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Stress degradation of Lisinopril as per ICH Guidelines & Characterisation

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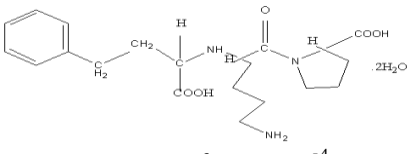
Abstract

Lisinopril an antihypertensive drug was subjected to stress degradation, since the drug is photosensitive undergo hydrolysis and oxidized in presence of oxygen. Hence the objective of the study was to stress degrade Lisinopril & to find out the pathway for stress degradation of Lisinopril. Stress testing methods are screening methods to be used to understand the degradation chemistry of a drug. Lisinopril was subjected to stress degradation under different conditions recommended by International Conference on Harmonization (ICH). The chromatographic separation of Lisinopril & its degradation products was done on C_{18} column & mobile phase was mixture of Methanol & Water in ratio 80:20, pH 3.5 adjusted with orthophosphoric acid at a flow rate of 1ml/min using UV detector with λ_{max} 220nm. The quantification & characterizations of degraded products were carried out by UV, IR spectroscopy & HPLC. The mechanism of degradation was confirmed by GC-MS fragmentation pattern.

Keywords: Stress degradation, Lisinopril, ICH guidelines

1. Introduction

Lisinopril, (LIS), (2*S*)-1-[(2*S*)-6-amino-2[[(1*S*)-1-carboxy-3-phenylpropyl] amino]-hexanoyl]-pirrole-2-carboxylic acid[1] used in the treatment of essential hypertension, symptomatic & asymptomatic left ventricular systolic dysfunction, post-myocardial infarction, renal failure & diabetic nephropathy. The analysis of Lisinopril was reported by RP-HPLC method in bulk & tablet dosage form. A novel RP-HPLC method was developed for simultaneous estimation of Lisinopril in combination for bulk & tablet dosage form[2]. The pharmaceutical products are prone to undergo degradation in various physical & chemical conditions & yield impurities which adversely affect the performance of drug substance. Hence, it has been mandated by regulatory agencies of various countries to submit the stability indicating data of the drug substance & the drug product before the approval for commercialization of products. Hence it is necessary to develop stability indicating method for analysis of drug substance & their impurities. There is no reported stability indicating analytical method for analysis of Lisinopril & its degradation products in bulk. The present work aimed at the stress degradation study of Lisinopril in bulk & establishment of structures of degraded product by sophisticated instrumental methods like UV, IR, RP-HPLC and GC-MS[3].



Structure of Lisinopril[4]

2. Materials and Methods

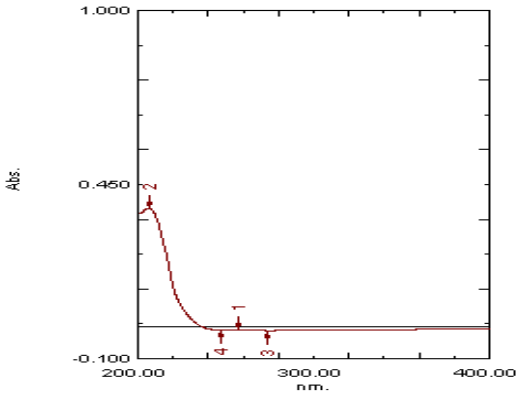
2.1 Instrumentation: The UV spectrophotometer was used of model SHIMADZU UV 1650-PC. IR spectrophotometer was used of model SHIMADZU 8400S. The high pressure liquid chromatographic (HPLC) system used was of model SHIMADZU 8400S Prominence SPD-20Am Gradient System equipped with Hamilton injector & UV visible detector. A Phenomex HPLC column C18 reverse phase, 5µm, (50×4.6mm) was used. The GC- MS instrument of model- Accu TOF GCV, model no-7890 with FID detector, head space injector & combipal autosamlper & mass range 10-2000amu, mass resolution 6000 was used for analysis.

2.2 Materials: Lisinopril bulk was obtained as a gift sample from Mylan Laboratories, Aurangabad, India. Methanol & water HPLC grade obtained from Fischer Scientific, Mumbai. Orthophosphoric acid (Sd fine chemicals).

2.3 Methods

2.3.1 Selection of Wavelength: The wavelength of maximum absorption for Lisinopril was recorded by UV spectrophotometer (figure 1).

Fig no.1 UV spectrum for Lisinopril



2.3.2 Preparation of mobile phase: The mobile phase was prepared in the ratio 80:20 v/v (methanol: water). The pH was adjusted to 3.5 with orthophosphoric acid, filtered & degassed with sonication for 10 mins.

2.3.3. Preparation of Standard solution: UV spectrum was recorded using spectroscopic grade water as solvent[5]. For HPLC analysis, a standard stock solution was prepared by dissolving 100 mg of Lisinopril in 100 ml of mobile phase. 1 ml of above solution was diluted to 100 ml with mobile phase to obtain a concentration of 10µg/ml & further it was diluted to 100ml with mobile phase to obtain a concentration of 1 µg/ml. Lisinopril chromatogram is given in fig 4.

2.3.4. Preparation of calibration curve: For calibration by UV spectrophotometer, six standards were prepared having concentrations in the range of 3-18µg/ml using water as a solvent. (Table 1 & fig 2). For HPLC analysis, six standards were used having concentrations in the range 0.6-1.6 µg/ml diluting with mobile phase. (Table 2 & Fig 5)

2.3.5. Recording IR spectra: The IR spectrum of Lisinopril was recorded using kBr pellet technique.[6] (fig no.3)

2.3.6. System suitability Tests: System suitability was verified by injecting working standard of 1µg/ml. Various parameters such as HETP, number of theoretical plates, tailing factor & asymmetry were recorded. (Table 3)

2.4. Validation parameters as per ICH guidelines: The UV & HPLC methods were validated in terms of precision, LOD, LOQ, linearity, range, ruggedness & robustness[7].

2.4.1. Precision: The precision of proposed methods were evaluated by carrying out six independent test values. Intraday & Interday precision were carried out.

2.4.2. Limit of Detection & Limit of Quantitation: The limit of detection (LOD) & the limit of quantitation (LOQ) for Lisinopril by UV & HPLC were reported from standard deviation of the response & the slope.

$$LOD = \sigma/S \times 3.3$$
$$LOQ = \sigma/S \times 10$$

2.4.3. Robustness: The robustness of the method was determined as measure of the analytical method capability to be unaffected by deliberate small change in method parameters. The changes made such as use of single beam instrument for double beam, variation in flow rate by $\pm$ 0.2 ml/min, variation in wavelength by $\pm$ 2nm. At these changed conditions the standard solutions were analyzed. S.D & % R.S.D were calculated.

2.4.4. Ruggedness: The ruggedness of the method was checked by changing analyst & expressed as S.D & % RSD.

2.4.5. Force degradation study: To check the stability, the drug was forced degraded under acid/base hydrolytic, oxidative & Photolytic stress conditions as per ICH recommendation[8].

The drug was subjected to acid hydrolysis by using 0.1 N hydrochloric acid for 6hrs at 40^0C; base hydrolysis by using 0.1N sodium hydroxide solution for 6 hrs at 40^0C; oxidation by using 6% solution of hydrogen peroxide for 6 hrs & photolytic stress using sunlight for 72hrs[9].

2.4.6. Characterization of degraded product by GC-MS study: The fragmentation pattern was used for mechanism of degradation by various degraded process. Fig no.10, 12, 14, 16 respectively.

3. Results and Discussion

3.1 Method development

3.1.1. UV Method development: Water was used as a solvent for recording UV spectrum. The λ max selected was 205 nm. The UV method was developed & validated as ICH guidelines. Table no. 1

Table No.1 Summary for UV Method validation of Lisinopril

Parameter		Observation
Linearity		3-30µg/ml
Slope		0.0291
Intercept		0.0259
Correlation Coefficient		0.988
Precision	S.D	0.0084
	% RSD	1.92
Intraday Precision	S.D	0.0074
	% RSD	1.60
Interday Precision	S.D	0.0072
	%R.S.D	1.58
Ruggedness	S.D	0.0049
	% RSD	1.20
Robustness	S.D	0.0076
	%RSD	1.64
LOD		0.95
LOQ		2.88

3.1.2. HPLC Method development: Several mobile phase compositions were tried to resolve the peaks of Lisinopril & its degradation products. The mobile phase containing methanol- water 80:20 (v/v) was optimized for analysis since it resolved the peaks of Lisinopril (RT = 2.28 ± 0.02) with resolution factor of 5.6. The pH was adjusted to 3.5 with orthophosphoric acid. The system suitability parameter was stated in Table no.3 Quantification was achieved with UV detection at 205nm on the basis of peak area. A typical chromatogram was obtained. The HPLC method developed & validated as per ICH guidelines for quantitation of force degraded products[10]. Table no. 2

Table No.2 Summary for method validation of Lisinopril by HLPC

Parameters		Observations
Linearity		0.6- 1.6 µg/ml
Slope		270.97
Intercept		10.79
Correlation coefficient		0.995
Precision	S.D	1.250
	%RSD	0.45
Intraday Precision	S.D	0.463
	%RSD	0.16
Interday Precision	S.D	0.652
	%RSD	0.23
Ruggedness	S.D	0.430
	%RSD	0.16
Robustness	S.D	0.726
	%RSD	0.30
LOD		0.015
LOQ		0.046

Table No.3 System suitability parameters for Lisinopril

Retention time	Tailing factor	Asymmetry	Theoretical plate	Resolution
2.28	1.23	1.89	4426.65	5.68

Table No.4 Comparative data for % degradation by UV & HPLC

Compounds	Hydrolysis Acid	Hydrolytic Base	Hydrolytic Base	Photolytic
% Degradation by UV	67.83%	69.95%	73.27%	59.83%
% Degradation by HPLC	92.81%	93.36%	94.50%	89.85%

3.2 Force Degradation

Forced degradation studies were carried out for Lisinopril in acid hydrolysis, alkaline hydrolysis, oxidation & photolytic stress[11]. The peaks of degradation components were well resolved & appeared at 2.3, 3.2, 2.8 & 2.9 respectively.fig no.6-9.

The % degradation of Lisinopril was quantified by UV & HPLC methods the comparative data is presented in table no.4. Since HPLC is more specific & selective method the % degraded amount is more for HLPC as compared to UV method.

3.3. Mechanism of degradation

The structure of degraded products was confirmed by GC-MS study. The fragmentation patter was correlated with the structure of degraded products[12].

For acid hydrolysis degraded product formed Benzenebutanoic acid & the pathway of degradation was stated in fig no.11

For base hydrolysis degraded product formed Benzene fragment & the pathway of degradation was stated in fig no.13

For oxidation degraded product formed P-Toluene & the pathway of degradation was stated in fig no.15

For photolysis degraded product formed & the pathway of degradation was stated in fig no.16.

Fig no.2 Graph for Linearity of Lisinopril

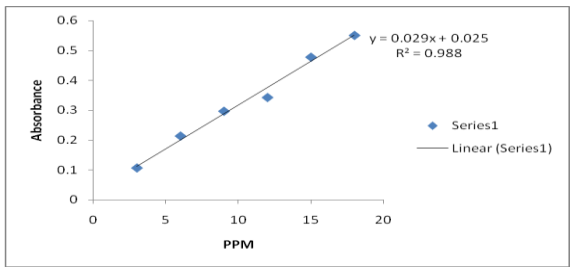


Fig no.3 IR spectrum for Lisinopril

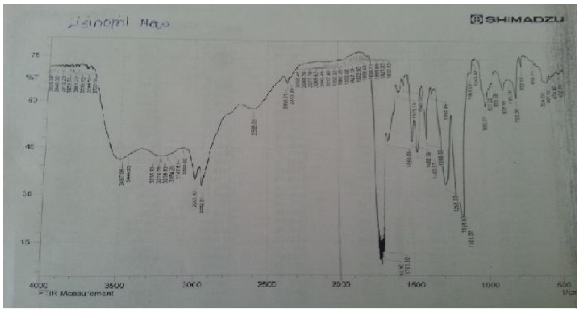


Fig no.4 HPLC chromatogram for Lisinopril

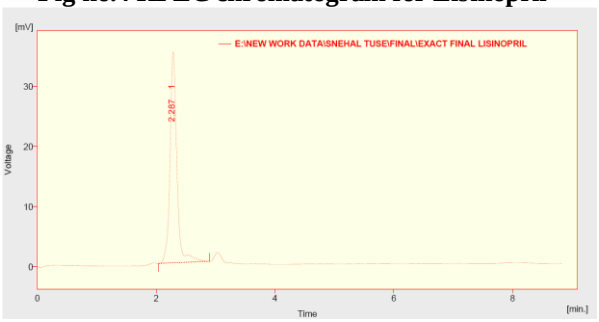


Fig no.5 Graph for linearity for Lisinopril by HPLC

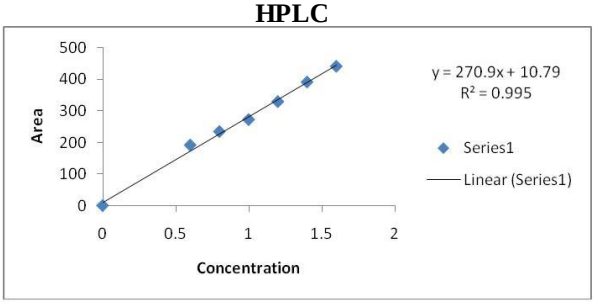


Fig no.6 HPLC chromatogram for acid hydrolytic degraded product

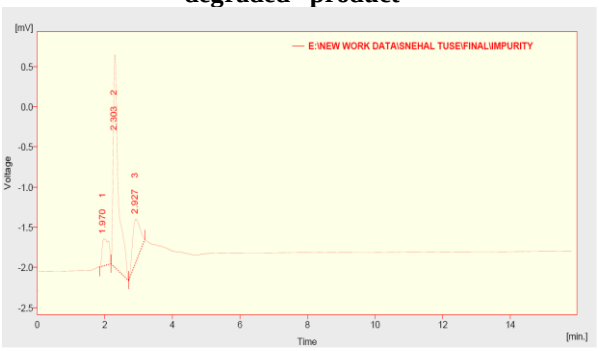


Fig no.7 HPLC chromatogram for base hydrolytic degraded product

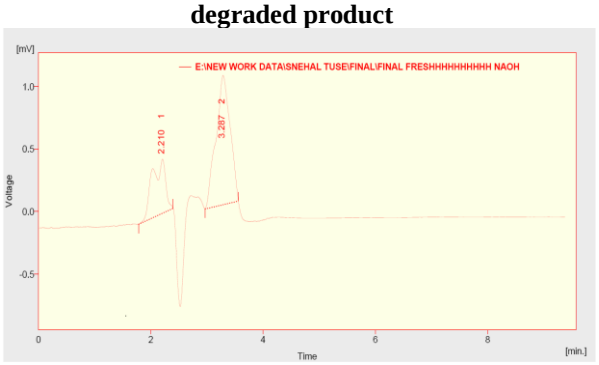


Fig no.8 HPLC chromatogram of oxidation degraded product

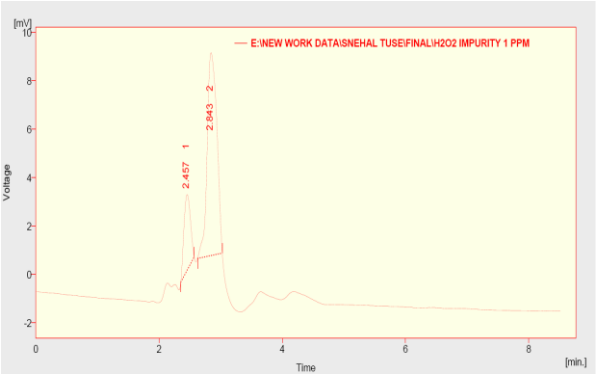


Fig no.9 HPLC chromatogram of photolytic degraded product

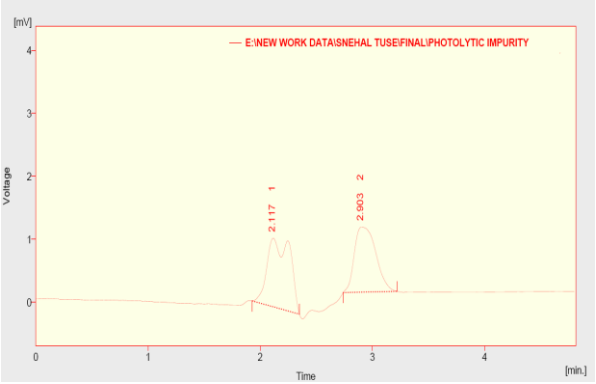


Fig no.10 GC-MS spectra of degradation of Lisinopril by acid hydrolysis

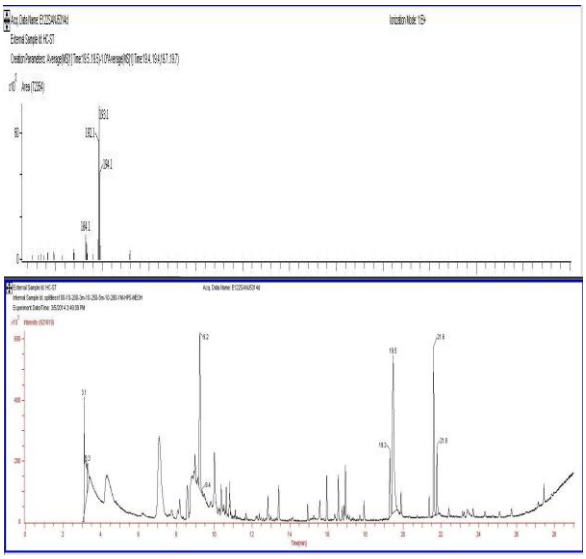


Fig no.11 Fragmentation pattern for acid hydrolytic degraded product

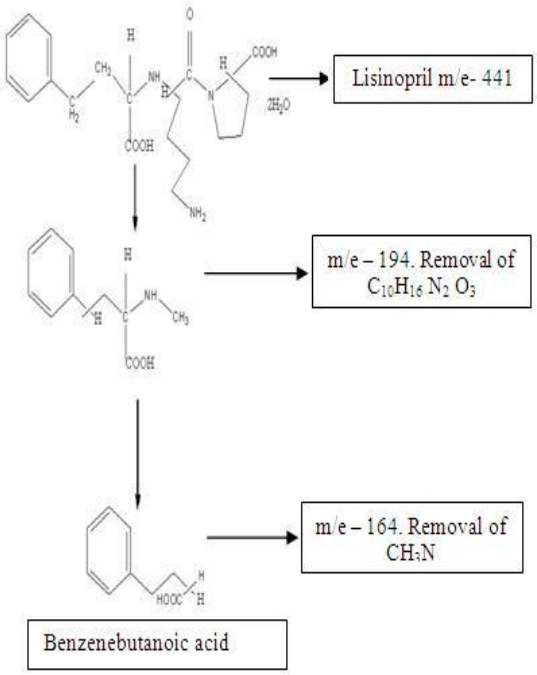


Fig no.12 GC-MS spectra of base hydrolytic degraded product

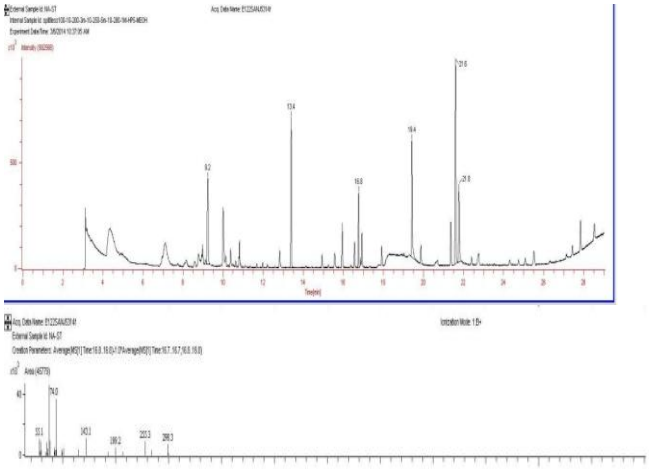


Fig no.13 Fragmentation pattern for base hydrolytic degraded product

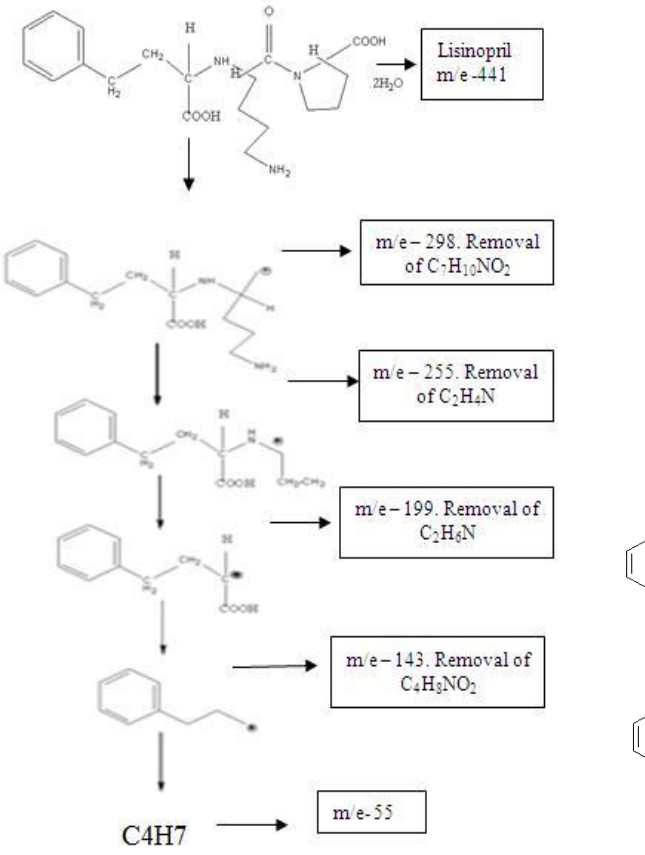


Fig no.14 GC-MS spectra oxidation degraded product

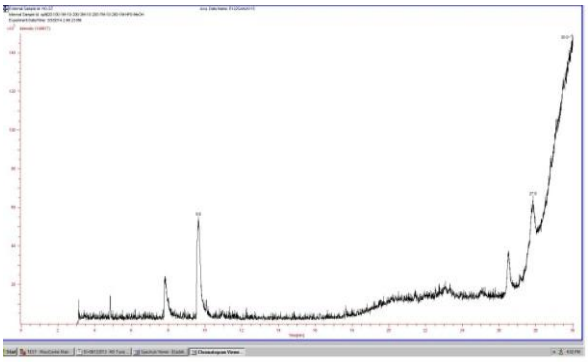
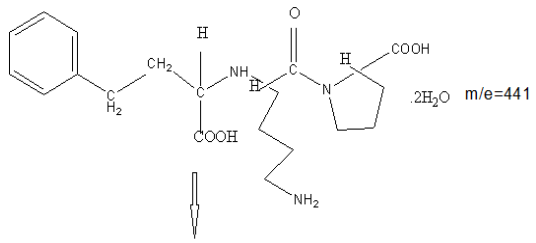
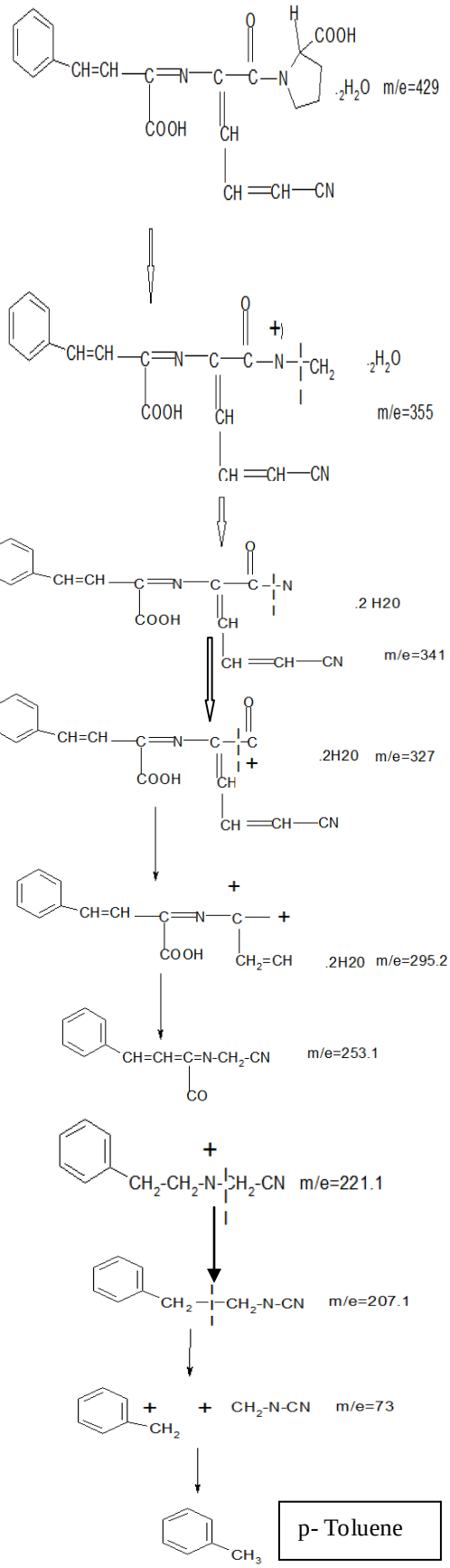


Fig no.15 Fragmentation pattern for oxidation degraded product





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