Comparative Stability Evaluation of Marketed Paracetamol IV Formulations in India

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ABSTRACT

Paracetamol is a well-established drug widely used around the world for analgesic and antipyretic purposes in oral solid dosage forms. While paracetamol is stable in solid dosage form, intravenous formulations have significant stability related issues, as the drug is susceptible to hydrolysis and oxidation in aqueous media. Formation of hydrolytic product can be well controlled by adjusting the pH of formulation. However, control of oxidation of the drug during manufacturing and packaging is a challenge, as it requires sophisticated operational controls to remove dissolved oxygen and/or addition of compatible anti-oxidant. Seven commercially available paracetamol intravenous formulations from the Indian market were evaluated for their oxidative degradation potential. The study was performed in two tiers: i) to compare formation of oxidative products in selected stress degradation conditions; and ii) to determine presence of cysteine, an anti-oxidant excipient. Firstly, all formulations were subjected to thermal stress condition at 80ºC for 7 days and oxidative stress condition with 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) at 40ºC for 48 hours. Of the seven formulations tested, one formulation (PerfalganTM, Manufactured by BMS) showed absence of oxidative products after both stress conditions. For tier II studies, a sensitive HILIC-MS/MS method was developed on API 4000 (AB Sciex) for the determination of cysteine presence. Only Perfalgan™ showed presence of cysteine. Thus, Perfalgan™ can be expected to have better stability compared to other marketed formulations. Overall, it is concluded that presence of an anti-oxidant in intravenous formulation could give additional advantage over stability of paracetamol.

Key words: HILIC, LC-MS, Oxidative stress, Paracetamol, Stability.

INTRODUCTION

Paracetamol, an analgesic and an antipyretic drug has been widely used in the last four decades by wide range of patients through different administration routes and via different pharmaceutical formulations. The most widely used oral solid formulations are considered chemically stable, as paracetamol in solid state is non-hygroscopic and well tolerated against hydrolysis and oxidation. However, intravenous formulations are absolutely necessary in case of post-surgery pain treatment or acute hyperthermia or inaccessible oral route of administration. Instability of paracetamol in aqueous medium is well known[1,2] Hydrolysis and oxidation are the primary mechanisms of drug degradation. Hydrolysis results in deacetylation generating p-aminophenol which quickly degrades further to produce p-benzoquinoneimine. This deacetylation takes places both at acidic pH and (much faster) and at basic pH when the phenolate form is present. The rate of hydrolysis can be well controlled by maintaining the pH of the formulation at a specific pH. The rate and extent of oxidation depends on dissolved active oxygen in the formulation. Appropriate anti-oxidant needs to be used as an excipient to quench active oxygen present in the formulation. Objective of the present study is to evaluate the oxidative potential of available intravenous formulations in Indian market through selective stress degradation, and to understand their potential for instability, if any, through presence/absence of anti-oxidant excipients(s).

Seven formulations were procured from the Indian Market, and were subjected to two selective stress studies to access

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oxidative potential. The first selected stress study is in the presence of AAPH (a water soluble oxidative agent), which can provide active oxygen to the formulation. Formulations with primary packaging were subjected to higher temperature (80ºC) as the second selective stress condition. The rationale of thermal stress for accessing oxidative potential is based on the relation of temperature with rate of reaction as the degradation rate would be higher at the elevated temperature. Stressed solutions were analysed with LC and LC-MS. While Perfalgan™ (Manufactured by BMS) was exclusively stable under these stress conditions, all other formulations showed degradation to different extent. In order to investigate superior stability of Perfalgan™ over other formulations, presence of anti-oxidants in formulation was studied. Common anti-oxidants for parenteral formulations are cysteine and sulfites. The sulfites are reported to induce hyper-sensitivity reactions in a few cases.[3] In some preparation of stable liquid paracetamol compositions, cysteine is used as an antioxidant agent and citrate buffer.[4] In the European patent application EP-A-1752139, it is disclosed an aqueous paracetamol composition which comprises an antioxidant agent selected form the group consisting of ascorbic acid, N-acetyl-L-cysteine, and other stabilizing compounds containing the-SH group, which is essentially free of organic solvents and buffering systems, and which contains less than 1 mg/l of oxygen.[5] In Perfalgan™ cysteine is used as the anti-oxidant. An HILIC LC-MS method was developed to understand the presence of cysteine in all formulations and, an effort was made to correlate instability of the formulations to the anti-oxidant level.

MATERIAL AND METHODS

Chemicals

Seven paracetamol IV formulations marketed by different pharmaceutical companies were procured and supplied by BMS India Private Limited to Vimta Labs, Hyderabad. All commercial formulations except Perfalgan™ were coded as A1, A2, A3, A4, A5 and A6. All other chemicals used were of a reagent grade.

Instruments

The stressed samples were injected on a HPLC system equipped with a photodiode array detector (1200 Series, Agilent Technologies, Waldbronn, Germany). LC-MS/MS studies were carried out on a system in which LC (1200, Agilent Technologies, Waldbronn, Germany) was hyphenated to API 4000 (AB Sciex, Toronto, Canada). Other small equipment used in the study were cyclomixer, sonicator, precision analytical balance, oven, refrigerator and centrifuge.

Selective Stress Study

Thermal stress samples were generated by storing the formulation as such (with primary packaging) at 80°C for 7 days. Oxidative stress samples were generated by storing 10 mL of individual formulation(s) containing ~5.00 ± 0.2 mg AAPH [(2, 2'-azobis (2-amidino-propane) dihydrochloride)] at 40°C for 48 hours in volumetric flasks. All stressed samples was diluted 25 times with Milli-Q water and injected for HPLC and LC-MS analyses.

Analyses of Stressed Samples

The HPLC separation of the paracetamol and its known hydrolytic product, 4-amino phenol was initially established on optimized method before sample analysis as a system check. For chromatographic elution, 95 volume 10 mM Ammonium formate in Mill-Q Water: 5 volume Acetonitrile (A) and 5 volume 10 mM Ammonium formate in Mill-Q Water: 95 volume Acetonitrile (B) were varied in a linear gradient program (T_{min}/A:B; T_{0-12}/100:0; T_{40}/50:50; T_{44}/30:70; T_{50-60}/100:0) at 0.8 mL/min flow rate. The column, Zorbax SB Aq (150 X4.6) mm, 3.5 µ was kept at 30ºC for entire LC run. The injection volume was kept 5 µL constant for all the stressed samples for HPLC analyses. For the determination of molecular mass, LC-MS analyses were performed on API 4000 mass spectrometer using the same HPLC method. The mass range during entire run was kept 50-1000 m/z in ESI positive mode. The divert valve was used to allow oxidative peaks in mass analyzer, whereas, paracetamol peaks was diverted to waste.

Determination of Cysteine

A specific method was used to prepare samples for LC-MS/MS analyses. A 200 µL of formulation sample was accurately added to 800 µL of acetonitrile and shaken for 5 min. The mixture was further incubated at 2 to 8°C for 10 min followed by centrifugation at 12000 rpm for 8 min at 4°C. The supernatant was collected and loaded on LC-MS/MS for analysis of cysteine (Figure 1). For the separation of cysteine from other peaks, HILIC mode was used. Mobile phase 95 volume 10 mM Ammonium formate in Mill-Q Water: 5 volume Acetonitrile (A) and 5 volume 10 mM Ammonium formate in Mill-Q Water: 95 volume Acetonitrile (B) were varied in a linear gradient program (T_{min} /A:B; T_{0-12}/100:0; T_{40}/50:50; T_{44}/30:70; T_{50-60}/100:0) at 0.4 mL/min flow rate. The column used for the study was ZIC-HILIC 150 x 4.6 mm; 5 µm, 200°A. Two MRM transitions were selected m/z 122→m/z 105 and m/z 122 →m/z 76. The mass parameter DP and EP were kept 39.0 and 8.0, respectively. The CE and CXP were maintained at

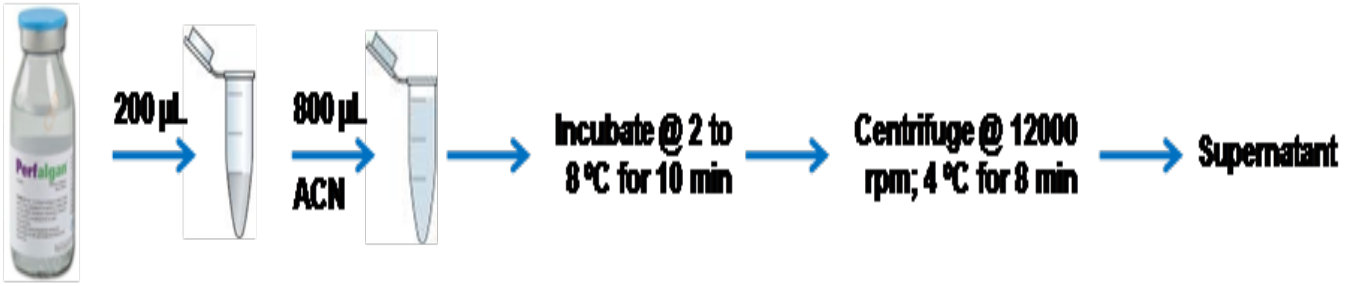


Figure 1: Extraction of Cysteine from formulation

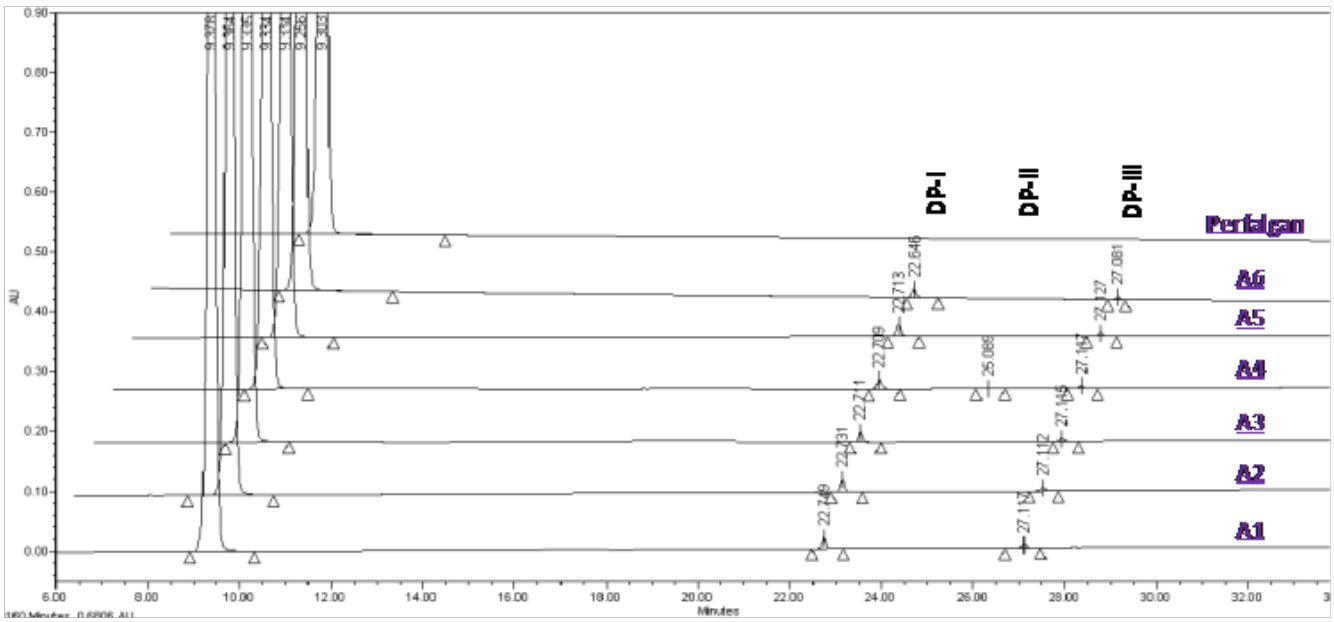


Figure 2: Overlaid HPLC chromatograms of tested formulations after thermal stress

14.22 and 9.00, respectively, for the first transition, whereas they were maintained at 18.27 and 5.34, respectively for second transition. Before analyses of samples, the standard solution of~300 ppm cysteine hydrochloride hydrate was injected as the sensitivity check solution.

RESULTS AND DISCUSSION

Selective Stress Study

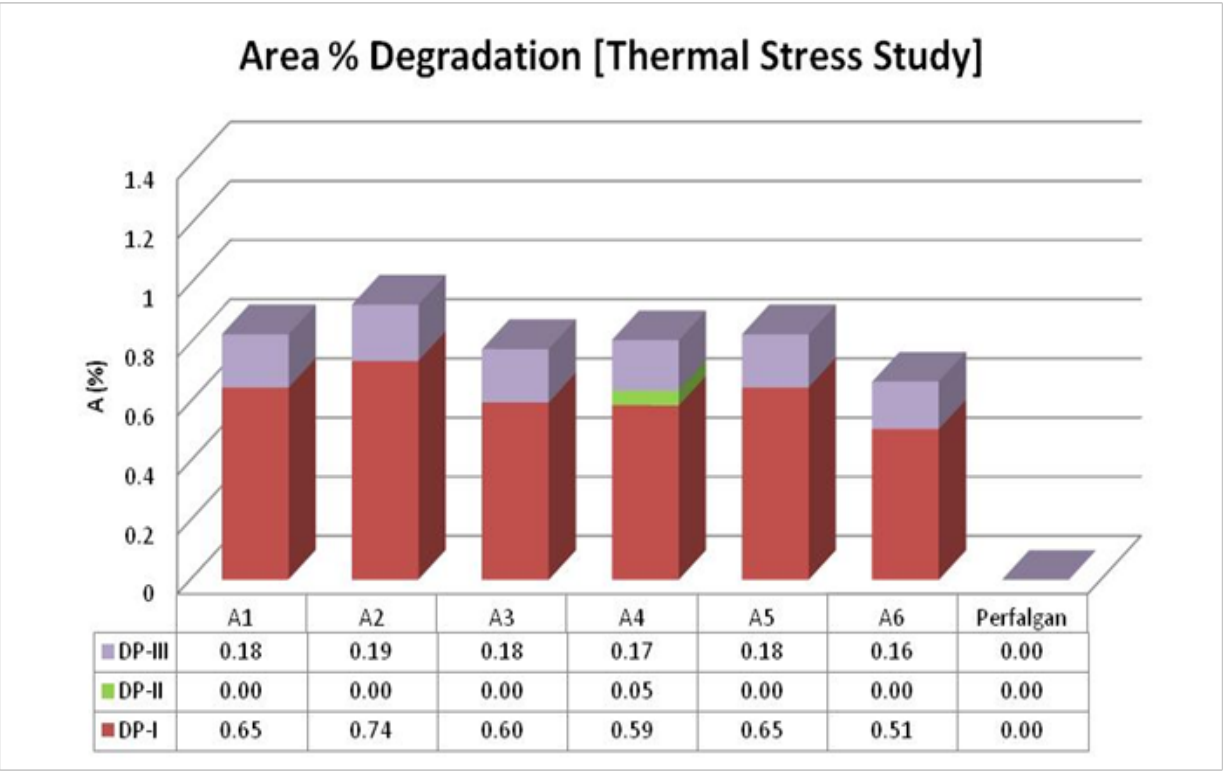
The stress studies with thermal condition and oxidative reagent were designed to compare the potential of paracetamol to be oxidized in the formulations. Thermal condition with primary packaging at 80ºC for 7 days was selected, as oxidative degradation rate due to dissolved oxygen would be higher at the higher temperature. AAPH, an oxidative agent was added to all formulations to understand the direct impact on stability through formulation strategy, if used. The gradient HPLC method was suitable to separate three major oxidative degradation products (DP-I to DP-III) eluting at RRT-2.35, RRT-2.58 and RRT-2.75. The LC-MS indicated masses for those degradation products as m/z 300, m/z 300 and m/z 450, respectively.

Thermal Stress Study

Figures 2 showed overlaid chromatograms of stressed formulations. All formulations except Perfalgan™ showed significant degradation in terms of DP-I, DP-II and DP-III. Figure 3 depicts extent of degradation (in terms of area percent) of all tested formulations. Out of seven formulations, Perfalgan™, proved to be the least susceptible to oxidative degradation during thermal stress.

AAPH Stress Study

Figures 4 shows overlaid chromatograms of stressed formulations with different extent of degradation. Figure 5 depicts extent of degradation of all the tested formulations. Perfalgan™ showed 0.03 A (%) degradation, whereas others showed significant degradation ranging from 0.86



	A1	A2	A3	A4	A5	A6	Perfalgan
DP-III	0.18	0.19	0.18	0.17	0.18	0.16	0.00
DP-II	0.00	0.00	0.00	0.05	0.00	0.00	0.00
DP-I	0.65	0.74	0.60	0.59	0.65	0.51	0.00

Figure 3: Comparative degradation profile of the tested formulations after thermal stress

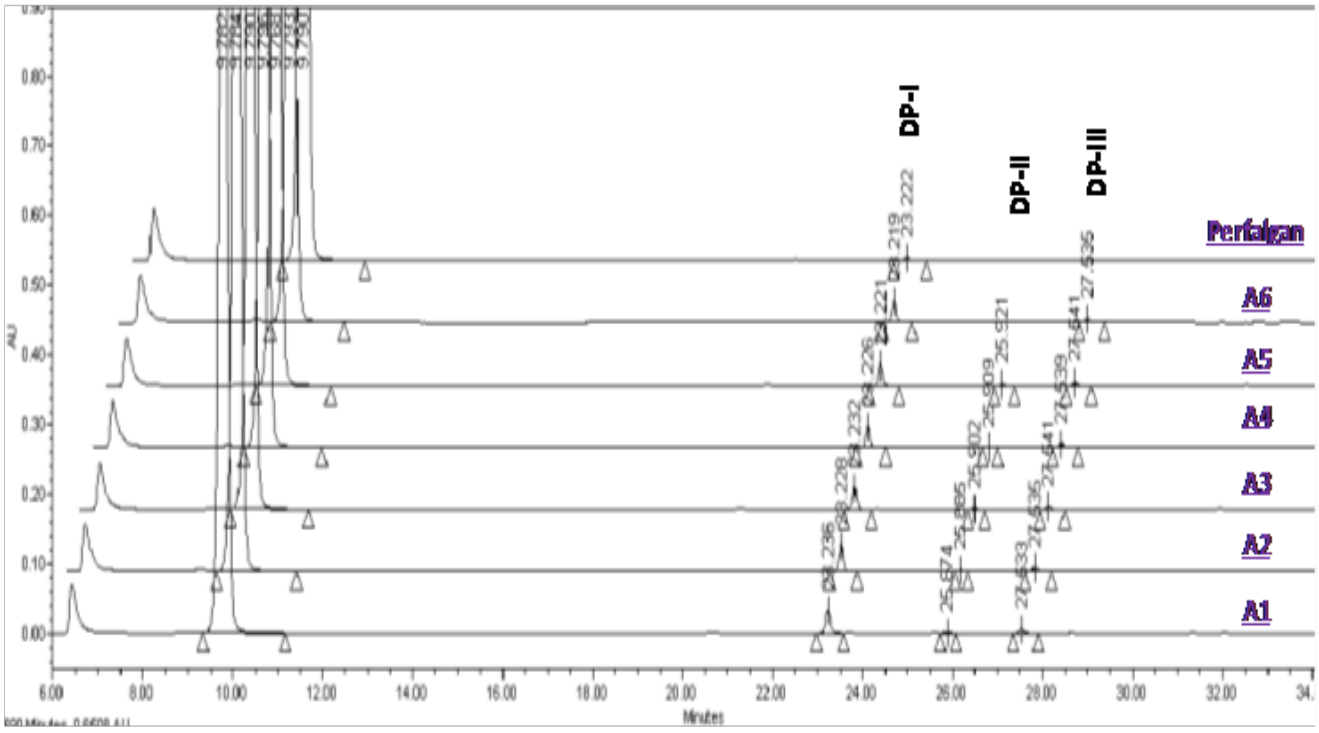
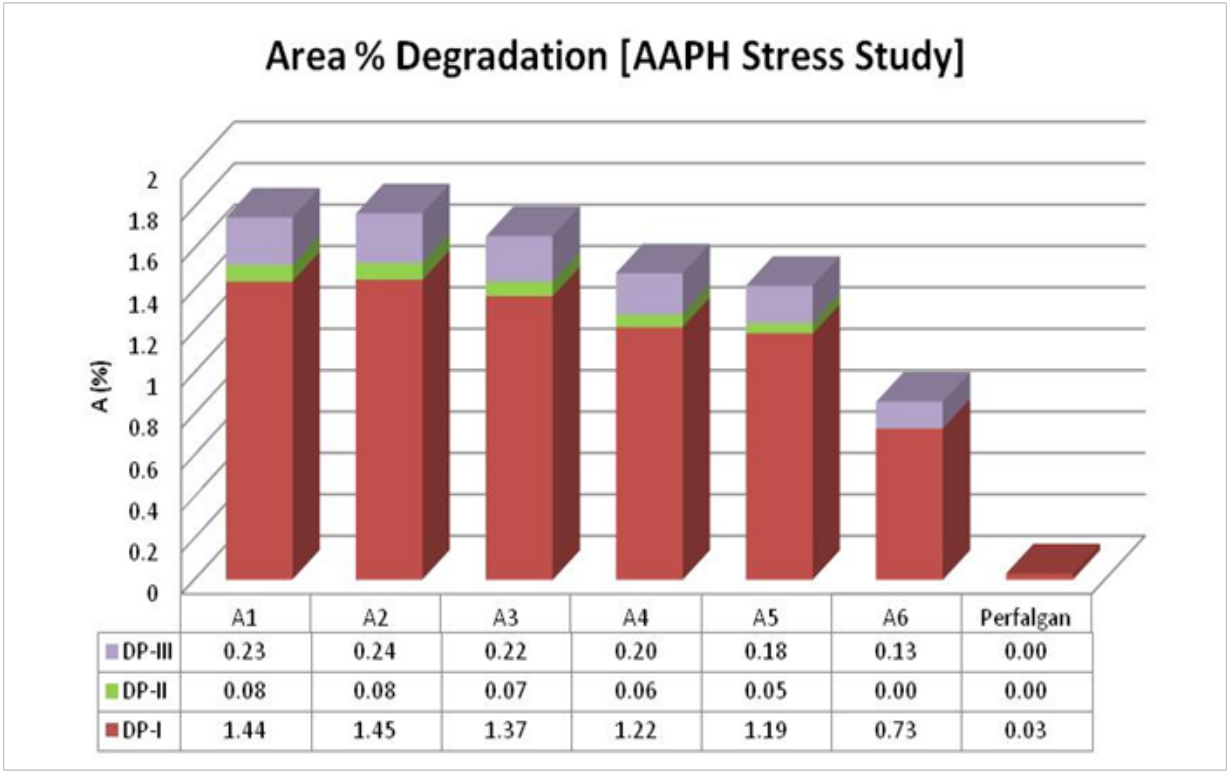


Figure 4: Overlaid HPLC chromatograms of tested formulations after 2,2' -azobis (2-amidino-propane) dihydrochloride (AAPH) stress



	A1	A2	A3	A4	A5	A6	Perfalgan
DP-III	0.23	0.24	0.22	0.20	0.18	0.13	0.00
DP-II	0.08	0.08	0.07	0.06	0.05	0.00	0.00
DP-I	1.44	1.45	1.37	1.22	1.19	0.73	0.03

Figure 5: Comparative degradation profile of the tested formulations after 2,2' -azobis (2-amidino-propane) dihydrochloride (AAPH) stress

A :Area; DP: Degradation product

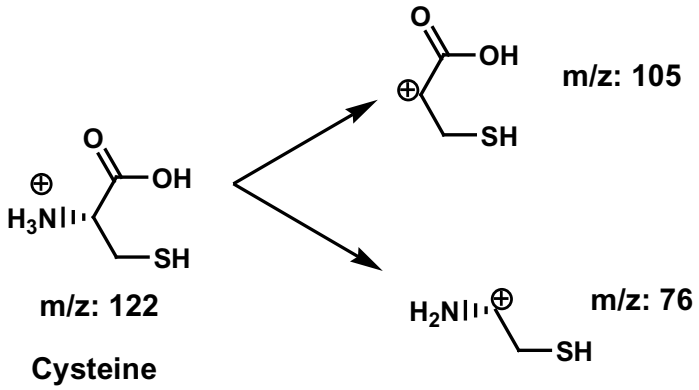


Figure 6: MRM transitions for cysteine LC-MS/MS

A (%) to 1.77 A (%). Of the seven formulations tested, Perfalgan™, proved to be the least susceptible to oxidative degradation even in the presence of oxidative agent, AAPH.

Cysteine Determination

Cysteine (as an excipient) determination through LC is challenging due to its polar property and its low content in formulation. Generally, parenteral formulations contain very high levels of isotonicity modifiers (excipients) such as mannitol and in the presence of such matrix, detection and quantification of cysteine is difficult and enrichment of cysteine in the sample is necessary. In order to achieve this, a sensitive and rapid solvent precipitation technique was developed. Acetonirile was used as an anti-solvent to facilitate precipitation of isotonicity modifiers. Moreover, low temperature (2 to 8°C) was maintained for 10 minutes to increase the rate of precipitation. Considering the level of cysteine typically used in the formulations, solubility of cysteine in acetonitrile: water (80: 20 v/v) was determined to be sufficient in the solution phase. The suspension was centrifuged at 4°C to remove precipitated isotonicity modifiers. The supernatant could easily be transferred to LC vials without additional filtration step, and this

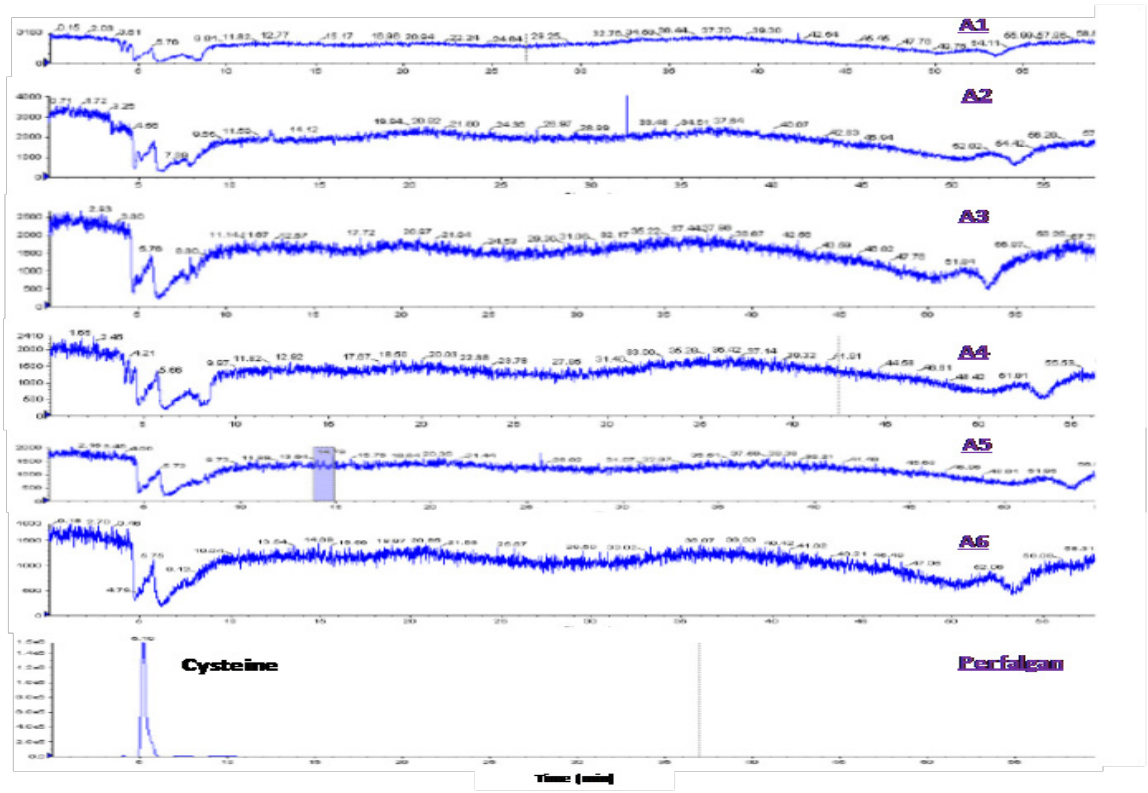


Figure 7: Overlaid LC-MS/MS data for cysteine presence of tested formulations

procedure eliminated the possibility of incompatibility with filters. The second challenge was to achieve suitable separation of cysteine from available other formulation excipients. HILIC separation mode was selected to get sufficient retention of cysteine. Moreover, MS/MS was selected in a selective detection mode to avoid any interference from unknown formulation excipients. Two transitions (Figure 6) m/z122→m/z 105 and m/z122→m/z76 gave additional selectivity for the detection. Figures 7 showed overlaid chromatograms for cysteine presence. Out of seven formulations, only Perfalgan™ showed presence of cysteine. It was not present in all other formulations.

CONCLUSION

This study depicts the experimental design to access oxidative potential of paracetamol IV formulations. Of the seven tested formulations marketed in India, Perfalgan™ was found to be the most stable under selective thermal and AAPH stress conditions. Presence of cysteine in Perfalgan™ is responsible for its extended stability. This study also proved necessity of an anti-oxidant in paracetamol solution formulations to avoid potential oxidative degradation during storage.

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