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Manuscript: Chemical Stability of Reconstituted Sincalide (Kinevac®) in Sterile Water under Two Different Storage Conditions

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IRB Exempt: No living subjects are included in this study

ABSTRACT

This study aimed to evaluate the chemical stability of sincalide at two common storage conditions; room temperature and refrigeration in an attempt to simulate the conditions faced during centralized reconstitution and subsequent distribution to regional clinical facilities. Sincalide is a peptide hormone product administered parenterally as an aid for diagnostic imaging of hepatobiliary conditions. With an estimated post-reconstitution shelf-life of 8 hours (updated by the manufacturer in 2014 with limited supporting data) and frequent shortages due to intermittent supply, there is both clinical and economic value in the experimental determination of true chemical stability of this agent. **Methods:** Sincalide was reconstituted and stored at both temperatures (n = 4 each) and samples were collected at pre-determined time points. A validated HPLC (high performance liquid chromatography) analytical method was employed for quantification of the active ingredient in these samples. **Results:** Little to no chemical degradation of sincalide was observed for the duration of study, over 8 days, following reconstitution and storage at room temperature. A trend of cyclic fluctuation in concentration was also shared between all samples. A similar trend of little to no chemical degradation and cyclic pattern was observed for the duration of study, over 8 days, following reconstitution and storage in refrigeration. **Conclusion:** This study supports that from a chemical standpoint, sincalide may potentially be used up to at least 8 days following reconstitution with sterile water, thus providing convenient and cost-saving benefits to medical institutions utilizing the product. The findings of this study, however, warrants microbial testing over this storage duration before any recommendations for extended usage can be made.

KEY WORDS: Sincalide, chemical stability, reconstitution, peptide stability

INTRODUCTION

Sincalide is an 8-amino acid, cholecystopancreatic-gastrointestinal peptide hormone used as an aid to contract the gallbladder for diagnostic imaging purposes in hepatobiliary diseases (*1*). Kinevac® (sincalide for injection by Bracco Diagnostic Inc.) is supplied in a single-use, reconstitutable vial containing 5 µg of sincalide. This

agent is dosed 0.02 or 0.04 μg/kg based on the diagnostic procedure (*1*), resulting in an average dose of 1.4 μg being administered to a prototypical 70-kg patient. This leads to a significant portion, approximately 67%, of the vial being wasted when administered to an average adult.

With an estimated post-reconstitution shelf-life of 8 hours (updated by the manufacturer in 2014 with limited supporting data) (*1,2*) and frequent shortages due to intermittent supply (*3*), there is both clinical and economic value in the experimental determination of true chemical stability of this agent.

Littleton et al reported a similar shelf-life of sincalide after 8 hours post-reconstitution; 80.05 ± 4.07% and 89.73 ± 2.49% in normal saline and sterile water, respectively. However, this study did not evaluate the stability of sincalide past 8 hours or under refrigeration, which is a common storage condition of reconstituted sincalide (*4*).

The chemical stability of similar agents has been studied. Octreotide, another 8-amino acid peptide hormone, has a stated 24-hour post-reconstitution shelf-life per manufacturer's package insert (*5*). The objective of this study was to determine the stability of sincalide in sterile water at both room temperature and refrigeration, and for an extended duration of several days post-reconstitution.

METHODS

Materials

For this study, we have used the following materials; Kinevac® (sincalide), sterile water for injection, (Tyr[SO3H]27) Cholecystokinin fragment 26-33 Amide, HPLC grade water, sodium monophosphate monobasic, sodium phosphate dibasic, and 1-propranol. The following is the supplier of materials. Sincalide was purchased from Bracco Diagnostics (Spokane, WA) and was the commercial product evaluated in this study. Sterile water for Injection was purchased from Hospira, Inc. (Lake Forest, IL) and was utilized in the reconstitution of commercial sincalide. Cholecystokinin fragment 26-33 Amide was acquired through Sigma-Aldrich (Milwaukee, WI) as pure

sincalide peptides. This was then diluted to varying concentrations and used in the construction of calibration curves for HPLC analysis.

Water, HPLC Grade was acquired from Avantor Performance Materials, LLC (Center Valley, PA) and was utilized in the preparation of mobile phase. Sodium phosphate monobasic was purchased from Sigma-Aldrich (St. Louis, MO) and sodium phosphate dibasic was acquired from Fisher Chemical (Fair Lawn, NJ) and both were used in the preparation of phosphate buffer (in mobile phase). 1-Propanol was purchased from Beantown Chemical (Hudson, NH) and was also utilized in the preparation of mobile phase.

Sample Collection

At t = 0, 8 vials of commercial sincalide were reconstituted with sterile water and stored at either room temperature (22.2˚C; labeled as vials 1 through 4) or refrigeration (2 – 8˚C; labeled as vials 5 through 8), n = 4 each. Room temperature vials were stored in a drawer to ensure equivalent protection from light as in a closed refrigerator. The drawer and refrigerator were only opened to retrieve the vials for sample collection, ensuring a controlled storage environment. At predetermined time points (0, 8, 16, 24, 32, 40, 52, 64, 76, 100, 124, 148, 172 and 196 hours), 100 µL samples were collected and stored at -80˚C until analyzed by HPLC. Tuberculin syringes (27G needle) were used on self-sealing vial stoppers to draw samples. The vials were also stored in resealable plastic bags to circumvent any significant diluent loss due to evaporation.

HPLC Method

A reverse-phase HPLC assay for quantification of sincalide in collected samples was employed and validated based on the method of LT Littleton et al. with slight modification (*4*). The method consisted of ultraviolet detection at 220 nm (Waters, Milford, MA, USA) and Waters Symmetry® C18 column (4.6 X 150 mm). Mobile phase consisted of a 4:1 ratio of 150 mM phosphate buffer and 1-propanol at a flow rate of 0.5 mL/min. The injection volume used was 50 µL.

Preparation of Calibration Curves

Using pure sincalide, intra- and inter-day calibration curves were constructed using the HPLC method described above. Each calibration curve consisted of four concentrations (0.25, 1, 1.25, 2.5 µg/mL) prepared through serial dilution. Mobile phase was used as the diluent. Intra- and inter-day variability in data was established. A straight-line equation of the general format y = mx + b was generated from all calibration curves constructed, which was subsequently used in data analyses. No data points were dropped.

Data Analysis

The calibration curve trendline equation was used to convert experimental absorbance peaks (mcV) to concentration (µg/mL). All experimental data was then standardized to an assumption of a concentration of 1 µg/mL at t = 0, per label claim. These values were then converted to percentage of concentration remaining compared to t = 0. For all data, mean values were produced, along with standard deviation (Std Dev) and coefficient of variation CV (defined as (mean /Std Dev) * 100%).

RESULTS

Calibration Curve

The HPLC assay was validated with intra-day (n = 3) and inter-day (n = 6) variability, expressed as CV, of 0.89% and 2.39%, respectively (**FIGURE 1**).

Little to no chemical degradation of sincalide was observed for the duration of study, over 8 days, following reconstitution and storage at room temperature (vials 1 through 4). A trend of cyclic fluctuation in concentration was also shared between all samples (**FIGURES 2 & 3**).

A similar trend of little to no chemical degradation and cyclic pattern was observed for the duration of study, over 8 days, following reconstitution and storage in refrigeration (vials 5 through 8; **FIGURES 3 & 4**).

DISCUSSION

The data collected suggests sincalide in sterile water, at both room temperature and refrigeration, is chemically stable for at least 8 days. Littleton et al reported the stability of sincalide to be shorter, however, that study analyzed stability over only 8 hours since the primary objective of that study was to compare sincalide stability upon reconstitution in normal saline versus sterile water (*4*). Octreotide, another 8-amino acid peptide, has an shelf-life of 24 hours post-reconstitution per manufacturer (*5*), which is supported by an independent accelerated stability study (*6*). As a similar molecule, it is plausible for sincalide to also demonstrate chemical stability beyond 8 hours.

Although the final outcome indicates significantly longer stability compared to the above sincalide study (*4*), the findings of this study demonstrates a similar and consistent pattern of decreased concentration by hour 8 (**FIGURES 2 & 3**). After that time point, though, the concentration of sincalide increased and continued to fluctuate in a repetitive pattern until it appeared to reach equilibrium.

A possible explanation for this concentration fluctuation pattern is a conformational change of sincalide in water. Sincalide may cycle through conformation changes while dissolved, thereby absorbing UV light in different capacities. This observation has not been explicitly reported with similar molecules, as there is limited data on long-term stability evaluations of reconstituted peptides/proteins with frequent sampling as in this study . However, changes in the folding pattern, if they occur, would result in a change in UV absorbance as previously reported; folded proteins do exhibit changes in UV absorbance at 230 nm; very similar to this studies absorbance of 220 nm (*7*).

Possible confounders were considered when observing and interpreting this data pattern. The drawer, where room temperature vials were kept, and refrigerator, were only opened to retrieve vials for sample collection, ensuring a controlled storage environment. This limited samples' exposure to light and temperature changes. Additionally, all samples collected were covered to prevent evaporation of reconstituted solution.

Although temperature was not directly monitored at each point of sample collection, both the room and refrigerator were kept at constant temperature settings and they do not have a tendency to deviate.

While this concentration fluctuation pattern warrants further research in an attempt to understand the mechanism behind it, this studies' data supports that sincalide is chemically stable for at least 8 days under both storage conditions evaluated. Based on these findings and that sincalide is a drug with frequent supply chain interruptions, one can conclude that prolonging the expiration time of sincalide may have a cost saving benefit as less product is likely to be wasted. Although, it is noteworthy that the objectives of this study did not include examination of bacterial growth or pyrogenicity on samples collected. Therefore, no recommendation in regard to continued use of sincalide beyond 8 hours post-reconstitution is suggested, as USP guidelines state that any reconstituted medication should not be used past the expiration time specified by the manufacturer (*8*).

CONCLUSION

Results from this study demonstrated little to no chemical degradation of sincalide for its entire duration at both room temperature and refrigeration. These findings support that from a chemical standpoint, sincalide may potentially be used up to at least 8 days following reconstitution with sterile water, thus providing convenient and cost-saving benefits to medical institutions utilizing the product. The findings of this study warrant microbial testing over this storage duration before any recommendations for extended usage can be made.

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No author has any conflict of interest, financial or otherwise, to disclose.

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FIGURES

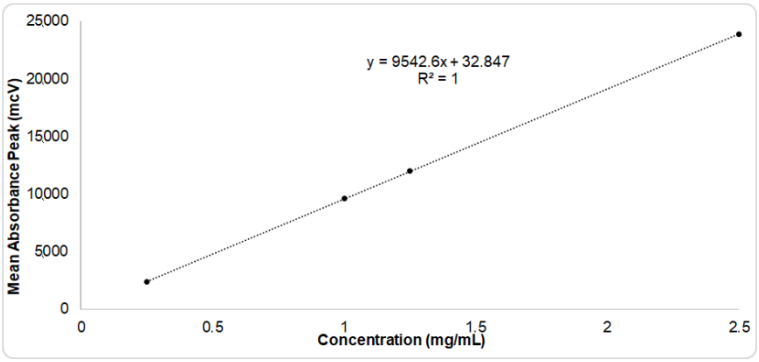

FIGURE 1. Mean data from absorbance peaks of pure sincalide dilutions were used to generate the calibration curve and trendline equation.

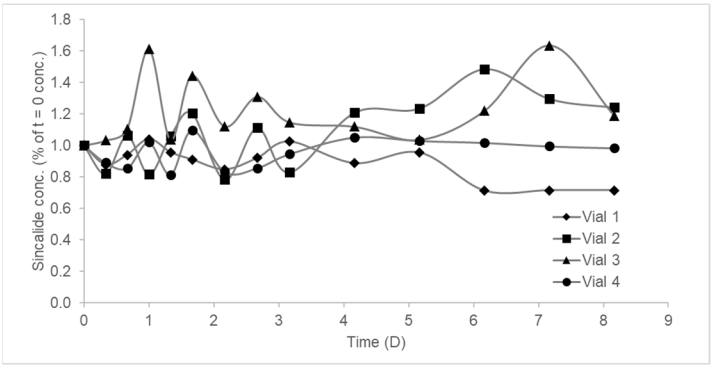

FIGURE 2. Percentage of concentration remaining at room temperature compared to that at t=0 plotted againstrespective time points.

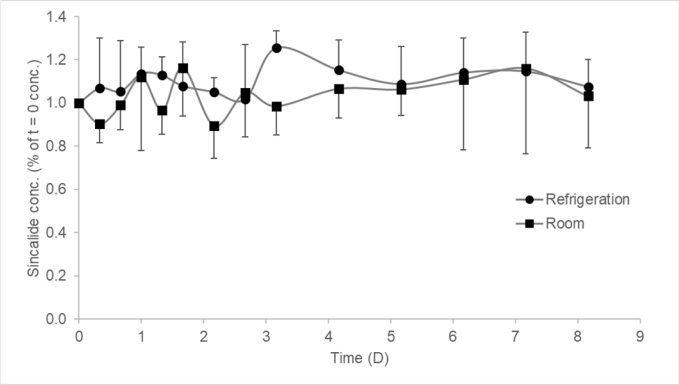

FIGURE 3. Mean values for percentage of concentration remaining at room and refrigerated temperatures, side-by-side, compared to that at t=0 plotted against respective time points. Error bars denote standard deviation.

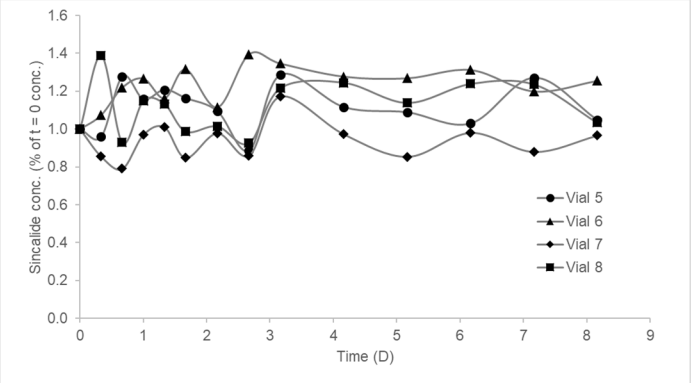

FIGURE 4. Percentage of concentration remaining at refrigerated temperature compared to that at t=0 plotted against respective time points.