ESTIMATION OF PHARMACEUTICAL CONCENTRATIONS IN WASTEWATER USING THE DATA VISUALISATION TOOL

Factors affecting measured and predicted influent pharmaceutical wastewater concentrations.

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Executive Summary

The ability to predict pharmaceutical concentrations in influent wastewater (and then in the environment) using prescription data can fill existing monitoring information gaps and prioritise locations and pharmaceuticals for targeted monitoring or intervention. Existing studies have found poor agreement between measured pharmaceutical concentrations in influent wastewater and those predicted using prescription data. A review of the literature was carried out to identify how predicted pharmaceutical concentrations using prescription data can be improved, and what information needs added to the Data Visualisation Tool to support this. A review of CIP monitoring approaches and literature was also undertaken to establish how accurate pharmaceutical concentrations can be obtained, to validate the predicted concentrations.

The recommendations for information to be included in the Data Visualisation Tool to enable prediction of pharmaceutical concentrations in influent wastewater are:

1. Total human excretion % (urinary and faecal) of pharmaceuticals and their major metabolites.
2. Degradation rates of pharmaceuticals and metabolites during sewer transport.
3. Average sewer residence time for WWTPs.
4. Average daily flows of wastewater.
5. Theoretical population figures for WWTPs (household only).
6. Ammonium concentrations determined in 24 h influent composite samples.

This information can then be used to predict pharmaceutical concentrations using the following equation:

$$Influent = \frac{Prescription \times CF_{population} \times F_{excretion} \times CF_{sewer} \times 10^6}{Flow \times d}$$

The equation utilises monthly drainage operational area prescription data (*Prescription*), real-time population estimates using ammonium in wastewater ($CF_{population}$), pharmaceutical excretion ($F_{excretion}$), and sewer degradation data (CF_{sewer}), and WWTP flow information (*Flow*). This can be used to estimate the average concentration of pharmaceuticals over a month, or the average daily concentration on individual days within that month. However, it should be noted that there is a lack of information on the transformation of pharmaceuticals and their metabolites in sewers to utilise literature data and would need determined experimentally to be incorporated. There is also notable variability in excretion data for some pharmaceuticals and a lack of metabolite excretion data that needs considered.

The recommendations for analysis and sampling (which differ from past CIP monitoring requirements) to obtain pharmaceutical concentrations directly comparable to the predicted concentrations are:

1. To sample in a discrete flow proportional manner over 24 hours with a sub-sample collection frequency of 15 min and cooled to 4 °C during collection.
2. To analyse the parent pharmaceutical and at least one major metabolite.
3. To determine the total pharmaceutical concentration (sum of liquid and particulate phase concentrations).

To validate the approach of predicting pharmaceutical concentrations using prescription data it is recommended to collect five random samples from a single WWTP within a single calendar month. This should be repeated at a total of three WWTPs comprising low, medium, and high household population numbers. The validation study could be completed without any changes to the Data Visualisation Tool to first establish the accuracy of predicted pharmaceutical concentrations in influent wastewater and the optimum way of utilising the pharmaceutical excretion information due to its variability. Assuming acceptable accuracy is achieved, the Data Visualisation Tool could then be used to automatically predict influent concentrations of pharmaceuticals upon entry of wastewater flow and ammonium data at WWTPs on interest.

Contents

1.0 Introduction

The One Health Breakthrough Partnership (OHBP) have developed the Data Visualisation Tool which is the first open access interactive tool in the UK to combine national environmental and prescribing data for pharmaceuticals (SEPA and OHBP 2023). Ideally, medicine prescription data could be used to accurately predict pharmaceuticals concentrations in wastewater (and then in the environment). Such an approach could fill data gaps where no monitoring data exists in Scotland, or be used to prioritise locations for further monitoring or intervention. This requires knowledge that these predicted concentrations are accurate by validating with measured pharmaceutical concentrations. Previous studies have found poor correlations between predicted and measured concentrations (**Table 1**). More than 50 % of the studied pharmaceuticals had correlations out with a two times concentration difference, with a considerable number having more than 10 times concentration difference. Poor agreement between measured and predicted concentrations is due to several factors. These factors lie in both the data used to predict pharmaceutical concentrations in wastewater as well as the monitoring used to assess the accuracy of those predicted concentrations. Therefore, a better understanding of what factors result in these poor correlations, and what is needed to improve their agreement is required.

Table 1. Examples of literary comparisons of predicted concentrations to measured concentration.

Sample (sub-sample collection frequency)	Annual	Population	Storage	Correlation[a]	Reference
24-hour time-proportional composite (10 min)	1 day	59,400 63,000 58,500 43,200 54,900	-20°C	1≤x<2= 40% 2≤x<3= 23.3% 3≤x<5= 10% 5≤x<10= 11.7% x≥10= 15% n=60 pharmaceuticals	Van Nuijs et al. 2015
24-hour time-proportional composite (20 min, pooled to create pseudo flow-proportional)	5 consecutive days	1,619,602 151,076	4°C autosampler and transport -20°C in laboratory	1≤x<2= 38.7% 2≤x<3= 21.3% 3≤x<5= 14.7% 5≤x<10= 6.7% x≥10 = 18.7% n=75 pharmaceuticals	Casas et al. 2021
24-hour time-proportional composite (60 min)	4 consecutive days	120,000	4°C autosampler and transport -20°C in laboratory	1≤x<2= 30% 2≤x<3= 0% 3≤x<5= 20% 5≤x<10= 10% x≥10 = 40% n=10 pharmaceuticals	Verlicchi et al. 2014

[a] – Largest/Smallest for measured and predicted concentrations

1.1 Aims and Objectives

Aim: To provide guidance on the Data Visualisation Tool and CIP 4 Scotland monitoring approaches to support the use of pharmaceutical prescription data to estimate influent wastewater pharmaceutical concentrations.

Objectives:

1. To identify literature data requirements for the Data Visualisation Tool to help estimate pharmaceutical concentrations in influent wastewater (**Part A**).

2. To examine sampling processes and data requirements from sampling that are needed to better assess the alignment of measured pharmaceutical concentrations with predicted concentrations based on prescription data (**Part B**).

A review of the literature and CIP sampling approaches was carried out to identify sampling requirements needed for better alignment of measured pharmaceutical concentrations and predicted concentrations using prescription data. Additional data requirements from sampling (wastewater flows etc) as well as the literature to be included in the Data Visualisation Tool (% excretion of unchanged pharmaceuticals following human metabolism etc) were identified. A simple algorithm to estimate pharmaceutical concentrations in wastewater and the environment based on the recommended data requirements is outlined for future use. Requirements of a validation study to establish the accuracy of predicted pharmaceutical concentrations in wastewater is provided (**Part C**).

Part A: Data Visualisation Tool requirements

2.0 Excretion information

A portion of a consumed pharmaceutical is excreted unchanged. To enable the prediction of pharmaceutical concentrations in influent wastewater the excretion rate of each pharmaceutical needs included in the Data Visualisation Tool. The inclusion of major metabolite excretion rates (and their analysis) can help corroborate the findings of the parent pharmaceutical as well as help quantify any direct disposal within the sewer network. Most studies use urinary excretion information for back calculations. Parent compounds and metabolites excreted in faeces is a relevant factor that requires considering as these compounds can still partition out and into the aqueous phase. It is unlikely that the excreted distribution of pharmaceuticals between urine and faeces will reflect that of the aqueous (e.g., filtered) and particulate phases of wastewater. For example, Casas et al. (2021) found that including faecal excretion rates improved the correlation of 19 out of 21 studied pharmaceuticals between predicted and measured wastewater concentrations.

Recommendation: All pharmaceuticals to be monitored in CIP4 Scotland as well as their major metabolites have their total excretion % (sum of urinary and faecal excretion) included in the Data Visualisation Tool. Available literature data on selected pharmaceuticals has been compiled in Appendix 1.

3.0 Pharmaceutical and metabolite transformation in sewers

Pharmaceuticals entering wastewater can undergo biotransformations between source and sampling point (during sewer transport), as well as deconjugation of glucuronide metabolites back to the parent compound (Choi et al 2020). The is due to the presence of biofilms on the inner surface of pipes. Although a lack of data exists on the stability of pharmaceuticals during sewer transport, available data suggests that parent pharmaceuticals are relatively stable during this process. For example, Choi et al. (2020) found that carbamazepine, ibuprofen and 11 other compounds were stable in a simulated gravity fed sewer reactor for up to 12 hours. However, there is very little information on the fate of pharmaceutical metabolites in sewers (including antibiotics). A considerable dose of a pharmaceutical (>50 %) is often metabolised in the body to glucuronide conjugates which increases the polarity of the compound and aids excretion in urine. These glucuronides do not tend to be stable in wastewater. Glucuronide metabolites are likely to undergo in-sewer and in-sample deconjugation back to the parent compound which can cause discrepancies between measured and predicted concentrations (Gao et al. 2021). Assuming that glucuronide metabolites fully deconjugate back to the parent compound may not be accurate. Gao et al. (2017) found that up to 80% of glucuronide metabolites of morphine and codeine were degraded after 2 hours and almost 100% after 6 hours, of which between 15-40% of the glucuronides converted back to the parent compound.

Recommendation: To investigate the degradation of pharmaceuticals included in CIP 4 Scotland (as well as their metabolites) in pilot-scale gravity sewers and incorporate their degradation rates in the Data Visualisation Tool. Available data is provided in Appendix 2. The likelihood of glucuronide metabolites partially deconjugating back to the parent compound adds an additional layer of complexity. However, it needs investigated and accounted for to improve the accuracy of estimated concentrations. Utilising this in prediction calculations would require an estimation of the average sewer residence time for drainage operational areas of interest. If the sewer residence time is not available it is possible to assume times of 1 h, 6 h and 12 h for small, medium, and large drainage operational areas (Guo et al. 2022).

4.0 Site specific data

4.1 Wastewater flow

The conversion of a pharmaceutical load (prescription data) to a wastewater concentration (e.g., µg L^{-1}) requires the flow of wastewater to be available in the Data Visualisation Tool.

Recommendation: To include the average daily flow of wastewater (L d^{-1}) per calendar month in the Data Visualisation Tool. This will enable the average predicted pharmaceutical concentrations in influent wastewater during that month to be calculated, which will align with the prescription data (pharmaceutical prescription in drainage operational area per month). Alternatively, flow data from individual days can be included in the Data Visualisation Tool and average pharmaceutical concentrations for each day of the month included. Including flow data for all WWTPs is unrealistic therefore it is recommended to do this for sites of greatest interest initially (e.g., lowest effluent dilution ratios), once the accuracy of predicting pharmaceutical concentrations can be ascertained.

4.2 Ammonium concentrations

The prediction of pharmaceutical concentrations in wastewater assume that all medicines prescribed within a drainage operational area are then excreted within that area. However, people are likely to move between drainage operation areas

for work etc. Real-time population numbers are likely to deviate from local census data, contributing to the uncertainty in the comparison of prescription data to wastewater concentrations (Castiglioni et al. 2013). Therefore, it is important to account for the movement of people as incorrect population figures have been estimated to account for between 7-55% error in such studies (Castiglioni et al. 2013). Authors report that this can be done most accurately using mobile device usage or ammonium concentrations/loads in wastewater (Lin et al. 2019; Baz-Lomba et al. 2019; Thomas et al. 2017). However, obtaining data on mobile device usage is particularly challenging. Ammonium is useful to predict the contributing population as it is the main form of ammonia in wastewater, which is hydrolysed from urea in water (Lin et al., 2019). It is less affected than other water quality measures (e.g., biological oxygen demand) by non-human activities.

Recommendation: Theoretical population figures need included in the Data Visualisation Tool. WWTP population equivalents (PE) included in the Annual Return submitted to the Water Industry Commission for Scotland include household PE, non-household PE, tourist PE and trade effluent PE. Only the household PE should be used in the Data Visualisation Tool to reflect the population which the prescription data relates to. Inclusion of ammonium concentrations similar to the flow data (i.e., daily average concentration per calendar month) can then be used to calculate the actual population contributing wastewater. This can then be used to account for the movement of people in and out of the drainage operational area. This assumes that there is little difference in prescription in the locations where people are moving between and these people are representative of that population (which is unlikely to be the case for small numbers).

5.0 Equation to predict pharmaceutical concentrations

Influent wastewater concentrations can be predicted using the simple algorithm expressed in the equation below:

$$Influent = \frac{Prescription \times CF_{population} \times F_{excretion} \times CF_{sewer} \times 10^6}{Flow \times d}$$

This equation incorporates the prescription data, population correction factor, fraction of pharmaceutical excreted unchanged, in-sewer biotransformation correction factor as well as wastewater flow data. The parameters of the equation, their description and associated units are in **Table 2**.

Table 2. Explanation of parameters in the equation used to predict influent wastewater concentrations.

Parameter	Description	Units
Influent	Pharmaceutical concentration in influent wastewater	µg L^{-1}
Prescription	Pharmaceutical prescription in drainage operational area	g $month^{-1}$
$CF_{population}$	Population correction factor (ammonium estimated/theoretical) $CF_{population} = \left(\frac{Concentration^a \times Flow^b}{6\ g\ day^{-1} person^{-1}\ ^c}\right) / theoretical\ population$	-
$F_{excretion}$	Fraction of pharmaceutical (or metabolite) excreted in urine and faeces $F_{excretion} = \frac{Excretion\ (\%)}{100}$	-
CF_{sewer}	Correction factor for pharmaceutical (or metabolite) changes in sewer[d] $CF_{sewer} = \frac{\left(degradation\ rate\ \left[\frac{\%}{h}\right] \times sewer\ residence\ time\ [h]\right)+100}{100}$ [e] $CF_{sewer} = \frac{1}{e^{-rate\ constant\ (h^{-1}) \times sewer\ residence\ time\ (h)}}$ [f] $CF_{sewer} = \frac{100}{(100-k \times \%excretion)}$ [g]	-
Flow	Influent wastewater flow	L day^{-1}
d	Number of days in month	days $month^{-1}$
10^6	Unit conversion (g to µg)	-

Key: [a]Concentration of ammonium in g L^{-1}; [b]wastewater flow in L day^{-1}; [c]See **Appendix 3**. [d]If the formed pharmaceutical (from deconjugation) is then degraded itself in the sewer then this needs modelled instead of using a simple read across equation; [e]parent pharmaceutical degradation if following zero order kinetics (assuming no conversion of metabolites to the parent pharmaceutical; [f]parent pharmaceutical degradation if following first order kinetics (assuming no conversion of metabolites to the parent pharmaceutical; [g]where the metabolites transform into the parent compound (assuming no degradation of the parent pharmaceutical) and *k* is the proportion of metabolites that converts to parent compound (assumed to be rapid and not time dependent).

Part B: Sampling and analysis requirements

6.0 Sampling processes

Pharmaceutical waste, either as parent compounds or metabolites, enters wastewater streams through domestic and hospital waste (i.e., toilet flushes, bath/showers, including direct disposal of unused medicines), and pharmaceutical industrial waste (Diwan, Lundborg and Tamhankar, 2013). The pharmaceutical loaded wastewater mixes with other wastewater sources from households (washing machine, dishwasher etc), industrial waste and rainfall between source and wastewater treatment plant (WWTP) influent. The flow of wastewater will vary over the course of 24 hours, typically having higher flows during the day and lower flows during the night. Pharmaceutical waste entering wastewaters can be broadly divided into two generalised groups: frequently and infrequently discharged pharmaceuticals (**Figure 1**). From **Figure 1** it is easy to visualise that frequently discharged pharmaceuticals are prone to less concentration variation over time than infrequently discharged pharmaceuticals. Therefore, the discharge frequency is important to know as this will influence the sampling frequency required to produce a representative sample of that pharmaceutical's influent concentration. To enable comparison with predicted concentrations from prescription data, the sampling must provide an average concentration (e.g., over 24 hours).

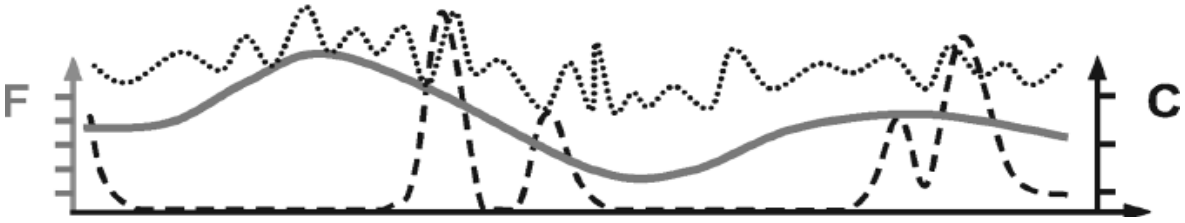
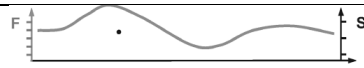
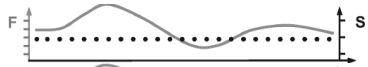
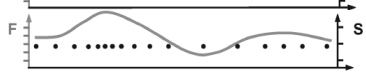
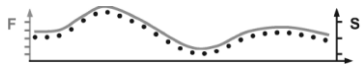


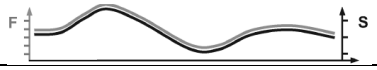
Figure 1. Theoretical visualisation of pharmaceutical concentration variation overlayed onto wastewater flow rate over time (adapted from Ort et al. 2010a).

Key: continuous grey line – wastewater flow (F, primary y-axis), dotted line – high use, frequently discharged pharmaceutical concentration (C, secondary Y-axis), dashed line – low use, infrequently discharged pharmaceutical concentration (C, secondary Y-axis).

The type of sampling performed can be divided into two modes, either continuous or discrete. Continuous sampling includes flow-proportional and constant-flow systems, these methods sample wastewater continuously during the whole sampling timeframe. Flow-proportional sampling adjusts the volume of sample collected proportionally to the volume of wastewater flowing at that location, whereas constant flow maintains the same sample volume over time independent of wastewater flow (**Table 3**). Discrete sampling methods include time-proportional, flow-proportional, volume-proportional and grab sampling. Time-proportional sampling takes a constant sample of volume at set time intervals, flow-proportional discrete sampling adjusts the volume of sample collected to the flow of wastewater at constant time periods (**Table 3**). Volume-proportional sampling collects a constant volume of sample at frequencies proportional to the flow of wastewater, sampling frequency increases with increasing flow and vice versa (**Table 3**). Grab sampling is the simplest sampling type and involves taking one or more constant volume samples irrespective of wastewater flow or time. There are currently no available on-line detectors for pharmaceuticals, and passive samplers are currently under development. Laimou-Geraniou, Heath and Heath (2023) found that passive samplers can significantly over- or under- report concentrations of pharmaceuticals in wastewater. A major limitation of passive samplers is the occlusion of the exposed membrane by particulates.

Table 3. Visualisation and brief description of different sampling modes (adapted from Ort et al. 2010a).

Sampling Mode		Brief Description	Illustration[a]	Requires
Discrete	Grab sampling	One or more individual samples at constant volume.		n/a
	Time-proportional	Constant sample volume at constant sample frequency.		Auto sampler
	Volume-proportional	A constant sample volume collected at a variable sampling frequency proportional to wastewater flow.		Auto sampler Flow meter
	Flow-proportional	Sample volume proportional to wastewater flow at constant sample frequency.		Auto sampler Flow meter

Continuous	Constant volume	Divert a constant sample volume over time.		Pump
	Flow-proportional	Divert sample volume proportional to wastewater flow.		Pump Flow meter

[a]Grey line representing the wastewater flow (F, primary y-axis), black line representing sampling points, their frequency of collection and volume (S, secondary y-axis).

6.1 Grab sampling.

Previous CIP studies have used grab sampling (with low collection frequency) which is the only method that does not require either an autosampler or flowmeter. Therefore, this method is the cheapest and easiest to perform. However, grab sampling only provides a snapshot of pharmaceutical concentration at the time of sampling and the cross-section of the sample point without considering inter- or intra- day variation (Brack et al. 2016; Diwan, Lundborg and Tamhanker, 2013). Grab sampling only produces representative samples for stagnant water but is does not produce representative samples for dynamic systems such as wastewater influent (Brack et al. 2016; Diwan, Lundborg and Tamhanker, 2013). Grab sampling at WWTPs is only considered useful if the concentration of pharmaceuticals is required for a single point in time (Diwan, Lundborg and Tamhanker, 2013). Grab sampling may contribute to an error rate of >100%, especially for smaller drainage operational areas when estimating pharmaceutical concentrations in wastewater from prescription data, therefore the use of grab sampling is not recommended (Ort et al. 2010a,b).

6.2 Composite sampling.

A review by Laimou-Geraniou, Heath and Heath (2023) states that in order to produce reliable estimations of wastewater pharmaceutical concentrations, it is crucial to create composite samples to ensure the collection of representative samples. Because the flow of wastewater is almost certain to vary over the required sampling period, and pharmaceutical wastewater concentrations varies independent to flow, then this flow variation must be considered during the sampling period to weight individual subsamples and obtain a representative composite sample (Ort et al. 2010a). Composite samples will contain an average concentration of pharmaceuticals over the monitoring time-period (Diwan, Lundborg and Tamhanker, 2013). For dynamic systems, such as wastewater influent, composite samples produce more representative samples for the monitored time-period (Diwan, Lundborg and Tamhanker, 2013). A representative 24-hour composite sample is often deemed appropriate since a longer time period can be covered at the same analytical cost if diurnal variation is less important (Ort et al. 2010a). A longer time period than 24 hours becomes more costly due to the requirement for frequent sampler maintenance in order to minimise potential biofilm growth in the sampling hose (Ort er al. 2010a).

6.3 Time-proportional sampling.

Time-proportional sampling only results in unbiased composite samples if flow variations are small (Castiglioni et al. 2013). A time-proportional method will over-represent influent concentrations when the flow is high (typically during the day) and underrepresent concentrations when the flow is low (typically during the night) (Ort et al. 2010a). Time-proportional sampling will systematically under- or over- estimate pollutant loads when either the flow varies over the sampling period or when flow and concentration are positively or negatively correlated (Ort et al. 2010a).

6.4 Volume-proportional sampling.

Volume-proportional sampling, often mistakenly referred to as flow-proportional, in combination with short sampling intervals can be considered acceptable but not ideal since the volume of sample recovered is constant (Castiglioni et al. 2013). If multiple samples are combined by this method, then the target analytes can be subject to the dilution effect.

6.5 Constant volume sampling

It is clear to see from **Table 2** that constant volume sampling would over-represent influent concentrations when the flow is high and underrepresent concentrations when the flow is low similar to time-proportional discrete sampling. This method would suffer the same detrimental dilution effects of volume-proportional sampling and would not produce representative composite samples.

6.6 Flow proportional sampling

It is almost certain that the flow rate in a sewer will vary significantly over time during the course of a day when sampling takes place. A flow-proportional sampling mode is the only sampling mode that correctly weights the individual subsamples, which are combined to form a composite sample, and analysed to obtain an average concentration (Ort et al. 2010a;b). Both continuous and discrete flow-proportional sampling produces the most representative samples (Ort et al. 2010b) and does not systematically under- or over- estimate pharmaceutical concentrations when compared to all other available methods

(Castiglioni et al. 2013). The availability of continuous flow-proportional autosamplers is lacking meaning that out of the two, discrete flow-proportional sampling appears to be the only viable option. For discrete flow-proportional sampling the accuracy of the determined concentrations only depends on the sample frequency (Ort et al. 2010b).

6.7 Sampling frequency

Toilet flushes and shower/bath water releases are expected to contain the majority of pharmaceuticals and should be considered as events with respect to sewage systems (Ort et al. 2010a). Such events can lead to significant short-term variation of pharmaceutical concentrations in combined and separate sewers in the range of minutes (Ort et al. 2010a). At source these events can last a few seconds up to a few minutes, these events can extend over 1-10 minutes by the time they reach the influent of WWTPs depending on hydraulic conditions and flow distances (Ort et al. 2010a). A sampling frequency should be determined on the basis of expected short-term variations which can be as little as 5 minutes up to 1 hour or more (Ort et al. 2010a).

An appropriate sampling frequency should be informed by the expected concentration variability throughout the sampling period and not by the limitations of the sampling device nor the amount of effort available for sampling and analysis (Ort et al. 2010b). Inadequately low sampling frequencies unnecessarily increase random sampling uncertainty or systematic over- or under- estimations of pollutant loads and cannot be compensated for with a large number of samples, or a sophisticated analytical technique or statistical analysis (Ort et al. 2010a).

For a small drainage operational area, the number of events (or 'pulses') expected over the course of a day will be low requiring a high sampling frequency. The opposite may not be the case for larger drainage operational areas as a large number of pulses can result in a highly fluctuating pattern which would require a high sampling frequency to capture such fluctuations in a representative manner (Ort et al. 2010a). If the concentration variability of target analytes is unknown a precautionary high sampling frequency (≤5 min) is necessary to minimise sampling uncertainty as much as possible (Ort et al. 2010b). Alternatively, a modelling approach can be used to calculate and approximate sampling frequency using the following equation:

$$N_{pulses\ in\ drainage\ operational\ area} = \frac{Prescribed_{monthly} \times n_T}{d \times dose_{day}}$$

Whereby $Prescribed_{monthly}$ is the amount of pharmaceutical consumed in the drainage operational area in a month, n_T represents the number of times a person goes to the toilet which is approximately five times a day (Rauch et al. 2003), d is the number of days in a month, and $dose_{day}$ represents the typical daily dose per person of the pharmaceutical. This approach suggests that there is no large difference if thousands to ten thousands of pulses are expected between sampling frequencies of 15 min to 2 h. However, if the number of pulses is smaller than 1,000 it is strongly recommended to use a sampling frequency of 15 min or smaller to minimize sampling uncertainty (Ort et al. 2010b). This is likely to be the case for WWTPs serving a small population or for an infrequently discharged pharmaceutical. A sampling frequency of 15 minutes would be adequate for both scenarios and is recommended to produce representative samples for the estimation of pharmaceutical concentrations in wastewater.

Most Scottish Water sewers are gravity fed and so less consideration is required for pump operated WWTPs. For pump operated pressurised sewers the sampling frequency should be less than the shortest pump cycle in order to capture fluctuations in a representative manner (Ort et al. 2010b). Missing such pumping events would imply that the pharmaceutical loads from entire sub-drainage operational areas will not be properly accounted for.

Recommendation: To sample in a discrete flow proportional manner over 24 hours with a sub-sample collection frequency of 15 min. At smaller WWTPs (e.g., ≤25,000) it is suggested that the number of 'pulses' is determined for lower use pharmaceuticals and if <1,000 then a 5 min sub-sample collection frequency is recommended.

7.0 Analyte procedures

7.1 Metabolites

Several studies have reported disposal of unused medicine in wastewater (Baker, Barron and Kasprzyk-Hordern 2014; Duan et al. 2022; Gao et al. 2021; Kasprzyk-Hordern et al. 2021; Verlicchi et al. 2014; Petrie et al. 2016). This can occur at the patient level (i.e., the user) but also by pharmacies. Qualitative studies using anonymous questionnaires have revealed that returned medicines can be disposed of inappropriately (Tong, Peake and Braund 2011). Analysing the metabolite as well as the parent pharmaceutical will theoretically enable quantification of any disposal. The parent pharmaceutical will be a sum of all excreted and disposed sources. Whereas the metabolite will only reflect excretion from the human body. However, the excretion data for metabolites is often less consistent than that for parent compounds and thus higher uncertainties can result (Casas et al. 2021). Furthermore, it must be known that the metabolites are not formed by microbes within the sewer

network. This should be captured during pilot sewer studies (Section 3.0). Metabolites included in back-calculations should have well-studied and documented excretion rates in both urine and faeces, have little variation in rate between healthy and unhealthy individuals, and be chemically stable in wastewater (Casas et al. 2021). Including multiple metabolites along with the parent compound can also reduce uncertainty arising from inaccurate excretion rates as the over-estimation of one compound would be negated by the under-expression of another (Casas et al. 2021). Care must also be taken to ensure that the metabolite is unique to the parent pharmaceutical. For example, 10,11 dihydroxycarbamazepine is the major metabolite of carbamazepine with excretion in urine being 32% (Bahlmann et al. 2014). However, it is also a major metabolite of another pharmaceutical (oxcarbazepine) (See **Appendix 4**).

Recommendation: At least one major metabolite of each pharmaceutical selected for monitoring in CIP 4 Scotland is also included. Selection of an appropriate metabolite will be reliant on the findings of stability assessment in pilot sewer studies (Section 3.0).

7.2 'Total' pharmaceutical concentrations in wastewater

As previously described in Section 2.0, pharmaceutical and metabolite excretion in urine as well as faeces needs incorporated into the calculation of their predicted concentrations. Therefore, to assess the accuracy of these predictions it is essential that the analytical methods employed capture the 'total' concentration of pharmaceuticals and metabolites. Analysis of wastewater is often on a prefiltered sample passed through a 0.45 or 0.70 µm glass fibre membrane and does not involve the particulate phase of wastewater. However, a significant proportion of a pharmaceutical can be associated with particulates in wastewater. More than 50 % of the total concentration of the fluoroquinolone antibiotics ofloxacin and ciprofloxacin have been found in the particulate phase (Petrie et al., 2014). Previous CIP monitoring of pharmaceuticals have involved a direct injection methodology. Typically, a sample is filtered and analysed directly without any further sample pretreatment. This would only capture the liquid phase concentration. Analysis of pharmaceuticals in CIP 2 Scotland was performed by ALS Laboratories (UK) LTD and it was not possible to obtain detailed information on the analysis. The summary of sampling requirements stipulated for CIP analysis stated that samples for wastewater analysis should not be filtered, but further information is not given.

The determination of total pharmaceutical concentrations in wastewater can be determined by extracting and analysing the particulates retained on filters. However, this is laborious and expensive. Alternatively, an isotopically labelled surrogate standard can be spiked into the sample prior to filtration. These behave the same way as the pharmaceutical in question, meaning it should have similar adsorption to particulates. The concentration of the pharmaceutical is then determined based on its ratio to the labelled standard. This would provide the total concentration in wastewater and has been applied in studies previously (Yao et al. 2021). Labelled standards are routinely used in analytical methods to ensure accurate results by accounting for any losses or errors during sample analysis. A further consideration for the filtration of samples is the loss of pharmaceuticals to the membrane filter. This is often overlooked in the validation of analytical methods. For example, the losses of the chemotherapy drugs paclitaxel and tamoxifen in wastewater effluent filtered using a 0.45 µm polyamide membrane was high due to their hydrophobicity (88% and 85%, respectively) (Cristóvão et al. 2021). Nevertheless, the addition of labelled standards prior to filtration is recommended to account for these losses (Laimou-Geraniou, Heath and Heath 2023).

Recommendation: The total concentration of pharmaceuticals in influent wastewater is measured by spiking the sample with isotopically labelled surrogate standards, filtered and then analysed.

7.3 Pharmaceutical stability following sample collection

Cristóvão et al. (2021) found that the degradation of a range of pharmaceuticals in wastewater stored at 4°C was <12% over seven days. They concluded that storage of wastewater samples at 4°C was appropriate and longer-term storage would require temperatures of -20°C. The analysis of samples as soon as possible is recommended, as is an assessment of pharmaceutical stability is wastewater (Riva et al. 2020). Previous CIP programmes have performed their own degradation studies and stipulated a cut off time for analysis to be complete for samples stored at 4 °C. This was 4 days for the analysis of ibuprofen, propranolol, erythromycin, ofloxacin, oxytetracycline, salicylic acid and fluoxetine, and 14 days for the steroid estrogens.

Recommendation: As a 24-hour composite sampling method is recommended then the autosampler needs refrigerated/cavity packed with ice to maintain samples ≤4°C. The stipulated cut off time for analysis outlined by CIP would then need to be shortened by one day to account for this.

Part C: Recommendations for validation

8.0 Validation study

Use of the Data Visualisation Tool to predict pharmaceutical concentrations in influent wastewater (**Part A**) needs to be validated using the proposed sampling and analysis methodologies (**Part B**). Inadequate sampling frequency during the year can lead to any temporal variation of pharmaceutical consumption affecting the deviation of predicted to measured concentrations (Gao et al. 2021). However, the availability of monthly prescription data in Data Visualisation Tool enables any bias in seasonal trends to be omitted and a shorter validation study possible. Ort et al (2013) sampled WWTP influent serving an average population of 7,160 for low usage substances (illicit drugs) and they concluded that for the estimation of representative annual loads that a minimum of 56 stratified random samples need taken. The uncertainty associated with 56 random stratified samples is approximately 10% for most substances and locations and rises for a lower number of samples (Ort et al. 2013). Concentration variations of pharmaceuticals in influent wastewater of smaller drainage operational areas are likely to have higher variability than larger drainage operational areas as a smaller number of consumers can lead to large inter- and intra- day fluctuations and shorter residence times in sewers diminishes the effect of dispersion (Ort et al. 2013).

Recommendation: As monthly prescription data is available; it is recommended to collect five random samples from the same WWTP within a single month for analysis. These should be analysed for the pharmaceuticals of interest and ammonium and have flow data available for those sampling days. Furthermore, it is suggested to sample three different WWTPs that represent a broad range of PE served (e.g., see Table 3). Due to the number factors used in the determination of predicted pharmaceutical concentrations in wastewater, and their variability, a sensitivity analysis needs performed. The overall uncertainty will enable a more representative comparison to the measured pharmaceuticals concentrations to be made.

Table 3. Example WWTPs to monitor in the validation study

Location	Population served	Currently included in Covid-19 monitoring?
Seafield WWTP	605,569	Yes
Dalmarnock WWTP	142,556	Yes
Newbridge WWTP	24,821	Yes

Note: these WWTPs were selected to represent a range of populations served and within the Covid-19 monitoring programme for ease of sample collection. They can be changed if adequate sampling equipment is not available on site.

8.1 Limitations

Despite the recommendations provided to improve the reliability of predicted pharmaceutical concentrations in influent wastewater, several uncertainties and limitations remain that will influence results and are largely uncontrollable. Some of these limitations are described below:

- Inaccuracy or high individual variability of pharmacokinetic data (i.e., excretion rates) can be a source of error for back-calculations (Causanilles et al. 2018). Excretion rates determined by studies of healthy Caucasians introduces a bias if the drainage operational area population deviates, which is almost certain (Gao et al. 2021). For example, the excretion rates of carbamazepine from healthy individuals differ to those of epileptic patients who are treated by the pharmaceutical, the metabolism and excretion of carbamazepine among epilepsy patients are dose-dependent (Gao et al. 2021).
- If the pharmaceutical has significant over the counter sales (e.g., ibuprofen) or is produced naturally (estrone and 17β-estradiol) there will be little agreement between the predicted and measured wastewater concentrations. Furthermore, self-medication of estrogens sourced from overseas by the transgender community for hormone replacement therapy may have an impact on measured concentrations (if this occurs in small drainage operational areas where individual's behaviour can have a greater influence on influent wastewater composition).
- Prescription data included within the Data Visualisation Tool does not include private GP practices or hospitals and their influence will be drainage operational area specific.
- Prescription data for rural drainage operational areas which have low population numbers and greater dispersion (high proportion of private septic tanks) may not be representative for the community served by the WWTP.
- The low concentrations of 17alpha-ethinylestradiol found in influent wastewater may not be quantifiable to make the comparison to predicted concentrations.
- 'Total' concentrations of pharmaceuticals in wastewater (i.e., sum of aqueous and particulate concentrations) are measured and predicted using the approaches described. The aqueous concentration can be estimated by applying the known aqueous/particulate distribution of the pharmaceutical in wastewater.

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Appendix

1. Excretion data and metabolite information for pharmaceuticals of interest

Pharmaceuticals	% urinary excretion	% faecal excretion	Metabolites	% urinary excretion	% faecal excretion
17-alpha ethinylestradiol (EE2)	10.1[1] 53.3[2] 3[3] 8.14-16.9[4]	23.1[1] 2.7[2] 5-10[5] 8.88[4]	EE2-3-glucuronide[6] EE2-3-sulphate[6] 2-hydroxy-EE2[6] 2-methoxy-EE2[6] 2-hydroxy-EE2-glucuronide[6] 2-methoxy-EE2-sulphate[6]	90-95 as conjugates[5] 14.5-30 as glucuronides[4] 2.58-5.35 as sulphates[4]	- - -
17-beta estradiol (E2)	0[7] 0.5[3] 3.3 µg/d/capita[5]	5-10[5]	E2-17-glucuronide[1] E2-3-glucuronide[1] E2-3-sulphate[1] Oestrone (E1)[8] Estriol (E3)[8] 2-hydroxyestradiol[8] 4-hydroxyestradiol[8]	90-95 as conjugates[5]	-
Azithromycin	6-12[9] 12[10] 6[11] 11-14[12] 6[3] 12[13] 14[14] 11-14[15]	90[9] 44.6[16]	Descladinose[16] 9a-N-desmethyl-Azithromycin[16]	<10[16] <10[16]	12.7[16] 2.4[16]
Carbamazepine	3[17] 0.5[3] 0.8[18] 0.5[19] 0.4[20] 2-3[13] 1-3[21] 12[22] 13[23] 1[24] 3[25] 1-2[26] 3[27]	12.5[18] 10-15[24]	10-Hydroxy-Carbamazepine[3] Carbamazepine-10,11-epoxide[3] 10,11-dihydroxy-carbamazepine[18] 9-hydroxy-carbamazepine[28] 9-hydroxymethyl-10-carbamoylacridan[18] 1-Hydroxy-Carbamazepine[18] 2-Hydroxy-Carbamazepine[18] 3-Hydroxy-Carbamazepine[18] 4-Hydroxy-Carbamazepine[18] 2-hydroxy-1-methoxy-carbamazepine[18]	27[3] <0.1[18] 0.5[3] 1.4[18] 32[18] 1-1.4[19] 1.1[20] 1.2[23] 1.2[24] 23[28] 32[28] 24[28] 32-61[19] 34.6[20] 32.3[23] - 5.2[18] 5.2-8.8[19] 3.1[20] 2-10[18] 4.3[18] 2.7[20] 2.3[29] 1[22] 5.1[18] 3.8[20] 1[22] <2[18] <2[18]	- - - - - - - -

			2-hydroxy-3-methoxy-carbamazepine[18]	<3[18]	-
			Carbamazepine-N-glucuronide[18]	11[18]	-
Ciprofloxacin	65[3] 44.7[13] 40-50[13] 65.3[30] 44.7[29] 40-50[29] 15[29] 70[22] 25.5-33.6[31] 53.2-57.4[31] 55[14] 20[27] 40-50[15] 0.1-79.6 (Av 37.66±17.55 n=169)[32]	11.4[30] 0.4-61.5 (Av 25.49±19.30 n=14)[32]	Desethylene-ciprofloxacin[32]	0-2.9 (Av 0.84±0.84 n=26)[32]	0.5-1.4[32]
			Sulociprofloxacin[32]	0.1-14.1 (Av 3.64±4.41 n=21)[32]	1.3-6[32]
			Oxo-ciprofloxacin[32]	0-6.3 (Av 2.34±2.16 n=20)[32]	1-6.2[32]
			Formyl-ciprofloxacin[32]	0.3[32]	-
Clarithromycin	35[3] 20-30[13] 18-36[33] 20-30[29] 58[22] 20[34] 25[14] 25[27] 18[35] 18.4[36] 24.5-36.2[36] 20-40[15] 14.4-51 (Av 29.81±9.77 n=16)[32]	4[29] 4[35] 4.4[36] 0.3-7.5[32]	14-hydroxy-clarithromycin[33]	9.6-12[33] 13.7[36] 9.6-15.4[36] 7.6-19.4[32] 10-15[15]	6.0[36] 2.8-6[32]
			Desmethyl clarithromycin[32]	0.9-1[3]	4.7-7[32]
			Des-cladinose-clarithromycin[32]	1-1.3[32]	0.9-3[32]
			Didemethyl-clarithromycin[32]	0.1[32]	0.6[32]
Diclofenac	<1[37] 6[38] 6.2[38] 0.2-0.77[38] 0.5-0.7[38] 2[38] 17.8[38] 13.6[38] 0.5[3] <1[13] 1[21] 4.8-11.5[34] 15[26] 1-15[39] 5-10[27] 5.81±2.65[40] 15[41]	<1[37]	4’-hydroxy-diclofenac[42]	11[38] 30[38] 16[38] 13.4[38] 38.6[38] 27.2[38] 27[22] 10.58±7.12[40]	10-20[38]
			3’-hydroxy-diclofenac[42]	2[38] 6.1[38] 1.7[38] 0.95±0.44[40]	-
			5’-hydroxy-diclofenac[42]	5.6[38] 2.0[38] 7.7[38] 6.7[38] 1[22] 4.76±0.53[40]	-
			4’,5-dihyrdroxy-diclofenac[42]	11[38] 10.13±0.66[40]	-
			3’-hydroxy-4’-methoxy-diclofenac[38]	0.009[38] 1.4[38] 0.01[40]	-
Erythromycin	5[3] 2-5[29] 5[29] 25[22] 2-15[34]	0-15[29]	N-desmethyl-erythromycin[56]	-	-
			Anhydro-erythromycin[57]		

	4[25] 4-10[39] 5[27] 5[43] <5[15] 1.9±1.0[58]		Des-cladinose-erythromycin[59] N-oxide-erythromycin[59]		
Estrone (E1)	0[7] 13.8 µg/d/capita[5]	5-10[5]	E1-3-glucuronide[1] E1-3-sulphate[1]	90-95 as conjugates[5]	-
Ibuprofen	0.5[3] 1[29] 1-10[29] 11[29] 12[29] 0[44] 10[22] 12[34] 10[25] 1-8[26] 1-10[39] 10[27] 10.59±2.56[40] 15[15]	1[45] 0.82[44]	2-OH-Ibuprofen[29] Ibuprofen-glucuronide[46] 1-OH-ibuprofen[44] 3-OH-ibuprofen[44] Carboxy-ibuprofen[44] 1-OH-ibuprofen-glucuronide[44] 2-OH-ibuprofen-glucuronide[44] 3-OH-ibuprofen-glucuronide[44] Carboxy-ibuprofen-glucuronide[44]	26[29] 20[29] 25[29] 11[29] 25[22] 25.64±3.6[40] 14[46] 12[44] 5-17[39] - - 28[44] 16[44] 30[44] 37[22] 38.97±5.98[40] - - - 19[44] 12[44]	- - - - - - - - - - - - - - - - - -
Metformin	99[3] 90[34] 100[25]	-	-	-	-
Propranolol	0.25[3] 0.5[13] 0.5[29] <1[29] <1[26] 0[47] 10[15]	1-4[48] 2.5[49]	naphthoxylactic acid[48] 4-hydroxy-propranolol[48] Propranolol-glucuronide[50] 4-hydroxy-propranolol-glucuronide[50] 4-hydroxy-propranolol-sulfate[50]	20-40[48] 20-30[51] 42[15] 10-25[47] 17.5[47] 17[15] - -	- - - - - - -
Ranitidine	50-70[52] 30-35[13] 30[29] 35[29] 30[25] 30-40[27] 16-36[53] 40[53] 30-70[15]	26[29]	Desmethyl ranitidine[52] Ranitidine-N-oxide[53] Ranitidine-S-oxide[53]	1-3[52] 2[53] 1[15] 4[53] <4[15] 1[53] 1[15]	- - - - - - -
Triclosan	<1[54] 0.3-1.2[55]	96[49]	Triclosan Sulphate[24] Triclosan glucuronide[49] 2-4-dichlorophenol[49]	5[49] 16-40[49] 0[49]	1[49] 0[49] 3[49]

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2. Pilot sewer stability study data for pharmaceuticals of interest and their metabolites

Analyte	Experimental	Residence time	Degradation	Kinetics	Reference
Azithromycin	Pressurised pump sewer	22 h	15%=22h (0.68 %/h)	-	Jelic et al 2015
Carbamazepine	Gravity	12 h	0	-	Li et al 2021
	Rising Main	12 h	0	-	
Ciprofloxacin	Pressurised pump sewer	22 h	20%=22h (0.91 %/h)	-	Jelic et al 2015
Clarithromycin	Pressurised pump sewer	22 h	25%=22h (1.14 %/h)	-	Jelic et al 2015
Diclofenac	Gravity Sewer	12 h	10%=7.1 h ($k=0.01476\ h^{-1}$)	First-order	Ahmed et al. 2021
	Rising Main	12 h	10%=3.2 h 20%=6.9 h ($k=0.03243\ h^{-1}$)	First-order	
Ibuprofen	Gravity	12 h	0	-	Choi et al 2020
	Rising Main	12 h	0	-	
	Pressurised pump sewer	22 h	5%=22h (0.23 %/h)	-	Jelic et al 2015
Carboxy-ibuprofen	Gravity Sewer	12 h	0	-	Ahmed et al. 2021
	Rising Main	12 h	10%=4.3 h 20%=9.1 h (2.26 %/h)	-	
2-OH Ibuprofen	Pressurised pump sewer	22 h	2%=22h (0.09 %/h)	-	Jelic et al 2015
Metformin	Lab sewer 25°C	24h	3.1%=24h	First-order[a]	Lin et al. 2021
	Aerobic sewer 25°C		10%=24h		
	Anaerobic sewer 25°C		9%=24h		
Propranolol	Pressurised pump sewer	22 h	18%=22h (0.82 %/h)	-	Jelic et al 2015
Ranitidine	Gravity Sewer	24h	50%=10h	First order[a]	Choi et al 2018
	Rising Main	24h	50%=3.1h		
Triclosan	Gravity sewer	30 days	33.4%=30d (0.046 %/h)	-	Shi et al 2022

[a]First-order constant k not given.

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3. Report ammonium emissions by people used in wastewater-based epidemiology studies

Ammonium (NH_4-N) emission (g/day/person)	Reference
12.5	Van Nuijs et al. 2011
6.9±0.4	Been et al. 2014
8.1±0.37	Been et al. 2014
7.0	Been et al. 2014
10	Rico, Andrés-Costa and Picó 2017
5.78-7.57	Lin et al 2019
6.4	Huan et al 2010
6	Zheng et al 2017
4-8	Zheng et al 2017

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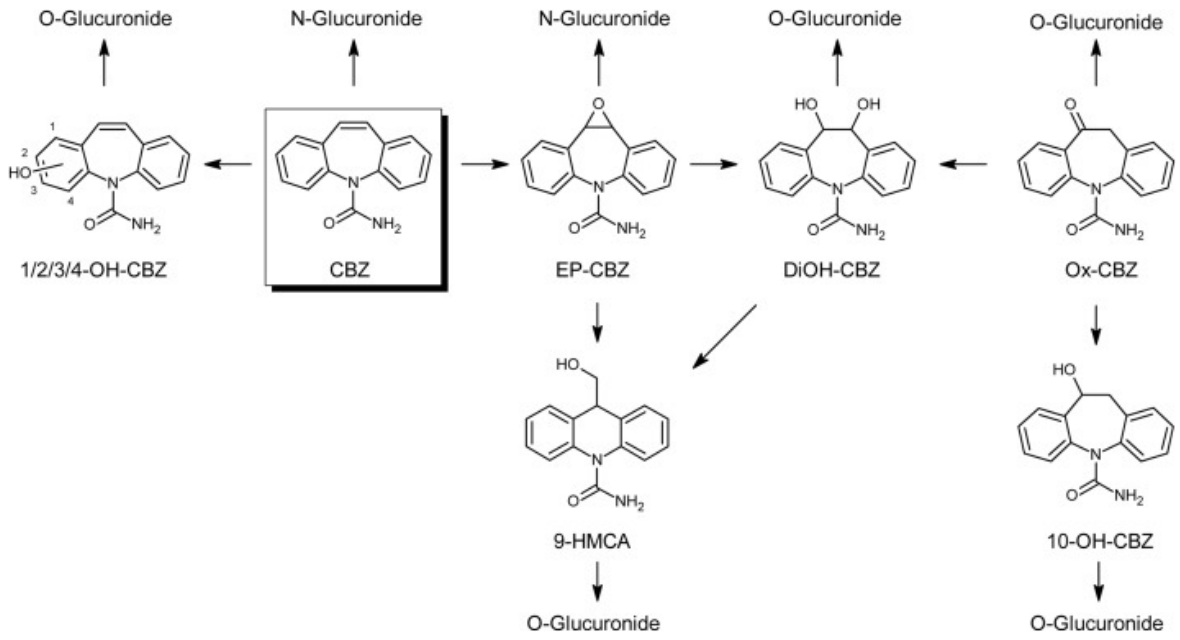
4. Example metabolism pathway of carbamazepine and oxcarbazepine (Bahlmann et al., 2014)



Key: OH-CBZ, hydroxy-carbamazepine; CBZ, carbamazepine; EP-CBZ, carbamazepine epoxide; DiOH-CBZ, dihydroxy-carbamazepine; 9-HMCA, 9-hydroxymethyl-10-carbamoylacridan; Ox-CBZ, oxcarbazepine.