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INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 11, November 2025, pp : 1598-1603

e-ISSN : 2583-1062
Impact Factor : 7.001

A COMPREHENSIVE REVIEW OF FORCED DEGRADATION STUDIES AND STABILITY INDICATING METHODS FOR PARACETAMOL AND METRONIDAZOLE

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ABSTRACT

In order to guarantee the safety, effectiveness, and quality of drug products over their entire shelf life, stability-indicating methods (SIMs) are an essential part of pharmaceutical analysis. This review thoroughly investigates the forced degradation behavior of two commonly used medications: metronidazole, an antimicrobial and antiprotozoal, and paracetamol, an analgesic and antipyretic. It explores the analytical techniques used to keep an eye on their stability, paying special attention to ultraviolet (UV) spectroscopy and reverse-phase high-performance liquid chromatography (RP-HPLC). In order to give a clear understanding of these techniques' role in quantifying drugs and their degradation products, a detailed explanation of their principles, applications, and practical considerations is provided. This article highlights the value of strong SIMs in directing formulation development and creating suitable storage conditions for these necessary medications by combining results from recent research.

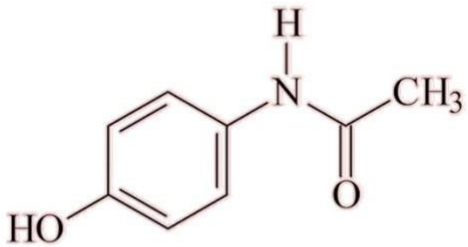
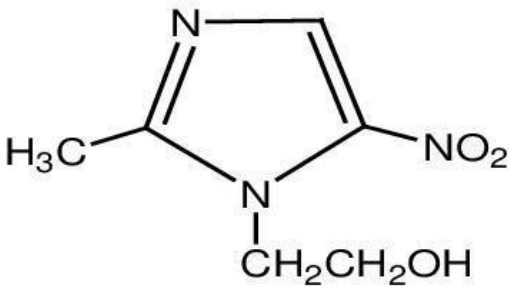
Keywords: Forced Degradation, Stability, Paracetamol, Metronidazole, UV Spectroscopy.

1. INTRODUCTION

Pharmaceutical products are prone to chemical deterioration over time, which can result in the development of potentially hazardous impurities, a decrease in bioavailability, and a loss of potency[1]. Therefore, forced degradation studies also referred to as stress testing—are an essential component of drug development. In these investigations, a drug substance is purposefully exposed to extreme environments, including heat, light, oxidation, acid, and base, in order to produce degradation products and clarify the molecule's intrinsic stability profile[5]. Developing a stability-indicating method (SIM), an analytical technique that can precisely measure the active pharmaceutical ingredient (API) while successfully separating and identifying its breakdown products, is the main objective[4].

Both metronidazole and paracetamol, which are included in the World Health Organization's List of Essential Medicines, are the subject of this review. For the safe of public health, it is essential to comprehend their degradation pathways. We also give a thorough look at two important analytical methods, RP-HPLC and UV spectroscopy, that are often used for these kinds of tests. We explain how they work and why they are so good for stability testing.

FORCED DEGRADATION BEHAVIOR OF PARACETAMOL AND METRONIDAZOLE

PARACETAMOL	METRONIDAZOLE
	
Studies on forced degradation show that paracetamol is especially vulnerable to alkaline, photolytic, and oxidative hydrolysis. It has been demonstrated that TiO_2 photocatalysis may break down over 95% of paracetamol in aqueous solutions in 80 minutes, with the effectiveness of this process depending on the initial drug load, light intensity, and dissolved oxygen concentration [12].	Metronidazole, a nitroimidazole antibiotic, has a slightly distinct degradation profile. It is extremely vulnerable to alkaline hydrolysis, but degrades more slowly under acidic, neutral, or weakly oxidative conditions [17].

Electrochemical oxidation is another effective pathway, completely eliminating both paracetamol and its intermediate oxidation products under controlled pH and current density [13].	A classical kinetic evaluation reported that metronidazole degradation between pH 3.1 and 9.9 follows pseudo-first-order kinetics, with catalysis attributed to H^+, OH^-, and water molecules [18].
Base-catalyzed hydrolysis plays a key role in its instability, with UV-spectrophotometric stress studies confirming reduced recovery compared to untreated controls [14].	Photolytic degradation alone is relatively limited, but UV/H_2O_2 advanced oxidation dramatically accelerates breakdown, where hydroxyl radicals act as the main reactive species responsible for up to 90% of the degradation [19].
Ozonation in alkaline conditions achieves approximately 85% degradation within 30 minutes, suggesting hydroxyl radical formation as the dominant pathway [15].	Oxidative stress conditions such as exposure to hydrogen peroxide also cause measurable drug loss, and recent catalytic wet peroxide oxidation (CWPO) studies using metal–organic framework catalysts report complete metronidazole removal in less than 20 minutes under optimized conditions [23].
In addition, dielectric barrier discharge plasma and solar photocatalysis yield low-molecularweight products such as quinones, aldehydes, and carboxylic acids, confirming that paracetamol undergoes stepwise oxidative fragmentation under severe stress [16].	Thermal stress studies show that metronidazole is stable under dry heat, but prolonged exposure to moist heat and elevated temperatures leads to detectable breakdown [21].

The forced degradation profiles of metronidazole and paracetamol, when combined, show how different their stability issues are. While metronidazole degrades more slowly but is driven by pH and radicals, paracetamol oxidizes quickly and breaks down into smaller aromatic byproducts when broken down by a base. These understandings are crucial for creating analytical techniques that indicate stability, ensuring the robustness of formulations, and creating efficient storage

CORE ANALYTICAL TECHNIQUES FOR STABILITY ASSESSMENT

Detection of Impurities in Paracetamol and Metronidazole by UV Spectroscopy

Introduction and Principle

Ultraviolet (UV) Spectroscopy is a valuable tool for quantifying specific, known impurities in Active Pharmaceutical Ingredients (APIs). Unlike chromatographic methods like HPLC, basic UV spectroscopy cannot separate mixtures. Therefore, its use in impurity analysis is limited to scenarios where the impurity has a distinct spectral feature that does not overlap with the main API, allowing for selective detection and quantification.

The fundamental principle relies on the Beer-Lambert Law: $A = \varepsilon * b * c$, where:

· A = Absorbance

· ε = Molar Absorptivity (L mol^{-1} cm^{-1})

· b = Path length (cm)

· c = Concentration (mol L^{-1})

By measuring the absorbance at a wavelength where the impurity absorbs significantly and the API has minimal or no absorption, the concentration of the impurity can be directly determined.

Part 1: **Detection of p-Aminophenol in Paracetamol**

p-Aminophenol (PAP) is the primary and most critical impurity in Paracetamol. It arises from the hydrolysis of the amide bond and is a known degradation product and precursor.

Principle of the Method: Paracetamol shows maximum absorption at around 243-245 nm.pAminophenol, however, has a significant absorbance peak at approximately 310-330 nm, where Paracetamol has very low absorbance. This allows for the direct measurement of PAP without significant interference from the parent compound.

Procedure:

1. **Standard Preparation:**

· Accurately weigh about 50 mg of high-purity p-Aminophenol standard and transfer it to a 100 mL volumetric flask.

· Dissolve and dilute to volume with the chosen solvent (e.g., 0.1 M NaOH or methanol) to obtain a stock solution of 500 µg/mL.

· Further dilute this solution to prepare a working standard solution of 5 µg/mL.

2. Test Solution Preparation:

· Accurately weigh about 500 mg of the Paracetamol API sample and transfer it to a 100 mL volumetric flask.

· Dissolve and dilute to volume with the same solvent used for the standard. The concentration of the test solution is 5 mg/mL (5000 µg/mL).

3. Instrumental Analysis:

· Use a double-beam UV-Visible spectrophotometer with matched quartz cells (1 cm path length).

· Set the wavelength range from 280 nm to 350 nm.

· Scan the solvent blank to baseline the instrument.

· Scan the p-Aminophenol working standard (5 µg/mL). A distinct peak at ~310-330 nm should be observed. Note the exact wavelength of maximum absorbance (λmax).

· Scan the Test Solution (5000 µg/mL) directly.

4. Calculation:

The concentration of p-Aminophenol in the Paracetamol sample is calculated by comparing the absorbance of the test solution at the λmax of PAP with that of the standard. Formula: % of p-Aminophenol = (A_u / A_s) * (C_s / C_u) * 100

Where:

· A_u = Absorbance of the Test Solution at λmax of PAP

· A_s = Absorbance of the p-Aminophenol Standard at its λmax

· C_s = Concentration of the p-Aminophenol Standard (µg/mL)

· C_u = Concentration of the Paracetamol Test Solution (µg/mL)

Example: If A_u = 0.125, A_s = 0.550, C_s = 5 µg/mL, and C_u = 5000 µg/mL: % PAP = (0.125 / 0.550) * (5 / 5000) * 100 = 0.0227%

Validation Parameters:

· Specificity: Confirm that a placebo or pure Paracetamol solution shows no significant absorbance at the λmax of PAP.

· Linearity: Prepare a series of PAP standards (e.g., 1, 3, 5, 7, 10 µg/mL) and verify a linear relationship ($R^2 > 0.995$) between concentration and absorbance.

· Limit of Detection (LOD) & Quantification (LOQ): Typically, this method can quantify PAP down to ~0.02%.[25-28].

Part 2: Detection of 2-Methyl-5-nitroimidazole in Metronidazole

2-Methyl-5-nitroimidazole (2-MNI) is a key starting material and potential impurity in Metronidazole.

Principle of the Method:

Metronidazole has a characteristic UV spectrum with λmax around277 nm and 320 nm. The impurity 2-Methyl-5-nitroimidazole also absorbs in the UV region but has a different spectral profile. The method often involves measuring the absorbance of the test solution at a wavelength (e.g., 260 nm or 310 nm) where the difference in absorptivity between the impurity and the API is greatest, as defined by pharmacopoeial methods.

Procedure (Based on Pharmacopoeial Methods):

1. Standard Preparation:

· Accurately weigh about 25 mg of 2-Methyl-5-nitroimidazole standard and transfer it to a 250 mL volumetric flask.

· Dissolve and dilute to volume with a 0.1 M hydrochloric acid (HCl) solution to obtain a stock solution of 100 µg/mL.

· Pipette 5 mL of this stock solution into a 100 mL volumetric flask and dilute to volume with

0.1 M HCl to obtain a working standard of 5 µg/mL.

2. Test Solution Preparation:

· Accurately weigh about 100 mg of the Metronidazole API sample and transfer it to a 100 mL volumetric flask.

· Dissolve and dilute to volume with 0.1 M HCl. The concentration is 1 mg/mL (1000 µg/mL).

3. Instrumental Analysis:

· Use a double-beam UV-Visible spectrophotometer with matched quartz cells (1 cm path length).

· Set the instrument to measure absorbance at the specified wavelength, typically 260 nm or 310 nm.

· Zero the instrument with a 0.1 M HCl blank.

· Measure the absorbance of the 2-MNI working standard (5 µg/mL).

· Measure the absorbance of the Test Solution (1000 µg/mL) at the same wavelength.

4. Calculation:

The calculation is similar to the one for Paracetamol. Formula: % of 2-MNI = (Au / As) X (Cs / Cu) X 100 Where:

· Au = Absorbance of the Metronidazole Test Solution

· As = Absorbance of the 2-MNI Standard

· Cs = Concentration of the 2-MNI Standard (µg/mL)

· Cu = Concentration of the Metronidazole Test Solution (µg/mL)

Example: If Au = 0.080, As = 0.400, Cs = 5 µg/mL, and Cu = 1000 µg/mL: % 2-MNI = (0.080 / 0.400) X (5 / 1000) X 100 = 0.10%

Important Considerations for Metronidazole:

· **Wavelength Selection**: The exact wavelength is critical and must be strictly adhered to as per the referenced pharmacopoeia method. The absorptivity of both the API and the impurity changes rapidly with wavelength in this region.

· **Solvent**: Using 0.1 M HCl provides a stable and consistent acidic medium for both compounds

[24-28].

2. HPLC METHODS FOR PARACETAMOL IMPURITY PROFILING

The following procedure is designed to separate and quantify Paracetamol (PAR) from three of its toxic impurities simultaneously.

Key Impurities: p-aminophenol (PAP), p-nitrophenol (PNP), and p-chloroacetanilide (PCA).

· **Analytical Column:** Xterra C8 column (150 mm × 4.6 mm, 5 µm)

· **Mobile Phase**: Isocratic mixture of acetonitrile and phosphate buffer (pH 7.5) in a 30:70 (v/v) ratio.

· **Flow Rate**: 0.7 mL/min.

· **Detection**: UV at 220 nm.

· **Injection Volume:** 50 µL.

· **Sample Concentration**: Working standard solutions of 100 µg/mL for each component, prepared in methanol.

This method has been validated as per ICH guidelines. The impurities PAP, PNP, and PCA are particularly hazardous, with regulatory limits set due to their toxic (teratogenic, hepatotoxic) effects.

For faster analysis, UHPLC methods using C18 columns with sub-2µm particles (e.g., 1.8 µm) can separate Paracetamol and up to ten impurities in under 2 minutes with a gradient elution.

3. HPLC METHODS FOR METRONIDAZOLE IMPURITY PROFILING

A modern, green HPLC method has been developed for the assay of Metronidazole and its related substances, suitable for quality control.

· **Key Impurity:** Metronidazole related impurity-A (also known as Tinidazole related compound A).

· **Analytical Column**: Zorbax SB C8 column (150 mm × 4.6 mm, 3.5 µm) is recommended for related substance testing.

· **Mobile Phase**: Isocratic mixture of isopropyl alcohol (IPA) and water in a 20:80 (v/v) ratio. This is noted as a more environmentally friendly approach.

· **Flow Rate:** 1.0 mL/min.

· **Detection**: UV at 315 nm.

· **Injection Volume**: 10 µL.

This method demonstrates excellent resolution (Rs = 9.0) between Metronidazole and its key impurity. The method is linear for Metronidazole from 75–225 µg/mL and for its impurity A from

0.055–7.5 µg/mL.[4,7].

Table 1: Comparison of RP-HPLC and UV Spectroscopy for Stability Testing Feature RP-HPLC UV Spectroscopy

FEATURE	RP-HPLC	UV SPECTROPHOTOMETRY
Principle	Separation based on hydrophobicity followed by detection	Direct measurement of light absorption by the sample.
Specificity	High (can resolve multiple components in a mixture).	Low (measures total absorbance, cannot distinguish co-eluting compounds).
Quantification	Accurate for individual components in a mixture	Accurate only for pure substances or simple mixtures.
Sensitivity	High (detects low concentrations).	Moderate to High.
Cost & Complexity	Higher cost and operational complexity.	Lower cost and simple to operate.

4. CONCLUSION

Forced degradation studies provide invaluable insights into the stability profiles of pharmaceutical compounds.For widely used drugs like paracetamol and metronidazole, understanding their degradation pathways is essential for ensuring patient safety. While UV spectroscopy offers a quick and economical tool for certain quantitative analyses, RP-HPLC stands as the gold standard for developing stability-indicating methods. Its unparalleled ability to separate, identify, and quantify a drug from its degradation products makes it an indispensable technique in the pharmaceutical analyst's toolkit. The continuous development and validation of such robust methods are paramount for maintaining the high quality, safety, and efficacy of pharmaceutical products in the market.

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