Evaluation of degradation profile of selected space medicine after accelerated gamma irradiation and its comparison with photodegradation as per ICH Q1(B)

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Abstract

Long-duration space missions requires, the stability of pharmaceuticals for maintaining astronaut health. Pharmaceuticals are exposed to a unique space radiation environment that alters their stability and efficacy during prolonged missions. Gamma radiation is one of the major radiation present in space and it can penetrate spacecraft and affect the stability of pharmaceuticals by inducing physical or chemical changes. To evaluate the effect of gamma radiation on the physical and chemical stability, two drugs were selected namely ciprofloxacin and promethazine with its marketed liquid formulations. Both were exposed to gamma and photo radiation in the present study. Gamma irradiation was carried out at varying doses from 25 Gy to 800 Gy and photo irradiation was conducted over 1.2 million lux hours as per ICH Q1(B) guideline. The chemical stability of the exposed samples was analyzed using RP-HPLC analysis. Ciprofloxacin and Promethazine aqueous API solution and formulations exhibited dose-dependent degradation on gamma exposure. Comparative degradation profile of photo and gamma-irradiated aqueous solution of ciprofloxacin and promethazine indicates the difference in the degradation pathways leading to the formation of degradation products.

Significant degradation was observed in the liquid formulation of ciprofloxacin with and without packing on gamma exposure. This is the first report to show that the primary packaging material used for ciprofloxacin eye/ear drops enables to control the degradation after irradiation with a low dose of gamma rays.

1. Introduction

Different space agencies and their global partners are preparing for extended human space exploration missions beyond low Earth orbit, such as roundtrip voyages to Mars lasting 2–3 years[1]. However, these deep-space missions pose increased health risks to crews due to prolonged exposure of space hazards. Maintenance of health of crew during space travel is essential, medicine plays a vital role in it[2]. Medicine have defined shelf life on Earth but in harsh environment of space they degrade much faster[3]. Currently, medicines are resupplied for short-duration space missions. However, for long-duration space missions, such resupply won't be feasible. Therefore, it's crucial to evaluate the stability of medicines for use in space to ensure their effectiveness over extended periods[4].

Du et al. compared the stability of 35 medications used by astronauts over 28 months on the International Space Station (ISS). The study revealed that the number of unstable formulations increased with long duration storage in space. Sulfamethoxazole/trimethoprim combination tablets, silver sulfadiazine cream, and levofloxacin tablets exhibited the least stability, degrading before their expiry after 353 days of exposure[5]. Recently NASA published a guidance document focussing on the stability of pharmaceuticals during long-duration space missions. Published data indicate that many formulations expired before the shelf-life period, exhibiting accelerated degradation in space than Earth. Studies have shown that more than half of the pharmaceuticals in the ISS formulary are expired within 36 months which raises issues of potency and safety of medicines used during long-duration space

programs like Mars mission[1]. Another case study carried out by Choung et al. assessed the stability of vitamin B complex formulations on the ISS, noting discrepancies in vitamin B1 strength[4]. Wotring observed deterioration and changes in active pharmaceutical ingredient (API) concentrations in medicines flown without ground controls[6].

According to the research carried out at NASA Space Radiation Laboratory (NSRL) by Daniel et al., emphasized the different medication susceptibilities to radiation exposure, especially for promethazine and clavulanate (as part of the amoxicillin-clavulanate combination). It is observed that API changes are dose-variable, with more deterioration shown at 10 Gy exposures than at 50 Gy doses given during equal time intervals. It raises the possibility that stability at high doses may not always transfer to stability at low doses and the changes in dosage form and dose rate may have a considerable influence on stability[7,8]. Very few studies are reported for degradation of drugs by ionizing radiation which includes a study on ciprofloxacin (CIP) in solid state exposed to different doses of 15KGy to 100KGy gamma radiation. The XRD pattern indicates the formation of amorphous CIP upon gamma irradiation. Analysis revealed that the physical properties of exposed powder samples changed[9]. Another study demonstrates the degradation of 0.1 mM CIP aqueous solution at various doses of 0 to 8KGy gamma radiation. The results indicate instability of CIP in the presence of hydroxyl radicals. Degradation was observed at lower doses such as 0.5 KGy and the extent of degradation increased as the radiation dose raised[10]. However, all these studies were carried out at a very high dose of gamma radiation which cannot directly correlate with gamma radiation exposure to medicine in space as the dose of gamma radiation will be very low if we calculate a total duration of 3–4 years for long duration mission[11]. Despite efforts, studies on drug stability during spaceflight have yielded inconsistent findings, with most medications failing to meet potency standards after long-term storage[12]. This highlights the critical need for carefully selecting stable medicines with suitable primary packaging for long-duration space missions.

The stability of pharmaceuticals is a critical aspect directly impacting a drug's shelf-life and its ability to deliver the intended efficacy. For a drug to be remain stable, it must maintain its physical and chemical properties over time[4]. Numerous factors influence pharmaceutical stability, including environmental conditions such as temperature, humidity, radiation, and oxygen, as well as product-related factors like excipients, manufacturing processes, dosage forms, and packaging materials[13]. Among these factors, photostability, or the susceptibility of a drug to degradation upon exposure to light, is one of the concerns. Photodegradation can occur through various mechanisms, including direct chemical reactions initiated by photon absorption or indirect processes involving the generation of reactive oxygen species[8].

To assess the quality of pharmaceuticals before market authorization, regulatory bodies such as the ICH, WHO, and FDA provide guidelines, Specific guideline of ICH Q1(B) addresses conditions to perform photostability testing[14]. As per the guideline, photostability is evaluated by exposing medicines to 1.2 million lux hours of visible light and not less than 200-watt hours/square meter near ultraviolet and cool white fluorescent and near ultraviolet lamps used as radiation sources. Space environment factors

such as radiation, pressure, microgravity, extreme temperatures, and carbon dioxide levels affect the stability of medicine[15].

However, the space radiation environment is a complex and dynamic system comprising various types of non-ionizing and ionizing radiation, including solar rays, cosmic rays, and trapped radiation[16]. Solar rays primarily originate from the Sun and consist of electromagnetic radiation, such as UV light, X-rays, and gamma rays, as well as energetic particles, such as protons and electrons, emitted during solar flares and coronal mass ejections[11]. Cosmic rays, on the other hand, are high-energy particles originating from outside the solar system, these particles include protons, and heavy ions. Trapped radiation refers to charged particles, primarily electrons and protons, trapped within Earth's magnetic field in the Van Allen radiation belts[17]. Space radiation interacts with the shielding material of spacecraft to create a variety of secondary particles, such as gamma rays, X-rays, heavy ion bremsstrahlung, neutrons, and proton recoils. That creates a complex environment inside the spacecraft. One of the major radiation present inside the spacecraft is gamma radiation. However, the radiation environment in space differs significantly from that on Earth. Currently, there are no established guidelines for the evaluation of space pharmaceuticals to be stored and used during space travel[17].

Given its prominence in space, gamma radiation highlights the need to assess medicine stability in its presence. Real-time stability studies in space face hurdles like time, cost, limited storage, and sample retrieval difficulties. Therefore, Simulated space radiation terrestrial studies are crucial to understand the impact of radiation on the stability of pharmaceuticals and its extrapolation to predict the shelf life of space medicine[18]. The author conducted a preliminary ground-based study to know the effect of different ionizing radiations such as gamma, proton, neutron, and heavy ions on the stability of CIP, Diclofenac, and metoprolol. They found that ionizing radiation can alter the physicochemical properties of drugs[19]. As per a published article related to the ISS formulary, it is reported that Ciprofloxacin and Promethazine are widely used space medicines in the form of tablets as well as liquid formulations like drops and injections, etc. during space travel[1].

So, in this research work, we have selected ciprofloxacin (CIP)and promethazine (PMZ) API and their liquid formulations to study. CIP is an antibacterial agent belonging to the fluoroquinolone class which is used for bacterial infections during space travel, while Promethazine is commonly utilized to alleviate symptoms of motion sickness[20,21].

2. Experimental work

2.1. Material

The reference standards of CIP and PMZ (purity ≥ 99.98%) were acquired from Yarrow Chem, Mumbai, India. Marketed formulations such as CIPLOX 0.3%w/v eye/ear drops (CIPLA Pvt, Ltd.), ZOXAN 0.3%w/v eye/ear drops (FDC Ltd, Maharashtra, India) and CIPLOX 500mg tablets (CIPLA Ltd, Sikkim, India),

CIPRODAC 500 mg tablets (CADILA Ltd, Ahmedabad, India) were purchased from local pharmacy stores. Marketed formulations of PMZ 25mg/mL Phenergan injection (Abbott Ltd, Maharashtra, India) and 25mg Phenergan tablet (Abbott Ltd, Uttarakhand, India) were also procured from the local market.

Analytical-grade sodium phosphate was obtained from Merck Mumbai, India. HPLC-grade methanol, acetonitrile, orthophosphoric acid, trimethylamine, and Milli-Q water were purchased from Merck, Mumbai, India. PVDF membrane filters and PVDF syringe filters with a pore size of 0.45 µm were procured from Millipore Ltd, Bangalore, India.

2.2. Methods

2.2.1. Sample Preparation

For Control samples of both drugs API in liquid and solid-state and formulations, were stored in glass bottles and wrapped in aluminum foil in a stability chamber at 25 ºC/45% relative humidity (RH) for storage.

Aqueous API solutions of CIP (100 ppm and 3000 ppm), solid CIP (100mg powder), aqueous API solutions of PMZ (100 ppm and 2500 ppm) and solid PMZ (100mg powder) were used for the radiation exposure study. All procured marketed formulations of both drugs are exposed to radiation with and without primary packaging.

2.2.2. Gamma Degradation Study

All samples are mentioned in section 2.2.1. were exposed to gamma radiation at dose 25, 50, 100, 400, and 800Gy using a Cobalt-60 source at the Inter-University Accelerator Centre (IUAC). The radiation was administered at a rate of 1.57 KGy/hr with an energy level of 1.2 MeV.

2.2.3. Photo Degradation Study

Following the ICH Q1(B) guideline, a photostability study was conducted using a photostability chamber (Thermolab Scientific Equipment, Mumbai, India). For each drug, API aqueous solutions, API powder, and formulations were placed in an uncovered quartz crucible placed inside the photostability chamber. The samples underwent simultaneous exposure to 200 W*hr/m2 UV radiation and 1.2 million lux hours of visible radiation.

All samples were exposed in duplicate sets to both photolytic and gamma radiation. All Control and exposed samples were stored in the refrigerator until HPLC analysis.

2.2.4. Organoleptic Evaluation

The physical attributes of each sample were evaluated for its color, odor, and the clarity of the solution.

2.2.5. High Performance Liquid Chromatography (HPLC)

The chemical stability of each drug was assessed for its percentage degradation utilizing the 1260 Infinity Quaternary HPLC system from Agilent Technologies. The system was equipped with a 100 µL capacity loop autosampler and is integrated with OpenLAB CDS EZChrom software (version A.01.03).

For the analysis of CIP samples, the official method outlined in the British Pharmacopoeia (BP, 2018) for chromatographic purity was employed. For PMZ the official BP method was tried but failed to give separation of all degraded impurities so, an in-house gradient method was developed for the separation of all degradation products for PMZ. The optimized chromatographic conditions are detailed in Table 1. All control and exposed samples were diluted with water to get the appropriate concentration for HPLC analysis.

Table 1
Chromatographic Conditions Employed in HPLC Analysis

Drug	Chromatographic Conditions	
CIP	**Mobile phase**: OPA (Ortho Phosphoric acid) buffer (0.025M; pH 3.0 ± 0.1 with TEA) and Acetonitrile (87:13, %v/v)	
	Column: Waters C18, (250×4.6) mm; 5 µm	**Sample concentration**: 100 µg/mL
	Diluent: Water	**Flow rate**: 1.5 mL/min
	Column temperature: 25◦C	**Injection volume**: 20µL
	Wavelength: 278 nm	**Runtime**: 15 minutes
PMZ	**Mobile phase**: Kh_2PO_4in buffer (0.025M; pH 5.5 ± 0.1 with KOH), Methanol and Acetonitrile in gradient mode ($Time_{min}$/%Methanol/%Acetonitrile: T_0/20/30; $T_{4.5}$/60/10; T_7/20/30	
	Column: Phenomenex C18, (150×4.6) mm; 5 µm	**Sample concentration**: 100 µg/mL
	Diluent: Water	**Flow rate**: 1.5 mL/min
	Column temperature: 45◦C	**Injection volume**: 20µL
	Wavelength: 254 nm	**Runtime**: 15 minutes

3. Results

3.1. Organoleptic evaluation

The samples were evaluated for the within two days for organoleptic assessment.

An aqueous solution of CIP API exhibited color changes from colorless to light brown when exposed to both photolytic and gamma radiation. However, color change was observed in 3000 ppm CIP aqueous solution which was not observed at 100 ppm CIP aqueous solution at 100Gy gamma radiation exposure. On analysis of photo-exposed samples, it was observed that 100 ppm and 3000 ppm CIP aqueous solution showed almost similar color changes while in aqueous formulations, color intensity was higher than in API solution.

No color change was observed in solid CIP API and all CIP formulations i.e., eye drops and tablets, while exposed to gamma radiation and photolytic radiation with packaging material.

Apart from that, in CIP eye drops, 25Gy and 50Gy irradiated samples exhibited turbidity and crystals which disappeared at higher radiation doses. An aqueous solution of PMZ API exhibited color changes from colorless to purple when exposed to both photolytic and gamma radiation. However, color change was observed in a 2500 ppm concentration solution at a starting dose of 25Gy which was observed in 100 ppm concentration at 400Gy gamma radiation exposure. On exposure to photolytic radiation, color change was observed in both concentrations of PMZ API solutions.

Solid samples of PMZ API showed stability on exposure to gamma radiation while exhibiting a light yellow color on photo exposure. The tablet formulation of PMZ did not show any color change on exposure to photolytic and gamma radiation with or without packaging. Surprisingly, the injectable formulation of PMZ showed color change at a very high radiation dose of 800 Gy but did not show any color change at a lower dose of gamma radiation (Fig. 1). Same concentration of PMZ API solution showed color change at a lower dose of 25Gy. Observation of both drugs indicates a dose and concentration dependent changes in color intensity of API solutions subjected to gamma radiation.

3.2. Chemical Stability of CIP

The HPLC chromatograms revealed the presence of a peak corresponding to CIP at approximately 8.7 ± 0.5 minutes which is well separated from all generated degradation products after gamma and photo irradiation in samples.

It is observed that almost the same percentage of degradation was observed for photo-exposed CIP as per ICH Q1(B) guideline and 400 Gy gamma-exposed CIP. However, the generated degradation products were different in both samples at any given concentration (Fig. 2). Detailed information about % degradation, and generated degradation products (CIP1-CIP8) along with its RRT are given in Table 2.

Table 2
Degradation summery of CIP irradiated by Photo and Gamma radiation.

Sample	Radiation/dose	%Degradation	Degradation Products RRT
100ppm	(1.2 lux M.hr/ 200-watt hours /square meter)	25.87%	0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.59(CIP6), 0.68(CIP7), 0.78(CIP8)
3000ppm		2.10%	0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.68(CIP7)
Formulation 1 without packaging		2.50%	0.39(CIP3), 0.44(CIP4), 0.68(CIP7)
Formulation 2 without packaging		4.60%	0.27(CIP2), 0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.68(CIP7)
Formulation 1 with packaging		No degradation observed	
Formulation 2 with packaging			
100ppm liquid	Gamma		
	25Gy	1.4%	0.44(CIP4),0.68(CIP7)
	50Gy	4.2%	0.44(CIP4),0.68(CIP7)
	100Gy	12.12%	0.44(CIP4),0.68(CIP7)
	400Gy	23.97%	0.27(CIP1),0.31(CIP2),0.44(CIP4), 0.51(CIP5), 0.68(CIP7),0.78(CIP8)
	800Gy	44.38%	0.27(CIP1),0.31(CIP2), 0.44(CIP4), 0.51(CIP5),0.68(CIP7),0.78(CIP8)
3000ppm liquid	25Gy	1.1%	0.68(CIP7)
	50Gy	1.3%	0.44(CIP4),0.68(CIP7)
	100Gy	1.53%	0.44(CIP4),0.68(CIP7)
	400Gy	2.26%	0.31(CIP2),0.39(CIP3), 0.44(CIP4), 0.68(CIP7)
	800Gy	2.31%	0.31(CIP2), 0.39(CIP3), 0.44(CIP4), 0.68(CIP7),0.78(CIP8)
Formulation 1 without packaging	25Gy	1.6%	0.68(CIP7)
	50Gy	2.1%	0.68(CIP7)
	100Gy	2.8%	0.44(CIP4),0.68(CIP7)

Sample	Radiation/dose	%Degradation	Degradation Products RRT
	400Gy	3.7%	0.31(CIP2), 0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.68(CIP7)
	800Gy	4.1%	0.31(CIP2), 0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.68(CIP7), 0.78(CIP8)
Formulation 1 with packaging	25Gy	1%	0.68(CIP7)
	50Gy	1.5%	0.68(CIP7)
	100Gy	2.1%	0.44(CIP4),0.68(CIP7)
	400Gy	4.3%	0.31(CIP2), 0.44(CIP4), 0.68(CIP7), 0.78(CIP8)
	800Gy	5.2%	0.31(CIP2),0.44(CIP4), 0.68(CIP7),0.78(CIP8)
Formulation 2 without packaging	25Gy	0.5%	0.68(CIP7)
	50Gy	1.2%	0.44(CIP4),0.68(CIP7)
	100Gy	1.9%	0.44(CIP4),0.68(CIP7)
	400Gy	3.3%	31(CIP2), 0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.68(CIP7)
	800Gy	4.5%	0.31(CIP2), 0.39(CIP3), 0.44(CIP4), 0.51(CIP5), 0.68(CIP7),0.78(CIP8)
Formulation 2 with packaging	25Gy	0.2%	0.68(CIP7)
	50Gy	1.1%	0.68(CIP7)
	100Gy	1.3%	0.31(CIP2), 0.44(CIP4), 0.68(CIP7)
	400Gy	2%	0.31(CIP2), 0.44(CIP4), 0.68(CIP7), 0.78(CIP8)
	800Gy	3.8%	0.31(CIP2), 0.44(CIP4), 0.68(CIP7),0.78(CIP8)

When the degradation profile of 400Gy gamma irradiated 100 ppm CIP API solution was compared with photo-exposed solution, it was observed that CIP1 and CIP2 were present in gamma irradiated samples but not in photo-exposed samples. CIP3 and CIP6 were not present in gamma-irradiated samples but present in photo-exposed samples. Apart from that, common degradation products (CIP5 and CIP8) have different peak intensities (Fig. 2A).

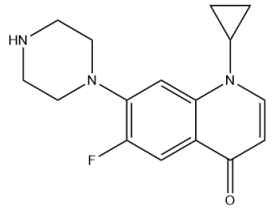
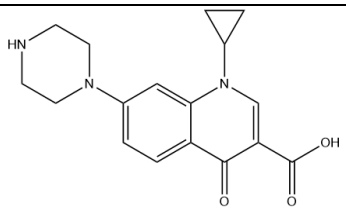
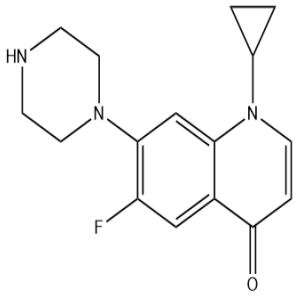
When a degradation profile of 3000 ppm CIP API solution was observed, it was noted that CIP2 was present in 400Gy gamma irradiated samples but not in photo-exposed samples. CIP5 was absent in the gamma-irradiated sample but present in the photo-exposed sample.

On comparison of the degradation profile of gamma-irradiated samples of 3000 ppm CIP API solution and CIP formulation, it was observed that CIP5 which was not present in CIP API solution but present in CIP formulations when exposed without packaging as shown in Fig. 2B, 2C and 2D. However, the intensity of CIP5 is very low in exposed CIP formulations. A total of five degradation products (CIP2-CIP5, CIP7) were observed in both aqueous formulations of CIP on gamma and photolytic irradiation without packaging. The aqueous formulations of CIP with packing material do not show any degradation when exposed to photolytic radiation, but degradation was observed while exposed to gamma irradiation. CIP7 was identified as the prominent peak in all gamma and photolytically irradiated samples, at any given concentration. In samples irradiated with 400 Gy of gamma radiation, both CIP API aqueous samples and CIP aqueous formulations displayed a higher intensity of CIP7 compared to other degradation products.

As shown in Table 2, both aqueous formulations of CIP showed a slightly higher percentage of degradation compared to the same concentration of API when exposed to photo and gamma radiation without packaging material. This might be due to the presence of excipients. Surprisingly, when both aqueous formulations were exposed to photo and gamma radiation in primary packaging material, significant degradation was observed in gamma-irradiated samples (Fig. 2E&F). CIP showed dose-dependent degradation in formulation with primary packaging material when exposed to gamma radiation (Fig. 3). It is worth to note that both CIP formulations have low-density polyethylene (LDPE) plastic bottles single-use containers as primary packaging material.

Upon comparison with the reported Relative Retention Times (RRT), it is observed that the degradation products CIP4, CIP7, and CIP8 might correspond to potential impurities labeled as IMPE, IMPB, and IMPC, respectively according to British Pharmacopoeia (BP), 2018. Structures of impurities are shown in Table 3.

Table 3: Structures and RRT of reported CIP impurities

RRT	Impurity	Structure
0.4 (CIP4)	IMPE	
0.6 (CIP6)	IMPB	
0.7 (CIP7)	IMPC	

3.3. Stability of PMZ

For analyzing PMZ, attempts were made to use the official pharmacopeial methods for sample analysis. Unfortunately, those methods proved ineffective in providing a clear resolution of degradation products. So, an in-house chromatographic method was developed (Table 1). The HPLC chromatogram showed the chromatographic peak of PMZ at approximately 7.1 ± 0.3 minutes.

When a 100 ppm PMZ API solution was exposed to photolytic radiation, around 40% degradation was observed, whereas gamma radiation exposure to PMZ API resulted in approximately 22% degradation. Due to over-degradation in photo-exposed samples, secondary degradation products may be produced. Therefore, 100 ppm concentration was excluded from the comparative degradation study of PMZ, and formulation concentration i.e., 2500 ppm was used for comparison of the degradation profile of PMZ. Detailed information about % degradation, and generated degradation products (PMZ1-PMZ8) along with its RRT are given in Table 4.

Table 4
Degradation summary of PMZ irradiated by Photo and Gamma radiation.

Sample	Radiation/dose	%Degradation	Degradation Products RRT
2500 ppm	(1.2 lux M.hr/ 200-watt hours /square meter)	15.2%	0.21(PMZ1), 0.30(PMZ2), 0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6), 0.82(PMZ7), 0.86(PMZ8)
Injectable Formulation without packaging		17.1%	0.21(PMZ1), 0.30(PMZ2), 0.41(PMZ3), 0.52(PMZ5), 0.75(PMZ6), 0.82(PMZ7), 0.86(PMZ8),
Injectable Formulation with packaging		--	No degradation observed
	Gamma radiation		
2500ppm	25Gy	0.6%	0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6), 0.82(PMZ7)
	50Gy	1.3%	0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6), 0.82(PMZ7)
	100Gy	1.7%	0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6), 0.82(PMZ7)
	400Gy	3.2%	0.21(PMZ1), 0.41(PMZ3), 0.45(PMZ4), 0.59(PMZ5), 0.75(PMZ6), 0.82(PMZ7), 0.86(PMZ8)
	800Gy	4.2%	0.21(PMZ1), 0.41(PMZ3), 0.45(PMZ4), 0.59(PMZ5), 0.75(PMZ6), 0.82(PMZ7), 0.86(PMZ8)
Injectable Formulation without packaging	25Gy	1.6%	0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6)
	50Gy	2.8%	0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6)
	100Gy	3.3%	0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6)
	400Gy	20.5%	0.21(PMZ1), 0.30(PMZ2), 0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6)
	800Gy	24.5%	0.21(PMZ1), 0.30(PMZ2), 0.41(PMZ3), 0.59(PMZ5), 0.75(PMZ6)

Sample	Radiation/dose	%Degradation	Degradation Products RRT
Injectable Formulation with packaging	25Gy	--	No degradation was observed.
	50Gy		
	100Gy		
	400Gy		
	800Gy		

With different peak intensities, PMZ1, PMZ3, PMZ5, and PMZ6 were found common in both gamma and photo-exposed samples. It was observed that PMZ3 showed similar intensity in 2500 ppm PMZ API irradiated solution in both conditions but a higher intensity of PMZ3 was observed in injectable formulation on gamma irradiation than photo irradiation. While the rest of the degradation products (PMZ5-PMZ8) showed higher intensity in the 2500 ppm PMZ API solution than injectable formulation. PMZ6-PMZ8 showed higher intensity in photo-degraded samples than gamma at any given concentrations. As shown in Table 3, the injectable formulation of PMZ showed a higher percentage of degradation compared to 2500 ppm concentration of API when exposed to gamma radiation without packaging material but showed lower degradation when exposed to photolytic radiation. This might be due to the presence of excipients and chemical structure which show more susceptibility toward particular radiation. PMZ showed dose-dependent degradation in the formulation without primary packaging material when exposed to gamma radiation but, showed stability with packaging material in both gamma and photolytic radiation (Fig. 4). It is worth to note that both PMZ formulations have ambered color glass in single-use containers as primary packaging material.

4. Discussion

Ensuring pharmaceutical stability is critical for extended space missions beyond Low Earth Orbit (LEO), where replacement options and emergency returns are limited. Prolong exposure to space conditions can lead to significant drug degradation, impacting their quality, effectiveness, and safety[3]. Thus, assessing the impact of space radiation on pharmaceutical stability is imperative before boarding on long-duration space missions. Utilizing forced degradation or accelerated stability studies through exposure to ionizing radiation can help anticipate potential effects.

Gamma radiation is a prominent form of radiation in space, capable of affecting pharmaceuticals through direct or indirect ionization[4]. Numerous articles have discussed the stability of drugs during gamma sterilization, typically using a standard dose of 25 kGy over a minimal period[22]. However, in Mars mission and Artemis program the duration of the mission will be 2–3 years during that time total dose of gamma radiation will be much lower but for a prolong period. It was reported that the drug behavior can vary depending on the total duration and dose of radiation. i.e., degradation profile of the drug observed at a higher dose applied for a fraction of time was different than lower dose applied for a prolong time.

So, it can be extrapolated that changes in dose and dose rate of gamma radiation may have different effects on the stability of drugs. Hence further experiments are required to derive a conclusion.

Articles regarding the stability of CIP during gamma exposure are reported. However, reported dose for gamma exposure includes high dose of 25KGy which is a sterilization dose or more than 25KGy dose applied for a short duration[9,10,23]. Here in this study, we have exposed drugs to low doses of gamma radiation from 25–800 Gy at a slow dose-rate. For the photostability study, dose of 1.2 lux million hours or 200 watts/meter square was applied as per ICH Q1(B) guideline. The generated degradation profile of CIP exhibited similar % degradation as 400 Gy gamma-exposed samples at all concentrations. So, the dose range between 300–600 Gy needs to be further optimized for an accelerated stability study of space medicines. In PMZ total % degradation was observed less in 400 Gy gamma exposed samples compared to photo-exposed samples so, further optimization of dose rate is required.

In both CIP and PMZ, a color change was observed in a solid API without any signs of chemical degradation. However, in aqueous liquid solutions both color change and chemical degradation were observed after exposure to gamma and photolytic radiation. In solid state or at higher concentrations shielding effect might occur, which restricts the amount of light or radiation that can penetrate a material and radical recombination, which stops reactive species from efficiently degrading the material.

No chemical degradation is observed in the solid API when evaluated using HPLC but, changes in the physical properties such as color change was observed. It indicates, further solid-state characterization is required by using techniques like X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR), etc.

While in aqueous liquid solutions high degradation is caused by the indirect ionization effect[7,8]. When radiation and water molecules interact, the water molecule is hydrolyzed and produces free radicals of water ($H_2O^{\cdot+}$, ·OH, H_3O^+, H·, O·, H_2, e –) that are stable for a longer period of time. Due to their extreme reactivity, these free radicals possess the ability to initiate chemical reactions that cause structural changes, bond breakage, or oxidative degradation in the presence of these reactive species[24]. This might alter the chemical stability, leading to loss of efficiency and generation of degradation products.

When CIP API was exposed to photolytic radiation according to ICH Q1(B), the degradation exhibited a percentage similarity to the sample exposed to 400 Gy gamma radiation at any given concentration. CIP7 which is the most common impurity in all CIP gamma and photo-exposed samples exhibited similar RRT as IMPC (Table 3.). The piperazine ring present in CIP which consists of the N-NH_2 group, undergoes ring opening phenomena and forms a hydrazine-like structure (NH-NH_2) in IMPC/CIP7[20,25]. This is the main mechanism for the generation of CIP7. Another common phenomenon is dehalogenation, which was observed in photodegradation and shown similarity to IMPB/CIP6 [26].

Whereas, in PMZ API and injectable formulation showed higher photolytic degradation compared to gamma irradiation at all tested concentrations. The molecule's electrons are excited by UV light, which

breaks bonds and accelerates photochemical processes like oxidation[27]. On the other hand, gamma radiation has a higher energy, it causes degradation by ionization, which is less specific. This indicates that as the chemical structure of a drug gets changed, degradation behavior gets changed in the presence of gamma radiation. There is essentially no evidence available on PMZ exposure to gamma radiation on Earth, despite reported papers suggesting that the drug is highly degradable in a space environment[5,8]. Therefore, a systematic detailed study is required to verify the degradation behavior of PMZ in the presence of gamma radiation.

It is very important to mention about degradation behavior of CIP in aqueous formulation with and without primary packaging material on irradiation of gamma radiation. At lower doses of gamma radiation (25 and 50 Gy), turbidity was observed in CIP samples which was not observed at higher dose exposure. Approx same % of degradation in the 400 Gy gamma exposed formulation of CIP with and without primary packaging material indicates that gamma radiation is penetrating in used primary packaging material and cannot protect CIP formulation from degradation. Here, low-density polyethylene (LDPE) plastic was used as a packaging material by the manufacturer which can efficiently protect CIP from photo-degradation. However, CIP aqueous formulations with packaging exhibited similar level of degradation as without packaging on exposure of gamma radiation. This highlights the need to explore alternative packaging materials for CIP to prevent its degradation.

For PMZ injectable formulation, ambered colored glass ampules were used as packaging material. No significant degradation was observed when PMZ was irradiated with photolytic and gamma radiation which indicates that ambered color glass can protect PMZ from gamma radiation. However, the use of glass as packaging material can further create problems in space. Glass packaging in space is susceptible to breakage due to temperature fluctuations and fragility, its weight adds to payload concerns.

The results of the present study showed that the gamma degradation profiles of CIP and PMZ differed from photo-irradiated profiles. Therefore, further LC-MS analysis is necessary to understand the degradation mechanism of the generated degradation products and to predict the toxicological profile of degradation products.

Abbreviations

API
Active Pharmaceutical Ingredient
BP
British Pharmacopoeia
CIP
Ciprofloxacin
FDA
Food and Drug Administration

FTIR
Fourier Transform Infrared Spectroscopy
GCR
Galactic Cosmic Rays
HPLC
High Performance Liquid Chromatography
ICH
International Council for Harmonisation
ISS
International Space Station
IUAC
Inter-University Accelerator Centre
LC-MS
Liquid Chromatography-Mass Spectrometry
LEO
Low Earth Orbit
LDPE
Low-Density Polyethylene
NASA
National Aeronautics and Space Administration
NSRL
NASA Space Radiation Laboratory
PMZ
Promethazine
PVDF
Polyvinylidene Fluoride
RH
Relative Humidit0079
SCR
Solar Cosmic Rays
SEM
Scanning Electron Microscopy
XRD
X-ray Diffraction
UV
Ultra-Violet
WHO
World Health Organization

Declarations

Competing interests

The authors declare no competing interests. This article does not contain any studies with human participants or animals performed by any of the authors.

Author Contribution

Manali Patel contributed for Methodology, Investigation, Data Curation, Writing Original Draft, and Review & Editing, Saif Khan and Birendra Singh contributed for Resources, and Radiation exposure, Priti Mehta contributed for Conceptualization, Methodology, Review, Visualization, and Supervision.

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Figures

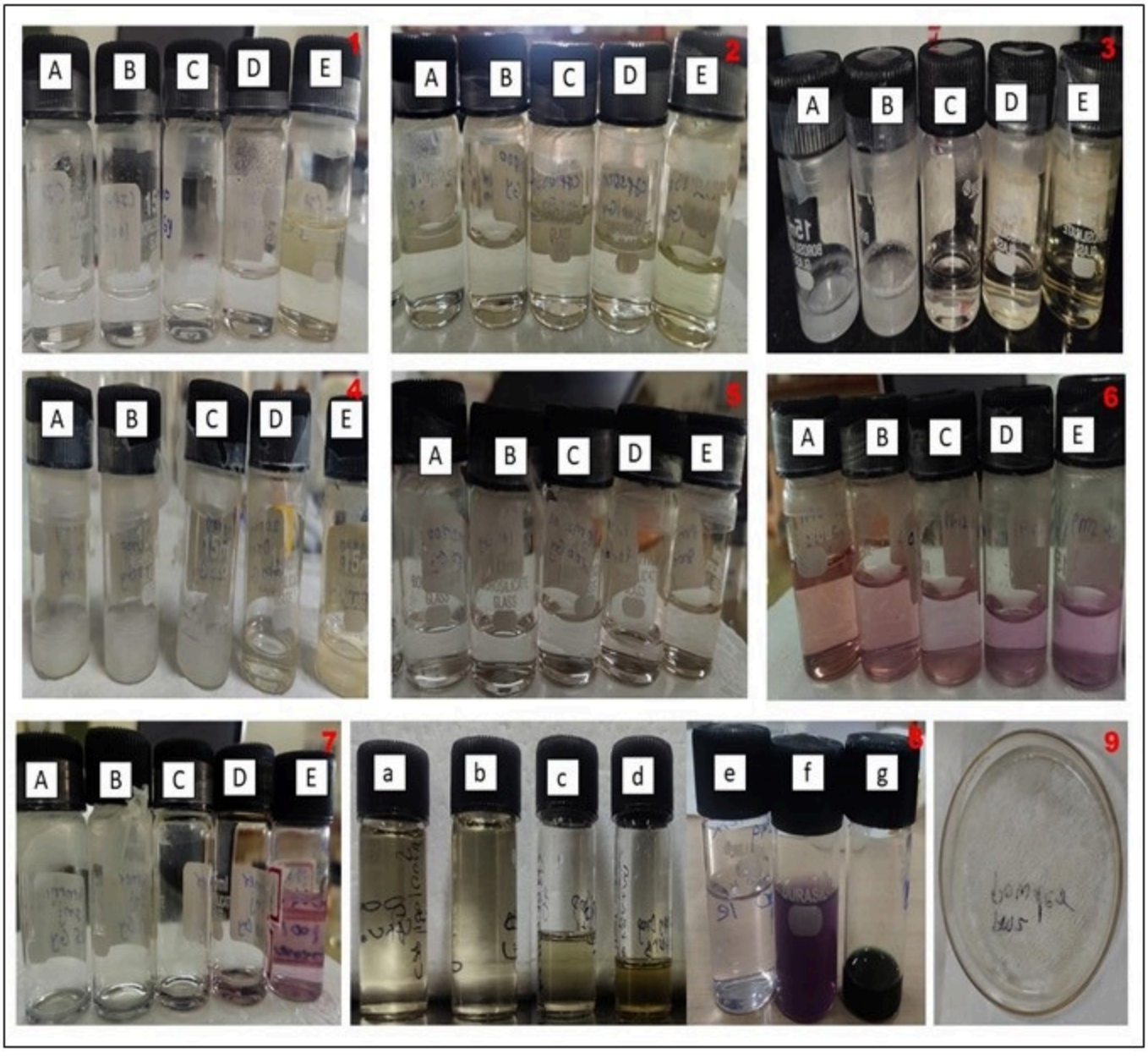

Figure 1

Physical changes in gamma exposed samples (1) CIP 100 ppm, (2) CIP 3000 ppm, (3) CIP aqueous formulation 1, (4) CIP aqueous formulation 2, (5) PMZ 100 ppm, (6) PMZ 2500 ppm, (7) PMZ injectable formulation, (8) Photo exposed API samples of CIP and PMZ, (9) Photo exposed solid API of PMZ.

(A. 25Gy, B. 50Gy, C.100Gy, D. 400Gy, E.800Gy; a. 100 ppm CIP API, b. 3000 ppm CIP API, c. CIP aqueous formulation 1, d. CIP aqueous formulation 2, e. 100 ppm PMZ API, f. 2500 ppm PMZ API, g. PMZ injectable formulation)

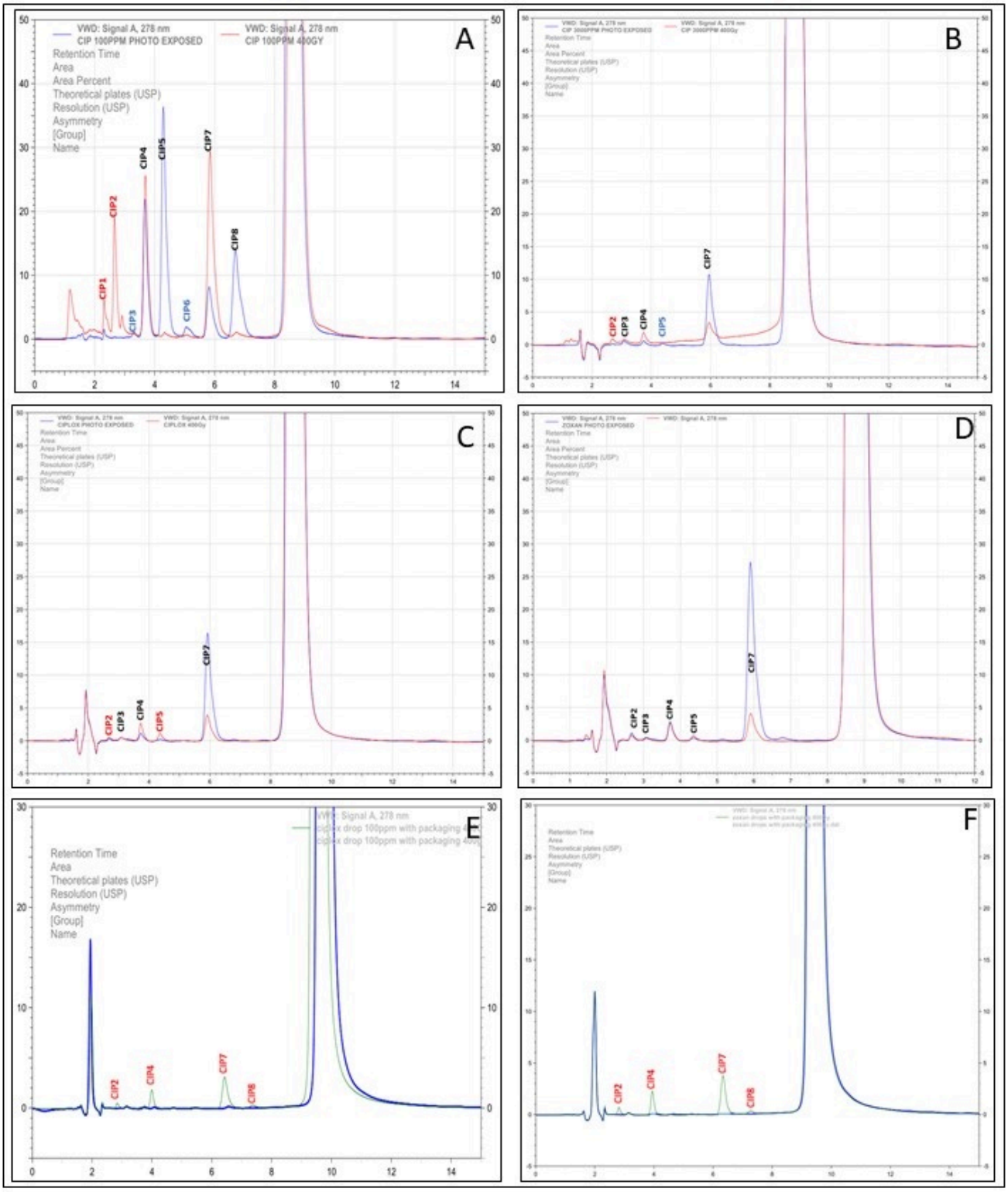

Figure 2

Overlay of gamma and photo-exposed samples (A. 100 ppm CIP API; B. 3000 ppm CIP API; C. CIP aqueous formulation 1 without packaging; D. CIP aqueous formulation 2 without packaging; E.CIP aqueous formulation 1 with packaging; F. CIP aqueous formulation 2 with packaging)

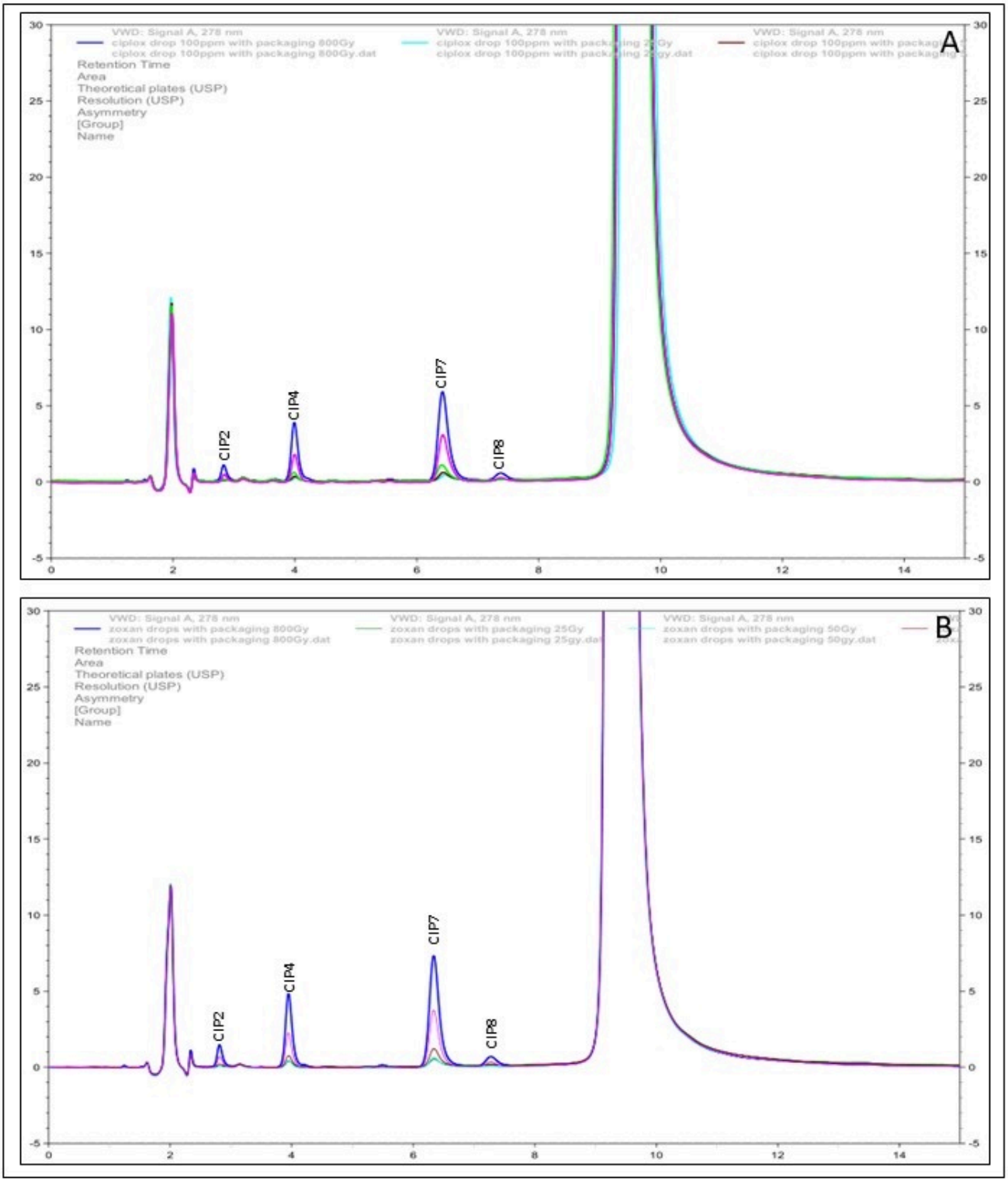

Figure 3

Overlay of 25 Gy-800 Gy gamma exposed sample (A. CIP aqueous formulation 1 without packaging; B. CIP aqueous formulation 2 without packaging)

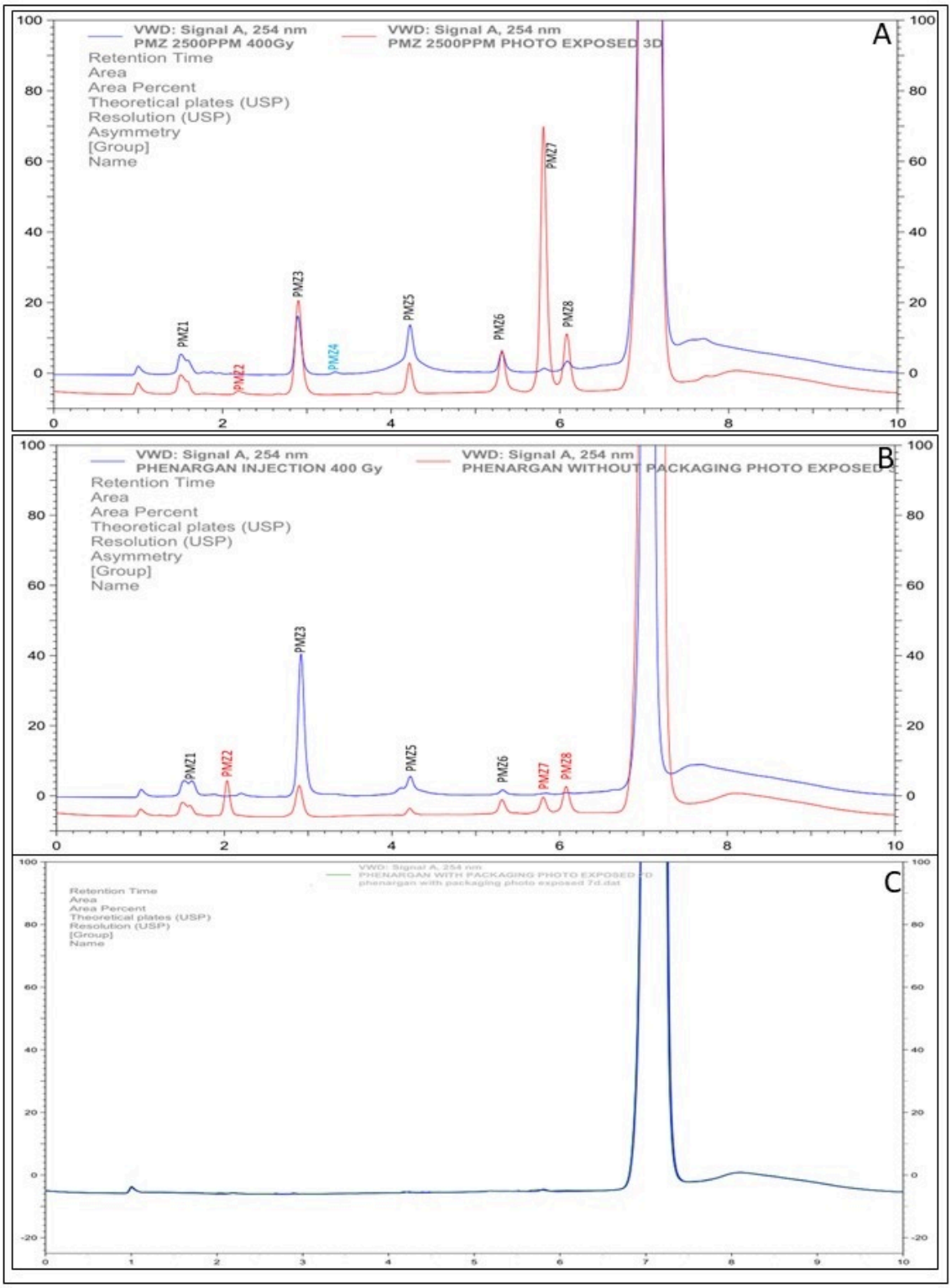

Figure 4

Overlay of gamma and photo-exposed samples (A.; 2500 ppm PMZ API; B. PMZ injectable formulation without packaging; C. PMZ injectable formulation with packaging)