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Research Article

IDENTIFICATION, SEPARATION AND CHARACTERIZATION OF POTENTIAL DEGRADATION PRODUCTS IN ACOTIAMIDE DRUG SUBSTANCE

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ABSTRACT

In the stress degradation studies of Acotiamide, two degradation products in alkaline conditions were identified by LC-MS. These impurities were isolated using preparative high performance liquid chromatography. By spectral data analysis (^{1}H NMR, ^{13}C NMR, MS, and IR), degradation products are characterized as 2-hydroxy-4,5-dimethoxy benzoic acid, and 2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxylic acid . The details of stress studies, identification, isolation, characterization, formation and mechanism of degradation products are discussed and presented here.

Keywords: Acotiamide, Degradation, Potential degradation product, Identification, Separation and Characterization.

INTRODUCTION

Acotiamide (ACOT) is chemically N-[2-[di(propan-2-yl)amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxamide;trihydrate;hydrochloride.

ACOT is used in functional dyspepsia[1]. It controls upper gastrointestinal tract to inhibit symptoms resulting from hypomotility and delay in gastric emptying. It produces action in stomach through inhibition of muscarinic receptors M1 and M2 and cause increase in release of acetylcholine and inhibits activity of acetylcholine esterase [2].ACOT is used in the combination of esomeprazole to improve the symptoms of functional dyspepsia [3,4].

The presence of impurities can have a significant impact on the product quality, safety and efficacy, hence the percentage level of impurities need to control in the drug substance as well as a drug product [5, 6]. The literature review reveals that there were few analytical methods available for determination of acotiamide by HPLC[7], LC-MS-MS[8], UPLC-Q-TOF-MS [9, 10], identification of degradation products of acotiamide by UPLC/ESI-quadrupole TOF-tandem MS[11].

Recently we have developed stability indicating method of acotiamide[12]. Major degradation products were formed in alkaline conditions. To the best of our knowledge there are no reports on isolation and characterization of degradation products of acotiamide. The present research work describes identification of degradation products in the alkaline condition after its isolation and characterization by LC-MS, NMR and IR.

MATERIALS AND METHODS

Chemicals, Reagents and Solutions

Acotiamide hydrochloride trihydrate (ACOT) bulk drug was provided by Hetero Drugs Pvt. Ltd. HPLC grade acetonitrile was procured from Rankem Ltd, Mumbai. HPLC grade triethylamine and formic acid were procured from Loba Chemie Pvt. Ltd., Mumbai. Analytical grade hydrochloric acid (HCl), sodium hydroxide (NaOH) were procured from S D Fine Chem. Ltd. Mumbai, India.

Mobile Phase was prepared by 1ml of Triethylamine in 1000 ml of double distilled water and adjusting the pH with 2 ml of formic acid.

Instrumentation and Chromatographic Conditions

HPLC-PDA

Waters Alliance 2695 separation module equipped with Waters 2996 Photo diode Array detector (PDA) was used. Data acquisition and integration was processed with Emchem 2 software. Thermo Hypersil BDS C-8 column (250 X 4.6 mm i.d., 5µ particle size) was used. The mobile phase was composed of 0.1 % triethylamine with 0.2 % formic acid: acetonitrile with the gradient program: time / % of MP-B was 20/20, 21/20, 25/40, 35/20. Detection was done at 282 nm with flow rate of 1ml/min. The column oven was maintained at 40ºC.

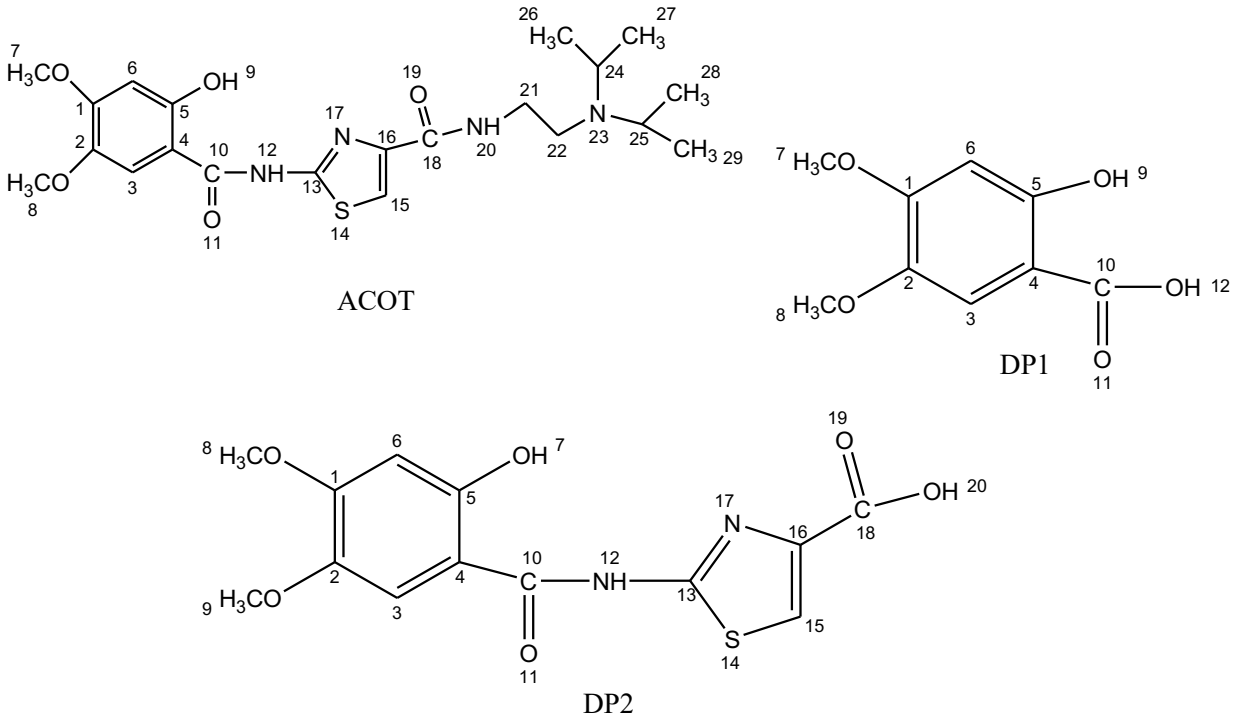

Figure 1 : Structures of ACOT, DP1 and DP2

High-performance liquid chromatography (Preparative)

Shimadzu LC-20 AP pump with SPD 20A detector was used for isolation of degradation products. Phenomenex Luna C-8(2) Column 250 X50 mm, 100Å, 10µ in diameter as used. Sample loading was through pump. Flow rate was kept at 60ml/min. UV detection was carried out at 282 nm. The mobile phase was composed of 0.1 % triethylamine with 0.2 % formic acid: acetonitrile /with the gradient program: time / % of MP-B was 20/20, 21/20, 25/40, 35/20.

NMR spectroscopy

The NMR spectra for ACOT and degradation products were recorded on Bruker 400 MHz NMR spectrometers in DMSO-d_6 and $CDCl_3$ solvents. The ^1H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ=0.00ppm), while ^{13}C chemical shift values were reported relative to DMSO-d_6 (δ=39.50ppm) and $CDCl_3$ (δ=77.0 ppm) as internal standards. Distortionless enhancement by polarization transfer (DEPT) spectral editing revealed the presence of methyl and methine groups as positive peaks while the methylene as negative peaks. The data were processed using Linux software.

LC-MS

LC-MS analysis was carried out on the alkaline degradation drug substance of ACOT using following conditions. Mobile phase 0.1 % triethylamine with 0.2 % formic acid: acetonitrile /with the gradient program: time / % of MP-B was 20/20, 21/20, 25/40, 35/20 and UV detection at 282 nm was used. The injected volume was 20µl. LC-MS system used was (Thermo Fischer Scientific LCQ Fleet) coupled with ion trap mass spectrometer with quaternary pump module. The nebulizer pressure was adjusted at 20psi with nitrogen gas. The gas temperature was adjusted at 250ºC with drying gas nitrogen at 30 psi pressure and capillary voltage of 5500V. The data acquisition was performed with Xcalibure software.

FT-IR spectroscopy

FT-IR spectra were recorded on Shimadzu ATR-FTIR spectrophotometer.

Preparation of alkaline degradation sample

1g of ACOT was taken in 50 ml volumetric flask, 10 ml of 1 N NaOH and 30ml of water was added .Solution was heated at 100°C for 8 hours. Solution was neutralised by 1 N HCl and volume was made up to 50 ml water.

RESULTS AND DISCUSSIONS

Analysis of alkaline degradation sample by HPLC

Alkaline degradation sample was diluted to the required concentration and was analysed with analytical HPLC. ACOT was eluted at retention time of 26.09 min while DP1 was formed at retention time of 8.11 min with 5.3% area by normalization and DP2 was formed at retention time of 15.52 min with 18.7% area by normalization (Figure 2).

Isolation of alkaline degradation samples by preparative HPLC

Degradation sample was subjected to preparative HPLC. Fractions of DP1 and DP2 were collected together and subjected to rotary evaporator to remove acetonitrile.

DP2 – DP2 was precipitated and was filtered and dried. DP2 was obtained as chromatographic purity of 99.8% (Figure 4).

DP1 – Filtrate was containing DP1 and API. This filtrate was purified by preparative HPLC with water and acetonitrile as mobile phase. Fractions of DP1 were collected and were purified with mobile phase of ammonium acetate buffer pH 6.5 and acetonitrile. The solution was lyophilized to obtain solid mass with chromatographic purity of 99.2% (Figure 3).

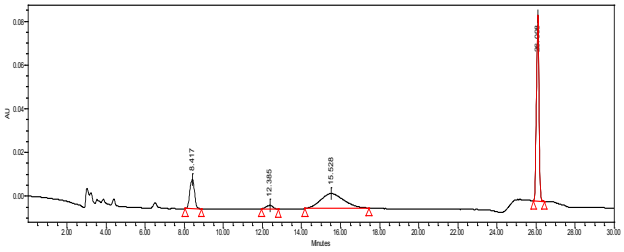

Figure 2 : HPLC chromatogram of stressed degradation sample by alkaline hydrolysis condition

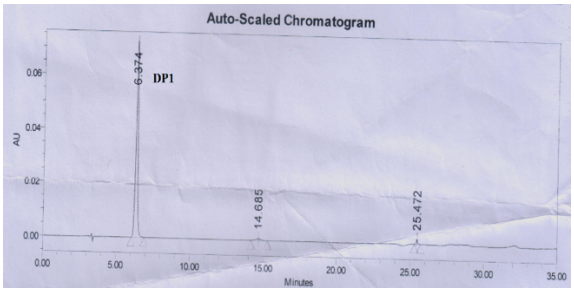

Figure 3: HPLC Chromatogram of Degradation product DP1

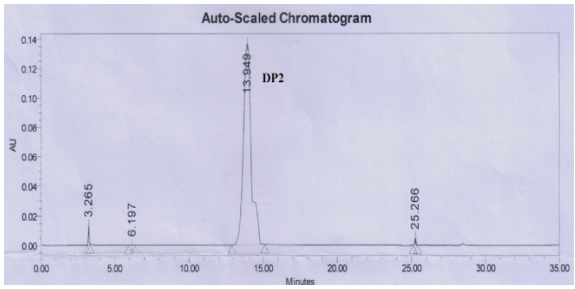

Figure 4: HPLC Chromatogram of Degradation product DP2

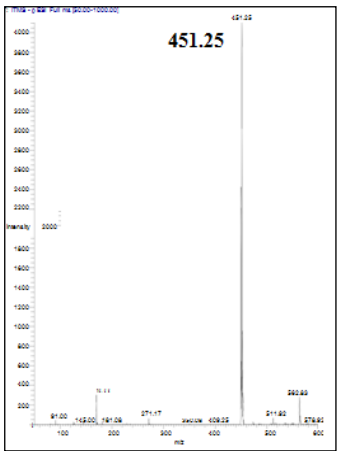

Figure 5: Mass spectrum of ACOT

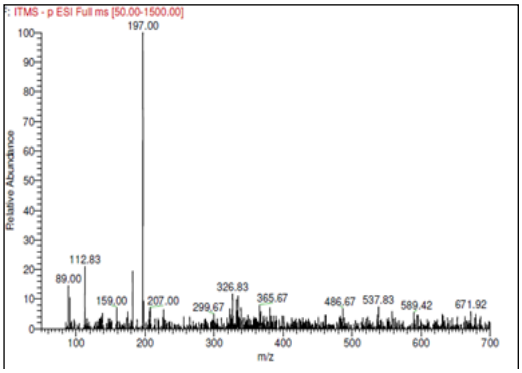

Figure 6: Mass spectrum of DP1

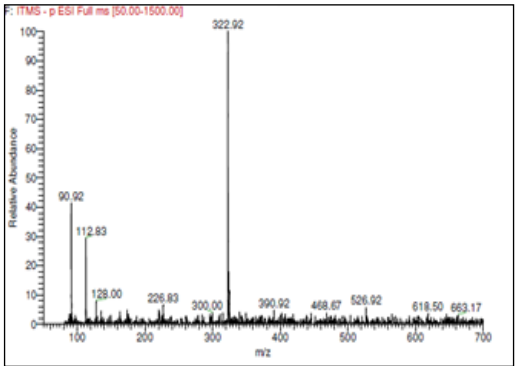

Figure 7: Mass spectrum of DP2

Table 1: NMR interpretation of ACOT

ACOT					
Position	^{1}H	Chemical Shift(δ ppm)	^{13}C	DEPT	D_2O exchange
1	---	---	154	Quaternary carbon	
2	---	---	142	Quaternary carbon	
3	1H	7.6, s	112	-CH-	
4	---	---	116	Quaternary carbon	
5	---	---	152	Quaternary carbon	
6	1H	8.0, s	102	-CH-	
7	3H	3.9,s	56.1	$-CH_3$	
8	3H	3.8,s	56.1	$-CH_3$	
9	-OH	9.5	---	---	Broad peak , absent in D_2O
10	---	---	162	ketone	
12	1H	11.8, s	---	---	-NH, absent in D_2O
13	---	---	160	Quaternary carbon	
15	1H	6.8, s	118	-CH-	
16	--	--	144	Quaternary carbon	
18	--	--	158	ketone	
20	1H	8.8, s	---	---	-NH, absent in D_2O
21	2H	3.5, t	38	$-CH_2$	
22	2H	3.2, t	46	$-CH_2$	
24	1H	3.5,m	53.5	-CH-	
25	1H	3.5,m	53.5	-CH-	
26	3H	1.4, m	18.1	$-CH_3$	$-CH_3$
27	3H	1.4, m	18.1	$-CH_3$	$-CH_3$
28	3H	1.4, m	19.2	$-CH_3$	$-CH_3$
29	3H	1.4, m	19.2	$-CH_3$	$-CH_3$

Table 2: NMR interpretation of DP1

DP1					
Position	^{1}H	Chemical Shift(δ ppm)	^{13}C	DEPT 135	D_2O exchange
1	--	--	158	Quaternary carbon	
2	--	--	160	Quaternary carbon	
3	1H	6.6,s	110	-CH-	
4	--	--	104	Quaternary carbon	
5	--	--	141	Quaternary carbon	
6	1H	7.2,s	100	-CH-	
7	3H	3.76,s	55	$-CH_3$	
8	3H	3.86,s	57	$-CH_3$	
9	---	12.5,s		--	AB
10			172	Carbonyl	
12	--	12.5,s			Broad peak , AB

Table 3: NMR interpretation of DP2

DP2					
Position	^{1}H	Chemical Shift(δ ppm)	^{13}C	DEPT	D_2O exchange
1	--	---	154	Quaternary carbon	
2	--	---	142	Quaternary carbon	
3	1H	7.8,s	119	-CH-	
4	---	---	104	Quaternary carbon	
5	---	---	152	Quaternary carbon	
6	1H	6.6,s	100	-CH-	
7	--	12.2	---	Broad peak	AB
8	3H	3.8,s	58.2	$-CH_3$	
9	3H	3.8,s	58.2	$-CH_3$	
10	---	---	160	Carbonyl	
12	1H	12,s	--	---	AB
13	--	--	158	Quaternary carbon	
15	1H	8.1,s	110	-CH-	
16	---	---	144	Quaternary carbon	
18			164	carbonyl	
20	1H	12,s	---	-OH	Broad peak, AB

Table 4: IR spectra interpretation of ACOT, DP1 and DP2

ACOT		DP1		DP2	
Wave number(cm-[1)]	Assignments	Wave number (cm^{-1})	Assignments	Wave number (cm^{-1})	Assignments
3481	N-H Secondary amide	2945	Aromatic C-H Stretch	3248	N-H Secondary amide
3298	N-H Secondary amide	2829	Methyl C-H Stretch	2972	Aromatic C-H stretch
3095	Aromatic C-H stretch	1643	C=O Acid	2749	Methyl C-H stretch
2953	Methyl C-H stretch	1510	C=C Ring Stretch	1678	C=O Acid
2673	Methylene C-H stretch	1456	C=C Ring Stretch	1641	C=O Amide
1639	C=O Amide I	1392	OH Bending	1612	N-H Bend
1564	C=O Amide II	1357	C-O Stretch	1516	C=C Ring Stretch
1519	C=C Ring Stretch	1205	C-O-C Stretch	1431	C=C Ring Stretch
1492	C=C Ring Stretch			1390	O-H Bend
1267	C-N Stretch			1213	C-O-C Stretch
1207	C-O-C Stretch			1163	C-N Stretch
1122	C-N stretch			775	C-S linkage
1080	C-O Stretch			702	Out of plane N-H bend
781	C-S linkage				
692	Out of plane N-H wagging				

STRUCTURAL INTERPRETATION OF DP1 AND DP2

To get structural insight, the LC-MS analysis was carried out on the stressed sample. The mass spectrum thus obtained was shown the molecular ion peak of degradation impurity DP1 at m/z 197(Figure 6), DP2 at m/z 322 (Figure 7) whereas the ACOT displayed protonated molecular ion at m/z 451(Figure 5).

The ^{1}H NMR, ^{13}C NMR and DEPT spectral data of degradation product DP1 and DP2 were compared with those of ACOT in Table-1. The numbering scheme for the NMR assignments is shown in Figure 1.

In DP1, protons of two isopropyl groups, two methylene groups, two protons of amide, protons of thiazole which are present in ACOT are missing. As well as in ^{13}CNMR presence of carbon of two isopropyl groups, methylene groups, carbonyl amide , thiazole are missing in DP1.Formation of carboxylic acid is indicated by formation of peak at 172 ppm in ^{13} CNMR and formation of –OH group is indicated by formation of broad peak at 12 ppm (Table 2).

IR absorption spectral data of degradation product DP1 (Table 4) support disappearance of amide bond and formation of carboxylic acid functional group at 1643 cm^{-1}. Two amide peaks which are present in ACOT at 3481 and 3298 cm^{-1} are absent in DP1. –OH bending is indicated by formation of peak at 1392cm^{-1}. It indicates that alkaline hydrolysis has taken place at amide bond between 10 and 12 position in ACOT. Based on this, DP1 is characterized as 2-hydroxy-4, 5-dimethoxy benzoic acid.

In DP2, protons of two isopropyl groups, two methylene groups, one protons of amide, protons of thiazole which are present in ACOT are missing. As well as in ^{13}CNMR presence of carbon of two isopropyl groups, methylene groups, one carbonyl amide , thiazole are missing in DP2.Formation of –OH group is indicated by formation of broad peak at 12 ppm (Table 3).

IR spectra of DP2 indicates one N-H stretch at 3248 cm^{-1} and carboxylic acid functional group with C=O stretch 1678cm^{-1}, N-H bending at 1612 cm^{-1} .There is absence of one –NH group (Table 4).

This indicates alkaline hydrolysis has taken place at amide group between 18 and 20 positions of ACOT. DP2 is characterized as 2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxylic acid.

DEGRADATION PATHWAY

The degradation products DP1 and DP2 formed in the presence of base stress degradation is due to the hydrolysis of amide bond. The probable degradation pathway of DP1 and DP2 is shown in Figure 8 and Figure 9.

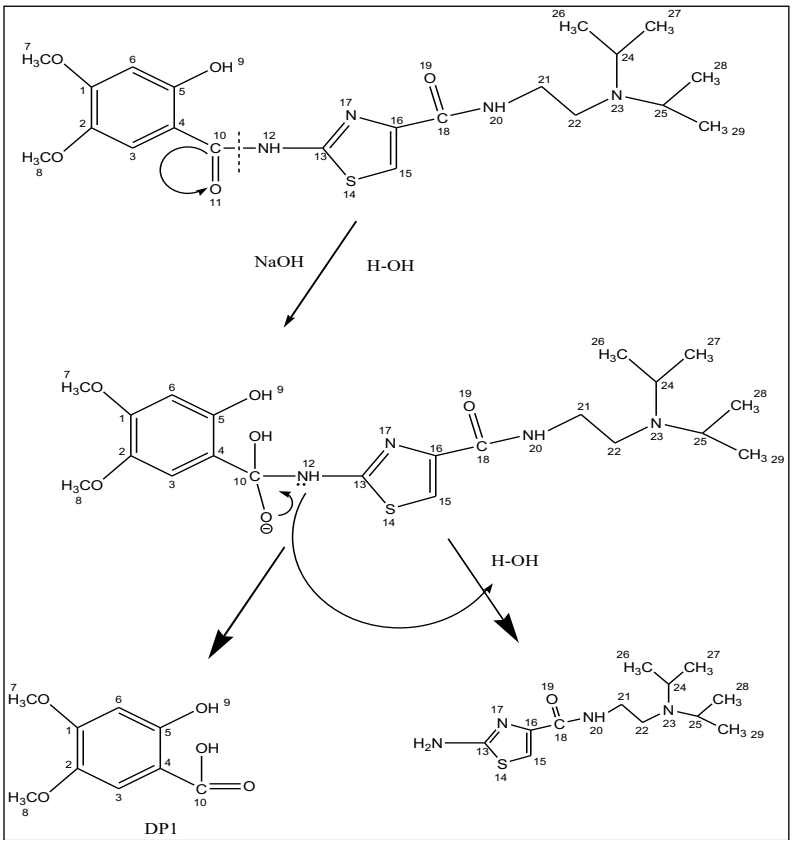

Figure 8: Degradation pathway of DP1

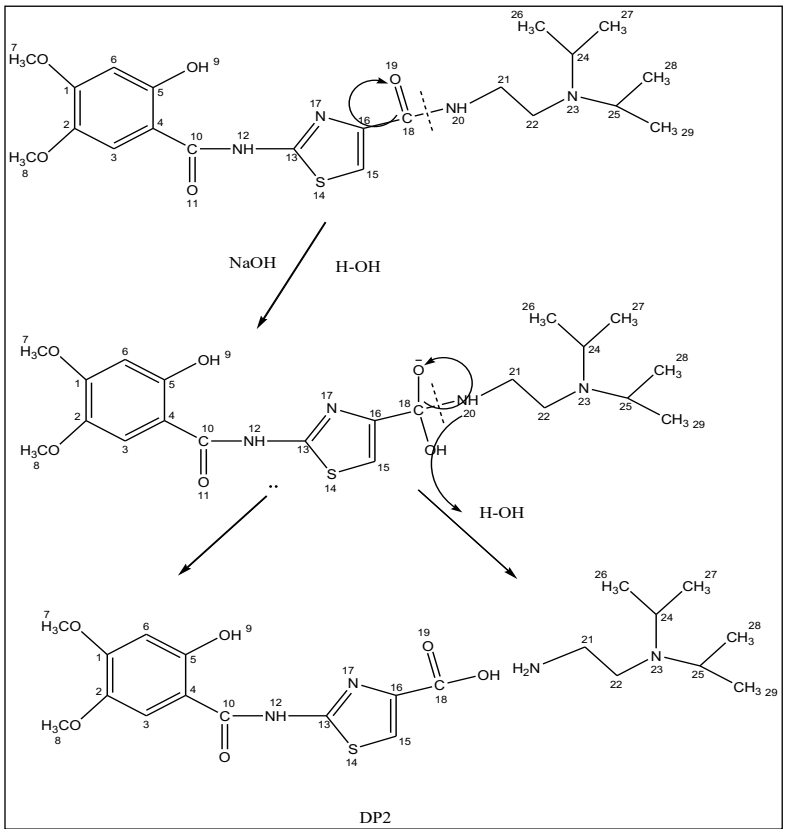

Figure 9: Degradation pathway of DP2

CONCLUSION

Two alkaline degradation products of ACOT were isolated by preparative HPLC and were characterized by using spectroscopic techniques namely NMR, ESI-MS and IR. The degradation products were characterized as 2-hydroxy-4,5-dimethoxy benzoic acid and 2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxylic acid .The present work is beneficial in applicability of NMR,LC-MS and IR in structural interpretation of novel degradation products in active pharmaceutical ingredients.

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