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# Pramlintide Injection Drug Product Robustness Studies

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**ABSTRACT** The article examines the effects of temperature excursions and actual dose withdrawal on the quality of pramlintide injection, a multidose liquid parenteral formulation. Studies were designed to demonstrate product robustness under conditions that may occur during patient use. Pramlintide %Purity was determined by two high-performance liquid chromatography (HPLC) methods, a reversed-phase (RP-HPLC) and a strong-cation exchange (SCX-HPLC) method. A second RP-HPLC method was used to determine pramlintide potency and the concentration of the *m*-cresol preservative. Antimicrobial preservative effectiveness testing was per USP and European Pharmacopeia (Ph. Eur.). Short-term stability studies were undertaken to probe the effects of the following conditions: 5 °C to 40°C and 5°C to –20° C temperature cycling over 10 days; once daily or four-times daily dose withdrawal over 12 or 42 days; and combined 30° C storage and four-times daily dose withdrawal over 42 days. In all cases, pramlintide %Purity and potency values remained essentially unchanged or unchanged relative to controls. Similarly, product appearance, and *m*-cresol concentration and preservative effectiveness were not significantly affected by the stress conditions used in the 5 studies. Pramlintide injection drug product is extremely robust to challenging stress conditions that may occur during patient use of this multidose product for chronic administration.

**KEYWORDS:** Pramlintide, Preserved Drug Product, Container Closure Robustness, Temperature Cycling, Preservative Efficacy

## INTRODUCTION

Amylin is a 37–amino acid peptide hormone that is produced in the pancreas and co-secreted with insulin in response to elevated plasma glucose concentrations [1-3]. Pramlintide, a synthetic analog of amylin, retains the biological activity of the hormone while offering superior physical and chemical properties that facilitate development of a stable drug product for parenteral administration [4]. Pramlintide is being evaluated as a drug treatment for people with type 1 or insulin-using type 2 diabetes [5-7].

An injectable, multidose liquid formulation for pramlintide drug product has been developed to permit chronic self-administration by the anticipated patient population. The formulation contains pramlintide at strengths of 0.30 to 1.0 mg/mL and *m*-cresol as an antimicrobial preservative. Previous investigations have(1) identified pramlintide hydrolysis products [8]; (2) demonstrated the performance of stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) and strong cation exchange (SCX-HPLC) analytical test methods [9]; and (3) established the stability of the drug product under realistic and stress conditions [10].

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In addition to stability testing of drug product in both long-term and accelerated conditions, various regulatory guidance documents stipulate that marketing applications must also include results of drug product robustness testing under patient-use conditions [11-15]. For example, in the case of preserved products, "the efficacy of the antimicrobial preservative under simulated in-use conditions must be established [12]." Similarly, multidose containers in which stoppers are subjected to multiple needle entries and product withdrawals require demonstration of ". . . product integrity after maximum entries/withdrawals have been made" [14].

This report describes studies that were undertaken to demonstrate drug product and primary packaging robustness and suitability for anticipated patient use conditions. The investigations subjected pramlintide injection drug product to (1) temperature cycling around recommended storage conditions; (2) temperature excursions from recommended storage conditions; and (3) stopper penetration and product withdrawal based on expected patient use patterns. Four product attributes were probed in these robustness studies, namely (1) pramlintide chemical stability, (2) *m*-cresol chemical stability, (3) *m*-cresol antimicrobial preservative effectiveness, and (4) product appearance.

## MATERIALS AND METHODS

### Test Articles

Sterile pramlintide injection samples were freshly prepared for this study by sterile filtration at a 0.6 mg/mL strength in pH 4.0 aqueous acetate buffer with 2.25 mg/mL *m*-cresol and 4.3% mannitol as iso-osmotic agent. The samples were stored in 5-mLUSP Type I borosilicate glass vials with 13-mm West 4416/50 bromobutyl rubber closures and flip-off aluminum seals.

### Sample Preparation

### Working Reference Standard

Pramlintide working reference standard solutions were prepared at 0.5 mg/mL in pH 4.0, 30-mM acetate buffer. *M*-cresol working reference standards were prepared at 2.25 mg/ml in pH 4.0, 30-mM acetate buffer.

### Drug Product Samples

For both the RP- and SCX-HPLC purity methods, pramlintide injection samples were subjected to a solid-phase extraction step to remove *m*-cresol and mannitol, as previously described [9]. For the RP-HPLC potency method, pramlintide injection samples were directly analyzed without additional workup.

### Test System

The analytical test system employed Waters (Milford, MA) equipment: Model 616 or 2690 solvent delivery, Model 717 autoinjector (with PEEK low dead-volume kit and refrigerated sample compartment), Model 486 detector, and Model 62079 column oven.

### Reagents

Buffers and solvents were HPLC grade or equivalent throughout. Chromatography reagents, sodium perchlorate, potassium phosphate monobasic, sodium phosphate, sodium hydroxide, potassium hydroxide, o-phosphoric acid, acetic acid, and acetonitrile were supplied by Fisher (Springfield, NJ). Trifluoroacetic acid (TFA) was supplied by Pierce (Rockford, IL).

### Test Methods

The RP-HPLC and SCX-HPLC methods for determining pramlintide %Purity have been described previously [9]. The RP-HPLC method for determining pramlintide potency and *m*-cresol concentration has also been previously described [10]. Visual inspection of the vials was conducted per procedures in *USP 23–NF 18* to detect the presence of any extraneous matter such as cores or fragments from the rubber stoppers. Antimicrobial preservative effectiveness testing was performed by AAI, Inc. (Wilmington, NC) according to USP <51>/Ph. Eur. 5.1.3.

### Experimental Design

**Table 1** summarizes the conditions used in the temperature cycling robustness studies (Studies 1 and 2).

**Table 1.** Experimental Conditions For Temperature Cycling Product Robustness Studies (Studies 1 and 2)

| Purpose | 5 to 40°C Temperature Cycling | 5 to –20°C Temperature Cycling |
|---|---|---|
| Study designation | Study 1 | Study 2 |
| Cycle dwelt Times | 16 h at 5°C and 8 h at 40°C | 16 h at 5°C and 8 h at -20°C |
| Test intervals (days) | 0, 5, 10 | 0, 5, 10 |
| # Dose withdrawals | 0 | 0 |
| Attributes tested | Pramlintide %Purity and Potency [*m*-cresol], visual appearance | Pramlintide %Purity and Potency [*m*-cresol], visual appearance |

**Table 2.** Experimental Conditions For Dose Withdrawal Product Robustness Studies (Studies 3 and 4)

| Purpose | Four-Times Daily Dosing | Once-Daily Dosing |
|---|---|---|
| Study designation | Study 4 | Study 5 |
| Storage temperatures | 5°C | 5°C |
| Test intervals (days) | 0, 6, 12 | 0, 14, 28, 42 |
| # Dose withdrawals per Day | 4 | 1 |
| Total volume withdrawn per day (mL) | 0.4 | 0.1 |
| Attributes tested | Pramlintide %Purity and potency [*m*-cresol], visual appearance | Pramlintide %Purity and potency [*m*-cresol], visual appearance, antimicrobial preservative effectiveness |

**Table 2** outlines the conditions used for the dose withdrawal studies (Studies 3 and 4). **Table 3** shows the conditions for the combined temperature excursion and dose withdrawal study (Study 5).

**Table 3.** Experimental Conditions For External Body Temperature and Dose Withdrawal Product Robustness Study (Study 5)

| Purpose | External Body Temperature and Dose Withdrawal |
|---|---|
| Study designation | Study 5 |
| Storage temperatures | 30°C |
| Test intervals (days) | 0, 14, 28, 42 |
| # Dose withdrawals per day | 4 |
| Total volume withdrawn per day (mL) | 0.1 |
| Attributes tested | Pramlintide %Purity and potency [*m*-cresol], visual appearance, antimicrobial preservative effectiveness |

## RESULTS

In the temperature cycling robustness studies (Studies 1 and 2; **Table 1**), pramlintide injection samples in vials were maintained 16 hours at 5° C (recommended long-term storage condition) then cycled to either 40° C (Study 1) or –20° C (Study 2) and held 8 hours before returning to 5° C. These temperature cycles were repeated for 10 days and samples were withdrawn for testing at 0, 5, and 10 days. In both studies, samples were visually inspected and also tested for pramlintide %Purity and potency, plus *m*-cresol concentrations.

**Table 4** summarizes the results for Studies 1 and 2. From **Table 4**, it is clear that pramlintide %Purity (as determined by two orthogonal HPLC methods [9]) remained essentially unchanged over the 10-day testing period. Similarly, pramlintide potency and *m*-cresol were not significantly affected by temperature cycling between 5° C and 40° C or temperature cycling between 5° C and –20° C over the 10-day interval. Visual inspection of product samples revealed that product appearance also remained unchanged throughout both Study 1 and Study 2.

**Table 4.** Pramlintide %Purity and Potency and *M*-cresol Concentration as a Function of Storage Interval for Temperature Cycling Studies (Studies 1 and 2)*

| Study #* (Description) | Storage Interval (days) | Pramlintide SCX-HPLC Purity (%)† | Pramlintide RP-HPLC Purity (%)† | Pramlintide RP-HPLC Potency (mg/mL)† | *m*-cresol RP-HPLC Concentration (mg/mL)† |
|---|---|---|---|---|---|
| 1 (5°C to 40°C) | 0 | 98.1 | 98.2 | 0.619 | 2.27 |
| 1 (5°C to 40°C) | 5 | 98.1 | 98.1 | 0.621 | 2.27 |
| 1 (5°C to 40°C) | 10 | 98.0 | 97.9 | 0.621 | 2.28 |
| 2 (5°C to -20°C) | 0 | 98.1 | 98.2 | 0.619 | 2.27 |
| 2 (5°C to -20°C) | 5 | 98.1 | 98.1 | 0.621 | 2.27 |
| 2 (5°C to -20°C) | 10 | 98.0 | 97.9 | 0.621 | 2.28 |

* See Table 1 for experimental conditions.
† See Experimental Details section for test method descriptions

Studies 3 and 4 probed pramlintide drug product robustness as a function of actual dose withdrawal. The expected dosing frequency of pramlintide injection could range between 1 and 4 times daily. The anticipated injection volume is 0.10 mL for the 0.6-mg/mL product, and the recommended storage interval during patient use is 1 month at room temperature. Thus, in Study 3, drug product samples in vials were maintained at 5° C and then equilibrated to room temperature before testing. Sample vials were penetrated 4 times daily with a 27-gauge needle and a 0.10-mL aliquot was withdrawn at each dose withdrawal. Similarly, in Study 4 the drug product samples were penetrated once daily with a 27-gauge needle and 0.10-ml aliquot was withdrawn. Product testing was as shown in **Table 2.**

**Table 5** shows the results for Studies 3 and 4. From **Table 5**, it is clear that pramlintide %Purity remained essentially unchanged over the 12-day testing period for Study 3 and the 42-day testing period for Study 4. Similarly, pramlintide potency and *m*-cresol were unaffected by the number of dose withdrawals over the 12- to 42-day testing intervals. Visual inspection of product samples revealed that product appearance also remained unchanged throughout both Study 3 and 4, with no evidence of stopper coring or fragmentation. Antimicrobial preservative effectiveness tests passed USP and Ph. Eur. criteria for samples withdrawn at 0, 28, and 42 days in Study 4.

In the combined temperature excursion/dose withdrawal study (Study 5) samples were maintained at a constant 30° C to simulate external body temperature and equilibrated to ambient temperature before dose withdrawal with a 27-gauge needle. Stoppers were penetrated 4 times daily and 0.10-ml aliquots withdrawn with each penetration. Samples were removed at 0, 14, 28, and 42 days for testing as described in **Table 3.** Control samples, maintained at 30° C and not penetrated 4 times daily, were tested concurrently at 0, 14, 28, and 42 days with the penetrated samples.

**Table 5.** Pramlintide %Purity and Potency and *m*-cresol Concentration Plus Preservative Effectiveness As a Function of Storage Interval for Dose-Withdrawal Studies (Studies 3 and 4)*

| Study #* (Description) | Storage Interval (Days) | *m*-cresol RP-HPLC Concentration (mg/mL) † | *m*-cresol Preservative Effectiveness (per USP and Ph. Eur.) | Pramlintide SCX-HPLC Purity (%)† | Pramlintide RP-HPLC Purity (%)† | Pramlintide RP-HPLC Potency (mg/mL) † |
|---|---|---|---|---|---|---|
| 3 (Once Daily | 0 | 2.27 | Not Tested | 97.5 | 97.3 | 0.618 |
| 3 (Once Daily | 6 | 2.25 | Not Tested | 96.9 | 96.8 | 0.615 |
| 3 (Once Daily | 12 | 2.24 | Not Tested | 96.9 | 96.6 | 0.612 |
| 4 (4X Daily) | 0 | 2.28 | Pass | 97.4 | 97.4 | 0.615 |
| 4 (4X Daily) | 14 | 2.24 | Not Tested | 97.5 | 97.5 | 0.621 |
| 4 (4X Daily) | 28 | 2.24 | Pass | 97.5 | 97.5 | 0.624 |
| 4 (4X Daily) | 42 | 2.22 | Pass | 97.4 | 96.9 | 0.624 |

* See Table 2 for experimental conditions.
† See Experimental Details section for test method descriptions

**Table 6**, which shows the results of Study 5, demonstrates that sample versus control results were identical (within the limits of experimental uncertainty) for pramlintide %Purity and potency, and for *m*-cresol at all timepoints tested. Visual inspection of product samples revealed that product appearance also remained unchanged throughout Study 5, with no evidence of stopper coring or fragmentation. Antimicrobial preservative effectiveness passed USP and Ph. Eur. criteria for samples withdrawn at 0, 28, and 42 days.

**Table 6** Pramlintide %Purity and Potency and *m*-cresol Concentration Plus Preservative Effectiveness as a Function of Storage Interval for External Body Temperature Study (Study 5)*

| Storage Interval (Days) | *m*-cresol | | Pramlintide | | | | | |
|---|---|---|---|---|---|---|---|---|
| | RP-HPLC Concentration (mg/mL)† | | SCX-HPLC Purity (%)† | | RP-HPLC Purity (%)† | | RP-HPLC Potency (mg/mL)† | |
| | Sample | Control ‡ | Sample | Control ‡ | Sample | Control ‡ | Sample | Control ‡ |
| 0§ | 2.24 | 2.24 | 95.5 | 95.5 | 96.5 | 96.5 | 0.619 | 0.619 |
| 14 | 2.23 | 2.22 | 94.4 | 94.8 | 95.6 | 95.8 | 0.614 | 0.614 |
| 28§ | 2.24 | 2.23 | 93.9 | 93.9 | 95.1 | 95.2 | 0.604 | 0.610 |
| 42§ | 2.22 | 2.23 | 93.8 | 93.9 | 94.2 | 94.2 | 0.604 | 0.603 |

*See Table 3 for experimental conditions.
†See Experimental Details section for test method descriptions
‡Samples were penetrated 4 times daily, controls were not penetrated except to withdraw aliquots for testing at 0, 14, 28, and 42 days
§Samples passed USP/EP antimicrobial preservative effectiveness testing at this time point.

## DISCUSSION

Previous studies identified 5° C as a suitable long-term storage condition for pramlintide injection and 25° C as an appropriate storage condition during a 1-month patient-use period [10]. The drug product vials, however, may experience temperature excursions (both above and below the recommended storage conditions) during patient use, or product shipping and warehousing. Consequently, Studies 1 and 2 were undertaken to examine the effects of 5° C to 40° C temperature cycling, and 5° C to –20° C temperature cycling on pramlintide injection quality.

The results of the "refrigerated-to-frozen" and the "refrigerated-to-higher temperature" cycling studies (**Table 4**) indicate that pramlintide injection is extremely robust, with no significant changes observed over a 10-day temperature-cycling interval.

Routine stability studies are necessary to determine drug product shelf life in intact, unopened containers. It is also necessary, however, to establish the 'in-use" lifetime of a multidose product for chronic self-administration by challenging the closure to multiple penetrations and sample withdrawals. Specifically, daily dose withdrawal over time could affect the antimicrobial effectiveness of the preservative or compromise the container-closure integrity. Studies 3 and 4 were conducted to evaluate the potential adverse effects of dose withdrawal and solution withdrawal on chemical, physical, and antimicrobial properties of pramlintide injection. As a worst-case test, the study period in Study 4 extended to 42 days (2 weeks beyond the recommended 1-month product expiry during normal patient use).

**Table 5** summarizes the results of the dose withdrawal studies and, again, demonstrates that pramlintide injection underwent no significant changes as a function of dose withdrawal over 12- to 42-day testing intervals. Antimicrobial effectiveness of the drug product was similarly unaffected by dose withdrawal in Study 4.

**Table 6** presents the results from Study 5, which evaluated the combined effects of external body temperature (estimated to be 30ºC) and 4-times daily dose withdrawals on pramlintide drug product quality. The drug potency and purity results indicated that the values remained well within acceptable limits for drug potency (limits of 90% to 110 % of label strength), purity (limit of 90 %Purity or higher), and preservative content (limits of 0.175 to 0.250 mg/mL) even when stored at 30°C during the 42-day testing interval. Additionally, when compared with the control samples (maintained at 30°C but not subjected to any punctures), no difference in the extent of drug degradation was observed, indicating that the 4 punctures per day did not affect the chemical stability of the product. Visually, the drug product vials did not show presence of extraneous matter, cores, or fragments. The results from the APE test for pramlintide injection in vials subjected to the 4-times daily needle penetration show that all samples met the Ph. Eur. "Criteria A" for preservative effectiveness. These APE results indicated that the stoppers penetrated multiple times remained sealed and prevented the loss of the volatile preservative, *m*-cresol, from the product.

## CONCLUSIONS

The 5 studies reported here show that pramlintide potency and %Purity, plus *m*-cresol concentration and antimicrobial preservative effectiveness of the product, all remained well within acceptable limits when subjected to simulated patient-use patterns and temperature excursions. Pramlintide injection was sufficiently robust to withstand stresses that may occur during its storage and patient use periods. This report thus presents an approach to address regulatory requirements for demonstrating the robustness of a multidose, parenteral drug product.

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