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RESEARCH PAPER

Development of a Performance Verification Test for USP Apparatus 4

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ABSTRACT

Purpose To evaluate salicylic acid tablets as a candidate reference material in a Performance Verification Test (PVT) when a USP performance test for dissolution (General Chapter <711>) relies on USP Apparatus 4 (flow-through cell).

Methods We developed a dissolution procedure relying on Apparatus 4 and salicylic acid tablets. Thereafter, a designed experiment was conducted to identify operational variables that significantly affect salicylic acid dissolution in this apparatus.

Results Four variables (size of glass beads, cell temperature, flow rate, level of deaeration) and one combination effect (deaeration/bead size) were significant for mean percent dissolved. Two variables (tablet orientation, level of deaeration) were significant for standard deviation results, but these effects were less pronounced than those for mean percent dissolved results. Three variables (analyst, tester manufacturer, amount of glass beads) had no statistically significant effects on either the mean or standard deviation of the responses.

Conclusions The proposed PVT is capable of probing effects of changes in several critical operational parameters of Apparatus 4. Salicylic acid tablets were shown to be a suitable reference material for the PVT. The PVT using salicylic acid tablets satisfies important aspects of a PVT.

KEY WORDS dissolution testing · flow-through cell · performance verification test · salicylic acid · USP Apparatus 4

INTRODUCTION

USP Apparatus 4 (flow-through cell) is a dissolution testing apparatus that uses the flow of dissolution medium through a cell containing a dosage form (1). Typically, a pulsating piston pump is used to deliver the medium. Apparatus 4 often is the preferred apparatus for drug release tests of controlled-release dosage forms and poorly soluble drugs. When operated in the open configuration, Apparatus 4 offers infinite sink conditions. When operated in the closed configuration, Apparatus 4 can use small volumes of medium to overcome limit of quantification issues. Because the dosage form is isolated from the medium reservoir, sampling and medium changes can occur without disturbing the hydrodynamics inside the flow-through cell. Various cells are available for tablets (1), powders and granulates, suppositories and soft gelatin capsules, implants, and semisolids. Methods for testing novel dosage forms such as microspheres (2) and liposomes (3) using Apparatus 4 are being developed. The FDA has approved Apparatus 4 methods for *in vitro* release testing of drug-eluting stents (Merciadez M, Alquier L. A novel method for the elution of sirolimus in drug-eluting stents. Paper presented at Sotax USP Apparatus 4 Workshop; June 14, 2007; Horsham, PA).

There is currently no performance verification test (PVT) for dissolution procedures that rely on Apparatus 4. A PVT is a means of assessing the integrity of the overall procedure, including not only the apparatus, but also the analytical procedure and the analyst (4,5). In the development of a PVT the following aspects are considered critical: 1) the test should be easy to perform in a short period of time; 2) the test should be repeatable, rugged, and reproducible; 3) the reference material should be stable, preferably should be nontoxic, and should contain an analytical marker that can be easily quantified; and 4) the results should be sensitive to changes in critical operational parameters of the apparatus. In this context, the PVT must be able to demonstrate sensitivity to instrument parameters that would not be assessed solely through mechanical validation.

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The scope of the current study was to assess a PVT for Apparatus 4 where the reference material for the PVT was salicylic acid tablets. Salicylic acid tablets have some desirable properties as a candidate for an Apparatus 4 PVT in that they are non-disintegrating, non-toxic, and have been shown to have good performance stability with other apparatus, and the analytical marker, salicylic acid, is easily quantified by UV analysis. Previous work conducted using salicylic acid tablets in Apparatus 4 had indicated that this material may be considered suitable for the performance verification test. However, these studies were very preliminary and did not include a rigorous evaluation of critical operational variables as the current study does (6,7).

MATERIALS AND METHODS

Materials and Reagents

Researchers used the following materials and reagents: potassium phosphate monobasic from Fisher Scientific, USA; Milli-Q water; sodium hydroxide solution 50% *w/w* from Ricca, USA; 5-mm beads and 1-mm borosilicate glass beads from Sotax, USA; 2-mm borosilicate glass beads from ChemGlass, USA; 25-mm glass microfiber filters from Whatman, USA; salicylic acid tablets produced for USP Lot Q0D200 and packaged in blister packs and USP Salicylic Acid Reference Standard, both from USP, USA; and HVLP 0.45-µm membrane filters from Millipore, USA.

Apparatus 4: PVT Procedure

The proposed PVT method uses salicylic acid tablets (nominal weight = 300 mg) packaged in blister packs. Six tablets are used for each run. The cells are filled by inserting one 5-mm bead in the bottom of the cell followed by two 12-mm dosing spoonfuls of glass beads. (One dosing spoonful is defined as the amount of glass beads required to fill the conical section of the flow-through cell.) The tablet is placed horizontally and centered on the bead bed, and two more 12-mm dosing spoonfuls of glass beads are added on top of the tablet to secure its orientation. The system is operated in a closed configuration by using separate 1-L glass bottles for each cell position. The medium is stirred at 300 rpm using a multistirrer. The tubing is filled with medium before the analyst starts the run. Following are the system parameters: dissolution mode: closed system with 12-mm cells; cell temperature: 37 ± 0.5°C; medium bottle (temperature not regulated); filter head: 1 Glass Microfiber Filter (GF/F); flow rate: 8 mL/min; dissolution medium volume: 1000 mL; dissolution medium: phosphate buffer pH 6.80 ± 0.05; medium deaeration: vacuum filtered and degassed for 5 min at room temperature; sampling time: 60 min; analysis: UV at 296 nm.

Experimental Design

For the experiment reported here, analysts varied some of the operational parameters just described. A design-of-experiment (DoE) approach (8) was used to probe the effect of eight variables (A–H, Table I) on salicylic acid drug release. A design with 38 runs was developed using Stat-Ease software (Design-Expert Version 7.0.2, Stat-Ease, Inc., Minneapolis, MN, USA). This was an incomplete factorial design in which all main effects of each variable and all two-factor interactions were not aliased with each other; i.e., the main effects and two-factor interactions could be estimated without being biased by other main and two-factor effects, known as a minimum resolution V design. The variables were set at a high (+ 1 value) and low (−1 value) level. Flow rate (A) and cell temperature (B) values were chosen to reflect normal operating conditions near the specified limits—36.0° or 38.0° for temperature and 7.6 mL/min or 8.4 mL/min for flow rate. The amount of glass beads (C) refers to the number of 12-mm dosing spoonfuls, 1 or 2, placed at the bottom of the cell on top of the 5-mm bead. Non-deaerated medium was used at room temperature. For deaeration level (D) deaerated medium was prepared by vacuum filtering room-temperature buffer through an HVLP 0.45-µm membrane filter. The receiving vessel then was capped, and vacuum continued for an

Table I Variables Included in Design of Experiment Study

Variable	Units	− 1 Value	+ 1 Value
A: Flow rate	mL/min	7.6	8.4
B: Cell temperature	°C	36.0	38.0
C: Amount of glass beads—bottom	Dosing spoonful	One	Two
D: Deaeration level	N/A	Deaerated	Non-deaerated
E: Size of glass beads	Diameter, mm	1	2
F: Tablet orientation	N/A	Horizontal	Vertical
G: Tester manufacturer	N/A	α	β
H: Analyst	N/A	1	2

additional 5 min. The measured pressure was always less than 100 mbar. Two diameters of borosilicate glass beads (E) were used: 1 mm and 2 mm. For experiments with a horizontal tablet orientation (F), the tablet was placed on top of the glass bead bed and adjusted, if necessary, using fine tweezers so that the tablet lay flat and was centered. For experiments with a vertical tablet orientation, the tablet was placed on its end, slightly embedded in the glass bead bed. Fine tweezers were used to adjust the orientation of the tablet, if necessary, so that it was vertical and centered. Two 12-mm dosing spoonfuls of glass beads were always placed on top of the tablet. Two different Apparatus 4 testers (G), α and β, from two different manufacturers were used. The two testers both used piston pumps of a similar design and a pulse rate of 120 pulses per minute. Two different analysts (H), Analyst 1 and Analyst 2, performed the work.

Table II Experimental Design and Results

	Variables								Responses	
Run	A mL/min	B Deg.	C #	D Deaer.	E Diam.	F Orient.	G Tester	H Analyst	Mean Percent (%)	SD
1	7.6	38°	2	No	1 mm	Hor	β	2	34.60	3.48
2	7.6	38°	1	No	1 mm	Ver	α	2	33.15	2.49
3	8.4	36°	1	Yes	2 mm	Hor	β	2	24.06	1.45
4	7.6	38°	2	No	2 mm	Hor	α	1	27.99	2.30
5	7.6	38°	1	Yes	1 mm	Ver	α	1	34.08	1.06
6	8.4	36°	2	No	2 mm	Ver	β	2	26.34	0.60
7	8.4	38°	1	No	2 mm	Hor	β	2	29.08	1.99
8	8.4	38°	1	Yes	1 mm	Ver	β	2	34.66	1.04
9	8.4	36°	2	No	2 mm	Ver	α	1	26.52	0.56
10	7.6	38°	2	Yes	2 mm	Ver	β	1	25.38	1.24
11	8.4	38°	1	No	1 mm	Hor	α	1	36.65	2.33
12	8.4	36°	2	Yes	1 mm	Ver	β	1	32.44	1.06
13	8.4	36°	2	No	1 mm	Ver	α	2	32.87	0.68
14	7.6	36°	2	Yes	2 mm	Hor	α	1	23.61	0.66
15	7.6	36°	1	Yes	2 mm	Ver	β	1	23.71	1.72
16	8.4	38°	1	Yes	2 mm	Ver	β	1	27.16	1.28
17	7.6	38°	1	No	2 mm	Ver	β	1	27.29	1.66
18	7.6	36°	2	Yes	1 mm	Hor	β	2	29.72	0.82
19	8.4	36°	1	No	1 mm	Ver	β	1	32.54	1.39
20	8.4	36°	2	No	2 mm	Hor	α	2	27.45	1.85
21	7.6	36°	1	No	2 mm	Hor	α	1	28.37	2.10
22	7.6	36°	1	Yes	1 mm	Ver	α	2	31.03	0.82
23	8.4	38°	2	No	1 mm	Ver	β	1	36.63	1.51
24	8.4	38°	2	Yes	1 mm	Hor	α	1	35.17	0.78
25	7.6	38°	1	Yes	2 mm	Ver	β	2	24.87	0.93
26	8.4	38°	1	Yes	2 mm	Hor	α	1	27.42	0.95
27	7.6	36°	2	No	2 mm	Hor	β	1	24.04	1.46
28	8.4	36°	1	Yes	1 mm	Hor	α	1	34.61	1.36
29	7.6	36°	2	No	2 mm	Ver	α	2	24.90	1.09
30	7.6	38°	1	Yes	1 mm	Hor	α	2	33.77	1.42
31	7.6	36°	2	No	1 mm	Hor	α	1	30.07	1.74
32	8.4	36°	1	Yes	2 mm	Ver	α	2	24.66	0.68
33	7.6	38°	2	Yes	1 mm	Ver	α	2	32.81	0.77
34	8.4	36°	2	No	1 mm	Hor	β	2	32.33	1.15
35	7.6	36°	1	No	1 mm	Hor	β	2	31.87	2.41
36	8.4	38°	2	No	2 mm	Ver	α	2	29.40	0.64
37	8.4	38°	2	Yes	2 mm	Hor	β	2	25.20	1.92
38	7.6	38°	1	Yes	1 mm	Hor	β	1	32.98	1.65

Statistical Analysis

Two responses were recorded for each run: mean percent dissolved at 60 min ($n=6$) and standard deviation (SD) of the six values at 60 min. The DoE software was used to analyze the data by a step-wise process. The step-wise process was repeated for both responses. The first step was to select an appropriate data transformation. For the mean percent dissolved response, no transformation was needed. This was confirmed using the Box-Cox Plot for Power Transforms. For the standard deviation response, a natural log transformation (lambda = 0) was selected. The second step of the process involved selecting the significant variables for each response. Analysts forced all eight main effects to be part of our final model to facilitate evaluation of each. In addition, the analysts added interactions (combination effects) if their importance as measured by percent sum of squares was comparable to that of the more important main effects. Pareto charts were used to illustrate the importance of the variables. These charts showed significance levels with Bonferroni correction and without adjustment for multiple testing. We emphasized results adjusted for multiple testing. The third step involved an analysis of variance (ANOVA, partial sum of squares–type III) test to quantify the contribution of each variable for both mean and standard deviation.

RESULTS

The DoE study was performed by setting each variable according to the experimental design (Table II). Analysts completed 38 runs and recorded the mean and standard deviation results. The DoE software was used to select significant variables and to quantify their contribution to both mean and standard deviation results as described in the Materials and Methods section. This is displayed in the effects lists (Tables III and IV) and Pareto charts (Figs. 1 and 2) for the two responses. Significance is shown using the Bonferonni corrected *t*-value limit and the *t*-value limit. Variables that are above the Bonferonni limit are definitely statistically significant, but those above the *t*-value limit but not above the Bonferroni limit are large effects that may be statistically significant with more data. The effect is the change in the response as the factor changes from its low level (−1 value, as indicated in Table I) to its high level (+1 value). For standard deviation, the effect is expressed as a percentage. In the Pareto charts, variables with dark-colored bars represent a negative effect (decrease) from the low level to the high level, and light-colored bars represent a positive effect (increase).

Four variables (size of glass beads, cell temperature, flow rate, level of deaeration) were significant for mean percent dissolved, and a combination effect (deaeration with size of the glass beads) was large, and thus worth noting, but did not reach statistical significance using the Bonferroni limit. Higher flow rate, higher temperature, non-deaerated medium, and smaller beads were associated with higher average values of percent of drug dissolved. The effect of the size of the glass beads was particularly large (see Fig. 1). The combination effect was that the impact of deaeration was greater with the 2-mm beads than with the 1-mm beads. The other four main effects were relatively unimportant.

Table III Effects List for Mean % Dissolved Results

Variable	Effect[a]	% Contribution
A: Flow rate	1.6	4.1
B: Temperature, cell	2.3	8.5
C: Amount of glass beads	−0.5	0.4
D: Deaeration level	1.5	3.5
E: Size of glass beads	−7.1	77.3
F: Tablet orientation	−0.2	0.0
G: Tester manufacturer	−0.8	0.9
H: Analyst	−0.5	0.5
DE: Deaeration/size of glass beads		1.4

[a] The effect size is the difference between the estimated average at the +1 value setting minus that at the −1 value setting. For main effects, the Effect and % Contribution are determined from the model containing only main effects. The % Contributions for the interaction are from the model with all main effects and the interaction; no effect size is provided for the interaction

Table IV Effects List for Standard Deviation Results

Variable	Effect[a]	% Contribution
A: Flow rate	−19.7	5.4
B: Temperature, cell	24.6	5.5
C: Amount of glass beads	−25.2	9.4
D: Deaeration level	50.7	19.1
E: Size of glass beads	−7.7	0.7
F: Tablet orientation	−33.0	18.0
G: Tester manufacturer	28.4	7.1
H: Analyst	−0.7	0.6
AD: Flow rate/deaeration		5.1
DF: Deaeration/tablet orientation		3.3

[a] The effect size is the difference between the estimated standard deviation at the +1 value setting minus that at the −1 value setting as a percentage of the standard deviation at the −1 value setting. That is, positive effects correspond to lower variability at the −1 value setting and negative effects to lower variability at the +1 value setting. For main effects, the Effect and % Contribution are determined from the model containing only main effects. The % Contributions for the interactions are from the model with all main effects and the interactions listed. No effect sizes are provided for the interactions

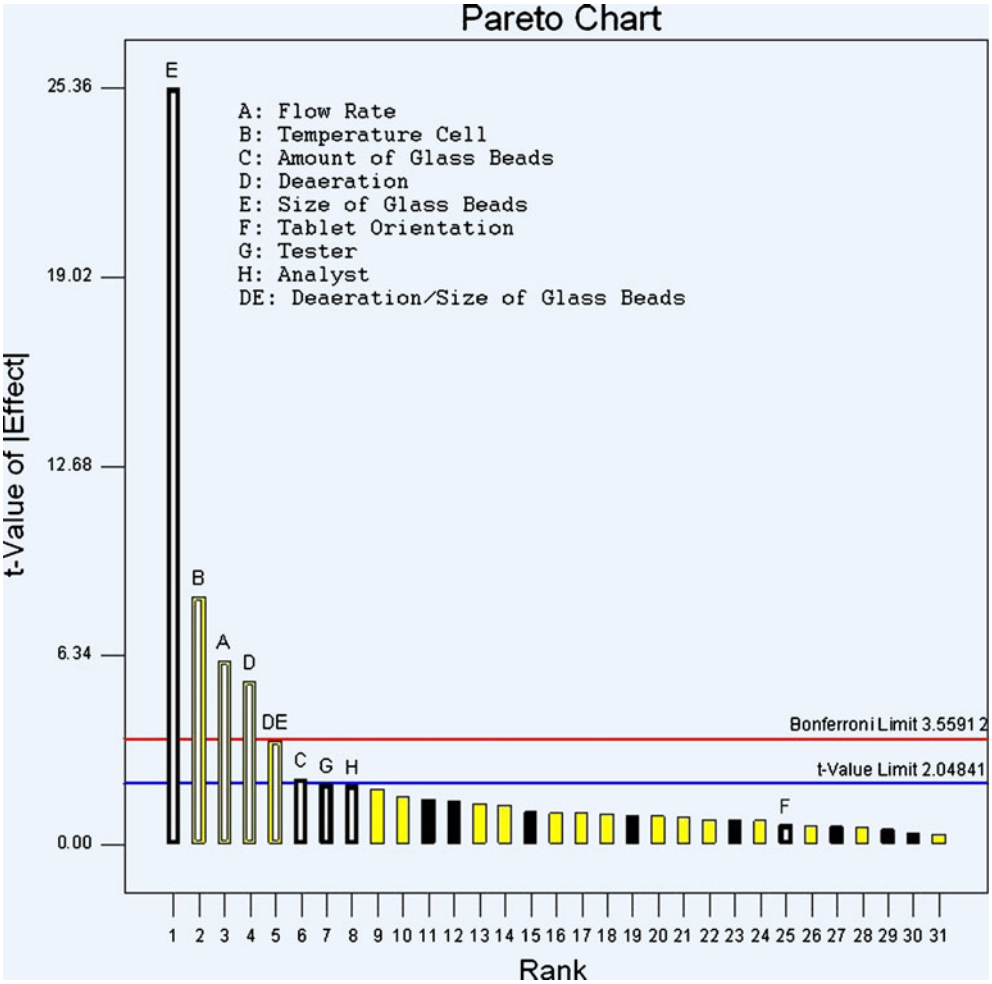

Fig. 1 Pareto chart for mean% dissolved results.

Two variables (tablet orientation and level of deaeration) were significant for standard deviation results. Lower variability was associated with deaerated medium and a vertical tablet orientation. Three variables (analyst, tester manufacturer, amount of glass beads) had statistically no effect on either of the two responses.

DISCUSSION

We established four criteria as critical aspects of an Apparatus 4 PVT procedure. The following summarizes what these studies have established for each criterion.

1. The dissolution test is easy to perform in a short period of time. The proposed Apparatus 4 PVT involves one sampling point at 60 min, uses a standard buffered dissolution medium, and is a straightforward procedure.
2. The dissolution test is repeatable, rugged, and reproducible. Three variables were found to have much smaller and statistically insignificant effects in this experimental design: amount of glass beads, instrument manufacturer, and analyst. The insignificance of instrument manufacturer and analyst to both mean percent dissolved and standard deviation results demonstrates the robustness of the dissolution test. Analysts obtained similar results and degree of variability regardless of which instrument they used or which analyst performed the test. The insignificance of the amount of glass beads is a measure of the ruggedness of the test. Because 12-mm dosing spoonfuls are not an exact amount, the results should be relatively unaffected by differences in amounts of glass beads from run to run. A future collaborative study will assess interlaboratory reproducibility of the PVT.
3. The reference material is stable, preferably is nontoxic, and contains an analytical marker that can be easily quantified. Salicylic acid tablets were selected as a potential reference material for the Apparatus 4 PVT for several reasons. First, because this is a nondisintegrating dosage form, tablet orientation can be fixed by

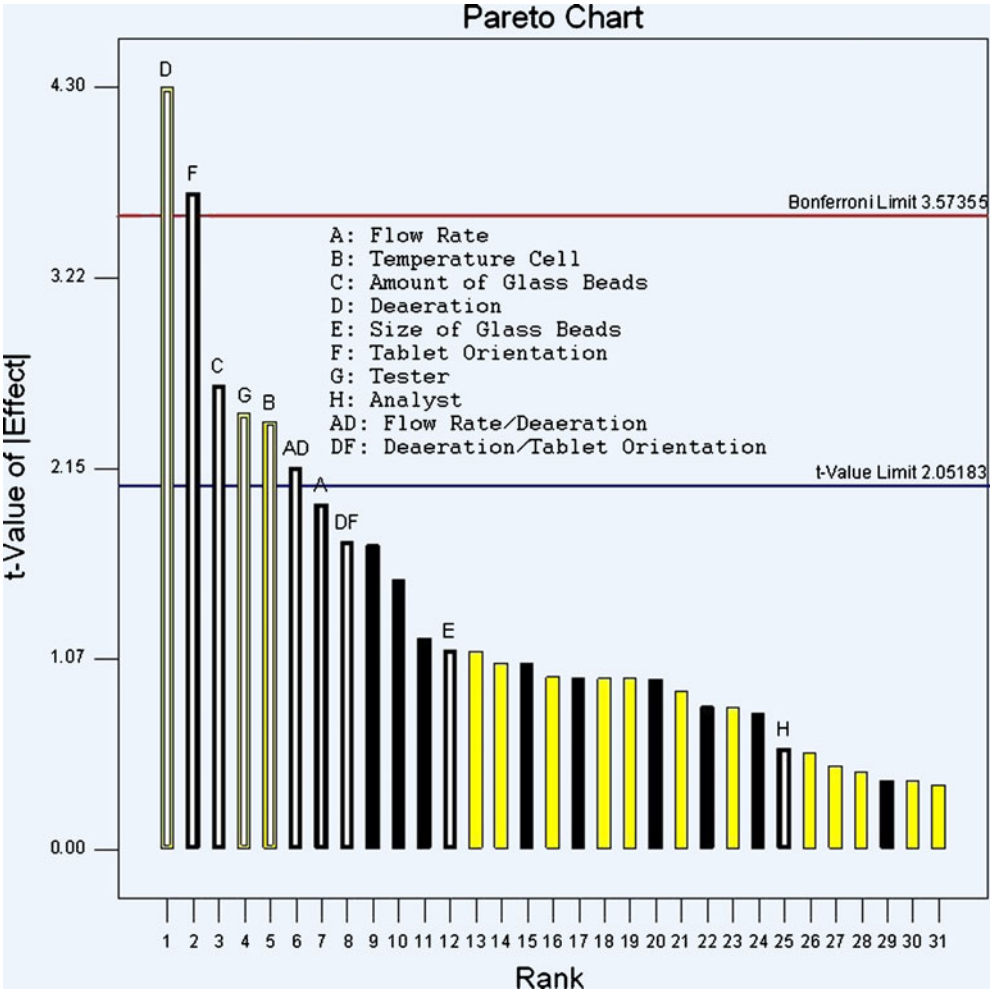

Fig. 2 Pareto chart for standard deviation results.

embedding the tablets in glass beads. Results affected by changing different operational parameters and changing hydrodynamic conditions inside the cell will not be confounded with results affected by changing orientation and thus exposed surface area of the dosage form. Second, although salicylic acid is classified as an irritant, it is not highly toxic and is easily quantified by UV analysis.

4. The results are sensitive to changes in critical operational parameters of the apparatus. Of the eight variables studied, five were shown to contribute significantly to either the mean or standard deviation results. Some of the significant variables also were significant as part of combination effects (two-factor interactions). The diameter of the glass beads was the most significant variable studied. Larger glass beads might have effectively reduced the exposed surface area of the tablets, thus lowering the dissolution rate. The hydrodynamic environment also is probably affected by the different sizes of glass beads. The salicylic acid tablets also showed sensitivity to cell temperature in the $37.0 \pm 1.0°$ range and flow rate in the 8.0 mL/min $\pm$ 5% range. This degree of sensitivity to flow rate is an important aspect of the PVT procedure. It provides a measure of the linear velocity inside the cell at the site of the dosage form that is not captured by simply measuring the average flow rate at the outlet of the pump. The standard deviation results show the importance of deaerating the medium and controlling tablet orientation.

The objective of this work was to evaluate a potential PVT and not exhaustively examine the hydrodynamic environment of the flow-through cell. However, some discussion of the results with regard to hydrodynamics is warranted. The large effect of glass beads size suggests that this variable has a large effect on the hydrodynamics inside the cell. Although procedures will state which size of glass beads to use, the size distribution of glass beads may be critical to obtaining consistent results with this apparatus. The number of dosing spoonfuls did not have a significant effect on dissolution results. The differing number of

spoonfuls (i.e., going from one scoop to two) on the bottom had the effect of raising the tablet up in the cylindrical portion of the cell. Achieving similar results at the two positions suggests that the hydrodynamic environment is consistent within the section of the cell where the dosage form is located.

Based on these results, we believe that a PVT can be developed to assure the integrity of a USP performance test when dissolution is the procedure of choice and the apparatus used in the procedure is USP Apparatus 4.

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