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*Without any complex sample preparation, an ion mobility spectrometer, coupled with a gas chromatograph, gives details about the flavouring agents and bitter-tasting compounds that contain a hops sample. An automated evaluation method helps in classifying the data. The results then allow conclusions about the hops variety and source.*

- **IN BRIEF**

- In contrast to GC-MS, ion mobility spectrometer (IMS) requires only less effort for apparatus technology, sample preparation and identification of substances.
- The detection limits are between 0.1 and 1 ng or one-billionth volume units (10 microlitre per m<sup>3</sup>) with a dynamic range up to three orders of magnitude.
- The coupling of GC and IMS detects flavouring agents in hops and clearly distinguishes also sensitive varieties of hops even from the same areas of cultivation.

Beer consists of an undetermined number of compounds, of which only a few hundred are known. Hops (*Humulus lupulus*) characterises the taste and smell of the beer. Terpenes, hop oils, polyphenols - including benzoic and cinammic acid derivatives, flavonoids, tannins and the less water-soluble, bacteriostatically active alpha and beta hop acids (humulones and lupulones) cause the mouthfeell.

### **Flavouring agents and bitter-tasting compounds**

Freshly harvested hops contains 3 - 17% alpha acids depending on the variety, area of cultivation and the season. They isomerise to water-soluble bitter-tasting compounds when boiling the wort. The iso-alpha acids almost reach the bitterness of quinine and also stabilise the beer froth. The water-soluble oxidation products of the beta acids (hulupones), which form during the storage of the hops, taste more or less twice as bitter as the iso-alpha acids as the bitterness of their oxidation products subsides.

Bitter-tasting compounds are assayed according to the European Brewery Convention (EBC) using HPLC (ESC 7.7) or as molecular parameter through conductometric titration (EBC 7.4). As magnitude of the bitterness of the beer, the International Bitterness Unit (IEU), measured as UV extinction of the iso-octane extract at 275 nm. The photometric hops storage index (HSI) is assayed from the toluene extract after dilution with methanolic alkaline solution and indicates the ratio of the extinctions between the decomposition products and the hop acids  $A(275 \text{ nm})/A(325 \text{ nm})$ . 8.9)

Flavours from modern hops extracts, e.g. hydrated isohumulones, enrich beer drinks, such as alcopops or beers with a fruity taste (flavour hops). Unwanted volatile aldehydes such as the cucumber-like fatty smelling trans-Non-2-enal form from hop lipids and other precursors during storage of bottled beer. The skunk-smelling 3-methylbut-2-en-1-thiol is caused by the photo-chemical decomposition of light-sensitive iso-alpha acids in the presence of riboflavin (vitamin B2).

In fresh hop oil, there are myrcene, beta caryophyllene, humulene (alpha caryophyllene) and other terpenes that form oxidation products during storage and in the beer. The terpene alcohols linaliol, geraniol, myrcene and their esters and oxidation products contribute to the flowery or resin-like flavour. The taste are at a few micrograms per litre.

Traces of aliphatic carbonyl compounds, acids and esters from aged hops oil are converted in the multistage brewing process partly into unpleasantly smelling components. The bitter-tasting 8-prenylningenin is the most effective phytoestrogen found in hops to date and is currently being tested for pharmaceutical applications

### Distinguishing Characteristics

Different varieties of hops are traditionally differentiated morphologically by characteristics of the hop umbels, DNA analysis and the composition of bitter-tasting compounds and essential oils. Although the flavonoid composition is considered variety-specific, European varieties differ only minimally. Headspace gas chromatograph of the essential oils occur in most of the hops varieties in the order of their volatility: alpha-pinene, 2-methyl-but-3-en-2-ol, beta-pinene, myrcene, limonene, alpha-ylangene, alpha-copaene, linalool, beta-caryophyll, aromadendrene, humulene, farnesene, gamma-murolene, viridiflorene, beta-selinene, alpha-selinene, cadinene and selinadiene, including a series of unidentified compounds. In the search for recognisable signs of odours and flavours which allow testing of identity and mix-up, we examined the hops using an ion mobility spectrometer with upstream GC column (GC-IMS). Further tests were done with flour, plastics and combustion residues.

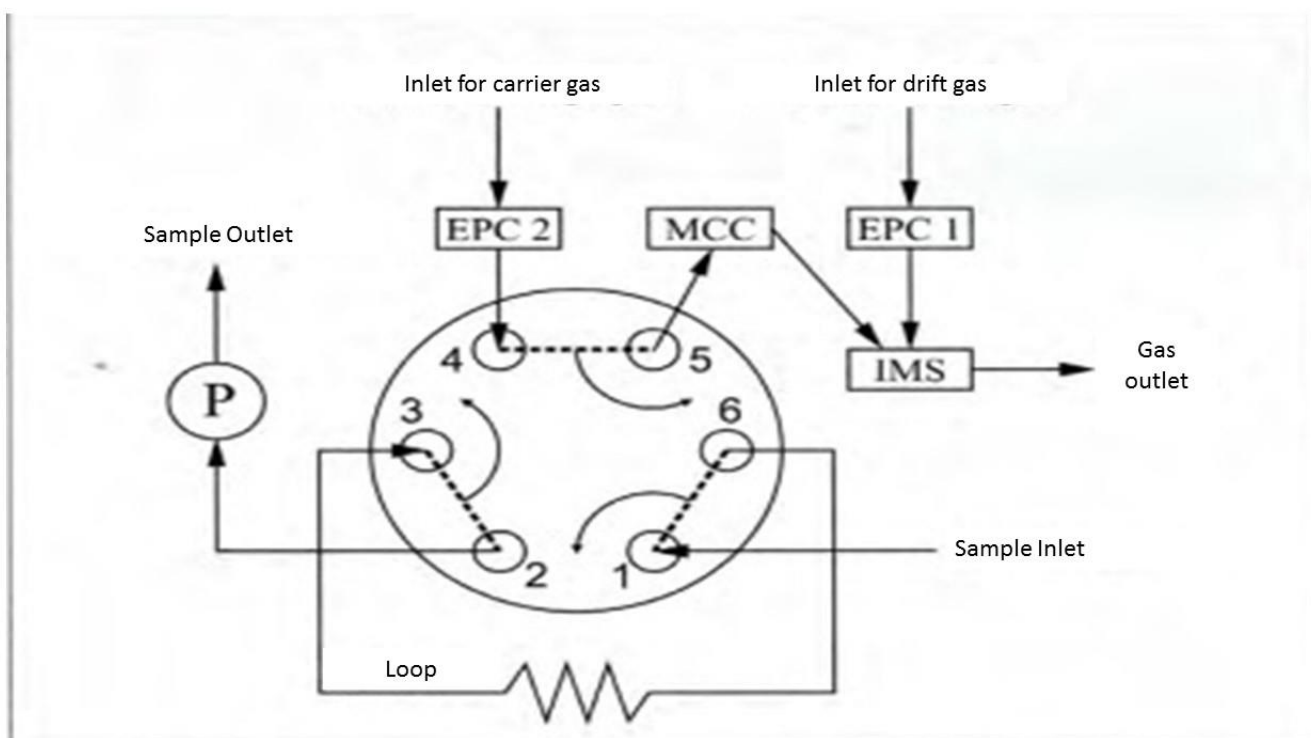


Fig. 1. Gas flow in a GC-IMS device during sampling in the sample loop: EPC = pressure regulator, P = pump. In the injection phase, the sample loop is emptied along the path 4-3-6-5 into the gas chromatograph column (MCC).

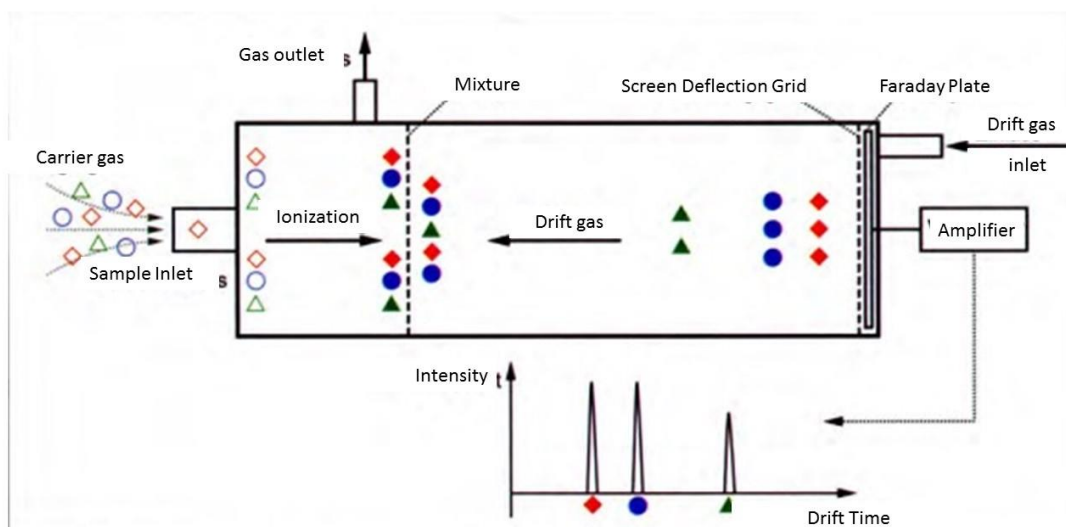


Fig. 3. Left: section from the GC-IMS spectrum of milled hop pellets. Right: coloured illustration and grey value illustration (fingerprint signature) of the marked topographic areas. Hop varieties: Hallertauer Northern Brewer (D), Perle (E), Spatter Select (F), Hallertauer Mittelfrueh from Spalt (G), Tettnanger (1). The colour coding illustrates the ion flow: black blue: null; bright blue: small; yellowsh white: medium; red: high.

### Ion mobility spectrometer

Ion mobility spectrometer has existed since 1970. Today they are considered as state of the art in the direct ultra-tracing analysis without sample preparation of chemical combatants, explosives, pesticides, safety-related hazardous substances at the workplace and drugs. Moreover, the IMS technology develops applications in food analysis and quality assurance. Examples are the purity test of special gases, the BTX (benzene, toluene and xylene) content of natural gas, the analysis of volatile organic compounds (VOC) indoors at concentrations of micrograms per cubic meter, detection of air contamination by microbes (MVOC), the medical analysis of respiratory air and the quality assessment of wine, coffee, olive oil, cola drinks, fish and beer.

In recent years, adsorption tubes and thermal desorption, solid phase micro-extraction (SPME), hollow fibre liquid phase micro-extraction (HF-LPME), flow injection systems and headspace gas chromatography with multicapillary columns (MCC) were tested for sample preparation. The coupling with multidimensional mass spectrometry (IMS-Msn) and laser desorption (MALD-IMS-MS) has advanced the analysis of isomeric, biological, polymeric or petrochemical samples.

The advantage of the ion mobility spectrometer when compared to the mass spectrometer is the basic option of re-isolating compounds of the same molar mass according to the molecular form and charge.

### Experimental information

For the hops test, we used a GC-IMS trace analyser of the Gesellschaft für analytische Sensorsysteme (G.A.S.) (Association of analytical sensor systems), Dortmund. Apart from a gas cylinder with drift gas (nitrogen or synthetic air) no other auxiliaries and is suitable in general for mobile applications when mounted on laboratory trolley. The device works with a tritium radiation source of 300 MBq, whose operation does not require any special radiation-related approval. A cannula transfers the substance from a headspace tube over the heatable 6-way valve into the gas-tight sample loop (1 up to 10 ml), the sample finally reaches the gas chromatographic multicapillary column and the IMS detector (Fig. 2, p. 657).

Ionisation using a radiation source causes positive and negative reactant ions  $R^+$  in the drift gas; depending

on the moisture in the gas, they are  $N_2^+$ ,  $N_4^+$ ,  $H(H_2O)^n+$ ,  $[NH_4(H_2O)]^n+$ ,  $[NO(H_2O)]^n+$  and  $CNO^-$ ,  $[O_2(H_2O)]^n-$ ,  $[OH(H_2O)]^n-$ ,  $Cl^-$ ,  $NO_2^-$  and others. Through the collision of  $[O_2(H_2O)]^n-$  and  $[H(H_2O)]^n+$  with the analyte molecules they respond to product ions such as  $M^-$ ,  $RM^-$  and  $MW$ ,  $MNO^+$ ,  $M_2H^+$ , etc. All molecules with a larger proton affinity than water (691 kJ/mol) are ionised by proton transfer, without requiring vacuum.

In the drift tubes, an electric pulse of 0.1 to 0.2 ms duration, injects the ions periodically through a witching grid (screen) into the drift zone fed with drift gas at atmospheric pressure in a counter current manner (approx. 200 ml/min), non-ionised molecules and contamination are flushed off the reaction space as a result.

In the homogeneous electric field of the metal rings and insulators arranged one behind the other (15 to 40 kV/m), the aqueous ion suspension is isolated, as the heavy ions with the same energy traverse the drift tubes with a length of a few centimeters slower than the ions of lower mass. The drift speed also depends on the substance-specific molecular cross-section of the sample substance, the mass of the drift gas molecules, the temperature and other factors.

When colliding on the Faraday plate (electrometer) at the end of the tubes, the ions cause a flow that is proportional to the concentration, which is amplified and applied as an intensity signal to the drift time. Depending on the polarity of the drift voltage, at an interval of 21 ms or many times thereof, positive or negative spectra are recorded continuously. The drift time is related to the peak of the reaction ions (RIP). This relative drift time  $tD/tD$ , RIP allows identification of substances; the peak intensity results in the concentrations.

The clear assignment of all substances and reaction peaks to compounds is difficult, but not necessary for an identity test. Without preliminary chromatographic isolation, additional peak overlaps and ion interactions occur. The often complicated, three-dimensional spectra are evaluated with an intuitive application known from the gas chromatography.

### Tested hops varieties

We tested hops pellets of different varieties and flavours from the cultivation areas of Hallertau, Spalt, Tettngang und Slovenia and two beer varieties from Hallertau. In the hops pellets of the type 90, 100 kg raw hops is pressed to 90 kg pellets, which still contain raw fibres from the sepals and stalks of the umbels.

1. Hallertauer Mittelfrueher: flowery fruity smell, harmoniously spicy bitter taste in the drink(A);

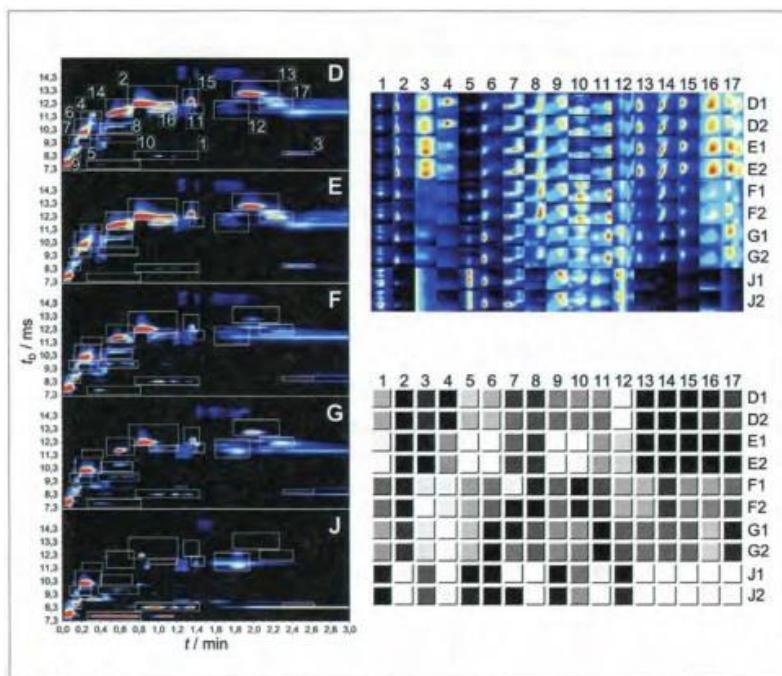
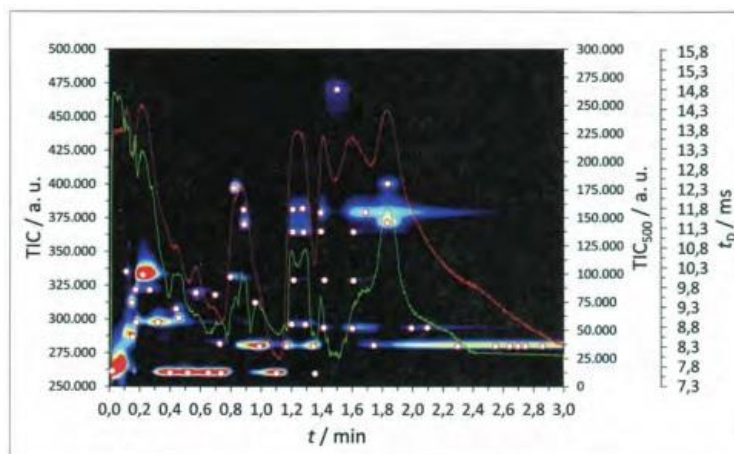


Fig. 3. Left: section from the GC-IMS spectrum of milled hop pellets. Right: coloured illustration and grey value illustration (fingerprint signature) of the marked topographic areas. Hop varieties: Hallertauer Northern Brewer (D), Perle (E), Spatter Select (F), Hallertauer Mittelfrueh from Spalt (G), Tettmanger (

2. Hallertauer Magnum (bitter variety): decent, flowery, fruity smell, harmonious bitterness (B);
3. Hallertauer Tradition: flowery scent, smell of green grass, intensively harmonious bitterness: (C);
4. Hallertauer Northern Brewer (bitter variety): harmoniously bitter, hop-spicy flavour (D);
5. Hallertauer Perle: harmoniously bitter, decent hop-spicy, fruity, flowery flavours (E);
6. Hallertauer Spalter Select: harmoniously bitter, hop-spicy Iruchug-blumige NOlen (F);
7. Hallertauer Mittelfrueher from Spalt (G);
8. Celeja from Slovenia (H);
9. Spalter from Spalt: aromatic smell, intensely harmonious bitterness (I);
10. Tettmanger from Tettmang: harmoniously bitter, hop-spicy, green-grassy flavours (J);
11. Hallertauer Saphir: flowery, fruity smell, harmonious bitterness (K).



*Fig. 4. Evaluation of the topographic GC-IMS spectrum of the hops variety Tettmanger (J) with a TIC molecular curve. Red: summation of all intensities, green: summation of the intensities greater than*

### Sampling

Sampling using a steel cannula directly from the vacuum packaging and from a 100-ml headspace glass (after 15 min waiting time) with 0.25 g milled hops or a hops pellet qualitatively show the same IMS spectrum. In the example of Hallertauer Magnum, the IMS spectra were exactly reproducible in several tests. Likewise, seven milled samples of the Spalt Select variety from different production lines of Hallertau (hops generator, production date, storage time) showed similar reproducible IMS spectra. For the less volatile components of lower intensity, the headspace method was beneficial, as they accumulate in the gas chamber. In milled samples that were exposed longer to air, the intensities of the IMS peaks changed. The best reproducibility was obtained in milled samples, while partly larger fluctuations occurred in the measurements directly from the packaging or on natural hop umbels.

At a flow rate of the carrier gas of 50 ml/min, the measurement time is about 30 min. The substance peaks were more frequent at the start and end of the time interval. At 20 ml/min only 7 min and then at 200 ml/min, the peaks are consistently separated after a measuring time of 16 min.

### Automated evaluation

The most important flavouring substances in the flavour of the hops were verified through separate measurements with reference substances: linalool, myrcene, alpha-humulene, beta-caryophyllene, geraniol, geranyl acetate, isobutyl isobutanoate, ethyl isovalerate, ethyl-2-methyl butanoate and methyl heptanoate.



The topographic IMS spectra (Fig. 3, p. 658) link - in each of the approximately 20 million (x,y) points - the ion current process along the GC retention time (x) and along the IMS drift time axis (y). The resolution of the GC retention time is 147 ms, the drift time is 6 to 7 microseconds. The areas in the GC range from 0 to 400 s that are significant for the flavouring substances can be correlated clearly with the hop varieties by comparing the sample or grey value. An optical correlation matrix shows the similar and different samples through grey values.

As this approach requires great effort in the routine operation, we have tested an automated evaluation method that illustrates the total ion current TIC (t) in will-arbitrary units versus the GC retention time t (Fig. 4, p. 659). The search and evaluation of the local peak maxima TICp(l) for each GC retention time is done numerically by external search in a continuous or specified drift time interval [tD1,tD2]:

$TICp(l) := \max TIC(t,tD)$  The so-obtained GC-IMS curves correlate with the peaks of the gas chromatographs, but show a partly greater depth of detail than the number of GC signals. The depth of detail can be monitored using a minimum peak height above which the summation starts. The total ion current of the five hops varieties shows, for example the number of drift time maxima (Fig 7)

The relative drift time  $r = tD/tD \cdot RIP$  correlated with the molar mass. A universal approximation polynomial, we cannot specify that esters, for instance, show relatively longer drift times than equally heavy terpenes.

The hardly volatile hops ingredients ingredients are also detected by the device due to lower detection limits of the device. Beta-caryophyllene appears at the GC retention time of 700 s and the IMS drift time of about 9 ms, humulene at 800 s or 10 rns (double peak). The volatile components appear earlier.

Suitable lead substances for the flavour quality are myrcene (resinous not desired) and linalool (flowery pleasant). Myrcene caused an IMS trace that went beyond the retention time of 3 min and flowed for several minutes (in Fig. 7, > 3 min). The summation solves the problem of the after current of components, which are not clearly isolated by the GC column, by increasing the basic signals, without affecting the peak positions. The quantification of the signals is but made difficult.

Fig. 7. Differentiation of related varieties from Hallertau. Northern Brewer (D), Perle (E).

Total ion current versus GC retention time  
Right scale: drift time of characteristic IMS peaks (+, 0).

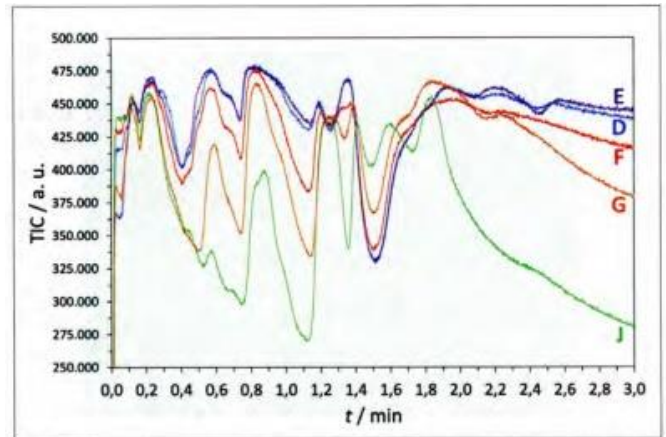


Fig. 5. total ion current versus the GC retention time of the hop varieties of Northern Brewer (D), Perle (E), Spalter Select (F), Hallertauer Mitte/friJher from Spalt (C), Tettnanger (J).

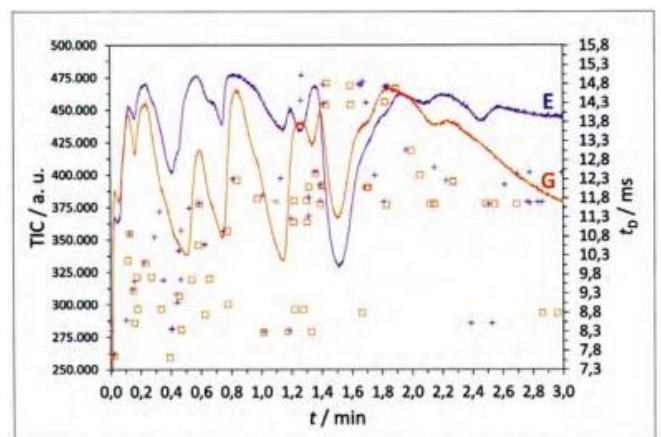
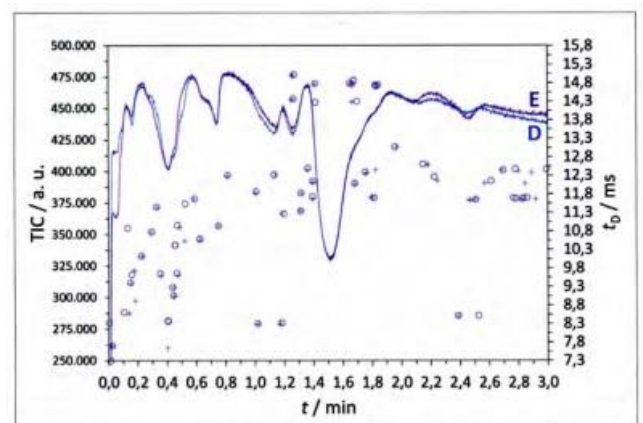


Fig. 6. Comparison of the varieties of Hallertauer Perle (E) from Hallertau and Hallertauer Mitte/frueh from Spalt (C). Total ion current versus GC retention time  
Right scale: drift time characteristic IMS peaks (+, 0).



## Summary

The charm of an ion mobility spectrometer (GC-IMS) coupled with a gas chromatography column is that no difficult sample preparation is required. Milled hop samples can be checked within 16 min for differences in their varieties. In the process, it is not required to know the largely unstudied ingredients, essential oils and polyphenols). The IMS method facilitates quality assurance during the production of hop products from several varieties with a defined composition of flavouring substances, for example, flavour hops, from which beers with unusual fruity tastes are brewed. IMS spectra are a handy tool to analyse the sensory characteristics of essential oils as a synergistic property of self-intensifying and extinguishing effects and not as a sum of single components

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  - 11) Institute of Plant Cultivation and Breeding of the Bavarian Institute of Agriculture annual report 2009: "Special hop cultures"; Wolnzach-Huell/2010.
  - 12) IMS Software Suite, LAV 2.0.0, Gallery plugin, G.A.S. Gesellschaft fuer analytische Sensorsysteme mbH.
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