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Original research paper

Impact of peroxide content in excipients and antioxidants on famotidine oxidative stability

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ABSTRACT

Famotidine, a widely used H_2-receptor antagonist, exhibits sensitivity to oxidative degradation, particularly in the presence of excipients containing peroxide impurities. This study explores the oxidative stability of famotidine under various storage conditions, with a specific focus on excipients with varying peroxide contents. A stability-indicating liquid chromatography-mass spectrometry (LC-MS) method was developed to identify and quantify famotidine degradation products, providing detailed insights into oxidative pathways. In addition, Zeneth software was employed to predict potential degradation products, and its predictive accuracy was evaluated against experimental findings. Antioxidants, including ascorbic acid, propyl gallate, and ethylenediaminetetraacetic acid (EDTA), were incorporated into compressed compatibility mixtures to assess their effects on peroxide-mediated degradation. While propyl gallate and EDTA consistently reduced peroxide levels and enhanced stability, ascorbic acid unexpectedly acted as a pro-oxidant under stress conditions, accelerating peroxide formation in povidone. These findings provide critical insights into mitigating oxidative degradation in famotidine and other solid dosage forms, emphasizing the importance of selecting appropriate excipients, antioxidants, and predictive tools to ensure product stability.

Keywords: peroxides, hydrogen peroxide, famotidine, Zeneth

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INTRODUCTION

Famotidine is an H_2 blocker that reduces the production of stomach acid. It is used to treat ulcers in the stomach and intestines, gastroesophageal reflux disease (GERD), erosive esophagitis, and hypersecretory conditions like Zollinger-Ellison syndrome. Famotidine works by blocking H_2 receptors in the stomach lining, thereby inhibiting histamine and decreasing acid production (1, 2).

In pharmaceutical development, the stability of an active pharmaceutical ingredient (API) and its formulations is a critical aspect of product quality, safety, and efficacy (3). A comprehensive understanding of stability is achieved through stability-indicating methods and forced degradation studies, which are essential in both the research and regulatory

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phases of drug development (4). Stability-indicating methods are specifically designed analytical techniques that can accurately differentiate between the intact API and its potential degradation products, excipients, and impurities. These methods are validated for their accuracy, precision, selectivity, and robustness to ensure they reliably track the stability profile of the API across various conditions (5, 6).

Prior to developing a new method, *in silico* tools offer a valuable means to assess the preliminary stability of APIs. Literature data, when available, can provide critical insights into stability profiles. However, for novel APIs, such data is often not publicly available. In these instances, reliance on foundational chemical knowledge becomes essential. Predictive tools, which have gained significant traction in recent years, particularly within the pharmaceutical industry, serve as a crucial resource. These tools can forecast degradation products of APIs, offering an initial evaluation of molecular stability under various stress conditions. For more information, see Supplementary materials.

Forced degradation studies, also known as stress testing, are a foundational part of stability testing, involving the deliberate exposure of the API and its formulation to extreme environmental conditions. Common stress factors include high temperatures, acidic and alkaline hydrolysis, oxidation, photolysis (light exposure), and high humidity (7). These conditions simulate potential degradation scenarios and accelerate the breakdown of the API, allowing researchers to identify degradation pathways and the resultant impurities. The insights gained from forced degradation studies are instrumental in developing stability-indicating methods, which must be capable of detecting and quantifying all relevant degradation products alongside the API (8). Together, stability-indicating methods and forced degradation studies enable researchers to define the stability characteristics of a pharmaceutical product, guiding the determination of optimal storage conditions, protective packaging, and expiration dating. These studies also provide critical data for regulatory submissions, satisfying requirements that ensure drugs remain safe and effective throughout their shelf life (3). Ultimately, the integration of these methods into the development process supports a thorough understanding of a drug's stability, enhancing product quality and patient safety. As such, stability-indicating methods and forced degradation studies are indispensable tools in modern pharmaceutical science, underpinning the rigorous standards required for drug approval and commercialisation (9).

In the development of new pharmaceutical products, a compatibility study with various excipients is essential. This process involves preparing blends or compressed mixtures of the API and excipients, which are then subjected to stress stability testing. The objective is to determine whether the API exhibits sensitivity to specific excipients and to identify any excipient components that may contribute to degradation (10–12).

Oxidation, following hydrolysis, is the second most prevalent degradation mechanism in the pharmaceutical industry. Oxidative degradation can be complex, often generating a range of degradation impurities (13). This study focuses on excipients with impurities known to induce oxidation in the API, particularly due to peroxides. These peroxides are critically significant in pharmaceutical formulations, as their control is necessary to prevent oxidation-related impurities from surpassing acceptable limits during the product's shelf life.

Traditionally, analytical methods have only quantified total peroxide content, measuring both hydrogen peroxide and organic peroxides collectively. However, to gain a more refined understanding of the distinct behaviors of hydrogen and organic peroxides – specifically their formation and decomposition under various conditions – an analytical

method capable of distinguishing these two classes of peroxides individually is required. For this purpose, a novel analytical method was developed, enabling separate detection and quantification of hydrogen peroxide and organic peroxides, thereby offering a more precise evaluation of oxidative stability in pharmaceutical excipient-API combinations.

This study investigates the oxidative degradation of famotidine in the presence of excipients with differing peroxide levels, aiming to elucidate the impact of hydrogen and organic peroxides on the stability of this API. A stability-indicating LC-MS method was developed to identify and quantify famotidine degradation products. Concurrently, *in silico* prediction tools were employed to forecast potential degradation pathways, and their reliability was evaluated against experimental data. The role of antioxidants, including ascorbic acid, propyl gallate, and EDTA, in modulating peroxide levels and oxidative degradation was systematically assessed under stress conditions. This comprehensive analysis provides insights into peroxide dynamics and the role of antioxidants in mitigating oxidative degradation in solid dosage forms.

EXPERIMENTAL

Chemicals and reagents

The following chemicals were used without any additional purification: hydroxydi-*p*-tolylborane (Toronto Research Chemicals, Canada), *p*-cresol (Sigma-Aldrich, USA), triphenylphosphine oxide (Sigma-Aldrich), triphenylphosphine (Merck KGaA, Germany), famotidine sulfoxide, famotidine (SynZeal, India).

For preparing the mobile phases the following solvents and chemicals were used: gradient grade acetonitrile, methanol and analytical grade 30 % aqueous hydrogen peroxide (J. T. Baker, Netherlands), analytical grade formic acid, hydrochloric acid solution, 4,4-azobis(4-cyanovaleric acid), copper(II) sulfate pentahydrate, di-*tert*-butyl peroxide and *tert*-butyl benzoat (Merck, Germany), ammonium acetate and ammonium formate (Honeywell, Germany), orto-phosphoric acid 85 % (Supelco Inc Germany), potassium phosphate dibasic (Honeywell, USA), potassium phosphate monobasic, iron(III) chloride, sodium hydroxide (Sigma-Aldrich). Purified water was obtained by a Milli-Q-POD® system (Merck Millipore, USA).

Equipment and software

Liquid chromatography (LC) analyses were performed on Vanquish UHPLC system (Thermo Fisher Scientific, USA) equipped with a binary solvent manager (BSM), a sample manager (SM), a temperature-controlled column compartment and a photodiode array (PDA) detector coupled with an Orbitrap Fusion Tribrid Mass Spectrometer. Thermo Fischer LC systems were equipped with Xcalibur™ chromatography data software (Thermo Fisher Scientific). Degradation products were predicted by Zeneth version 10 (Lhasa Limited, Leeds, United Kingdom).

The weighing was done on either an XPE205 analytical balance or an MX5 microbalance (Mettler Toledo, USA). pH was measured using a SevenMultiTM pH meter (Mettler Toledo). Pipettes used were HandyStep® touch automatic pipettes (Brand, Germany). The ultrasonic bath used was Sonic 20 (Iskra Pio, Slovenia). Magnetic stirrers used were IKA RO 15 (IKA®-Werke GmbH & Co. KG, Germany).

Conditions of forced degradation study

All samples were prepared in a dark room. Approximately 100 mg of famotidine was accurately weighed and transferred into a 100.0 mL volumetric flask, then dissolved in acetonitrile to obtain a stock solution with a concentration of approximately 1.0 mg mL^{-1}. From this stock solution, 10.0 mL was mixed with 10.0 mL of a selected stress medium. A total of nine different aqueous stress media were evaluated: 0.2 mol L^{-1} HCl, 0.2 mol L^{-1} NaOH, 0.6 % H_2O_2, and solutions containing oxidative agents at a molar concentration equivalent to 40 % of that of famotidine. These oxidative agents included 4,4'-azobis(4--cyanopentanoic acid) (ACVA), $FeCl_3$, $CuSO_4$, *tert*-butyl peroxybenzoate, di-*tert*-butyl peroxide, and cumene hydroperoxide. The samples were poured into HPLC crimp vials, the vials were sealed and put in a standard incubator chamber with a regulated temperature of 40 ± 2 °C. The samples were sampled at three different time points (4, 24 and 48 hours).

LC-MS conditions and parameters

High-performance liquid chromatography-mass spectrometry (HPLC-MS) was performed using a reverse-phase Xbridge C18 column (3.5 μm particle size, 4.6 mm diameter, 150 mm length). The operational parameters included a 1 μL injection volume. Gradient elution employed a mobile phase of 0.1 % formic acid in water and acetonitrile. The initial isocratic phase consisted of 5 % acetonitrile, which increased to 30 % between 3.0 and 10.0 minutes, followed by 2 minutes at 30 % acetonitrile, with the final 3 minutes dedicated to equilibration. The column temperature was maintained at 40 °C, the autosampler temperature was maintained at 10 °C, and the flow rate of the mobile phase was 1.0 mL min^{-1}. The parameters for the mass spectrometer were as follows: for full MS spectra, the scan range was *m*/*z* 100 to 600 with a resolution of 120.000 at *m*/*z* 200, the resolution for MS/MS was 15.000 at *m*/*z* 200. The RF (radio frequency) Lens was set to 60 %, and the maximum injection time was 50 ms. The AGC (automatic gain control) target was set to 20.000. Typically, the ion source spray voltage was set to 3.5 kV, the vaporiser temperature to 320 °C, and the sheath and auxiliary nitrogen flow rates to 60 and 20 arbitrary units, respectively.

RESULTS AND DISCUSSION

Development of the stability indicative method

Famotidine, with high polarity (logD = –1.5 at pH 7.5), elutes rapidly on reverse-phase columns. A method using an initial gradient with 5 % acetonitrile was developed to optimise separation. Degradation products, which are usually more polar and have a smaller molecular weight, tend to elute near the dead volume. Maintaining a low organic phase enabled adequate retention, resolving famotidine and its degradation products. The UV (ultraviolet) spectrum showed a maximum absorption (λ_{max}) at 260 nm, which was selected for HPLC detection. Optimised chromatographic conditions were established, effectively separating famotidine and degradation products.

Under various stress conditions, famotidine degraded into nine primary degradants, labelled IMP-1 through IMP-9 (Fig. 1), corresponding to the sequence in which peaks appeared from left to right in the chromatogram. We focused on major degradants, defined as those constituting at least 0.1 % of the total degradation profile. Fig. 2 shows the struc-

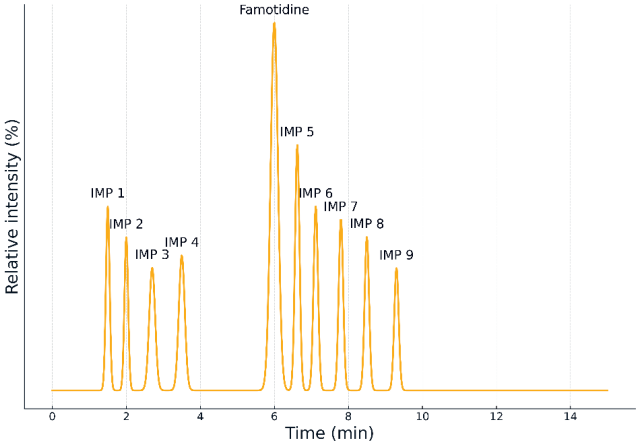

Fig. 1. Schematic chromatogram of famotidine with impurities from stress testing

tures of individual impurities, incorporating those associated with the terminologies of the European Pharmacopoeia (Ph. Eur.).

Degradation products

The degradation products were systematically characterised using high-resolution mass spectrometry (HRMS) and detailed mass fragmentation analysis. Proposed structures for each identified degradation product were deduced based on the mass spectral data, providing insights into their molecular composition and fragmentation pathways. Detailed mass spectra and structural proposals for all degradation products are available in the Supplementary Materials.

Under neutral conditions, degradation analysis of famotidine identified impurities IMP-5, IMP-6, IMP-7, and IMP-9, with an overall impurity formation of 2.6 % observed after 48 hours at 40 °C. This limited impurity formation under neutral pH conditions suggests that famotidine is relatively stable in non-reactive environments, exhibiting minimal degradation over the study period.

Under acidic conditions, degradation patterns shifted: impurities IMP-5, IMP-6 and IMP-8 were identified, with complete degradation of famotidine into IMP-6 within 24 hours. Notably, IMP-6 subsequently degraded further, resulting in the formation of IMP-8 over time, indicating an ongoing breakdown cascade within acidic conditions (Fig. 3).

Under alkaline conditions, an extensive degradation profile was observed, with impurities IMP-1, IMP-3, IMP-5, IMP-6, IMP-7, IMP-8, and IMP-9 detected within 24 hours. Similar to acidic conditions, famotidine underwent complete degradation within 48 hours. Among the degradation products, IMP-5 emerged as the predominant species, indicating that alkaline environments significantly accelerate famotidine breakdown. This enhanced degradation is likely mediated by hydrolytic or base-catalysed reactions, facilitating the formation of multiple degradation products. The findings underscore the susceptibility of famotidine to alkaline-induced chemical instability and highlight the formation of a complex impurity profile under such conditions (Fig. 4).

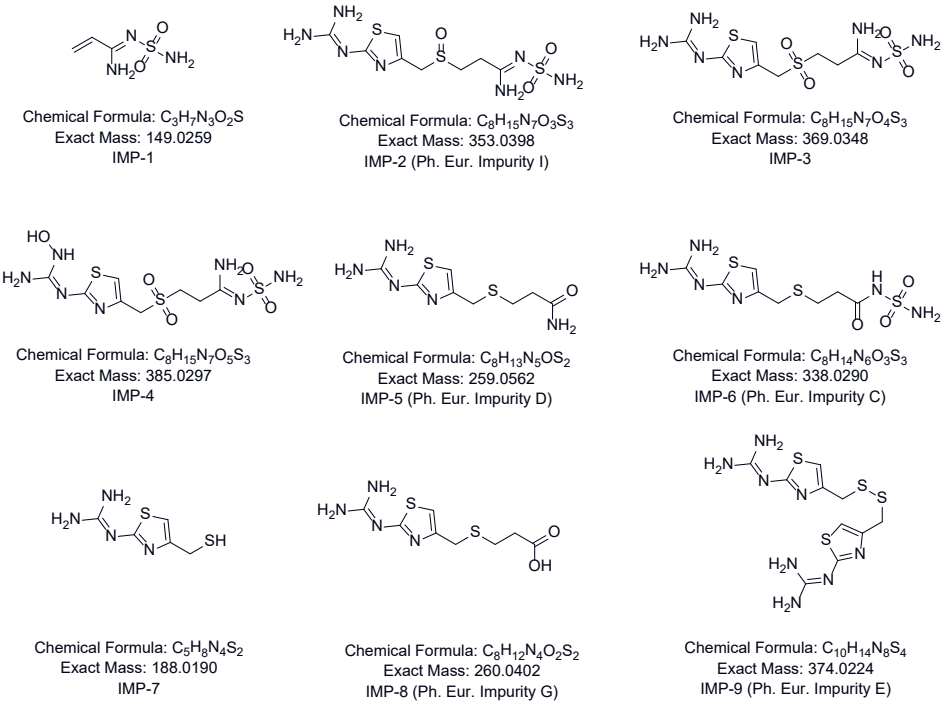

Fig. 2. Famotidine impurities.

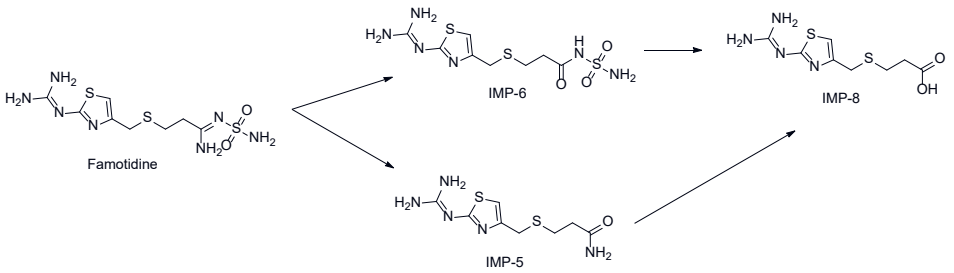

Fig. 3. Degradation of famotidine in acidic conditions (0.1 mol L^{-1} HCl).

Under conditions of oxidative stress induced by hydrogen peroxide, famotidine underwent rapid and complete degradation into impurities IMP-2 and IMP-3 within 4 hours at room temperature. Over time, IMP-2 was entirely converted into IMP-3, and IMP-4 formation was observed after 48 hours, indicating a sequential degradation pathway. Notably, no oxidative impurities were detected when famotidine was exposed to other radical oxidative stressors, including ACVA, di-*tert*-butyl peroxide, *tert*-butyl benzoate, and cumene peroxide. This highlights hydrogen peroxide as a uniquely potent oxidative agent for famotidine, driving specific degradation pathways not observed with other oxidative stressors (Fig. 5).

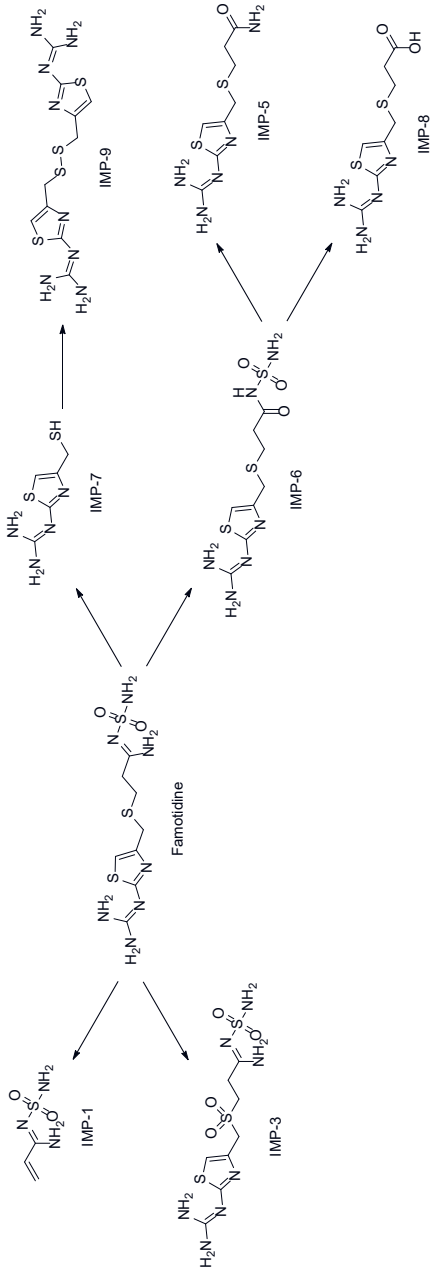

Fig. 4. Degradation of famotidine in alkaline conditions (0.1 mol L^{-1} NaOH).

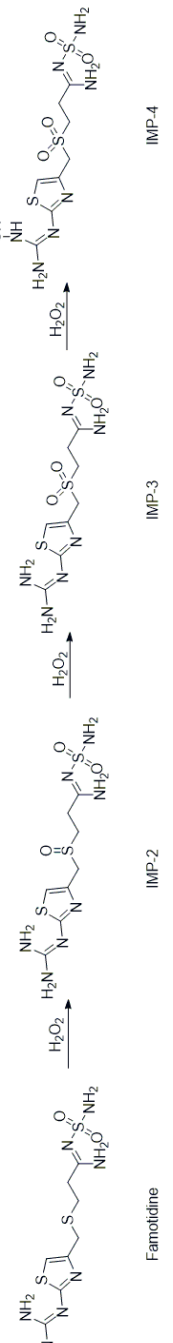

Fig. 5. Degradation of famotidine with hydrogen peroxide.

This comprehensive profiling of degradation behaviours under various conditions provides critical insights into the stability and transformation pathways of famotidine. Furthermore, famotidine sulfoxide, a known oxidative degradation product, was obtained from commercial sources to enable a direct comparison with the impurity profiles generated in oxidative stress tests. This comparison confirmed the presence and stability of famotidine sulfoxide in the oxidative degradation profile, supporting its identification as a primary oxidation by-product in stressed conditions.

Famotidine, an extensively studied and well-characterised API described in the Ph. Eur., served as the basis for a comparative analysis of impurities. This study juxtaposed the impurities specified in the famotidine monograph with those identified in the active substance. While the Pharmacopoeia 11th edition monograph largely aligns with the identified impurities, it does not account for certain additional impurities. Notably, two innovative impurities, designated as IMP-1 and IMP-7, were identified exclusively within the famotidine substance. Interestingly, IMP-7 is absent from the Pharmacopoeia specifications despite its significant presence under alkaline conditions, where its content exceeds 1 %. This discrepancy underscores the importance of ongoing impurity profiling to ensure comprehensive quality control and alignment with modern manufacturing conditions.

In silico *prediction of degradation products*

To aid in the identification of major degradation products and to evaluate its predictive accuracy and reliability, we utilised Zeneth software from the outset of the study. This approach provided a preliminary assessment of famotidine's chemical stability, enabling an early understanding of its susceptibility to degradation under various conditions.

Under neutral conditions at 40 °C, the Zeneth did not predict the formation of any degradation products. However, experimental analysis revealed the presence of impurities IMP-5 and IMP-9. After 48 hours, only 1 % of both degradation products were detected, indicating minimal degradation. This low level of degradation likely explains why Zeneth did not predict the formation of these products under the given conditions.

Under acidic conditions, Zeneth predicted the formation of three degradation products. However, none of these predicted impurities were observed during experimental analysis under acidic conditions. Interestingly, two of these impurities were later detected under alkaline conditions, suggesting that their formation may be more favourable in an alkaline environment rather than in acidic media.

Under alkaline conditions, famotidine exhibits the highest sensitivity to degradation, a finding corroborated by predictions from the Zeneth program. However, the experimentally observed yields of the predicted impurities were lower than expected, with only the formation of IMP-1 and IMP-7 being confirmed. Notably, while Zeneth primarily predicts degradation targeting the thiazole ring, experimental results indicate that the majority of degradation occurs at the *N*'-sulfamoylpropanimidamide moiety of the molecule.

Under oxidative conditions, the Zeneth program predicted the formation of two degradation impurities. Experimental analysis, however, identified three degradation products (IMP-2, IMP-3, and IMP-4), with the formation of the first two being confirmed as genuine. This suggests that, while Zeneth accurately predicts some degradation pathways, additional products may form under experimental conditions. For more information, see Supplementary materials.

Influence of antioxidants on the peroxide content

Given that oxidative impurities are major decomposition products, we conducted an in-depth investigation into the influence of excipients on the oxidation of famotidine. To achieve this, a specialised analytical method was employed to quantify hydrogen peroxide and organic peroxides, as no comparable analyses appear in existing literature. Additionally, we examined the effects of various antioxidants on peroxide levels within excipients. For this purpose, excipient mixtures were prepared with different antioxidant classes (ascorbic acid, propyl gallate, butylated hydroxytoluene (BHT)/butylated hydroxyanisole (BHA), and EDTA), covering reducing agents, radical reaction inhibitors, and chelating agents. Due to the large number of samples requiring stress testing and subsequent analysis, only a single replicate was included per condition.

Povidone was selected as a key excipient due to its susceptibility to peroxide formation; two grades were used, one with lower and one with higher peroxide content, alongside hydroxypropyl cellulose (HPC) and microcrystalline cellulose (MCC). A 5 % (*m*/*m*) ratio of each antioxidant to excipient was utilised. Samples were subjected to stress conditions, both with and without antioxidants, and each was prepared in triplicate under three different environments: open to air, oxygen atmosphere, and nitrogen atmosphere. This approach enabled a comprehensive evaluation of antioxidant effects under oxidative stress. Detailed protocols for excipient preparation and handling are provided in the Supplementary material.

The peroxide content in HPC and MCC was found to be minimal, with no significant changes in levels of hydrogen peroxide or organic peroxides observed even under stress conditions. These findings suggest that HPC and MCC are not critical contributors to the oxidative degradation of API, as they do not contain or generate compounds that facilitate hydrogen peroxide or organic peroxide formation when subjected to stress.

The behavior of peroxide content in povidone LP (low peroxide) under stress conditions presents a notable distinction between hydrogen peroxide and organic peroxides (Fig. 6). In povidone LP, which initially contains fewer peroxides, most of the total peroxides arise from hydrogen peroxide, with organic peroxides contributing only minimally. When subjected to the first stress condition (60 °C/75 % relative humidity (RH) for one week), a marked increase in peroxide content was observed in open containers. This trend persisted in other open conditions, particularly after 14 days at 50 °C/75 % RH and after one month at 40 °C/75 % RH, though the rate of increase gradually diminished with decreasing temperature and extended exposure time. Interestingly, at the earliest high-temperature time points, when samples were exposed to oxygen, there was a significant increase in organic peroxide levels. However, as the temperature decreased and the exposure duration lengthened, the impact of these conditions on peroxide formation diminished. This suggests that elevated temperatures and oxygen exposure are critical factors in organic peroxide formation, with the effect lessening as conditions moderate. Furthermore, in samples purged with nitrogen, the hydrogen peroxide content remained stable across all temperatures. This stability implies that without moisture, essential reactants for initiating peroxide formation, hydrogen peroxide levels did not increase. These results underscore the role of humidity in peroxide formation within povidone LP.

Povidone HP (high peroxide) exhibits a higher total peroxide content than LP povidone, with notably elevated levels of organic peroxides (Fig. 7). Similar to LP povidone, the formation of organic peroxides in HP povidone is strongly influenced by high tempera-

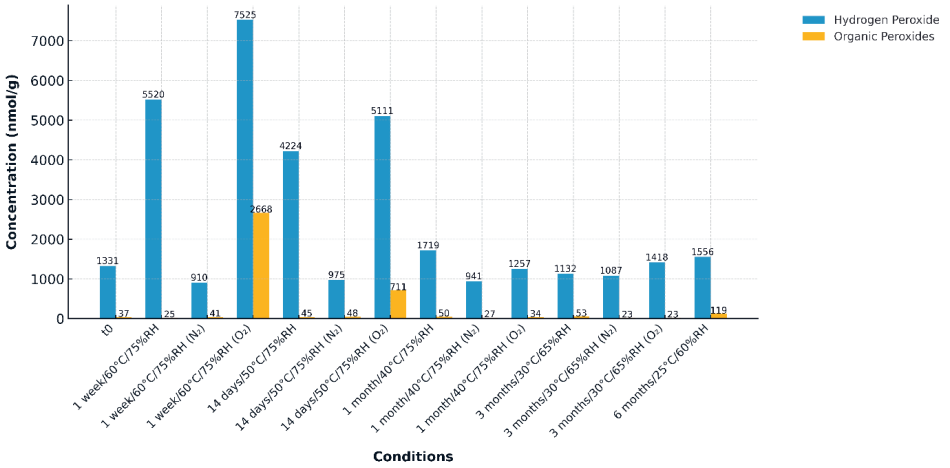

Fig. 6. Content of hydrogen peroxide and organic peroxide in povidone with low peroxides under stress conditions.

tures and the presence of oxygen. However, this effect diminishes at lower temperatures. Additionally, peroxide content in samples stored under nitrogen remains stable, with no significant variation in hydrogen peroxide formation or degradation observed.

Under open conditions, a notable distinction arises between LP povidone and HP povidone regarding peroxide levels. While the peroxide content in LP povidone increases over time, the peroxide levels in HP povidone decrease, irrespective of temperature. This inverse behaviour between the two grades of povidone remains unexplained. Similar observations were reported by Narang *et al.*, who noted a decrease in peroxide content under conditions of higher humidity (14). This phenomenon highlights the complexity of peroxide dynamics in different povidone grades and environmental conditions, warranting further investigation into the underlying mechanisms.

It is possible that hydrogen peroxide formation in LP povidone may be triggered by an undetected oxidative species, potentially catalysed by moisture and high temperatures, which serve as a reactive medium. This peroxide formation gradually diminishes as temperature decreases, eventually becoming negligible at 30 °C. In contrast, HP povidone appears to lack these reactive impurities, with peroxide content declining due to hydrogen peroxide hydrolysis facilitated by moisture.

To simulate typical conditions in the pharmaceutical industry, a final time-point measurement was taken for both samples after six months in a closed container at 25 °C and 60 % RH. This setup reflects real-world conditions where bulk excipients are sampled from large containers, which are exposed to a nitrogen environment but still subject to ambient air and oxygen upon reopening. Results indicate that peroxide content in the closed povidone sample (high and low peroxide) increased by 10 % over six months, a rise that is not as critical as reported in some sources but still suggests incremental oxidative changes during prolonged storage (15, 16). For APIs with higher susceptibility to oxidation, these findings support the use of nitrogen chambers during dispensing to minimise exposure to atmospheric oxygen and mitigate oxidative degradation.

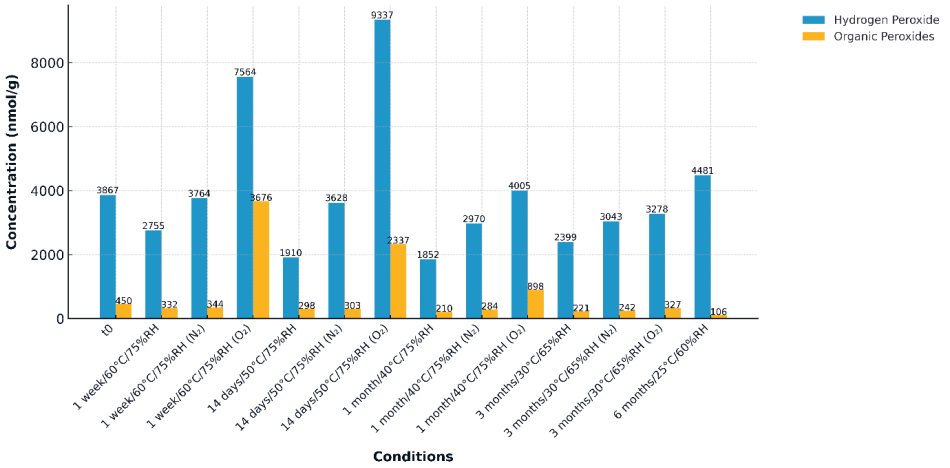

Fig. 7. Content of hydrogen peroxide and organic peroxide in povidone with high peroxides under stress conditions.

In excipients with added antioxidants, the effects of BHT/BHA and EDTA were comparable to those observed in excipients without antioxidants. However, the addition of ascorbic acid resulted in a significant increase in peroxide levels across all excipients, including HPC and MCC, which initially showed no detectable levels of hydrogen peroxide or organic peroxides. This suggests that at higher concentrations, ascorbic acid may exert a pro-oxidative effect, likely generating reactive species that promote hydrogen peroxide formation.

Similarly, the inclusion of propyl gallate also led to an increase in both hydrogen peroxide and organic peroxides. We hypothesise that these elevated levels may be due to an excessive concentration of antioxidants, potentially leading to unintended oxidative reactions. To further investigate, the experiment was repeated with a reduced antioxidant concentration of 1 %, focusing on ascorbic acid, propyl gallate, and EDTA. Since BHT/BHA did not significantly affect peroxide content, it was excluded from further experiments. For consistency, only povidone samples with both high and low initial peroxide levels were used as the excipients in this modified setup.

Reducing the antioxidant concentration did not mitigate the elevated peroxide levels associated with ascorbic acid, which remained high even at lower dosages. Interestingly, in experiments with povidone HP, stress conditions led to a notable increase in organic peroxide formation. However, lower concentrations of propyl gallate and EDTA exhibited a stabilising effect, particularly in povidone samples with initially low peroxide content. In these cases, the peroxide behaviour mirrored that observed in excipients without antioxidant additions: peroxide levels rose in an oxygen-rich environment, remained stable under nitrogen, and decreased in open conditions. This suggests that while ascorbic acid may act as a pro-oxidant under certain conditions, propyl gallate and EDTA can offer stabilisation, especially in excipients with minimal starting peroxide levels.

To evaluate the oxidative stability of famotidine in formulations, we examined the effects of high-peroxide (HP) and low-peroxide (LP) povidones, as well as their mixtures

Table I. Content of famotidine sulfoxide (in ppm) in compacts in closed conditions

	t_0	14 days 50 °C/ 75 %RH	28 days 40 °C/ 75 %RH	3 months 30 °C/ 65 %RH
Famotidine + povidone LP	354	799	637	323
Famotidine + povidone HP	885	2753	2261	1308
Famotidine + povidone LP + 1 % ascorbic acid	412	1526	1238	772
Famotidine + povidone HP + 1 % ascorbic acid	2389	2585	24231	21530
Famotidine + povidone LP + 1 % propyl gallate	349	616	506	348
Famotidine + povidone HP + 1 % propyl gallate	728	1664	1361	983
Famotidine + povidone LP + 1 % EDTA	292	745	611	527
Famotidine + povidone HP + 1 % EDTA	816	2166	1678	1330

with various antioxidants. Compacts were prepared and subjected to stress conditions, including both open and closed environments (Tables I and II). The study aimed to establish a correlation between peroxide content and the formation of famotidine sulfoxide, the primary oxidative degradation product. HP povidone demonstrated a higher baseline level of oxidative degradation products, with significant increases under stress, particularly in open conditions. Conversely, LP povidone exhibited greater stability, generating minimal formation of famotidine sulfoxide under similar conditions.

Unexpectedly, the inclusion of ascorbic acid led to elevated levels of oxidative products, especially in HP povidone, suggesting a pro-oxidant effect at higher concentrations (up to 22,676 parts per million (ppm) in open conditions). In contrast, antioxidants such as propyl gallate and ethylenediaminetetraacetic acid (EDTA) consistently mitigated oxidative degra-

Table II. Content of famotidine sulfoxide (in ppm) in compacts in open conditions

	t_0	14 days 50°C/ 75 %RH	28 days 40°C/ 75 %RH	3 months 30°C/ 65 %RH
Famotidine + povidone LP	354	1646	2146	1731
Famotidine + povidone HP	885	6514	8252	9796
Famotidine + povidone LP + 1 % ascorbic acid	412	8070	12468	7379
Famotidine + povidone HP + 1 % ascorbic acid	2389	21747	22676	10947
Famotidine + povidone LP + 1 % propyl gallate	349	4422	1941	1444
Famotidine + povidone HP + 1 % propyl gallate	728	5295	6439	4613
Famotidine + povidone LP + 1 % EDTA	292	1992	3913	1692
Famotidine + povidone HP + 1 % EDTA	816	6361	8535	7357

dation. For instance, the combination of HP povidone with propyl gallate resulted in significantly reduced oxidation levels (6,439 ppm) compared to ascorbic acid.

Overall, HP povidone is more prone to oxidative degradation than LP povidone, and while ascorbic acid can exacerbate oxidation, propyl gallate and EDTA provide effective stabilisation. For formulations requiring oxidative stability, LP povidone combined with stabilising antioxidants like propyl gallate and EDTA is recommended to minimise the formation of oxidative degradation products.

The incorporation of antioxidants into famotidine and povidone in these experiments was conducted at a concentration of 1 %. The observed increases in oxidative impurity levels align with the results obtained from the assessment of hydrogen peroxide and organic peroxide content in excipients containing a 1 % addition of individual antioxidants.

CONCLUSIONS

This study provides a comprehensive evaluation of the oxidative degradation pathways of famotidine, emphasizing the role of peroxides in excipients as key contributors to instability. The novel analytical method developed for quantifying hydrogen peroxide and organic peroxides enabled a precise assessment of peroxide dynamics under stress conditions. Experimental findings revealed that ascorbic acid can act as a pro-oxidant, promoting peroxide formation and accelerating famotidine degradation. In contrast, propyl gallate and EDTA demonstrated effective antioxidant properties, significantly reducing peroxide levels and oxidative degradation in both excipient mixtures and tablet formulations. One limitation of this study is the extensive number of samples that required stress testing and analysis, which involved considerable manual effort and instrument time. As a result, only a limited set of antioxidants and excipients could be evaluated. To improve efficiency and avoid repeating the entire procedure for different APIs, it would be advantageous to establish a standardized screening protocol for identifying optimal antioxidant options. Additionally, future research should aim to expand the scope of excipients tested and systematically investigate the synergistic potential of excipient combinations in mitigating API oxidation. Furthermore, comparative studies with HP and LP povidone highlighted the critical influence of excipient quality on oxidative stability. While HP povidone exhibited greater susceptibility to peroxide formation and subsequent degradation, the inclusion of stabilizing antioxidants mitigated these effects. This study underscores the importance of carefully selecting excipients and antioxidants to minimize oxidative degradation, thereby ensuring the stability and efficacy of famotidine formulations. These findings offer valuable guidance for the development of robust pharmaceutical formulations and contribute to the broader understanding of API-excipient interactions under oxidative stress.

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REFERENCES

1. A. S. Taha, N. Hudson, C. J. Hawkey, A. J. Swannell, P. N. Trye, J. Cottrell, S. G. Mann, T. J. Simon, R. D. Sturrock and R. I. Russell, Famotidine for the prevention of gastric and duodenal ulcers caused by nonsteroidal antiinflammatory drugs, *N. Engl. J. Med.* **334**(22) (1996) 1435–1439; https://doi.org/10.1056/NEJM199605303342204
2. A. S. Taha, C. McCloskey, R. Prasad and V. Bezlyak, Famotidine for the prevention of peptic ulcers and oesophagitis in patients taking low-dose aspirin (FAMOUS): a phase III, randomised, double-blind, placebo-controlled trial, *Lancet* **374**(9684) (2009) 119–125; https://doi.org/10.1016/S0140-6736(09)61246-0
3. S. Bajaj, D. Singla and N. Sakhuja, Stability testing of pharmaceutical products, *J. Appl. Pharm. Sci.* **2**(3) (2012) 129–138; https://doi.org/10.7324/JAPS.2012.2322
4. M. Bakshi and S. Singh, Development of validated stability-indicating assay methods—critical review, *J. Pharm. Biomed. Anal.* **28**(6) (2002) 1011–1040; https://doi.org/10.1016/S0731-7085(02)00047-X
5. M. Blessy, R. D. Patel, P. N. Prajapati and Y. K. Agrawal, Development of forced degradation and stability indicating studies of drugs – A review, *J. Pharm. Anal.* **4**(3) (2014) 159–165; https://doi.org/10.1016/j.jpha.2013.09.003
6. M. Dong, K. Huynh-Ba and J. Ayers, Development of stability-indicating analytical procedures by HPLC: An overview and best practices, *Pharm. Technol.* **38**(8) (2020) 440–456.
7. A. Roy, J. Henry, A. Wilken and L. Collazo-Malave, Current practices and considerations for a stability-indicating method in pharmaceutical analysis, *Pharm. Technol.* **32** (2014) 568–575.
8. J. M. Campbell, C. Foti, C. Wang, N. Adams, L. R. Allain, G. Araujo, R. Azevedo, J. R. Franca, S. R. Hicks, S. Hostyn, P. J. Jansen, D. Kotoni, A. Kuemmell, S. Marden, G. Rullo, A. C. O. Santos, G. W. Sluggett, T. Zelesky and S. W. Baertschi, Assessing the relevance of solution phase stress testing of solid dosage form drug products: A cross-industry benchmarking study, *J. Pharm. Sci.* **111** (2022) 298–305; https://doi.org/10.1016/j.xphs.2021.06.012
9. S. Singh, M. Junwal, G. Modhe, H. Tiwari, M. Kurmi, N. Parashar and P. Sidduri, Forced degradation studies to assess the stability of drugs and products, *Trends Anal. Chem.* **49** (2013) 71–88; https://doi.org/10.1016/j.trac.2013.05.006
10. V. Dave, R. Haware, N. Sangave, M. Sayles and M. Popielarczyk, Drug-excipient compatibility studies in formulation development: Current trends and techniques, *AAPS Formul. Des. Dev. Sect. Newsl.* (2015) 9–15.
11. G. Bruni, L. Amici, V. Berbenni, A. Marini and A. Orlandi, Drug-excipient compatibility studies: Search of interaction indicators, *J. Therm. Anal. Calorim.* **68** (2002) 561–573; https://doi.org/10.1023/a:1016052121973
12. R. Chadha and S. Bhandari, Drug-excipient compatibility screening – Role of thermoanalytical and spectroscopic techniques, *J. Pharm. Biomed. Anal.* **87** (2014) 82–97; https://doi.org/10.1016/j.jpba.2013.06.016
13. A. Gabrič, Ž. Hodnik and S. Pajk, Oxidation of drugs during drug product development: Problems and solutions, *Pharmaceutics* **14**(2) (2022) Article ID 325 (19 pages); https://doi.org/10.3390/pharmaceutics14020325
14. A. S. Narang, V. M. Rao and D. S. Desai, Effect of antioxidants and silicates on peroxides in povidone, *J. Pharm. Sci.* **101**(1) (2012) 127–139; https://doi.org/10.1002/jps.22729
15. W. R. Wasylaschuk, P. A. Harmon, G. Wagner, A. B. Harman, A. C. Templeton, H. Xu and R. A. Reed, Evaluation of hydroperoxides in common pharmaceutical excipients, *J. Pharm. Sci.* **96**(1) (2007) 106–116; https://doi.org/10.1002/jps.20726
16. Y. Wu, J. Levons, A. S. Narang, K. Raghavan and V. M. Rao, Reactive impurities in excipients: profiling, identification and mitigation of drug-excipient incompatibility, *AAPS PharmSciTech* **12** (2011) 1248–1263; https://doi.org/10.1208/s12249-011-9677-z