

INVESTIGATION OF THE *IN-VITRO* METABOLITES OF ETODOLAC USING LC-MS/MS TECHNIQUE

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1. INTRODUCTION:

The study of the metabolic fate of drugs is an essential and important part of the drug development process.

Metabolism is a process of biotransformation, where drugs are transformed into a different chemical form by enzymatic reactions. The metabolic reactions are mainly categorized into two types: **Phase I** and **Phase II reactions**⁽¹⁾.

In this work, the phase I metabolites of Etodolac were studied using two *in-vitro* models: **Human Liver Cell lines** and **Chicken Liver Tissue**. The metabolites formed were analyzed on **Triple Quadrupole Mass Spectrometer**.

Etodolac (Fig.1) is a non-steroidal anti-inflammatory drug approved by the U.S. Food and Drug Administration. This group of drugs has anti-inflammatory, analgesic and antipyretic activities. It is

mostly used in conditions like osteoarthritis and rheumatoid arthritis, as well as for general pain relief. It is rapidly metabolized in the liver followed by renal elimination as the primary route of excretion.

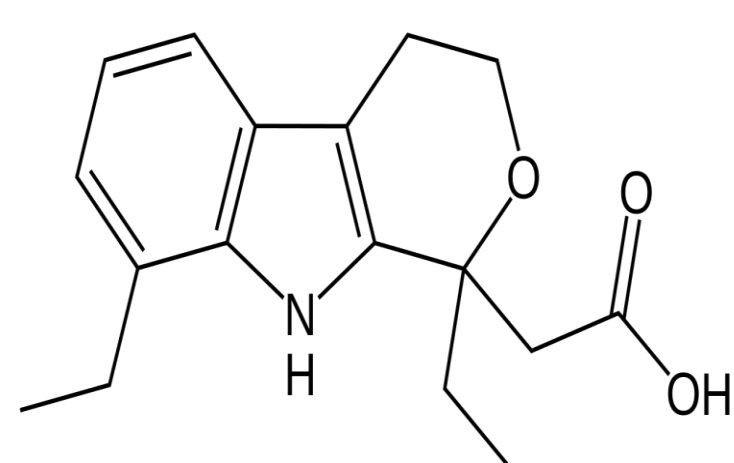


FIGURE 1: CHEMICAL STRUCTURE OF ETODOLAC

2. METHODOLOGY:

2A. SAMPLE PREPARATION:

The metabolic profile of Etodolac was studied in the following two *in-vitro* models.

A: Normal Human Immortalized Liver Cells

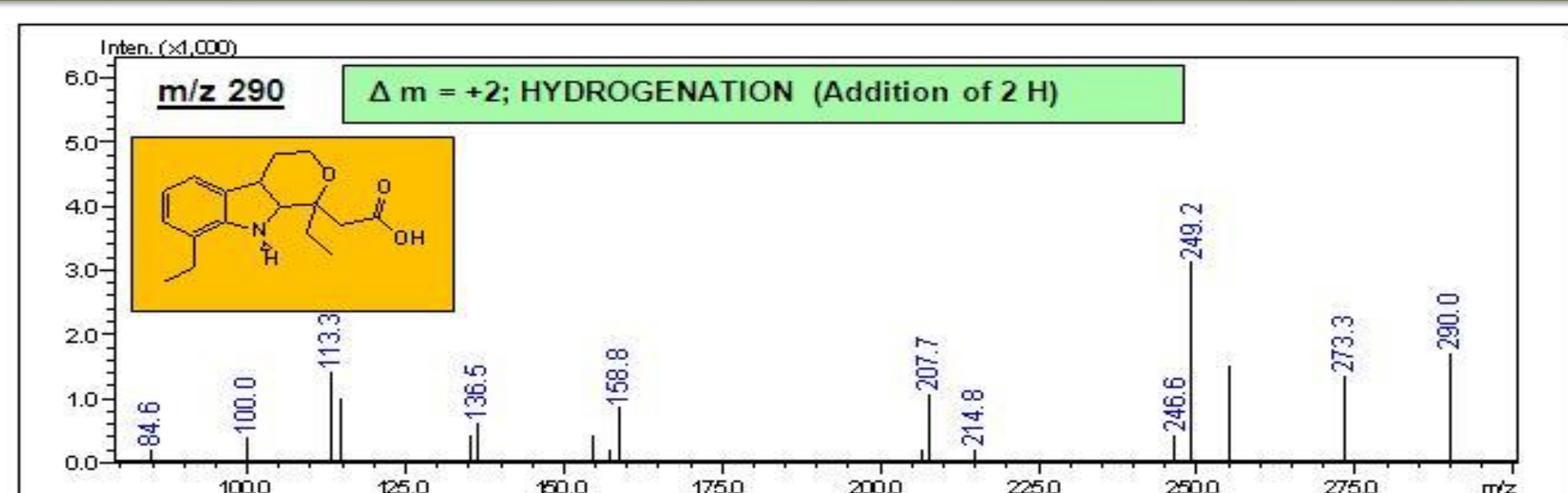
B: Chicken Liver Tissue

- A:** Liver Cells were thawed & then cultured for a week & 2 passages.
- B:** A fresh chicken liver tissue was procured & stored in PBS-A.
- A:** Liver Cells were seeded with density of 10^4 cells/ml in 96 well plate.
- A:** Cultured cells were observed for normal morphology after 16h.
- B:** Tissue was aseptically cut into medium sized pieces and maintained in DMEM (10% FBS).
- Cells and tissue were dosed with required concentration of drugs and incubated for 24h.
- Supernatant medium was discarded and cells & tissue were washed with PSBA.
- Cells were lysed using sonication. The lysate was treated with Acetonitrile to precipitate the protein, centrifuged at 10,000rpm for 15mins at 10°C, supernatant was filtered through 0.45µ millipore membrane and analyzed on LC-MS/MS.

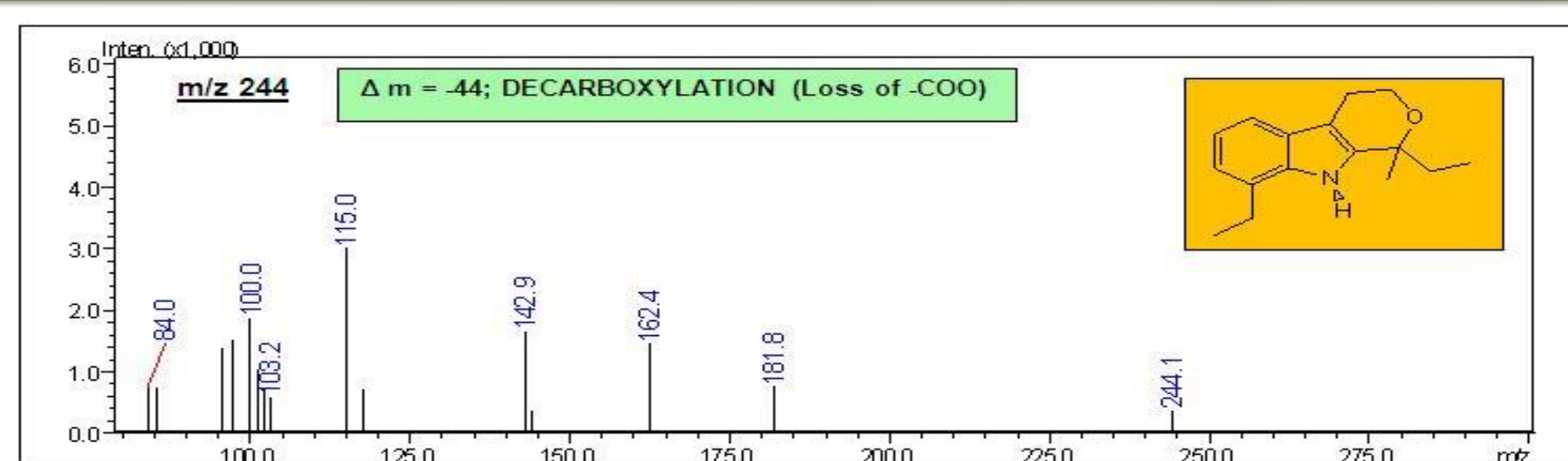
2B. ANALYSIS PARAMETERS USED:

- System Used** : Shimadzu's Triple Quadrupole Mass Spectrometer LCMS-8040
- MS interface** : Electrospray Ionization (ESI)
- Column Used** : Shimpak XR ODS (100 mm * 2mm, 3µ)
- Mobile phase** : Water (pH 3.0, adjusted with formic acid): Acetonitrile, gradient mode

3. RESULTS:



(a) Metabolite 1: m/z 290



(e) Metabolite 5: m/z 244

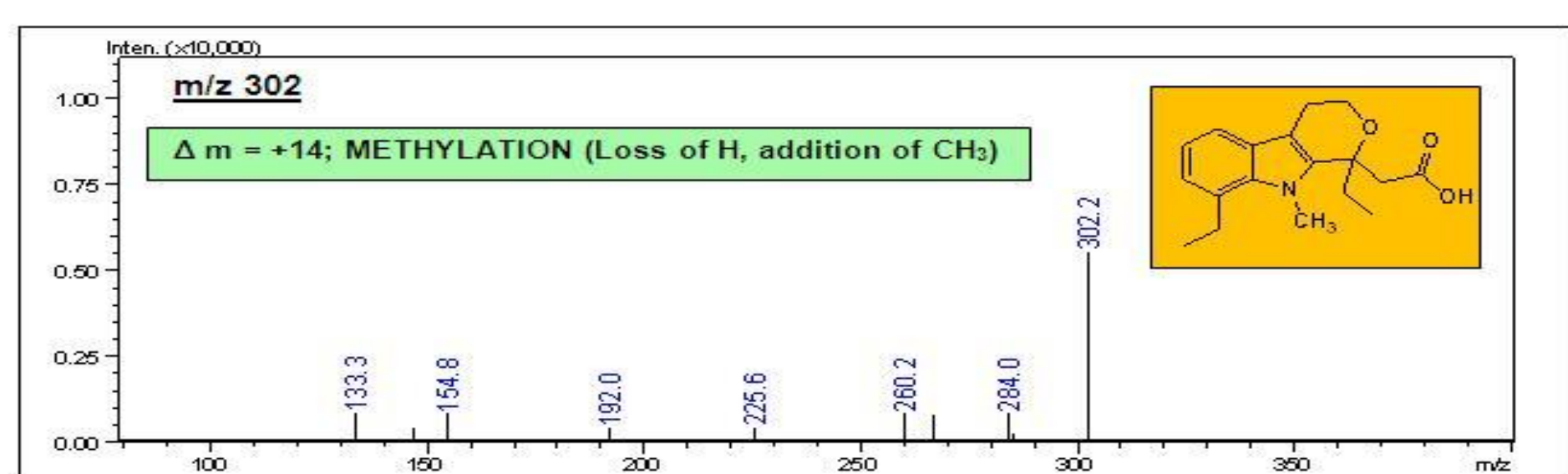
FIGURE 2 (a-e): The probable phase-I reactions, product ion scan of the metabolites obtained and their tentative structures.

4. DISCUSSION AND CONCLUSION:

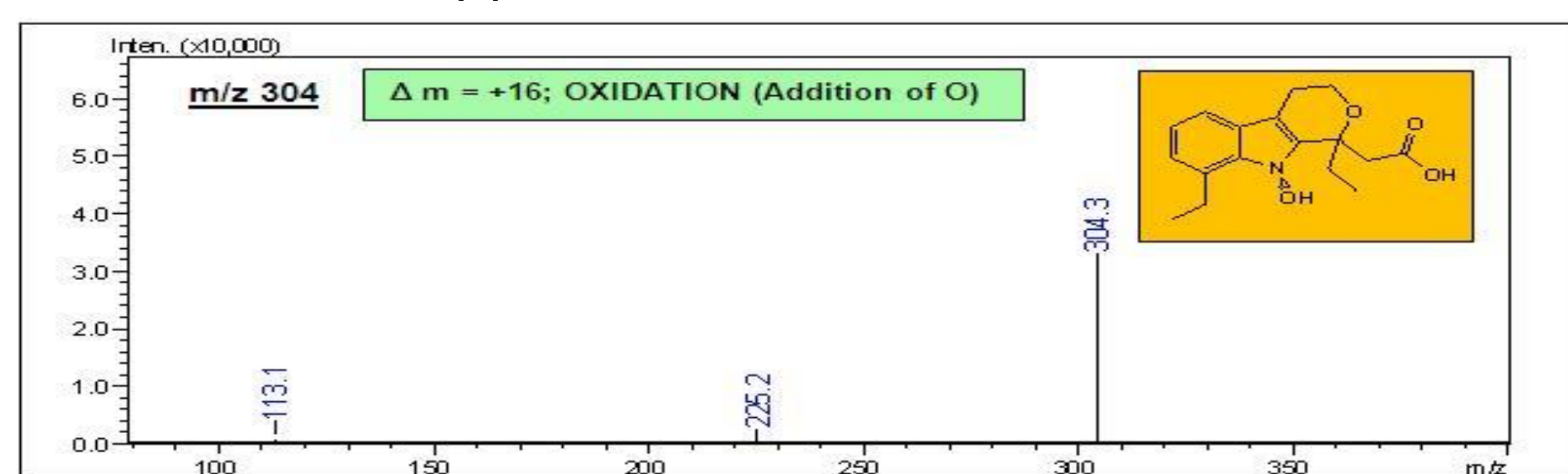
- In the present study, the metabolic profile of Etodolac was investigated in two *in-vitro* models and the tentative structure of the metabolites obtained was elucidated, employing LC-MS/MS technique.
- Mainly phase-I metabolic changes were observed including Hydrogenation (m/z 290), Methylation (m/z 302), Oxidation (m/z 304), N-acetylation (m/z 330) and Decarboxylation (m/z 244) (Fig.2a-e). These metabolites were observed in both the *in-vitro* models used for the study.
- So far, only the hydroxylated metabolites of Etodolac (-OH group at the 5,6,7,8 positions) have been reported^(2,3). The metabolites investigated in this study are not published in any literature till date, to the best of our knowledge.

5. REFERENCES:

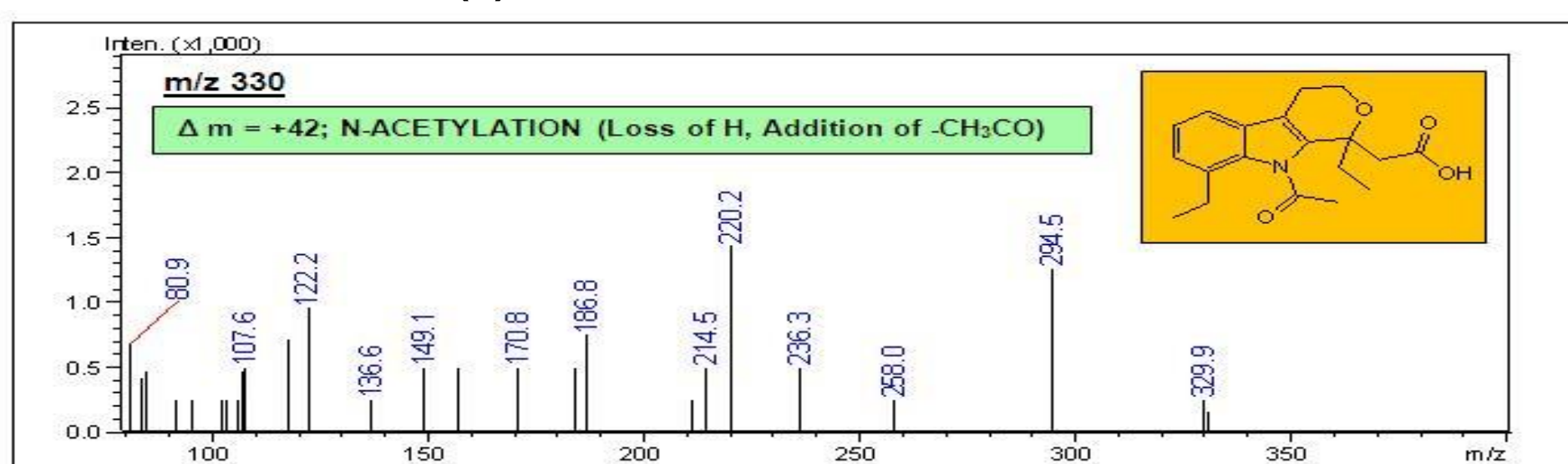
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(b) Metabolite 2: m/z 302



(c) Metabolite 3: m/z 304



(d) Metabolite 4: m/z 330