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Application Note

Automating Sample Preparation to meet USP <232>/<233> Guidelines

Jacob Herrington
Teledyne CETAC Technologies
Omaha, NE USA
Jacob.Herrington@Teledyne.com

Figure 1: SimPrep Liquid Handling Station

Introduction

USP <232> specifies that pharmaceutical drug products must be tested for specific elemental impurities to establish the safety of the product. Elemental impurities can be naturally present in a drug product or can be added during its manufacture. Each element has its own permitted daily exposure (PDE) limit based on the element's toxicity as well as whether the drug product has an oral, parenteral, or inhalation route of administration. USP <233> specifies sample preparation and analysis requirements. Liquid oral drug products such as cough syrups and nighttime sleep aids are diluted and analyzed using either ICP-OES or ICP-MS.

Calibration standards and sample spike amounts for the drug products are determined by calculating the J value for each element.

$$J = \frac{Permitted\ Daily\ Exposure}{Maximum\ Daily\ Dose \times Dilution}$$

As the maximum daily dose (MDD) varies from one product to another, the calibration standards and spike amounts can vary as well. By increasing the sample dilution, J values can be standardized. However, adding dilutions increase sample prep time. The SimPrep is an automated dilution system that can dilute and spike liquid samples for USP compliance. For this application note, two common oral liquid drug products were prepared by the SimPrep to demonstrate its effectiveness.

Sample Preparation

Two over-the-counter syrups with different MDDs were selected for analysis:

- Sample A: OTC daytime cold/flu remedy, MDD 147 g/day
- Sample B: OTC nighttime sleep aid, MDD 34 g/day

In order to minimize sample matrix effect and interferences, sample A was diluted at 100×. The diluent was 1% HNO_3, 0.5% HCl, and 100 µg/L of Au. Gold was added in order to stabilize Hf and improve washout. Sample B has an MDD approximately 5× lower than sample A, therefore sample B was diluted at 500×. The final J values are in Table 1. Oral drug products only require Class 1A and 2A elements to be analyzed unless other elements are known to be present. For this application note, only the mandatory elements were tested.

Table 1: J Values per Element

Element	J (µg/L)
Cadmium	0.3
Lead	0.3
Arsenic	0.9
Mercury	1.8
Cobalt	3
Nickel	12
Vanadium	6

The SimPrep spiked all of the samples as well as prepared the calibration curve. The sequence is shown in Figure 2. On line 6, 100 µL of sample A (from position R2-01) along with 300 µL of the Cal Parent are added to a vial and filled to a final volume of 10000 µL. Sample B was diluted at 500× so that the samples had the same J value. On line 18, a 5× dilution was performed. The sample was mixed. Then, 100 µL of the 5x dilution was added to 10000 µL to make a 500x dilution. The SimPrep can spike and dilute the sample in the same preparation.

		Sample		Dilution 1				
	Status	Position	Name	Position	Volume [µl]	Vol Sample	Vol Std	Name Std
1	▶			R1-01	10000			
2	▶		0.5 J	R1-02	10000		300	Cal Parent
3	▶		1.5 J	R1-03	10000		900	Cal Parent
4	▶		1.5 J	R1-04	10000		900	Cal Parent
5	▶	R2-01	A	R1-05	10000	100		
6	▶	R2-01	A 0.5 J	R1-06	10000	100	300	Cal Parent
7	▶	R2-01	A 0.5 J	R1-07	10000	100	300	Cal Parent
8	▶	R2-01	A 0.5 J	R1-08	10000	100	300	Cal Parent
9	▶	R2-01	A 1.5 J	R1-09	10000	100	900	Cal Parent
10	▶	R2-01	A 1.5 J	R1-10	10000	100	900	Cal Parent
11	▶	R2-01	A 1.5 J	R1-11	10000	100	900	Cal Parent
12	▶	R2-01	A 1J	R1-12	10000	100	600	Cal Parent
13	▶	R2-01	A 1J	R1-13	10000	100	600	Cal Parent
14	▶	R2-01	A 1J	R1-14	10000	100	600	Cal Parent
15	▶	R2-01	A 1J	R1-15	10000	100	600	Cal Parent
16	▶	R2-01	A 1J	R1-16	10000	100	600	Cal Parent
17	▶	R2-01	A 1J	R1-17	10000	100	600	Cal Parent
18	▶	R2-02	B 5x	R1-18	10000	2000		
19	▶	R1-18	B 500x	R1-19	10000	100		
20	▶	R1-18	B 0.5 J	R1-20	10000	100	300	Cal Parent
21	▶	R1-18	B 0.5J	R1-21	10000	100	300	Cal Parent
22	▶	R1-18	B 0.5J	R1-22	10000	100	300	Cal Parent
23	▶	R1-18	B 1.5J	R1-23	10000	100	900	Cal Parent
24	▶	R1-18	B 1.5J	R1-24	10000	100	900	Cal Parent
25	▶	R1-18	B 1.5J	R1-25	10000	100	900	Cal Parent
26	▶	R1-18	B 1J	R1-26	10000	100	600	Cal Parent
27	▶	R1-18	B 1J	R1-27	10000	100	600	Cal Parent
28	▶	R1-18	B 1J	R1-28	10000	100	600	Cal Parent
29	▶	R1-18	B 1J	R1-29	10000	100	600	Cal Parent
30	▶	R1-18	B 1J	R1-30	10000	100	600	Cal Parent
31	▶	R1-18	B 1J	R1-31	10000	100	600	Cal Parent
32	▶			R1-32	10000		900	Cal Parent

100 µL of R2-01 (Sample A) is added to vial R1-06

300 µL of Cal Parent is added to R1-06

R1-06 filled to a final volume of 10000 µL

2000 µL of R2-02 (Sample B) is added to R1-18 to make 5× dilution

Serial dilution: 100 µL of R1-18 (5x dilution) added to R1-19 to make 500× dilution

Figure 2: SimPrep Sequence

Sample A was much more viscous than Sample B. By altering the user settings for the SimPrep, it can effectively handle the difficult matrix. First, the sample probe depth was altered such that only a small portion of the probe contacts the sample (Figure 3). This prevents liquid from sticking to the sides of the probe and dripping (Figure 4). The software allows the user to set the probe depth for any position. In our example, Sample A is in spot R2-02.

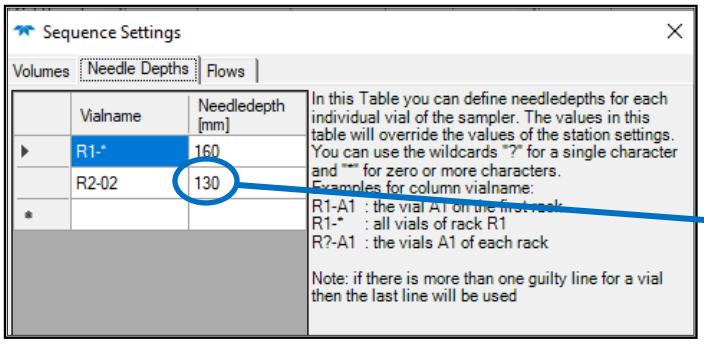
Next, the aspirating and dispensing flows were adjusted to prevent air bubbles during aspiration and to allow any sample adhering to the sample pathway more time to dispense. For this application, the flow was set to 100 µL/sec for Sample A (Figure 5).

Finally, the user can add a no drop volume as well as a dip into the wash port. The no drop volume was set to 100 µL. After the sample was aspirated, air equivalent to 100 µL was aspirated to prevent the sample from dripping. The probe then went to the rinse station (wash port) to remove any sample that may have adhered to the side before proceeding to the sample vial (Figure 6).

Instrumentation

Samples were analyzed using a Thermo RQ ICP-MS with a Teledyne CETAC ASX-560 Autosampler. The instrument was tuned according to manufacturer guidelines to determine operating conditions. All elements were analyzed in Kinetic Energy Discrimination (KED) mode using Helium as the reaction gas. The internal standards—Scandium (Sc), Yttrium (Y), Terbium (Tb), and Bismuth (Bi)—were added in-line at a concentration of 5 ppb.

The instrument was calibrated using a blank and two standards – 0.5J and 1.5J. Calibration coefficients were greater than 0.999 for all elements. The calibration curves for Hg and Cd are shown in Figure 7.



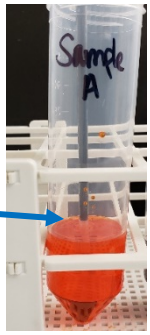


Figure 3: SimPrep Probe Depth Settings (left)

Figure 4: Probe at 130mm (right)

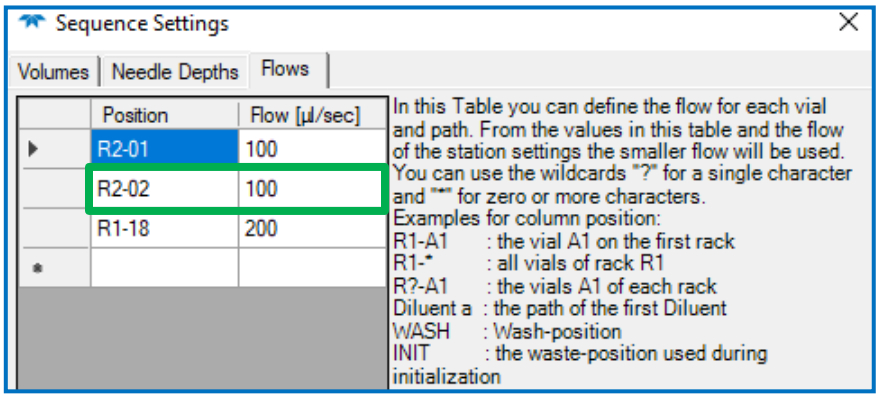


Figure 5: Flow Settings

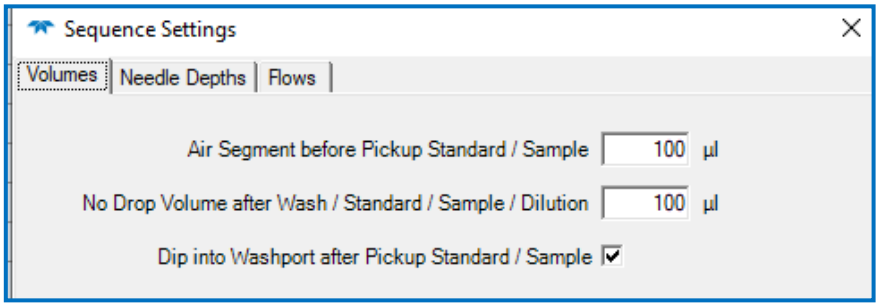


Figure 6: Volume Settings

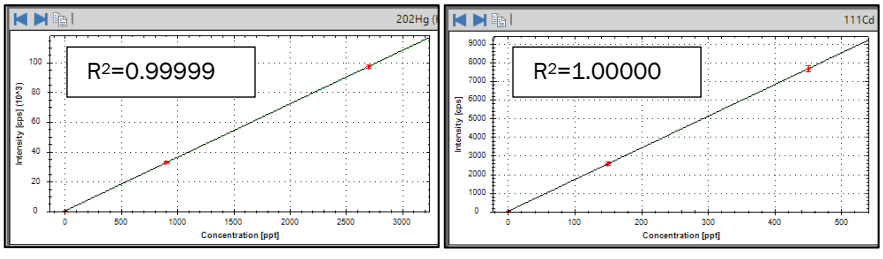


Figure 7: Calibration Curves for 202Hg and 111Cd

Results

Sodium (Na) is not an element required for testing by USP <232>. However, in these samples, the Na concentration after dilution was between 1-5 mg/L. Therefore, Na was used to monitor the precision of the 6 1J sample spikes as prepared by hand and by the SimPrep. The samples were prepared fresh each day for analysis. On Day 2, 50 mL of 1J Sample A was prepared and all 6 aliquots were taken from that preparation. This 50 mL preparation served as a control to determine instrument performance irrespective of preparation technique. As shown in Figure 8, the SimPrep results met or exceeded the Hand Prep results. The higher RSDs for the Day 2 hand prep are likely the result of contamination. Using the SimPrep, user created contamination can be avoided while maintaining the precision of hand pipetting.

USP <233> has four requirements to validate the analysis: accuracy, repeatability, ruggedness, and system suitability.

Accuracy

To determine accuracy, both samples were spiked in triplicate at 0.5J and 1.5J. Sample recoveries must be within 70-150%. The average recoveries for each element are in Figure 9. Spike recoveries for Arsenic (As) in Sample A, while passing, are likely high due to matrix effect.

Repeatability and Ruggedness

Repeatability is determined by analyzing six dilutions of each sample spiked at 1J. These dilutions were prepared on a second day and analyzed again for ruggedness. RSDs of the 6 spikes must be below 20% while the 12 analyses must have an RSD below 25%. All RSDs are below 4%, easily meeting these criteria (Figure 10).

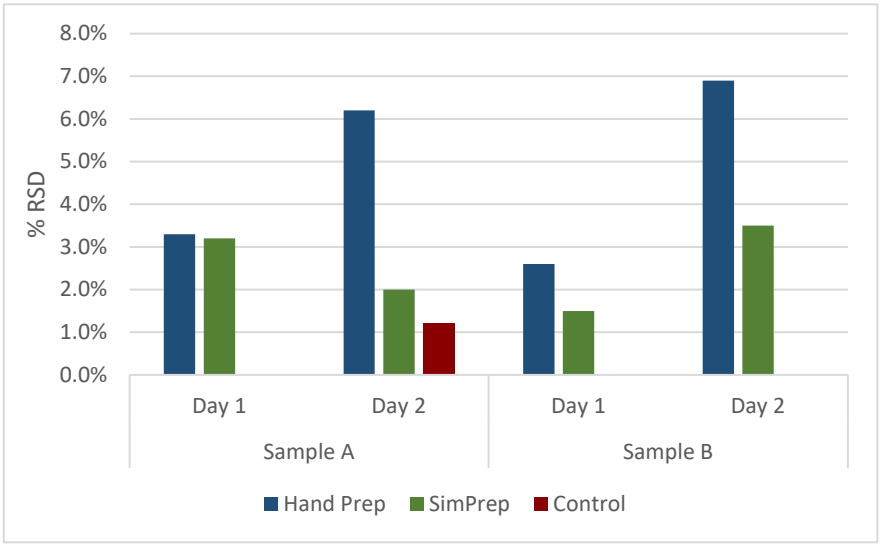


Figure 8: RSD of Na Intensities for 6 Preparations of 1J Sample A and Sample B

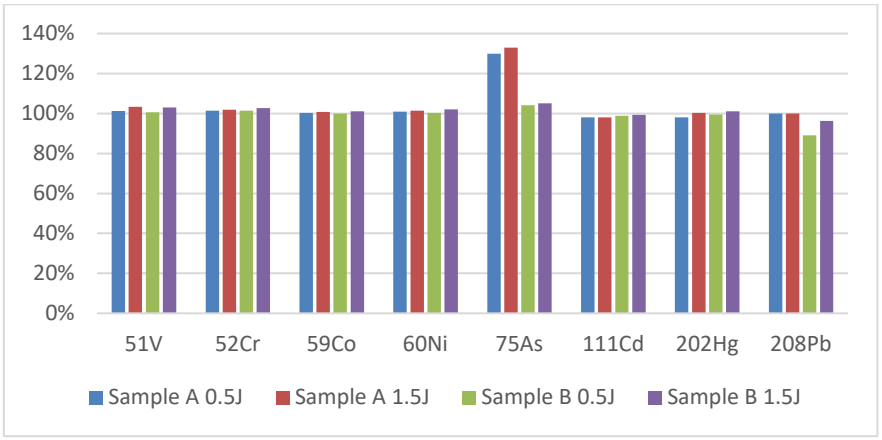


Figure 9: Accuracy – Average Recoveries 0.5J and 1.5J Sample Spikes

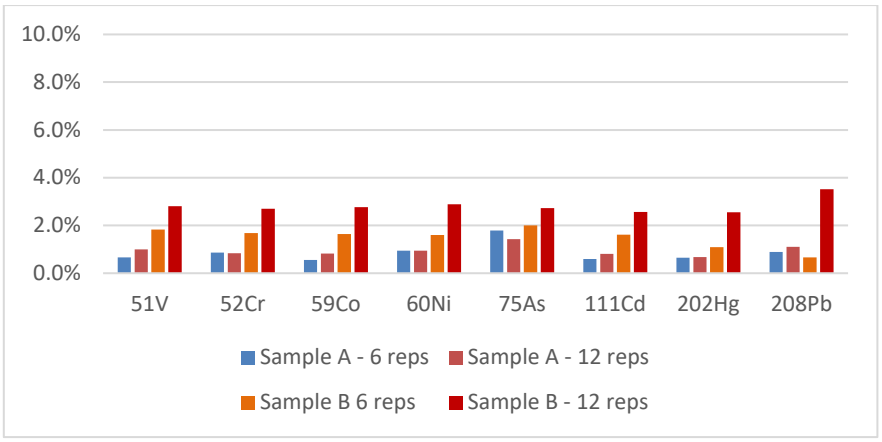


Figure 10: Repeatability and Ruggedness

System Suitability

The 1.5J standard is analyzed before and after the samples. Drift from the analysis must be less than 20%. The drift for all elements is below 5% as seen in Figure 11.

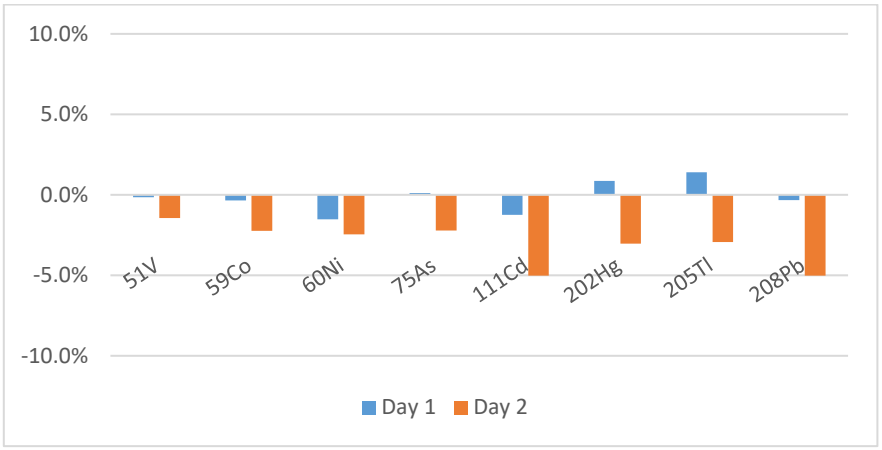


Figure 11: System Suitability

Conclusion

The SimPrep diluted and spiked oral liquid drug products accurately and precisely achieving the guidelines for USP <233>. Due to the flexibility of the features in the software, the viscosity of the cough syrup sample did not affect the analysis. Incorporating the SimPrep Autodilution system into your everyday sample preparation can save time and money while maintaining quality control.

References

1. General Chapter <232> Elemental Impurities – Limits: 2nd Supplement of USP 35-NF 30.
2. General Chapter <233> Elemental Impurities – Procedures: 2nd Supplement of USP 35-NF 30.

Teledyne CETAC Technologies
14306 Industrial Road
Omaha, NE 68144 USA
+1.402.733.2829
teledynecetac.com

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