WHITE PAPER

Implementation of USP New Chapters <232> and <233> on Elemental Impurities in Pharmaceutical Products

Author

PerkinElmer, Inc.
Shelton, CT

Introduction

For more than 100 years, the standard method for measuring elemental impurities in pharmaceutical products sold in the United States has been the "Heavy Metals Test," described in Chapter 231 of the United States Pharmacopeia's (USP) National Formulary (NF).[1] This test is based on a sulfide precipitation of the analyte elements with a thioacetamide (C_2H_5NS) solution, and assumes that all analytes behave in a similar manner to a lead standard with which samples are compared. When the USP heavy metals method was first published, it was only intended as a screening tool with results being reported as < 10 ppm Pb. Additionally, although USP Chapter <231> is listed as a "Heavy Metals Test," it was initially intended to detect a larger group of elements like Pb, Hg, Bi, As, Sb, Sn, Cd, Ag, Cu, Mo, and Se, but there was no clear definition of which individual elements the method was expected to detect.

One of the many limitations of this approach is the assumption that the reaction mechanism for the formation of the sulfides in the sample is very similar to the formation of lead sulfide in the standard solution and is not impacted significantly by the sample matrix. However, since many metals' sulfides can form colloids, which behave very differently to solutions, the method requires that the visual comparison is performed in a relatively short period of time (< 5 mins.) after the precipitate has formed but before the sample starts to become unstable. The problem is that different analysts can differ in their interpretation of a result by how they perform the visual comparison, and it is fairly typical that inexperienced analysts may not understand the subtleties of how to accurately and consistently read the sample and standard solutions each time.

Another limitation of the technique is that ~ 2 g of sample is required in order to achieve the desired detection capability. Such a large sample weight is often difficult to acquire at the early stages of drug development due to the very limited supply. This is additionally compounded by the sample preparation procedure, involving ashing at 600 °C and acid dissolution of the sample residue, which is notoriously prone to sample losses. In fact, some studies have shown that up to 50% of the metals may be lost during the ashing process, particularly the volatile elements like selenium (Se) and mercury (Hg). The loss of metals is also matrix-dependent, and because the procedures are time-consuming and labor-intensive, recoveries can vary significantly among differing analysts.

Expert Committee Findings

The general consensus by a panel of experts in a 2008 workshop organized by the Institute of Medicine (IOM) was that the current methodology for metals testing was inadequate and should be replaced by instrumental methods of greater specificity and sensitivity for a wide range of metals of interest. The challenge, however, was finding a suitable analytical method and combining it with risk assessment studies to get a better understanding of what metals have a negative impact on public health. Due to known toxicity effects and the potential for contamination in pharmaceutical ingredients, there was agreement that lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) would need to be measured at toxicologically-relevant concentrations. In addition, metal catalysts such as the platinum group metals (PGMs) – platinum (Pt), palladium (Pd), ruthenium (Ru), rhodium (Rh), and rubidium (Rb) – used in the production of many pharmaceuticals, should be included based on the likelihood of them being present. Also, a wider range of metals used as organometallic reagents were used in the manufacturing process and therefore at risk of being present. An important consideration was the form of the metal, particularly with arsenic and mercury. For example, dietary supplements that contain kelp and other natural constituents have very high concentrations of organic arsenic, which is relatively harmless compared to the highly toxic inorganic form of the element. Similarly, metallic mercury is relatively innocuous, whereas methyl mercury is highly toxic and is known to be concentrated in some fish.

PerkinElmer®
For the Better

Chapters <232> and <233>

The workshop participants came away with a clear need for a revision of USP <231>, not only for selecting which metals needed to be tested, but also for setting the toxicity limits for each of the metals. They also wanted to ensure there was harmony across the major Pharmacopeias, such as the European and Japanese Pharmacopeias, because of the need for a set of global standards for the industry. They proposed the formation of an Expert Committee on Elemental Impurities to initiate a public process, where various stakeholders, including the general public, pharmaceutical industry, academia and regulatory agencies, would be asked for their input at each step of the process. For the next four years, this USP committee had regular meetings and workshops, where it received input and feedback from all the interested stakeholders. Based on this lengthy process, two new Chapters – <232> and <233> – were eventually finalized in April of 2012. Chapter <232> specifies the list of elements and their toxicity limits, defined as maximum daily doses of different drug categories, such as oral, parenteral (intravenous injection), inhalation and large volume parenteral,[2] while Chapter <233> deals with the analytical procedure, sample preparation and instrumental method for measuring the elements, including the choice of two plasma-based spectrochemical techniques – inductively coupled plasma atomic emission spectroscopy (ICP-AES or ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).[3] General Chapters <232> and <233> became official February 1, 2013 in the Second Supplement to USP 35–NF 30. Until General Notices 5.60.30 Elemental Impurities in USP Drug Products and Dietary Supplements becomes official on January 1, 2018, however these General Chapters would be applicable only if they are referenced in a particular monograph. It is important to note that revisions to General Chapters <232> and <233> are proposed in Pharmacopeial Forum 40(2) [March-April 2014. General Chapters <232> and <233> will remain official in their current form until the revisions published in PF 40(2) become official, or are themselves updated to reflect the limits published in the ICH Q3D step 4 document. On 14th Jan 2015 the USP issued notice of intent to remove General Chapter <231> and its references on January 1st 2018 to align with the applicability of General Chapters <232> and <2232>. This means all interested stakeholders will have 18 months to change their analytical methodology for carrying out the determination of elemental impurities to be consistent with the limits and procedures described in these two new chapters.

Let's take a brief look at these two new chapters to get a better understanding of how ICP-AES/OES and ICP-MS can be used to achieve the desired limits in a range of pharmaceutical products.

Chapter <232> Elemental Impurities – Toxicity Limits

Table 1 shows the maximum permissible daily exposure (PDE) values (in µg/day) for the administration of drug products based on an "average" 50 Kg person. The toxicity of an elemental impurity is related to its bioavailability. The extent of "chronic" exposure has been determined for each of the elemental impurities of interest for three routes of administration: orally, intravenously (parenteral) and inhalational. When the daily dose of an injection is > 100 mL, the amount of element must be controlled through the individual components used to produce the drug product. This is known as the large volume parenteral (LVP) component limit (in µg/g) and is shown in the last column of Table 1. It should also be noted that the other two routes of administration – mucosal (nose) and topical (skin) – are considered to be the same as the oral PDE except where otherwise stated in the individual monograph. For more detailed information about these delivery techniques, refer to Chapter <1151> Pharmaceutical Dosage Forms – Routes of Administration, described in the USP National Formulary (NF).[4]

Table 1. Maximum permissible daily exposure (PDE) values (in µg/day) for the administration of drug products, based on an "average" 50 Kg person

Element	Oral Daily Dose PDE (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalation Daily Dose PDE (µg/day)	LVP Component Limit (µg/g)
Cadmium	25	2.5	1.5	0.25
Lead	5.0	5.0	5.0	0.5
Inorganic Arsenic	1.5	1.5	1.5	0.15
Inorganic Mercury	15	1.5	1.2	0.15
Iridium	100	10	1.5	1.0
Osmium	100	10	1.5	1.0
Palladium	100	10	1.5	1.0
Platinum	100	10	1.5	1.0
Rhodium	100	10	1.5	1.0
Ruthenium	100	10	1.5	1.0
Chromium	*	*	25	*
Molybdenum	100	10	10	1.0
Nickel	500	50	1.5	5.0
Vanadium	100	10	30	1.0
Copper	1000	100	100	10

Note: * = Not a safety concern

The arsenic limits are based on the inorganic (most toxic) form. Arsenic can be measured using a total-arsenic procedure under the assumption that all arsenic contained in the material under test is in the inorganic form. Where the limit is exceeded using a total arsenic procedure, it may be possible to show via a procedure that can determine the different forms (species), such as HPLC coupled to ICP-MS, that the inorganic form meets the specification. The mercury limits are based on the inorganic Hg^{2+} oxidation state, as studies have shown methyl mercury (CH_3Hg), the most toxic form, is rarely found in pharmaceutical products.

This chapter also defines the maximum limit of the elemental impurities based on the materials' final use. This is to ensure the drug manufacturers determine an acceptable level of impurity in the drug compound and excipient material (filler) used to produce the final product. A table is included, listing the limits of oral-, parenteral- and inhalational-administered drugs based on a maximum daily dose of ≤10 g/day.

Chapter <233> Elemental Impurities – Procedures

This chapter describes two analytical procedures with associated sample preparation steps for the determination of the elemental impurities in the drug products described earlier. The chapter also describes criteria for any alternative procedure that can be used as long as it meets the necessary validation requirements. The first analytical procedure describes an ICP-AES/OES method, while the second one describes an ICP-MS method. Let's first take a closer look at the sample preparation section as it is applicable to both techniques.

Sample Preparation can be approached in four different ways:

- Use neat, undiluted sample, if in suitable liquid form
- Dilute in aqueous solution, if soluble in water
- If not soluble in water, dilute in appropriate organic solvent
- Use closed-vessel microwave acid digestion for insoluble samples – an example procedure is given

The ICP-AES/OES and ICP-MS analytical instrumental procedures are very generic in nature, with no details about instrumental parameters or the best wavelengths (ICP-AES/OES) or masses (ICP-MS) to use. They basically include a number of QC/QA protocols to ensure the method is working correctly. Let's take a brief look at the main points of each procedure:

Procedure 1: ICP-AES/OES

- Two calibration standards are required – a high standard at 2x target limits and a low standard at ½ target limits
- Target limits are defined as the acceptance value for the elemental impurity being evaluated, based on the frequency of taking/administering the drug
- Matrix-match standards to samples – dissolution method should be the same for calibration standards and samples
- Dilute sample solution so concentration does not exceed 2x target limits
- As a signal stability check, run ½ target limits standard before and after analyses of sample solutions – drift should not be greater than ± 20%
- Analyze according to manufacturer's suggestions for instrumental conditions and wavelengths, making sure to correct for any spectral overlaps
- More detailed information can be found in Chapter <730>, the USP Plasma Spectrochemistry Method[5]

Procedure 2: ICP-MS

- Two calibration standards are required – a high standard at 2x target limits and a low standard at ½ target limits
- Target limits are defined as the acceptance value for the elemental impurity being evaluated, based on the frequency of taking/administering the drug
- Matrix-match standards to samples – dissolution method should be the same for calibration standards and samples
- Dilute sample solution so concentration does not exceed 2x target limits
- As a signal stability check, run ½ target limits standard before and after analyses of sample solutions – drift should not be greater than ± 20%
- Analyze according to manufacturer's suggestions for instrumental conditions and analyte masses, taking appropriate measures to correct for matrix-induced polyatomic interferences, such as $^{40}Ar^{35}Cl$ on ^{75}As
- A collision/reaction cell may be beneficial to reduce the polyatomic spectral interferences
- More detailed information can be found in Chapter <730>, the USP Plasma Spectrochemistry Method[5]

Alternative Procedure

If a specified procedure does not meet the needs of a specific application, an alternative procedure may be used. However, any alternative procedure must be fully validated and must be acceptable and equivalent to the procedure for the purpose of this test. For example, if ICP-AES/OES or ICP-MS is not available, another atomic spectroscopic technique, such as flame or graphite furnace atomic absorption (AA); can be used instead, as long as it meets all the specificity, accuracy, precision, repeatability, linear-range, and detection-capability performance requirements. Full details of the validation process are given in Chapter <1125>, Validation of Compendial Procedures.[6]

Let's now take a look at PerkinElmer's suggested plasma-based instrumental solutions and microwave digestion system for the implementation of these two new USP chapters.

Optima 8x00 Series ICP-AES/OES Instruments

As an appropriate multi-element technique, ICP-AES/OES can achieve most of the PDE limits for the platinum group elements and transition metals in the list of elemental impurities shown in Table 1. However, the kind of sample preparation technique required for the drug product can be a factor in meeting the necessary limits for some of the elements. If the mode of delivery is in a liquid form, which requires minimal dilution, the majority of the elemental limits can be achieved. Alternatively, if any significant sample dilution is needed, either by simple dilution or closed-vessel digestion, the limits could be difficult to reach on a routine basis.

With regard to the toxic suite of elements (Cd, Pb, As and Hg), which are mostly specified at much lower levels than the other metals, it could be more challenging to determine them with good accuracy and precision using ICP-AES/OES. However, ICP-AES/OES detection capability for As and Hg can be improved quite significantly using a hydride generation/cold vapor sampling accessory, so even if the sample preparation step requires a 100-fold dilution, even the lowest large volume parenterals (LVP) limits can be achieved for these two analytes. The bottom line is that depending on the element and the mode of administering the drug, careful evaluation of the ICP-AES/OES technique is needed before selecting it for this analytical procedure.

With the lowest detection limits of any ICP-OES and a full suite of enhanced data security features, PerkinElmer's Optima® 8x00 series ICP-OES makes it easy to comply with stringent regulatory requirements. Dual viewing of the plasma allows the Optima 8x00 ICP-OES to provide a wide calibration range for enhanced productivity. Added features, such as superior interference correction and patented Flat Plate™ plasma technology make the Optima 8x00 ICP-OES easy to use and easy to maintain.

NexION 350 Series ICP Mass Spectrometers

There is no question that ICP-MS is the most suitable multielement technique for determining elemental impurities at these levels in pharmaceutical products. The desired limits, even for the large volume parenterals (LVP), which are the lowest specifications of all the different drug delivery methods, can be reached with relative ease. The LVP values and levels following a sample preparation method involving 0.2 g of sample made up with 100 mL of solvent are listed in Table 2. It can be seen that the PerkinElmer NexION® 350X ICP-MS detection capability is approximately 2-5 orders of magnitude lower than these, depending on the element of interest. The added benefit of ICP-MS for this application is that it can be seamlessly coupled to a liquid chromatographic (LC) separation system to determine the different forms of arsenic and mercury, if required.

Figure 1. PerkinElmer Optima 8300 ICP Optical Emission Spectrometer.

Figure 2. PerkinElmer NexION 350 ICP Mass Spectrometer.

Table 2. NexION 350X detection capability compared with large volume parenteral (LVP) component limits and levels (in ppb) after a sample preparation method of 0.2 g of sample dissolved 100 mL of solvent

Element	LVP Component Limit (µg/g)	Level (ppb) Based on Sample Prep of 0.2 g/100 mL (500-fold dilution)	NexION 350 ICP-MS Detection Capability Lower than LVP Limit
Cadmium	0.25	0.5	10^2
Lead	0.5	1.0	10^3
Inorganic Arsenic	0.15	0.3	10^2
Inorganic Mercury	0.15	0.3	10^3
Iridium	1.0	2.0	10^3
Osmium	1.0	2.0	10^3
Palladium	1.0	2.0	10^3
Platinum	1.0	2.0	10^3
Rhodium	1.0	2.0	10^4
Ruthenium	1.0	2.0	10^4
Molybdenum	1.0	2.0	10^3
Nickel	5.0	10	10^3
Vanadium	1.0	2.0	10^2
Copper	10	20	10^5

It should be noted that even though there are four different models of NexION 350 ICP mass spectrometers, the configuration recommended for this application is the NexION 350X, which includes a single-channel universal collision/reaction cell. This enables the instrument to be used in either the Collision (KED) mode or the Reaction (DRC) mode using one cell gas, in addition to the Standard/normal ICP-MS mode. In fact, the detection limits in Table 2 were carried out by a combination of Collision mode and Standard mode. The Collision mode was used for elements like arsenic, which have the potential to be negatively impacted by the argon-chloride (ArCl) interference in a sample digested/diluted with hydrochloric acid, while the elements like the PGM's, which are known to be free of polyatomic spectral interferences, were determined using the Standard mode.

Additionally, one of the many unique features of the NexION's Universal Cell Technology™ is the capability known as Extended Dynamic Range (EDR) – this patented feature is very useful if the requirement is for both trace metal impurities and major nutritional elements in pharmaceutical or nutraceutical products. With EDR, the dynamic range can be extended for elements which are present at high concentrations. This means that for the analysis of dietary supplements, the nutritional elements like Ca, Mg, Na and K can be determined in the same multielement method as the suite of toxic contaminants (Cd, Pb, As, Hg) described in the proposed new Chapter <2232>, Elemental Contamination of Dietary Supplements, which is still in the review/comments stage.[7]

Another advantage of using NexION 350 ICP-MS technology for this application is the extremely good, long-term signal stability. The patented Triple Cone Interface translates into a well-defined ion beam, providing less dispersion of the ions, therefore preventing deposition on internal components. When combined with the novel Quadrupole Ion Deflector ion optics, which ensures particulate and neutral species never enter the Universal Cell or mass analyzer, the result is unsurpassed stability with real-world samples.

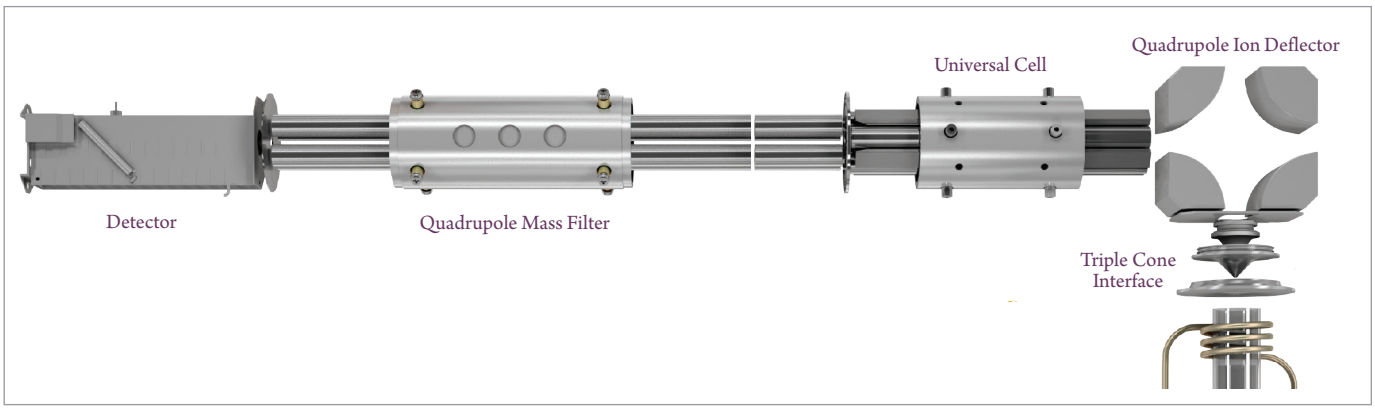

Figure 3. NexION 350 ICP-MS ion optics.

With NexION's design, no matrix particulates enter the mass spectrometer, dramatically reducing routine maintenance. The only components that need cleaning are the interface cones. When compared with other systems, which require tedious and time-consuming cleaning of the ion lens, cell and cones, the NexION 350 ICP-MS is ideally suited to the demands of the high-throughput QC pharmaceutical lab. And to keep the system running at peak performance, NexION ICP-MS Software provides alarms that can be set to remind the operator when it's time for the few preventative maintenance tasks required, such as roughing pump oil changes and tubing replacement. The system will even display how many hours various components have been used and when they might need attention. There is no question that the design of the interface and ion optic region on the NexION 350 ICP-MS is a direct result of PerkinElmer's proven experience in the development of ICP-MS instrumentation for real-world applications over the past 25 years.

Microwave Digestion

PerkinElmer also offers the Titan MPS™ Microwave Sample Preparation System, capable of the high-performance closed-vessel digestion required by USP <233>. Using the unique DTC™ and DPC™ contact and connection-free sensing technologies, the Titan MPS system accurately monitors the sample temperature in each digestion vessel to provide outstanding reaction control and deliver consistent digestion results. The TFM™ vessels employed in the Titan MPS are robust and simple to use, come with a one-year warranty and deliver the lowest background available to ensure the ability to meet the USP <232> detection requirements.

Figure 4. Titan MPS Microwave Sample Preparation System.

Regulatory Compliance

It should also be mentioned that all USP drug standards are enforceable in the United States by the Food and Drug Administration (FDA), as these standards are developed and relied upon in more than 140 countries. So the importance of regulatory compliance in pharmaceutical manufacturing cannot be over-emphasized. In March 1997, Title 21 of the Federal Regulations, which govern foods and drugs, issued the final Part 11 regulations that provided criteria for acceptance of electronic records, electronic signatures, and handwritten signatures executed electronically, as equivalent to paper records and handwritten signatures executed on paper. These regulations, which apply to all FDA programs, are intended to permit the widest possible use of state-of-the-art electronic technology, compatible with FDA's responsibility to protect the public health and to ensure that abuse, falsification, or inadvertent corruption of electronic data is prevented. As such, 21 CFR Part 11 sets forth the detailed requirements that computerized systems need to fulfill in order to allow electronic signatures and records in lieu of handwritten signatures on paper records. In summary, the regulations apply to: validations for closed and open computerized systems; controlled access to the computerized system; content integrity; use of electronic signatures for authentication of electronic documents; audit trails for all records/signatures; and access to electronic records.

Procedures complying with these requirements must include the appropriate administrative controls to ensure that personnel, who develop, maintain or use electronic records and signature systems, have the education, training and experience to perform their assigned tasks. Compliance with 21 CFR Part 11 also requires designing and embedding appropriate technical controls in the instrumentation to ensure the integrity of system operations and information stored in the system as well as maintain appropriate audit trails.

For that reason, it's important to emphasize that the NexION and Optima Enhanced Security™ (ES) Software packages equip laboratories to be fully compliant with regard to the integrity, safety and traceability of all data generated in the identification and testing of pharmaceutical compounds and their raw materials. In addition, PerkinElmer also offers both Installation Qualification (IQ) verification by way of documented proof that the equipment has been installed in accordance with relative drawings and specifications under the guidelines of the appropriate safety regulations, and Operational Qualification (OQ) verification, which establishes that all process equipment and sub-components are fully capable of operation within the limits and tolerances specified. It is well-recognized that with such a wide array of software and hardware tools, PerkinElmer provides the most comprehensive range of compliance offerings for regulated laboratories.

OneSource Laboratory Services – The ONE You Can Count On

As the most experienced, most complete provider of laboratory services worldwide, PerkinElmer OneSource® is uniquely positioned to offer a more valuable, customizable and profitable partnership. Far beyond the traditional model of a laboratory services company, OneSource becomes an integral part of your business, providing a level of technical support and scientific expertise that's truly unique in the industry. Having a single, consolidated care and repair program that covers all your instrumentation offers enormous benefits and cost efficiencies. But we don't stop there — OneSource brings the most experienced people and advanced technologies to bear on the operational issues you face every day, streamlining workflows, consulting on scientific challenges, even supporting the computer systems behind your instruments.

In Summary

This overview is intended to keep the pharmaceutical community abreast of the latest developments with regard to the new chapters on elemental impurities in pharmaceutical products. It has provided an overview of the salient points described in Chapter <232> on Elemental Limits and Chapter <233> on Analytical Procedures and offered suggestions as how best to approach the determination of elemental impurities in pharmaceutical products using PerkinElmer ICP-AES/OES and ICP-MS instrumentation. It is meant to be the first step in educating pharmaceutical laboratories and explaining why the old "Heavy Metals Test", described in Chapter <231> is being replaced with these two brand new chapters. The approval of Chapter <2232> –"Elemental Contamination of Dietary Supplements" – became official August 1, 2013. Until General Notices 5.60.30 Elemental Impurities in USP Drug Products and Dietary Supplements becomes official on January 1, 2018, however this General Chapter would be applicable only if it is referenced in a particular monograph.

PerkinElmer, a trusted leader in trace metal analysis for 50+ years, now a secure partner to meet the requirements of USP Chapters <232>, <233> and <2232>.

References

1. General Chapter <231> *Heavy Metals Test* in USP National Formulary (NF)
2. General Chapter <232> *Elemental Impurities – Limits:* 2nd Supplement of USP 35-NF 30
3. General Chapter <233> *Elemental Impurities – Procedures:* 2nd Supplement of USP 35-NF 30
4. General Chapter <1151> *Pharmaceutical Dosage Forms – Routes of Administration* in USP National Formulary (NF)
5. General Chapter <730> *Plasma Spectrochemistry Method* in USP National Formulary (NF)
6. General Chapter <1225> *Validation of Compendial Procedures* in USP National Formulary (NF)
7. General Chapter <2232> *Elemental Contamination in Dietary Supplement* (still in the USP review/comments stage)
8. "ICP-MS with Auto Dilution and Auto Calibration for Implementing the New USP Chapters on Elemental Impurities", *Spectroscopy* magazine ICP & ICP-MS Supplement, November 2012.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

011179C_01