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Extensive Study of Aspirin and Its Related Impurities Under Various Stressed Conditions in Low Dose Aspirin and Esomeprazole Magnesium Capsules

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ABSTRACT

The objective of the present research work is to develop a isocratic reversed-phase liquid chromatographic (RP-HPLC) method for the determination of Aspirin in pharmaceutical pharmaceutical dosage forms for its related impurities in presence of esomeprazole. The chromatographic separation was achieved on a RP 18 column (100mm×4.6mm, 5 µm). The isocratic LC method employs mixture of buffer methanol and isopropyl alcohol in the ratio of (84:13:3 v/v) solutions as mobile phase. The buffer solution contains 6.8g of Potassium dihydrogen orthophosphate adjusted to pH 2.5 with orthophosphoric acid .The flow rate was 1.5 ml/min and the detection wavelength was 275 nm. In the developed HPLC method, the resolution between Aspirin and its potential impurity salicylic acid was found to be greater than 4.0. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation in presence of esomeprazole. Considerable degradation was found to occur in basic medium and mild degradation observed in acid hydrolysis stress conditions. Degradation product formed during acidic hydrolysis was salicylic acid. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed RP-HPLC method was validated with respect to linearity, accuracy, precision and robustness.

Keywords: RP-HPLC; Forced degradation; Validation; Aspirin, Salicylic acid Method development

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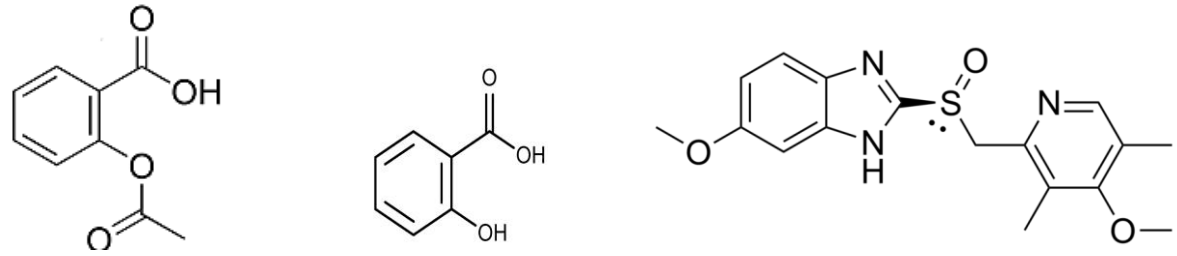
INTRODUCTION

Aspirin (ASP), also known as acetylsalicylic acid, is a salicylate drug, often used (Figure. 1) as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory Medication. Esomeprazole is a proton pump inhibitor which reduces acid secretion through inhibition of ATPase in gastric parietal cells[1]. By inhibiting the functioning of this enzyme, the drug prevents formation of gastric acid.Aspirin, by irreversibly acetylating cyclo-oxygenase (COX), reduces the production of thromboxane A2 (TXA2) in platelets and prevents platelet aggregation[2]. Aspirin can also reduce prostacyclin (PGI2) production in endothelial cells and cause vasoconstriction. One of the side-effects associated with this use of aspirin is gastrointestinal ulcers. Aspirin has a long history of therapeutic use, not only for its analgesic, antipyretic and anti-inflammatory properties but also for its anti-thrombotic properties, which are of value in states of platelet hyperaggregability. Aspirin binds irreversibly to the enzyme cyclo-oxygenase-1 (COX-1) in platelets, leading to its antiplatelet effect[3]. Side effects of aspirin treatment are mainly dyspeptic symptoms, gastrointestinal (GI) lesions and increased gastrointestinal and overall bleeding, which are consequences of the blockage of prostaglandin synthesis through inhibition of various COX enzymes. This leads to a decrease in mucosal protection, which in turn predisposes the patient to mucosal lesions such as peptic ulcers and peptic ulcer bleeding. Esomeprazole is a proton pump inhibitor (PPI) which is indicated, amongst other indications, for the prevention of gastric and duodenal ulcers associated with NSAID therapy (including aspirin therapy). There are very many drug products containing aspirin 100 mg strength enteric coated tablets. The latter are the only low-dose aspirin monotherapy drug products apart from breaking a 300 mg tablet in half which is probably done by a small proportion of patients taking low dose aspirin for cardiovascular protection [4-6].



Aspirin **Salicylic acid** **Esomeprazole**

Figure: 1: Chemical Structures of Aspirin, Salicylic acid and Esomeprazole

Combination of esomeprazole and aspirin assay/related impurities method was traditionally difficult due to their stability in aqueous solutions, dosage variations and their absorption

differences in the UV region. Literature reveals that aspirin is stable in acidic form where as esomeprazole stability was found in basic solutions. The objective of the present research work is to establish specificity and stability of aspirin in presence of esomeprazole and its related impurities which gives precise and accurate quantitation of aspirin in the pharmaceutical dosage forms of aspirin and esomeprazole. There were significant number of analytical methods for the determination of aspirin using HPLC[7-18], column switching chromatography [19] LC-MS/MS [20] and electrophoresis [21] have been reported for ASP in single form and in combination with other drugs. There were several reported methods in human plasma also reported including its stability in biological matrices. Recently Vijaya Bharathi D *et al* 2012, reported a new collection procedure in the research article Low dose aspirin estimation: an application to human pharmacokinetic study states that aspirin stability in acidic diluents rather than basic solutions due to its sensitivity toward hydrolysis in aqueous and also in biological matrices(Figure-2).

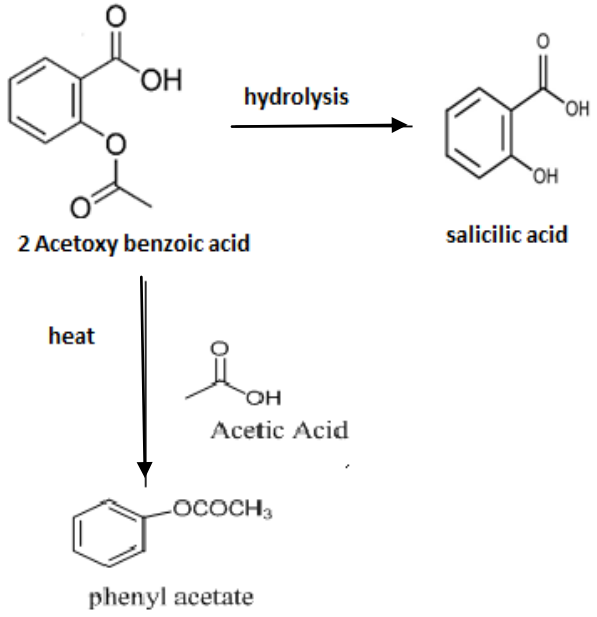


Figure: 2:Degradation Pathway of Aspirin

MATERIALS AND METHODS

Chemicals

Samples of aspirin, esomeprazole and its related impurities were obtained from Drreddys laboratories limited (Hyderabad, India) HPLC grade acetonitrile, analytical reagent grade potassium dihydrogen orthophosphate and ortho phosphoric acid, Isopropyl alcohol was purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurities used in this study were of greater than 95.0% purity.

Equipment

The HPLC system, used for method development, forced degradation studies and method validation was waters HPLC system equipped with a diode array detector, from Waters Corp. (Milford, MA, USA). The output signal was monitored and processes using Empower software (Waters) Water bath equipped with temperature controller was used to carry out degradation studies for all solution. Photo stability studies were carried out in a photo stability chamber and thermal stability studies were performed in a dry air oven (Mack Phar-matech, Hyderabad, India).

Chromatographic Conditions

The chromatographic column used was water XTerra RP C18 Column 100mm×4.6 mm, 5µm, all obtained from Waters Corp. (Milford, MA, USA). The isocratic LC method consists buffer: methanol: isopropyl alcohol in the ratio of (84:13:3 v/v) as mobile phase. The buffer solution contains 6.8gms of potassium dihydrogen orthophosphate pH adjusted to 2.5 with ortho phosphoric acid (Buffer).The flow rate of the mobile phase was 1.5 ml/min. The column temperature was maintained 50°C and the detection was monitored at a wavelength of 275nm. The injection volume was 20µl. [Buffer pH 2.5: Acetonitrile: Ethanol in the ratio of (50:30:20)] was used as a diluent.

Preparation of Solutions

A stock solution of aspirin and its related impurity salicylic acid (1 mg·mL–1) was pre-pared by dissolving appropriate amount in the diluent [Buffer pH 2.5: Acetonitrile: Ethanol in the ratio of (50:30:20)].

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities[23]. Stress testing of the drug impurities can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the developed LC method for aspirin was determined in the presence of esomeprazole and its related impurities. Forced degradation studies were also performed on aspirin to provide an indication of the stability indicating property and specificity of the proposed method. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), Dry heating done at 105° C for about 2 hrs., acid hydrolysis (Refluxed with 1N HCl solution for about 30 minutes at 60ºC), base hydrolysis (Refluxed with 1N NaOH solution for

about 30 minutes at 60ºC), water hydrolysis and oxidation (Treated with 30% Hydrogen peroxide (H_2O_2) for about 30 minutes at 60ºC). Sunlight, thermal and UV degradation was also performed and purity of stressed samples was checked by using Photo diode array detector (PDA). The purity factor is within the threshold limit obtained in all stressed samples demonstrates the analyte peak homogeneity. Specificity of the aspirin was shown by spiking all the esomeprazole and its related impurities the specification level (i.e. 0.15% of analyte concentration which is 1 mg/mL).

ANALYTICAL METHOD VALIDATION

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and system suitability [22-24].

Precision

The precision of test method was evaluated by analyzing six test samples of Low dose Aspirin and Esomeprazole magnesium capsules 325/40,325/20, 81/40 and 81/20 mg by spiking test preparation with Salicylic acid impurity at 3.0% and analyzed.

Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection and limit of quantification were established based on signal to noise ratio. A series of solutions having aspirin impurity were injected. Limit of detection for impurity was established by identifying the concentration which gives signal to noise ratio about 3. Limit of quantification was established by identifying the concentration which gives signal to noise ratio about 10.

Precision of aspirin impurity at about Limit of Quantification level was conducted. Six test preparations having impurities at the level of about Limit of quantification were prepared and injected into the HPLC system.

Linearity and Range

The linearity for aspirin and its impurity salicylic acid from Limit of quantification level to 200% of the target concentration (3.0 %). Linearity shall be established for Salicylic acid from Limit of quantification level to 200% of the target concentration (0. 2 %) target concentration of aspirin unknown salicylic acid impurity and injected into the HPLC system.

Accuracy

Accuracy of aspirin and aspirin impurities at about Limit of Quantification and at 150% of target concentration level was conducted. Test solutions spiked with Aspirin impurities at about Limit of Quantification and at 150% of target concentration were prepared in triplicate and injected into HPLC system

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (*Rs*) between aspirin and its impurity salicylic acid was evaluated. The flow rate of the mobile phase was 1.5 mL·min–1. To study the effect of flow rate on the developed method, 0.2 units of flow was changed (*i.e.* 1.3 and 1.7 mL·min–1). The effect of column temperature on the developed method was studied at 45°C and 55°C instead of 50°C. In the all above varied conditions, the components of the mobile phase were held constant.

Solution Stability and Mobile Phase Stability

The solution stability of aspirin and its related impurity salicylic acid were carried out by leaving both spiked sample and unspiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content impurity was determined at every 6 h interval, up to the study period. Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions, for every 6 h interval. Salicylic acid impurity was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

Method Development and Optimization

The main complexity of the present research work is to develop a stability indicating method for the estimation of aspirin in esomeprazole and aspirin pharmaceutical dosage forms in presence of esomeprazole. The main target of the chromatographic method is to get the separation of critical closely eluting degradable peaks of esomeprazole that can interfere with aspirin and its related impurities. Impurities were co-eluted by using different stationary phases like C18, Phenyl and cyano and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2–8) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. After several logical trails and optimization of stationary phase, column temperature and flow rate and mobile phase pH the chromatographic separation was achieved on a Waters X terra RP 18 Column 100mm×4.6 mm, 5 µm column, The isocratic LC method consists buffer: methanol and isopropyl alcohol in the ratio of (84:13:3 v/v) as mobile phase. The buffer solution contains 6.8 gms potassium dihydrogen orthophosphate in 1000 mL pH adjusted to 2.5 with Potassium hydroxide solution (Buffer).The flow rate of the mobile phase was 1.5 ml/min. The column temperature was maintained 50°C and the detection was monitored at a wavelength of 275 nm. The injection volume was 20µl. Mixture of [Buffer pH 2.5: Acetonitrile: Ethanol in the ratio of (50:30:20)] was used as a diluent. The concentration is 1.0 mg·mL–1 for

related impurities method. The peak shape of aspirin was found symmetrical. In the optimized conditions aspirin and salicylic acid impurity was well separated with a resolution of greater than 4.0 and the typical retention times of aspirin and its related impurity was3.72 and 6.02 min respectively. The system suitability results are given in Table 1 and the developed HPLC method was found to be specific for aspirin and its three impurity salicylic acid.Figure-3, Figure-4 and Figure-5 shows the chromatograms of Diluent, Impurity blend solution and test sample solution.

Table-1: System suitability Report

System suitability parameters	Observed value	*Acceptance limit*
From System suitability solution		
The resolution between Aspirin and Salicylic Acid peaks.	3.7	NLT 2.5
From Standard preparation		
% RSD for Aspirin and salicylic Acid Peak areas for six replicate injections.	2.6/0.5	NMT 10.0 %
The Tailing factor for Aspirin and salicylic Acid peaks.	1.1/1.0	NMT 2.0

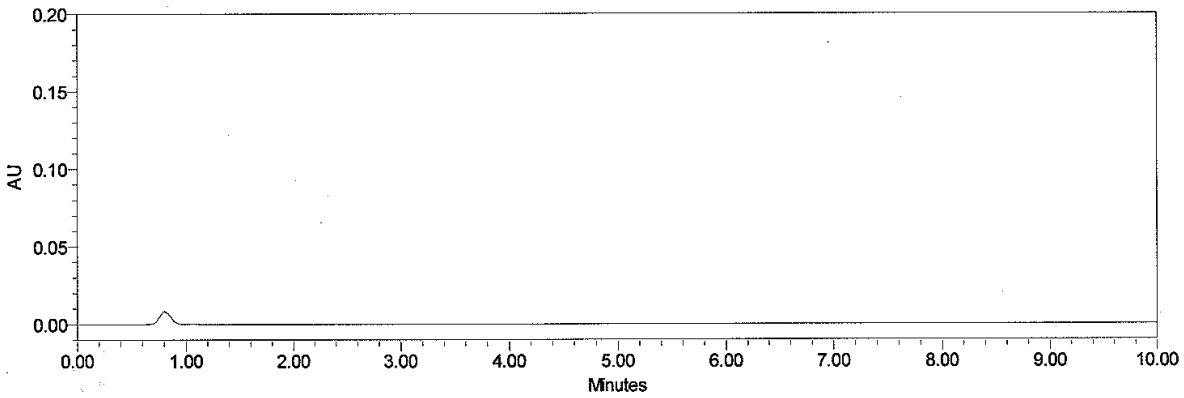


Figure:-3 Typical chromatogram of Diluent

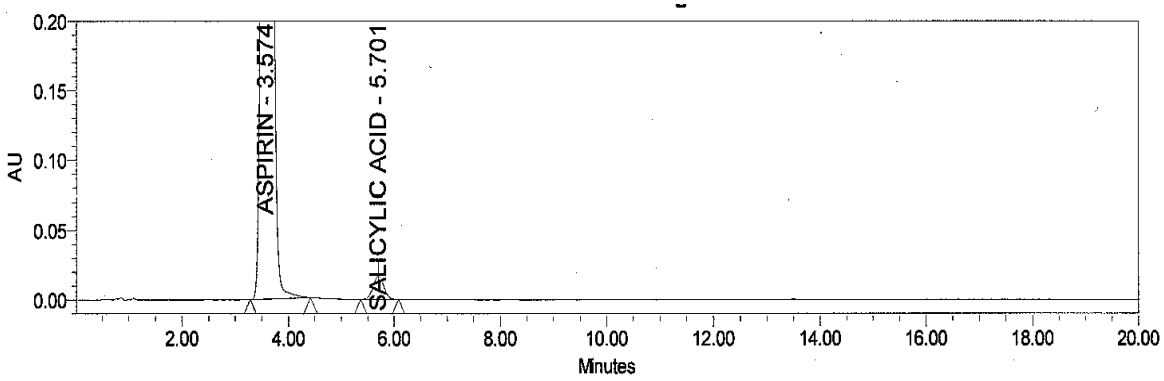


Figure:-4 Typical chromatogram of Impurities blend solution

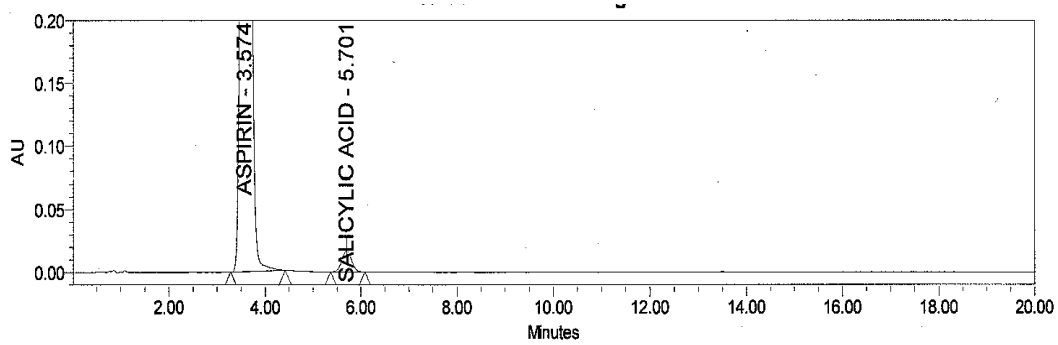


Figure:-5 Typical chromatogram of Sample

Results of Forced Degradation Studies

The drug was exposed to 1N HCl at 60°C for 30 min. aspirin has shown mild sensitivity towards the treatment of 1N HCl. The drug gradually undergone degradation with time in 1N HCl and prominent degradation was observed (~22%). The representative chromatogram present in Figure 6.

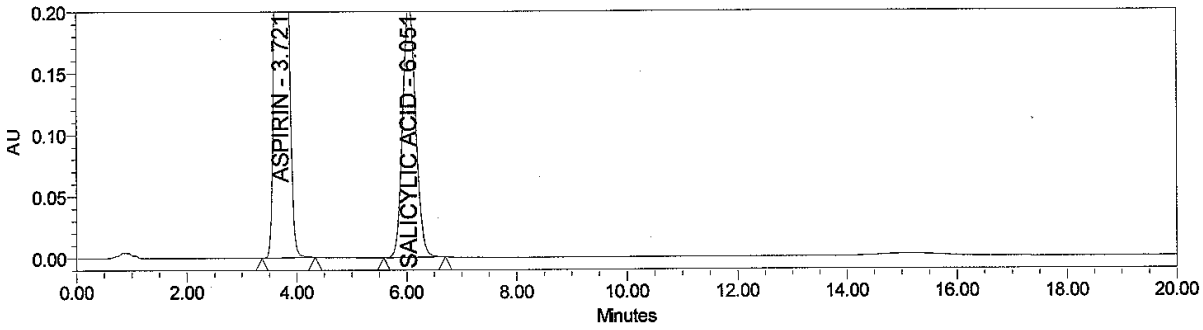


Figure 6: Typical chromatogram of Acid degradation

Degradation in Basic Solution

The drug was exposed to 1N NaOH at 60°C for 30 h. Aspirin has shown significant sensitivity towards the treatment of 1N NaOH. The drug gradually undergone degradation with time in 1N NaOH and degradation was observed (~8%). The representative chromatogram present in Figure7.

Oxidative Conditions

The drug was exposed to 30% hydrogen peroxide at room temperature for 60 min.Aspirin has shown no significant sensitivity towards the treatment of 30% hydrogen peroxide and the drug shown mild sensitivity in oxidative conditions.

Aspirin was stable under forced photo and thermal degradation. From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the Aspirin peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.5%. After exposing aspirin in sunlight(1.2 mn lux hours) and UV light(200 wt

hours per sq meter) 1.33 and 0.14 % degradation was observed. After dry heating at 105°C for 2hours and in the presence exposed humidit at 25°C /90%RH about 7 days 0.33 and 0.68 % degradation was observed. The forced degradation study results are given in **Table 2.** The representative chromatogram presents in Figure 7 to Figure 13.

Table-2: Summary of Forced degradation study report

Stress Condition	Drug Product			
	% Degradation	Purity angle	Purity threshold	Purity flag
Refluxed with 1N HCl solution for about 30 mins at 60ºC and neutralized with 1N NaOH	22.15	2.373	90.0	No
Refluxed with 1N NaOH solution for about 30 mins at 60ºC and neutralized with 1N HCl	8.79	2.983	90.0	No
Treated with 30% Hydrogen peroxide (H_2O_2) for about 30 minutes at 60ºC	4.07	3.161	90.0	No
Refluxed with purified water for about 5 hrs at 60ºC	2.73	0.277	0.661	No
Exposed to Sunlight for about 1.2 Million Lux hours.	1.33	0.380	0.746	No
Exposed to UV light both at shorter and longer wavelengths for about 200 watt hours / square meter.	0.14	0.295	0.640	No
Dry heating done at 105° C for about 2hrs.	0.68	0.280	0.58	No
Exposed to humidity at 25°C, 90% RH for about 7 days	0.33	3.524	90.0	No

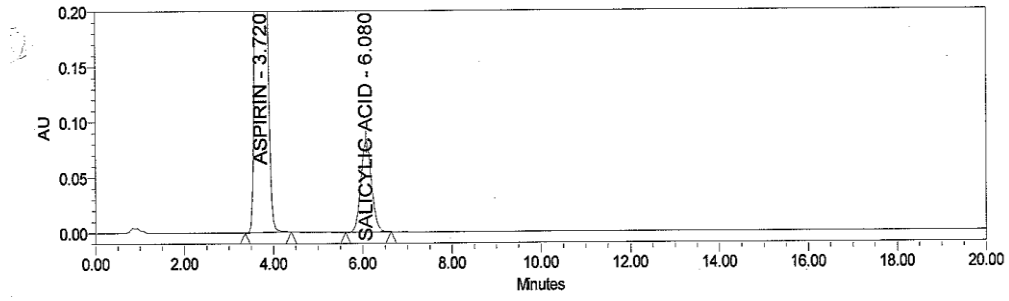


Figure 7: Typical chromatogram of Base degradation

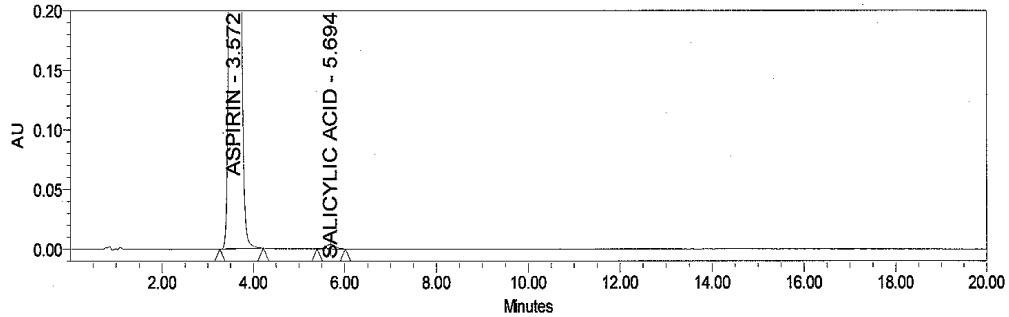


Figure 8: Typical chromatogram of oxidation degradation

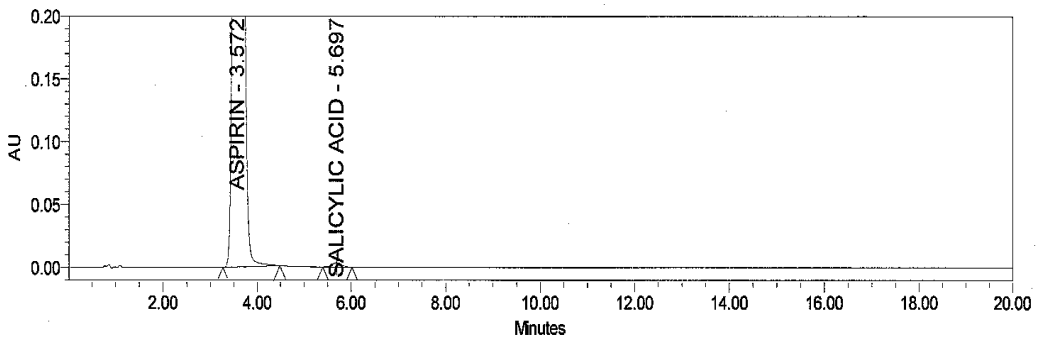


Figure 9: Typical chromatogram aqueous stressed preparation

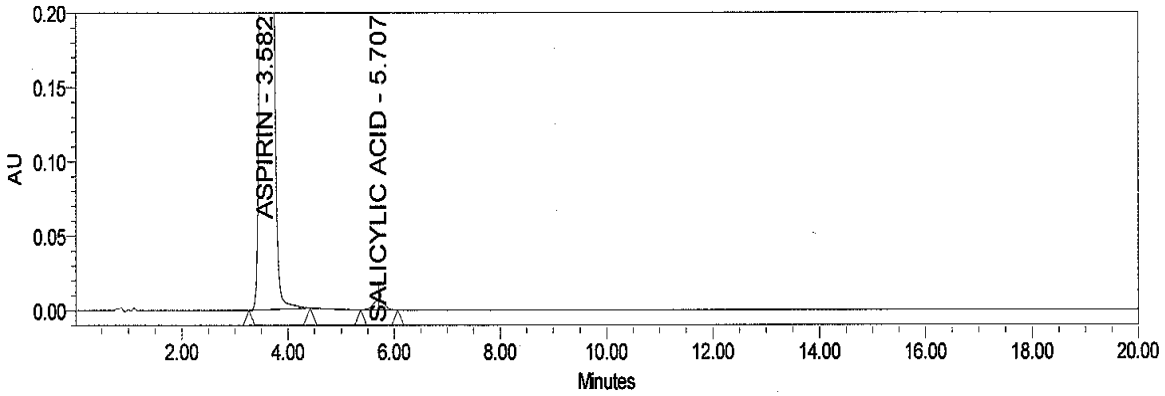


Figure 10: Typical chromatogram of Thermal degradation

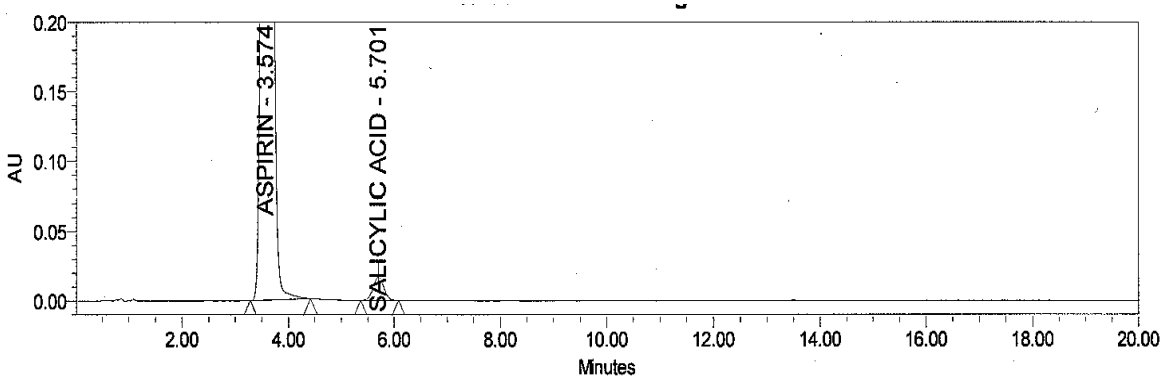


Figure 11: Typical chromatogram of sunlight stressed degradation

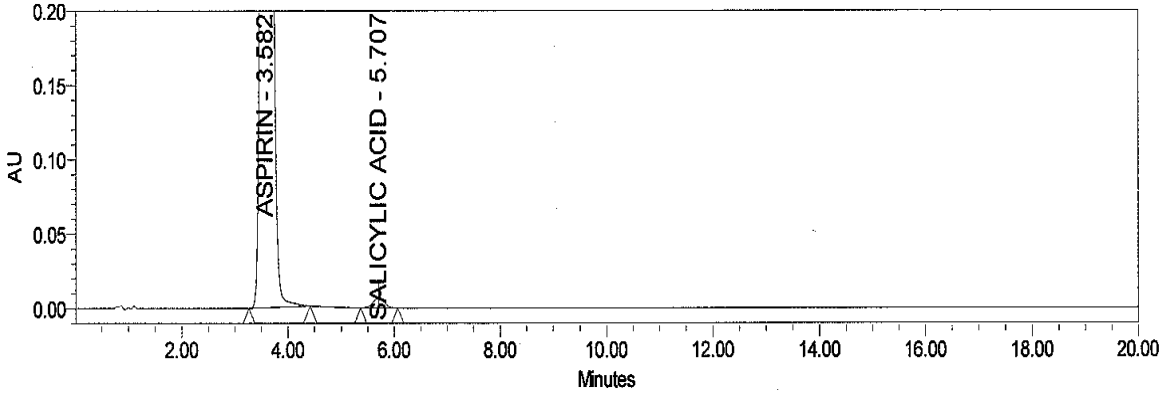


Figure 12: Typical chromatogram of dry heat stressed degradation

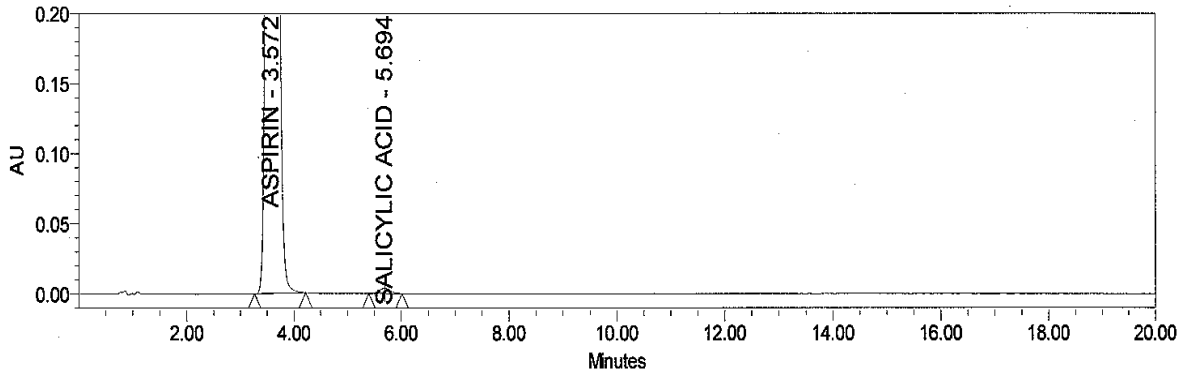


Figure 13: Typical chromatogram of UV Light stressed degradation

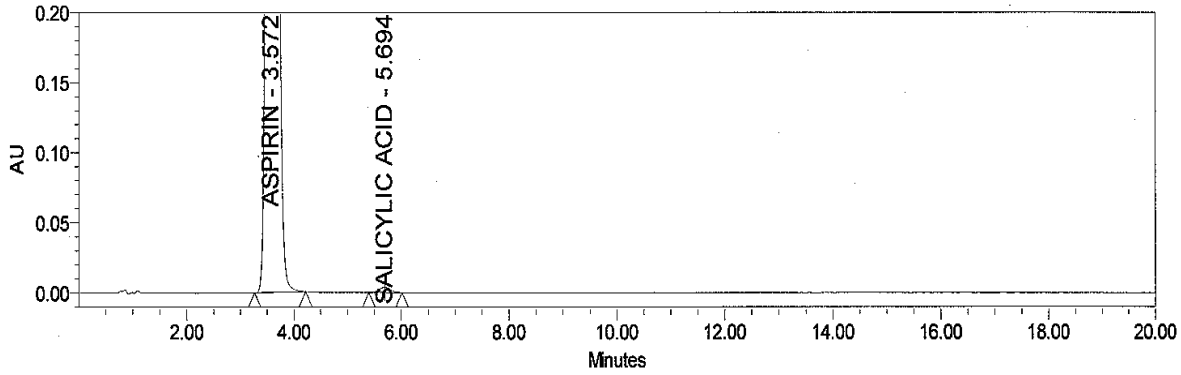


Figure 14: Typical chromatogram of humidity stressed degradation

Table 3: Precision and Accuracy Data of Salicylic acid

Sample No.	Spike level	'ppm' added	'ppm' found (recovered)	% Recovery	Mean % recovery
1.	20%	39.7256	38.5450	97.0	
2.	20%	39.7256	39.2600	98.8	
3.	20%	39.7256	38.6750	97.4	97.7
1.	50%	99.3141	104.2600	105.0	
2.	50%	99.3141	105.0400	105.8	
3.	50%	99.3141	104.9100	105.6	105.5
1.	100%	198.6281	204.7500	103.1	
2.	100%	198.6281	205.5300	103.5	
3.	100%	198.6281	205.1400	103.3	103.3
1.	120%	238.3537	245.8950	103.2	
2.	120%	238.3537	253.6950	106.4	
3.	120%	238.3587	249.6650	104.7	104.8
1.	150%	297.9422	327.4050	109.9	
2.	150%	297.9422	311.6100	104.6	
3.	150%	297.9422	311.7400	104.6	
4.	150%	297.9422	310.4400	104.2	
5.	150%	297.9422	311.1550	104.4	105.3
6.	150%	297.9422	309.8550	104.0	

Method validation

Precision and Accuracy

The %RSD of area of a method precision study were 1.5. This aspirin and its related impurity the good precision of the developed analytical method for related impurities. Experimental data given in Table 3 and Table 4.

The percentage recovery of aspirin and salicylic acid in pharmaceutical dosage forms ranged from 103% - 112%. The percentage recovery of aspirin and its impurity ranged from 97% to 105.5% (Table 4).

Table 4: Precision and Accuracy Data of Aspirin

Sample No.	Spike level	'ppm' added	'ppm' found (recovered)	% Recovery	Mean % recovery
1.	20%	3.2144	3.6400	113.2	
2.	20%	3.2144	3.5750	111.2	
3.	20%	3.2144	3.6400	113.2	112.5
1.	50%	6.4288	6.9550	108.2	
2.	50%	6.4288	6.7600	105.2	
3.	50%	6.4288	6.7600	105.2	106.2
1.	100%	12.8575	13.5200	105.2	
2.	100%	12.8575	13.5200	105.2	
3.	100%	12.8575	13.5850	105.7	105.4
1.	120%	16.0719	16.5100	102.7	
2.	120%	16.0719	16.1200	100.3	
3.	120%	16.0719	17.0950	106.3	103.1
1.	150%	19.2863	21.6450	112.2	
2.	150%	19.2863	20.3450	105.5	
3.	150%	19.2863	20.2150	104.8	
4.	150%	19.2863	20.2800	105.2	
5.	150%	19.2863	20.2150	104.9	107.1
6.	150%	19.2863	21.1900	109.9	

Table 5: Limit Of Quantification - Precision

Name	Sample No.	% Impurity	Mean	%RSD
Salicylic acid	1	0.015	0.014	4.5
	2	0.014		
	3	0.014		
	4	0.013		
	5	0.014		
	6	0.014		
Aspirin	1	0.009	0.009	8.2
	2	0.009		
	3	0.010		
	4	0.008		
	5	0.010		
	6	0.009		

Sensitivity

The limit of detection of aspirin and salicylic acid was 0.003% (of analyte concentration, i.e. 1

mg/ml) for 20µl injection volume. The limit of quantification of salicylic acid was 0.015 % and 0.010 (of analyte concentration, i.e. 1.0 mg/mL) for 20µl injection volume. The precision at LOQ concentration were below 10 %.Experimental data shown in Table 5 and Table 6.

Table 6: Limit of Quantification – Accuracy

Sample No.	Spike level	(ppm) added	(ppm) found (recovered)	% Recovery	Mean % recovery
1	LOQ	0.7960	0.8450	106.2	107.6
2	LOQ	0.7960	0.8450	106.2	
3	LOQ	0.7960	0.9100	114.3	
4	LOQ	0.7960	0.8450	106.2	
5	LOQ	0.7960	0.8450	106.2	
6	LOQ	0.7960	0.8450	106.2	
Aspirin					
1	LOQ	0.5994	0.5850	97.6	99.4
2	LOQ	0.5994	0.5850	97.6	
3	LOQ	0.5994	0.6500	108.4	
4	LOQ	0.5994	0.5200	86.8	
5	LOQ	0.5994	0.6500	108.4	
6	LOQ	0.5994	0.5850	97.6	

Linearity and Range

Linear calibration plot for related impurities method was obtained over the calibration ranges tested, *i.e*. 0.8 to 397 ppm for salicylic acid. The correlation coefficient obtained was greater than 0.999 for salicylic acid impurity. The result shows an excellent cor-relation existed between the peak area and concentration of impurity. Experimental data presented in Table 7

Table 7: Linearity

Sample Name	Sample ID	Concentration 'ppm'	Peak Area
Salicylic acid	01	0.8	4633
	02	39.7	223647
	03	99.3	530509
	04	194.7	938616
	05	238.4	1305587
	06	297.9	1581388
	07	397.3	2146851
Aspirin	01	0.6	3482
	02	2.5	12309
	03	6.5	30841
	04	13.0	55836
	05	16.0	74782
	06	20.0	92427

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow

rate, pH and column temperature) revealed that the resolution between closely eluting peaks, namely aspirin and its related impurity was always greater than 2.0, illustrating the robustness of the method.

Solution Stability and Mobile Phase Stability

No significant changes were observed in the content of salicylic acid impuruty during solution stability and mobile phase stability experiments for related impurities. The %RSD of assay of aspirin during solution stability and mobile phase stability experiments was within 1.0.The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related impurities determination were stable up to the study period of 48 h.

Assay Analysis

Analysis was performed for different batches of Esomeprazole and low dose Aspirin dosage forms in (n = 3) ranged from 99.95% - 99.96%.

CONCLUSION

In this present research article complete degradation stress studies were reported for aspirin and its related impurity salicylic acid in presence of esomeprazole. The RP-HPLC method developed for related impurities was linear, precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested as per ICH guidelines. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of aspirin samples same procedures on a different day (inter day precision). To the best of our knowledge the specified method presented in the article successfully measures aspirin and its related impurities by HPLC in presence of esomeprazole.

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