The analysis of caffeine in soft drinks

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This essay details the history, development, importance and applications of high-performance liquid chromatography (HPLC), and discusses its use alongside other analytical techniques in determining the concentration of caffeine in soft drinks. Methods for the removal of caffeine from soft drinks are compared.

Introduction

Coffee is one the most important commodities in the global economy, and millions of tonnes of it are produced and consumed every year[1]. Caffeine is an important substance in coffee, responsible for its stimulant effects and bitter taste. Consuming too much caffeine can cause people to experience unpleasant short-term symptoms (such as restlessness, insomnia and tachycardia), and there may be more serious long-term effects such as increased risk of cardiovascular disease[2]. There is a large market for decaffeinated drinks, which allow consumers to avoid some of the problems of caffeine-containing products, and trade organisations and governments regulate the level of caffeine permitted in such drinks[3]. Accurate and reliable methods to monitor and control the caffeine content of decaffeinated drinks are of great importance to industry.

There are a variety ways to determine the concentration of caffeine in drinks, and high-performance liquid chromatography (HPLC) is an effective one[4]. Caffeine can be removed from drinks in a process termed decaffeination. Due to its economic importance, there is an extensive range of methods of decaffeination, although by far the most common ones use water, organic solvents or supercritical carbon dioxide[2].

Caffeine

Caffeine is a purine alkaloid of the methylxanthine class and is synthesised in some plants, probably as a pesticide and perhaps also to prevent other plants growing nearby[5]. It generally occurs alongside its demethylated analogues theobromine and theophylline[2].

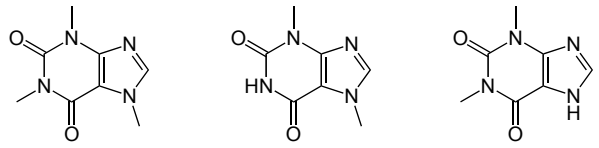

Figure 1. Skeletal formulae of caffeine[6], theophylline and theobromine[7].

In humans, caffeine has the pharmacological effects of central nervous system stimulation, diuresis, stimulation of cardiac muscle, and relaxation of smooth muscle, particularly bronchial muscle. It is not used clinically, except in over-the-counter remedies, although theophylline finds use as a bronchodilator in the treatment of asthma. Caffeine is generally safe even in very high doses[8]: 10 g, equivalent to around 70 cups of coffee, constitutes a fatal dose[2]. Roasted ground coffee contains around 2% caffeine, which is reduced to less than 0.1% after decaffeination[2].

Type of coffee	Typical caffeine concentration / mg ml^{-1}
decaffeintated	0.01–0.02
espresso	1.7–2.2
instant	0.3–0.5
brewed	0.4–0.7
filter	0.5–0.9

Figure 2. Representative caffeine content of different types of coffee[9].

Basic principles of chromatography

The term *chromatography* encompasses a wide variety of different separation methods, but all chromatographic techniques involve the same underlying process[10]:

A chemical mixture, the analyte, is exposed to a stationary phase and a mobile phase. The different components in the analyte then interact with and partition themselves between the two phases. The mobile phase moves in a definite direction relative to the stationary phase, carrying along fastest those components with the greatest affinity (strength or extent of interaction) for it. The components of the analyte with a greater affinity for the stationary phase are carried more slowly by the mobile phase, thus leading to the separation of different components based on their relative affinities to the two phases.

Classical liquid chromatography and its limitations

The mobile and stationary phases can be solids, liquids or gases, and the various possible combinations give rise to several different types of chromatography with greatly differing capabilities. Chromatography employing a liquid mobile phase is called liquid chromatography (LC) and can have either a solid stationary phase (liquid-solid chromatography, LSC) or a liquid stationary phase coating a solid support (liquid-liquid chromatography, LLC)[11]. Classical liquid chromatography suffers limitations such as low flow rates due to the slow rate of diffusion of liquids – a longer time is required to establish equilibrium between the stationary and mobile phases, as analyte molecules have to travel long distances to move from the stationary to the mobile phase[12].

History of high-performance liquid chromatography

At the beginning of the 1960s, liquid chromatography involved long, wide-diameter columns, powered by gravity and was slow and inefficient[13].

Gas chromatography (GC), first developed in 1952, is fast and sensitive[12]. The understanding gained by chromatographers during the development of GC led to the realisation that much more efficient liquid chromatography separations could be achieved by replacing the 150 μm-diameter stationary phase particles with small porous stationary-phase particles of around 3-10 μm in diameter and by pressurising the mobile phase to make it flow faster[13], using pressures of up to 5000 psi (350 atm)[14]. The method developed from these theoretical predictions first emerged in the late 1960s[15] and was initially known as high-pressure liquid chromatography[14] but was subsequently renamed high-performance liquid chromatography because it is much faster and has much better resolving power than traditional liquid chromatography[10].

This was a real breakthrough, because HPLC can analyse a much wider variety of substances than can gas chromatography[13], which can only be used with compounds that evaporate easily. For many molecules, this is not the case – a high molecular weight or high polarity may make the molecule non-volatile, or it may undergo thermal decomposition at temperatures necessary to achieve evaporation. HPLC combines the best features of liquid chromatography and gas chromatography to provide a separation technique that is very rapid and sensitive, and yet can be used with almost any kind of molecule and any complexity of mixture.

How HPLC works

In HPLC, the stationary phase typically consists of very fine micron-sized particles of silica or alumina packed into a stainless steel column, around 3-5 mm in diameter and 10-30 cm in length.[10] A liquid mobile phase of one or more solvents is pumped from reservoirs into the column. The sample is injected into the mobile phase as it flows into the column. As the sample is forced through the column packed with stationary phase particles, its various components separate as they each have a different average rate of passage through the column due to their different partition coefficients, K_D (ratio of concentrations in stationary and mobile phases) and are washed out of the column (eluted) at different times.

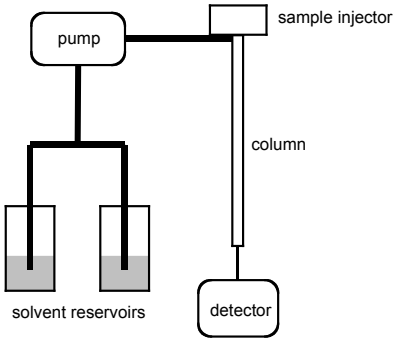

Figure 3. Simple diagram of the components of a high-performance liquid chromatograph, based on a figure in Christian[12].

In the original HPLC setups, the stationary phase had polar groups on its surfaces (e.g. silanol groups on unmodified silica) and a non-polar mobile phase such as hexane. This arrangement is normal phase HPLC (NP-HPLC) and is suitable for polar analytes which interact well with the polar stationary phase. Non-polar components of a sample do not interact well with the polar stationary phase and will elute more quickly without being well resolved.

Switching the polarities of the stationary and mobile phases leads to reverse-phase HPLC. RP-HPLC is much more common than NP-HPLC. RP has a non-polar stationary phase (e.g. silica functionalised with long alkyl chains) and a polar mobile phase, acetonitrile, water or methanol, for instance. To attach non-polar chains such as octyl and octadecyl groups to a silica particle, a reactive silicon electrophile such as an alkyldimethylsilyl chloride, RMe_2SiCl, is used. A lone pair on the oxygen atom of a terminal silanol (Si-OH) group on the surface of a silica particle attacks silicon, displacing a good leaving group such as chloride. The silanol proton is abstracted by any basic species present and the product is a functionalised silica particle with Si-O-Si-R units. The long hydrocarbon chains protrude from the silica particle and provide a tethered non-polar stationary phase.

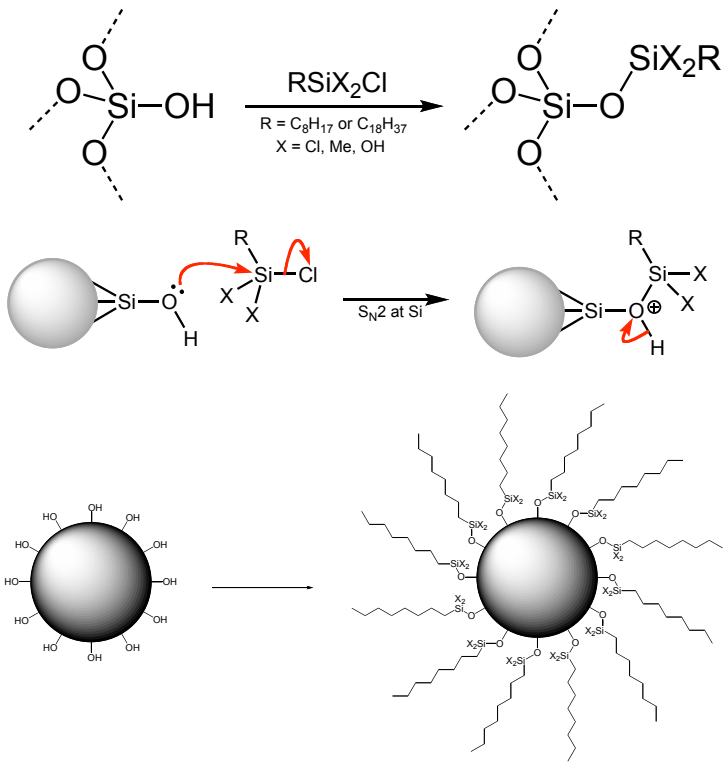

Figure 4. Functionalisation of terminal silanol groups on the surfaces of silica particles for reverse phase HPLC.

The mechanism of the interaction of analyte molecules with silica-bound alkyl chains has been investigated computationally and is found to be very different to the interaction free liquid hydrocarbon solvents, with small non-polar molecules interacting by adsorbing to the ends of chains rather than embedding themselves in between chains as previously assumed[16].

Solvent gradients

The mobile phase in an HPLC experiment can consist of a solvent or mixture of solvents whose composition does not change as the separation takes place. Such a solvent system is termed isocratic.

To maximise the effectiveness of HPLC, the composition of the mobile phase can be varied during the separation, a process known as gradient elution. The mobile phase may, for example, consist of a very polar solvent at the start of the experiment and steadily decrease in polarity as a greater proportion of a non-polar solvent is mixed in to the mobile phase. The advantage of gradient elution is that it avoids trade-off between high resolution of the least well retained compounds and quick elution of the best retained compounds – the molecules with a low affinity for the stationary phase are well resolved as they elute, and then the mobile phase changes polarity and molecules that previously interacted very little with the mobile phase can then be eluted quickly.

Applications of HPLC

Research and development of HPLC proper has slowed in the past twenty years – technique has matured[15]. It remains very widely used for both analysis and preparative separation.

It is invaluable in the biological sciences, where complicated mixtures of molecules including high molecular weight proteins and other biopolymers need to be separated efficiently and quickly, but where gas chromatography is unsuitable. The pharmaceutical industry uses HPLC for quality control and for monitoring the rates of dissolution and degradation of medicines.

HPLC is very useful for determining levels of pollutants in samples collected from the environment, and has many applications in forensics where complex mixtures of biological fluids, drugs or explosives can be analysed. Similarly, HPLC provides a valuable tool to research in food and agriculture, again where mixtures with many components, of widely varying molecular weight and chemical behaviour are common.

Detectors for HPLC

The fractions eluted from an HPLC experiment can be identified in a number of ways, the most common being ultraviolet-visible spectroscopy (UV-vis) and photodiode absorption detection. UV-vis is popular because it is sensitive and is applicable to most organic compounds of interest, but it can only measure the absorbance of one wavelength of light at any particular moment in time. This limitation is not a problem if the components of interest all strongly absorb UV or visible light of a similar wavelength, but if this is not the case, repeat experiments at different wavelengths may be necessary.

Photodiode arrays (PDAs), made of semiconducting materials, are able to detect the intensity of light at a wide range of wavelengths simultaneously and are therefore more versatile than UV-vis detectors as they can identify the presence of molecules with different characteristic wavelengths of absorption at the same time. PDAs thus provide a three-dimensional data set from an HPLC experiment (time, wavelength, absorbance) and decrease the need for multiple experiments, saving time, effort and money[10].

For mixtures containing components whose retention times and UV-vis spectra are very similar, UV-vis and photodiode array detectors may be unable to identify the different compounds separately. In such cases, mass spectrometry (MS) may provide invaluable in analysing the sample, as it provides more direct structural data on the molecules present[17]. HPLC-MS necessitates the removal of solvent from the eluent, and this can be done in a hot steel capillary tube called a thermospray interface[10], before the analyte is presented to the mass spectrometer.

Many other detectors are suitable for HPLC use, and each have their own advantages, depending on the nature of the analyte. Fluorescence detectors can be extremely sensitive when fluorescent analytes are present. Ions can be detected with electrochemical detectors, and infra-red spectroscopy is sometimes useful despite the fact that most solvents absorb IR strongly[10].

Determination of caffeine content in soft drinks

It is quite possible to analyse caffeine with non-HPLC methods, and indeed many techniques have been successfully utilised, including UV-vis and IR spectroscopy, GC, ion chromatography, thin-layer chromatography and capillary electrophoresis. A method employing gas chromatography-mass spectrometry and using only one drop of solvent has been developed [4]. Its benefits include low cost, negligible solvent waste, simplicity and speed. These methods are all quantitative, providing they are calibrated with caffeine samples of known concentration. Internal standards can be added to check no sample is lost in during the process, or to account for any that does. For the analysis of caffeine in biological matrices such as blood or sweat, samples may need pre-treatment to remove protein[18].

Methods for caffeine extraction and industrial decaffeination

Decaffeination was first performed in 1900 and for the next 70 years, the method of choice was solvent extraction. The most effective solvent was dichloromethane (methylene chloride, CH_2Cl_2), but its use began to be phased out in the 1970s due to concerns about its possible carcinogenicity and its detrimental effect on the ozone layer. Ethyl acetate has enjoyed some success as a replacement for dichloromethane, since it is non-toxic and of much less environmental concern[2]. Water can extract caffeine from coffee, and several such procedures are in use in industry, but caffeine's solubility in water is much greater in hot or boiling water, which is detrimental to the flavour of the decaffeinated coffee produced. The problem has been overcome by saturating the caffeine-extracting water with coffee compounds.

Above its critical point[7] of 305 K and 7.4 MPa, carbon dioxide becomes a supercritical fluid, and has the desirable properties of high density combined with low viscosity. Supercritical CO_2 is a very selective solvent for caffeine, producing in much better tasting decaffeinated coffee than is obtained by other methods. It is non-toxic and easily separated from the decaffeinated product.

The main drawback to supercritical CO_2 is the high cost of the machinery and the expertise required to operate it safely, so it is most suited to large-scale production plants. More exotic decaffeination methods exist, including the use of microorganisms which feed on caffeine, but these are not yet commonplace in industry[2].

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