PSCI PIE&AMR TEAM

Sampling and Analysis of Pharmaceutical Industry Wastewater for Active Pharmaceutical Ingredients

The following document provides guidance on potential techniques, methodologies, and available data sources. The correct approach to follow is dependent on specific aspects of the risk assessment to be conducted. The PSCI does not advocate a single correct approach or data source, but aims to provide information to help risk assessors design assessments suitable for their requirements.

Section 1. Introduction

Measuring the Active Pharmaceutical Ingredient (API) content in pharmaceutical industry wastewater is another risk assessment tool for evaluating discharges from manufacturing. The data can be used to supplement API losses estimated by mass balance methods (see section 4.4.1 and Appendix A1 in section 8).

There are some challenges with generating meaningful data when sampling pharmaceutical wastewater, so this document is designed to give the user some practical guidance when developing sampling and analytical plans, and it offers guidance for evaluating analytical results.

The principals and procedures described in this guidance are not substitutes for any of the specific sampling and analysis provisions required by regulatory authorities.

Section 2. Sampling Plan

A well-designed wastewater sampling plan will ensure that representative samples are collected. Choosing the sample location, sample type/sampling equipment, number of samples and sample dates will depend on several factors, including:

- API of interest
- Sampling objectives
- Analytical target and Data Quality Objectives
- Site production schedule
- Wastewater discharge temporal variations and residence times in collection/treatment systems

2.1 Pre-Planning

Understanding the general production process flow for the API of interest is paramount. A process flow diagram showing the point of generation (POG) and fate (wastewater treatment, off-site incineration, recovery, etc.) for all liquid losses will help determine what, where, how and when to sample.

The sampling objective should be clearly understood. Wastewater samples are typically collected to help quantify API losses associated with a production line or specific unit operation. When quantifying API losses for an entire production line, sampling the total wastewater discharged from the site or a building is most common. Although not always practical, sampling a dedicated process wastewater line (no sanitary or utility wastewater) is desired to minimise analytical matrix interferences. Sampling a specific unit operation at the POG is useful in distinguishing high API waste streams from low API waste streams. Understanding the relative strength of API-containing waste streams can help drive targeted control strategies.

The analytical target (detection limit) and data quality objectives (DQOs) should be established with the contract laboratory well in advance of sampling. For wastewater sampling purposes, the DQOs and analytical detection limits will help determine how much (volume) and how many samples should be collected for analysis. Guidance on setting analytical detection limits and DQOs is provided in more detail in Section 3.

Given the typical campaign operation of pharmaceutical manufacturing, aligning wastewater sampling with the production schedule can be challenging. Know the schedule well in advance and be prepared for changes. Special emphasis may be needed on short-run campaigns where opportunities to sample may be limited.

Typical batch operations in pharmaceutical production usually generate wastewater discharges that are temporal in nature (i.e. equipment cleaning). Understanding when and where in the process the target API wastewater discharges occur will help establish a sampling timeframe. Include the residence times of wastewater collection and wastewater treatment systems when establishing sample dates.

2.2 Choosing a Sample Location

Choosing the sample location will depend on the sampling objective. Targeting specific production processes at or near the point of generation (POG) or quantifying API in the total wastewater discharged from the site are the two basic scenarios.

A challenge when sampling at POGs is accessibility. There may be access restrictions due to GMP protocol or there may be no simple means to divert targeted waste streams to a sample collection point. Use of totes/IBCs and temporary piping may be necessary, and this could be disruptive to the normal production process. Careful planning with production personnel is necessary.

When sampling at the POG does not fit the sampling objective or when it is not practical, sampling the total wastewater discharge from the site is an option. Accessibility becomes less of an issue, particularly for sites required to collect routine samples required by permit or license. The challenge with this scenario is with the laboratory analysis because a total wastewater effluent sample is more

complex, and it can present more matrix interferences that could impact analytical detection limits (see section 3). On the other hand, dilution means you may need a more sensitive analytical method.

Choose a location that meets the sampling objective and is the least intrusive to production operations.

2.3 Choosing Sample Type/Sampling Equipment

There are two types of wastewater samples: grab or composite.

A grab sample is a single sample collected over a short period of time (usually instantaneously). Analysis of a grab sample will indicate the characteristics of the wastewater sampled at a location and point in time. It cannot usually be extrapolated to longer averaging times.

Grab samples are useful and typically most practical when collecting at the point of generation (POG) in the production process, and when a wastewater discharge occurs over a short period of time (i.e. equipment cleaning), or where routine discharges have quality criteria (i.e. an aqueous mother liquor). However, it is important to ensure that a grab sample is representative of the entire discharge. In cases where it is not, collecting a series of grab samples to form a composite is acceptable.

Composite samples are intended to represent the composition of a wastewater over a specified averaging period (e.g., typically 24-hours). There are two types of composite sample:

- *Flow-weighted* – the composite consists of multiple grab samples collected during the averaging period and whose volume added to the composite sample is calculated based on the wastewater flow at the time that the grab sample was collected.
- *Time-weighted* – grab samples collected at specified time intervals during the averaging period are added in equal volume to the composite sample.

In general, flow-weighted composites are the preferred method for sampling continuous wastewater discharges because they usually provide the most representative sample. However, flow-weighted composite samples require special equipment that may not be readily available or practical, unless there is an existing permit or license requirement. In cases where it is possible to collect a flow-weighted composite sample, collecting a time-weighted composite sample is acceptable.

Sample collection can be performed either manually or automatically. The decision as to the type of sampling equipment to use is generally site-specific and depends upon the type of samples required to meet the project objectives and the planned duration of the sampling program (e.g., routine monitoring versus one-time sampling). Programmable automatic samplers that can collect either grab samples or composite samples simplify sample collection and minimise the amount of manual intervention.

The following principles should be considered when selecting sampling equipment:

1. Physical conditions for obtaining the samples – Accessibility to the sampling point is typically more challenging when sampling at the POG, especially when working in GMP areas or where there is no convenient means to divert targeted waste streams to a sample collection point. The use of totes/IBCs and temporary piping may be necessary

2. Volume of sample required for all specified analyses – The analytical lab requirement is typically small (<1 litre), but it is important to collect a large enough sample that is representative of the entire discharge; size collection equipment appropriately.
3. Compatibility of sample containers with the type of analyses to be performed – Glass amber bottles are preferred to minimise API adherence to sample container walls and to minimise photolysis; establish specific requirements with the contract laboratory.
4. Requirements for preservative addition and holding times – Most samples must be preserved to prevent changes in chemical composition between the times of sampling and analysis. Maximum holding times for preserved samples should also be established to assure that the analysis is conducted before chemical composition changes occur in the stored samples. The required preservation and holding times for samples collected will either be supplied by the approved analytical method or determined when a method is developed using your sample matrix.
5. Sample refrigeration – provisions should be made to keep samples cool during collection (composite samplers), during interim storage and during shipping.
6. Programming capabilities of automatic samplers – ideally capable of collecting flow-weighted samples when flow monitoring can be integrated.

In summary, when choosing sample type and sampling equipment:

- Collecting representative samples is required
- Choose sample type depending on the project objective and wastewater discharge duration
- Use grab samples typically at POG and/or when discharge duration is short
- Use composite samples typically when total wastewater effluent from the site is sampled
- Composite samples are usually preferred (most representative)
- Automatic samplers offer the most flexibility and minimal manual intervention
- Establish sample container type, sample volume, preservatives, holding times and packaging/shipping requirements with the contract laboratory

2.4 Determining Number of Samples

The number of samples to collect will depend on several factors: project objectives, the nature and frequency of the wastewater discharge, the duration of the discharge and the residence time through the wastewater collection system and treatment plant, where applicable.

When sampling at or near the point of generation, sample sets should be defined based on the nature and discharge frequency of the process operations. A typical POG sampling scenario involves collecting equipment cleaning samples. Wastewater discharges from equipment

cleaning will vary widely in volume and duration depending on equipment size and cleaning methodology (manual vs. clean-in-place). Also, the discharges may vary from batch-to-batch, so consider collecting multiple samples.

When sampling wastewater effluent, collecting a minimum of three consecutive daily composite samples is recommended. In some cases, consider extending sampling to more than three days if discharges to wastewater occur over an extended period. Conversely, smaller sample sets may be justified given site-specific conditions, such as batch operations where all activities (including

equipment cleaning) may occur on one day. However, a larger dataset is usually more desirable because it can capture variability and peak discharges.

2.5 Establishing Sample Dates

Establish sample dates based on the production schedule for each API of interest, the process knowledge predicting process steps with API losses, the nature and duration of the discharge (batch vs. continuous), and the residence time through wastewater collection systems and treatment plants, where applicable.

Sampling dates should be directly linked to the discharge activity and its duration. Collecting samples at the POG should be straightforward. Often these discharges are of a batch nature and short duration, so timing the collection with production personnel is critical. Otherwise, a sampling opportunity could be missed or samples not representative of the actual discharge could be collected.

Sampling dates at the site wastewater effluent involves a little more planning. It is still important to understand the timing of API-containing discharges associated with the process, but when to start and stop collecting daily composites depends on the residence time through wastewater collection system and treatment plant if sampling downstream from the POG. For process operations where there is a primary source of API-containing wastewater (equipment cleaning), start sampling when the activity is expected to occur and ensure that the sampling event is long enough to account for any residence time. For example, if cleaning starts on Monday and the residence time is 24 hours, the recommended 3-consecutive day sample period would be adequate. Consider extending sampling to more than 3 days if equipment cleaning activities or other discharges to wastewater occur over a period of several days.

2.6 Recordkeeping Considerations

Maintaining complete records is very important. When samples are collected and how they are handled until receipt by the lab is typically captured on a chain-of-custody form provided by the contract lab. It will typically provide:

- The sample identification number.
- The container description (material, volume).
- The analyses to be performed on the sample.
- Any preservatives added to the sample.
- Any special instructions for sample handling or analysis.
- The date, time, and signature of everyone that is responsible for and has possession of the sample, beginning with the individual collecting the sample and ending with the individual at the laboratory that takes custody of the sample.

If not using a standard chain-of-custody form, maintain a sampling log that captures the information above. In the sampling log, it is also recommended that production activities generating wastewater for the API of concern are recorded, and that wastewater flow rates (either at the POG or total site discharge) are recorded so that mass discharge rates can be calculated.

Section 3. Analytical Plan

The combination of low PNECs and lack of standard analytical methods to measure APIs in wastewater presents a challenge.

It is not typical to monitor APIs in wastewater unless there is a permit or license requirement. The limited regulatory framework means that few commercial labs have the capacity or expertise to test API in a complex wastewater matrix at the low concentrations typically needed (ng/L) to make meaningful risk assessments. Internal Quality Control labs can test for API but typically in a clean matrix and at a high method detection limit (mg/L).

Given these limitations, it is often necessary to partner with a lab (commercial or academic) to develop analytical methods sensitive enough to measure an API concentration that would result in a PEC lower than the PNEC based on site specific flow rates and receiving water dilution factors. It doesn't necessarily mean that the analytical method detection limit must be less than the PNEC.

Determining which analytical method is most appropriate should be discussed with the laboratory. It is also important to understand the quality assurance/quality control (QA/QC) specifications for analytical methods that will be used, detection and quantitation limits, and matrix interferences.

3.1 Analytical method selection

Analytical method selection involves, at a minimum, selecting methods that meet the following:

1. The quality control (QC) tests in the method must be an integral part of the method;
2. The QC acceptance criteria in the method must be part of the method; and
3. The method detection limit (MDL) should be at least one third (1/3) the concentration limit being targeted.

The concentration limit being targeted can be calculated from the PNEC value and dilution factors. For example, if measuring API in total site wastewater effluent discharging directly to a surface water with a dilution factor of 50 and a PNEC of 0.01 mg/L, an MDL of 0.2 mg/L would be appropriate (0.01 mg/L × 50 × 0.33). Note that there may be instances where an MDL of 1/3 of the concentration limit is not achievable. These instances should be handled on a case by case basis.

3.2 Detection and Quantitation Limits

Detection and quantitation limits are essential components of an analytical method. Many different names are given to detection and quantitation limits by the different organizations that develop analytical methods. However, all of them can be simplified into two basic definitions:

- A *detection limit* is the lowest concentration of a substance that can be identified in a sample matrix. The concentration is so low that the concentration of the chemical present in the sample is uncertain and cannot be reported with acceptable accuracy.
- A *quantitation limit* (also called quantification limit, LoQ) is the lowest concentration of a substance in a sample matrix that can be measured at a specified level of precision (e.g., ± 30%).

The quantitation limit for a substance in a sample matrix is always greater than the detection limit for that substance in the same matrix.

The difference between detection limits and quantitation limits is very important. At a quantitation limit, there is a much lower chance of a false positive measurement (i.e., reporting a substance as present when it is not) than there is at a detection limit. While not always possible, it is best to assure that the quantitation limit is enough for determining whether a PNEC value is being met.

3.3 Quality Assurance/Quality Control

Discuss Data Quality Objectives (DQOs) with the lab so that they can integrate quality assurance/quality control (QA/QC) measures into the analysis. There are several QA/QC measures that can be used to interpret the quality of the laboratory data.

3.3.1 Analysis of Spikes

The term spike refers to a known quantity of a target analyte that is added to a sample before analysis. The recovery of a spike from a sample (expressed as a percent of the spike concentration) is a measurement of the accuracy of the analysis. Accuracy is defined as how close a measurement is to the true concentration of the target analyte in a sample. The lab should establish a range of recoveries that is acceptable. Sometimes, spikes before sampling to cover the whole process (preservation, sampling, cooling, etc.) make sense. Also, field blanks should be taken.

3.3.2 Analysis of Duplicates

Duplicate analyses are used to evaluate precision, which is the variance in measured concentrations. Discuss with the lab what duplicates analysis is appropriate. If field duplicates are desired, then that should be programmed into the sampling plan. Otherwise, the lab can perform method or instrument duplicates on random samples that are received provided that enough sample volume is collected. The lab should establish a precision range that is acceptable.

3.3.3 Analysis of Blanks

A blank is a sample that should be completely free of the target API. The objective of the blank is to detect contamination and/or interference problems, or to document their absence. As with duplicate samples, blanks can be introduced at various points in the sampling and analytical process. There are several types of blanks commonly used: trip, field, equipment, method, instrument. If field or equipment blanks are desired, then that should be programmed into the sampling plan. At the very least, field blanks should be considered for analysis.

3.3.4 Analysis of Standards

Standards are used to assess instrument calibration and method performance. Most instrumental test methods require analysis of calibration standards every day the instrument is used, and one or more

check standards are processed with every batch of samples analysed. The lab will typically prepare the standards and it will establish acceptance criteria.

3.3.5 Matrix interferences

Both the physical properties and chemical composition of a sample can influence the ability of an analytical method to measure a target analyte. Typically, matrix interferences will cause poor precision, poor recovery, and/or elevated MDLs and quantitation levels in a sample. If the interference is severe, the method may be unable to achieve the method performance requirements.

Matrix interferences most often occur in complex samples, and particularly in untreated and/or partially treated process wastewaters. Dilution of the sample is one approach to remove high concentration interferences, but it can elevate detection limits to concentrations that exceed target values. Most analytical methods include procedures that laboratories can implement to try to reduce matrix interferences. Be sure to identify samples where there is a higher risk of matrix interferences.

Section 4. Data Evaluation

How the data will be used will vary depending on the project objective. Are total API losses from the site being quantified? Are select processes being targeted to isolate and control a part of the API loss?

No matter the case, and as a first step, use the maximum measured API concentration in your risk analysis. If this worst-case condition indicates that the PEC is less than the PNEC, generally no further action is required. If the PEC is greater than the PNEC under these worst-case conditions, additional statistical analysis of the sample results should be performed to determine the appropriate indicator value for the risk assessment. For example, it may be appropriate to average the sample results. Also, if additional treatment occurs downstream of the discharge, it may be appropriate to perform modelling of the treatment system.

It is not uncommon to see API concentrations measured in wastewater that result in Predicted Environmental Concentrations (PECs) lower than those derived from mass balances, especially when conservative assumptions are made in the mass balance analysis. An order of magnitude difference should not be an alarm. When there is a big difference between the two methodologies, re-examine mass balances, validate the representativeness of the samples collected and consider additional wastewater testing.

Wastewater Sampling & Analytical Plan Checklist

For recordkeeping purposes, consider using this checklist to capture details for each sampling event.

Wastewater Sampling Plan	
API of Interest	Click here to enter text.
Sampling Objective	Click here to enter text.
Process Flow Diagram	Click here to enter text.
Sample Location	Click here to enter text.
Sample Type	Click here to enter text.
Sample Equipment	Click here to enter text.
Number of Samples	Click here to enter text.
Sample Dates	Click here to enter text.
Recordkeeping Log Content	Click here to enter text.
Analytical Plan	
Lab Name	Click here to enter text.
Analytical Method	Click here to enter text.
Method Detection Limit	Click here to enter text.
Method Quantification Limit	Click here to enter text.
Sample Volume Required	Click here to enter text.
Sample Container Type	Click here to enter text.
Sample Preservative	Click here to enter text.
Holding Time	Click here to enter text.
Quality Control Measures	☐ Spikes ☐ Duplicates ☐ Blanks ☐ Standards
Quality Control Details	Click here to enter text.