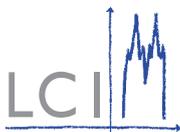


"Humps" – Challenges in Chromatographic Separation

When determining the ingredients and composition of a food an analyst's objective is to isolate and quantify the substances it contains. Chromatography offers just such a possibility.



Food Chemistry
Institute of
the Association
of the German
Confectionery Industry

Institute Director:
Prof. Dr.
Reinhard Matissek

Adamsstraße 52 - 54
51063 Köln

phone
+49 221 62 30 61
fax
+49 221 61 04 77
web
www.lci-koeln.de

A "normal" chromatogram shows sharply differentiated and clearly recognisable individual signals, also known as peaks (cf. Fig. 1).

A measure of how well two peaks are separated from each other is provided by so-called "resolution". This is understood to mean the correlation between a system's selectivity and its efficiency. Selectivity provides the difference between the retention times of two components, i.e. the bigger the difference, the better the chromatographic separation. Efficiency of a separation describes the peak broadening of the component: the narrower the

rated peaks but instead show broad humps. These humps are also referred to as unresolved complex mixtures (UCMs). Humps partially have spiked peaks on top of them, indicating resolved substances (cf. Fig. 2).

The term "unresolved complex mixture" was first coined by Farrington and Quinn in 1973 when describing chromatograms of sediment extracts taken from the Narragansett Bay in the USA. A hump is comprised of thousands of individual substances which, due to their great number, cannot be separated and resolved using simple chromatographic methods. This renders

by way of a quantification as the sum total of all components, integrating the entire hump from trough to trough.

If one assumes, for example, that the number of possible mineral hydrocarbons is 40, the various combination possibilities mean that theoretically over 60 trillion different constitutional isomers are possible. If one then hypothetically presumes that a fraction of these 60 trillion individual compounds is present and all compounds occur in the same concentration, a real MOAH content of 0.6 mg/kg leads to a notional content of $1 \cdot 10^{-18}$ mg/kg per individual substance, i.e. 10^{-3} femtograms or

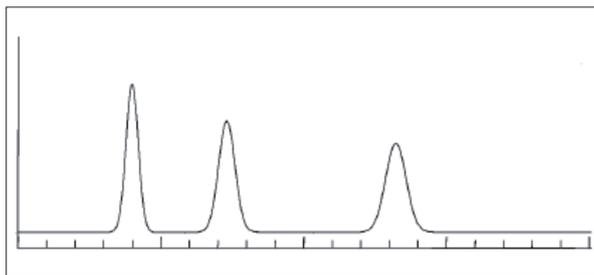


Figure 1 - Typical chromatogram

peak, the more successful the separation. To influence the resolution, for example, the separation column can be lengthened. This leads to a greater number of so-called theoretical plates. In this connection, plate theory divides the separation column into consecutive sections with a corresponding height. The smaller the differences in retention times of substances are, the more theoretical plates are required for separation. Accordingly, many theoretical plates leads to a high resolution. Mathematical models enable calculation of which height equivalents to one theoretical plate are achievable at best and hence where the performance limits of a separation column lie. Usual values for the number of plates in gas chromatography come to 10,000, enabling 200 to 500 peaks to be separated from each other. In liquid chromatography, typical separation columns have between 6,000 and 15,000 plates, resulting in a separation of 50 to 100 peaks.

Some gas and liquid chromatograms, however, do not reveal sharply separated

identification and quantification either difficult or impossible.

In liquid chromatography these humps appear, for example, in the analysis of black tea and are also termed "thearubigin humps". Thearubigins are largely composed of polyphenols formed during fermentation. According to estimates, a cup of black tea contains at least 30,000 different chemical compounds. In terms of its complexity, the composition of black tea is only exceeded by that of mineral oil.

Hence gas chromatography particularly reveals characteristic humps in chromatograms of samples contaminated with mineral hydrocarbons due to the great number of components contained therein. As a result of this complexity the determination of MOSH (mineral oil saturated hydrocarbons) and MOAH (mineral oil aromatic hydrocarbons) cannot be conducted in the form of an individual component analysis but, using GC-FID (gas chromatography with flame ionisation detection), are determined

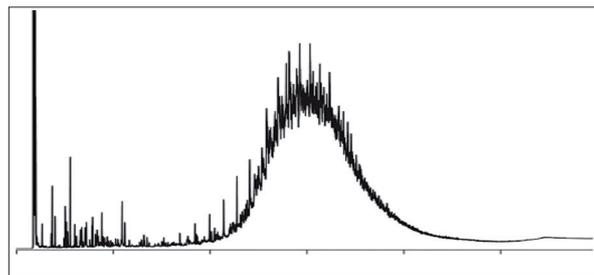


Figure 2 - Characteristic "hump" showing in a gas chromatogram

an atogram of the individual compound per kilogram of the sample.

Analytical determination of MOSH and MOAH as a cumulative parameter was successfully developed and validated by the LCI and can be commissioned in our sister institute, the Institute of Quality Promotion in the Confectionery Industry (IQ.Köln) in Cologne.

Within the course of developing modern analysis methods, such as two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOF-MS) or Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ever higher resolution capacities can still be achieved, however. This has partially enabled the composition of UCMs to be resolved. The existence of UCMs can, however, be used in less sophisticated lower resolution chromatography as a screening tool to discover samples containing complex mixtures of mineral hydrocarbons that require more extensive analyses.