseline-resolved from each other and from all eleven degradation products generated during stress testing. Photodiode array spectral purity analyses confirmed peak homogeneity for both active analytes without co-eluting degradant interference. The method complied with validation criteria for selectivity, linearity, sensitivity, precision, and accuracy across commercial capsule matrices, providing a validated analytical method for routine commercial batch release and regulatory stability monitoring of fixed-dose combination formulations.

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