Chapter 3

We saw in Chapter 3 that there is a need to prepare or process our “representative” sample in order to ‘measure’ our analyte(s) or property of interest. This should be performed in such a way, so as to maintain the integrity of the sample, it’s analyte(s) or property.
We also saw that while there are a number of stages involved in the sample preparation process, it helps first to identify from our Analytical Choices flowchart (Figure 3.1) what we are dealing with (sample and analyte or property types) and what we are measuring in terms of particular categories. This process will influence our decision making process and as we have seen in chapter 3, there is a close link between the analyte(s) or property to be measured, the sample preparation process and the final measurement technique.

Feedback for problem 1

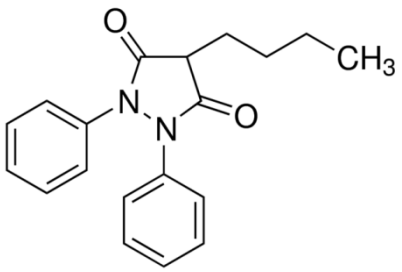
i) Example Feedback: Using Figure 3.1 and the relevant section in Chapter 3:
A solid, organic-based sample (wet, biological tissue) requiring a qualitative and quantitative measurement of an organic molecular compound phenylbutazone; ($C_{19}H_{20}N_2O_2$ – a veterinary, anti-inflammatory drug used for treatment in large mammals).
This drug is not allowed in meat for human consumption but may be present in meat products for other animals. This scenario became a potential issue in Europe in 2013 when horsemeat was found in meat products labelled as beef, edible for human consumption. This was in addition to the breach of labelling regulations. See Food Standards Agency and https://www.gov.uk/government/news/processed-beef-products-and-horse-meat

Using Figure 3.1 and the relevant section in Chapter 3, all the scenarios (ii to x) can be identified in terms of their ‘categories’ as shown in the above example. As further guidance, scenario v) may be considered in terms of:
A solid, organic-based sample (wet, biological plant tissue) and a solid, mixed inorganic and organic-based sample (wet, soil) requiring qualitative and quantitative measurements of selected (radiochemical, isotopic) elements.

Feedback for Problem 2

i) Example Feedback: We start with the physical properties and chemical make-up of the analyte of interest which is readily available on-line; e.g. see: https://pubchem.ncbi.nlm.nih.gov/compound/phenylbutazone

Phenylbutazone (PBZ, Its chemical structure and elemental constituents are shown below) is a non-volatile organic molecular solid, soluble in some alcohols, acetone, DMSO / DMF etc. In water <1 mg/mL (at room temp.). Some solubility in diethyl ether, acetonitrile dichloromethane. Limited in benzene / toluene / cyclohexane, etc. Its molecular absorbance in the UV part of the spectrum is shown to be; λ_{max} = 239 to 244 nm. It is also a strong absorber in the IR region around 1690 -1720 cm^{-1} due to the carbonyl groups.



Looking at Chapter 3 and section 3.3, we would need **to consider** various sample processes, such as:

Homogenisation of the meat sample,

Drying of the meat sample (?),

Particle size reduction of the meat sample (after drying?)

Sieving of the meat sample (after drying and size reduction?) ,

Dissolution / extraction of analyte (see Figure 3.3)

'Clean-up' (and if necessary Pre-concentration) to reduce possible interferents and improve selectivity.

To be in a 'form' ready for a suitable measurement technique.

? above is to indicate that this particular process is not always necessary.
It is noted that different meats have very different fats content and therefore a different approach may be required depending upon the meat-type.

It is necessary to extract the PBZ from the 'representative' meat product, so we would first need to homogenise the sample.
The options are:
to blend (homogenise) the wet meat and then extract the PBZ from a representative quantity of this blended material, usually between 1 and 4 g using a suitable solvent (noting that sample is ~70% water and will vary).
Or
To blend (homogenise) the wet meat first and then freeze-dry to remove water (note weight before and after freeze-drying to correct back to wet weight at the end). Then gently grind (agate pestle and mortar) the dried sample to reduce particle size to aid later extraction of the PBZ from a representative quantity of dry ground material (say ~0.5 to 1 g) using a suitable solvent. Sieving may not be necessary as this dried material is usually quite soft and general grinding will reduce the particle size down to <500 µm.

For either of the above:
Extract the PBZ from the sample using a suitable solvent system. You would therefore use a solvent that readily dissolves the organic molecular analyte but is also compatible with the organic sample (matrix) itself. It should be readily absorbed by the sample matrix and should "wet" it. Using the physico-chemical information found above, there are various solvent options

PBZ is soluble in organic solvents like ethanol, ethanenitrile (acetonitrile) and acetone (propan-2-one). This extraction can be achieved efficiently by use of i) multiple shaking and sonication (ultrasonic bath) of the sample with the solvent + then centrifuging (up to three times) and combining the separated supernatent extracts (→ 3 x) to form one final extract or ii) by a gentle Soxhlet process. In each case you are looking for → 100% extraction efficiency. See section 3.6 and Table 3.8
In both cases, whether cold or hot extractions are used, the presence of various other organic moieties such as fats or proteins (a range of polar and non-polar materials present in

the meat sample) will be noted alongside the analyte of interest. In order to reduce the problems of complex compound measurement later on, and their possible interferences, a 'clean up' process would be of benefit. As noted in Chapter 3, this process can be achieved by taking out those compounds through the judicious use of solvent solubility factors and / or, the use of solid phase extraction (SPE) media. See section 3.6.2 and Table 3.7

This process is itself quite complex and will depend upon i) the relative solubility of the analyte in a particular solvent system compared with the undesirable matrix components and / or ii) the relative retention factors of the analyte for a given SPE media compared with the undesirable matrix components. These are based around the range of polar and non-polar properties for many organic analytes / sample matrices, and are often tailor-made for a given pairing. At this stage, an appreciation of the processes involved and how they work, is all that is needed. Fine tuning can come from a literature search or experimentation.

To provide some detail to this process as an example, using the information shown in section 3.6 and Table 3.7, we could consider first a solvent that is good (high solubility) for the analyte (phenylbutazone, PBZ), but also one that allows the more polar, less desired components that are also extracted from the meat, to be separated. To remove the more polar, undesirable matrix components a simple silica SPE column would suffice (normal phase system). Passing the extract-solvent solution through the silica SPE column, the more polar impurities will be retained by the silica SPE column while the analyte would remain with the solvent. However, so would the less polar undesirable matrix components. The good solubility properties of the chosen solvent mean that the analyte (PBZ) will remain in solution and be difficult to compete with. One could then use a simple liquid-liquid extraction process, where a less polar, immiscible solvent (which has little analyte-solubility properties in comparison) is added and shaken with the analyte-containing good solvent, such that the less polar undesirable matrix components from the meat will pass over to the less polar solvent, leaving a further cleaned good solvent solution of the analyte.

One other clean-up approach could be to choose an extraction solvent and SPE system with properties that allow the analyte to be retained on the SPE cartridge (e.g. C18 type; octadecylsilane coated silica) and allow the other less desirable matrix components to pass through. With 100% retention of the PBZ on the SPE, a simple washing with a small quantity of a good PBZ solvent will result in a step that concentrates up the analyte.

Stepwise we see the summary as:

Solvent / Extractant – Chosen to be compatible with (to 'wet') and absorbed by the matrix;
Analyte of interest to be highly soluble in this extractant; → 100 % efficient extraction.
Some of the matrix is soluble in this extractant; (a consequence of being compatible with matrix; like dissolves like).
Can consider separating matrix components from analyte to reduce / remove complex undesirable components (possible interferents) from later measurement step – a "clean-up" stage. Not always needed.
Consider adding another solvent to the original extract but one that is immiscible. This other solvent having the property of either greater solubility for the analyte and less for the matrix components or vice versa. Liquid-liquid extraction I (see table 3.7);
Consider using SPE which either retains the analyte and lets through the matrix or vice versa. Solid phase extraction (SPE) by polarity forces (see table 3.7).
Both routes can offer the possibility of pre-concentration (section 3.6.2) by adjusting volumes of the solvents used.

At this stage, the analyte of interest (Phenylbutazone) is stable and in a suitable solvent ready to be considered for the analytical measurement step.

Feedback (brief) to scenarios 'ii' to 'x' of **problem 2**.

Scenario →	i	ii	iii	iv	v	vi	vii	viii	ix	x
Stage in sample preparation step ↓	PBZ in Horse-Meat	PBA in Face Cream	Manuka in Honey	BPA in Printed Till and Card receipts	Radio-nuclides in plants and soils	TiO_2 in sun-screen	Scrap-metals from waste facility	Elemental composition in mineral supplements	Fatty Acids in Soya-Beans	NH_4^+ ions in Waste water dis-charge
Homogenisation,	☑	☑	☑			☑		☑	☑	
Drying*	☑	☑	☑		☑	☑				
No-drying*	☑	☑	☑							☑
Size reduction*,	☑	☑	☑	☑?	☑			☑	☑	

Sieving,	?	?	?		?			☑	?	
Dissolution / extraction of analyte (see Figure 3.3 **and** ‡ below)	☑	☑	☑	☑	☑+?	☑ + ?	?	?	☑	?
[‘Clean-up’ and Pre-concentration]	☑	☑	☑	?			?		☑	

*** = Samples may be processed directly, without drying and grinding stages. This option is available for example with ELISA measurement techniques**

? = Not always necessary – depends on measurement technique used and / or extraction technique / efficiency employed. See also feedback to “on-line problem 4: Chapter 4”; for possible measurement techniques, after attempting Problem 4 on-line for chapter 4.

‡ Use the information from Part A to help identify the next step: i.e. identification of the possible categories for each of the above analytes and sample scenarios, using Figure 3.1 and the relevant sections in Chapter 3 from Figure 3.3. To consider, is it an extraction / dissolution or a decomposition to release the analyte? Will a decomposition step affect the analyte and if so, is this detrimental? If it is detrimental, then an extraction approach should be used. Will the extraction be efficient and allow all the analyte to be released? In order to release the analyte, the extraction process will usually need to permeate the matrix holding the analyte and allow that release process. The need to identify the solubility of the analyte in the “extractant” and its compatibility with the matrix to release the analyte is required.

Feedback to Problem 3

As before, with any new analytical problem, we first define the problem and then categorise (define) the sample and the analyte / property, to be measured. Therefore we have:

A solid, organic-based sample (plastic mug, dry) requiring i) a qualitative measurement of an organic-structural analyte (identification of the plastic(s) matrix of the sample mug, itself) and ii) a qualitative and quantitative measurement of selected inorganic elemental constituents (metal, metalloid and non-metallic) in terms of both their total content and their ‘available’ content (leachable or able to migrate).

a) See the question and feedback to part a) of Question 5 in chapter 4 (on-line) for any direct measurement techniques to aid in the identification of the plastic mug.

b) It is noted that the secondary evaluation (total and leachable elemental content) using techniques of i) direct measurement, ii) decomposition and iii) extraction, on separate samples from the mug to get these into a suitable form for measurement will be required (see feedback to Question 3 in chapter 3, on-line). This will allow measurement of their total elemental content and their leachable elemental and leachable compound content, for comparison with any regulatory or guidance values. For preparation of the sample to allow the identification and measurement of the total content of selected elements (not their compounds), in this case may be achieved using a direct solids technique (with little sample preparation required - see feedback to question 5, part b) in chapter 4 on-line) and, if required a decomposition technique (see fig. 3.3 in chapter 3 to start the process). Sections dealing with sampling the plastic, in order to reduce the size of the sample to be processed – e.g. cryogenic milling of fragments taken from all over the mug, or possible drilling of the mug to acquire plastic 'swarf' or cryogenic fracture of the mug as a whole to acquire smaller fragments for decomposition, can all be considered. Decomposition techniques such as "dry ashing" (with care, to avoid losses – see section 3.3.3.2 in chapter 3) and the techniques outlined in section 3.3.3.3 of chapter 3 on "Digestion" should be considered. This will allow the organic back-bone of the plastic matrix to be converted to CO_2, H_2O and CO_3^{2-} and leave the selected elements under study to be in a suitable solution ready for their 'total' measurement.

The preparative technique for the later measurement of leachable content from "migration of elements and compounds" present in the mug under identified analytical test conditions can be found on-line. The European joint research council has published a document which covers the earlier methodology and can be found under: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC51601/guidelines%20test%20conditions_final_ed2009.pdf

There is an updated amendment (EU) 2017 / 752 which supersedes the earlier document: Regulation (EU) No. 10/2011 (including amendment 1282/2011) relating to the migration of materials in contact with food, with regards to certain procedures. Please see:

https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0752&rid=6