Sections in This Document

1. Purpose

This document provides a framework for the analysis of samples which are obtained during the investigation of consumer complaints or which are associated with alleged instances of product tampering.

2. Scope

The Agency's consumer complaint system is a crucial source of information on potentially unsafe products in the US marketplace. Complaints involving death, serious illness, or injury are given the highest priority and demand an immediate response. Complaints about infant formula, baby food and medical foods are especially sensitive due to the vulnerability of the population that consumes these products. Product tampering is an intentional adulteration of a product that is frequently brought to the Agency's attention through consumer complaints.

Laboratory examination and analysis of complaint/tampering samples allows the Agency to begin to assess the severity and scope of the potential problem. Producing analytical results in a timely manner is paramount to developing a response that will minimize risk to public health.

3. Responsibility

A. Analyst

1. Review available background information, including complaint form and collection report
2. Obtain appropriate control/comparison samples as needed
3. Take appropriate safety precautions
4. Document sample examination and analysis in analytical worksheet(s)

B. Supervisor/Management

1. Communicate results to investigator, Office of Emergency Operations, District/Program Compliance Branch, Complaint Coordinators as applicable

4. Background

A complaint serves as notification that a product in commercial distribution may be in violation of FDA laws and regulations. FDA is authorized to investigate reported tampering of FDA regulated products under the Federal Anti-Tampering Act, Title 18, United States Code, Section 1365. Note that the Act not only makes tampering a crime but also identifies as criminal "whoever knowingly communicates false information about product tampering" and "whoever threatens." The Office of Criminal Investigations (OCI) has the primary responsibility for all criminal investigations of tampering/threat incidents.

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6. Procedure

6.1. Preliminary Information and the Analytical Strategy

Many complaints allege product tampering; however, facts uncovered during the investigation may not support the allegations. Unless there is prevailing investigational information prior to laboratory examination and analysis, it is difficult to differentiate manufacturing problems from intentional tampering. Consequently, a similar analytical approach is taken to both.

There are several different ways or points in the supply chain at which a product may be adulterated, either unintentionally or intentionally:

6.1.1. During the manufacturing process

Instances initially thought to be tampering (because only a few units were affected) but were eventually linked to manufacturing problems could include the contamination of a product with cleaning solution due to incomplete rinsing of processing equipment, or the presence of foreign objects such as metal fragments or machine parts due to equipment failure. Other problems may result from an incorrectly labeled or contaminated component used as an ingredient, or an incorrect product inside a container due to labeling mix-ups.

One example of a contaminated ingredient occurred in 1996. Drums labeled "USP Glycerine" were used in Haiti to produce children's cough syrup. The glycerin was contaminated with 24% diethylene glycol, the main component of antifreeze, and caused the deaths of many children.

6.1.2. During distribution or at the retail location

The appearance of adulteration may result from improper storage conditions. If the product is intentionally adulterated, it may often be disguised so that the product appears "normal". One of the most infamous examples is the 1982 case in which seven people in the Chicago area died after taking Tylenol capsules containing cyanide.

6.1.3. After the product is in the possession of the consumer

"Copycat" or false report cases may follow a highly publicized tampering. Product substitution is another type of tampering, in which the consumer will empty the contents of the container, refill it with another substance, and return it to the retail store for a refund. Intentional poisoning of a person is another example. There can also be instances in which the consumer unknowingly contaminates the product himself, when the suspicious tablet found in a beverage is determined to be the same as the tablet the consumer was attempting to swallow.

The objectives of the investigation and analysis of complaint/tampering samples are to determine if the product is adulterated or substandard, determine the nature/identity and extent of the adulteration, and evaluate, if possible, which adulteration scenario is most likely and/or which can be ruled out. This assessment represents an ideal situation. In reality, the ability to resolve these issues with absolute certainty is often limited by the details of the complaint and the history and condition of the sample received by the laboratory.

6.1.4. Prior to starting an analysis

Review the available background information. Read the complaint form, collection report and affidavits and discuss the sample with the investigators. It is important to differentiate facts from allegations and theories. Facts include when a product was manufactured (as determined from code/lot number/expiration date) and when the complaint was made. Allegations, e.g. the product tasted bitter or made the consumer nauseous, may or may not be accurate or true. Information obtained from the manufacturer on previously encountered packaging or product defects or additional complaints is beneficial. Medical records can provide important clues in cases involving death, serious illness, and injuries. For example, medical tests may indicate

that the consumer ingested a reversible cholinesterase inhibitor in which case it would be sensible to screen the sample for carbamate pesticides. Some guidance in this area can be obtained from toxicology textbooks or through consultation with local Drug and Poison Information Centers, toxicology laboratories or appropriate FDA Center subject matter experts.

Whenever possible, obtain a control sample of the product for comparison to the suspect sample. For the most meaningful comparison, the control should closely match the suspect with respect to product, packaging, manufacturing location, and time. There should also be some assurance that the control is intact and free of contamination. In the absence of a well-matched control, one alternative is to use a sample with nominally the same suspect sample matrix as a comparison control for chemical tests.

General guidance on the examination of materials for forensic purposes can be found in various monographs. It is also important to plan an analytical approach while considering that many times the amount of sample available for analysis is limited. The choice of destructive versus non-destructive techniques and the possibility of using one sample preparation for more than one analysis are options that should be considered.

The FDA Forensic Chemistry Center (FCC) located in Cincinnati, Ohio, has extensive experience in dealing with product tampering samples. Contact them whenever there are questions about an analytical approach, methods to use, or whether the FCC has prior experience with the type of complaint/tampering situation under investigation.

Forensic Chemistry Center:

Phone 513/679-2700

FAX: 513/679-2761

http://inside.fda.gov:9003/ORA/Labs/FCC/default.htm

6.2. Safety

Treat complaint/tampering samples with extra precaution because they may present unidentified/unforeseen hazards. Background information from the complaint form and collection report may help the analyst anticipate hazards including biological (e.g., bloodborne pathogens like HIV), chemical (e.g., cyanide, fentanyl or toxic or carcinogenic compounds) and/or physical (e.g., needles, syringes, razor blades, etc.). Choose the laboratory location or analysis site for the suspect sample with care. Use biological safety cabinets, chemical fume hoods or sealed glove boxes as needed. Personal protection should include safety glasses, gloves, respiratory protection if needed, and protective clothing ranging from laboratory coats to safety suits and face shields. Before starting the initial

examination all containment, clean-up and disposal supplies should be ready and on-hand. Special care should be exercised if any odors, leaks or discoloration associated with the sample container are noted. Be aware that some adulterants may react with the product containers and alter the sample if the sample is not transferred to a resistant container for storage. Suspect samples should only be examined/analyzed when other trained laboratory personnel can monitor the analyst in the event of an accident.

Although detectable odors may be unavoidable, the sample should be moved immediately to a chemical fume hood. Organoleptic testing, i.e., purposefully smelling the sample, is not recommended. Particulates that are present may also be inhaled, and compounds such as fentanyl and carfentanil are highly toxic in very low quantities. *Do not, under any circumstances, taste the sample!*

6.3. Sample Handling

It is important that judicious attention to detail occur in sample handling to maintain sample integrity and avoid overlooking or damaging evidence. Carefully document chain of custody and storage conditions. Preserve as much suspect material and forensic evidence as possible. Unless otherwise notified by submitting officials, protect fingerprint evidence by wearing cloth gloves over vinyl or latex gloves and using tongs or forceps to handle the sample on edges, seams, and corners (i.e., away from potential fingerprint regions). As much of the packaging and product as possible should be left undisturbed. It is imperative to document each step of the examination and every observation in writing or through photographic documentation.

Perform a thorough physical examination (Section 6.4) before proceeding to chemical analysis (Section 6.5). There may be a point at which it is best to stop the examination and refer the sample to another law enforcement agency or laboratory with more familiarity with certain forensic techniques, such as fingerprinting, analyzing adhesives, or identifying particles *in situ*. Any suspicious findings should be discussed with laboratory management immediately to determine a best course of action.

6.4. Initial Observations

6.4.1. Comparisons between suspect and control samples

It is often advantageous to examine the suspect sample in parallel with the control or comparison sample but is imperative that the sample be separated physically from the control, and care taken to minimize the risk of cross-contamination (for example by changing gloves between handling of items). Control samples should be processed using the same procedures as the suspect sample, and any discrepancies should be noted and documented.

Record the manufacturing code and any codes printed on the labels, containers, and closure systems for both the suspect and the control.

Obtain a gross sample weight before proceeding with the analysis. The sample may be weighed in the original container and the container weight subtracted later if needed. The weight/volume may be needed for comparison to control samples, to determine the amount of product remaining, or to substantiate the amount consumed, the amount of adulterant added, etc.

6.4.2. Examination of packaging

Intact suspect sample cartons, boxes, bags, plastic film covers, etc. should be opened in a region that is not suspected as being the site of entry. Avoid opening along manufacturer seams or seals. Record and document any observations and sample treatment used in the examination of suspect samples.

The observation process should proceed in a logical manner working from the external surfaces to the inside, only opening the product container after all external examinations are completed. Written and photographic documentation of the suspect product should be obtained for the product packaging (box, carton, package, wrapper, bottle, etc.) and any other sample-related items. Include a measurement scale (preferably metric units) in every photographic image at the same focus level as the sample. Examination may include visual observations using the unaided eye and closer examination using a hand lens, stereoscopic light microscope (SLM) and/or compound light microscope. The suspect sample should be examined using short-wavelength and long-wavelength ultraviolet light and/or a variable wavelength light source (e.g., Crimescope®, Video Spectral Comparator® or CDx device) and all findings documented.

Check for any surface anomalies such as punctures, tears, cuts, holes, slits and abrasions. Thoroughly examine all container closure seams, folds, crimps, caps, tops, lids and liners, and note any irregularities. Document the condition of tamper-evident closures such as safety seals, tear-away ring bottle caps and vial flip-off caps. Look for chips or cracks in glass and damaged threads on screw caps. Examine packaging joints and seams closely for excess glue, glue smears, glue tear pattern and multiple glue types. Any extraneous surface marks (paint, ink, pencil, marker, scratches) should be documented. Note any discoloration, dust, powders, crystals, debris, or leakage. Evaluate the product for color, clarity, fluidity, layering, clumping, marks, chips, stains, orientation (e.g., capsule parts askew, units out of place in a sectioned package) and foreign objects.

6.4.3. Documentation of anomalies

The location of any unusual findings or foreign objects should be diagrammed or photo documented. Removal of any portion of the suspect sample should take place only after the initial observations and photo-documentation have been completed. Once photographed in situ, the object or questionable portion of the sample may be isolated for additional characterization and/or chemical analysis.

6.4.4. Additional considerations

When sampling any portion of the suspect sample for analysis, use as little as possible to conserve the sample for confirmatory analysis or further testing that may need to be performed by other specially equipped laboratories such as the Forensic Chemistry Center. Ensure that the condition of the sample has been well documented before sampling. Ordinarily, the sample should not be homogenized. For example, if the sample consists of a liquid in a bottle with some particulate on the bottom, remove a portion of the liquid and remove a portion of the particulate and analyze them separately.

If portions of the sample are removed prior to homogenizing, quantitative work will not be able to be performed.

If an odor is detected unavoidably, move the sample to a chemical fume hood immediately to prevent exposure to toxic vapors or gases (see Section 6.2). Make a note of the odor because it may suggest the presence of bleach, ammonia, amines or a petroleum product such as gasoline, thinner or a vehicle for an emulsifiable concentrate of a pesticide.

6.5. Analytical and Instrumental Methods

6.5.1. Preliminary considerations

Experience with tampering cases has shown that the contaminant is typically present in relatively large amounts. However, when the examination reveals no remarkable differences, it is important to be aware of the adequacy of the screening procedure. The toxicity of a wide variety of substances has been described based upon ranges for the probable oral lethal dose. Significant clinical illness can be expected at doses on the order of 10% of the probable lethal dose. If a margin of safety of 3% of the probable oral lethal dose is set, then a target concentration for the detection of a contaminant in a product can be defined by the following equation:

$$\text{Target Concentration} = 0.03 \times \text{Probable Oral Lethal Dose} \times \text{Body Weight (kg)} \times \frac{1}{\text{Portion Size (kg or L)}}.$$

(mg/kg or L) (mg/kg body wt.)

Using a body weight of 70 kg (average adult) with a portion size of 355 mL (*e.g.*, a 12 oz. beverage), the table below can serve as a guide to the needed sensitivity of the analytical methods. This equation can be adjusted in keeping with additional information, for example, a good estimate of the amount of product ingested by a child of known body weight and an identified poison with a known lethal dose.

Description	Toxicity Class	Probable Oral Lethal Dose (mg / kg body wt)	Warning Concentration (mg/L)
Non-toxic	1	> 15000	
Slightly Toxic	2	5000 to 15000	30000
Moderately Toxic	3	500 to 5000	3000
Very Toxic	4	50 to 500	300
Extremely Toxic	5	5 to 50	30
Super Toxic	6	< 5	1*

* Note: the minimum lethal dose for some super toxic materials can be as low as 0.1 mg/kg body weight and this is used to calculate this number.

6.5.2. Chemical analyses

6.5.2.1. Methodology

Complaint/tampering samples can require numerous analytical approaches, including physical examinations, visual analyses, chemical spot tests, micro/molecular biological analyses, and instrumental techniques such as microscopy, chromatography, mass spectrometry and spectroscopy. Published methods such as those in the Pesticide Analytical Manual (PAM), Microbiological Methods & Bacteriological Analytical Manual (BAM), Elemental Analysis Manual for Food and Related Products (EAM), United States Pharmacopoeia (USP) and the Official Methods of Analysis of AOAC International are good resources. Frequently, however, the analytes of interest are unknown, and these established methods may not be applicable to, or may require modification for, the sample matrix received. Sources for alternate analytical methods include technical organizations (ASTM International, ISO, etc.), scientific journals, Laboratory Information Bulletins (LIBs), internet resources, Food Emergency Response Network (FERN), and technical procedures. When non-standard matrices or sample requests are received,

the analyst must verify the accuracy and reliability of the chosen method for the intended purpose. Method validation studies will demonstrate the method can be used to support the conclusions of the analysis and the situation to which it was applied.

6.5.2.2. Analytical approach

Depending on the nature of the suspected contaminant, the sample-related information supplied by the submitter, or the information needed to address public health aspects, it must be determined whether the sample is to be analyzed by single or multiple disciplines. If multiple analytical disciplines are required it will be necessary to determine whether the analyses should run consecutively or concurrently. Consecutive analyses will require that one type of analysis be completed before another may be initiated. For example, a suspect vial of liquid may require consecutive analyses if determination of evidence of tampering and screens of the liquid were requested, or if the product must be sampled for microbiological contamination before aliquots are removed for chemical analyses. Concurrent analyses of the same suspect vial might be appropriate if the analyses to be performed do not depend on completing one before initiating another, such as examination of an opened carton and identification of the liquid contents.

6.5.2.3. Sampling considerations

Sample preparation should be minimized to speed the progress of analyses and retain information. Non-destructive techniques may be preferred initially, particularly if the amount of suspect sample is limited. Whenever possible, direct analysis is preferred to extraction/clean-up. Good judgment on the part of the analyst(s) should be exercised when deciding which methodologies will be used and the order in which they will be performed.

6.5.2.4. Initial characterizations

If moderate shaking of the sample (see Section 6.4 before shaking) produces a persistent foam, this may indicate the presence of a surfactant which might be associated with a cleaning product. This form of tampering is relatively common and there are a variety of tests to determine which class of surfactant is present. If the formation of foam is suppressed when the sample is acidified, this may indicate the presence of a soap. If, on the other hand, foaming is relatively unaffected by pH, then the presence of a detergent is likely. Additional characterization utilizing color tests can distinguish among nonionic, anionic or cationic surfactants. Further analysis, if necessary, can proceed after consulting the chemical literature.

Another chemical test is the measurement of pH. A significant difference in sample pH relative to a control sample indicates a change in the sample which should be explored. If the pH is less than 2 or greater than 10, then the presence of a corrosive acid or base is likely. Further investigation might include examination by ion chromatography (IC) for anions such as chloride or nitrate which would correspond to the acids HCl or HNO3, respectively, or inductively-coupled plasma atomic emission spectroscopy (ICP-AES) or mass spectroscopy (ICP-MS) for metals or cations to indicate the presence of a metalloid and/or complex anion (such as PO43-). IC can also be used to detect some organic acids and cations including ammonium, and gas chromatography-mass spectrometry (GC-MS) can be used to detect organic acids or bases.

Exposing the sample to ultraviolet light may indicate that a component of the contaminant is a fluorescent material. Identification of the fluorescent compounds may help focus the analytical approach. For example, dyes which are associated with antifreeze may suggest the sample should be analyzed using GC-MS for the presence of ethylene glycol or diethylene glycol.

Another simple chemical test might include a cyanide screen using Cyantesmo test paper. Positive results for the presence of cyanide would require confirmatory analysis, for example, using ultraviolet-visible spectroscopy.

Spot tests for the presence of oxidizing agents using diphenylamine in sulfuric acid or Starch/Iodide paper can provide useful information.

6.5.2.5. Instrumental methods and techniques

The following sections contain brief overviews of the use of instrumental methods to compare the suspect sample and the control sample. It is recognized that not all the instrumentation that will be discussed is found in every laboratory. A basic understanding of each technique on the part of the analyst is assumed.

A. Particle analyses using stereoscopic light microscopy (SLM), polarized light microscopy (PLM), scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM/EDS) or Fourier-transform infrared spectroscopy (FT-IR)

B. Vibrational spectroscopic techniques (FT-IR and Raman)

C. Gas chromatography-mass spectrometry (GC-MS) with headspace sampling for volatile materials

D. GC-MS for volatile and semi-volatile materials

E. Ion chromatography (IC) or IC-MS for anions and cations

F. Liquid chromatography-mass spectrometry (LC-MS) with DART/ESI/APCI/other ion sources

G. Inductively-coupled plasma-atomic emission spectroscopy (ICP-AES) or –mass spectrometry (ICP-MS) for minerals, metals and metalloids

It is also necessary to be aware that detected compounds may be indicative of other contaminants that may not be found using the instrumental methods described above. For example, the detection of ricinine using LC-MS, or ricinoleic acid using GC-MS, may indicate the presence of ricin. The determination of this protein requires specific tests such as enzyme-linked immunosorbent assay (ELISA) or protein/peptide LC-MS.

6.5.2.5.1. Stereoscopic Light Microscopy (SLM), Polarized Light Microscopy (PLM) and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDS)

In general, SLM and PLM are used to examine solids, semi-solids, powders, liquids and packaging materials visually. Samples can be examined independently or compared to controls and standards. Stereoscopic and polarizing light microscopes can be equipped with cameras to document observations with digital images.

Stereoscopic light microscopes are designed to examine large areas of a sample using low magnifications and various lighting conditions. SLM is used typically to determine the heterogeneity or homogeneity of a sample, examine packaging for evidence of tampering (*e.g.*, additional adhesives or tears) and isolate particles and materials physically from samples for further analysis. Individual or small groups of particles can be examined at higher magnifications using PLM, to determine physical characteristics (*e.g.*, morphology, surface texture) and other optical properties which can be used to identify a sample when compared to known standards or literature references.

SEM is used to visualize samples at even higher magnifications (10X-500,000X). An SEM image can provide morphological information about a sample. An EDS attachment to an SEM can be used to obtain qualitative elemental information about the sample.

If the sample is limited in size or volume, SLM, PLM, and SEM/EDS are useful techniques for initial characterizations because they are often non-destructive to the sample and can be helpful in preparing the sample for further analyses.

6.5.2.5.2. Vibrational Spectroscopic Techniques (FT-IR and Raman Spectroscopy)

The selection of an FT-IR or Raman analytical technique is generally dependent on the physical form of the sample, the quantity or size of sample,

and sample information available. These techniques can be used to analyze single crystals, amorphous solids, conglomerate/ aggregate particles, films, fibers, semi-solids, single- and multi-component solids, liquids and gases. In general, the instrument's lack of sensitivity limits the usefulness for the analysis of solutions except in instances of gross contamination. However, FT-IR may be utilized in conjunction with a microscope for the examination of individual particles or for characterization of substances isolated by physical means. FT-IR can determine the composition of samples by comparing spectral features with libraries of IR spectra from reference compounds. FT-IR can also provide indications of the presence of functional groups to supplement other analytical information.

In certain instances, some sample preparation (*e.g*., liquid extraction) may be necessary to isolate the analyte(s) of interest from the sample matrix. Therefore, FT-IR or Raman spectral information about the sample matrix, as well as literature information about the specific analyte(s), should be used to determine the optimum sample preparation conditions.

6.5.2.5.3. Headspace Sampling combined with Gas Chromatography-Mass Spectrometry (GC-MS)

The technique is used for the detection and characterization of volatile materials (boiling points approximately below 200°C). It is very useful for detecting solvents (*e.g*., alcohols, chlorinated hydrocarbons, other organic solvents), fragrances associated with cleaning products which frequently appear as contaminants, and petroleum products. Petroleum products may occur as contaminants in their own right or may be associated with pesticides in emulsifiable concentrates.

This technique can also be used to screen for toxic anions, such as azide, cyanide, or fluoroacetate by forming their ethyl derivatives prior to analysis.

Mass spectra which are associated with observed differences between the suspect sample and control sample are compared to reference spectra to obtain tentative identification. This identification may be subsequently confirmed through the analysis of standards concurrently with the sample.

6.5.2.5.4. Gas Chromatography-Mass Spectrometry (GC-MS)

This technique is applicable to a variety of matrices being screened for evidence of tampering or adulteration. Direct injection of the sample or an extract/solution of the sample in methanol or acetonitrile is the first choice, with the understanding that other volatile solvents could be utilized as needed. The range of applicability can be extended to functionally non-volatile materials by silylating the extracts to form trimethylsilyl (TMS) derivatives. Silyl derivatives

are formed by replacement of active hydrogens from acids, alcohols, thiols, amines, amides, and enolizable ketones and aldehydes with the TMS group. Derivatization is typically performed after solvent exchange into pyridine by the addition of N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) which contains 1% trimethylchlorosilane with incubation at 70°C for 15-30 min and repeating the analysis. Other derivatization reagents *(e.g*., N-methyl-bis(trifluoroacetamide), MBTFA) may be used when selective replacement of active hydrogens is desired.

In addition, an acid/base extraction should be considered for samples with high fat, sugar, and/or protein content. The sample solution should be between pH 5 and 9 to minimize column damage. Minimizing the amount of water in the sample/extract solution will limit the creation of active sites in the injection port liner, which may prevent loss in sensitivity of an analyte. If the nature of the sample precludes this or if some pre-concentration is needed, then the sample may be extracted with acidified aqueous acetonitrile (pH = 3) and the acetonitrile subsequently isolated by "salting out" for analysis by GC-MS. Isolation of a basic extract is obtained using the same procedure with basic aqueous acetonitrile (pH = 10). Additional sensitivity can be obtained by evaporative concentration of the acetonitrile extracts.

The suspect and control samples are compared to expose differences. The mass spectra associated with these differences are compared to reference spectra to provide tentative identification. This identification may be subsequently confirmed through the analysis of standards concurrently with the sample. Occasionally, available mass spectral libraries do not contain spectra of the compound(s) observed, and tentative identification proceeds from mass spectral interpretation.

6.5.2.5.5. Ion Chromatography (IC)

The role of this technique is to detect anions such as those in the table below. A number of common anions (*e.g.*, bromide, chloride, cyanate, iodide, nitrate, phosphate) are in Toxicity Class 3 with a warning concentration of 3000 mg/L and can be determined using IC. Those that are more toxic, such as anions in Classes 5 and 6, can also be detected using anion exchange chromatography with suppressed conductivity detection.

Toxicity Class 6	**Toxicity Class 5**	**Toxicity Class 4**
WL = 1 mg/l	**WL = 30 mg/l**	**WL = 300 mg/l**
Azide	Bromate	Chlorate
Fluoroacetate	Fluoride	Fluoroborate

Cyanide	Fluorosilicate	Iodate
	Nitrite	Lactate
		Oxalate
		Thiocyanate
		Thioglycolate

It is worth noting that a reactive contaminant, such as bleach, may produce additional peaks in a suspect sample that can only be properly characterized through experiments that monitor the impact of the addition of the nominal contaminant to a control sample through time. The probable presence of bleach in the suspect sample at some point in time is implied by elevations in the chloride and chlorate levels along with high pH and the presence of excess sodium.

Ion chromatography is also useful for identifying sugars, sugar alcohols, and amino acids. The wrong label on an infant formula which actually contains lactose might put a child who is lactose intolerant at risk. For pharmaceutical formulations in which lactose, mannitol, or glycine is used, IC may be a useful tool to verify the authenticity of a manufacturer based on the profile of components detected. Lower levels of components in a formulation may be an indication that the product has been diluted or counterfeited.

6.5.2.5.6. High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)

HPLC- or ultra-HPLC (UHPLC)-MS offers versatility, sensitivity, selectivity, mass accuracy, and rapid screening of samples for drugs, poisons, toxins, peptides, and proteins. It is versatile because it offers many different ionization sources (*e.g.,* ESI (electrospray ionization), APCI (atmospheric pressure chemical ionization), DART (direct analysis in real time)) and positive or negative ionization modes.

HPLC-MS is the method of choice for detecting non-volatile or thermally unstable compounds (*e.g.*, cardiac glycosides, alkaloids, proteins) which are not amenable to determination using GC-MS. HPLC-MS is especially useful as a screening tool if a comparison sample is available. Due to the ever-growing compilation of reference spectra (MS/MS libraries), mass spectra which are associated with observed differences between the suspect and control sampled can be compared to obtain tentative identification with subsequent verification through the analysis of standards.

If still needed and available, high-resolution accurate mass (HRAM) can be utilized to aid in determination of unknowns by generating potential chemical formulae with subsequent verification through the analysis of standards.

6.5.2.5.7. Inductively-Coupled Plasma (ICP)-Atomic Emission Spectroscopy (AES) or -Mass Spectrometry (MS)

Methods and procedures outlined in the EAM should be followed in principle to the extent they are appropriate. Elevated levels of minerals, metals and metalloids relative to the native levels in comparison samples may serve as an indicator of adulteration. In contrast, the concentration of minerals, metals or metalloids "missing" relative to the native levels in closely matched comparison samples can sometimes be useful in estimating the extent to which the adulterant has diluted the original sample.

While some minerals, metals and metalloids have significant toxicity, in tampering scenarios the adulterant would typically be added as a salt or molecular complex which may have significantly different toxicity than the elemental or ionic form.

Toxicity Class 6	Toxicity Class 5	Toxicity Class 4
WL = 1 mg/l	**WL = 30 mg/l**	**WL = 300 mg/l**
Arsenic	Antimony	Boron
Selenium	Barium	Cobalt
	Cadmium	Copper
	Chromium	Gold
	Mercury	Lead
	Silicon	Lithium
	Tellurium	Manganese
	Thallium	Nickel
	Tin	Zinc
	Vanadium	

The chemical form of the element defines its toxicity. For example, phosphorous as yellow phosphorous (Toxicity Class 6) is a significant hazard but phosphorous as phosphate (Toxicity Class 3) is commonly encountered in foods. Barium (Toxicity Class 5), when present as the insoluble sulfate salt, is not considered harmful. Inorganic arsenic is highly toxic (toxicity class 6), but arsenic as arsenobetaine, which is found in seafood, is considered non-toxic. Although most forms of silicon are innocuous, silicon is significant as an

indicator of the possible presence of fluorosilicate. Consequently, it may be necessary to couple separation techniques with atomic spectroscopy, often called speciation, or to compare the total elemental content with results from ion chromatography or molecular mass spectrometry to fully evaluate the associated risk.

Additionally, vulnerable populations, may be more sensitive to high levels of certain elements or salts. For example, very high levels of sodium chloride or iron in infant formula have resulted in significant toxicity.

6.5.3. Suspected biological contamination

If the suspected contamination is unknown or microbiological in nature, the way in which the sample is processed and analyzed will depend on whether the contamination is deemed routine or high-risk (Biosafety Level 3 [BSL-3]). Guidance can be found in the BAM and FERN.RES.0001.01.

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7. Glossary/Definitions

A. AES – Atomic Emission Spectroscopy
B. AOAC – Association of Official Analytical Chemists
C. APCI – Atmospheric Pressure Chemical Ionization
D. ASTM – American Society for Testing and Materials
E. BAM – Microbiological Methods & Bacteriological Analytical Manual
F. BSL-3 – Biosafety Level 3
G. BSTFA – N,O-bis(trimethylsilyl)trifluoroacetamide
H. CDx – Counterfeit Detection Device, version x
I. DART – Direct Analysis in Real Time
J. EAM – Elemental Analytical Manual for Food and Related Products
K. ELISA – Enzyme-linked Immunosorbent Assay
L. ESI – Electrospray Ionization
M. FCC – Forensic Chemistry Center
N. FDA – United States Food and Drug Administration
O. FERN – Food Emergency Response Network
P. FT-IR – Fourier-transform Infrared Spectroscopy
Q. GC – Gas Chromatography

R. HPLC – High-Performance Liquid Chromatography
S. HRAM – High-Resolution Accurate Mass
T. IC – Ion Chromatography
U. ICP – Inductively-Coupled Plasma
V. ISO – International Organization for Standardization
W. LIB – Laboratory Information Bulletin
X. MBTFA – N-methyl-bis(trifluoroacetamide)
Y. MS – Mass Spectrometry
Z. OCI – Office of Criminal Investigations
AA. PAM – Pesticide Analytical Manual
BB. PLM – Polarized Light Microscope/Microscopy
CC. SEM/EDS – Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy
DD. SLM – Stereoscopic Light Microscope/Microscopy
EE. TMS – Trimethylsilyl
FF. UHPLC – Ultra High-Performance Liquid Chromatography
GG. USP – United States Pharmacopeia

8. Records

A. Analytical Worksheets
B. Sample Summary Reports

9. Supporting Documents

A. None

10. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
1.2	R	09/01/2005	LMEB	LMEB
1.3	R	02/02/2010	LMEB	LMEB
1.4	R	02/06/2012	LMEB	LMEB
1.5	R	02/14/2013	LMEB	LMEB
02	R	05/05/2020	LMEB	LMEB

* - D: Draft, I: Initial, R: Revision

11. Change History

Revision #	Change
1.2	• Table of Contents – Section 14.11 added. • Section 14.8 II. – revised fourth paragraph; Section 14.8 III. removed headers A. & B and 1-7 now A-G; revised Section 14.8 III. D. & E. • References formatted with numbers 22. – 35.
1.3	14.5 – paragraphs 1, 5, and 6 revised 14.8 I. – deleted last sentence 14.8 II. – revised 14.8 III. – paragraphs 1, 2, and 3 revised 14.9 – revised 14.10 - Changed “Division of Emergency Operations” to “Office of Crisis Management”

Revision #	Change
1.4	14.3 – deleted “FATA” in second paragraph 14.4 – updated contact information with weblink 14.7 – added “in order” and updated Section 14.4 to 14.5, Safety in third paragraph 14.8 II – deleted supplier name in fourth and fifth paragraph 14.8 III – revised first sentence in first paragraph 14.8 III A. – added (GC_MS) in heading; defined LODs 14.8 III B. – added (GC-MS) in heading; defined FID 14.8 III C. – added (IC) in heading; defined ICP 14.8 III E. – added (ICP-AES) in heading; corrected spelling of Thallium in Table 14.8 III F. – added (FT-IR) in heading 14.8 III G. – added (UV-VIS) in heading 14.9 – deleted second sentence in second paragraph
1.5	Header – Division of Field Science changed to Office of Regulatory Science 14.11 – Division names removed; ORO change to OO; Division of Field Science changed to Office of Regulatory Science
02	Reformatted SOP to follow current template
	“14.1 Objective” renamed “1. Purpose”
	“14.2 Introduction” renamed “2. Scope” and updated content
	“14.3 FDA Laws and Regulations” renamed “4. Background” and updated content
	“14.11 References” renamed “5. References” and updated content
	“14.4 Preliminary Information and the Analytical Strategy” moved to section 6.1 under “6. Procedure” and updated content
	“14.5 Safety” moved to section 6.2 under “6. Procedure” and updated content
	“14.6 Sample Handling” moved to section 6.3 under “6. Procedure” and updated content
	“14.7 Initial Observations” moved to section 6.4 under “6. Procedure” and updated content. Subheadings created for: Comparisons between suspect and control samples; Examination of packaging; Documentation of anomalies; Additional considerations
	“14.8 Analytical and Instrumental Methods” moved to section 6.5 under “6. Procedure” and updated content
	Subheadings under previous 14.8 “I. Simplify the problem if possible”, “II. When there isn't much to go on”, and “III. Application of instrumental techniques” updated to “6.5.1 Preliminary considerations” and “6.5.2 Chemical analyses” under “6.5 Analytical and Instrumental Methods.” Updated content.

Revision #	Change
	Under 6.5.2, added subheadings “6.5.2.1 Methodology”, “6.5.2.2 analytical approach”, “6.5.2.3 Sampling considerations”, “6.5.2.4 Initial characterizations”, “6.5.2.5 Instrumental methods and techniques”. Under 6.5.2.5 created “6.5.2.5.1 Stereoscopic Light Microscopy (SLM), Polarized Light Microscopy (PLM) and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDS)”, “6.5.2.5.2 Vibrational Spectroscopic Techniques (FT-IR and Raman Spectroscopy)”, “6.5.2.5.3 Headspace Sampling combined with Gas Chromatography-Mass Spectrometry (GC-MS)”, “6.5.2.5.4 Gas Chromatography-Mass Spectrometry (GC-MS”, “6.5.2.5.5 Ion Chromatography”, 6.5.2.5.6 High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)”, “6.5.2.5.7 Inductively-Coupled Plasma (ICP)-Atomic Emission Spectroscopy (AES) or -Mass Spectrometry (MS)”. Deleted “G. UV-Visible Spectrophotometry (UV-VIS).” Updated content in all sections.
	Added “6.5.3 Suspected biological contamination” under “6.5 Analytical and Instrumental Methods.”
	Deleted “14.9 Analytical Documentation” and “14.10 Reporting”.
	Added “Analytical Worksheets” and “Sample Summary Reports” to “8. Records”

12. Attachments

None