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# Quality Assurance Report for the National Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue



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# Chapter 1 Introduction

This report documents the quality of data generated for pharmaceuticals and personal care products (PPCPs) under the National Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue (hereafter referred to as the "pilot study"). Below is a brief summary of the pilot study design and implementation. The PPCP Fish Pilot Study final report, which is referenced at the end of this document, provides additional information (USEPA, 2023).

### Section 1.1 Background

PPCPs are a large and diverse group of chemicals consisting of all prescription drugs and over-thecounter medications, as well as non-medicinal chemicals in consumer products, such as fragrances in soaps and lotions and active ingredients in sunscreens and insect repellants. Studies completed by 2005 documented the occurrence of PPCPs primarily in surface waters, sediment, and effluents (discharges) from wastewater treatment plants. Little data were available on PPCPs in fish at this time, and early studies of PPCPs in fish focused on sample collection from a single site and analysis of tissue for a specific chemical or chemical class. In 2006, EPA's Office of Science and Technology within the Office of Water decided to expand investigation of the presence and accumulation of PPCPs in fish from locations across the United States.

## Section 1.2 Study Design

The pilot study is a screening-level study designed to investigate the occurrence of a broad suite of PPCPs in freshwater fish. It incorporated the following design elements, which are summarized in Table 1:

- Selection of sampling sites on five effluent-dominated streams in population centers across the country and at a remote reference site in an area not influenced by wastewater discharges
- Collection of six composite samples of resident fish species at each sampling site
- Analysis of fish tissue composite samples for 36 PPCPs

	not Study Design Liement Summary
Number of Sites	5 Effluent-dominated streams + 1 reference site
Site Selection Method	Targeted
Sampling Year	2006
Fish Composite Samples/Site	6
Fish Tissue Sample Types	Fillets and Livers for Pharmaceuticals Fillets only for Personal Care Products
Number of Target Analytes	24 Pharmaceuticals 12 Personal Care Products
Total Tissue Samples Analyzed	72 for Pharmaceuticals 36 for Personal Care Products

 Table 1.
 PPCP Fish Pilot Study Design Element Summary

#### 1.2.1 Site Selection

EPA selected fish sampling locations on five effluent-dominated streams in densely populated areas of the U.S. based on the assumption that PPCPs were more likely to occur in these areas. Other factors considered for site selection included a larger percentage of elderly residents, higher median incomes (as

a surrogate for volume of PPCP sales), availability of fish, and representation of different wastewater treatment technologies to evaluate their potential impact on PPCP removal.

The sampling sites for the pilot study are as follows:

- North Shore Channel in Chicago, Illinois
- Trinity River in Dallas, Texas
- Little Econlockhatchee River in Orlando, Florida
- Salt River in Phoenix, Arizona
- Taylor Run in West Chester, Pennsylvania (a suburb of Philadelphia)

In addition, EPA received assistance from staff at New Mexico Environment Department to identify a suitable reference site on the East Fork Gila River in the Gila River Wilderness Area of New Mexico. The remote location of this reference site ensured minimal impacts of human influences.

#### 1.2.2 Fish Sample Collection

Field sampling teams applied consistent methods to collect 18 or 24 adult fish of the same species and similar size from each sampling location during the late summer and fall of 2006. Fish collection from effluent-dominated streams focused on resident fish species that were continually exposed to discharges from wastewater treatment plants. The field teams divided the fish from each site into six composites of either 3 or 4 fish before shipping whole fish to the laboratory for tissue sample preparation and analysis.

#### 1.2.3 Tissue Sample Analysis

Baylor University's Center for Reservoir and Aquatic Systems Research prepared and analyzed the fish tissue samples for the pilot study. Laboratory technicians removed fillets and livers from individual fish in each of the six composite samples collected at every site, and they homogenized the tissue to prepare 36 fillet and 36 liver composite samples for analysis (6 composites/site x 6 sites x two tissue types for a combined total of 72 tissue composite samples for analysis). At the time of this study, Baylor University's analytical methods for PPCPs in fish tissue could screen tissue samples for 24 pharmaceuticals using high performance liquid chromatography (HPLC) with a tandem mass spectrometric (MS/MS) detector and for 12 personal care product chemicals using gas chromatography (GC) with a tandem ion trap mass spectrometric (MS/MS) detector. Table 2 provides the name, CAS Registry Number, and analytical method for each of the 36 PPCPs included in the pilot study. Fillet composites were analyzed for both the pharmaceuticals and personal care product chemicals. Analysis of the 36 liver composites was limited to pharmaceuticals only due to problems the laboratory encountered with interferences from the high lipid content in the liver tissue.

Pharmaceuticals by HPLC-MS/MS			
Analyte	CAS No.	Analyte	CAS No.
Acetaminophen	103-90-2	Ibuprofen	15687-27-1
Atenolol	29122-68-7	Lincomycin	154-21-2
Caffeine	58-08-2	Metoprolol	37350-58-6
Carbamazepine	298-46-4	Miconazole	22916-47-8
Cimetidine	51481-61-9	Norfluoxetine	54910-89-3
Codeine	76-57-3	Propranolol	525-66-6
Diltiazem	42399-41-7	Sertraline	79617-96-2
1,7-Dimethylxanthine	611-59-6	Sulfamethoxazole	723-46-6
Diphenhydramine	58-73-1	Thiabendazole	148-79-8
Erythromycin	114-07-8	Trimethoprim	738-70-5
Fluoxetine	54910-89-3	Tylosin	1401-69-0
Gemfibrozil	25812-30-0	Warfarin 81-81-2	
Personal Care Products by GC-MS/MS			
Analyte	CAS No.	Analyte	CAS No.
Benzophenone	119-61-9	Nonylphenol	104-40-5
Celestolide	13171-00-1	Octocrylene	6197-30-4
Galaxolide	1222-05-5	Octylphenol	1806-26-4
4-Methylbenzylidine camphor	36861-47-9	<i>m</i> -Toluamide	618-47-3
Musk ketone	81-14-1	Tonalide	1506-02-1
Musk xylene	81-15-2	Triclosan	3380-34-5

Table 2. Target Analytes by Analytical Method

### Section 1.3 Study Participants

EPA's Office of Science and Technology (OST) conducted the pilot study with support from two agency contractors (Tetra Tech and GDIT) and the analytical laboratory at Baylor University. Figure 1 (on the following page) identifies roles and responsibilities for the primary study participants from the initiation of the study through 2012. OST also received voluntary logistical and field sampling assistance from EPA's Great Lakes National Program Office and the Metropolitan Water Reclamation District of Greater Chicago for the North Shore Channel site in Chicago and from the New Mexico Environment Department for the reference site in the Gila Wilderness Area of southwest New Mexico.





# Chapter 2 Quality Assurance Program

Environmental measurements always contain some level of uncertainty, and decision makers must recognize the uncertainty associated with the data on which their decisions are based. In planning the pilot study, EPA managers recognized that there was significant potential that the study data might be used by others within EPA and by other interested parties responsible for making environmental, economic, and policy decisions. Therefore, the study managers established a quality assurance (QA) program intended to ensure that data produced during the pilot study would meet defined and documented standards of quality.

The pilot study QA program prescribed minimum requirements to which all organizations that gathered data were required to adhere. Data quality was defined, controlled, assessed, and documented through these QA program activities. The remainder of this chapter presents highlights of the QA program employed during the study.

#### Section 2.1 Quality Assurance Project Plans

EPA decided to develop separate quality assurance project plans (QAPPs) to support field sampling and laboratory analysis for the pilot study. This decision allowed the QAPP for sample collection activities to be prepared and approved on an accelerated schedule, so field teams could complete sampling at all study sites during the summer and fall of 2006. The analytical activities QAPP was completed and approved about a month before field teams finished fish sample collection for the study. Tetra Tech provided support for development of the sample collection activities QAPP, and both GDIT and Tetra Tech supported development of the analytical activities QAPP.

#### 2.1.1 Sample Collection Activities QAPP

The sample collection QAPP is formally referred to as the *Quality Assurance Project Plan for Sample Collection Activities for a Pilot Study to Investigate the Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue* (USEPA, 2006a). This QAPP established data quality goals for all sample collection and handling activities and described quality assurance/quality control (QA/QC) techniques employed by field sampling teams to support those goals. EPA based the sampling procedures for the pilot study on the approach successfully applied for sample collection in the agency's National Lake Fish Tissue Study. Technical and QA staff from EPA and Tetra Tech approved the sample collection QAPP on August 1, 2006. Everyone involved in the sample collection process received a copy of this QAPP.

#### 2.1.2 Analytical Activities QAPP

The analytical activities QAPP is officially known as the *Quality Assurance Project Plan for Laboratory Sample Preparation and Analysis Activities in the National Pilot Study of Pharmaceuticals and Personal Care Products (PPCPs) in Fish Tissue* (USEPA, 2006b). This QAPP established measurement quality objectives (e.g., QC acceptance criteria) for laboratory data generated during the pilot study and described the QA/QC procedures applied by laboratory staff and the other contractors supporting the study to ensure these goals were met. Scientists and QA managers from EPA, GDIT, Tetra Tech, and Baylor University approved the analytical activities QAPP on October 19, 2006. All contractors responsible for fish tissue sample preparation and analysis, data quality review, or database development received copies of this QAPP.

### 2.1.3 QAPP Revisions

Field sampling teams successfully implemented the sample collection activities QAPP as initially written, so this QAPP did not undergo revision during the pilot study. In contrast, some approaches that were planned and documented in the initial analytical activities QAPP were refined as work on tissue sample analysis progressed during the study. Since this was a pilot study, all of the study participants anticipated that changes to the original version of this QAPP would be necessary. Following is a list of the substantive revisions to the analytical activities QAPP and a brief summary describing each change:

- The laboratory revised the list of target analytes as follows:
  - After the original analytical activities QAPP was signed, the laboratory refined their HPLC-MS/MS procedure and added diphenhydramine as a target analyte
  - The laboratory dropped clofibric acid from the target analyte list due to instrumental difficulties encountered while switching ionization modes on the HPLC-MS/MS
  - The laboratory dropped three nonylphenol monoethoxylate isomers as target analytes due to problems obtaining a suitable standard. The readily available standard was a mixture of all three isomers, and the vendor could not certify the concentration of each isomer in the mixture or supply a separate standard for each isomer
- The laboratory revised the analytical techniques for the personal care products, particularly to incorporate the use of a tandem mass spectrometric detector rather than using selected ion monitoring on a single MS detector. Section 3.4 of this report provides more information about these changes
- The laboratory extracted and analyzed only the fillet samples for personal care products after encountering problems with lipid interferences in multiple attempts to analyze liver samples and modify the extract cleanup procedures to address these interferences
- Analytical results for the tissue samples were reported down to the laboratory's method detection limits (MDLs) rather than the laboratory's project quantitation limits

For each case, EPA approved changes to the analytical approach described in the original QAPP prior to their implementation. The laboratory delivered the final data package for pharmaceuticals in May 2007 and for personal care products in February 2008. At EPA's request, Tetra Tech updated the analytical activities QAPP to document changes to the analytical procedures implemented during tissue sample analysis and submitted the revised analytical activities QAPP in July 2010.

## Section 2.2 Field Sampling QA/QC

EPA incorporated a number of QA/QC procedures to ensure consistency in fish sample collection and to produce complete and accurate documentation of field sampling data. Collectively these procedures contributed to reducing sampling variability and to improving sample representativeness and sampling completeness. Key field sampling QA/QC procedures include the following:

- Preparation and implementation of the sample collection activities QAPP
- Development and application of standard operating procedures for sample collection and handling activities
- Preparation and use of standardized sampling kits containing field sampling supplies to control contamination of fish samples and forms to allow consistent documentation of fish collection and sample shipping
- Use of the same experienced fisheries biologist to lead field sampling teams of fully trained technicians and to ensure proper implementation of procedures

- Daily tracking and coordination of fish sample shipments through a centralized source during fish sampling operations
- Implementation of standardized procedures for review and documentation of field data quality

Field teams documented sample collection and shipping information using four standardized forms and labels: a field record form, a sample identification label, a chain-of-custody form, and a chain-of-custody label for sealing each shipping container. Field data reviewers used a fifth standardized form, the resolved action form, to record results and decisions for field data quality assessments. Assessment of field data quality showed that sampling teams met the goal of collecting six fish composite samples that adhered to composite criteria specified in the QAPP at each of the six study sites by November 2006. No fish samples were lost or compromised during shipment to the laboratory at Baylor University, so field teams achieved the sampling completeness goal of 100%.

# Section 2.3 Sample Analysis QA/QC

EPA integrated several QA/QC activities and laboratory requirements into the pilot study to ensure data comparability and generate analytical data of known quality during preparation and analysis of the fish tissue samples and evaluation of analytical data quality. Following is a summary of critical QA/QC components to achieve analytical data quality goals:

- Development and implementation of the analytical activities QAPP
- Use of one laboratory for sample preparation (filleting and liver extraction, tissue homogenization, and preparation of tissue aliquots) and tissue sample analysis
- Requirement for triplicate lipid analyses to test for tissue homogeneity during sample preparation
- Identification of quantifiable measurement quality objectives
- Use of pure and traceable reference standards
- Demonstration of instrument calibration and system performance
- Periodic calibration verification
- Analysis of QC samples to assess performance of analytical methods
- Specification of MDLs and method/chemical QC acceptance criteria that applied throughout the study
- Use of a standardized data quality assessment process

The general measurement quality objective (MQO) for the pilot study was to satisfy method-specific performance criteria. The analytical activities QAPP provides a summary of the method performance criteria and specifies MQOs and QC acceptance criteria to assess the bias and precision associated with the analytical methods used for this study. Chapter 4 of this report describes the process for data quality assessment and presents the results of these assessments, which includes data from the following laboratory QC samples or measures: blanks, recoveries for spiking surrogate chemicals into field-based tissue samples, matrix spiking (matrix spike/matrix spike duplicate (MS/MSD)), laboratory control samples, and calibration verifications. Chapter 4 also includes a discussion of data completeness for the pilot study.

# Section 2.4 Training

Planning for the pilot study included using experienced staff for sample collection, laboratory analyses, and data validation and providing project-specific training, as necessary, to staff responsible for these activities. The QAPPs covering the respective sampling and analysis activities describe training for these

areas of responsibility. Prior to initiating field sampling operations, sample collection staff received training on pilot study sample collection and handling procedures. Laboratory personnel at Baylor University consisted of senior research associates supervising technical staff with the education and skill levels required to maintain consistent measurement system performance throughout the study. GDIT assigned data reviewers to evaluate pilot study results that were trained in the application of data review guidelines developed for EPA's National Lake Fish Tissue Study and adopted for this study. They were also experienced in reviewing data generated with the instrumentation used in the pilot study. Each data reviewer received a copy of the analytical activities QAPP, which specified the performance criteria and MQOs applicable to this study.

## Section 2.5 QA Oversight of Laboratory Operations

Tetra Tech was responsible for providing analytical services for the pilot study, and subcontracted with Baylor University to obtain these services. As part of their contractual oversight, Tetra Tech assigned a QA chemist to maintain oversight of laboratory operations for the duration of the contract and to work collaboratively with laboratory staff to ensure optimal completion of tissue sample analysis for the pilot study. The QA chemist provided technical and QA support to the laboratory through a variety of activities. These activities included the following:

- Conducting collaborative reviews of laboratory operations at critical points in the process of preparing and analyzing the tissue samples, such as:
  - Visiting Baylor University in December 2006 to observe and provide guidance on the preparation of fish tissue samples. Laboratory staff did not have prior experience preparing fillet samples from the size of fish collected for the pilot study, and they improved the process for homogenizing fillet tissue as a result of this visit. The laboratory also implemented actions to improve freezer temperature monitoring and cataloging freezer contents.
  - Returning to the laboratory in February 2007 to observe the extraction of tissue samples and review the HPLC-MS/MS and GC-MS instrumentation and analytical procedures. Discussions between the Tetra Tech and Baylor University culminated in the laboratory adopting use of GC-MS/MS techniques for personal care product analysis after EPA's concurrence.
  - Traveling to an onsite meeting with laboratory staff in September 2007 to discuss approaches for effectively addressing the extensive lipid interferences the laboratory was encountering during analysis of fillet samples for personal care products. The goal of the meeting was to identify a solution that would allow the laboratory to complete these analyses and deliver analytical results of acceptable quality. Using gel permeation chromatography (GPC) as a cleanup technique in the analyses of fillet samples for personal care products provided adequate resolution of this issue.
- Involving the laboratory in regular comprehensive reviews of the analytical procedures for both the pharmaceuticals and personal care products. An outcome of these reviews was Tetra Tech working with the laboratory to fine-tune several aspects of the laboratory's analytical effort and obtaining EPA approval for the revised procedures.
- Working extensively with the laboratory to resolve QA and data reporting issues, including:
  - Identifying and testing options to address lipid interferences in analyzing tissue samples for personal care products. After this effort, Tetra Tech and Baylor University agreed that the use of gel permeation chromatography (GPC) would be required as a cleanup technique for all GC-MS/MS analyses in this study.
  - Participating in discussions with Baylor University and other study participants for resolving a data reporting error for galaxolide results

# Chapter 3 Analytical Methods

When EPA initiated the pilot study, several agency programs, including those within the Office of Water and the Office of Research and Development, were beginning to develop methods for the analysis of PPCPs in various matrices. At that time, however, there were no formal EPA analytical methods for the PPCPs<sup>1</sup>, nor were methods available from any voluntary consensus standards bodies. Therefore, the project team sought the assistance of Baylor University's Center for Reservoir and Aquatic Systems Research because researchers in the center had developed separate analytical methods for analyzing pharmaceuticals and personal care products in fish tissue. Scientists from Baylor joined the project team when the university agreed to provide laboratory services for the pilot study, which included preparation of fish tissue samples and analysis of the tissue samples for lipid content and for 24 pharmaceuticals and 12 personal care products. Section 3.1 summarizes the process for preparation of fish tissue samples, and Section 3.2 describes the method for analyzing the lipid content of each tissue sample. Sections 3.3 and 3.4 outline procedures for the PPCP methods, respectively, that Baylor University used to analyze fish tissue samples for the pilot study. Section 3.5 provides information about quality control procedures for the PPCP methods.

#### Section 3.1 Preparation of Fish Tissue Samples

The laboratory prepared 36 fillet composite samples (from 6 fish composite samples per site at 6 sites) following procedures described in EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis, Third Edition, 2000* (USEPA, 2000). During the homogenization process, Baylor staff ensured that there was minimal loss of tissue mass, and that each sample was uniformly mixed, using lipid analysis to test for tissue homogeneity

The process for preparing fillet samples included the following steps:

- Removing the entire fillet (including the skin and belly flap) from both sides of each fish in the composite sample and using all the available tissue to prepare the fillet composite sample (i.e., the batch method)
- Grinding frozen cubes of fillet tissue to a fine powder using a high-speed blender and adding small amounts of dry ice during grinding to facilitate consistent blending of the tissue
- Applying quartering, mixing, and re-grinding techniques described in the guidance document to produce a homogeneous composite mixture of fillet tissue and
- Storing the homogenized fillet composite samples in a freezer at a temperature of -20°C until the laboratory was ready to analyze them for PPCPs

To prepare the 36 liver composite samples, the laboratory applied tissue dissection and homogenization techniques developed for prior studies conducted by Baylor University to characterize concentrations of PPCPs in fish tissue (Brooks et al., 2005). These techniques involved the following steps:

• Removing the liver from each fish in the composite (a total of three or four livers, depending on the sampling location) and placing all of them in a clean glass container

<sup>&</sup>lt;sup>1</sup> The Engineering and Analysis Division (EAD) of the Office of Water released a draft EPA method for PPCPs in December 2007, well after the start of the pilot study. *Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS*, addresses the analysis of a large suite of PPCPs in water and biosolids, but the method does not include any procedures for the analysis of fish tissues, nor has EAD evaluated the method in tissue matrices.

- Homogenizing the liver tissue using a Tissuemiser<sup>®</sup> set to rotate at 30,000 revolutions per minute (rpm) and
- Storing the liver homogenate samples in the freezer at a temperature -20°C until the laboratory was ready to analyze them for pharmaceutical compounds

### Section 3.2 Determination of Lipid Content

The laboratory analyzed three replicate aliquots (individually consisting of approximately 2 g) from each of the 36 fillet composite samples for lipids using the method described in Mottaleb et al. (2009). This method was modified slightly for liver samples. Due to limited sample mass, triplicate lipid measurements were made for only one of the six liver composite samples from each sampling site. Lipid analysis involved the following steps:

- 1) weighing out 2 g of tissue and combining it with 15 mL of a 1:1 mixture of dichloromethane:hexane in a borosilicate vial
- 2) homogenizing each mixture for 3 minutes using a Tissuemiser®
- 3) Placing the vials in an incubator for 24 hours at 35 °C and periodically agitating by gentle end-overend rotation
- 4) adding 2 g of solid anhydrous sodium sulfate to each 1 g of sample following extraction
- 5) filtering the mixture through Grade 415 filter paper
- 6) washing the solid residue with an additional 15 mL of 1:1 dichloromethane:hexane
- 7) collecting the combined filtrate for each sample in a pre-weighed test tube
- 8) evaporating the solvent with dry nitrogen for 8 hours at 45 °C using a Zymark<sup>®</sup> Turbovap LC Concentration Workstation and
- 9) drying the lipid residue after evaporation to a constant weight in a vacuum oven at 40  $^{\circ}$ C

Lipid content was determined gravimetrically by weighing three replicates of each sample. Percent lipid determinations were then calculated as shown in the following equation:

% lipid= 
$$\frac{\text{weight of lipid residue (g)}}{\text{weight of tissue (g)}} \ge 100$$

## Section 3.3 Analysis of Pharmaceuticals by HPLC-MS/MS

Baylor analyzed for 24 pharmaceuticals in fish tissue by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using the method described in Ramirez et al. (2007). Table 2 (Chapter 1) provides a list of the target analytes. This method utilizes matrix-matched calibration standards (aliquots of control matrix from outside of the study area that are expected to be reasonably free of target compounds) spiked at a minimum of five concentrations, and extracted and analyzed along with study samples. By extracting standards, matrix effects and bias were minimized in the final analytical results.

#### 3.3.1 Sample Extraction for Pharmaceuticals

Prior to extraction, each sample was spiked with a set of surrogate compounds to assess extraction efficiency. Extraction of tissue homogenates for analysis involved the following steps:

- 1) weighing out 1.0 g  $\pm$  0.01 g of fillet tissue and 0.5 g  $\pm$  0.01 g of liver tissue for each standard or sample composite and placing each tissue aliquot into a 20-mL borosilicate glass screw-cap vial
- 2) spiking the samples with the appropriate surrogates (acetaminophen-d<sub>4</sub>, diphenhydramine-d<sub>3</sub>, carbamazepine-d<sub>10</sub>, fluoxetine-d<sub>6</sub>, and ibuprofen- $^{13}C_3$ )
- 3) combining sample homogenates with 8 mL of 1:1 mixture of 0.1 M acetic acid buffer (pH 4) and methanol extraction solvent, and tightly replacing the cap
- 4) sonicating the mixture in an ultrasonic bath for 15 minutes at 25  $^{\circ}$ C
- 5) shaking the mixture vigorously by hand for 20 seconds to further ensure mixing and extraction
- 6) quantitatively transferring each extract to a separate 50-mL polypropylene copolymer roundbottomed centrifuge tube with several rinses of the extraction solvent
- 7) centrifuging the extracts at 16,000 rpm for 40 minutes at 4 °C to achieve a full separation of residual solid and liquid phases
- 8) transferring the supernatant into a clean 18-mL disposable borosilicate glass culture tube with rinses of methanol using disposable Pasteur pipettes
- 9) evaporating the sample extracts to dryness under a stream of dry nitrogen at 45  $^{\circ}$ C
- 10) reconstituting the extracts in 1 mL (for fillets) or 0.5 mL (for livers) of mobile phase (0.1% formic acid in reagent water)
- 11) adding internal standards (7-aminoflunitrazapam-d<sub>7</sub>, fluoxetine-d<sub>6</sub>, and meclofenamic acid)
- 12) sonicating for 1 min at 25  $^{\circ}$ C
- filtering extracts using a 0.2-μm PTFE-membrane syringe filter into an amber HPLC injection vial and
- 14) sealing the vial with a fluoropolymer-lined cap

To maintain similar method sensitivity for both tissue types, the final extract volumes were 1.0 mL for fillet samples and 0.5 mL for liver samples.

The laboratory prepared samples in batches of 20 or fewer samples, which were accompanied by the required batch quality control samples, including a method blank, low- and high-level control samples, and a pair of MS/MSD samples from each site.

#### 3.3.2 Preparation of Calibration Standards for Pharmaceuticals

Fish tissue samples generally contain large amounts of lipids and other materials that can interfere with the tissue analyses. Cleanup techniques, such as gel permeation chromatography (GPC), can remove much of the lipid material and other interferences. However, significant "matrix effects" can remain, which may limit the overall accuracy of the measurement process. To address potential matrix effects, the laboratory prepared their instrumental calibration standards in a clean tissue matrix, extracted those standards in the same manner as samples were extracted, and analyzed the extracted standards. Some EPA methods apply this approach for other analytes and matrices, such as the analysis of herbicides in drinking water. The laboratory used tissue from smallmouth bass samples collected at the New Mexico reference site to prepare separate extracted calibration standards for liver and fillet tissues. Each standard

for liver analysis required a 0.5-g aliquot of clean liver tissue and each standard for fillet analysis required a 1.0-g aliquot of clean fillet tissue.

## 3.3.3 HPLC-MS/MS Analysis of Pharmaceuticals

HPLC-MS/MS has advantages over gas chromatographic (GC) methods for the analysis of pharmaceuticals because GC methods involve introducing the analytes into the instrumentation in a gaseous form and many of the pharmaceuticals are not easily volatilized. For example, some pharmaceuticals have boiling points that are above the operating temperatures of a GC system, while others will break down when heated.

Tandem mass spectrometry (MS/MS) involves the use of two quadrapole mass spectrometers in series, with a collision cell between them, such that selected ions produced in the first MS unit are directed into the collision cell and further fragmented before being sent to the second MS for detection. The only ions passed through the collision cell are those selected by the instrument as representing the analytes of interest, thus minimizing the effects of many potential interferences. These fragments, or "daughter" ions, are characteristic of the "parent" or "mother" compound, and they are used to positively identify the analyte in the presence of other analytes and potential interferences. The MS/MS detector can be operated in several ionization modes, including one that produces positive ions and another that produces negative ions from the analytes of interest.

Table 3 provides a brief summary of Baylor University's instrumental operating conditions. These conditions may not be applicable to instruments from other manufacturers or to different lists of target analytes.

HPLC	Varian ProStar Model 210			
MS/MS	Varian Model 1200L triple quadrapole mass analyzer equipped with an electrospray interface			
Guard column	Agilent Extend-C18 column, 12.5 mm x 2.1 mm, 5 μm particle size			
Analytical column	Agilent Extend-C18 column, 15 cm x 2.1 mm, 5 μm particle size			
Injection volume	10 μL			
Elution gradient	0.1% (v/v) formic acid in water and 100% methanol at 350 $\mu$ L/min and 30 °C			
Collision gas	as Argon, at 2.0 mTorr			
Run time	50 minutes			

Table 3. HPLC-MS/MS Operating Conditions

Baylor staff selected the optimal electrospray ionization (ESI) mode (either positive or negative) and MS/MS parameters by infusing standards of each individual target analyte and selecting the conditions that yielded the most intense precursor ion for each analyte. Immediately prior to analysis, they fortified each sample extract with internal standards, adding 7-aminoflunitrazapam- $d_7$  and fluoxetine- $d_6$  as the internal standards for the ESI+ analyses and meclofenamic acid for the ESI- analyses. For routine sample analyses, laboratory staff identified the target analytes on the basis of chromatographic retention time (compared to an authentic standard) and the presence of both the parent and daughter ions for each analyte. They quantified each analyte by an internal standard calibration approach using the extracted calibration standards.

# Section 3.4 Analysis of Personal Care Products by GC-MS/MS

Baylor determined 12 personal care products in fillet tissue samples by gas chromatography-tandem mass spectrometry (GC-MS/MS) using procedures described by Mottaleb et al. (2009). Baylor originally planned to use a single MS unit and monitor selected ions (i.e., GC-MS with SIM), but modified those plans during the method development efforts before the study samples were analyzed. Table 2 (Chapter 1) provides the list of target analytes. Unlike the pharmaceutical method, the personal care product (PCP)

method did not involve preparation and extraction of matrix-matched calibration standards. Instead, standards were prepared in solvent, as is common practice for most GC procedures.

### 3.4.1 Sample Extraction for Personal Care Products

Prior to extraction, each sample was spiked with a set of surrogate compounds to assess extraction efficiency. The extraction procedures include derivatization of sample extracts to enhance measurement response. This involved use of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) to derivatize some target analytes to more volatile forms (e.g., musk ketone and triclosan). Extraction of fillet homogenates for analysis involved the following steps:

- 1) weighing out  $1.0 \text{ g} \pm 0.01 \text{ g}$  of fillet tissue for each sample composite and 1.0 g of control matrix for each QC sample individually to the nearest 0.01 g into a 20-mL borosilicate glass screw-cap vial
- 2) spiking the samples with the appropriate surrogates (benzophenone- $d_{10}$ , and *p*-n-nonylphenol-<sup>13</sup>C<sub>6</sub>)
- 3) adding 10 mL of acetone to each spiked homogenate aliquot
- 4) sonicating samples for 15 min at 25 °C
- 5) shaking the samples vigorously by hand for 20 seconds to further ensure mixing and extraction
- 6) transferring extracted samples into 50-mL polypropylene copolymer round-bottomed centrifuge tubes using 1mL acetone as a rinse
- 7) centrifuging extracted samples at 16,000 rpm for 40 min at  $4 \,^{\circ}\text{C}$
- 8) transferring the supernatant into 18-mL disposable glass test tubes
- 9) evaporating the solvent to dryness under a stream of nitrogen at 30 °C
- 10) reconstituting the samples in 200  $\mu L$  of 65:35 (v/v) hexane:acetone in preparation for silica gel clean-up
- 11) loading sample extracts onto a preconditioned (8 mL of 65:35 hexane:acetone by volume) silica gel column (1 g), and eluting with 30 mL of hexane:acetone
- 12) exchanging the solvent by evaporating the resultant extract to near-dryness and reconstituting in 700  $\mu$ L of methylene chloride
- 13) injecting one half (350 μL) of the extract into the GPC, which was equipped with a cross-linked styrene divinylbenzene copolymer guard column (30 mm × 4.6 mm) and a similar analytical (150 mm × 19 mm) column connected in series
- 14) eluting the GPC column with methylene chloride and collecting the fraction eluting between 11.4 and approximately 19.4 minutes
- concentrating the methylene chloride extract to near dryness and reconstituting it to approximately 200 μL in hexane:acetone in a GC injection vial with a PTFE-lined septum
- 16) adding 100 μL of MSTFA derivatizing agent, capping the GC vial, and heating the mixture in an oven at 60 °C for 45 minutes
- 17) concentrating the derivatized extract to near dryness at room temperature under a stream of nitrogen then reconstituting it in 180 μL of n-hexane and
- 18) spiking the extracts prior to analysis with 20  $\mu$ L of the internal standards (phenanthrene-d<sub>10</sub> and mirex)

The laboratory prepared samples in batches of 20 or fewer samples, which were accompanied by the required batch quality control samples, including a method blank, low- and high-level control samples, and a pair of spiked MS/MSD samples from each site.

#### 3.4.2 GC-MS/MS Analysis of Personal Care Products

Table 4 provides a brief summary of Baylor University's instrumental operating conditions. These conditions may not be applicable to instruments from other manufacturers or to different lists of target analytes.

GC	Varian Model CP3800		
MS	Varian Model 1200 triple-quadrapole mass spectrometer		
GC column	Restek XTI-5, 30 m × 0.25 mm × 0.25 µm film thickness		
Carrier gas	Helium, at 1.0 mL/min		
Injection volume	1 µL		
1st Temperature gradient	100 -180 °C at 15 °C/min		
2nd Temperature gradient	180 - 290 °C at 6 °C/min		
Final temperature	290 °C for 6 min		
Ionization potential	250 eV		

Table 4. GC-MS/MS Operating Conditions

For routine sample analyses, laboratory staff identified the target analytes on the basis of chromatographic retention time (compared to an authentic standard) and the presence of a primary quantitation ion and at least one secondary ion. Baylor researchers investigated the use of extracted calibration standards during the development of their GC-MS/MS procedure for the personal care products, but found that it did not offer the same advantages that it did for the HPLC-MS/MS analyses of the pharmaceuticals. Therefore, they quantified the target analytes by an internal standard calibration approach using traditional calibration standards prepared in pure solvent.

### Section 3.5 Quality Control

Although the procedures used by Baylor University were not formal EPA or voluntary consensus standard body methods, they did include the same types of quality control parameters found in these types of methods. Laboratory staff implemented the following procedures:

- Multi-point calibration of all instruments (minimum of five points) with a linearity requirement
- Calibration verification (each analysis shift)
- Method blanks prepared with each batch of samples
- Addition of surrogate compounds to every sample as a measure of extraction efficiency
- Spiked sample analyses in duplicate (e.g., matrix spike and matrix spike duplicate samples)
- Laboratory control samples in duplicate (e.g., blank spikes)

Results from all of these QC operations were reported in the final data packages submitted to Tetra Tech and evaluated by GDIT.

# Chapter 4 Data Quality Assessment

#### Section 4.1 Data Review

All of the data from the pilot study were subjected to three distinct levels of review by different study participants. First, all of the laboratory data were reviewed by Baylor's Laboratory Quality Manager, Kevin Chambliss, before they were reported to Tetra Tech. Tetra Tech staff reviewed the data that the laboratory submitted to track progress and completeness (e.g., all samples sent to the laboratory were analyzed) and to assess the effectiveness of the analytical procedures. Finally, the data reviewers examined the results for each field-based tissue sample and the available quality control data to assess and document the quality of the data relative to the objectives of the pilot study.

Each data package was thoroughly reviewed to ensure the following:

- All samples were analyzed and results were provided for each sample analyzed, including results for any dilutions and reanalyses, and for all associated QC samples
- All required QC samples were analyzed and these QC samples met specified acceptance criteria
- Data reporting forms and/or electronically formatted data were provided for each of the field-based tissue samples and/or associated QC analyses
- Raw data associated with each field-based tissue sample and QC sample were provided with each data package, and the instrument output (peak height, area, or other signal intensity) was traceable from the raw data to the final result reported
- Any problems encountered and corrective actions taken were clearly documented

When anomalies were identified, GDIT contacted the laboratory through Tetra Tech and asked them to provide the missing data, clarifications, and/or explanations so that a comprehensive data review could be performed to verify the quality of their results.

GDIT developed a database to capture results for each sample and entered results of the data reviews directly in the database through the application of standardized data qualifier flags and descriptive comments concerning the reliability of the flagged results. Table 5 contains the individual data qualifiers that were applied to results from the pilot study and provides an explanation of the implications of each qualifier for the use of the data.

*Note:* The presence of data qualifiers is not intended to suggest that data are not useable; rather, the qualifiers are intended to caution the user about an aspect of the data that does not meet the acceptance criteria established in the project QAPP.

SCC Code	Definition	Implication		
В	Blank       If the amount detected in the sample was at least 5x the blank level, indicating that the sample was greater than 1x and le blank level, then it was likely that the sample result was inflated contamination in the lab that showed up in the blank. In that ca was assigned, indicating that the sample result may have been due to the blank contribution. Data users should consider this in about RMAX values.         If the amount detected in the sample was less than or equal to the sample result was reset to a nondetect at the method detection sample size and percent solids. If applicable			
EXC	Excluded	This flag was applied to the results for analytes where the laboratory determined that analytical difficulties indicated that results could not be reported with any reliability. For the personal care products analyses by GC-MS/MS, the analytes benzophenone and octocrylene were excluded. For the pharmaceuticals analyses by HPLC-MS/MS, the results for miconazole in liver tissue samples were excluded. (The		
HLCS	High LCS recovery	The lab control sample (LCS) was a clean reference matrix. If recovery in the LCS was high, there may be a high bias for that analyte. If the analyte was not detected in a field-based tissue sample, there was no concern and the RNAF flag was applied as well.		
HMSR	High matrix spike recovery	High matrix spike (MS) recovery indicated a positive interference or a high bias. Isolated instances of high recovery are not uncommon, and patterns across multipl MS samples are more of a concern. When high matrix spike recovery was observe for an analyte, the results for that analyte in all of the samples in the batch with the matrix spike sample were qualified. However, if the analyte was not detected in a given sample in the batch, then there was no concern and RNAF was added to the HMSR flag.		
HSSR	High surrogate spike recovery	Assignment of this flag indicated surrogate recovery above the acceptance limits. Large exceedances suggested a positive interference or "matrix effect." If the analyter was not detected in a field-based tissue sample, there was no concern and the RNAF flag was added to the HSSR flag. Even for detected analytes, exceedances of a few percent were not cause for concern, given that the methods were being developed during the study, and therefore, reasonable method performance criteria and acceptance limits were not well established.		
HVER	High VER recovery	Results for the calibration verification were above the acceptance limit. If the analyte was not detected in a field-based tissue sample, there was no concern and the RNAF flag was added to the HVER flag. Results for detected analytes may have a possible high bias.		
LLCS	Low LCS recovery	If the recovery in the LCS was low, there may have been a low bias for that analyte. Nondetects in field-based tissue samples may be false negatives and detects may have a low bias.		
LMSR	Low matrix spike recovery	Low recovery in the matrix spike indicated a potential low bias for the analyte, possibly due to poor extraction efficiency in the sample matrix. Isolated instances of low recovery are not uncommon, and patterns across multiple MS samples are more of a concern. When low matrix spike recovery was observed for an analyte, the results for that analyte in all of the samples in the batch with the matrix spike sample were qualified.		

# Table 5. Individual Data Qualifiers Applied to Pilot Study Data

Table 5. Individual Data Qualifiers Applied to Pilot Study Data

SCC Code	Definition	Implication		
LSSR	Low surrogate spike recovery	This flag was assigned for surrogate recovery below acceptance limits. Large failures suggest a "matrix effect." If extraction efficiency was not sufficient, then all analytes may have a low bias. Recoveries outside of the acceptance limits by a few percent were not a serious concern, given that the methods were being developed during the study, and therefore, reasonable method performance criteria and acceptance limits were not well established.		
LVER	Low VER recovery	Results for the calibration verification were below the acceptance limit. If the analyte was detected in a field-based tissue sample, there may be a low bias for that analyte. Nondetects in field-based tissue samples may be false negatives.		
REXC	Result exceeded calibration range	This flag was applied when the reported result exceeded the instrument calibration range, but the laboratory noted that it did not saturate the detector. Results above the demonstrated calibration range may have greater uncertainty associated with the numerical value.		
RMAX	Result is a maximum value	This flag was applied when other qualifiers indicated a potential positive bias, such as method blank contamination. Data users should consider these values as upper limits of the actual concentration.		
RNAF	Result not affected	Assignment of this flag indicated that the overall assessment of the qualifier applie was that the sample result was not affected. Common examples included analytes not found in the sample, but found in the associated blank or with high recovery in the associated matrix spike.		
RPDX	DX Relative percent difference (RPD) between MS and MSD exceeded criteria This flag was applied when the precision of the recoveries in the MS and analyses, measured as the RPD, exceeded the acceptance limits. This m been due to a failure in one of the two analyses, and is often the result of a interference in one of the two analyses. The poor precision may have been sample nonhomogeneity, or it may have been an analytical issue. While of should be aware of this issue, it is often not a serious data quality concern			

When the 14 individual codes were applied to results from the study, there were a total of 41 unique combinations of codes. Of those 41 combinations, 22 were applied to the HPLC-MS/MS results for the pharmaceuticals, 23 were applied to the GC-MS/MS results for the personal care products, and 4 of the codes overlapped between the two types of analyses. Appendix 1 presents a summary of these qualifier flag combinations, which includes the frequency of occurrence of each combination by analysis type (e.g., a list for pharmaceuticals and a list for personal care products).

# Section 4.2 Analysis of Blanks

Blanks are used to verify the absence of contamination that may occur at any point in the measurement process. In the pilot study, target analytes were expected to occur at low concentrations. Therefore, frequent analysis and assessment of blanks was critical to determine if measured sample concentrations were biased by the presence of contamination during analysis.

The data reviewers evaluated each sample result in comparison to the result for that analyte in the method blank prepared in the same extraction batch. For those analytes reported as present in the method blank, the data reviewers applied the 5x rule described in Table 5 to determine the potential impact of the blank contamination on the study results.

The impacts of blank contamination are discussed separately for the two analytical procedures (HPLC-MS/MS and GC-MS/MS) in Sections 4.2.1 and 4.2.2.

# 4.2.1 Blanks for the HPLC-MS/MS Analyses

Overall, there were few data quality issues with the blanks from the HPLC-MS/MS analyses of the pharmaceuticals, as illustrated in Figure 2 on the following page.

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Figure 2 shows that more than 99% of the pharmaceutical results were not affected by blank contamination, either because the analyte was not detected in the blank (83.74%) or because the concentration in the sample was more than 5x times the level observed in the blank (16.20%). For 0.06% of the results (1 result for 1 analyte), the data reviewers judged that the sample result is likely a maximum value (RMAX) because there is some chance that the sample result was inflated by the background contamination from the lab that is evident in the blank. None of the pharmaceutical results were changed to nondetects (RNON) because of blank contamination.

#### 4.2.2 Blanks for the GC-MS/MS Analyses



Figure 3. Impacts of Blank Contamination on the Personal Care Product Results

## Section 4.3 Surrogate Spiking



Figure 2. Impacts of Blank Contamination on the Pharmaceutical Results

Figure 3 illustrates the impacts of blank contamination on the results for the personal care products. There were even fewer issues with the blanks from the GC-MS/MS analyses than for the HPLC-MS/MS analyses. More than 97% of the personal care product results had no blank qualifier at all, and all of remaining results (2.78%) were more than 5x times the level observed in the blank, and therefore not affected by blank contamination. These percentages do not include two analytes, benzophenone and octocrylene, that were detected often in method blanks associated with a number of samples. Due to other analytical considerations, all results for these two analytes were excluded from the project data set, including these assessments.

As noted in Chapter 3, the laboratory spiked each sample with a set of surrogate compounds that were used to assess extraction efficiency. Surrogates often are isotopically labeled variants of the target analytes or compounds with similar structures that are not expected to be found in environmental samples. The analytical activities QAPP (USEPA, 2006b) specified acceptance limits of 60–150% for the recovery of the surrogates. The impacts of surrogate recoveries are discussed separately for the two analytical procedures (HPLC-MS/MS and GC-MS/MS) in Sections 4.3.1 and 4.3.2.

#### 4.3.1 Surrogate Recoveries for the HPLC-MS/MS Analyses

During the pilot study, the laboratory used five surrogate compounds in the analysis of the pharmaceuticals: acetominophen- $d_4$ , carbamazephine- $d_{10}$ , diphenylhydramine- $d_3$ , fluoxetine- $d_6$ , and ibuprofen- $^{13}C_3$ . These five surrogates were recovered within acceptance limits for all but a small percentage of the samples. Figure 4 illustrates the impacts of surrogate recoveries on the pharmaceuticals.

There were no occurrences of low surrogate recoveries for the pharmaceutical analyses. Figure 4 shows that over 99% of the samples were not affected by surrogate recoveries. This includes 92.65% of the samples that met the surrogate recovery acceptance criterion and 6.89% of the samples that were associated with higher-than expected surrogate recovery, but there was no impact on data quality because the corresponding analytes were not detected in those samples. Only 0.46% of the samples had detected results that are associated with high surrogate recoveries. In these cases, the recovery exceeded the upper acceptance criterion of 150%. This may indicate either a positive interference in the sample that inflates the apparent result for the surrogate, or an issue with the extracted internal standards where a lower-than-



Figure 4. Impacts of Surrogate Recoveries on the Pharmaceutical Results

expected internal standard response could cause similar inflation of the results.

#### 4.3.2 Surrogate Recoveries for the GC-MS/MS Analyses

The laboratory used two surrogates in the analysis of personal care products: benzophenone- $d_{10}$ , and *p*-nonylphenol-<sup>13</sup>C<sub>6</sub>. More surrogate recovery problems were observed with the personal care products analyses than for the pharmaceutical analyses. Figure 5 illustrates the impacts of surrogate recoveries on the personal care products.



Figure 5. Impacts of Surrogate Recoveries on the Personal Care Product Results

Figure 5 shows that more than 63% of the personal care product results were not affected by surrogate recovery problems. Of these, 43.52% of the sample results met the acceptance criterion for surrogate recoveries and 19.91% were associated with high surrogate recoveries which did not affect sample results because the associated analytes were not detected in the tissue samples.

Surrogate recoveries exceeded the upper acceptance criterion of 150% for 1.85% of the samples in which the associated analyte was detected. For these detected results, the high surrogate recovery may indicate positive interferences that could inflate both the surrogate result and the target analyte result.

As noted in Section 3.4, the laboratory did not use extracted internal standards in the GC-MS/MS analyses, and the laboratory calibrated the target analytes using solutions prepared in pure solvent. As a result, quantifying the surrogates in each GC-MS/MS analysis may have been subject to more instrument variability than in the LC-MS/MS analysis of the pharmaceuticals, yielding more recoveries outside of the acceptance limits.

Approximately one third (34.7%) of the sample results were affected by low surrogate recoveries. The low recoveries are indicative of either difficulties in extracting the sample, or unexpected losses of analytes during any cleanup steps. Both suggest the potential for low bias in the target analyte results, and an increased chance of not detecting a target analyte that is present at low concentration.

### Section 4.4 Matrix Spiking

The laboratory used matrix spike (MS) and matrix spike duplicate (MSD) samples to assess the bias and precision of the analytical methods in a tissue matrix. The laboratory included a MS/MSD pair with each batch of samples prepared for analysis, spiking the samples with all of the analytes of interest at concentrations in the upper third of the calibration range. The pilot study analytical activities QAPP (USEPA, 2006b) specified acceptance limits of 60-150% for MS/MSD recovery. The acceptance limit for the precision of the MS/MSD analyses was a relative percent difference (RPD) of 40%. The impacts of matrix spike recoveries and precision are discussed separately for the two analytical procedures (HPLC-MS/MS and GC-MS/MS) in Sections 4.4.1 and 4.4.2.

#### 4.4.1 Matrix Spike Recoveries and Precision for the HPLC-MS/MS Analyses

Figure 6 shows that more than 95% of the pharmaceutical analytes were associated with MS/MSD samples that met the acceptance criteria for recoveries of the spiked compounds (88.77%) or were nondetects (6.71%) that were not affected by the high recoveries. Another 3.1% of the results were detected analytes associated with high matrix spike recoveries that suggest a potential high bias for the target analytes. Low matrix spike recoveries were associated with 1.39% of the results. As with the surrogate recoveries, the low matrix spike recoveries are indicative of either difficulties in extracting the sample or unexpected losses of analytes during any cleanup steps. Both suggest a potential for low bias in the target analyte results, and an increased chance of not detecting a target analyte that is present at a low concentration.



Figure 6. Impacts of Matrix Spike Recoveries on the Pharmaceutical Results

For the pharmaceuticals, none of the results are associated with MS/MSD results where the precision did not meet the acceptance criterion for the relative percent difference (RPD).

A large portion of the 3.13% of the detected results with high matrix spike recoveries is related to the amount of analyte spiked into the MS/MSD samples relative to the "background" concentration in the fish liver samples. Although the laboratory spiked the target analytes at concentrations that would be in the upper third of the calibration range, some of the unspiked liver samples had background concentrations of a few analytes that were greater than the spike amounts.

The situation was particularly difficult for sertraline, where a number of samples contained 5 to 15 times the amount of this analyte that was spiked into the MS and MSD aliquots. In these cases, the variability in background concentration can mask the amount spiked, resulting in the calculation of very large matrix spike recoveries. For sertraline, an unspiked sample contained over 500 ppb of this analyte, but the spike level was only 40 ppb. Fluoxetine, norfluoxetine, and gemfibrozil also exhibited high matrix spike recoveries in some liver MS/MSD samples, although not as high as those for sertraline. Given the analytical schedule for this project, it was not possible to determine an exact cause for these very high matrix spike recoveries in the liver samples. Therefore, data users should take the high matrix spike data qualifiers into account in interpreting the liver sample results for the pharmaceuticals.

#### 4.4.2 Matrix Spike Recoveries and Precision for the GC-MS/MS Analyses

Figure 7 shows that the matrix spike results for the personal care products exhibited different patterns than the pharmaceuticals. About 85% of the personal care product analytes were associated with MS/MSD samples that met the acceptance criteria for recoveries of the spiked compounds (83.33%), or were nondetects (1.39%) that were not affected by the high recoveries.

Only 1.39% of the detected results were associated with high matrix spike recoveries that suggest a potential high bias for the target analytes. None of the matrix spike recoveries for the personal care products were as high as those noted above for certain pharmaceuticals.

Low matrix spike recoveries were associated with 9.72% of the results, indicative of either difficulties





in extracting the sample or unexpected losses of analytes during any cleanup steps. Both suggest the potential for low bias in the target analyte results and an increased chance of not detecting a target analyte that is present at a low concentration.

An additional 4.17% of the results are associated with MS/MSD results where the precision did not meet the acceptance criterion of 40% for the RPD. In all those instances, the actual MS/MSD recoveries met the acceptance criteria, but the RPD between the recoveries in that MS/MSD pair did not meet the precision criterion.

## Section 4.5 Other Quality Control Checks

As part of the data review effort, the data reviewers examined all of the other QC results generated during the analyses, which included results for laboratory control samples (LCS) and calibration verifications (VER). the data reviewers compared these QC results to the acceptance criteria in the analytical QAPP and, when appropriate, flagged the associated sample results using qualifiers shown in Table 5. As with the QC results described above, there were generally fewer data quality issues with these other QC checks for the pharmaceuticals analyzed by LC-MS/MS than for the personal care products analyzed by GC-MS/MS. For example, there were no instances of low LCS results for pharmaceuticals, while LCS and VER issues were associated with 5.56% of the qualified data for the personal care products. Pharmaceutical results associated with calibration verifications that did not meet the acceptance criteria accounted for about 1.57% of the results (1.22% high and 0.35% low), and the personal care products had 2.31% of the results similarly affected (all low).

Given the relatively low occurrences of the qualifiers for the LCS and VER, particularly for the pharmaceutical analyses, this section of the report does not present pie charts similar to those in Figures 2 through 7. However, Appendix I reports the percentages of affected results for each analysis type.

## Section 4.6 Overall Data Quality Assessment

The approach that EPA used to review the data is designed to maximize the amount of useful data, while maintaining a high degree of transparency in the process. The end result is that EPA applies a data qualifier flag to a measurement result when one aspect of the analytical process does meet the QA acceptance criteria to advise data users. However, many of the data qualifier combinations in Appendix I

also include the "RNAF" qualifier, indicating that the measurement result is not affected by the QA concern. Common, readily understandable examples include instances where an analyte is found in a method blank at a very low level, and the sample result for that analyte is much higher (see Section 4.2), or when the recovery of a spiked analyte is higher than expected, but analyte is not detected in the unspiked sample (see Section 4.4).

Table 6 illustrates the breakdown of analytes and samples across the two types of analyses (pharmaceuticals, and personal care products). EPA, Baylor, and Tetra Tech ultimately abandoned attempts to analyze the fish liver samples for the personal care products, therefore, that cell in Table 6 is blank.

	Tissue Type	
Analyte Class	36 Fillets	36 Livers
24 Pharmaceuticals	864	864
12 Personal Care Products	432	
Total	1296	864
Grand Total		2160

 Table 6. Sample/Analyte Combinations

Overall, approximately 93% of the pharmaceutical results either had no qualifiers at all (68.68%) or had all qualifiers that were applied and also include the "RNAF" code which signifies that the results were not affected (24.08%). Approximately 2% of the pharmaceutical results were excluded. The excluded results represent the decision to not pursue analysis of miconazole in the liver samples because of analytical problems associated with the high lipid content of the liver samples.

In contrast, approximately 34% of the personal care product results either had no qualifiers at all (18.76%) or had all qualifiers that were applied and also include the "RNAF" code which signifies that the results were not affected (15.28%). Also, based on pervasive problems with the levels of two personal care products observed in the laboratory method blanks, all the results for benzophenone and octocrylene were excluded. These two analytes account for 16.67% of the results for the personal care products. Considering all the analytes and samples in both analysis types, over 81% of the results had no data quality issues that affect their use in meeting EPA's study objectives. Appendix I provides additional details regarding the frequency at which each flag or flag combination was applied to the study results.

## Section 4.7 Completeness

Completeness is a measure of the amount of data that are collected and deemed to be acceptable for use the intended purpose. The sample collection QAPP (USEPA, 2006a) and the analytical activities QAPP (USEPA, 2006b) for the pilot study identified three measures of completeness:

Sampling Completeness:	The number of samples collected relative to the number of samples planned for collection
Analytical Completeness:	The number of valid sample measurements relative to the number of valid samples collected and
Overall Completeness:	The number of valid sample measurements relative to the number of samples planned for collection

The completeness goal in this study was to obtain valid measurements from 95% of the samples planned for collection.

#### 4.7.1 Sampling Completeness

The sample collection QAPP (USEPA, 2006a) states that "the completeness goal is achieved when five effluent-dominated sites and one reference quality site found to contain target fishes are sampled, and the fish tissue samples are shipped with no errors in documentation or sample handling procedures."

Despite the challenges encountered by the sampling team, particularly at the remote reference site, samples were collected at all 6 sites as planned, (yielding 36 fillet composites and 36 liver composites). Therefore, , sampling completeness was 100%.

#### 4.7.2 Analytical Completeness

For multi-analyte methodologies, analytical completeness is best calculated on the basis of the number of possible sample/analyte combinations. Otherwise, a problem with a single analyte could be seen as invalidating an entire field sample.

For the pilot study, there were 36 fillet composites and 36 liver composites, for a total of 72 samples. There were 24 pharmaceutical analytes and 12 personal care product analytes. Thus, the number of planned sample/analyte measurements was  $2592 (72 \times 36)$ .

However, as noted elsewhere in this report, there were significant difficulties with the analysis of the liver samples for the personal care products. Therefore, EPA made an informed decision not to pursue the liver analyses for the personal care products. For the purposes of this report, the total number of planned observations is 2160, as shown in Table 6 above.

Baylor reported the results for miconazole in the liver samples as "NA" for not analyzed. However, that decision was based on significant analytical issues for this one pharmaceutical that affected data quality. During the data quality assessment, these 36 miconazole results from the liver samples were excluded from the data set. Similarly, as noted in Section 4.6, all 36 results for two personal care products, benzophenone and octocrylene, were excluded from the data set (72 results in total). Therefore analytical completeness was calculated based on 2052 valid sample/analyte combinations out of a possible 2160, which is 95%.

#### 4.7.3 Overall Completeness

Overall completeness can be calculated as the product of the sampling completeness and the analytical completeness. Overall completeness for this study is 95% (e.g., 100% sampling completeness times 95% analytical completeness). Thus, EPA met its completeness goal.

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# Appendix I

# Unique Qualifier Combinations Applied to Pilot Study Data by Analytical Method

SCC Code	% of Results	% Not Affecting Data Quality
B, RMAX	0.06	
B, RNAF	10.76	10.76
B, RNAF; HMSR	2.43	
B, RNAF; HMSR, RNAF	1.39	1.39
B, RNAF; HMSR, RNAF; HSSR, RNAF	0.23	0.23
B, RNAF; HMSR; HSSR	0.35	
B, RNAF; HMSR; HSSR; HVER	0.23	
B, RNAF; HMSR; HVER	0.12	
B, RNAF; HSSR	0.23	
B, RNAF; HSSR, RNAF	0.12	0.12
B, RNAF; HSSR, RNAF; LVER	0.06	
B, RNAF; LVER	0.29	
EXC	2.08	
HLCS, RNAF; HSSR, RNAF	0.35	0.35
HLCS, RNAF; HVER, RNAF	0.12	0.12
HMSR, RNAF	4.51	4.51
HMSR, RNAF; HSSR, RNAF	0.41	0.41
HMSR, RNAF; HSSR; RNAF; HVER, RNAF	0.17	0.17
HSSR, RNAF	5.21	5.21
HSSR, RNAF; HVER, RNAF	0.35	0.35
HVER, RNAF	0.46	0.46
LMSR	1.39	
No qualifier codes	68.68	
No qualifier codes, plus those not affecting data quality	92.76	

Table A-1. Unique Qualifier Combinations Applied to Pharmaceutical Data

The codes are listed in this table in alphabetical order. A detailed explanation of each code can be found in Table 5. The frequencies are based on a total of 1728 sample/analyte combinations for this analysis type. All frequencies are rounded to two decimal places.

If all of the data qualifiers in a particular combination of codes are followed by the "RNAF" code, then the data quality of the results for that sample/analyte combination is not affected, and the frequency appears in the third column as well.

For HPLC-MS/MS analyses, 68.68% of the results were not qualified at all (e.g., the "SCC Code" field in the database is blank). An additional 24.08% of the results had the "RNAF" code for each qualifier, and therefore, the net effect of the qualifiers did not affect data quality.

SCC Code	% of Results	% Not Affecting Data Quality
B, RNAF; LSSR; REXC; RPDX	0.23	
B, RNAF; LSSR; RPDX	1.85	
B, RNAF; RPDX	0.69	
EXC	16.67	
HMSR	0.46	
HMSR, RNAF	1.39	1.39
HMSR; HSSR	0.93	
HSSR	0.93	
HSSR, RNAF	13.89	13.89
HSSR, RNAF; LLCS	2.31	
HSSR, RNAF; LMSR	1.39	
HSSR, RNAF; LMSR; LVER	0.93	
HSSR, RNAF; LVER	1.39	
LLCS	1.39	
LLCS; LMSR	0.46	
LLCS; LMSR; LSSR	2.31	
LLCS; LSSR	1.39	
LMSR	1.16	
LMSR; LSSR	3.47	
LSSR	26.62	
LSSR; REXC; RPDX	0.46	
LSSR; RPDX	0.69	
RPDX	0.23	
No qualifier codes	18.76	
No qualifier codes, plus those not affecting data quality	34.04	

Table A-2. Unique Qualifier Combinations Applied to Personal Care Product Data

The codes are listed in this table in alphabetical order. A detailed explanation of each code can be found in Table 5. The frequencies are based on a total of 432 sample/analyte combinations for this analysis type. All frequencies are rounded to two decimal places.

If all of the data qualifiers in a particular combination of codes are followed by the "RNAF" code, then the data quality of the results for that sample/analyte combination is not affected, and the frequency appears in the third column as well.

For GC-MS/MS analyses, 18.76% of the results were not qualified at all (e.g., the "SCC Code" field in the database is blank). An additional 15.28% of the results had the "RNAF" code for each qualifier, and therefore, the net effect of the qualifiers did not affect data quality.