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A validated stability-indicating high-performance liquid chromatography-photodiode array method for the evaluation of extemporaneous compounded formulations prepared from isoniazid substances and tablets

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ABSTRACT

Objectives: The objectives of the study are to develop and validate a stability-indicating method for evaluating the stability of extemporaneous oral liquid formulations containing 50 mg/mL isoniazid (INH) prepared from INH drug substances and INH tablets. Materials and Methods: The optimized high-performance liquid chromatography (HPLC) conditions included a C18 column (250 mm × 4.6 mm, 5 µm), a flow rate of 1 mL/min, a column temperature of 30°C, and a detection wavelength of 254 nm. The separations were performed using a gradient HPLC system, employing acetonitrile and water as the mobile phases. The method was validated according to ICH Q2(R1) guidelines. The validated method was applied to assess the INH concentration in extemporaneous oral liquid preparations stored under refrigerated conditions (4 ± 2°C) for duration of 180 days. Results: The optimized method enabled the simultaneous determination of INH and its degradation products in lactose-containing samples. The peak purity index of INH was 1.0000. The linearity assessment over a concentration range of 10–150 µg/mL yielded correlation coefficients (r) surpassing 0.999. Accuracy was observed within the range of 99.24%–100.53%, and the relative standard deviations for intra-day and inter-day precision remained below 1.0%. The investigated formulations contained >93% of the labeled amount of INH throughout the study period, indicating acceptable stability. In addition, no significant changes in pH or color were detected. It was also discovered that the examined commercially available INH tablets are lactose-free formulas. Conclusion: The study reveals that the use of INH substances and lactose-free tablets for oral liquid formulations results in a prolonged stability period than indicated by previous research. This study not only contributes to the validation of a reliable stability-indicating method but it also provides stability data for an extended shelf life of the extemporaneous INH oral liquids, which can improve patient convenience and consequently patient compliance.

Keywords: Extemporaneous, high-performance liquid chromatography, isoniazid, stability-indicating, tuberculosis
INTRODUCTION

Isoniazid (INH), also known as isonicotinic acid hydrazide or isonicotinyl hydrazide, is the first-line drug for the treatment and prophylaxis of tuberculosis (TB) in adults and children.\textsuperscript{[1-4]} INH is used therapeutically either alone or in combination with rifampicin, ethambutol, and pyrazinamide. In many countries, INH is available in various pharmaceutical dosage forms, including tablets, oral solutions, and injectable solutions. However, in Thailand, only INH tablets (100 mg/tablet) are commercially available. Notably, the administration of INH tablets poses inconvenience for patients with swallowing difficulties, especially among the elderly and children under the age of five. Consequently, to address these concerns, the Ministry of Public Health (Thailand) advocates the use of a 10-mg/mL extemporaneous INH oral suspension prepared from crushed INH tablets and a sugar-free vehicle (referred to as vehicle type 2) – a recommendation particularly pertinent for pediatric applications.\textsuperscript{[5]} The implementation of this approach adheres to clinical practice guidelines for TB treatment in children, necessitating refrigeration and consumption of extemporaneously prepared INH oral suspension within a span of 21 days. However, long-term therapy is often required in TB treatment. The frequent renewal of medications at health-care facilities due to the short shelf-life of the suggested INH formulation raise concerns about patient inconvenience, particularly for patients who reside in rural and border areas of Thailand. These concerns may lead to the discontinuation of medication and therapeutic inefficacy. Therefore, a formulation with an extended shelf life is necessary to improve patient compliance through improving patient convenience.

Lactose is a common diluent in INH tablet formulations. The presence of lactose in extemporaneous INH oral liquids has been associated with rapid INH degradation due to the formation of isonicotinoyl lactotinoyl hydrazine.\textsuperscript{[6,7]} This has led the British pharmaceutical codex to recommend substituting INH substances for INH tablets when compounding INH oral liquid formulations.\textsuperscript{[7]} In addition, Haywood et al. (2005) demonstrated that an INH mixture containing INH substance at a concentration of 10 mg/mL exhibited acceptable stability for up to 30 days when stored at temperatures of 4°C and 25°C.\textsuperscript{[7]} Previous studies demonstrated that replacing INH tablets with INH substances improved the stability of extemporaneous INH oral liquids. Consequently, Thai hospital pharmacies in Phichit and Uttaradit provinces consider using INH substances and vehicle type 2 in extemporaneous preparations. However, there is insufficient proof of stability in this formulation. Therefore, the formulations examined in this study were developed in two key aspects that differed from the conventional approach. First, the concentration of INH was increased from 10 mg/mL to 50 mg/mL to enhance patient compliance. This modification was made because employing a conventional 10 mg/mL extemporaneous INH suspension could lead to impractically large oral volumes for pediatric patients. Second, INH substances were considered for use as the active ingredient in extemporaneous INH oral solutions to improve stability. However, this study also considered the stability investigation of a formulation containing crushed INH tablets and vehicle type 2 to compare the stability with INH substances and to be an alternative in extemporaneous preparations. Altering the origin of active pharmaceutical ingredients and the strength of pharmaceutical products can affect their stability. Therefore, the purpose of this study is to evaluate the stability of 50 mg/mL extemporaneous INH oral liquids that were formulated using vehicle type 2, INH substances (F1), and INH tablets (F2).

To carry out the chemical stability investigation, we required a reliable analytical technique that was unaffected by the degradation products of INH and pharmaceutical excipients, including parabens. Regarding the literature reviews, the determination of INH was frequently accomplished using HPLC methods coupled with electrochemical, ultraviolet (UV), fluorescence, and photodiode array (PDA) detectors.\textsuperscript{[8-15]} In addition, the stability studies of lactose-containing INH tablets were conducted using a variety of analytical techniques, such as HPLC,\textsuperscript{[6,7]} UV/VIS spectrophotometry,\textsuperscript{[16]} and GC with pre-column derivatization.\textsuperscript{[17]} Furthermore, the United States Pharmacopeia (USP) monograph employs nitrite titration to assay INH in INH oral solution.\textsuperscript{[18]} However, nitrite titration is a method that is non-specific and time-consuming. Among these methodologies, HPLC-PDA offers the advantage of peak purity determination. Thus, the stability-indicating method employing HPLC-PDA was developed and validated in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q2(R1).\textsuperscript{[19]} The chemical stability study of the investigated formulations was evaluated over a 180-day period under refrigerated storage conditions (4 ± 2°C). As described in the USP monograph for INH oral solution, the stability of the examined INH formulations was acceptable if the labeled amount of INH fell within the specified range of not <93.0% and not more than 110.0%. In addition, physical stability, such as pH and color changes, was also investigated.

MATERIALS AND METHODS

Chemicals

Standard INH (99.9% purity, calculated on a dried basis) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). INH tablets (100 mg/tablet) and INH substances were received from the Government Pharmaceutical Organization (GPO), Bangkok, Thailand. Sodium carboxymethylcellulose (CMC), methylparaben USP, propylparaben USP, and citric acid USP were bought from B.L. Hua and Co., Ltd. (Bangkok, Thailand). Glycerine, sorbitol 70% solution, and the essence of banana were purchased from Triumph Supply Co. Ltd. (Bangkok, Thailand). Sterile water was obtained from A.N.B. Laboratories Co. Ltd. (Bangkok, Thailand). Methylparaben, propylparaben, and sodium hydroxide were of analytical grade and purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Hydrogen peroxide was bought from Merck KGaA (Darmstadt, Germany) and Siribuncha Co. Ltd. (Nonthaburi, Thailand). All other reagents were of analytical or pharmaceutical grade. Lactose was ordered from Krungthepchemi Co. Ltd. (Bangkok, Thailand). Acetonitrile, methanol, hydrochloric acid, and deionized water were purchased from RCI Labscan (Bangkok, Thailand). All organic solvents were of HPLC grade.

HPLC Conditions

The analysis was carried out using a RP-HPLC system (Nexera LC-40 series, Shimadzu Corporation, Japan) equipped with a
photo-diode array detector (SPD-M40, Shimadzu Corporation, Japan). Chromatographic separations were performed on a C18-column (Shim-pack G1S, 250 mm × 4.6 mm i.d., particle size 5 µm) at a temperature of 30°C. The mobile phase is a combination of acetonitrile and deionized water, with acetonitrile ranging from 20% to 100% by volume. The flow rate was maintained at a constant 1.0 mL/min, and analytes were detected at a UV wavelength of 254 nm. Before injection, analytical solutions were degassed and filtered through a 0.45-µm porous membrane filter. The injection volume was 20 µL, and the total run time for each injection was 11 min.

Preparation of Standard and Sample Solutions

A stock solution of 1.0 mg/mL INH was prepared by accurately weighing 25 mg of the INH standard and diluted with deionized water to 25 mL. Calibration standards and quality control samples were diluted from the stock solution to desired concentrations and adjusted in volume with deionized water. Standard solutions of methylparaben and propylparaben were prepared, diluted in methanol, and used for peak identification.

Formulation F1 was prepared by combining 3.0 g of INH substance with 60 mL of vehicle type 2. Formulation F2, the comparative formulation, was prepared by grinding 30 INH tablets into a fine powder and combining it with 60 mL of vehicle type 2. The vehicle type 2 composition included 14% v/v CMC mucilage (100 mL of CMC mucilage contains 1.4 g of CMC 1500, 1.4 mL of 10% w/v methylparaben and 2% w/v propylparaben in propylene glycol, and water to make up 100 mL), 20% v/v diluted sorbitol solution, 1.5% v/v glycerol, 0.2% v/v banana flavoring, and 1% v/v paraben concentration (methylparaben and propylparaben combined). The pH of the vehicle was adjusted to between 5 and 6 with citric acid, and the volume was completed to 100% v/v using purified water. In addition, a sample containing 20% w/v lactose was prepared using 3.0 g of INH substance, 12 g of lactose, and 60 mL of the vehicle. All investigational formulations were prepared and stored in amber glass bottles with screwed plastic caps prior to evaluation.

Analytical sample solutions were prepared by pipetting 0.1 mL of each compounded INH oral liquid formulations into a 50-mL volumetric flask and adjusting the volume with deionized water. All sample solutions were filtered through a 0.45-µm syringe filter before injection.

Method Validation

The optimized HPLC-PDA method was validated for specificity, the limit of detection (LOD), the limit of quantitation (LOQ), linearity and range, accuracy, precision, and robustness.

Specificity was established to verify the absence of interference from solvents, formulation excipients, or degradation products. This involved analyzing solvents and vehicles and comparing their corresponding HPLC chromatograms against the INH standard. In addition, the peak purity index of INH in the presence of its degradation product resulting from the reaction with lactose was evaluated using a photo-diode array detector. Thus, the lactose-containing samples refrigerated for 0–14 days were analyzed for the specificity tests.

The LOD and LOQ were evaluated using the signal-to-noise ratio. The working standard solutions at low concentrations were analyzed and compared to blank samples. Acceptable signal-to-noise ratios for LOD and LOQ are 3:1 and 10:1, respectively.

Linearity was accessed by plotting a calibration curve between INH standard concentrations and peak areas. The least-squares method was used to determine the linear regression of the calibration curve. Standard solutions of INH at concentrations of 10, 30, 60, 90, 130, and 150 µg/mL were prepared and analyzed independently in triplicate. A correlation coefficient (r) > 0.999 was acceptable. The y-intercept and the slope were presented. The selected concentration ranges should provide linearity, accuracy, and precision.

The accuracy was obtained by analyzing quality control samples at concentrations of 80, 100, and 120 µg/mL. Triplicate preparations of each concentration were independently analyzed on the same day, and the mean percent recovery derived from added and actual INH quantities was reported as accuracy. The acceptable range for mean recovery was 98% to 102% across all concentrations.

Precision was evaluated through repeatability (intra-day precision) and intermediate precision (inter-day precision) assessments of the analytical method. In addition, the intermediate precision was also determined through the combination of results from two different analysts. The standard solutions at a concentration of 100 µg/mL were individually prepared. To establish repeatability, a minimum of six determinations were performed and analyzed on the same day. Inter-day precision was established by repeating the process for six determinations over 3 consecutive days. Both intra-day and inter-day precision assessments were deemed acceptable if the percent relative standard deviation (%RSD) did not exceed 2%.

Robustness was determined to demonstrate the reproducibility of the analytical procedure when the optimized HPLC conditions are deliberately altered. Standard solutions of INH at a concentration of 100 µg/mL were employed to evaluate typical variations in flow rate (0.8–1.2 mL/min), column temperature (28–32°C), and UV wavelength (252–256 nm). RSD values of <5% were considered acceptable.

Forced Degradation Study

Forced degradation studies were conducted to demonstrate specificity and provide information regarding the stability of INH under stress conditions. INH standard solution was forced to degrade by exposure to acid (1 N hydrochloric acid (HCl), 60°C, 3 h), alkaline (1 N sodium hydroxide (NaOH), 60°C, 3 h), oxidation (3% hydrogen peroxide (H₂O₂), 60°C, 3 h), thermal (60°C, 3 h), and direct UV radiation (4500 LX, 72 h). The chromatograms of stressed samples were compared to freshly prepared samples in water that were not subjected to stress. In addition, the peak purity index of the control and stressed samples was determined to confirm the results.
Stability Study

The concentration of F1 and F2 was evaluated as part of the chemical stability investigation. Each formulation was prepared in triplicate, stored at a refrigerated temperature (4 ± 2°C), and analyzed independently. Samples were analyzed immediately after compounding (day 0) and at designated intervals, i.e., days 7, 14, 21, 28, 35, 45, 60, 90, 120, 150, and 180, respectively. The quantity of INH remaining in oral liquid formulations was determined, with acceptable labeled amounts ranging from 93.0% to 110.0%.

The physical stability of both formulations, including apparent changes in pH, color, and the presence of air bubbles, was monitored throughout the study duration.

RESULTS AND DISCUSSION

Method Development and Optimization

A simple and rapid HPLC-PDA method was established by configuring initial conditions involving a mobile phase composed of deionized water, methanol, and acetonitrile, a flow rate of 1.0 mL/min, a column temperature of 25°C, and a UV wavelength of 254 nm. Isocratic HPLC employing the mobile phase containing water and 10%-30% organic solvents was evaluated for the separation of INH and formulation excipients. Initial experiments revealed that a mobile phase containing 20% (v/v) acetonitrile in water facilitated rapid separation of INH (t_p = 2 min). However, the total analysis time was extended to 30 min due to the detection of methylparaben and propylparaben at 25 min and 30 min, respectively. Increasing the proportion of acetonitrile accelerated the elution of parabens, but excessive acetonitrile (above 20% v/v) distorted the INH peak. Subsequently, a gradient elution was employed to accelerate the analysis time, with the column temperature raised to 30°C for faster elution. Based on the results of the initial isocratic HPLC method, a gradient analysis utilizing solvent A (acetonitrile) and solvent B (water) at specific time intervals was developed. The amount of acetonitrile was systematically altered from 20% v/v to 100% v/v at various time intervals using a stepwise gradient elution approach. Initially, based on the results of isocratic elution, it was determined that INH could be effectively separated using 20% v/v acetonitrile within a retention time of 2 min. Therefore, the acetonitrile concentration was maintained at 20% v/v from 0 to 2 min. Subsequently, the concentration of acetonitrile was systematically increased to expedite the elution of parabens from the column. Table 1 provides a summary of the optimized gradient elution program. Figure 1a and b demonstrates that the gradient HPLC-PDA method enabled the detection of INH, methylparaben, and propylparaben at 2.7, 6.0, and 6.6 min, respectively, with a total analysis time of 11 min. System suitability tests affirmed favorable performance with a peak area RSD of 0.31% (n = 6), INH peak tailing factors between 0.98 and 1.09, and the number of theoretical plates ranging from 16,000 to 18,000.

Considering the potential interference arising from the interaction between INH and lactose in tablets, an evaluation was conducted on a sample containing INH substance and 20% w/v lactose. HPLC chromatograms on days 7 and 14 showed an additional peak at 2.3 min (Figure 1c and d). The resolution of INH and the adjacent peak was >1.5. Although the peak at t_p 2.3 min was not further identified, it was hypothesized from the study of Butterfield et al. (1980) that this peak could be attributed to 1-isonicotinyl-2-lactosylhydrazine. On day 14, more than 50% of INH had transformed into its degradation product (Figure 1d). This study highlighted that the INH content could be drastically decreased when combined with lactose.

In addition, in comparison to the HPLC method developed by Haywood et al. (2005), the method investigated in this study allows simultaneous determination of the preservatives, degradation product, and INH peak.

Method Validation

Specificity was investigated through the HPLC analysis of the vehicle, acetonitrile, water, and INH standard solution. The HPLC chromatogram of the vehicle (Figure 1a) showed that there was no interfering peak at the retention time of the INH peak. In addition, the peak purity index of INH in lactose-containing and lactose-free samples was 1.0000, indicating that the developed method was not interfered with by the degradation product and formulation excipients.

The method demonstrated LOD and LOQ values of 0.05 µg/mL and 0.1 µg/mL, respectively. The correlation coefficients for linearity over a concentration range of 10–150 µg/mL were >0.999, indicating acceptable linearity in the examined concentration range. In addition, the specified concentration ranges demonstrated linearity with accuracy ranging from 99% to 101% and precision of <2% RSD at each concentration point. The accuracy of analytical procedures was confirmed by mean percent recoveries ranging between 99.0% and 101.0%. In addition, both intra-day and inter-day precision exhibited RSD values below 1.0%. In addition, the results obtained by two different analysts were essentially identical, demonstrating the intermediate precision of the method. The validation data showed that the optimized method was accurate and precise. The validated parameters are summarized in Table 2.

The method was robust to minor changes in column temperature and wavelength, as the RSD of the peak area did not exceed 2.0%. In contrast, varying flow rates caused the variation in peak area to exceed 20%. The results demonstrated that flow rates affected peak area in gradient HPLC and must be precisely controlled to achieve reliable quantitative analysis. The robustness of the method is expressed in Table 3.

Finally, the validated method aligned with ICH guideline Q2(R1), confirming its applicability for determining the INH concentration in the investigated formulations.

Forced Degradation Study

HPLC chromatograms and peak purity index of samples exposed to acid, alkaline, oxidative, thermal, and UV light
degradation are illustrated in Figure 2. According to the results, INH was stable under acid, heat, and UV light exposure conditions, with <5% degradation and no additional peak being detected. Unexpectedly, oxidative degradation yielded an extra peak at $t_R = 2.5$ min, showing similar intensity to the INH peak; however, the INH peak area remained largely
unchanged. It was apparent that INH exhibited resistance to oxidative stress, a finding confirmed by repeated tests with different hydrogen peroxide sources. The results were identical to the prior examination. Then, stress samples containing only H₂O₂ without INH were prepared and analyzed. Interestingly, the presence of the peak at tᵣ 2.5 min was detected even in H₂O₂ samples without added INH, as shown in Figure 3, suggesting its origin from H₂O₂ components rather than a degradation product. Thus, INH was resistant to 3% hydrogen peroxide without the formation of any degradation products, a result comparable to that reported by Bhutani et al. (2007). The alkaline degradation obtained two additional peaks at tᵣ 2.3 min and 2.4 min. The study demonstrated that INH could degrade rapidly under alkaline stress conditions. The chemical

![Typical high-performance liquid chromatography chromatogram of control INH sample without stress exposure (a), INH exposed to acid stress (b), INH exposed to alkaline stress (c), INH exposed to oxidative stress (d), INH exposed to thermal stress (e), and INH exposed to ultraviolet light stress (f). The retention time of INH was 2.7 min. Each stress condition was assigned a peak purity index of 1.0000 (a), 1.0000 (b), 0.9868 (c), 0.9137 (d), 1.0000 (e), and 1.0000 (f). INH: Isoniazid](image)

### Table 2: Method validation data

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<tr>
<th>Parameters</th>
<th>Values</th>
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<td>Range (µg/mL)</td>
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<td>Correlation coefficient (r) (n=3)</td>
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<tr>
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<td>Limit of quantitation (µg/mL) (n=3)</td>
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<tr>
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<td>Intra-day, %RSD (n=6)</td>
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<tr>
<td>Intermediate precision</td>
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<tr>
<td>Inter-day, %RSD (n=6)</td>
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<tr>
<td>Inter-analyst, %RSD (n=6)</td>
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<tr>
<td>Accuracy, % recovery* (n=9)</td>
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<td>At concentration 80 µg/mL</td>
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<td>At concentration 100 µg/mL</td>
<td>100.09±0.60</td>
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<tr>
<td>At concentration 120 µg/mL</td>
<td>100.53±0.32</td>
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%RSD: Percent relative standard deviation, *Mean±SD

### Table 3: Method robustness

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<td>30</td>
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<td></td>
<td>32</td>
<td>3604930, 3608332, 3607127</td>
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<td>3531808, 3537973, 3538081</td>
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<td>3659152, 3659204, 3659315</td>
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structures of these degradation peaks were not further elucidated. However, according to the research of Bhutani et al., (2007) the degradation products of alkaline stress may refer to isonicotinic acid and isonicotinamide. Table 4 provides a summary of the results of the forced degradation study. The method’s specificity remained unaffected by the stated stress conditions, except for the alkaline stress condition.

**Stability Study**

Chemical stability evaluations were conducted on formulations F1 and F2 in a sugar-free vehicle. According to the USP monograph for INH oral solution, the labeled amount of both formulations was acceptable. Table 5 shows the chemical stability data of formulations F1 and F2 stored at refrigerated temperatures for 180 days. The results demonstrated that the labeled amount of INH in F1 was at least 95% throughout the study period. F2 maintained a minimum INH content of 93% throughout the 180-day period, exhibiting greater stability than previously reported by Haywood et al. (2005). Regarding the method development section, the lactose-containing samples demonstrated that INH rapidly degraded in the presence of reducing sugars. Consequently, we contacted the manufacturer (GPO, Bangkok, Thailand) to figure out if INH tablets contain lactose. The manufacturer claimed that lactose has not been used as a pharmaceutical excipient in INH tablet production for several years. Therefore, the INH tablets used in this study did not contain lactose. Clearly, the study demonstrated that oral liquid formulations of INH prepared extemporaneously from INH substances and lactose-free tablets were stable for at least 180 days when stored in a refrigerator (4 ± 2°C). Both investigated INH formulations exhibited comparable chemical stability profiles. However, difficulties in resuspension were observed in formulation F2, whereas formulation F1 maintained a clear solution throughout the study period. Furthermore, spanning from day 35 to day 180, the labeled amount of INH in F1 consistently surpassed that of F2. The findings indicated that the components of tablets may influence the stability of F2.

In parallel with chemical stability, the physical stability of F1 and F2 was assessed. Their pH ranged between 6.30 and 6.91, without observed color changes throughout the experimental period. However, air bubble formation was observed in F2 on days 120, 150, and 180. The presence of air bubbles observed on the surface of F2 may indicate potential deterioration of the INH oral suspension; thus, the stability of F2 should be investigated further in terms of microbiological stability.

**CONCLUSION**

In this study, we have successfully developed and validated a rapid and robust HPLC-PDA method. In addition, the method allows simultaneous determination of INH and its degradation product in formulations containing lactose. The validation parameters were in accordance with ICH guidelines, enabling the optimized method to be used as a stability-indicating method. This method not only serves as an essential tool for the quality control of extemporaneous INH compounding but it also shows promise for evaluating the stability of INH in future extemporaneous sugar-containing preparations.

The evaluation of the stability of 50 mg/mL INH oral liquid formulations, prepared extemporaneously using either pure drug substances or lactose-free tablets in a sugar-free vehicle, has yielded crucial insights. Our findings indicate that these formulations maintain stability for a considerable
duration of up to 180 days when stored in a refrigerator post-compounding, with a minimum of 93% of the labeled amount remaining. Throughout the experimental period, no significant alterations in pH or color were observed, suggesting robust physical stability. However, it is noteworthy that the occurrence of air bubble formation in formulations containing ground INH tablets, specifically between day 120 and day 180, raises the need for supplementary microbiological assessments to ensure formulation stability.

In conclusion, our study contributes valuable evidence supporting the potential for extended shelf life of INH oral liquid formulations prepared extemporaneously using INH substances and lactose-free tablets. This extended shelf life surpasses the stipulated 21-day period outlined in Thailand’s TB guidelines, signifying a step forward in enhancing patient convenience and access to effective treatments.

REFERENCES