Author: Kyle Uhlmeyer and Andrew Kavan

SampleSense prep*FAST* – Automated Inline Sample Dilution and J Spiking for Pharmaceutical Analysis

Abstract

Determination of trace elemental impurities in drug products by ICPMS is well defined in the United States Pharmacopeia (USP). The standard procedure requires significant sample dilutions as well as calibrations at specific concentration levels for each product based on the maximum daily dose, resulting in a unique calibration for each dose level and sample dilution factor. For traditional sample introduction systems, these requirements lead to excessive manual preparation steps for samples and calibration standards. An automated dilution system greatly simplifies these analyses by reducing manual dilution steps and automatically preparing calibration standards inline, dramatically increasing laboratory efficiency. Besides requiring excessive dilutions, many oral drug products also vary in viscosity, making it difficult to consistently introduce the sample to the ICPMS. The SampleSense prep*FAST* described in this application note automatically calibrates, dilutes and spikes samples, and adjusts sample loading for variable sample volume and viscosity to improve efficiency, consistency, and performance for the analysis of pharmaceutical products.

Figure 1. SampleSense valve

SampleSense prep*FAST*

The prep*FAST* is a syringe-driven, inline, automated sample dilution system that can automatically calibrate the ICPMS, dilute samples at the time of analysis, and auto-spike samples for QC verification. Many pharmaceutical labs already use the prep*FAST* to automate USP elemental impurities analysis, allowing them to reduce manual labor and increase efficiency by reducing time spent diluting samples and preparing calibration standards.

The prep*FAST* uses a vacuum pump to load sample onto a valve loop located near the ICPMS nebulizer. After loading the sample, two syringes rapidly dilute the sample up to 200x inline to a second valve loop while a third syringe may be used to add calibration standard, adding the ability to create calibration curves or spike samples for QC without manual preparation of those solutions. The prepared sample, blank, or standard is then injected into the ICPMS nebulizer for analysis.

SampleSense enhances the performance of the prep*FAST* by adding integrated optical sensors to automatically detect the presence of a non-segmented liquid sample at the entrance and exit of the vacuum-loaded loop. Upon detection, the sensed sample is diluted by the prep*FAST* and injected into the ICPMS nebulizer, followed by automatic triggering of the ICPMS analysis. Sample throughput is improved, sample consumption is reduced, and viscosity effects on sample uptake are eliminated. SampleSense also detects and reports sample loading failures. Unsensed samples – for example, empty tubes or capped tubes – are identified and logged, saving the operator the time and hassle of deciphering ICPMS data from non-sample events.

Instrumentation

All samples were analyzed using SampleSense prep*FAST* in combination with an ICPMS.
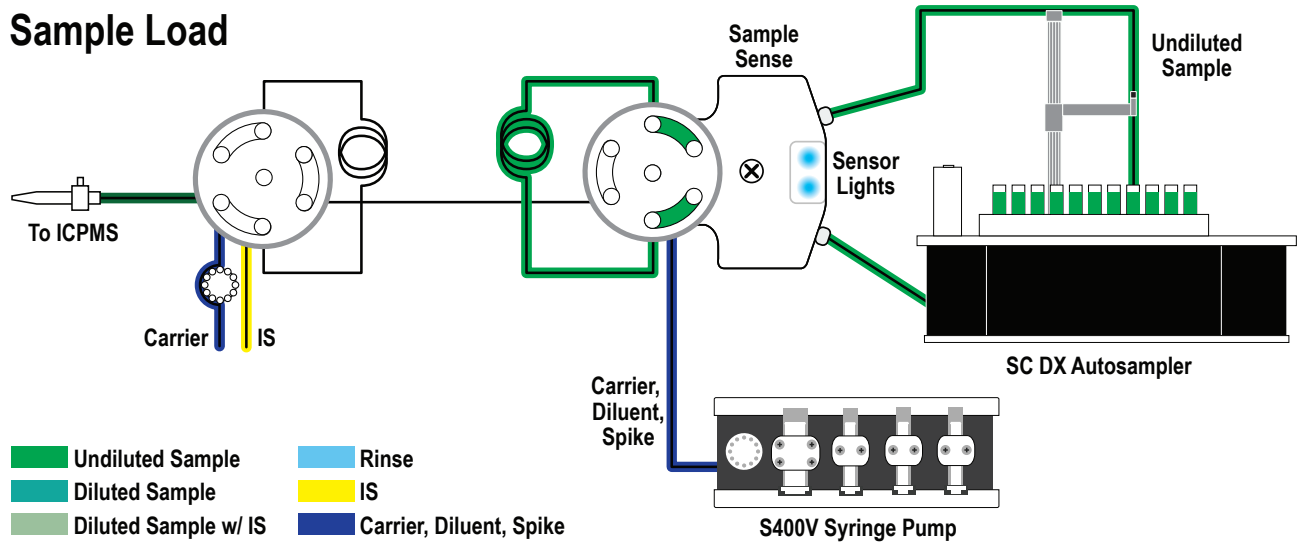
Features

- Automatic sensing and injection of samples – even viscous ones such as syrups and suspensions

- Automatic triggering of the ICPMS analysis

- Automatic dilution, calibration, and inline spiking of samples prior to analysis

- Detection and reporting missing or empty sample tubes as "unsensed" samples

- Integrated valve assembly

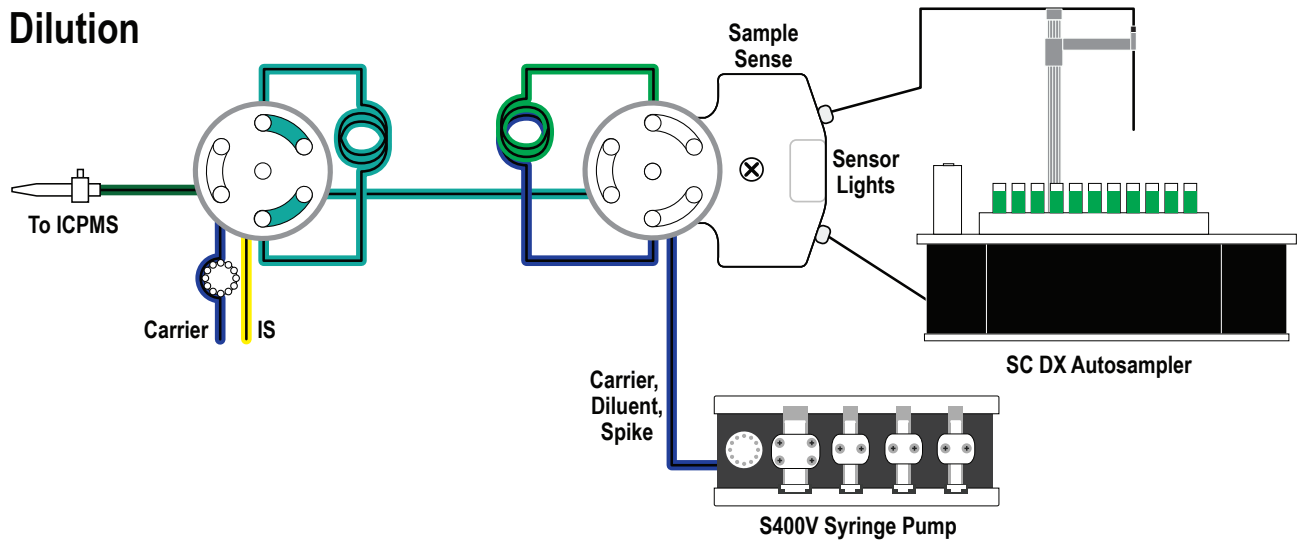## SampleSense prep*FAST* for USP

USP chapters <232> and <233> outline the analytical requirements for determining elemental impurities in pharmaceutical products. USP <232> defines a permissible daily exposure (PDE) limit in µg/day for each element and exposure pathway (oral, inhalation, or parenteral) (Table 1). Chapter <233> further defines the analytical limit, "J," as the PDE divided by the maximum daily dose of the product and the sample dilution factor. This complicates analysis since each product may have a different daily dose and dilution factor, leading to a unique J for each product. In addition, USP <233> defines the calibration standards to be 0.5 J and 1.5 J for each product, requiring a separate calibration curve for each product and dilution factor.

SampleSense prep*FAST* simplifies USP-compliant analyses by both automating inline sample dilution and automatically creating calibration curves at the necessary J levels for each product. It also allows the analysis of pharmaceutical samples with differing viscosities in the same analytical run without making any method or timing changes. Figure 2 shows the basic analysis routine for the SampleSense prep*FAST*.

Sample Load

To ICPMS

Carrier   IS

Sample Sense

Sensor Lights

Undiluted Sample

SC DX Autosampler

Carrier, Diluent, Spike

S400V Syringe Pump

▇ Undiluted Sample	▇ Rinse
▇ Diluted Sample	▇ IS
▇ Diluted Sample w/ IS	▇ Carrier, Diluent, Spike

Dilution

To ICPMS

Carrier   IS

Sample Sense

Sensor Lights

SC DX Autosampler

Carrier, Diluent, Spike

S400V Syringe Pump

Sample Analysis and Wash

To ICPMS

Carrier   IS

Sample Sense

Rinse

Sensor Lights

SC DX Autosampler
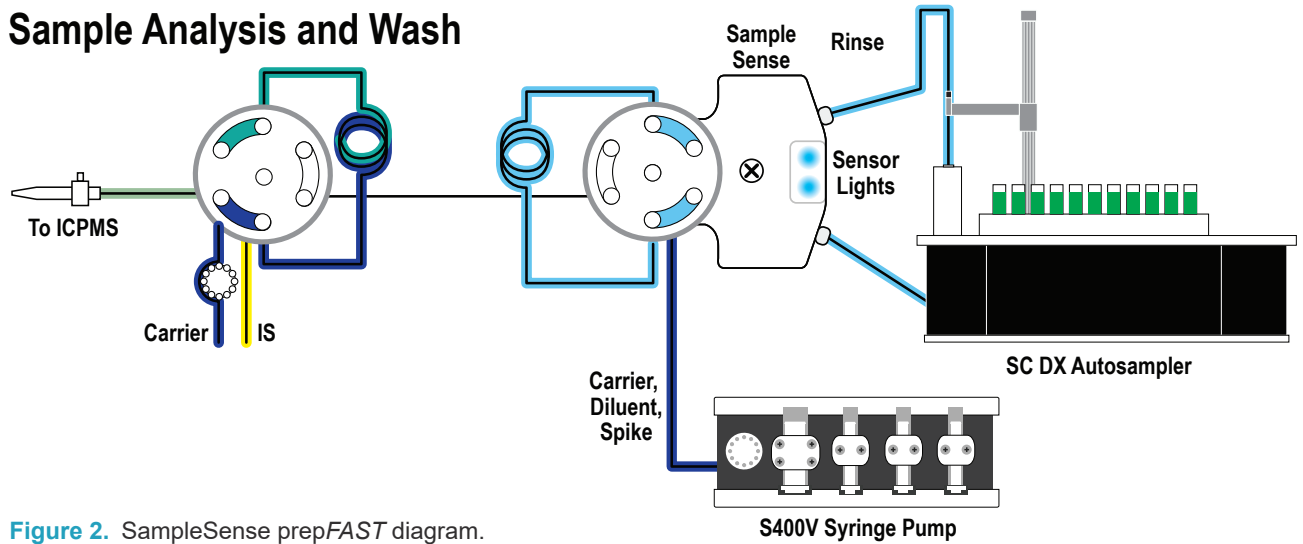
Carrier, Diluent, Spike

S400V Syringe Pump

Figure 2. SampleSense prep*FAST* diagram.

Sample Preparation

Four pharmaceutical products were selected for analysis in this study: two syrups (a children's cough syrup and a daytime flu remedy syrup), an immune system supplement, and normal saline solution. The syrups and saline solutions were poured into tubes neat and diluted inline by the prepFAST. The immune supplement was prepared according to the package instructions – in DIW and poured into tubes for analysis. Sample dilution factors and associated J values can be seen in Table 3.

Table 1. Experimental conditions.

Experimental Conditions	
System	SampleSense prepFAST with Inline Spiking
Rinse Solution	4% HNO_3/1% HCl
Carrier/Diluent Solution	2% HNO_3/0.5% HCl
Internal Standard	20 ppb Ge, In in 2% HNO_3/ 0.5% HCl/10% Methanol (to account for matrix differences)
Dwell Time (ms)	As, Ge: 150 All others: 50
Analysis Flow Rate (µL/min)	300
Internal Standard Flow Rate (µL/min)	100
Analysis Injection Method	Peristaltic Pump

Table 2. Permitted Daily Exposure (PDE) limits for oral, parenteral and inhalation drugs, along with risk class.

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
Cd	1	5	2	2
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rh	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

Table 3. Sample prep table with J values for each product.

Element	Children's Cough Syrup	Daytime Flu Remedy	Immune Supplement	Normal Saline
prepFAST Dilution Factor	50	100	20	20
Element	J (ng/mL)	J (ng/mL)	J (ng/mL)	J (ng/mL)
Cd	1.67	0.42	1.41	0.09
Pb	1.67	0.42	1.41	0.23
As	5.00	1.25	4.23	0.68
Hg	10.0	2.50	8.47	0.14
Co	16.7	4.17	14.1	0.23
V	33.3	8.33	28.2	0.45
Ni	66.7	16.7	56.5	0.90
Tl	2.67	0.67	2.26	0.36
Au	33.3	8.33	28.2	4.50
Pd	33.3	8.33	28.2	0.45
Ir	33.3	8.33	28.2	0.45
Os	33.3	8.33	28.2	0.45
Rh	33.3	8.33	28.2	0.45
Ru	33.3	8.33	28.2	0.45
Se	50.0	12.5	42.3	3.60
Ag	50.0	12.5	42.3	0.45
Pt	33.3	8.33	28.2	0.45
Li	183.3	45.8	155.2	11.3
Sb	400	100	339	4.05
Ba	467	117	395	31.5
Mo	1000	250	847	67.5
Cu	1000	250	847	13.5
Sn	2000	500	1694	27.0
Cr	3667	917	3105	49.5

Table 4. Instrument parameters.

Instrument Parameters	
Instrument	PerkinElmer NexION 2000
ICP RF Power (W)	1600
Plasma Gas Flow (L/min)	15
Auxillary Gas Flow (L/min)	1.2
Nebuilzer Gas Flow (L/min)	1.16
Spray Chamber	C3X Glass Cyclonic
Torch	Demountable Quartz
Injector	2.5 mm Demountable Quartz
Sampler/Skimmer Cones	Nickel
Peltier Cooler Set Point	2 °C

Calibrations

USP <233> prescribes the calibration range to include 0, 0.5 J, and 1.5 J for each product being analyzed. Based on this requirement, the prep*FAST* prepared a calibration curve for each analyte by loading a blank solution (2% HNO_3 / 0.5% HCl) from the autosampler deck and automatically spiking it with different amounts of calibration standard to achieve concentrations at 0, 0.5 J and 1.5 J. Due to the higher daily dose and resulting lower J value, a separate calibration standard was used for the saline sample. Correlation coefficients for all calibrations can be seen in Table 5. Figures 3 and 4 show calibration curves for As and Cd at the saline levels and Hg and Pb at the immune supplement levels. The prep*FAST* makes highly accurate calibration curves even at low levels by utilizing precise syringe movements and enclosed reagent bottles to maintain purity.

Table 5. Calibration Correlation Coefficients

Calibration R for Common Pharmaceuticals				
Element	Children's Cough Remedy	Daytime Flu Remedy	Immune Supplement	Saline
Cd	1.0000	1.0000	0.9998	0.9996
Pb	0.9999	0.9999	0.9999	1.0000
As	1.0000	1.0000	0.9999	1.0000
Hg	1.0000	1.0000	0.9999	0.9999
Co	1.0000	1.0000	1.0000	0.9999
V	1.0000	1.0000	1.0000	1.0000
Ni	1.0000	1.0000	1.0000	1.0000
Tl	1.0000	1.0000	1.0000	1.0000
Au	0.9999	0.9999	1.0000	1.0000
Pd	1.0000	1.0000	1.0000	1.0000
Ir	0.9999	0.9999	1.0000	1.0000
Os	0.9999	0.9999	1.0000	0.9998
Rh	1.0000	1.0000	1.0000	1.0000
Ru	1.0000	1.0000	0.9999	1.0000
Se	1.0000	1.0000	1.0000	1.0000
Ag	0.9999	0.9999	1.0000	0.9998
Pt	0.9999	0.9999	1.0000	1.0000
Li	0.9992	0.9992	1.0000	1.0000
Sb	0.9999	0.9999	1.0000	1.0000
Ba	0.9998	0.9998	1.0000	1.0000
Mo	0.9997	0.9997	0.9999	1.0000
Cu	0.9999	0.9999	1.0000	1.0000
Sn	0.9995	0.9995	1.0000	1.0000
Cr	1.0000	1.0000	1.0000	1.0000

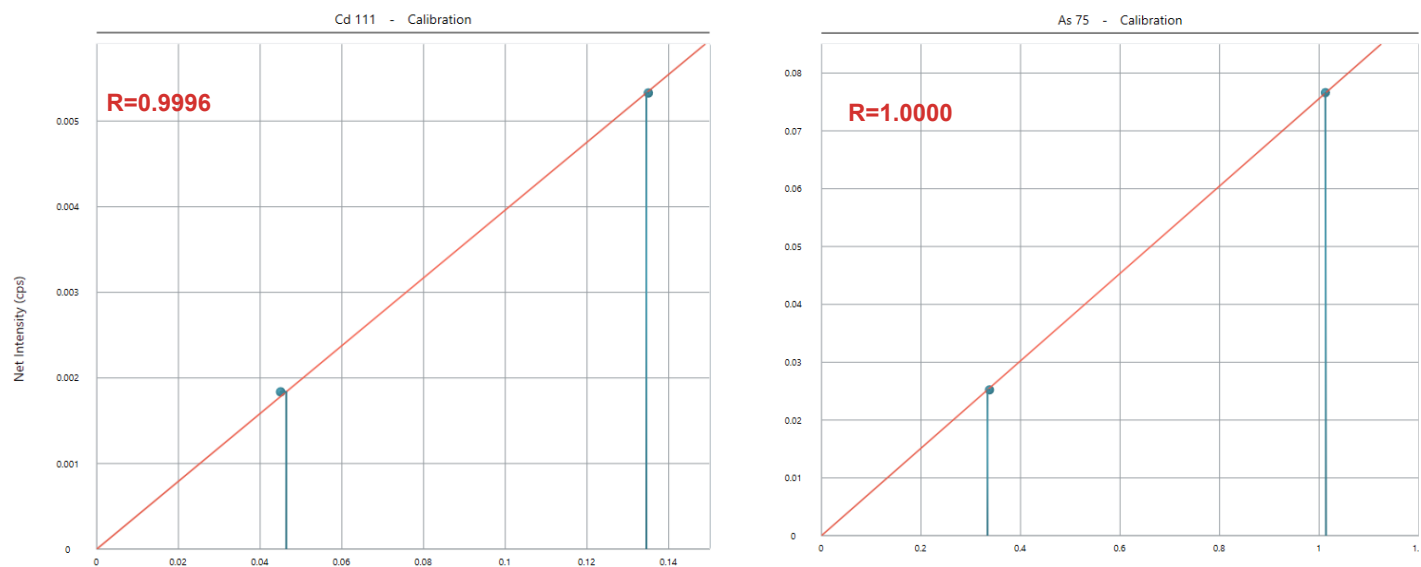## As and Cd Calibration Curves in Saline

Figure 3. Calibration curves for Cd (0, 0.045, 0.14 ppb) and As (0, 0.34, 1.01 ppb) in 20x diluted saline created automatically by inline spiking of calibration standard into blank solution.

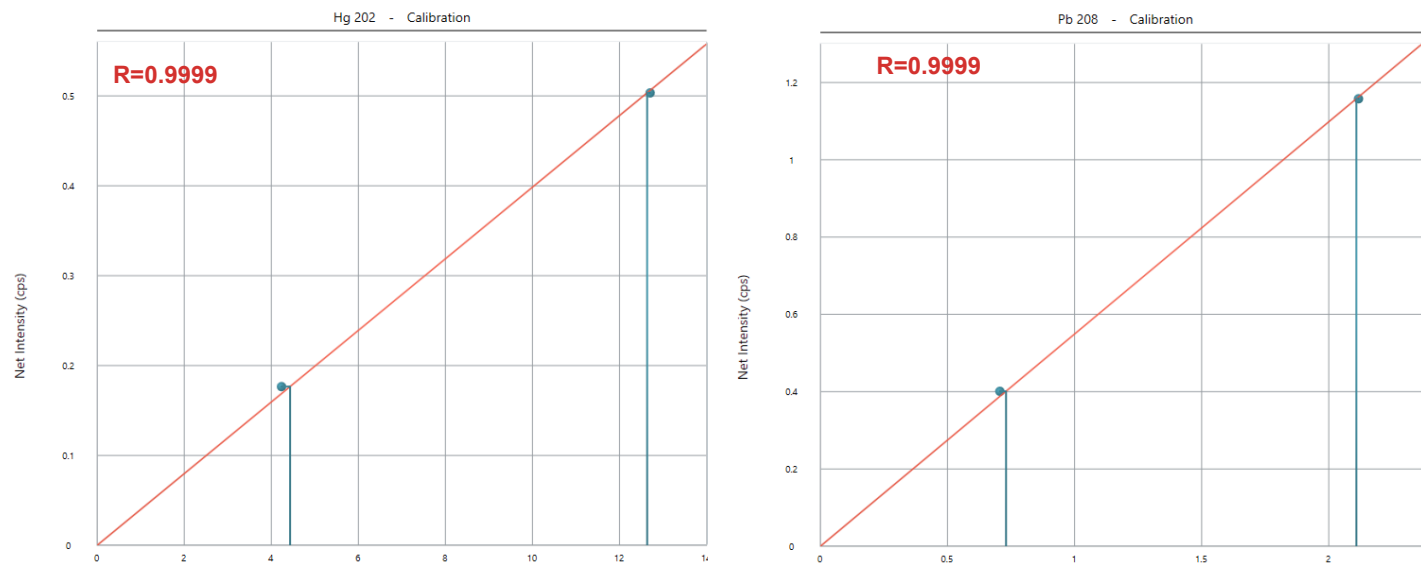## Hg and Pb Calibration Curves in Immune Supplement

Figure 4. Calibration curves for Hg (0, 4.43, 12.6 ppb) and Pb (0, 0.73, 2.11 ppb) in 20x diluted immune supplement created automatically by inline spiking of calibration standard into blank solution.

Inline J Spiking Stability

After calibration, analytes were determined in each product followed by a 1 J spike to calculate spike recovery accuracy and precision. Determined concentrations can be seen in Tables 6-7. Performing the 1 J spikes inline proved to be accurate and precise, with all elements in each product recovering within the range specified by USP <233>. The 1 J spike recoveries for Class 1 elements (Cd, Pb, As, Hg) in 24 inline spiked samples are highlighted in Figures 5-8, demonstrating the capability of the prep*FAST* to efficiently and accurately perform inline spikes for system validation. Additionally, Figures 9-10 show the average 1 J spike recovery and precision for all elements in each product, with most elements recovering within 90-110% with precision better than 3% *(n = 24)*.

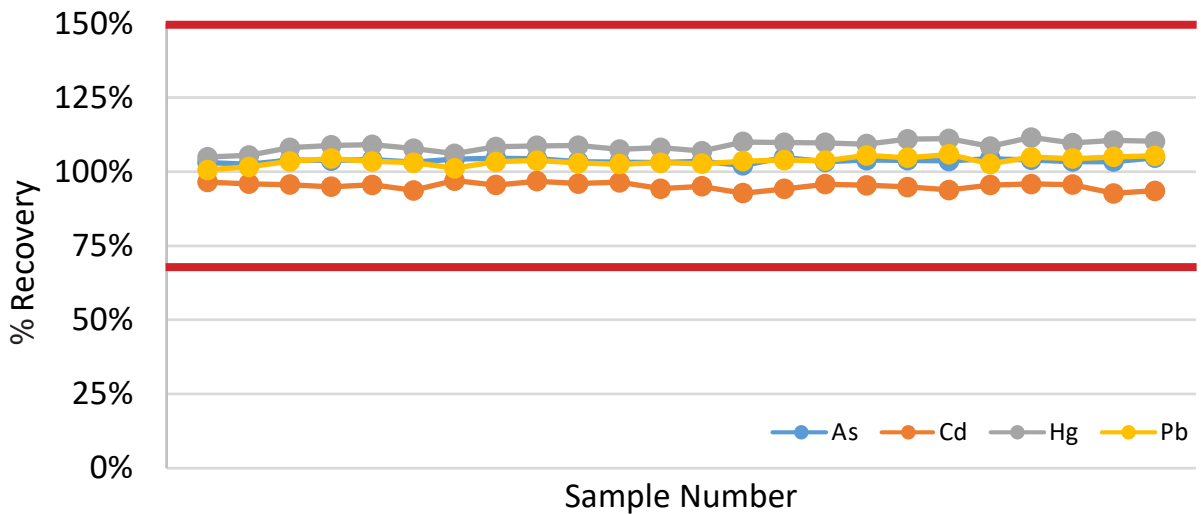### 1 J Spike Recovery in Children's Cough Syrup

Figure 5. Spike recoveries in 24 children's cough syrup samples with inline 1 J spike. Class 1 elements are shown, which must be measured in all products (As, Cd, Hg, Pb).
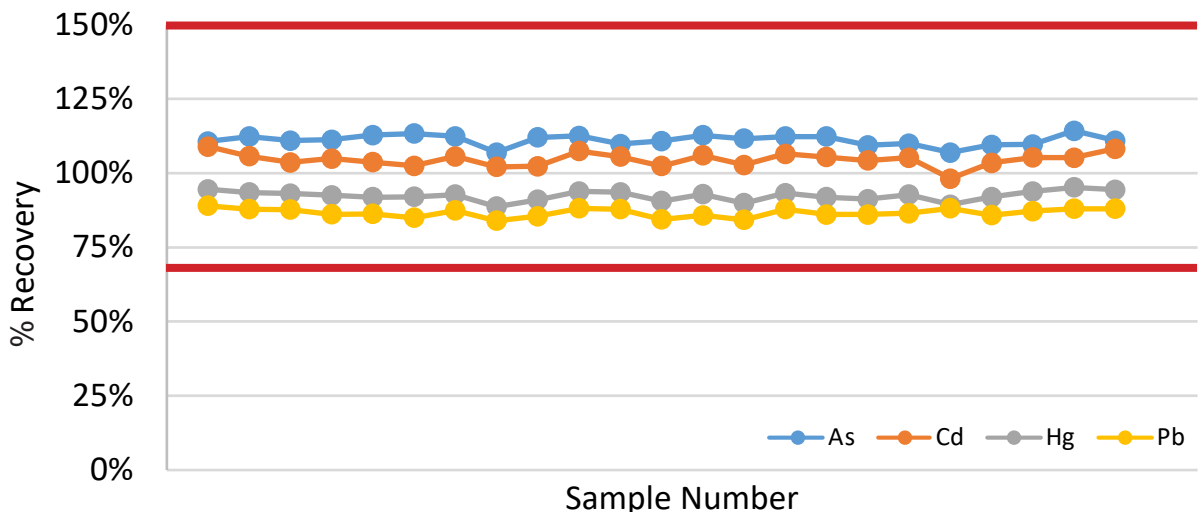
1 J Spike Recovery in Immune Supplement

Figure 6. Spike recoveries in 24 immune supplement samples with inline 1 J spike. Class 1 elements are shown, which must be measured in all products (As, Cd, Hg, Pb).
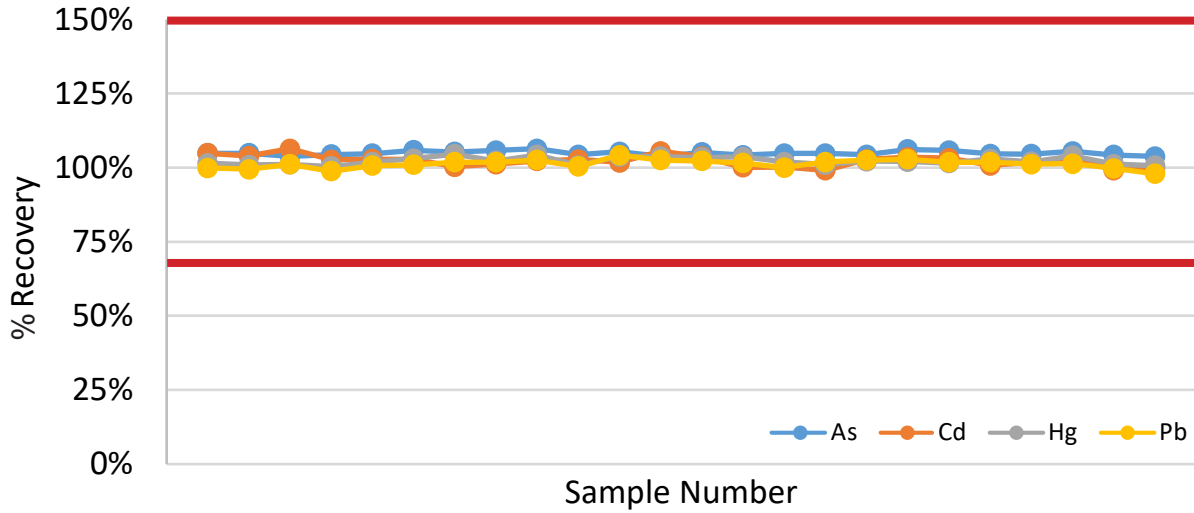
1 J Spike Recovery in Daytime Flu Remedy

Figure 7. Spike recoveries in 24 daytime flu remedy samples with inline 1 J spike. Class 1 elements are shown, which must be measured in all products (As, Cd, Hg, Pb).

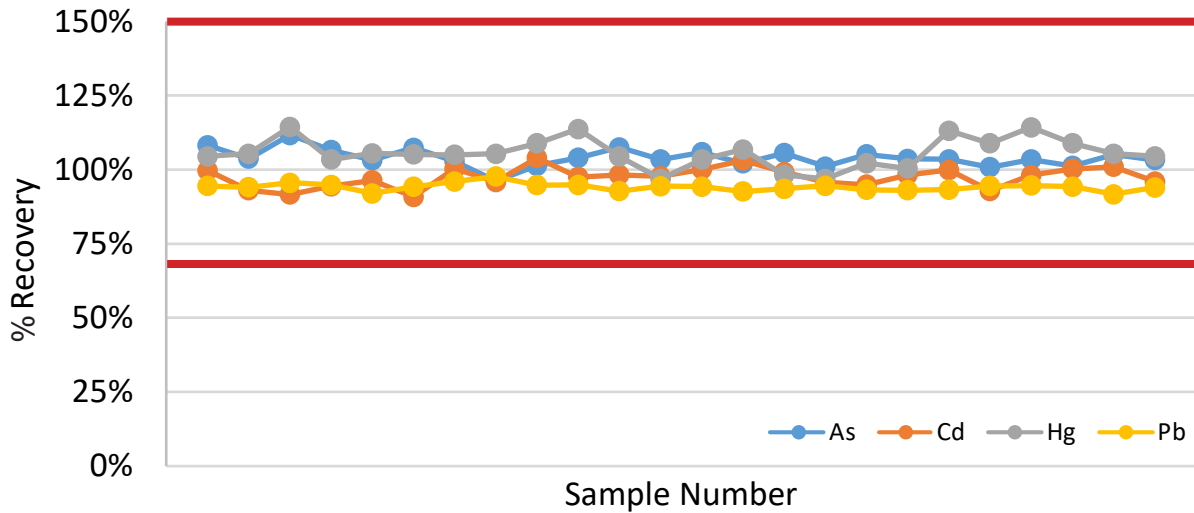## 1 J Spike Recovery in Saline

Figure 8. Spike recoveries in 24 saline samples with inline 1 J Spike. Class 1 elements are shown, which must be measured in all products (As, Cd, Hg, Pb).
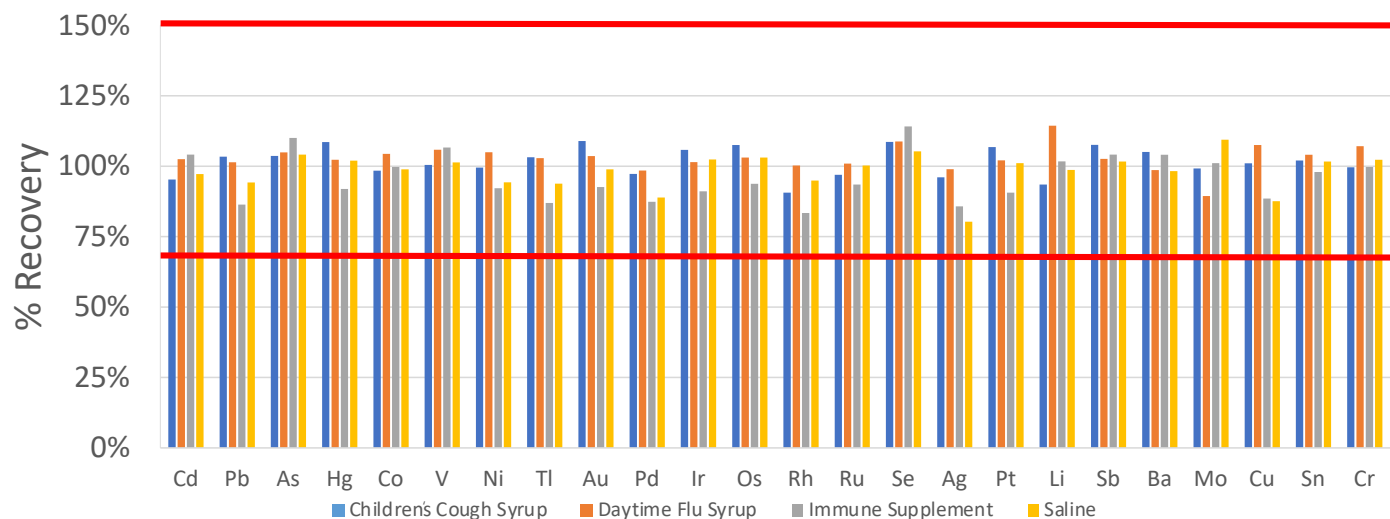
1 J Average Spike Recoveries

Figure 9. Average recovery of inline 1 J spiked samples for all elements *(n = 24)*. All elements fall within USP accuracy test limits (70-150%).
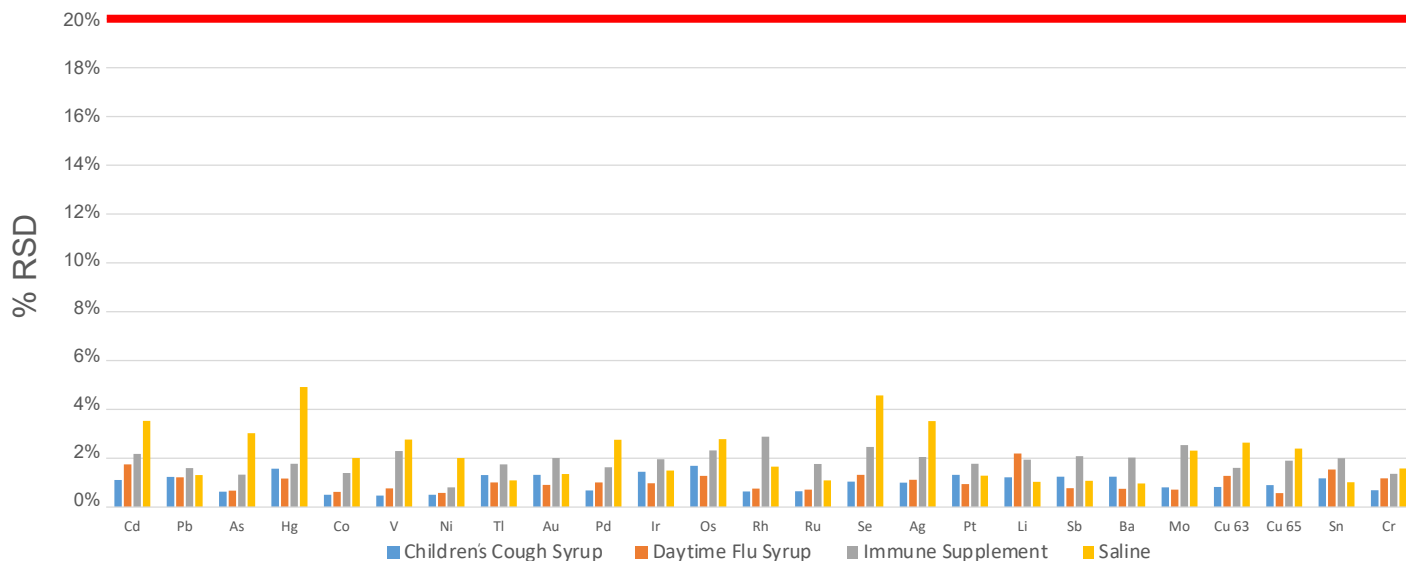
1 J Spike Precision

Figure 10. Average precision for samples with inline 1 J spike *(n = 24)*. All elements easily fall within the USP precision test limit (maximum 20%).

Conclusion

SampleSense prep*FAST* with inline spiking and autocalibration improves analytical efficiency, accuracy, and instrument performance for pharmaceutical analyses using USP methods. The ability to automatically calibrate for different products at different J values reduces manual labor and produces linear calibrations over a wide analysis range, and automatic sample spiking allows a lab to easily validate and QC a new product with minimal startup time. Autodilution of samples eliminates manual sample preparation steps and improves instrument stability through the reduction of deleterious ICPMS matrix effects. SampleSense eliminates method customization and removes guesswork for sample uptake and instrument delays by sensing the sample in the valve loop and triggering the ICPMS analysis at the correct time, making the analysis of samples with varying viscosity and volume easy and routine. These features make SampleSense prep*FAST* the ideal sample introduction system for the determination of elemental impurities in pharmaceutical products.

Analysis Results

Table 6. Determined concentration and 1 J spike recovery and precision *(n = 24)* for all elements in autodiluted cough syrups. Calibrations and 1 J spikes performed inline with SampleSense prep*FAST*. Excellent accuracy and precision were achieved in both sample products.

Element	Children's Cough Remedy			Daytime Flu Remedy		
	Concentration (ng/mL)	1 J Spike Recovery	1 J Spike Precision	Concentration (ng/mL)	1 J Spike Recovery	1 J Spike Precision
Cd	< 0.002	95%	1.1%	0.01	102%	1.8%
Pb	< 0.002	103%	1.2%	< 0.002	101%	1.2%
As	0.05	104%	0.6%	0.01	105%	0.7%
Hg	< 0.01	109%	1.6%	< 0.01	102%	1.2%
Co	< 0.0007	98%	0.5%	0.004	104%	0.6%
V	< 0.006	100%	0.5%	0.03	106%	0.8%
Ni	0.06	100%	0.5%	0.02	105%	0.6%
Tl	< 0.0001	103%	1.3%	0.002	103%	1.0%
Au	< 0.003	109%	1.3%	< 0.003	104%	0.9%
Pd	< 0.001	97%	0.7%	< 0.001	98%	1.0%
Ir	< 0.0007	106%	1.4%	0.001	101%	1.0%
Os	0.002	108%	1.7%	< 0.001	103%	1.3%
Rh	< 0.0003	91%	0.6%	0.001	100%	0.8%
Ru	< 0.0006	97%	0.7%	0.002	101%	0.7%
Se	7.2	109%	1.0%	0.8	109%	1.3%
Ag	< 0.001	96%	1.0%	< 0.001	99%	1.1%
Pt	< 0.0004	107%	1.3%	0.001	102%	0.9%
Li	0.03	94%	1.2%	< 0.003	114%	2.2%
Sb	0.09	108%	1.2%	0.06	103%	0.8%
Ba	0.52	105%	1.2%	0.02	99%	0.8%
Mo	< 0.009	99%	0.8%	0.03	89%	0.7%
Cu	< 0.02	101%	0.8%	0.72	108%	1.3%
Sn	< 0.009	102%	1.2%	0.06	104%	1.5%
Cr	0.73	100%	0.7%	0.36	107%	1.2%

Analysis Results

Table 7. Determined concentration and 1 J spike recovery and precision *(n = 24)* for all elements in autodiluted Immune Supplement and Saline. Calibrations and 1 J spikes performed inline with SampleSense prep*FAST*. Excellent accuracy and precision were achieved in both sample products.

Element	Immune Supplement			Saline		
	Concentration (ng/mL)	1 J Spike Recovery	1 J Spike Precision	Concentration (ng/mL)	1 J Spike Recovery	1 J Spike Precision
Cd	0.05	104%	2.2%	< 0.002	97%	3.5%
Pb	0.01	86%	1.6%	< 0.002	94%	1.3%
As	0.14	110%	1.3%	< 0.006	104%	3.0%
Hg	< 0.01	92%	1.8%	< 0.01	102%	4.9%
Co	0.52	100%	1.4%	< 0.007	99%	2.0%
V	0.36	107%	2.3%	< 0.006	101%	2.8%
Ni	0.24	92%	0.8%	< 0.009	94%	2.0%
Tl	0.001	87%	1.7%	< 0.0001	94%	1.1%
Au	0.01	93%	2.0%	< 0.003	99%	1.4%
Pd	< 0.001	87%	1.6%	< 0.001	89%	2.8%
Ir	< 0.0007	91%	2.0%	< 0.0007	102%	1.5%
Os	< 0.001	94%	2.3%	< 0.001	103%	2.8%
Rh	< 0.0003	83%	2.9%	< 0.0003	95%	1.7%
Ru	< 0.0006	93%	1.8%	< 0.0006	100%	1.1%
Se	< 0.5	114%	2.5%	< 0.5	105%	4.6%
Ag	0.003	86%	2.1%	< 0.001	80%	3.5%
Pt	< 0.0004	91%	1.8%	< 0.0004	101%	1.3%
Li	0.28	102%	1.9%	0.03	99%	1.0%
Sb	0.04	104%	2.1%	< 0.004	102%	1.1%
Ba	2.8	104%	2.0%	0.004	98%	1.0%
Mo	0.22	101%	2.5%	< 0.009	109%	2.3%
Cu	0.11	89%	1.6%	< 0.02	88%	2.6%
Sn	0.02	98%	2.0%	< 0.009	102%	1.0%
Cr	7.8	100%	1.4%	0.02	102%	1.6%

Description	Part Numbers
SampleSense prep*FAST* 2DX	2PF-SS8
SampleSense prep*FAST* 4DX	4PF-SS8
SampleSense prep*FAST* 8DX	8PF-SS8
SampleSense prep*FAST* 14DX	14PF-SS8