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# Characterization of products formed by forced degradation of Etodoalc using LC/MS/MS

ASMS 2015 ThP 426

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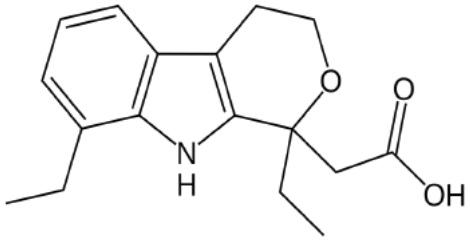
## Introduction

Forced degradation is a process, whereby the natural degradation rate of a drug product or drug substance is accelerated by the application of an additional stress. Understanding of these products qualitatively and at times quantitatively, can assist in predicting their toxicity and in deciding shelf life of the drug[1].
The ICH guidelines indicate that stress testing is designed to determine the stability of the molecule by knowing degradation pathways in order to identify the likely degradation products. The degradation products are those formed under different stress conditions such as temperature, humidity, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH value.




(*RS*)-2-(1,8-Diethyl-4,9-dihydro-3*H*-pyrano[3,4-b]indol-1-yl)acetic acid

Figure 1. Structure of Etodolac

Even though ICH and FDA ask to include this study at Phase III level, it is recommended to start this study as early as possible to be able to provide valuable information to assess inherent stability of a drug, and to improve formulation and the manufacturing process[2].
In this work, acid, base, peroxide, thermal and UV degradation products of Etodolac were studied using a Triple Quadrupole Mass Spectrometer LCMS-8040. Ultra fast scanning and Ultra fast polarity switching of LCMS-8040 enabled higher data points and identification of degradation impurities, present in low concentrations in a single run. Prior to LC/MS/MS analysis, the degradation products were isolated using Shimadzu preparative HPLC.

**Etodolac** (shown in Figure 1) is a member of the pyranocarboxylic acid group of nonsteroidal anti-inflammatory drug approved by the U.S. Food and Drug Administration. It is used to relieve the inflammation, swelling, stiffness, and joint pain in conditions like osteoarthritis and rheumatoid arthritis, as well as for general pain relief. The therapeutic effects of etodolac are achieved via inhibition of cyclooxygenase (COX) activity resulting in decreased synthesis of prostaglandin involved in fever, pain, swelling and inflammation [3].
It is rapidly metabolized in the liver followed by renal elimination as the primary route of excretion. Etodolac is official in Indian pharmacopoeia, British, European and United State Pharmacopoeia.

## Method of analysis

### Forced degradation

As per the ICH guidelines[4], the study of effect of temperature is suggested to be done in 10 °C increments above the accelerated temperature conditions and the humidity level of 75 % or greater. No details are, however, provided for the study of oxidation, photolysis and hydrolysis at different pH. In absence of guidelines, it is difficult to decide on stress conditions to be employed for the study.
Etodolac was received from commercial sources in pure form and was used for the degradation study at different conditions as shown in Table 1.

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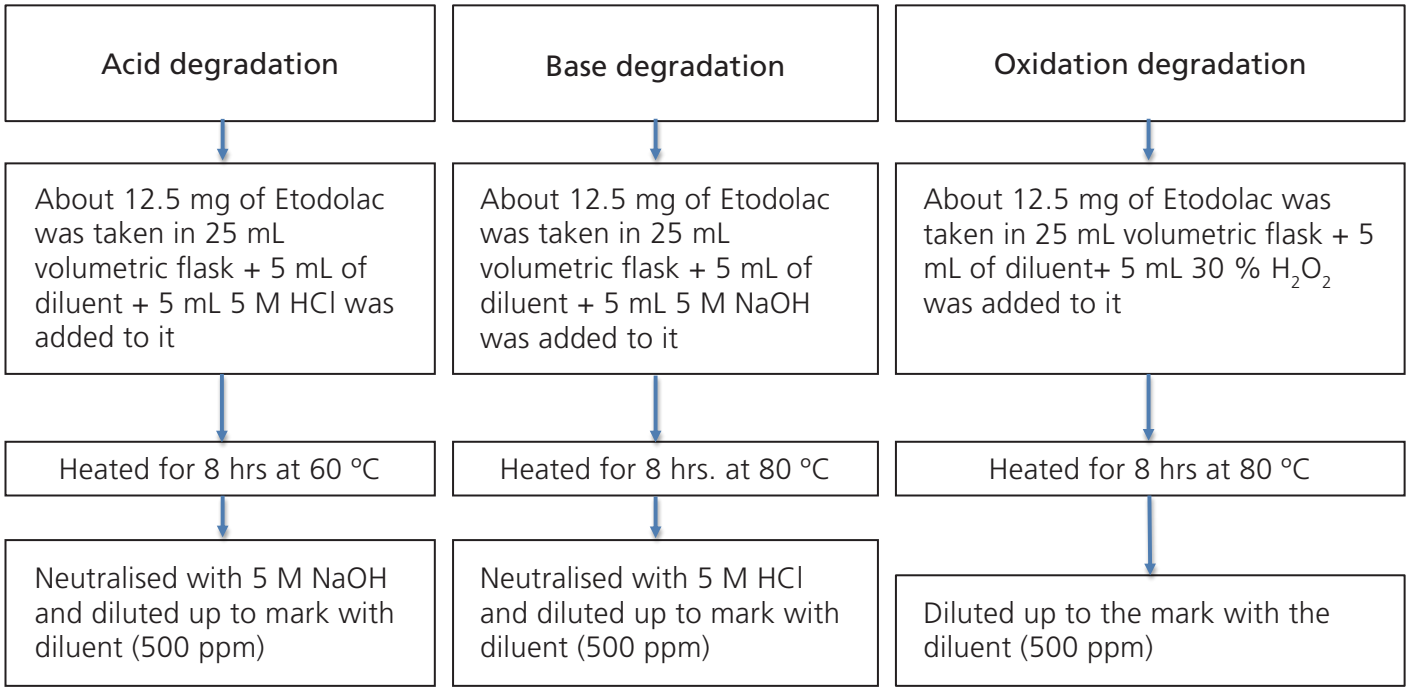
Table 1. Degradation condition

| | |
|---|---|
| Acid degradation | : 5 M HCl for 8 hrs at 60 °C in water bath. |
| Base degradation | : 5 M NaOH for 8 hrs at 80 °C in water bath. |
| Oxidative degradation | : 30 % $H_2O_2$ for 80 °C for 8 hrs in water bath. |
| Thermal degradation | : 100 mg of drug substance was placed in petridish in temperature controlled oven at 80 °C for 48 hrs. |
| UV degradation | : 500 ppm solution of Etodolac was exposed to UV radiations for 1.2 million lux hrs. |

Etodolac was subjected to degradation by above conditions and degradation products were analyzed on LC/MS/MS with UV detector for determination of extent of degradation and mass of degradation samples.

## Sample Preparation

**Degradation samples were prepared in the following way for different stress conditions:**




**Thermal degradation:** About 100 mg of Etodolac was placed in petri dish in a temperature controlled oven at 80 °C for 48 hrs. and 500 ppm solution was prepared from it.

**UV degradation:** 500 ppm of Etodolac solution, prepared in the diluent was exposed to UV radiations for 1.2 million lux hours. Water : acetonitrile (50:50 v/v) was used as diluent for sample preparation.

All the degradation samples were injected on Shimadzu Nexera system to know the extent of degradation. The analytical method was optimized and transferred on Shimadzu preparative HPLC system with automatic fraction collector and collected the degradation products in pure form. The purified degradation products were injected on LCMS-8040 to identify degradation products and extent of degradation (shown in Table 2). After knowing the degradation products' m/z the same were used as a precursor ion for further fragmentation.

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Figure 2. LCMS-8040 triple quadrupole mass spectrometer by Shimadzu

## LC/MS/MS analysis

Degradation samples were analyzed on LCMS-8040 (shown in Figure 2) for identification and confirmation of degradation products. The analytical conditions are as follows.

| | |
|---|---|
| Column | : Shim-pack XR ODS (100 mm L x 2 mm I.D. x 3 µm) |
| Flow rate | : 0.4 mL/min |
| Oven temperature | : 40 °C |
| Detector | : UV detector at 225 nm |
| Mobile phase | : A: water (pH 3.0 adjusted with formic acid)<br>B: acetonitrile [A:B (50:50 v/v)] |
| Injection volume | : 5 µL |
| MS interface | : Electro Spray Ionization (ESI) |
| Nitrogen gas flow | : Nebulizing gas 2 L/min; Drying gas 15 L/min |

# Results

## LC/MS/MS analysis

The purified degradation products were injected on LCMS-8040 and identified their m/z and same were selected for product ion scan (shown in Figures 3 and 4) and fragmentation products observed were used for probable structure identification (shown in Figure 5).

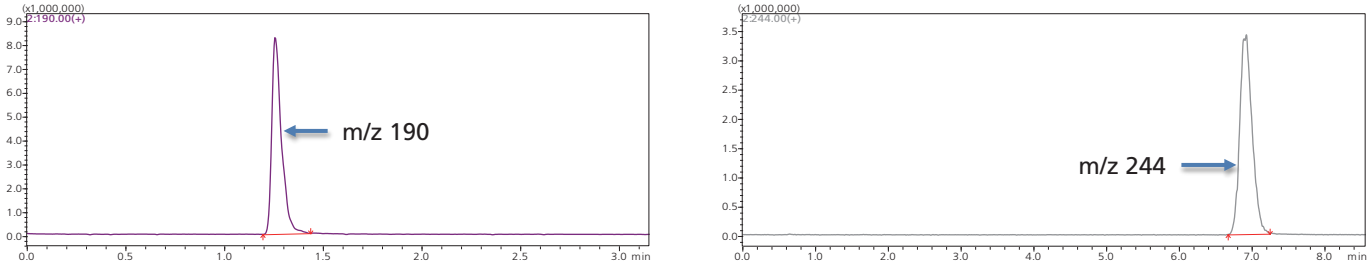


Figure 3. Acid degradation products

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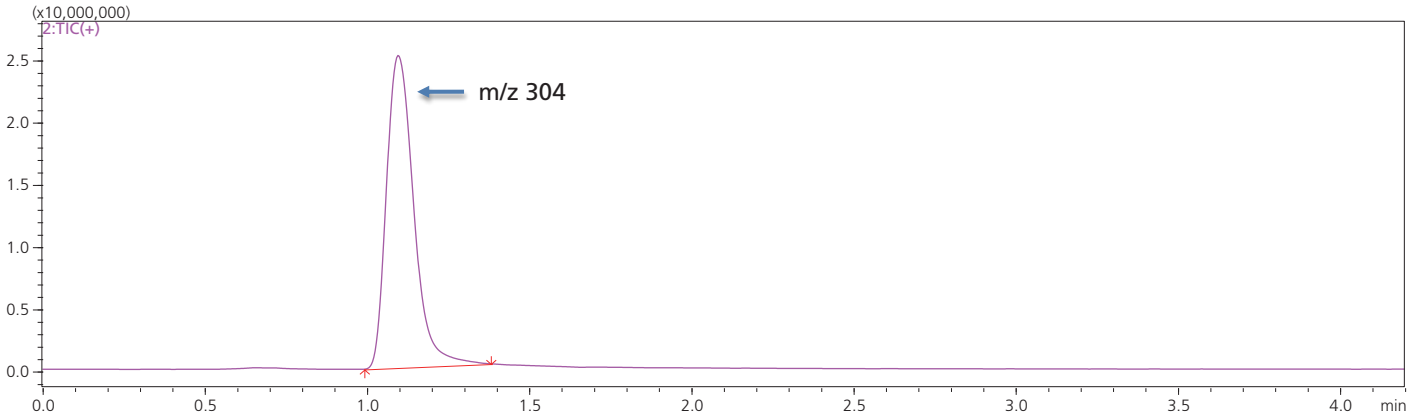


Figure 4. Oxidation degradation product

## Product ion spectra

A) Acid degradation

B) Oxidative degradation

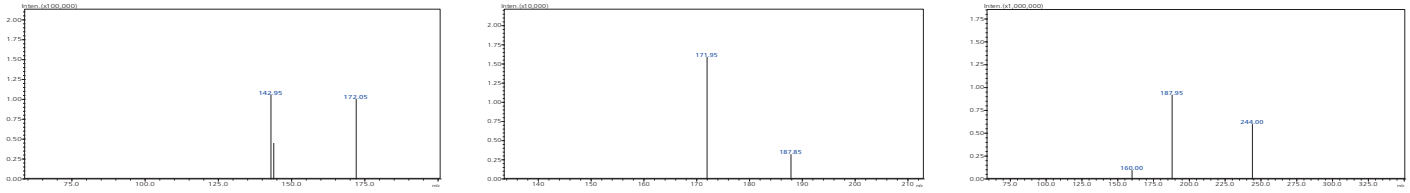


Figure 5. Product ion scan spectra of different degradation samples

Table 2. Degradation process and percentage of degradation

| NO | Degradation type | Experimental conditions | Degradation observed |
|---|---|---|---|
| 1 | Acid Hydrolysis | 5 M HCl, 60 °C for 8 hrs | 100% |
| 2 | Base Hydrolysis | 5 M NaOH, 80 °C for 8 hrs | 5% |
| 3 | Oxidation | 30 % $H_2O_2$ 80 °C for 8 hrs | 68% |
| 4 | Thermal | 80 °C for 48 hrs | 1% |
| 5 | UV | Exposed for 1.2 million lux hrs | 6% |

Etodolac was subjected to degradation under different conditions to achieve maximum degradation. The main purpose of this study was to identify the degradation products of Etodolac by LC/MS/MS.
The identification of degradation products was also very effective for knowing the pathways of degradation of drug substance. Use of LC/MS/MS technique made it possible to obtain detailed structural information rapidly on small quantities of substances without isolation of impurities.

Degradation products with m/z 190 and 244 in acid degradation (shown in Figure 6) and m/z 304 (shown in Figure 7) in oxidative degradation were subjected to product ion scan at different collision energies. Probable structures of product ions were drawn to confirm the degradation products.
UV degradation gave products with m/z 304 which was not subjected to product ion scan as it was same as obtained under oxidation indicating catalytic oxidation of the molecule on exposure to UV radiations.

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## Forced degradation products

### A) Acid degradation

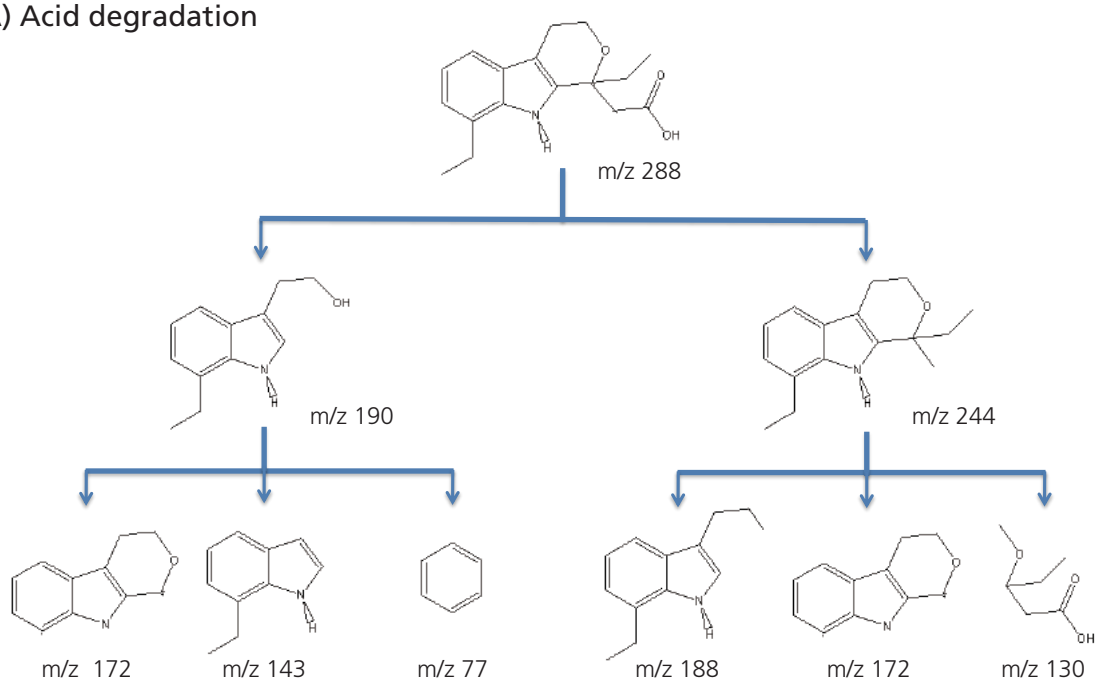


Figure 6. Acid degradation products

### B) Oxidative degradation

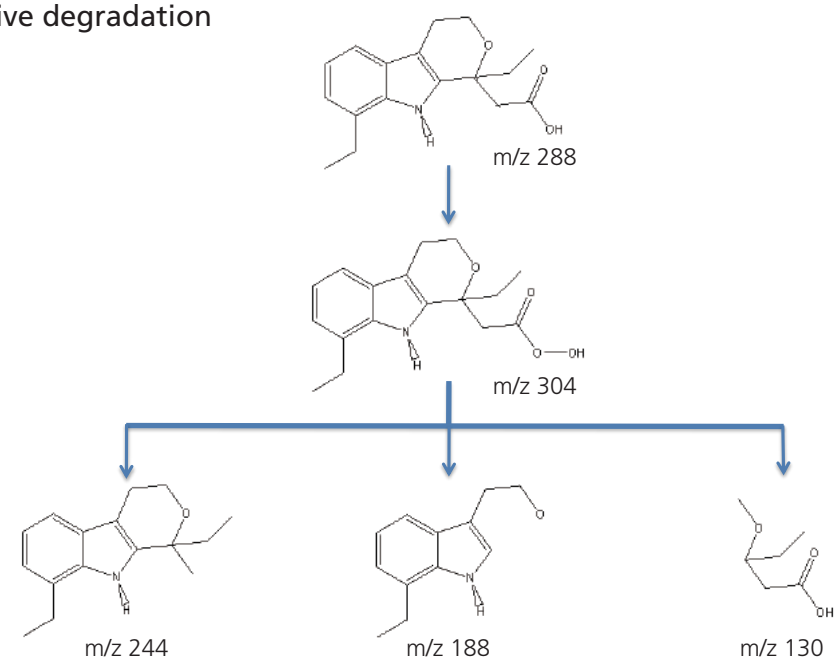


Figure 7. Oxidation degradation products

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## Conclusion

- A fast LC/MS/MS method was developed to identify degradation products at different stress conditions. Etodolac was found to degrade completely in acidic condition; 68 % in 30 % $H_2O_2$, 6 % due to UV radiations, 5 % under basic condition and only 1 % under thermal degradation.
- Major degradation products were identified using LCMS-8040 and product ions were scanned after fragmentation to get structural information.
- Fragment ion with m/z 172 was common in both the degradation products of acid while fragment ions with m/z 188 and 130 were found to be present in oxidation and one of the acid degradation products.
- The method was fast and reliable to identify different products at the same time and at low concentration levels.

## References

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First Edition: May, 2015

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