

Original Research Article

Stability Studies of a Mixture of Paracetamol and Ascorbic Acid, Prepared Extempore, at Elevated Temperature and Humidity Conditions

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Abstract

Purpose: To determine the effect of the temperature of water used for the preparation of paracetamol and ascorbic acid mixture on its stability, as well as to assess the influence of humidity on the stability of single components and their mixtures.

Methods: The stability of the mixtures in aqueous medium was evaluated with the aid of UV-Vis spectrophotometer interfaced with a computer. Spectral analysis was adapted to monitor changes in the aqueous medium of a commercial paracetamol and ascorbic acid mixture, an extemporaneously prepared mixture of paracetamol and ascorbic acid, and the individual preparations of paracetamol and ascorbic acid.

Results: The degradation rate was lower in commercial preparation ($6.80 \times 10^{-3} \text{ min}^{-1}$), compared to that of the extemporaneously prepared ascorbic acid/paracetamol mixture ($2.30 \times 10^{-2} \text{ min}^{-1}$). The decomposition of the commercial product in aqueous medium was 3.38 times slower than that of the extemporaneously prepared mixture. Ascorbic acid, tested under the same conditions as the commercial product, was unstable in aqueous solutions, with a degradation rate of $1.17 \times 10^{-2} \text{ min}^{-1}$. Ascorbic acid, dissolved in water, degraded completely within 4 h at room temperature, whereas paracetamol remained stable under the same conditions for 11 days.

Conclusion: The individual drugs in their original form retained their stability for 72 h, but some of the mixtures, in particular, the extemporaneously prepared ones showed more rapid degradation. Extemporaneous preparation of paracetamol/ascorbic acid liquid mixtures should not be encouraged.

Keywords: Paracetamol, Ascorbic acid, Stability, Degradation, Over the counter drugs, Extemporaneous preparations

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INTRODUCTION

Among numerous medicinal products from over the counter (OTC) class available without a medical prescription, there are analgesic and antipyretic products. Some of them are available in the form of effervescent tablets and powders for dissolution in water. If properly stored, medicinal products authorised for marketing

retain their stability during their storage – in the manufacturer's warehouse, pharmacy, or patient's house.

Drug stability is the ability to maintain the required characteristics and properties under the conditions determined by tests [1]. Usually, it is limited to 10 % degradation of an active pharmaceutical ingredient (API), that manufacturers consider fundamental in

determining the medicinal product's expiry date [2]. However, the stability of ex tempore medicinal products, prepared at home or at the patient's bed, is considerably limited and literature sources are often insufficient. Physical factors, such as sorption, and water evaporation, may adversely affect the drug's appearance, and above all, lead to API degradation, or its irregular distribution in the drug form, which negatively influences dosing [3,4].

Chemical reactions, such as hydrolysis, oxidation and reduction may result in the loss of activity of the ingredients, and consequently, hindered efficacy or increased toxicity [5]. Information on storage and preparation conditions is particularly important for patients living in tropical countries in III or IV climate zone, where climate conditions differ from those of I and II climate zone [6]. Paracetamol and ascorbic acid are commonly used organic ingredients of OTC medicinal products. Paracetamol is a widely used analgesic and antipyretic [7,8] while ascorbic acid, due to 2,3-endiol group in the molecule, may act both as an antioxidant or pro-oxidant, depending on the concentration [9]. As a result of ascorbic acid oxidation to dehydroascorbic acid one hydrogen atom is removed and an intermediate product is formed – the semi-hydroascorbic radical or two hydrogen atoms from the hydroxyl groups bonded to the 2nd and 3rd carbon atom [10].

Increased moisture content in commercial medicinal powders increases molecular mobility, and chemical reactivity [11]. Additionally, paracetamol contains the amide group which may be sensitive to hydrolytic degradation. Research on medicinal products stored in different temperatures and humidity has shown that physical properties of paracetamol in the form of powder and solution remain unaffected for even as long as three months [12-14]. It is different in the case of ascorbic acid's stability which decreases with increased temperature, sun exposure and pH. The dissociation constant of the compound is $pK = 4.04$. The oxidation process involves the elimination of two protons at pH 1-4 and one proton at pH > 5. With pH increasing up to 8.4, dissociation rate remains almost constant [15-19]. A higher number of ingredients in the product may prevent ascorbate from oxidation as a result of oxygen exposure [20]. The influence of the preparation conditions on the stability of the solutions, prepared by the patient at home are of high importance for the effectiveness and safety of the therapy [21].

The aim of this paper was to evaluate the stability of ascorbic acid and paracetamol, as well as mixtures of them in ambient temperature and various humidity conditions, and to determine the effect of the temperature of water used for the preparation of the solution of commercial mixture of paracetamol and ascorbic acid.

EXPERIMENTAL

Materials

The commercial mixture of paracetamol and ascorbic acid, manufactured by Bristol – Meyers, was obtained from the commercial stock of commune pharmacy (Fervex D). The experiments were conducted with the use P2153 and P3724 batches. Ascorbic acid was purchased from Pharma Cosmetic, Poland. Paracetamol of pharmaceutical grade according to European Pharmacopoeia 8.0 was used in the study.

Test solutions

The starting solution was prepared by dissolving the contents of one sachet of the product, containing 500 mg of paracetamol, 200 mg of ascorbic acid, 25 mg of pheniramine maleate, and excipients, in a 250 ml volumetric flask. The final concentration of ascorbic acid in the starting solution was 4.54×10^{-3} mol/l, and that of paracetamol was 1.32×10^{-2} mol/l. In order to compare the solution of paracetamol and ascorbic acid without excipients and additional pheniramine maleate, a mixture of those substances was prepared in the quantity corresponding to the contents of one commercial sachet. Parallel individual solutions of ascorbic acid and paracetamol, respectively, were prepared. The solutions were diluted 100 times for the spectrophotometric measurements. Each measurement was repeated three times.

Spectrophotometric and potentiometric assays

The stability of the paracetamol and ascorbic acid mixture in water solutions was evaluated with the use of PG Instruments UV – VIS T60 spectrophotometer (USA) interfaced with the PC to enable recording of the results. The pH of the starting solutions was measured with the use of the Mera Elwro type 517 pH-meter (Poland).

Effect of exposure of aqueous solutions to air

Four starting solutions were tested: solution of commercial product, solution of mixture of

paracetamol with ascorbic acid, solution of paracetamol, solution of ascorbic acid, all of them were exposed to air at room temperature for eleven days. The air temperature was 25 °C and humidity approx. 25 %. The absorbance in the 190 - 350 nm range was measured daily at the same time for eleven days.

Degradation studies

To analyse the kinetics of the degradation of the paracetamol and ascorbic acid mixture in water, the starting solutions were prepared as described above. The absorbance was first measured in the eleventh minute. Then, the content of API was assessed in regular 5-minute intervals until the plateau phase was reached. All spectra were collected in 190-350 nm range every second, at temperature of 25 °C. Respective wavelength was established due to the literature [22,23].

Effect of temperature of water on product stability

For each type of test samples, five starting solutions were prepared with distilled water of the following temperature respectively: 8, 25, 30, 50, 70 and 90 °C. After 60 minutes, the absorbance was measured by UV-Vis spectrophotometry at 190 - 350 nm.

Effect of humidity on the stability of ascorbic acid

The tests were conducted with the use of following powders: (1) a commercial mixture of paracetamol and ascorbic acid, with pheniramine maleate added, available in Polish pharmacies, (2) ascorbic acid, (3) mixture of ascorbic acid and paracetamol prepared in the laboratory in the form of powder. The effect of humidity on stability of assessed powders was tested using eight samples of powder, reflecting 80 mg of API – ascorbic acid. Four of them were placed in open weighing bottles, exposed to air, at room temperature, while the other four were placed in a desiccator containing hydrophilic silica, and incubated in the period 1 – 72 h. After 1, 2, 3, 24, 48, and 72 h the samples were weighed and used to prepare solutions. The absorbance of samples dissolved in 100 ml of water was measured.

Statistical analysis

The analysis of the data obtained was conducted with a Statistica 10.0 software. The Wilcoxon test was performed, $d < 0.05$. The kinetic degradation rate constants were evaluated as a function $y =$

$A_1 + A_0e^{-kt}$ estimated with the use of the Gauss–Newton algorithm.

RESULTS

Effect of storage time on the stability of the mixture of paracetamol and ascorbic acid in water

Changes in the aqueous solutions of the commercial paracetamol and ascorbic acid mixture (A), mixture of paracetamol and ascorbic acid (B), paracetamol (C) and ascorbic acid (D) were monitored spectrophotometrically and the results are shown in Fig 1.

The results obtained for the solutions of commercial mixture and the prepared mixture are similar (Fig 1A and B). After one day, ascorbic acid in both solutions degraded and only paracetamol remained unchanged (Fig 1C), whereas in the case of ascorbic acid, degradation was very rapid (Fig 1D). In the analysis of the effect of storage time on the stability of paracetamol in water solution for 11 days, the absorbance remained largely the same, and the spectral shape did not change, which proves that the paracetamol solution was stable in that period of time. The difference between the level of the paracetamol solution absorbance measured on the last day (day 11), and the absorbance obtained on the 1st day was below 5 % ($p < 0.05$, Wilcoxon test) showed that those differences were not statistically significant.

Degradation of paracetamol and ascorbic acid in aqueous solution

Based on Fig 2A, the maximum absorbance at the fixed wavelength, decreases with time. The decrease is very fast, both in the case of the paracetamol and ascorbic acid solution, as well as in the case of Fervex D. Initially the absorbance decreases rapidly, while after a longer time, it enters into the plateau phase. To compare the kinetics of the degradation process in aqueous solution of both samples, the function graph, $y = A_1 + A_0e^{-kt}$, was estimated using Gauss–Newton algorithm. The slopes of the straight lines reflect the variability of the degradation processes. The degradation rate was lower for the commercial preparation ($6.80 \times 10^{-3} \text{ min}^{-1}$), than the extemporaneously prepared mixture of ascorbic acid and paracetamol ($2.30 \times 10^{-2} \text{ min}^{-1}$). The degradation of commercial product in the water solution was 3,38 times slower than the degradation of the extemporaneously prepared mixture of ascorbic

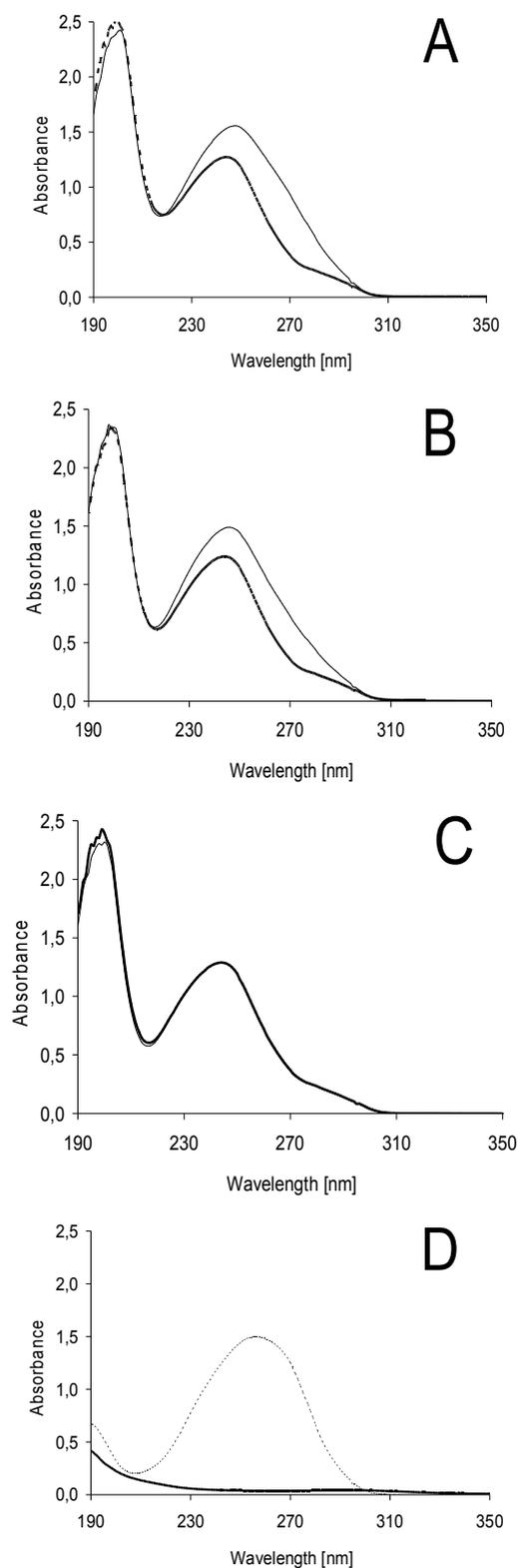


Fig 1: UV-VIS spectra of the solutions of: commercial paracetamol and ascorbic acid mixture (A), mixture of paracetamol and ascorbic acid (B), paracetamol (C), ascorbic acid (D) recorded every 24 hours for 11 days. The thin line (-) represents the absorbance at the initial stage, whereas the thick line (—) represents the absorbance after 11 days

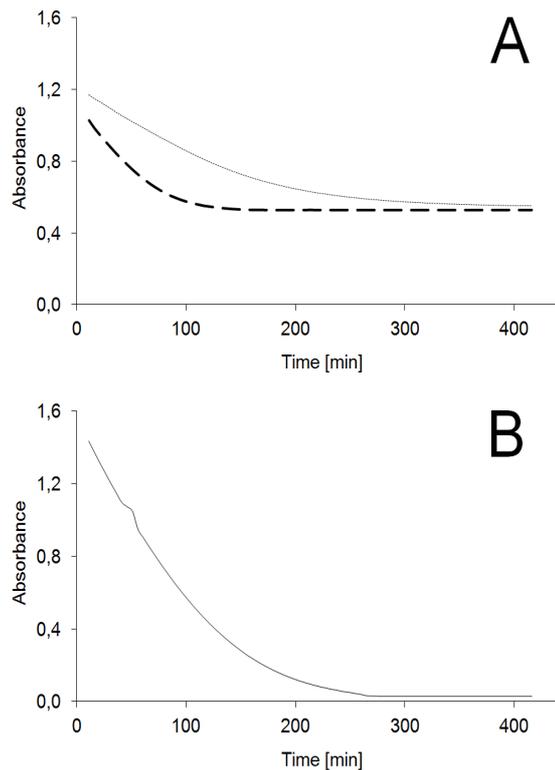


Fig 2: (A) Changes of absorbance vs. time, of the commercial product solution, thin line (-) and the “in-house” prepared paracetamol and ascorbic acid solution, dotted line (· ·) measured at the maximum signal, 256 nm wavelength, and time, presented as a function $y=A_1+A_0e^{-kt}$ estimated with the use of the Gauss–Newton algorithm. (B) Correlation of the absorbance of the ascorbic acid solution and time measured at the 256 nm wavelength, presented as a function, $y=A_1+A_0e^{-kt}$, estimated with the use of the Gauss–Newton algorithm

acid and paracetamol . Ascorbic acid, tested under same conditions as the commercial product was unstable, with a degradation rate of $1.17 \times 10^{-2} \text{ min}^{-1}$.

Effect of the temperature of water used on stability of ingredients

According to the results, the temperature of water used for preparation of the solutions has no significant effect on the stability of ascorbic acid, paracetamol and their mixture produced in laboratory. Almost 18 % increase in absorbance was observed in the case of the solutions of commercial mixtures prepared at extreme temperatures: 8 °C and 90 °C, whereas in the case of intermediate temperatures of 25, 35, 50 and 70 °C the values of the absorbance were lower. Differences between the maximum absorbance values were < 5 %, and statistically not significant for the temperature range 25 – 70

°C. The influence of temperature on the assessed absorbance in the case of the commercial mixture of ascorbic acid and paracetamol is presented in Fig 3.

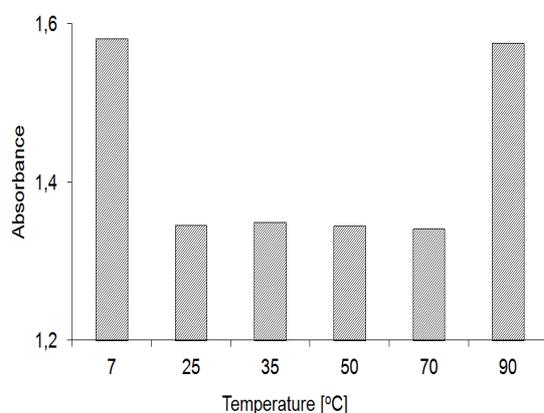


Fig 3: Influence of temperature of used water on the absorbance of the solutions of commercial mixture (paracetamol + ascorbic acid) at maximum wavelength of 256 nm

Effect of humidity on stability of paracetamol and ascorbic acid mixture

The absorbance value of the maximal signal for: the individual solutions of ascorbic acid, paracetamol, mixture of both ascorbic acid and paracetamol, as well as commercial mixtures remained at a constant level for 4 consecutive days. The maximal absorbance of the solution of paracetamol alone was approximately 0.3154, whereas the other solutions maintained their absorbance at 0.6219, 0.5958 and 0.4771 for commercial formulation, extemporaneously prepared mixture, and ascorbic acid, respectively. Variation in maximal absorbance and weight of sample in the test period was statistically not significant (Wilcoxon test, $p < 0.05$).

DISCUSSION

Based on the results obtained, degradation was retarded in the commercial mixture of paracetamol and ascorbic acid, compared to the solution of ascorbic acid and paracetamol without excipients. Due to the high instability in water solution, the ascorbic acid, when evaluated as single component of the solution, degraded completely after ca. 250 min of the initiation of the reaction. Degradation of ascorbic acid in the solution of commercial product proceeded slowly. Conversely, the ascorbic acid in the mixture of paracetamol and ascorbic acid, as well as the solution of ascorbic acid itself degraded rather fast. Ascorbic acid contains a dienol group with

strong reducing activity, which leads to the fast degradation of the structure. The product of its oxidation is dehydroascorbic acid which is even less stable. The stability of the commercial mixture of paracetamol and ascorbic acid in the solution was enhanced due to the excipients contained in the pharmaceutical product, such as citric acid and magnesium citrate, stabilizers of the ascorbic acid [24]. Citric buffer, which is formed in situ after dissolution of the commercial product, ensures the optimal pH of the water solution, which was ca. 4.8 in the performed measurements. The increased pH results in prolonged degradation of ascorbic acid in the aqueous solution, comparing to the solution of mixture of ascorbic and paracetamol prepared in laboratory, with a pH of 3.7. The absorbance of the solution of paracetamol remained at the same level for 450 min, which supports the idea of stability of paracetamol under the test conditions, in the absence of ascorbic acid.

It can be assumed that even water evaporation, which might have occurred during the test, did not affect significantly the stability of the assessed single/individual substances, but for the mixture of ascorbic acid and paracetamol in the commercial product, the temperature of the water influenced the content of ascorbic acid. The obtained results correspond to the recommendations of the pharmaceutical product's manufacturer in the product leaflet: "dissolve the content of the sachet in cold or hot water". According to the European Pharmacopoeia, "cold" water temperature ranges 8 – 15 °C, while "hot" water is at least 80 °C. Polyvinyl pyrrolidone (PVP), as an excipient may influence the amount of drug in solution. The increased temperature of the solution favours decrease of PVP chain length, as well as decrease of the viscosity [25]. This may contribute directly to the decrease in the number of hydrogen bonds between polymer molecules and drug molecules, which may then result in decreased solubility of the drug [26]. Therefore increased absorbance may be observed in the commercial mixture prepared in water at 8 °C, compared to other temperatures.

PVP may increase the solubility of paracetamol, as is the case for other active substances, by forming easily soluble complexes [27,28]. There is a correlation between the increase in solubility of paracetamol in water solution, and the increase in the number of hydrogen bonds between polymer hydroxyl groups and paracetamol carboxyl groups confirmed [29]. Polymer-solvent interactions depend upon the polymer molecular structure, chemical composition, solution concentration, solvent

molecular structure, and solution temperature. As temperature rises, the entropy of the system increases, which may contribute to the formation of intercyclic and intracyclic reactions. Also intermolecular hydrogen bonds may appear, resulting in the increased UV absorbance at 90 °C. Previous tests did not reveal any effect of evaporation on the results.

Air humidity did not exert any effect on the stability of ascorbic acid, paracetamol and mixtures of ascorbic acid and paracetamol, whether commercial and extemporaneously prepared. Based on literature, the weight of vitamin C remains the same for 8 weeks when exposed to an atmosphere of 0 and 75 % relative humidity at room temperature [30]. In another study, under normal storage conditions, commercial tablets of ascorbic acid were stable for > 5 years [31].

CONCLUSION

The findings of this study indicate that the conditions of preparation, viz, temperature of water, amount of active ingredient in the aqueous commercial mixture of ascorbic acid and paracetamol did not affect product stability. Ascorbic acid in aqueous solution degrades completely within 4 h at room temperature, whereas paracetamol remains stable under the same conditions for 11 days. Relative humidity under the test conditions does not affect significantly the stability of individual components, and their mixtures.

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