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Evaluation of physicochemical characteristic of rifampicin extemporaneous suspension in specific time and conditions

Chiv Sinly[1], Rutchyaporn Anurach[1], Rachadaporn Benchawattananon[3], Pansu Chumworathaya[1], Chernporn Navanukraw[4], Kiettipum Phontree[1], Chantana Boonyarat[1], Thatsanand Xayavong[1] and Theera Rittirod[1,*]

[1]Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand
[2]Faculty of Pharmacy, University of Health Sciences, Vientiane, Lao
[3]Program in Forensic Science, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
[4]Program of Aesthetic Sciences and Health, Graduate School, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand
[*]Corresponding author: theera@kku.ac.th

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Abstract

Rifampicin is one of the first line medicines for treatment of tuberculosis (TB). Some patients such as children cannot take the solid dosage form orally and need oral liquid preparations. In those cases, pharmacists prepare rifampicin extemporaneous suspensions. However, the stability of rifampicin extemporaneous suspensions in a tropical country such as Thailand has not been investigated. A lack of stability information means that the compounding pharmacist is responsible for ensuring the stability efficacy and safety of the suspension. Rifampicin extemporaneous suspension was prepared at 25 mg/mL and kept under 3 temperature conditions: refrigerated (7±3°C), room temperature and accelerated temperature (45±5°C). The color, pH and viscosity of the extemporaneous solution was recorded after 0, 7, 14, 30, 45 and 60 days and the rifampicin content were determined by HPLC analysis. There was no change in the color of suspensions stored at room temperature and refrigerated temperature, but the color did change under 45±5°C. The pH remained stable under all three conditions of storage. The suspensions decreased in viscosity as viscometer spindle speed was increased, and this was unaffected by the different storage conditions. All samples stored under refrigerated temperature and room temperature retained more than 90% of their initial rifampicin concentration for 60 days. However, under the accelerated temperature, only 19.51% of the initial concentration of rifampicin remained on day 60 (p<0.05). Thus, testing for stability at a range of temperatures is important when extemporaneously preparing pharmaceutical suspensions in tropical climates.

Keywords: Extemporaneous suspension, Stability test, Physicochemical, Temperature, Rifampicin

1. Introduction

Pharmaceutical formulation is the process of combining active pharmaceutical ingredients and excipients to produce a medicinal product [1]. Most drugs are available in tablet and capsule forms suitable for use in adults, while dosage forms specifically formulated for pediatric use are typically lacking except for diseases common or specific to children [2]. In many practice settings, there is a shortage of commercially available oral liquid dosage forms [3]. In these cases, patients who are unable to swallow solid dosage forms such as tablets or capsules can be given an oral suspension of the drug that is extemporaneously prepared by a compounding pharmacist [6]. Information about the stability, physical properties, chemical properties, resistance to microbial contamination and toxicity of extemporaneously prepared drug formulations is lacking [4]. The stability of a pharmaceutical formulation under different storage conditions is an important consideration to ensure the safety, efficacy and quality of pharmaceutical formulations and to determine the shelf-life of a preparation [5].

Tuberculosis (TB) is a respiratory disease caused by Mycobacterium tuberculosis. Rifampicin is the first line medicine for treatment of TB, but it is poorly soluble in water and vulnerable to photodegradation, and is therefore

rarely produced in liquid form [7]. However, a liquid dosage form of rifampicin is important for effective treatment of TB in debilitated patients and very young children. In a previous study, it was found that the rifampicin content of an Extemporaneous suspension of 25 mg/mL was above 90% for 90 days under refrigerated and room temperature. In addition, the appearance, color, odor and pH remained unchanged and there was no microbial contamination. In another study, the concentration of rifampicin in a 25 mg/mL extemporaneous suspension was between 90 and 110% after storage for 60 days at both refrigerated and room temperature [8]. However, the stability of an extemporaneously prepared suspension of rifampicin has not previously been determined in a tropical country such as Thailand where average daytime temperatures range from 32-36ºC (91-97ºF) year-round with some daily maximums exceeding 40ºC (104ºF) between March and May.

The aim of this study was to develop a suitable vehicle for extemporaneous suspension of rifampicin and evaluate its physicochemical properties under different storage conditions such as refrigerated (7±3°C), room temperature and accelerated temperature (45±5°C).

2. Materials and methods

2.1 Chemicals and reagents

Capsules containing 300 mg of rifampicin (lot number: 1076303-1) were supplied by Siam Pharmaceutical Manufacturer. Acetonitrile (Lab-scan, Thailand), citric acid anhydrous (Lot no: AA-1710-30731, S. Tong Chemicals Co. Ltd, Thailand), sodium citrate (Smart sciences, Thailand), glycerin 99.50% USP (Lot BGPXWC1126, MANUFACTURER/SUPPLIER, Thailand), sucrose (Pure refined sugar ®, Thailand).

2.2 Instruments

Automatic balance (Model PL-402-L, Mettler Toledo, Switzerland), pH meter (Ezedo/PL600, Taiwan) Brookfield viscometer (Model DVNXLVTJG programmable rheometer, LabX, USA), high-performance liquid chromatography (HPLC auto agilent 1100 series, Japan), hot air oven (HW-HP01, Thailand), vortex mixer (Model S0100-220, National Labnet Co. Inc, USA) were used in this study.

2.2.1 Preparation for development formulation

Formulation was prepared by weighing rifampicin powder and transferring it to a mortar (concentration 25 mg/mL) as shown in Table 1. Tween 80 was added as a wetting agent to the mortar and stirred for a smooth paste. Sodium carboxymethyl cellulose (SCMC, 10 mL) was dispersed and added to the mortar then mixed about 5 min. Ten milliliters of xanthan gum (dispersed) with 10 mL syrup was added to the mortar and stirred. Then paraben was added and the mixture was mixed until it was homogeneous. Citrate buffer (pH = 5) was added as buffering agent to control pH. The mortar was filled with distilled water to a final volume of 50 mL and the mixture was transferred from the mortar to 9 plastic bottles and stored in dark place and covered with aluminium foil.

Table 1 Ingredients of rifampicin extemporaneous suspension.

Ingredient	Formula	Function
Rifampicin (g)	1.25	Active pharmaceutical ingredient
0.1 % Tween 80 (mL)	4	Wetting agent
Carboxy methyl cellulose sodium (g)	0.75	Flocculating agent
Xanthan gum (g)	0.25	Suspending agent
Syrup USP (mL)	10	Sweetening agent
Citrate buffer (mL) pH: 5	5	Buffering agent
Paraben concentrated (mL)	2	Preservative
Purified water qs. (mL)	50	Vehicle

2.2.2. Quality assessment

2.2.2.1 Ease of redispersibility

It is also an important parameter, which reflects the quality of suspensions. It was checked after 1 week. This was done in over spaced volume bottle. The stored suspensions in a measuring bottle were inverted through 180 degree and a number of inversions necessary to restore a homogeneous suspension was determined on inversion was considered 100% redispersibility.

2.2.2.2. Appearance

Color changes were assessed by visual inspection. The original suspensions were red/orange in color.

2.2.2.3. pH measurement

The pH of the rifampicin extemporaneous suspensions was measured in triplicate for each sample by pH meter (Ezedo/PL600, Taiwan) on the first and the last days of evaluation.

2.2.2.4. Viscosity

Viscosity was recorded using a rotational viscometer using spindle LV 1 at speeds of 25, 50, 100, 150 rpm.

2.2.2.5. High-performance liquid chromatography (HPLC)

HPLC was used to determine the content of rifampicin according to the method of a previous study [9]. Rifampicin was detected at the wavelength 250 nm. The mobile phase consisted of acetonitrile, distilled water (DI) ratio (40:60) in buffer phosphate pH 5.80. The injection volume was 20 µL with a C18 column 4.6-mm × 10-cm; 5-µm packing L7 and flow rate 1.20 mL/min. For analysis of the drug suspension, 1 mL of the rifampicin suspension (25 mg/mL) was withdrawn from 50 ml and transferred to a microtube. A 200 µL aliquot was mixed with 800 µL acetonitrile. The mixture was centrifuged and 20 µL of the supernatant was added to the mobile phase for analysis in HPLC.

A standard curve was prepared by serially diluting the stock solution with mobile phase. Standard solution was made from rifampicin standard 0.5 mg/mL of USP Rifampin RS in diluent. The calibration of concentration was 0, 5, 10, 100 and 200 µL/mL.

2.3 Statistical analysis

All results are expressed as mean ± standard deviation. Suspensions with the content of rifampicin in the acceptable range from 90 to 110 % were considered stable. One-way Anova was performed to analyze the data.

3. Results and discussion

3.1 Ease of redispersibility

Resuspendibility parameters can be observed for all samples, because the sediments were easily redispersed after 5 sec of vigorous and manual agitation, resulting in a homogeneous system for all temperatures and times.

3.2 Appearance

The acceptance criterion for the appearance of the suspension was retention of the original red/orange color. The rifampicin extemporaneous suspension samples stored at 7±3°C and at room temperature showed no change in color over 2 months (Figure 1, columns 1 and 2). The color of the rifampicin extemporaneous suspension samples stored at 45±5°C was not stable. The color changed from red/orange to red/black after only 7 days at 45±5°C (Figure 1, columns 3)

Figure 1 Change of color of rifampicin extemporaneous suspensions after storage at 7±3°C (A), room temperature (B), and 45±5°C (C). First row: day 0, second row: day 7, third row: day 14, fourth row: day 30, fifth row: day 45, sixth row: day 60.

3.3 pH measurement

All rifampicin extemporaneous suspension samples showed no significant changes in pH under any conditions. Samples stored at refrigerated temperature (7±3°C) showed pH values in the range of 5.58 – 5.77 ($p > 0.05$; Figure 2, blue line). The pH range for samples stored at room temperature was 5.70 – 5.78 ($p > 0.05$; Figure 2, green line) and 5.67 – 5.72 ($p > 0.05$; Figure 2, red line) for samples stored at 45±5°C. This indicates that the buffering agent successfully kept the formulation stable at these temperatures.

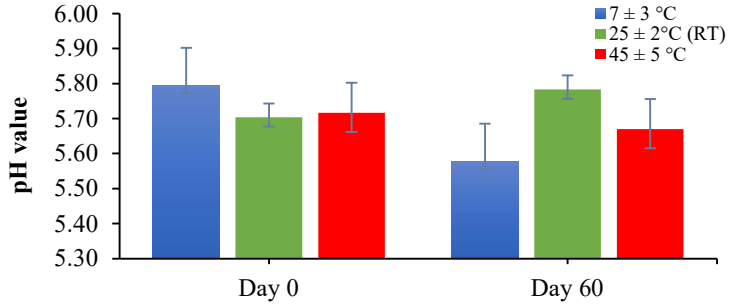

Figure 2 pH value under three conditions in day 0 and day 60.

3.4 Viscosity

Viscosity was tested at day zero and after 60 days. A shown in Figure 3A, viscosity values decreased when the speed was increased in the same manner for all suspensions on day zero. After storage for 60 days, viscosity significantly decreased at all speeds for the suspensions stored at 45±5°C (Figure 3B).

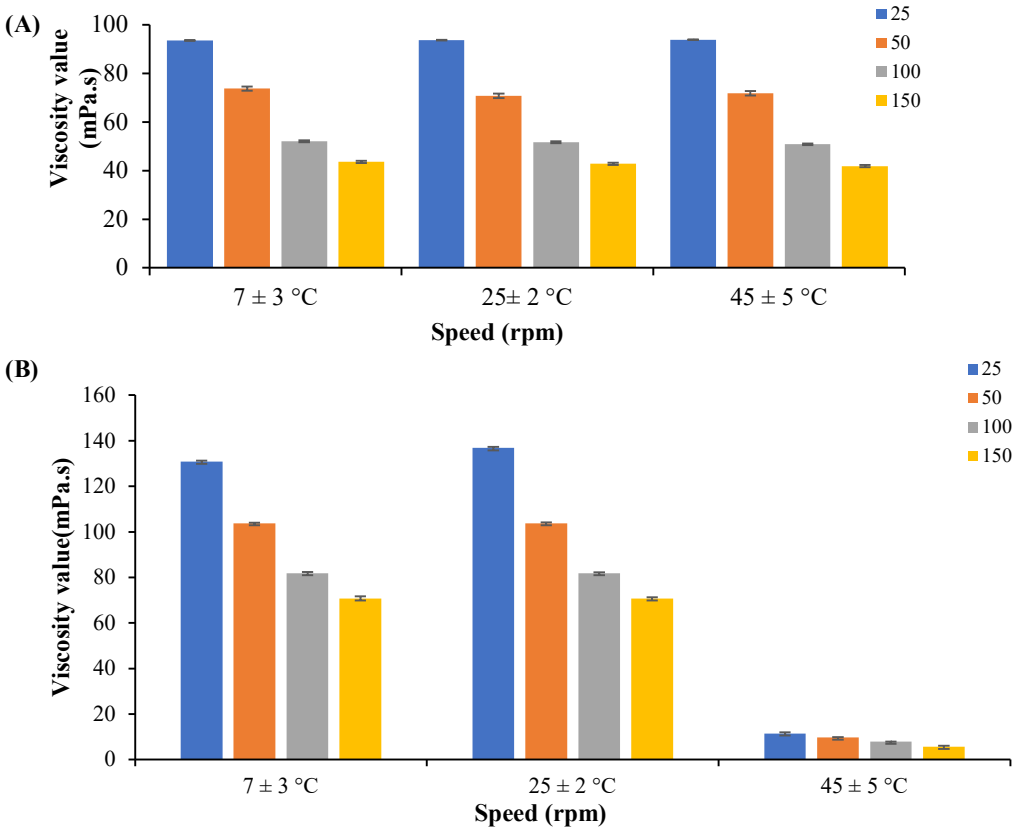

Figure 3 Viscosity value in day 0 under 3 conditions (A) and in day 60 under 3 conditions (B).

3.5 Rifampicin content

The standard curve for rifampicin is shown in Figure 4. The linear regression of standard curves was Y= 27.157x - 13.572. The correlation coefficient (R^2) was 0.9995 (n=3). The peak areas of samples were measured, and the concentrations of samples were calculated from the standard.

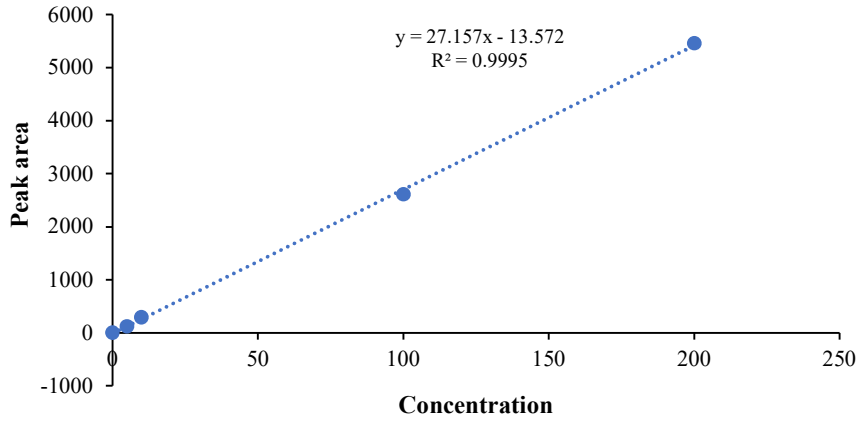

Figure 4 Standard curve of Rifampicin (n=3).

Representative HPLC chromatograms of rifampicin standard and rifampicin samples are shown in Figure 5 A and Figure 5 B, respectively.

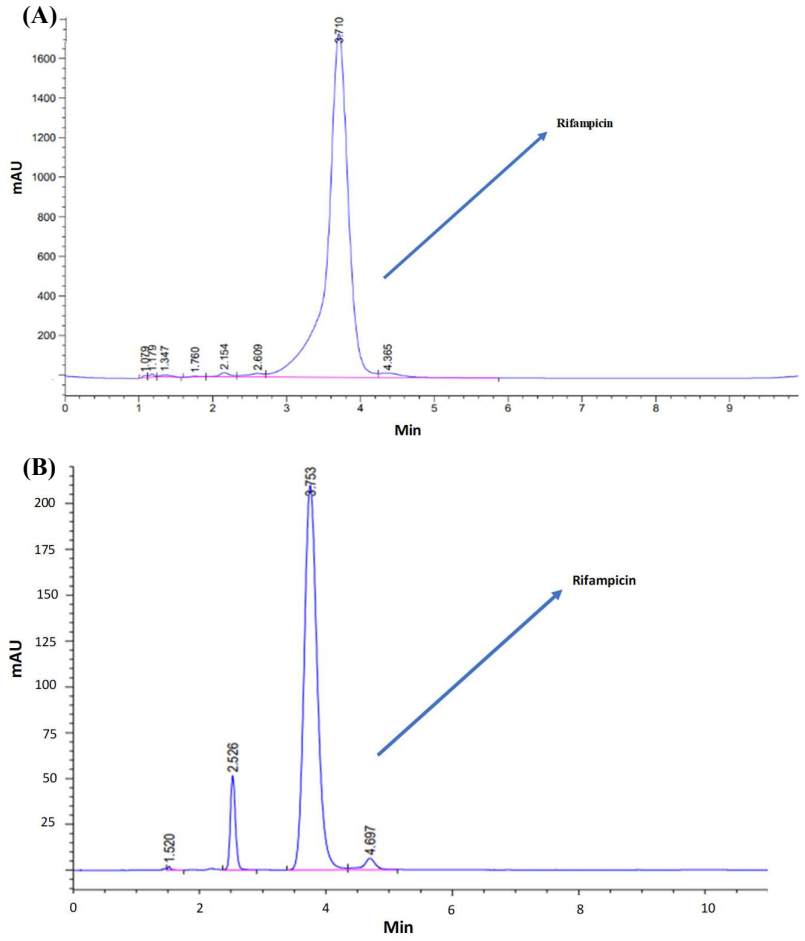

Figure 5 Chromatogram of (A) rifampicin standard and (B) rifampicin sample.

The initial rifampicin contents in the extemporaneous suspensions were 102%, 101.74% and 101.55% for samples stored at refrigerated, room temperature and 45±5°C, respectively. The results show small reductions in the rifampicin concentration over 60 days under refrigerated and room temperature storage conditions (102% to 101.55% and 100.74% to 94.73%, respectively). Samples stored at 45±5°C showed a significant reduction in rifampicin content from 101.55% on day zero to 19.51% on day 60 ($p < 0.05$, Figure 6).

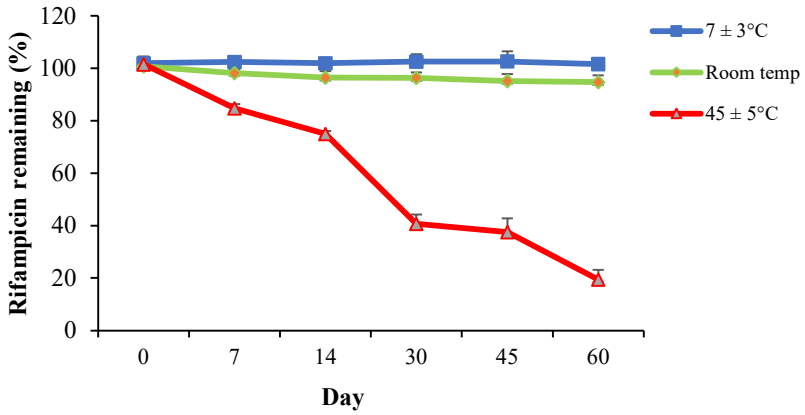

Figure 6 Rifampicin content in extemporaneous suspensions under 3 storage conditions.

3.6 Discussion

All samples were easily redispersed to homogeneity after 5 sec of vigorous and manual agitation for all tested temperatures and times. Our formulation contained 1.5% SCMC and 0.5% xanthan gum in concordance with Shayoub et al.,[10] who reported that formulated suspensions containing 0.4 to 0.6% xanthan gum have sufficient viscosity to resist sedimentation and re-suspend easily.

Color is of importance in pharmaceutical products because it is a major component of the first impression formed by consumers and patients. Color changes may also provide some information about the condition of the drug. In this study, the color did not change for samples stored at refrigerated and room temperature, but there was a distinct change from red/orange to black caramel under accelerated temperature conditions. This change of color reflects the decomposition of rifampicin by hydrolysis or oxidation to 3- formyl rifampicin or rifampicin quinone, respectively [11].

The pH value of the rifampicin extemporaneous suspensions showed no significant differences ($p > 0.05$) under the three different storage conditions over 2 months. This result shows that the buffering agent (citrate buffer) contained in the formulation successfully maintained the pH of this extemporaneous suspension [12].

Viscosity values decreased when the force of agitation (speed) of the viscometer was increased which means the extemporaneously prepared suspensions behaved as thixotropic fluids. Samples stored at refrigerated and room temperatures maintained this viscosity for 60 days, but samples stored at 45±5°C, showed a significant decrease in viscosity after 60 days. At the higher temperature, the structure of cellulose is destroyed. SCMC and xanthan gum are both forms of cellulose. The measurement of viscosity showed that carboxymethylcellulose changed viscosity when temperature was increased [13-14].

There was a small reduction in rifampicin concentration over 60 days under refrigerated and room conditions, but a major reduction in drug concentration for samples stored at 45±5°C. This is the first report that high storage temperatures have an effect on the drug content of extemporaneously prepared rifampicin suspensions. This study is in concordance with a previous study that reported no change in the color of an extemporaneous rifampicin suspension stored at room and refrigerated temperature for 60 days, although the color did change in that study after 60 days. The concentration of rifampicin also remained above 90% in that study [15]. Another study about the stability of rifampicin in an extemporaneous suspension found that the appearance did not change and the rifampicin content was 90 to 110% after storage fort 60 days at refrigerator and room temperatures [8].

4. Conclusion

Rifampicin extemporaneous suspensions should be put-on long-term stability studies to provide data for a definitive decision to be made on their stability. Rifampicin extemporaneous suspensions can be stored at 7± 3°C and room temperature up to 60 days because the drug content remained above 90 percent. This assay content is within the acceptable criteria. However, the same formulation was unstable when stored at 45±5°C. Rifampicin extemporaneous suspension stored at 45±5°C was less stable, showing significant color changes and drug content below 90% within 7 days of storage.

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