

Trace Elements in Over the Counter Cough Syrup by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

INTRODUCTION

Recognition of the adverse effects of human exposure to toxic elements in drugs has increased over the last two decades. Elements such as arsenic, cadmium, lead and mercury are commonly found in drug substances and the elemental analysis procedures outlined by the USP do not allow these elements to be reliably quantified at toxicologically relevant concentrations.

The USP has identified two classes of elemental impurities that must be quantified in all substances involved in the manufacturing of a drug including: raw materials, catalysts, active pharmaceutical ingredients (API), excipients and the finished product. Class 1 elemental impurities are considered to be hazardous to the environment and toxic to humans, even when present at low concentrations. All four of these elements must be quantified, regardless of whether they were used during the manufacturing process. Class 2 elemental impurities are considered to be less toxic and hazardous than those outlined in the first class. Class 2 impurities need only be measured when they have been added to the drug manufacturing process.

The USP has set limits on the concentrations of Class 1 and Class 2 impurities that can be

present in drug substances [1] and those limits are outlined in Tables I and II below. The limits are reported as total permissible daily exposure (PDE) limits in units of $\mu\text{g day}^{-1}$. The impurity limits for each class are defined separately for drug substances that are consumed orally as well as those consumed parenterally (injected or inhaled).

Class 1 Elemental Impurities		
Element	Oral Daily Dose PDE ($\mu\text{g day}^{-1}$)	Parental Daily Dose PDE ($\mu\text{g day}^{-1}$)
As	15	1.5
Cd	5	0.5
Pb	10	1.0
Hg	15	1.5

Table I. Class 1 elemental impurity limits set by the USP.

Class 2 Elemental Impurities		
Element	Oral Daily Dose PDE ($\mu\text{g day}^{-1}$)	Parental Daily Dose PDE ($\mu\text{g g}^{-1}$)
Cr	250	25
Cu	2500	250
Mn	2500	250
Mo	250	25
Ni	250	25
Pd	100	10
Pt	100	10
V	250	25
Os	Combination not to exceed 100	Combination not to exceed 10
Rh		
Ru		
Ir		

Table II. Class 2 elemental impurity limits set by the USP.

Protocols for quantifying Class 1 and Class 2 elemental impurities are outlined in USP Chapter <231> - Procedures. Established in 1905, these procedures involve colorimetric reactions based on metal sulfide precipitation. Not only was the chemistry in these protocols developed well over 100 years ago, but the reactions have many challenges and drawbacks associated with them. For example, the reaction itself produces hydrogen sulfide (H_2S), which poses a substantial health threat to the personnel performing the analysis. The reaction is also challenging to reproduce which affects the reliability of the results. The heavy metals are quantified via visual comparison with a colored, lead-based standard which produces results that are subjective, hard to reproduce and potentially inaccurate. The lead standard itself is unstable and changes color over time which makes the procedure time-sensitive and makes spike recovery studies and duplicate analyses difficult to perform. The reactions are not element specific unless the elements of interest are separated prior to the sulfide precipitation. Furthermore, the sensitivity of the reaction varies by element and some elements may not react at all [2].

The USP is working to update current protocols to include more modern instrumental methods for elemental analysis. The new protocols outline the use of spectroscopic techniques for determining elemental impurities and require adherence to specific validation procedures to ensure analytical quality control. The validation requirements for analysis by ICP-OES are outlined below.

The instrument must be calibrated at concentrations equal to $0.0J$, $0.1J$ and $2.0J$ where J represents the appropriate limit for each elemental impurity of interest (refer to Tables I and II for elemental impurity limits). The standards must be prepared in a matrix that matches the acid concentration and content of that in the prepared samples. Once calibrated, three check standards must be analyzed at concentrations equal to $0.5J$, $1.0J$ and $1.5J$. The measured concentration of each check standard must be within 80-150% of the true (i.e. known) concentration.

After passing check standard results have been recorded, the precision and accuracy of the method must be determined. Accuracy is determined via spike recovery results of samples spiked at concentrations equal to $0.5J$, $1.0J$ and $1.5J$. Each spiked sample must be prepared in triplicate and the average spike recovery at each concentration must be between 80-150%.

The precision of the method is determined based on six replicate sample preparations, each spiked at a concentration equal to 1.0J. Short-term precision, termed “repeatability,” is calculated based on the %RSD of these six spiked samples, measured as a single, consecutive data set. The calculated RSD value must be less than 20%. Longer-term precision, termed “intermediate precision,” is based on two non-consecutive measurements of the six spiked samples. In order for these measurement sets to be considered non-consecutive, the second analysis must be performed according to one of the three following options: samples can be measured by a different analyst, samples can be measured by the same analyst using a different instrument, or the analysis can be performed on a different day. The %RSD is calculated for the twelve sample results and must be less than 25%.

This work will demonstrate the ability of the Teledyne Leeman Labs *Prodigy High Dispersion ICP* to analyze commercially available cough syrup. The sensitivity and large linear dynamic range of the instrument in the axial view mode will be used to determine a wide range of elements in several different non-prescriptive cough syrup products.

Experimental

A Dual View *Prodigy* High Dispersion Inductively Coupled Plasma (ICP) (Teledyne Leeman Labs, Hudson, NH) equipped with an 88-position autosampler was used to generate the data for this manuscript. The sample introduction system consisted of a four-channel peristaltic pump, glass cyclonic spray chamber with a center knockout tube, a single piece quartz torch and a Conikal concentric nebulizer (Glass Expansion, Pocasset, MA). Instrument conditions used for all data collection are listed in *Table III*.

Parameter	Setting
RF Power	1.3 kW
Coolant Flow	18 L min ⁻¹
Auxiliary Flow	0.0 L min ⁻¹
Nebulizer Pressure	34 psi
Nebulizer Type	Conikal concentric
Spray Chamber	Glass cyclonic with center knockout tube
Torch	Single piece axial
Sample Pump Tubing	Tygon black/black
Sample Uptake Rate	1.0 mL min ⁻¹

Table III. Instrument parameters used for all sample analyses.

All solutions were prepared using >18 MΩ cm² water (Barnstead, Dubuque, IA), reagent grade hydrochloric acid (VWR, West Chester, PA) and reagent grade nitric acid (VWR). Multi-element calibration standards were prepared by dilution from single-element stock solutions (PlasmaPure® Standards, Teledyne Leeman Labs, Hudson, NH) containing 1000 µg mL⁻¹ of As, Cd, Cr, Cu, Hg, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, V and Zn. All dilutions were performed on a w/w basis.

Calibration standards were prepared in two groups to represent the two classes of elemental impurities. For applications in which Class 2 elemental impurities do not need to be quantified, only standards 1-5 need to be prepared. Each group of standards was prepared at concentrations equal to 0.0*J*, 0.1*J*, 0.5*J*, 1.0*J* and 2.0*J* where *J* represents the oral daily dose permissible daily exposure concentration (refer to Table I). All standards were prepared in a matrix of 1% nitric acid and 1% hydrochloric acid. The concentrations used for calibration are listed in *Tables IV and V*.

Element	Std 1 ($\mu\text{g mL}^{-1}$)	Std 2 ($\mu\text{g mL}^{-1}$)	Std 3 ($\mu\text{g mL}^{-1}$)	Std 4 ($\mu\text{g mL}^{-1}$)	Std 5 ($\mu\text{g mL}^{-1}$)
As, Hg	0	0.015	0.075	0.15	0.3
Cd	0	0.005	0.025	0.05	0.1
Pb	0	0.01	0.05	0.1	0.2

Table IV. Calibration standard concentrations for Class 1 elemental impurities, prepared in a 1% nitric and hydrochloric acid matrix.


Element	Std 6 ($\mu\text{g mL}^{-1}$)	Std 7 ($\mu\text{g mL}^{-1}$)	Std 8 ($\mu\text{g mL}^{-1}$)	Std 9 ($\mu\text{g mL}^{-1}$)	Std 10 ($\mu\text{g mL}^{-1}$)
Cr, Mo, Ni, V	0	0.25	1.25	2.5	5.0
Cu, Mn	0	2.5	12.5	25	50
Pd, Pt	0	0.1	0.5	1.0	2.0
Ir, Os, Rh, Ru	0	0.025	0.125	0.25	0.5

Table V. Calibration standard concentrations for Class 2 elemental impurities, prepared in a 1% nitric and hydrochloric acid matrix.

Since all elemental impurities were present in the check standards and spiked samples, an interfering element correction (IEC) factor was required to correct for the interference of Pt on the emission from As at 189 nm. If left uncorrected, the spectral overlap from Pt would produce biased results for As. The interfering element correction factor was calculated using a single element standard containing 20 $\mu\text{g mL}^{-1}$ of Pt. After calibrating the instrument, the Pt solution was analyzed and the measured concentration of As was recorded. The Prodigy software used this concentration to automatically compute and apply the appropriate correction factor.

The analytical method described above was applied to an over the counter cough syrup for the determination of trace elements. The product used was a non-alcoholic cough suppressant containing dextromethorphan HBr and guaifenesin. Fifteen replicate preparations of the cough syrup sample were prepared for analysis, as outlined in USP Chapter <231>. Four of the preparations were spiked with the elements of interest such that the concentrations of the spiked elements were at 0.0*J*, 0.5*J*, 1.0*J*, and 1.5*J*. This set of spiked samples was prepared in triplicate for purposes of calculating an average spike recovery at concentrations equal to 0.5*J*, 1.0*J*, and 1.5*J*.

The remaining three preparations were spiked with all elements of interest such that the concentrations of the spiked elements were at 1.0*J*. These three preparations, along with the three preparations spiked at 1.0*J* described above, were analyzed consecutively to determine the repeatability of the sample preparation procedure.



All samples were prepared for analysis according to the following procedure. Approximately 9 grams of each cough syrup sample was weighed into a 50 mL polypropylene vial with a screw cap top and the exact weight was recorded. To each sample, approximately 20 grams of a 2% nitric acid solution was added. Appropriate aliquots of a multi-element standard containing As, Cd, Cr, Cu, Hg, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, V and Zn were added to twelve of the sample preparations. The other three preparations remained unspiked. All samples were brought to a final mass of 50 grams with a 2% nitric acid solution and the final masses were accurately recorded. All samples were tightly capped and manually shaken for 30 seconds.

Three check standards were prepared at concentrations matching those in standards 4, 5 and 6. The check standards were prepared by dilution on a w/w basis following the procedure outlined above for preparing the calibration standards. Each check standard was prepared in a matrix of 1% nitric and hydrochloric acid to match the acid concentration and content of the calibration standards.

Once the plasma was ignited the instrument was allowed to warm up for 15 minutes before the torch observation height was set using standard 1.5J (see Table IV). Following calibration, a check standard was analyzed to confirm the accuracy of the calibration curves. After the check standard measurement, a study was performed to determine the method detection limits (MDL) for the elements of interest. The MDLs were measured in a prepared cough syrup sample spiked with all elements of interest at concentrations three times the calculated instrument detection limits (IDLs). The MDLs were calculated to be the concentration equal to three times the standard deviation of seven replicate measurements of this spiked sample.

Results and Discussion

Results for the MDL study are reported in Table VI in units of $\mu\text{g mL}^{-1}$. Detection limits are reported for the prepared solution as well as for the original sample after the appropriate dilution factor was corrected for. Results in Table VI indicate that the detection limits are more than sufficient for determining whether the analytes of interest are present at concentrations that exceed the regulatory requirements outlined in Tables I and II on the previous pages.

Element	Wavelength (nm)	MDL ($\mu\text{g mL}^{-1}$)	MDL in Original Sample (μg)
As	193.759	0.006	0.0356
Cd	226.502	0.0001	0.0008
Pb	220.353	0.006	0.0350
Hg	194.227	0.004	0.0206
Cr	267.716	0.001	0.0033
Cu	324.754	0.006	0.0328
Ir	224.268	0.0002	0.0011
Mn	257.610	0.0004	0.0022
Mo	277.540	0.008	0.0433
Ni	231.604	0.001	0.0078
Os	225.585	0.003	0.0178
Pd	340.458	0.005	0.0289
Pt	265.945	0.014	0.0767
Rh	233.477	0.003	0.0178
Ru	240.272	0.003	0.0161
V	292.401	0.001	0.0067

Table VI. Detection limits in a 1% nitric and hydrochloric acid matrix.

Results for the analysis of the cough syrup sample are listed in Table VII. Results are presented for the measured concentrations in the unspiked sample, along with the average spike recovery of each spiked sample that was prepared in triplicate. All results are listed in units of $\mu\text{g g}^{-1}$ and reflect the concentration in the original sample after appropriate weight and volume correction factors were applied.

Element	Avg Measured Conc (μg)	%RSD	Avg Spike Recovery at 0.5J (%)	Avg Spike Recovery at 1.0J (%)	Avg Spike Recovery at 1.5J (%)
As	0.35	3.3	123.4	124.1	125.8
Cd	<0.0008	---	106.6	107.4	103.4
Pb	<0.035	---	103.5	99.8	95.3
Hg	<0.021	---	100.5	104.9	103.7
Cr	<0.003	---	96.2	95.7	94.1
Cu	<0.033	---	83.6	84.0	84.5
Ir	<0.001	---	109.5	112.1	110.0
Mn	0.35	0.1	90.5	93.7	95.7
Mo	<0.04	---	95.2	97.1	96.5
Ni	<0.0078	---	95.6	96.3	94.6
Os	<0.018	---	93.6	96.7	97.2
Pd	<0.029	---	80.9	82.9	82.7
Pt	<0.077	---	86.4	86.8	86.1
Rh	<0.018	---	96.8	98.9	97.8
Ru	<0.016	---	93.0	94.8	93.9
V	<0.007	---	95.5	95.0	93.4

Table VII. Results for cough syrup analysis.

Results for the analysis of six individual sample preparations, all spiked with the elements of interest at concentrations equal to 1.0J, are presented in Table VIII.

Element	Spike Recovery at 1.0J (%), Day 1							Spike Recovery at 1.0J (%), Day 2							Intermediate Precision (n=12)
	Prep 1	Prep 2	Prep 3	Prep 4	Prep 5	Prep 6	%RSD (n=6)	Prep 1	Prep 2	Prep 3	Prep 4	Prep 5	Prep 6	%RSD (n=6)	
As	126.3	126.5	119.5	126.3	126.3	123.8	2.04	125.6	125.8	125.9	125.9	124.3	122.4	1.04	1.69
Cd	111.6	108.3	102.4	99.1	100.5	102.6	4.22	102.2	102.8	105.3	97.9	98.1	97.6	2.92	4.19
Pb	102.5	102.2	94.7	102.4	103.7	103.8	3.08	93.8	95.9	96.2	99.4	98.6	98.8	2.04	3.62
Hg	110.5	105.8	98.5	97.9	96.4	103.8	4.91	102.5	103.4	105.2	98.5	97.7	99.3	2.73	4.19
Cr	97.9	96.7	92.6	94.9	95.5	97.3	1.84	95.0	94.2	93.1	95.2	95.7	94.4	0.87	1.65
Cu	84.9	84.7	82.3	85.0	85.5	86.7	1.56	87.2	84.8	81.5	86.2	86.5	84.8	2.21	2.01
Ir	117.1	113.1	106.0	98.3	97.0	98.7	7.40	107.5	109.7	112.9	98.6	96.7	95.9	6.49	7.31
Mn	95.3	94.8	91.2	93.2	93.3	94.9	1.50	95.4	96.4	95.1	95.1	94.8	93.0	1.07	1.51
Mo	98.5	98.5	94.4	96.8	97.8	99.7	1.73	97.5	96.9	95.1	97.9	98.6	97.3	1.14	1.54
Ni	99.4	97.0	92.3	94.5	94.9	96.4	2.33	94.6	94.6	94.7	94.5	94.5	93.5	0.45	1.91
Os	100.8	97.4	91.9	91.6	90.7	91.6	3.99	98.5	97.3	95.7	94.8	93.7	91.9	2.30	3.47
Pd	83.4	84.1	81.4	76.3	76.9	77.5	3.93	84.7	83.0	80.4	64.9	65.7	63.2	12.5	10.2
Pt	86.3	88.2	85.9	90.2	90.7	92.7	2.72	89.1	86.4	82.9	90.7	92.7	90.7	3.62	3.34
Rh	102.3	99.8	94.7	93.1	91.9	93.0	4.07	97.3	97.6	98.4	93.8	92.0	91.0	3.03	3.77
Ru	97.3	95.8	91.2	92.8	93.3	95.2	2.15	94.4	94.0	93.1	94.0	94.3	93.3	0.52	1.65
V	95.9	96.0	92.9	94.6	95.3	96.4	1.23	95.2	93.7	91.3	94.4	94.9	93.4	1.37	1.55

Table VIII. Results for cough syrup spiked at 1.0J.

Results from the analysis of the check standards prepared at concentrations equal to 0.5J, 1.0J and 1.5J (data not shown) indicate that all analytes were recovered between 80% and 150%, which meets the requirements in the new USP guidelines. The lowest recovered element was Pt which recovered at 88% of the true (i.e. known) concentration in the 0.5J standard. The highest recovered element was Cd which recovered at 105% of the true concentration in the 0.5J standard.

Results in Tables VII and VIII indicate that all elements of interest were recovered well within the 80-150% recovery range outlined by the USP. The RSD values in Table VIII indicate that the repeatability and intermediate precision of the method easily comply with the maximum allowed RSD values of 20% and 25%, respectively. Results also indicate that if regulated under the new USP guidelines for elemental impurities, the sample tested in this work would comply with all regulatory limits.

Conclusions

The determination of sixteen toxic elements in commercially available cough syrup has been presented. The results presented in this application note demonstrate that the Prodigy ICP produces results that meet the proposed requirements outlined in USP Chapter <233>, making the Prodigy a valuable tool for compliance monitoring of pharmaceutical samples. The instrument consistently produces accurate and reliable results, making it an excellent choice for the analysis of pharmaceutical samples on a routine basis.

The Prodigy's high precision, accuracy and versatility derive from its stable, free-running 40 MHz power supply and high sensitivity sample introduction system. In addition, a reliable autosampler provides flexibility and confidence in unattended operation.

The heart of the Prodigy is in its large format programmable array detector (L-PAD) and an advanced high dispersion Echelle spectrometer. The high resolution and dispersion inherent in the design result in a compact optical system that offers outstanding long-term stability. The use of the L-PAD detector allows all the analytes to be measured simultaneously and gives the largest dynamic range with true simultaneous background correction.

References

- (1) DeStefano, A.; Zaidi, K.; Cecil, T.; Giancaspro, G.; USP Elemental Impurities Advisory Panel. (2010) *Pharma. Forum*, **36**, 2-9
- (2) Borer, et al. PharmTech Webcast, June 4, 2009.