

Stability Testing of Compounding Capsule Combination between Paracetamol and Tramadol in a Private Hospital Semarang

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Abstract

A combination of Paracetamol and Tramadol is used in mild to severe pain management. In a private Hospital Semarang, this combination is included in fast-moving drugs that it is frequently compounded and prepared in advance. The study aims to determine Beyond Use Date (BUD) in compounding capsules combination between Paracetamol and Tramadol samples. Beyond Use Date is a time limit indicating that a medicine beyond the expiration date must not be used and determined based on the results of stability testing. Samples were stored for 14 days in a tightly closed container far from direct sunlight in room temperature without AC (28°C) and without silica gel, room temperature with AC (25°C) without silica gel, and room temperature with AC (25°C) with silica gel. Furthermore, samples underwent physical and chemical stability testing. Physical stability testing was conducted using organoleptic testing by observing direct changes from the powder color in capsules, capsules form, and scent on the samples. Meanwhile, chemical stability testing was conducted by determining the content of active ingredient from the samples using reversed-phase HPLC method and C18 as the stationary phase, methanol and aquabidest (40:60) as the mobile phase, wavelength 271 nm, flow rate 0.6 mL/min and injection volume 10 µL. The result shows that samples were physically stable for being able to retain the original physical properties showed by consistent powder color and capsules form, and there was no available scent. However, the chemical stability testing method was unable to separate and quantify the content of Paracetamol, Tramadol, and formed degradation products. It can be concluded that Beyond Use Date based on organoleptic and chemical stability testing for 14 days of compounding capsules combination between Paracetamol and Tramadol could not be determined. Nevertheless, this study showed favorable storage conditions in a tightly closed container far from direct sunlight in Room Temperature with AC (25°C) with Silica Gel.

Keywords: Beyond use date; Paracetamol; Reversed-Phase HPLC; Tramadol

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INTRODUCTION

Compounding is the process of combining, admixing, diluting, pooling, reconstituting other than as provided in the manufacturer's labeling, or otherwise altering a drug or bulk drug substance to create a non-sterile medication.¹ Limitations of extemporaneous compounding practice in drug formulas are not available on the market, limitations of the patient's condition in consuming the dosage forms, limited supply of drug preparations, patient compliance, and medical costs in the market.² Stability is the extent to which a product or preparation retains physical and chemical properties and characteristics within specified limits throughout its expiration or Beyond Use Date.¹ The stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective, and informational specifications.³ Stability studies are carried out to establish a Beyond Use Date for the compounded preparation. The Beyond Use Date indicates the days after the compounded formula is prepared in which the product is no longer safe to be used.¹

High-Performance Liquid Chromatography (HPLC) is one of the separation methods in a column containing a stationary phase that will be flowed by the mobile step.⁴ Reversed-Phase will elute samples with higher polarity earlier as the characteristic of the mobile phase is more polar than the stationary phase.⁵ HPLC method is used since the chromatography technique can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture

with better, faster, and more efficient compound separation results.^{4,6} Reversed-phase of HPLC with a UV detector provides the best available reliability, analysis time, repeatability, sensitivity, and capable of monitoring several wavelengths concurrently.⁷

Combination of Paracetamol and Tramadol works synergistically with different mechanisms of action and used as a second-line medication for the treatment of moderate to severe pain management.⁸ In a private Hospital, Semarang, this combination is included in fast-moving drugs. Yet, the prices are quite high on the market, that it is frequently compounded to lower the cost of the medication. Real-time stability testing was carried out to assign Beyond Use Date; thus, it can be used as a reference in storing and maintaining compounding capsules combination between Paracetamol and Tramadol.

METHODS

The materials were working standards of the pharmaceutical-grade of Paracetamol (batch no. WS-QC-400395378) and Tramadol (batch no. 100209203). It was used without further purification and certified to contain 99.3 % of Paracetamol and 99.2 % of Tramadol on a dry weight basis. The materials were such as paracetamol tablet 500 mg (Promedrahardjo Pharmaceutical Industries, batch no. 00818L0110) and Tramadol capsules 50 mg (Otto Pharmaceutical Industries, batch no. 8J 0935). Methanol gradient grade for liquid chromatography E.Merck®, Aquabidest was obtained from Water Purification System Easy Pure II RF, hard capsules shell, and silica gel.

Instrumentation and chromatographic conditions used was the HPLC system (Shimadzu®, LC-2010HT), consisting of a Pump with a dual serial plunger, micro-volume (10 µL on the primary side, 5 µL on secondary along with autosampler injector programmed at 10 µL capacity per injection, was used. The detector consisting of UV. LC separations was performed on a Phenomenex C₁₈ column (250×4.6 mm i.d., 5 µm particle size). The mobile phase consisted of 40: 60 (v/v); methanol: aquabidest. The flow rate was set to 0.6 mL/min, and UV detection was carried out at 271 nm. Other materials and tools were such as ultrasonication Retsch® T460, analytical scales Ohaus® PAJ1003 (max 120g, min 0.001g), micropipette Socorex® (0.1-2µL; 10-100µL; and 100-1000µL), millipore filter (0.45µm), solvent membrane filter Whatman® (0.45µm), microtube 1mL, injection sput 5mL, Pyrex glassware, tightly closed container, mortar, and pestle.

There were two kinds of samples made, which were compounding capsules combination between Paracetamol and Tramadol and compounding capsules of Tramadol. The first samples were prepared by finely powdered a tablet of Paracetamol and a pill of Tramadol separately. The accurate weight of the powder, which was 25 mg of Paracetamol and 10 mg of Tramadol, was weighed and mixed before being inserted into an empty capsule shell. The second samples were made by 10 mg of a finely powdered capsule of Tramadol and were inserted into an empty capsule shell. Furthermore, samples were stored for 14 days in a tightly closed container far from direct sunlight with three different storage conditions such as room temperature without AC (28°C) and without silica gel, room temperature with AC (25°C) without

silica gel, and room temperature with AC (25°C) and with silica gel. The third condition conformed to private hospital Semarang conditions. Physical and chemical stability testing was conducted to the samples on Day - 0, 1, 3, 7, 10,14.

Organoleptic testing

The characteristic of the physical stability of the samples was determined by observing direct changes from the powder color in capsules, capsules form, and scent on the samples. The parameter used was the result on Day - 0 as the sample was freshly made and expected to have higher stability than the sample with a longer storage period.

Chemical stability testing

The chemical analysis performed in this study was conducted by determining the content of active ingredient from the samples using reversed-phase HPLC method and using Phenomenex C₁₈ (5 µm) as the stationary phase, methanol and aquabidest (40:60) as the mobile phase, wavelength 271 nm, flow rate 0.6 mL/min and injection volume 10 µL [9].

Standard solutions and calibration

A stock standard solution containing Paracetamol (2500 µg/mL) and Tramadol (1000 µg/mL) was prepared by dissolving 25 mg of Paracetamol and 10 mg of Tramadol in 10 mL volumetric flask with methanol. It was further diluted to obtain working standard solutions in a concentration range of 0.05 – 2.45 µg/mL (i.e. 0.05; 0.45 ; 0.85 ; 1.25 ; 1.65 ; 2.05 ; and 2.45 µg/mL) for Paracetamol and 1 µg/mL for Tramadol. This solution was filtered through a 0.45 µm millipore then transferred to HPLC vials and sonicated for 10 min. A constant volume of 10 µL injections was made for each concentration of three replications and chromatography under the above-

mentioned conditions. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. Linear calibration curves were generated using least-squares linear regression analysis.

Sample preparation

Samples that had been stored were taken and transferred into 10 mL volumetric flask and diluted to 10 mL with mobile phase methanol and aquabidest (40:60). 0.286 μL of compounding capsules combination between Paracetamol and Tramadol was transferred to 1 mL microtube, and 999.714 μL of the mobile phase was added. 1 μL of compounding capsules of Tramadol was then transferred to 1 mL microtube, and 999 μL of the mobile phase was added. The concentration achieved after the above-mentioned dilution was 1 $\mu\text{g}/\text{mL}$. This solution was filtered through a 0.45 μm millipore, then transferred to HPLC vials and sonicated for 10 min. A constant 10 μL volume of sample solution was injected under the above-mentioned

conditions. The peak areas were measured at 271 nm, and their contents in the samples were determined using a multilevel calibration curve developed on the same HPLC system under the same conditions using the linear regression equation.

RESULTS AND DISCUSSION

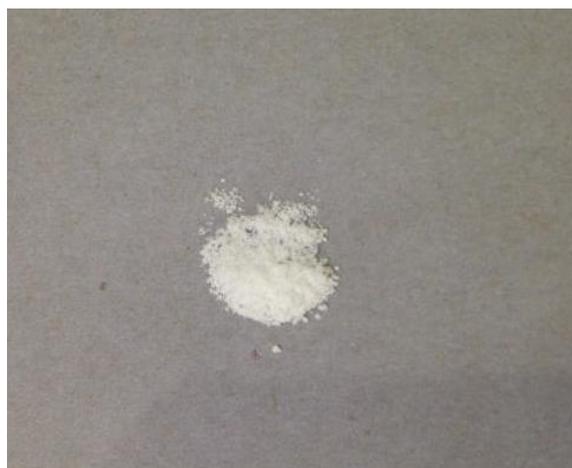
Organoleptic testing

This test is carried out to identify the physical stability of the compounded preparation and to establish a Beyond Use Date. Table 1 and Figure 1 show that within 14 days of storage, compounding capsules combination between Paracetamol and Tramadol, compounding tablet of Paracetamol, and compounding capsules of Tramadol remained consistent in powder color and capsules form, and there is no available scent on the samples. It indicates that the compounded preparation is physically stable for being able to retain the original physical properties.¹⁰

Table 1. Organoleptic studies

Sample storage time	Room temp. without AC (28°C) and silica gel	Room temp. with AC (25°C) and without silica gel	Room temp. with AC (25°C) and silica gel
Day - 0	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-
Day - 1	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-
Day - 3	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-
Day - 7	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-
Day - 10	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-
Day - 14	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-	TB : white PC : white CF : rigid Scent :-

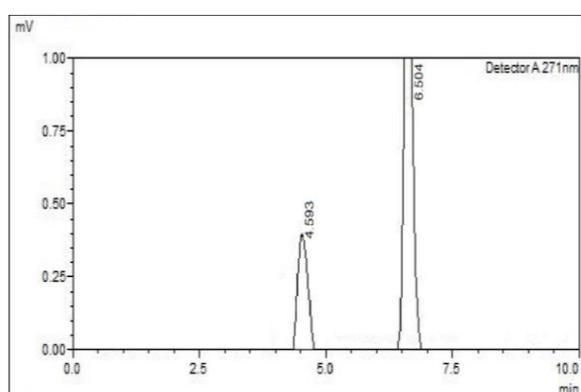
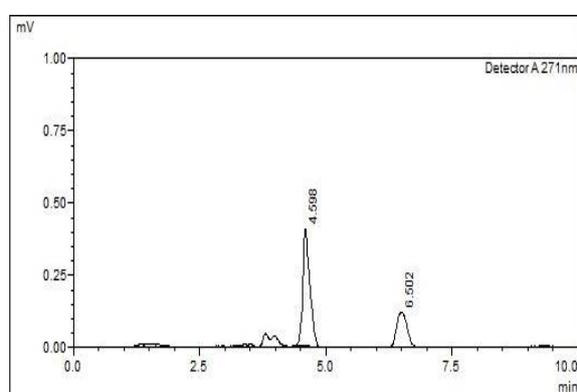
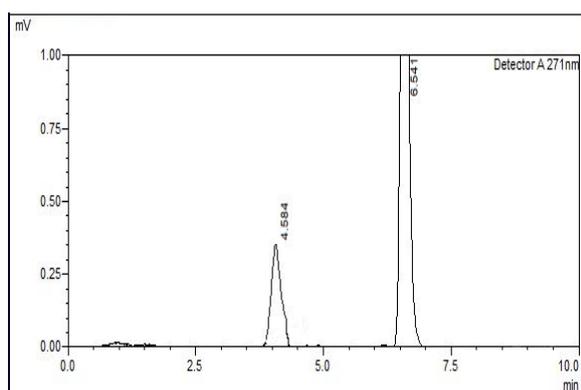
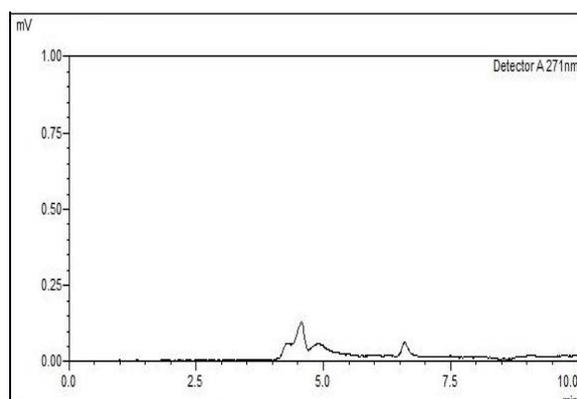
Information: Powder color in tablets (TB), capsules (PC), capsules form (CF)



Prior to Storage



After Storage

Figure 1. The physical appearance of the sample**Figure 2.** Chromatogram of Paracetamol standard 0,45 µg/mL**Figure 3.** Chromatogram of Tramadol standard 1 µg/mL**Figure 4.** Chromatogram of compounding capsules combination between Paracetamol and Tramadol 1 µg/mL**Figure 5.** Chromatogram of compounding capsules of Tramadol 1 µg/mL

However, this testing has a high subjectivity; thus, chemical stability testing is required to determine the stability of compounded preparation.

Chemical Stability Testing

Chemical stability is the ability of each active ingredient to retain its chemical integrity and labeled potency within the

specified limits.¹⁰ This chemical stability testing is carried out to establish a Beyond Use Date.

Linearity

Linear relationships were observed by plotting Paracetamol concentration against obtained peak areas. Paracetamol showed linear response in the concentration of 0.05; 0.45; 0.85; 1.25; 1.65; 2.05; and 2.45 µg/mL. The corresponding linear regression equation was $y = 22746x + 9210.3$ with correlation coefficient (r) of 0.9927 indicating the linear relationship between increased concentration and instrument response.

The retention time for Paracetamol standard 0.45 µg/mL, Tramadol standard 1 µg/mL, compounding capsules combination between Paracetamol and Tramadol 1 µg/mL, and compounding capsules of Tramadol 1 µg/mL was found to be 6.504; 6.502; 6.541; and 0 minutes respectively. Retention time is a measure of the time taken by solute to pass through a column to the detector. In this

study, reversed-phase chromatography was used with the mobile phase, which was more polar than the stationary phase; thus, the more polar compounds would have earlier retention time. The retention time variation requirement was ≤ 0.05 minutes.¹¹ The time difference obtained from the time retention of Paracetamol standard and compounding capsules combination between Paracetamol and Tramadol was 0.037 minutes; therefore, Paracetamol was found in the compounded preparation samples. In Figure 5, the AUC and the time retention cannot be determined as, at 1 µg/mL, the Tramadol samples also contain excipients, which make the concentration measured lower than 1 µg/mL.

Based on Figure 6, it can be concluded that Paracetamol is more stable at room temperature with AC (25°C) and silica gel as, in the other two conditions, there is an increased Paracetamol content in Day - 7, 10, 14.

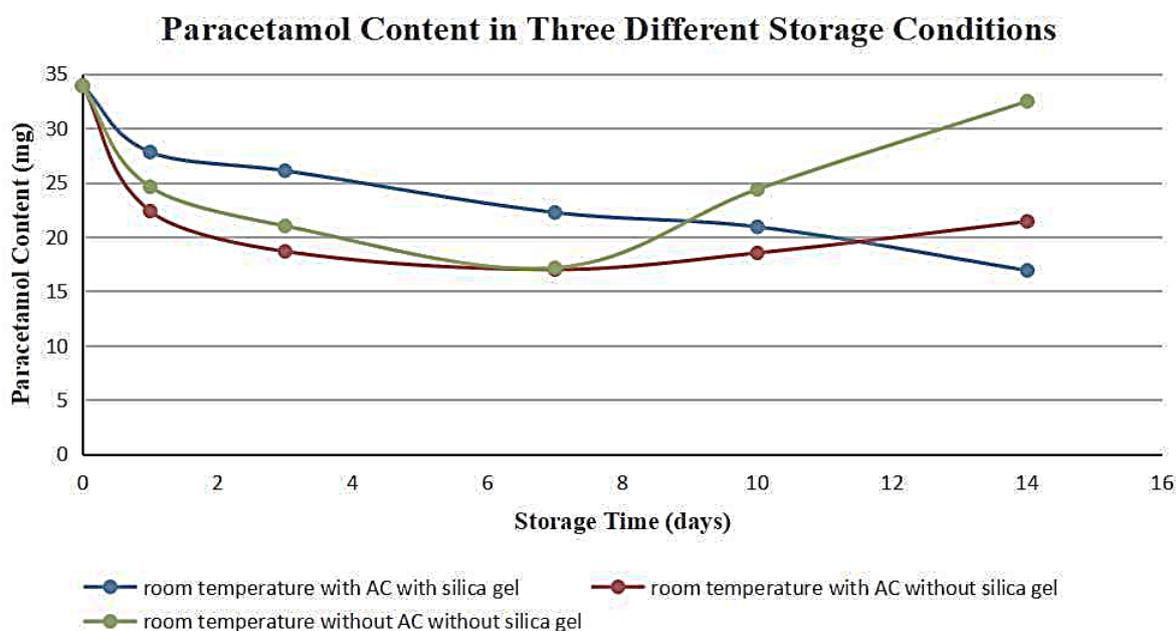


Figure 6. Paracetamol content in three different storage conditions

Decreased Paracetamol content

There is a decreased Paracetamol content since the physical or chemical characteristics of the compounded preparation can change over time.¹ Humidity will be more controlled at room temperature with AC (25°C) because AC will absorb humid air in the room. Humidity in room temperature without AC (28°C) will vary based on the environmental humidity. Moreover, silica gel can help to slow down decreased Paracetamol content as the microporous structure in silica gel has the capacity to absorb the water vapor into those cavities. There is no chemical reaction taken place in the way silica gel adsorbs moisture. It happens due to the difference in water vapor gradient between the surrounding environment and the microcavities in silica gel. The moisture absorption is taken place until the cavities are saturated or until the water vapor pressure of the surrounding environment, and microcavities have gained an equilibrium, which is mostly around 12 weeks.¹²

Increased Paracetamol content

There is increased Paracetamol content because, during storage, gelatin capsules can absorb or release moisture. When stored in a high relative humidity environment, hard gelatin capsules can absorb moisture and lose their rigid shape and become distorted. Whereas, in a different environment of extreme dryness, capsules may become too brittle.¹³ Paracetamol contains the amide group, which may be sensitive to hydrolysis degradation.¹⁴ Hydrolysis takes place in labile carbonyl function groups, which include amides, esters, arylamines, imides, imines alcohols, and carbamates.¹⁵ During the storage process, hydrolysis degradation of Paracetamol may occur and produce *p*-

aminophenol, which is stated to be a very toxic substance and has the ability to cause nephrotoxicity, teratogenicity, and methemoglobinemia.¹⁶

Paracetamol degradation product can be measured in the HPLC system as it has a similar structure to Paracetamol and because the HPLC system for degradation product is not optimized. Mechanism of the Paracetamol oxidation pathway is favored by the hydroxylation of the benzene ring, leading to the formation of 3-hydroxyacetaminophen and releasing acetamide as a reaction of byproduct. The breakdown of the bond between the amino group and the substituted methanol in the paracetamol molecule can lead to degradation of byproducts with substituted nitrogens, such as 4-aminophenol and nitrophenol. The mineralization of paracetamol appears through ortho, meta, and para-substituted oxidation pathways, leading to the formation of dihydroxy aromatic rings such as catechol, resorcinol, hydroquinone as well as trihydroxylated rings such as pyrogallol, phloroglucinol, and hydroxyhydroquinone.¹⁷

Moreover, the time difference obtained from the time retention of Paracetamol standard (6.504) and Tramadol standard (6.502) is 0.002 minutes. Therefore, there is a possibility that Tramadol is also measured in the area of Paracetamol due to the slight difference in time retention, which is ≤ 0.05 minutes.¹²

Beyond Use Date

In the absence of a USP-NF compounded preparation of monograph or specific stability information for solid dosage forms (capsules, tablets, granules, powders), the maximum BUD packaged in tight, light-resistant containers in controlled room temperature (20°-25° C)

is 180 days.¹ In this study, Beyond Use Date based on organoleptic and chemical stability testing for 14 days of compounding capsules combination between Paracetamol and Tramadol is unable to be determined as the chemical stability testing method cannot separate and quantify the content of Paracetamol, Tramadol, and formed degradation product. Nevertheless, this study showed favorable storage conditions in a tightly closed container far from direct sunlight at room temperature with AC (25°C) and silica gel.

CONCLUSION

Beyond Use Date based on organoleptic and chemical stability testing for 14 days of compounding capsules combination between Paracetamol and Tramadol is unable to be determined. Nevertheless, this study showed favorable storage conditions in a tightly closed container far from direct sunlight in Room Temperature with AC (25°C) and Silica Gel.

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CONFLICT OF INTEREST

The authors declare there is no potential of conflict of interest with the research, authorship, and article publication.

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