Application Note

Pharmaceuticals

Determination of Extractable and Leachable Elements Using ICP-MS

Analysis of elemental impurities from plastic ophthalmic drug containers using an Agilent 7900 ICP-MS

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Introduction

The measurement of extractable and leachable compounds (E&Ls) is an important part of the overall risk assessment that is needed before the launch of a new drug product. E&Ls can enter a drug product during manufacturing, storage, and from the packaging system—also known as the container closure system (CCS). The CCS comprises primary packaging components (those in contact with the drug product, such as vials and vial caps, blister packs, etc.) and secondary packaging. Further components such as labeling and drug delivery components—droppers, dosage measuring spoons, inhalers, syringes, etc.—should also be included in the risk assessment if included with the product (*1*). Some organic and inorganic E&L contaminants present a direct risk due to their inherent toxicity, while other compounds may adversely affect the efficacy, stability, and shelf-life of the drug. An E&L study shows both the potential for the drug product to become contaminated under extreme conditions (extractables), and the actual contamination that occurs during normal and extended storage (leachables).

Extractables are elements and other compounds that could be transferred from the container into the drug product under worst case (extreme) conditions. The extraction approach should replicate the harshest conditions that might occur if, for example, a drug package was left in the sun on the parcel shelf in a car for several hours. Extraction conditions such as high or low pH, raised temperature, or sonication make it more likely that impurities could migrate from the container, potentially contaminating the drug product. Sources of extractables include plastic and elastomeric packaging components (monomers, polymeric initiators, plasticizers, etc.), ink and adhesives used in labels, and degradation products related to container processing, storage, and sterilization.

Leachables are elements and other compounds that migrate from the container into the drug product under normal storage conditions. To measure leachable contaminants, a pre-analyzed drug product is placed in the drug container for a given period under normal ambient conditions. The drug product is then remeasured to assess any changes in the elemental content of the drug material. Extended storage—up to the normal shelf-life of the product—can be simulated by modifying the storage conditions.

Given the diversity of potential impurities in packaged drugs, measuring E&Ls is a complex challenge that requires multiple analytical techniques and produces large amounts of data (*2*). A typical analytical workflow for the analysis of E&Ls is summarized in Figure 1.

Worldwide regulations typically recommend that elemental impurities in pharmaceutical products are analyzed using a multi-element instrumental technique such as ICP-MS or ICP-OES. Both techniques are approved for use in the United States Pharmacopeia and National Formulary (USP–NF) general chapters on the control of elemental impurities in drug products (*3, 4*). USP<232> defines the limits for elemental impurities, and USP<233> defines sample preparation and analysis options, with the use of ICP-MS or ICP-OES recommended. Other atomic spectroscopy techniques can be used if they can be shown to meet the method validation requirements. International guidelines for controlling elemental impurities in pharmaceutical products are closely aligned with the USP methods. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) released equivalent standards defined in ICH guideline Q3D (*5*). The harmonized methods were developed in collaboration with the European, Chinese, and Japanese Pharmacopoeias.

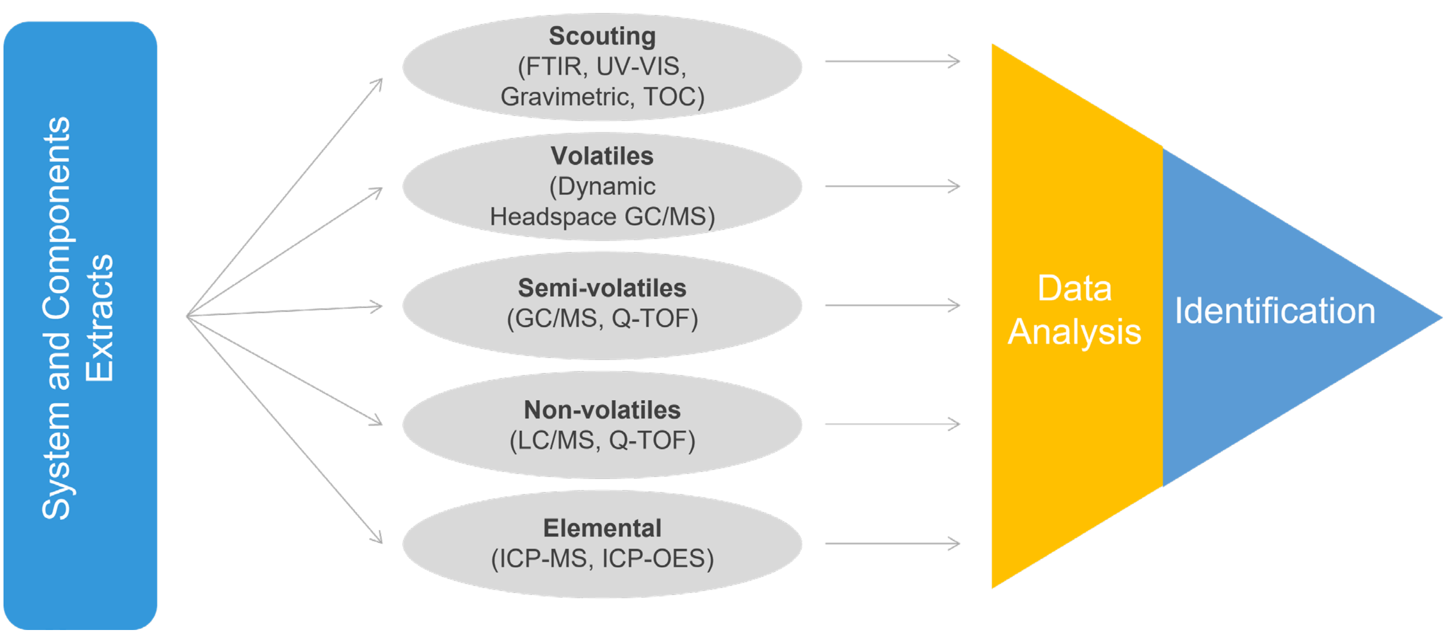

Figure 1. Analytical approaches used for E&L analysis.

The US Food and Drug Administration (FDA) classifies E&L contaminants based on the likelihood of the compound being transferred from the packaging to the drug product, and the level of risk associated with the route of administration. Aerosols and solutions intended for inhalation or injection are considered among the highest risk, while the risk associated with other routes of administration such as oral or topical is lower. There are no separate permitted daily exposure (PDE) limits for ophthalmic drug products, so the FDA recommends that ophthalmic leachables should be managed case by case (*6*). Given the potential for damage to the eye, the FDA suggests that ophthalmic drug products are risk assessed in a similar way to injectable drugs. Following this recommendation, the lower elemental impurity limits that apply to parenteral drugs were used in this work, rather than the higher oral or topical PDEs (*7*). The assessment of elemental impurities, including E&Ls, in ophthalmic solutions is an important application that requires low-level analysis of a range of elemental impurities.

In a previous study, the Agilent 7900 ICP-MS successfully completed the suitability tests for USP <232>/<233> for the analysis of the 24 USP/ICH elements in sterile artificial tear eye drops (SATED) (*8*). The method outlined in that study demonstrates the suitability of the 7900 ICP-MS for the analysis of elemental impurities in the eye drops product. The effect of storage on the level of elemental impurities in the eye drops was also investigated, generating the leachables data included in this work.

In this study, elemental impurities extractable from a low-density polyethylene (LDPE) eye drop container were investigated. The container was treated with various extraction solutions—including an organic solvent, strong acid, and alkali—with and without sonication and heat, as outlined in the workflow in Figure 2. Elements in each of the extraction solutions were quantified using a 7900 ICP-MS. The 7900 uses the ORS^4 collision/reaction cell (CRC) to control the common polyatomic interferences that can affect the measurement of many elements by ICP-MS. The ORS^4 is optimized for removal of polyatomic overlaps using helium (He) collision mode through the physical process of kinetic energy discrimination (KED). He KED uses the same cell settings for all typical analyte elements, providing a simple methodology that delivers the high-quality data sets needed for the routine monitoring of elemental impurities.

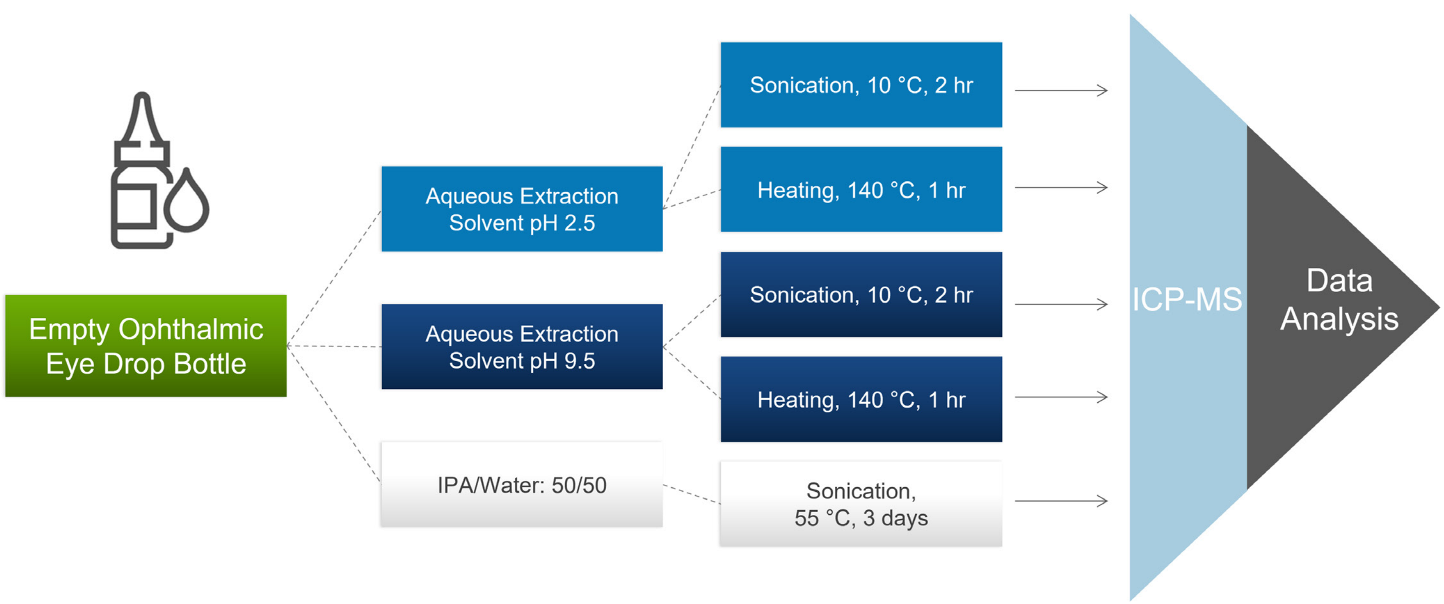

Figure 2. The analytical workflow used to extract elemental impurities from empty plastic ophthalmic eye drop bottles for determination using ICP-MS.

Experimental

The sample preparation procedure was adapted from a method developed for the Product Quality Research Institute (PQRI) Leachables and Extractable Working Group (*9*). PQRI, which includes representatives from the pharmaceutical industry, academia, and regulatory agencies, was established in 1999 to develop regulatory guidance for pharmaceutical analysis. The E&L Working Group's guidance is also recognized by the US FDA. The extraction solvents should cover a wide range of polarity and should mimic the drug product formulation.

Reagents

Ophthalmic eye drops were bought from a local store in Berkeley, California, USA. Because of the large number of elements of interest in the E&L study, calibrations were prepared for a wider range of elements than those listed in the USP/ICH guidance. Agilent Elemental Standard solutions 1–4 (part numbers 8500-6940, 8500-6942, 8500-6944, 8500-6944) and Agilent Environmental Quality Control solution (p/n 5183-4686) were used to prepare calibration and quality control solutions. An Agilent Internal standard (ISTD) mix containing ^{6}Li, ^{45}Sc, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb, and ^{209}Bi was used (p/n 5183-4681). The ISTD mix was diluted to 1 ppm in 2% nitric acid (HNO_3) and added to the extract solutions using the standard online mixing T-connector. Optima grade HNO_3, isopropyl alcohol (IPA), potassium chloride (KCl), 37% hydrochloric acid (HCl), mono- and dibasic hydrogen phosphate ($H_2PO_4^-$, HPO_4^{2-}), and sodium hydroxide (NaOH) were bought from Sigma Aldrich. De-ionized water (DIW, 18 MΩ.cm, EMD Millipore Billerica, MA, USA) was used.

Extraction solutions

Acidic aqueous extraction solution: 1 M KCl and 37% HCl stock solutions were prepared and diluted by mass to produce an extraction solution with a final concentration of 0.01 M KCl and 0.003 M HCl. The pH of the solution was 2.29.

Basic aqueous extraction solution: a solution of 0.0045 and 0.007 M concentrations of monobasic and dibasic sodium phosphate salts, respectively was prepared by mass in DIW. The solution was titrated with 1 M NaOH to a final pH of 9.47.

Polar extraction solution: IPA was added to DIW 1:1 (v:v).

Standards, quality control, and sample preparation

Calibration standards were prepared by mass from the standard stock solution serially diluted into the appropriate aqueous or organic diluent solution. The diluent solutions were 5% HNO_3 in DIW for aqueous (acidic) extractions and 5% HNO_3/5% IPA in DIW for IPA extractions. Standards were prepared from 0.01–10 ppb for all elements.

Two quality control (QC) solutions were prepared at 0.5 and 5 ppb together with a blank solution. To meet Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) requirements, each QC solution was measured every 10 samples.

The extract solutions were diluted by a factor of 1:10 with the appropriate diluent and analyzed in triplicate. Also, aliquots of the acidic (pH 2.29) and organic (IPA) extracts were spiked with 0.1 ppb QC standard for further validation of each data set.

Instrumentation

The Agilent 7900 ICP-MS includes a glass concentric nebulizer, quartz double-pass spray chamber, Ultra High Matrix Introduction (UHMI) system, 2.5 mm injector quartz torch, Ni interface cones, and ORS^4 cell as standard. An Agilent SPS 4 autosampler was used for sample introduction. The 7900 settings for the sample introduction system, ion lens voltages, and detector were automatically optimized using the Agilent ICP-MS MassHunter software autotuning functions. Typical instrument operating parameters are given in Table 1. For data acquisition settings, the preset method 'USP<232>/<233> Elemental Impurities in pharma products' was used. This analysis was run on the Agilent 7900 ICP-MS, but the method is also compatible with the Agilent 7850 ICP-MS.

Table 1. Typical Agilent ICP-MS operating conditions.

Parameter	Setting
RF Power (W)	1550
Sampling Depth (mm)	10
Nebulizer Gas Flow (L/min)	1.05
Lens Tune	Autotune
He Cell Gas (mL/min)	5.0
KED (V)	5

Results and discussion

Calibration curves were generated for all elements. Example 7900 ICP-MS calibration curves for As, Cd, Hg, and Pb are shown in Figure 3.

The Limits of Detection (LODs) and Limits of Quantitation (LOQs) given in Table 2 were calculated from the standard deviation (SD) of seven blank runs (LOD = 3.14 x SD; LOQ = 10 x SD).

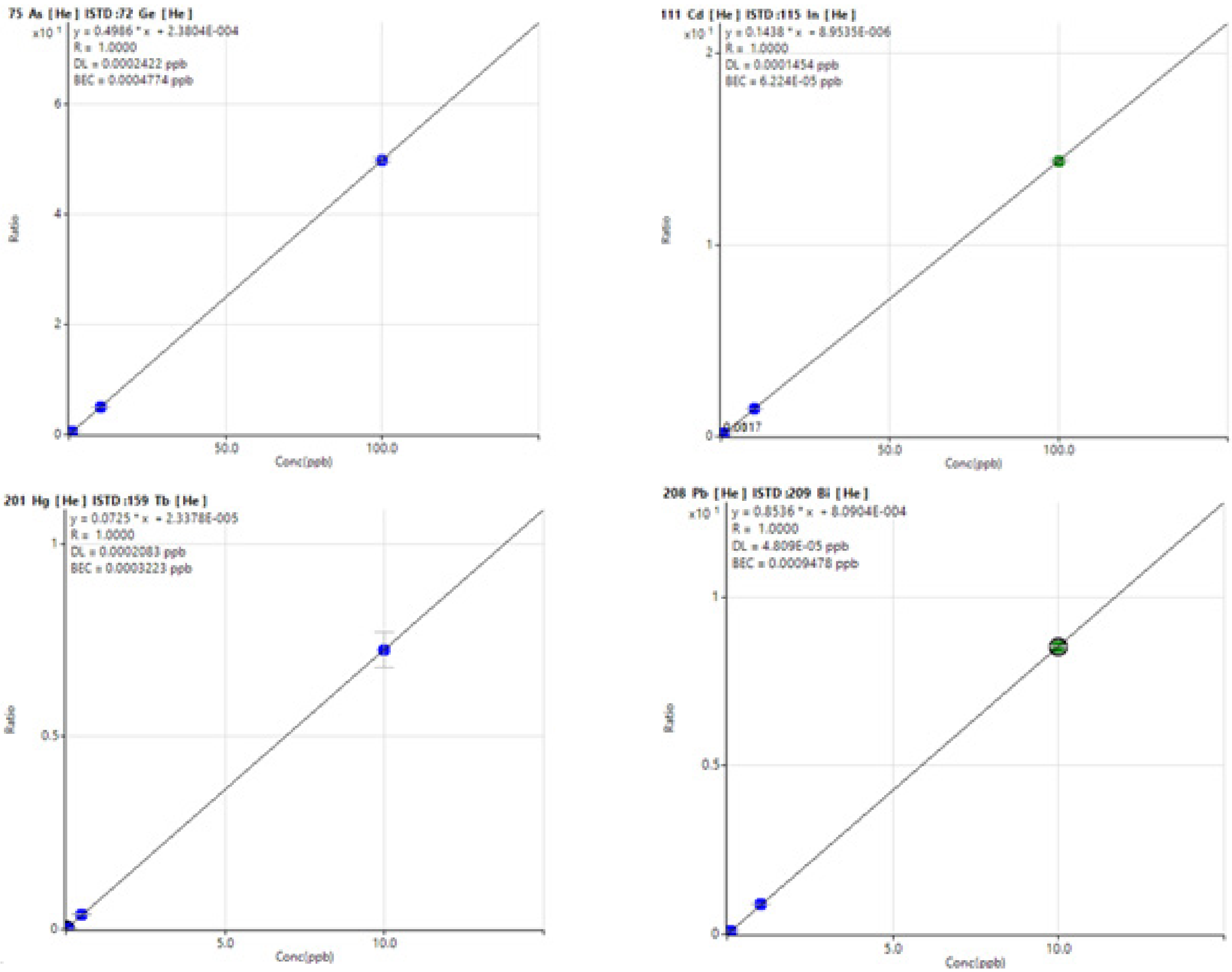

Figure 3. Representative calibration curves for As, Cd, Hg, and Pb, showing extremely low, sub-ppt instrument DLs and good linearity (R = 1.0000) across the calibration range.

Ophthalmic solutions do not have designated PDEs set by USP and Q3D so parenteral PDEs were used (*7*). Using a daily dose of 5 g/day, the J values for some elements were calculated, and are shown in Table 2. The J value is the PDE limit value converted to a concentration in solution taking into account the sample dilution and the daily dosage, as described in a previous publication (*10*).

To monitor the precision and accuracy of each analytical run, a set of QC solutions consisting of CCB, CCVs (low-level (0.5 µg/kg) and mid-level (5.0 µg/kg)) run every 10 samples, and spike samples (level (1.0 µg/kg)). Sample spike and QC recoveries were almost all within ± 20%, as shown in Table 2.

Table 2. Parenteral daily exposure limits for SATED, J values based on 5 g/day daily dose and a dilution of 50x, limits of detection (LOD), limits of quantification (LOQ), and CCV mean recovery (1 µg/kg), n=4; low- and mid-level QCs (0.5 µg/kg and 5.0 µg/kg, respectively, except where indicated), n=5 each; and spike recovery data (1.0 µg/kg), n=3.

ICH/USP Class	Element	Parenteral PDE, µg/day	J Value (µg/L)	LOD	LOQ	CCV Mean Recovery (%)	Low-Level QC Mean Recovery (%)	Mid-Level QC Mean Recovery (%)	Spike Mean Recovery (%)
				(µg/L)	(µg/L)				
Class 1	111 Cd	2	8	0.0001	0.0002	100	104	104	106
	208 Pb	5	20	0.0002	0.0005	101	103	105	110
	75 As	15	60	0.0003	0.0011	100	104	105	104
	201 Hg	3	12	0.0009	0.0172	72	*100*	*99*	*104*
Class 2A	59 Co	5	20	0.001	0.0193	100	102	104	103
	51 V	10	40	0.0002	0.0005	99	103	103	*104*
	60 Ni	20	80	0.0009	0.003	98	105	106	*100*
Class 2B	205 Tl	8	32	*0.0107*	*0.0340*	103	100	103	*103*
	107 Ag	10	40	*0.0179*	*0.0571*	83	*93*	*91*	*99*
	78 Se	80	320	*0.0193*	*0.0613*	73	100	100	110
	197 Au	100	400	*0.9631*	*3.0672*	104	*93*	*96*	*98*
	105 Pd	10	40	*0.1176*	*0.3746*	95	*95*	*94*	*97*
	193 Ir	10	40	*0.0463*	*0.1475*	98	*95*	*96*	*99*
	189 Os	10	40	*0.0311*	*0.0991*	*99*	*100*	*97*	*102*
	103 Rh	10	40	*0.0047*	*0.0149*	98	*93*	*96*	*99*
	101 Ru	10	40	*0.0203*	*0.0648*	96	*95*	*96*	*98*
	195 Pt	10	40	*0.0096*	*0.0305*	95	*97*	*95*	*99*
Class 3	7 Li	250	1000	0.0194	0.0619	88	*98*	*90*	*97*
	121 Sb	90	360	0.0002	0.0005	103	101	101	*103*
	137 Ba	700	2800	0.0005	0.0014	100	104	104	105
	95 Mo	1500	6000	0.0002	0.0005	81	107	106	79
	63 Cu	300	1200	*0.4245*	*1.3520*	105	99	102	*99*
	118 Sn	600	2400	0.0004	0.0012	102	*97*	*101*	*104*
	52 Cr	1100	4400	0.0011	0.0034	100	103	104	100
Other	24 Mg			0.0081	0.0258	105	97	100	115
	27 Al			0.0145	0.0462	110	91	96	105
	47 Ti			0.0078	0.0249	84			
	55 Mn			0.0017	0.0056	106	97	98	102
	56 Fe			0.0035	0.0113	106	100	102	99
	66 Zn			0.0033	0.0105	103	99	101	*
	71 Ga			0.0005	0.0016	106			109
	85 Rb			0.0007	0.0021	106			94
	88 Sr			0.0002	0.0006	105			117
	90 Zr			0.0001	0.0003	83			
	93 Nb			0.0001	0.0003	83			
	133 Cs			0.0004	0.0012	104			
	181 Ta			0.0007	0.0023	79			
	182 W			0.0002	0.0006	80			
	185 Re			0	0.0002	81			
	238 U			0	0.0001	88	104	108	93

Data in italics: concentrations in the low and medium level QCs were at 0.5J and 1.5J, respectively. Spike level was at 1J.
**Spike concentration was too low compared the native level in solution.*

Leachable elemental contaminants

To illustrate the potential for leachable contaminants to be transferred into the eye drop solution from the plastic container bottle, the eye drops were analyzed as received (no treatment). To simulate extended storage conditions, the eye drops were also analyzed after a short period of heating to 120 °C, and after simulated long-term storage (sonication at 55 °C for three days). The results are presented in Table 3. None of the levels of elements leached from the eye drop container caused the impurity levels to exceed the J values based on the PDE for the eye drops.

Table 3. Quantitative results for leachable elements measured in SATED after different storage times and conditions in plastic ophthalmic eye drop bottle.

Element	J Value for SATED (µg/L)	Eye Drops as Supplied (µg/L)	After Heating to 120 °C (µg/L)	After Sonication at 55 °C for 3 Days (µg/L)
7 Li	1000	<LOQ	<LOQ	1.65 ± 0.13
24 Mg	–	0.17 ± 0.19	0.10 ± 0.08	<LOQ
27 Al	–	<LOQ	<LOQ	<LOQ
47 Ti	–	0.27 ± 0.20	0.68 ± 0.22	<LOQ
51 V	40	<LOQ	<LOQ	<LOQ
52 Cr	4400	<LOQ	<LOQ	<LOQ
55 Mn	–	<LOQ	<LOQ	0.08 ± 0.01
56 Fe	–	<LOQ	0.15 ± 0.05	0.24 ± 0.30
59 Co	20	<LOQ	<LOQ	<LOQ
60 Ni	80	<LOQ	0.28 ± 0.03	<LOQ
66 Zn	–	<LOQ	84.47 ± 15.73	<LOQ
71 Ga	–	<LOQ	<LOQ	0.04 ± 0.01
75 As	60	<LOQ	<LOQ	<LOQ
78 Se	320	<LOQ	<LOQ	<LOQ
85 Rb	–	0.0934 ± 0.046	0.3656 ± 0.0191	0.1541 ± 0.0608
88 Sr	–	<LOQ	<LOQ	0.0446 ± 0.0529
90 Zr	–	0.0369 ± 0.023	0.0211 ± 0.0066	0.0163 ± 0.0085
93 Nb	–	0.0012 ± 0.001	0.0011 ± 0.0009	<LOQ
95 Mo	6000	<LOQ	<LOQ	<LOQ
111 Cd	8	<LOQ	<LOQ	<LOQ
118 Sn	2400	<LOQ	<LOQ	<LOQ
121 Sb	360	<LOQ	<LOQ	0.0016 ± 0.0009
133 Cs	–	0.0195 ± 0.003	0.0227 ± 0.0021	0.0501 ± 0.0385
137 Ba	2800	<LOQ	<LOQ	<LOQ
181 Ta	–	0.0049 ± 0.002	<LOQ	<LOQ
182 W	–	0.0110 ± 0.003	0.0118 ± 0.0004	0.0175 ± 0.0044
185 Re	–	0.0019 ± 0.000	<LOQ	<LOQ
201 Hg	12	0.01 ± 0.00	<LOQ	<LOQ
208 Pb	20	<LOQ	0.0151 ± 0.0038	<LOQ
238 U	–	0.0054 ± 0.002	0.0049 ± 0.0003	0.0052 ± 0.0008

Extractable elemental contaminants

Each combination of extraction solutions and conditions produced a unique profile of extracted elements, as shown in Table 4. The results confirm that improper storage conditions can affect drug contamination, potentially compromising consumer safety. Many elements were below the LOQ, but some contaminants were detected under one of more of the extraction conditions, including Ni, Zn, Rb, Zr, Nb, Cs, W, and U.

The results show that heat has a significant impact on the level of contamination, including for Fe (under alkaline conditions), Zn, Sr, and Ba. While all concentrations were below USP <232> exposure limits, these findings still warrant public health consideration, especially as Zn and Fe have been implicated in the development of cataracts (*11, 12*).

Table 4. Concentrations of elements where significant differences were observed for the different extraction solutions and conditions (µg/kg, n=3).

	pH 2.5		pH 9.5		IPA/Water
Element	**Sonicated**	**Heated**	**Sonicated**	**Heated**	**Sonicated**
7 Li	<LOQ	<LOQ	0.07 ± 0.12	0.15 ± 0.12	<LOQ
24 Mg	<LOQ	<LOQ	0.1171 ± 0.1224	0.5128 ± 0.1241	<LOQ
27 Al	<LOQ	<LOQ	0.9226 ± 0.8848	1.7875 ± 0.1769	<LOQ
47 Ti	<LOQ	<LOQ	<LOQ	1.1500 ± 0.2356	<LOQ
51 V	<LOQ	<LOQ	<LOQ	0.0101 ± 0.0003	<LOQ
52 Cr	<LOQ	<LOQ	<LOQ	0.0825 ± 0.0071	<LOQ
55 Mn	<LOQ	<LOQ	0.0266 ± 0.0075	0.0567 ± 0.0033	<LOQ
56 Fe	<LOQ	<LOQ	0.0840 ± 0.1787	0.9264 ± 0.3489	<LOQ
59 Co	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
60 Ni	0.1798 ± 0.004	0.2162 ± 0.0236	0.1088 ± 0.1011	0.0727 ± 0.0573	<LOQ
66 Zn	0.3521 ± 0.119	106.4095 ± 17.9431	0.8106 ± 0.8306	80.3392 ± 16.7258	<LOQ
71 Ga	<LOQ	<LOQ	0.0043 ± 0.0056	0.0106 ± 0.0032	0.0122 ± 0.0062
75 As	<LOQ	<LOQ	<LOQ	0.0061 ± 0.0015	<LOQ
80 Se	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
85 Rb	0.0934 ± 0.046	0.3656 ± 0.0191	0.1541 ± 0.0608	0.1952 ± 0.0353	0.1048 ± 0.0219
88 Sr	<LOQ	<LOQ	0.0446 ± 0.0529	0.2363 ± 0.0307	<LOQ
90 Zr	0.0369 ± 0.023	0.0211 ± 0.0066	0.0163 ± 0.0085	0.0401 ± 0.0038	0.0242 ± 0.0016
93 Nb	0.0012 ± 0.001	0.0011 ± 0.0009	<LOQ	0.0007 ± 0.0003	0.0028 ± 0.0005
95 Mo	<LOQ	<LOQ	<LOQ	0.0157 ± 0.0057	<LOQ
111 Cd	<LOQ	<LOQ	<LOQ	0.0018 ± 0.0006	<LOQ
119 Sn	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
121 Sb	<LOQ	<LOQ	0.0016 ± 0.0009	0.0044 ± 0.0015	<LOQ
133 Cs	0.0195 ± 0.003	0.0227 ± 0.0021	0.0501 ± 0.0385	0.0657 ± 0.0187	0.0195 ± 0.0271
137 Ba	<LOQ	<LOQ	<LOQ	0.3391 ± 0.0175	<LOQ
181 Ta	0.0049 ± 0.002	<LOQ	<LOQ	<LOQ	<LOQ
182 W	0.0110 ± 0.003	0.0118 ± 0.0004	0.0175 ± 0.0044	0.0176 ± 0.0033	0.0228 ± 0.0078
185 Re	0.0019 ± 0.000	<LOQ	<LOQ	<LOQ	0.0165 ± 0.0013
201 Hg	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
208 Pb	<LOQ	0.0151 ± 0.0038	<LOQ	0.0117 ± 0.0095	<LOQ
238 U	0.0054 ± 0.002	0.0049 ± 0.0003	0.0052 ± 0.0008	0.0054 ± 0.0009	<LOQ

Conclusion

An Agilent 7900 ICP-MS was used to analyze elemental impurities extracted from a plastic ophthalmic drug bottle under various stressed conditions. Extraction conditions included raised temperature, high and low pH, organic solvent, and extended time. Some of the elements detected in the extraction solvents were of concern for ophthalmic drugs, in particular iron and zinc. Even at trace levels, Fe and Zn have been implicated in the development of cataracts. Further studies are needed to determine safe elemental exposure limits for ocular medicines.

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