The Art of Separation

To those looking for help and guidance with navigating the often-tricky process of divorce or the end of a romantic entanglement, my apologies. This article deals not with maintaining a healthy and cordial relationship for the sake of the kids, but the role of chromatography in the commercial analytical laboratory.

If we try and break down the majority of chemical tests routinely performed in an environmental lab (note the specific reference to chemistry – microbiology and geological tests follow a different road) we can split the process into four parts:

1) Extraction
2) Separation
3) Detection
4) Calculation

Point 1) deals with the process of taking the things you are interested in from the matrix in which they are submitted. Environmental samples cover soils, waters, Incinerator Bottom Ash, made ground, landfill waste, waste materials intended for disposal or re-use, vegetation and in some cases biota (animal tissue). Contrary to the indications of American TV forensics shows in the 2000's it is not possible to simply sample these materials (or matrices) and feed directly into an analytical instrument. In the main, they require a very specific type of prepared subsample, often a very low volume of organic solvent, to avoid damage to instruments and ensure viable data can be obtained. A large part of all laboratories is made up with the process of turning that tub of soil that comes into the front end of the process into a very small amount of suitable solvent containing all the chemicals you are interested in testing for, and hopefully none of the ones you don't!

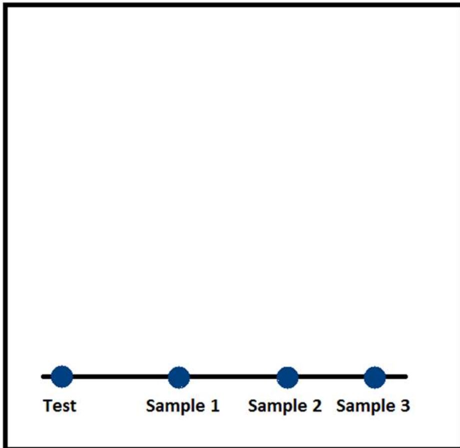
Point 3) we will touch on later in the article, but is basically ensuring that you are using the most appropriate method of ascertaining the presence and concentration of the chemicals you are interested in. Getting everything right up to this point then selecting a detection system that physically can't "see" the chemicals you are looking for won't help – at the risk of stretching an analogy you can't measure the intensity of light with a decibel meter! The choice of detector is down to the chemistry of the compounds you are interested in, and often have multiple options at which points considerations of cost and fit for purpose design enter the fray…

Point 4) rounds off the process and ensures that the customer receives data that relates back to the originally submitted material and provides relevant real-world information rather than the purely empirical data generated during the process.

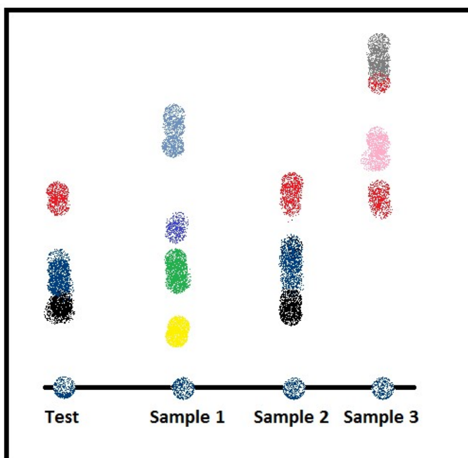
Which brings us back to point 2) and the reason we are here: Separation. Or to give the scientific term, Chromatography. Every sample received is potentially packed with all sorts of chemical contaminants and the role of the lab is to provide detail the presence and concentration of specific ones. If we are doing our extraction process right, by the time we get to instrumental analysis we should have an extract that contains (should they be present, we'll assume they are) everything we want. But if the intention is to measure each component individually, how do we do that?

Time to take you back to school…

You may recall being introduced to chromatography by what's normally referred to as the "ink test"



The above shows 3 'samples' of ink, all of which look the same.



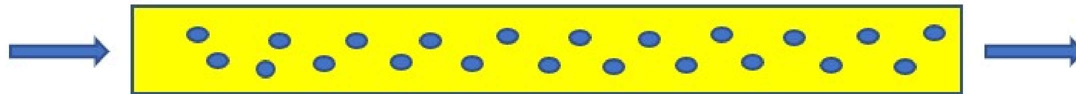
Through chromatographic separation a solvent passes up through the ink dots and 'dissolves' the component colours which are then split out as the solvent passes up the test, highlighting just how each 'identical' ink dot is actually made up.

In our more advanced analytical lab, the basic principle of separating the component parts remains the same but the means by which we do it can vary.
During a chromatographic process there is:

- A Mobile Phase: this carries the sample through the process
- A Static Phase: through which the mobile phase passes and by its interaction causes the separation

Imagine a long thin corridor with a door at each end. Should two people (one adult, one child) enter at the same time and pass down the corridor they would likely get to the end at roughly the same time.

If, however we fill the corridor with other people first:



the adult would be impeded while the child could pass more easily and get to the end much quicker. This is the basis of Size Exclusion Chromatography, separating chemicals based on size.

To stretch the analogy further (to look at other forms of separation) imagine two adults but one knows every other person in the room; they would likely slow to ***interact*** with people thereby slowing their journey while the second person moves quickly through.
To bring this back to actual chemistry, what we do is create a static phase that chemically interacts with the analytes of interest creating the impediment to passage and separation.

So, what are the real life uses and applications in the lab as they process environmental samples?

The two main uses are GC (Gas Chromatography) and LC (Liquid Chromatography), the gas and liquid indicating the mobile phase used in each instance.
GC: used in conjunction with MS (Mass Spectrometry), FID (Flame Ionisation) detectors, covers a wide range of compounds but most commonly used in TPH, PAH, SVOC/VOC, PCBs, etc.

The extracted sample, typically 5-25µl in an organic solvent, is volatalised at high temperature and the compounds pushed into the chromatographic column under a constant flow of gas (usually nitrogen, hydrogen or helium). The columns used are now capillary columns, very thin copper with sub-micron internal diameter up to 60m in length:

The columns are packed usually with a polymeric material to interact with the compounds and generate the separation, but the analyst can also use gas flow rates and temperature variations to increase separation. The specific nature of the column packing varies by application.

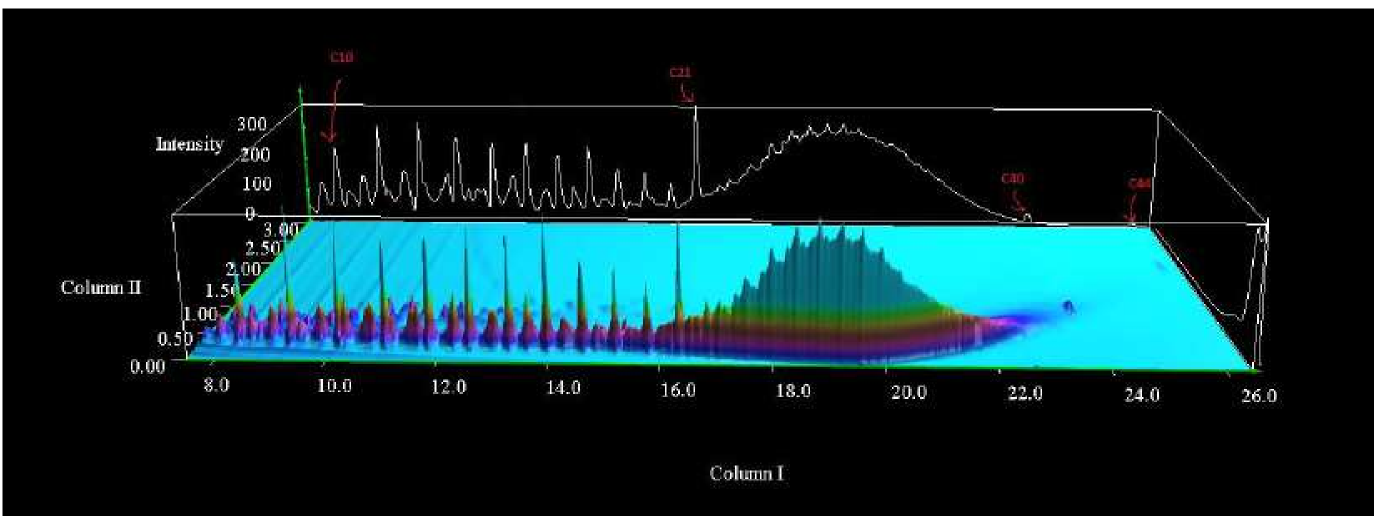
LC: used in conjunction with MS, UV (ultraviolet) Absorbance or Fluorescence, ECD (Electrochemical Detector) and covers a range of compounds including Phenoxy Acid Herbicides, Phenyl Urea's, PFAS, Phenolics and Fluorinated surfactants amongst many others.

Often larger injection values than GC, with aqueous mobile phase as well as organic solvents. The columns vary in length and width, with various options for packing material.

The polarity of the static phase and mobile phase help create the separation, with the analyst also able to directly control pressure, flow rates, relative concentrations of multiple solvents within the mobile phase to vary parameters and further aid the chromatography.

In more recent years, the use of 2-Dimensional chromatography (GCxGC and LCxLC) has become more prevalent, the most common application being the use of GCxGC for investigatory analysis and now routine quantitative analysis of Hydrocarbons.

The principle is that two different columns of different chemistries' (different static phases) are connected prior to the detector with the flow of mobile phase/carrier gas modulated following the first column then into the second. In the case of TPH and GCxGC, the second column is much shorter in length and separates based on polarity rather than just the boiling points as done through column one.



Adding this second dimension gives rise to chromatograms like the above where we can see horizontally on the x-axis the separation from column one based on size/boiling point and now also a z-axis where the second column further separates the peaks based on the polarity.

In this instance the chromatogram shows the separation of aromatic from aliphatic elements of TPH, something that previously would be done by a secondary separation process during the extraction (Solid Phase Extraction – SPE) where the sample is loaded onto a static phase, usually silica or florisil and the components selectively eluted by modifying the elution solvent which would then generate two separate samples for instrumental analysis. Allowing the instrument to perform the live separation gives immediate operational efficiencies as also allows for more detailed levels of data to support an investigatory process.

The development of newer detection systems (Triple Quadrupole MS systems for example) allows for more selectivity and sensitivity at the back end of the process, pushing detection limits and allowing analysis of a broader range of matrices. But these and many other developing techniques will still rely on the appropriate application of good chromatographic principles to succeed.