

Chromatography Standards – Context, Relevance and Application

– Part 1

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Abstract

The following paper is the first of a series on organic standards applicable to the calibration, control, qualification and validation of Gas Chromatography (GC) and Liquid Chromatography instrumentation. The context includes, a brief introduction to the theory behind these measuring techniques, the application of various standards, detection systems and a brief overview of standards that facilitate the measurement of Volatile Organic Compounds (VOCs), Phenols, Polycyclic Aromatic Hydrocarbons (PAHs) and Pesticides. Particular emphasis is placed on informing the reader on where such substances are to be found as contaminants, their environmental and health impact and how the various types of standards (external, internal and surrogate) are used in practice. Information on how such standards are regulated and how these standards should be prepared and certified is presented. There are many high-quality producers of these products, but in the examples presented, the preparation and range of Reagecon products is used for example purposes as these are most familiar to the author. There is no suggestion that these Reagecon products are superior to others. The information in terms of the preparation and functionality of standards and the calibration and instrumental techniques is applicable to the measurement of many other analytes that include Fatty Acid Methyl Esters (FAMES), Fatty Acid Ethyl Esters (FAEEs), Azo Dyes, Nitrosamines, Polybrominated Biphenyls (PBBs), Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ethers (PBDEs), Phthalates, Semi Volatile Organic Compounds (SVOCs), PIANOs, PONAs, PNAs and Petrochemicals. Appropriate standards for such analytes will be covered in Part 2 and Part 3 of our series of papers on Chromatography Standards.

Chromatography Theory

Chromatography is the collective term for a set of laboratory techniques used for the separation of mixtures. The mixture (i.e. the sample) is carried by a liquid or gas and is separated into its respective components as a result of the different affinities of the components as they flow through a stationary liquid or solid phase, known as a column. Two of the main techniques of analytical chromatographic analysis are Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), both of which are instrumental.

The output of all instrumental chromatographic techniques, whether GC or HPLC, is a chromatogram, which is the visual output of the GC or HPLC instrument. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture. (See Figure 1)

Chromatogram of a Mixture of Polycyclic Aromatic Hydrocarbons (PAHs)

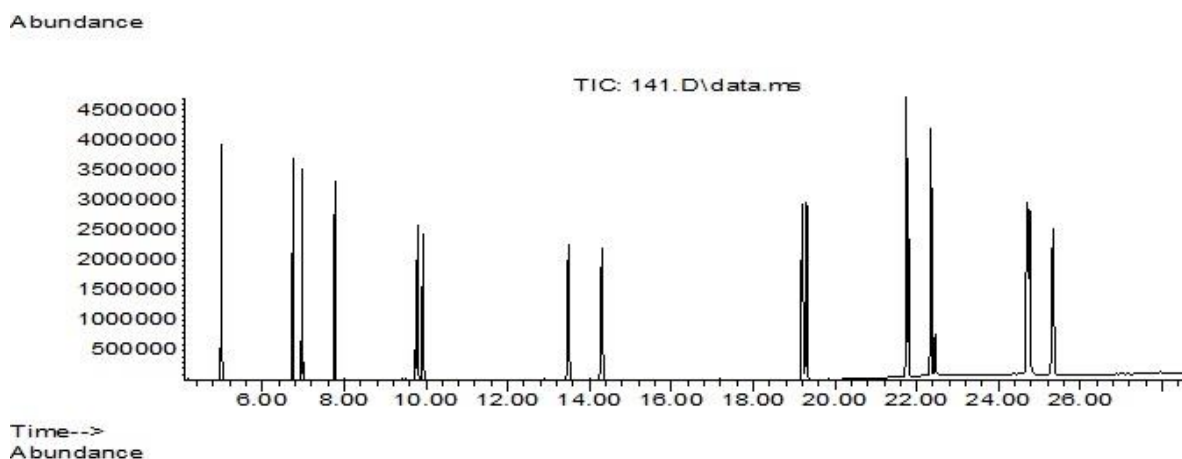


Figure 1

In Figure 1, plotted on the x-axis is the retention time and plotted on the y-axis a signal (a mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

Standards – External (Calibration), Internal and Surrogate

To quantify the amount of analytes in an unknown test sample, the analyst tests the sample in the same way as the standards and uses the comparison of instrument response for standards and samples to determine the identity and quantity of analytes in samples. The analyst may use a number of types of standards for this purpose as follows:

The Calibration Standard/External Standard

This is the standard that contains the analytes of interest. To quantify the amount of analytes in an unknown test sample, the analyst prepares a set of dilutions, called calibration curves. The dilutions are then tested by the GC or HPLC instrument. The result is a calibration curve, which is a graph of detector response (peak area) v concentration. Figure 2 overleaf shows a typical calibration curve.

A Typical Calibration Curve

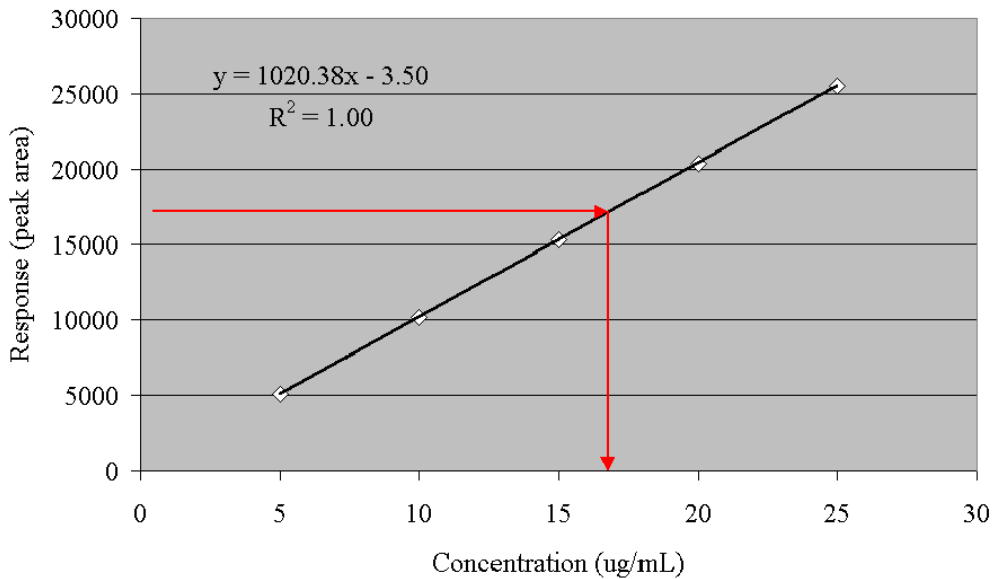


Figure 2

In Figure 2 the y-axis depicts the instrument response (peak area) and the x-axis contains the various calibration curve standards. The intercept line shows how an unknown sample is quantified from the peak area detected, against the set of standards.

Internal Standards

Very often in chromatographic systems the response of the detector over repeat tests is not constant/stable. This can lead to erroneous, imprecise results and non-linear calibration curves. To fix this, the analyst uses an internal standard. An internal standard is a compound or compounds that are chemically similar to the analytes of interest but are not being tested for. They are added to both the calibration curve dilutions and the test samples in the same known amount. Instead of then plotting a calibration curve of concentration of external standard v detector response, as in Figure 2, the analyst plots the ratio of internal standard response/external standard response v concentration. Because both the internal and external standards, fluctuate in response at the same rate, the response ratios, rather than the response alone, will remain the same. This has the effect of greatly improving both linearity of the calibration curve and the precision of the results. Internal standards are used in almost all chromatographic techniques in this way.

Surrogate standards

A surrogate is a material which is similar to the test analyte(s) in chemical composition and behaviour in the analytical process, but which is not normally found in real test samples. Surrogate standards are added in known amounts to test sample and their quantities determined by testing. The amounts recovered should be within a specification e.g. 90-110%. If the correct amounts are recovered then it can be assumed that the test method is working for the real analytes, which are under test. Calibration (External), Internal and Surrogate standards are routinely used in almost all chromatographic methods.

Types of Instruments

Gas Chromatography (GC)

Gas chromatography is used to separate organic compounds, that are volatile, or can be made to be volatile (i.e. are thermally labile). A gas chromatograph is an instrument which consists of a flowing gas mobile phase (called the carrier gas), an injection port, a separating column containing the stationary phase, a detector such as a Mass Spectrometer (MS), Flame Ionisation Detection (FID) or Thermal Conductivity Detection (TCD) and a data recording system. The organic compounds are separated due to differences in their partitioning behaviour, between the mobile gas phase and the stationary phase in the column and are thus separated. They are then detected by the detector system. Mobile phases are generally inert gases such as helium, argon, or nitrogen. The injection port consists of a rubber septum, through which a syringe needle is inserted, to inject the sample. The injection port is maintained at a higher temperature, than the boiling point of the least volatile component in the sample mixture. Since the partitioning behaviour is dependent on temperature, the separation column is usually contained in a thermostatically controlled oven. Separating components with a wide range of boiling points are accomplished, by starting at a low oven temperature and increasing the temperature over time, to elute the high-boiling point components.

Typical uses of GC include, separating the different components of a mixture of thermally labile compounds (the relative amounts of such components can also be determined) or, testing the purity of a particular substance. In some situations, with the use of selective detectors such as MS, GC may help in identifying a compound. GC covers the analysis of vast number of different types of compounds, including Volatile Organic Compounds (VOCs), Phenols, Polycyclic Aromatic Hydrocarbons (PAHs) and Pesticides (including herbicides and insecticides), among many others.

High Performance Liquid Chromatography (HPLC)

HPLC involves a liquid sample being passed over a solid adsorbent material packed into a column using a flow of liquid solvent mobile phase under high pressure. Each analyte in the sample interacts slightly differently with the adsorbent material in the column, thus changing the flow of the analytes. If the interaction is weak, the analytes flow off the column quickly, and if the interaction is strong, then the elution time is longer. A detector system is required to register the presence of various components within the sample but must not detect the solvent. For this reason, there is no universal detector that works for all separations. Examples of detectors that may be used include Ultra Violet/Visible (UV/Vis) as many organic molecules absorb UV radiation, detectors that measure fluorescence (FLV) as well as Mass Spectrometry (MS). HPLC is especially useful when an analyte or analytes are not thermally labile or where complex mixtures require separation that gas chromatography cannot achieve. HPLC techniques are used by the medical, pharmaceutical and research sectors for the analysis of drugs, Phenols, Polycyclic Aromatic Hydrocarbons (PAHs), Leachates, Pesticides and Food Residues and a wide variety of other types of compounds.

Chemistry/Analyte Groups

The following narrative briefly describes four of the major classes of products for which either GC or HPLC (or both) are particularly useful techniques.

Volatile Organic Compounds

Volatile organic compounds, or VOCs, are organic chemical compounds that are volatile (i.e. become a gas and evaporate) at room temperatures and are emitted as gases from solids or liquids. VOCs include a variety of chemicals, some of which may have short and long-term adverse health effects, and are emitted by a wide array of everyday products. Examples include: paints and lacquers, paint strippers, cleaning supplies, building materials and furnishings, office equipment such as copiers and printers, correction fluids and carbonless copy paper, graphics and craft materials including glues and adhesives, permanent markers, and photographic solutions. The level of certain VOC's in the environment and drinking water are monitored by national Environmental Protection Agencies (EPAs), and the level of some VOCs in the environment is controlled by legislation. The US EPA and the European Union have created a large number of reference analytical test methods, in particular GC, GC-MS and HPLC methods, for the testing/analysis of VOCs in the environment. These methods are used as standard methods by national EPA, governmental and contract laboratories all over the world for the determination of VOCs.

Bromoform, a haloalkane VOC that is routinely tested for in the environment and in drinking water.

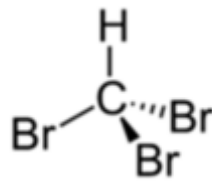


Figure 3

Adverse Health Effects of VOCs include:

- Liver, kidney and central nervous system damage
- Damage to the respiratory system.
- Some VOCs are carcinogenic/mutagenic/teratogenic. Benzene is an example of a well-known carcinogen.

VOC Standards/Internal Standards and Surrogates have applications in:

- Environmental Testing - US EPA/EU methods, EPA laboratories, Government Laboratories, University Laboratories, Contract Testing Laboratories
- Petrochemical Analysis – BTEX, Aromatics/Aliphatics testing
- Food Analysis/Residues testing
- Air/water/soil analysis
- Environmental forensics

Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of chemicals that occur naturally in coal, crude oil and gasoline. PAHs also are present in products made from fossil fuels, such as coal-tar, pitch, creosote and asphalt. When coal is converted to natural gas, PAHs can be released, for example. Although hundreds of PAHs exist, two of the more common and familiar ones are Benzo(ghi)perylene and Naphthalene. PAHs are found throughout the environment in the air, water and soil, and can remain in the environment for months or years. Levels of PAH in urban air may be 10 times greater than those found in rural areas. As is the case with VOCs, the levels of PAHs are routinely monitored in the environment, drinking water and foods with the aid of EPA Standard GC and HPLC methods. Some PAHs have been identified as carcinogenic, mutagenic and teratogenic and as such their analysis is of significant consequence. PAHs vary in size from the smallest,

naphthalene, to very large compounds such as benzo(ghi)perylene, as shown in Figure 4, although, there are also much larger PAH molecules than benzo(ghi)perylene.

Naphthalene and Benzo(ghi)perylene

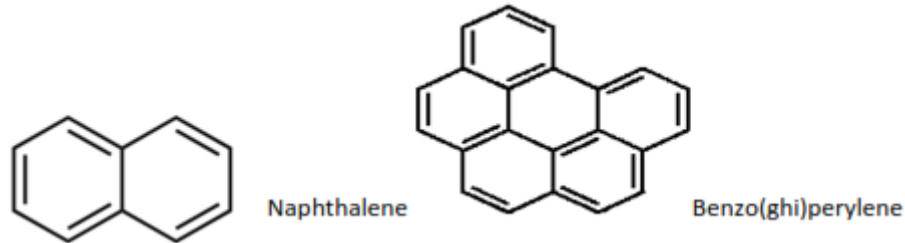


Figure 4

Both the US and the European Union EPAs have lists of controlled PAHs.

Adverse Health Effects of PAHs include:

- Gastrointestinal, pulmonary, renal and dermatological damage from short-term acute exposure or long-term exposure
- Some PAHs are confirmed carcinogens. Others are identified as possible carcinogens.
- Some PAHs also cause damage to the hematopoietic and immune system as well as neurological effects.
- Keto/Nitro/Alkyl PAHs and PAH metabolites are all likely to cause similar health effects that are caused by unsubstituted PAHs.

PAH Standards/Internal Standards and Surrogates have applications in:

- Environmental Testing – US EPA/EU methods, EPA Laboratories, Government Laboratories, University Laboratories, Contract Testing Laboratories
- Petrochemical Analysis – PAH Content
- Food Analysis/Residues Testing
- Air/Water/Soil Analysis
- Environmental Forensics

Phenols

Phenols are a manufactured class of weakly acidic water-soluble chemical compounds related to the organic chemical compound phenol which is naturally present in many foods. Phenols and phenolic

compounds are present in food dyes, artificial flavours, preservatives and in highly coloured fruits and vegetables and in bioflavonoids. Phenols are used as disinfectants, in medical products, and as a reagent in research laboratories and as a precursor or intermediate during the manufacture of phenolic resins, bisphenol A, adipic acid, alkylphenols, aniline, and chlorinated phenols. They are readily absorbed by inhalation, ingestion and skin contact and with other dietary chemicals, have been implicated in behavioural disorders. As is the case with VOCs, the quantity of phenols is routinely monitored in the environment, drinking water and foods with the aid of EPA GC and HPLC methods. Shown in Figure 5 is 2,3,4,5 Tetrachlorophenol, a common phenol. The hydroxyl (OH) group is always denoted as carbon No. 1

2,3,4,5 Tetrachlorophenol

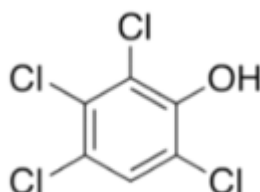


Figure 5

Adverse Health Effects of Phenols include:

- Respiratory irritation, burning of the eyes and headaches occur due to short-term, acute exposure
- Long term exposure effects include respiratory cancer, heart disease and effects on the immune system

Phenol Standards/Internal Standards and Surrogates have applications in:

- Environmental Testing – US EPA/EU methods, EPA Laboratories, Government Laboratories, University Laboratories, Contract Testing Laboratories
- Food Analysis/Residues Testing
- Air/Water/Soil Analysis

Pesticides

A pesticide is a chemical or biological agent that through its effect deters, incapacitates, kills or otherwise discourages pests. They may also inhibit the action or growth of animal, insect or plant pests. Pests include plants or animals which may prevent the successful growth/production of

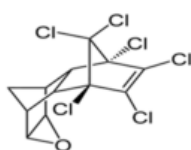
food, manufacture, processing, storage or transport of food, wood or wood products, feedstuffs or other agricultural commodities. Defoliant, desiccant, plant growth regulator or fruit thinning agents are all pesticides, as well as substances used to for the control of insects, arachnids or other insect/animal pests. Pesticides are also frequently used to protect a commodity from deterioration during storage and transport.

All pesticides must be assessed by either the US EPA (for use in the USA) or the EU before they can be licensed for use. The EPA/EU will only grant licenses after the manufacturing company has met stringent regulatory requirements, achieved by extensive scientific evaluation. The US EPA also works with OECD in the licensing of pesticides for use outside the US. As well as issuing licenses for use, the EU and the US EPA have banned many pesticides from use, owing to their unintended harmful effects to humans and flora/fauna.

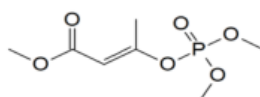
Pesticides must be tested for in both the environment and in food stuffs or agricultural commodities. The US, the EU and national governments have limits on the quantities of pesticides permissible in the environment and also have MRLs (Maximum Residue Limits) on the amount of residual pesticides in food. This means that a huge amount of pesticide testing is carried out throughout the world, not only for licensed pesticides but also for pesticides which have previously been banned. Pesticide testing is of paramount importance in food safety and environmental analysis.

Pesticides fall broadly into three main chemical categories: Organochlorine, Organophosphorous and Organonitrogen.

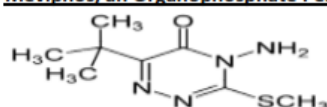
Examples of the three main categories of pesticides



Endrin, an Organochlorine Pesticide



Meviphos, an Organophosphate Pesticide



Metribuzin, an Organonitrogen Pesticide

Figure 6

As is the case with VOCs, the level of pesticides is routinely monitored in the environment, drinking water and foods with the aid of EPA and food safety GC and HPLC methods.

Adverse Health Effects of Pesticides include:

- Birth defects, nerve damage and cancer from both long- and short-term exposure.
- Death in humans/animals. Category I pesticides may be fatal on ingestion of quantities of greater than 5 grams.
- Severe environmental damage including pollution of rivers, wells and streams. More than 90% of US environmental water is polluted by one or more pesticides. Pesticides can also extensively damage soil and thus affect agricultural output.
- Pesticides have had a major negative on wildlife, especially birds such as hawks, eagles, falcons and other species at the top of the food chain. Pesticides are also lethal to large marine and land predators, and thus they upset/damage the food chain. More than 72 million birds die each year in the US due to pesticides.
- Bees – pesticides have been and are still reducing the amount of pollination performed by bees each year. This affects the whole ecosystem and has the potential to seriously disrupt food production.

Pesticide Standards/Internal Standards have applications in:

- Environmental Testing - US EPA/EU methods, EPA laboratories, Government Laboratories, University Laboratories, Contract Testing Laboratories
- Food Analysis/Residues testing
- Air/Water/Soil Analysis

Reagecon Chromatography Standards

Weighing/Preparation

All Reagecon [Chromatography Standards](#) are prepared gravimetrically on a weight/weight basis. Both solute and solvent are weighed on a balance calibrated by Reagecon engineers using OIML traceable weights. Reagecon holds ISO17025 accreditation for calibration of laboratory balances (INAB ref 265C). The resulting Balance Certificate of Calibration is issued in accordance with requirements of ISO/IEC17025. The concentration of each standard is verified using a high-performance Gas Chromatograph Mass Spectrometer (GC-MS). The calibration of the GC-MS Instrument is completed

using high purity ISO Guide 34 accredited Chromatography Standards similar in composition and concentration to the manufactured Chromatography standard. The Mass Spectrum of each of the analytes is confirmed by comparison with the National Institute of Standards and Technology (NIST) Mass Spectral library.

Analyte Groups, Singles and Mixes

Reagecon produce a wide range of [VOC](#), [Phenol](#), [PAH](#) and [Pesticide](#) Standards in many mixes and configurations, either single compounds, multi-elemental compounds or customised formulations. As already stated, there are many good producers of organic standards, Reagecon is one such producer and are cited of how a good organic standard should be produced and certified, for example purposes.

Industrial Applications

Environmental Testing

US EPA specified methods are used worldwide by both public and private environmental Laboratories. These methods were initially developed in the 1970s by the US EPA to create an approach to standard methods for the analysis of pollutants in the environment. The methods are continuously revised and more and more new methods added to reflect changes in both types and quantities of analytes, changes in industrial and agricultural chemicals being used and also improvements/additions to instrumentation. The main series of tests are outlined in Table 1:

Main Series of EPA Test Methods

EPA Method	Analysis
500 Series	Pollutants in drinking water
600 Series	Pollutants in municipal or industrial wastewater
8000 Series	Pollutants in sludge's, sediments and solid wastes

Table 1

Within each series a wide spectrum of analyte groups are encountered e.g. VOCs, phenols, PAHs and pesticides. Unlike standard methods such as ASTM, EPA methods are for guidance only and need not be followed exactly. Therefore, laboratories can create in-house methods based loosely

on the EPA methods, but with local method flexibility. ISO17025 accreditation was made mandatory for all laboratories reporting to national EPAs across the EU from 2014. Auditors generally require that all purchased standards used are from ISO17025 accredited manufacturers.

Conclusion

The narrative presented in this paper, is an attempt to provide the reader with an overview, of the optimal methodology for measurement of families of organic compounds that include VOC's, PAH's, Phenols and Pesticides. The overview is not designed to be exhaustive, but merely, to provide guidelines on that methodology, description of the various organic families and possible adverse health effects. Because the expertise and experience of the author pertains particularly to standards and metrology, significant attention is paid to that aspect of the analytical process, with guidance as to how a good quality standard should be prepared, by a commercial producer. Significant additional detail on specification, metrology and availability of organic and inorganic standards is to be found in other [publications](#) from this author.