Analysis of Artificial Colorants in Various Food Samples using Monolithic Silica Columns and LC-MS

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This work describes a simple and sensitive high performance liquid chromatography method with UV or mass spectrometry detection for the analysis of artificial colorants from dye classes such as azo or chinophthalone in various food samples. After a short sample preparation procedure all samples were separated on C18 reversed phase monolithic silica columns via a gradient elution profile and directly transferred to UV or MS for the analysis of the main components. This setup enabled the identification of dyes in real life samples such as beverages or sweets within very short analysis times and with a minimised sample preparation step.

In the nineteenth century chemicals such as mercury sulphide, lead oxide, copper salts or fuchsine were utilised to artificially colour food such as cheese, confectionary, pickles [1] or wine [2]. In the end of that century the discovery of many synthetic organic food colorants allowed for more brilliant colours than traditional natural dyes. One large group out of this new substance class is the family of azo dyes, which are among the most hazardous colorants existing. It is clear, that some 150 years ago nutrition was as dangerous as it could be.

While from the late 19th century, food additives containing heavy metals were banned by a food law, azo dyes were not covered by this act. Therefore they were still used, e.g. dimethylanilinazobenzene for the colouring of cheese[3].

Since that time, many colorants have been banned. This includes a group of yellow to red azo dye food colorants (e.g. 'Sudan', 'Para Red'), which are highly hydrophobic and therefore water insoluble. They are degraded to carcinogenic amines in the body and are forbidden in the EU since 1995. However, they can still be found in samples such as chilli powders, curcuma or palm oil as well as in tomato-containing food, making fast and reliable identification necessary.

Today approximately 45 colorants are permitted for use in food in the European Union. Of these, six are metals, metal oxides or metal salts such as iron oxide, gold or calcium carbonate and another 23 are of natural origin (e.g. anthocyane from blackberries, carotinoids from tomato or paprika, betanin from beets) or based on natural compounds ('semi-synthetic', e.g. chlorophyllin from algae or caramel colouring). All other colorants are synthetic and mainly belong to the substance class of azo compounds (see Tables 1 and 2 and Figure 1). They are utilised as a single colouring ingredient or as a mixture with other colorants in a wide variety of foods and beverages. All listed dyes are nontoxic and water soluble and can therefore be excreted by an organism very easily.

Most of the current artificial colorants can now be replaced by natural dyes very easily. Nevertheless, for economic reasons they are still used to improve the attractiveness of sweets or soft drinks towards children or of

Table I: List of all artificial food colorants permitted for use in the EU (digest, without pigments and natural dyes): names, CAS number and substance class.

Colorant	CAS number	E number	Substance class
Allura Red AC	25956-17-6	E129	azo
Amaranth	915-67-3	E123	azo
Azorubine	3567-69-9	E122	azo
Brillant Blue FCF	3844-45-9	E133	triphenylmethane
Brillant Black BN	2519-30-4	E151	azo
Brown FK	8062-14-4	E154	azo
Brown HT	4553-89-3	E155	azo
Cochineal Red A	2611-82-7	E124	azo
Erythrosine	568-63-8	E127	xanthen
Green S	3087-16-9	E142	triphenylmethane
Indigo carmine	860-22-0	E132	indigo
Lithol Rubine BK	5281-04-9	E180	azo
Patent blue V	20262-76-4	E131	triphenylmethane
Quinoline Yellow WS	8004-92-0	E104	quinophthalone
Sunset Yellow FCF	2783-94-0	E110	azo
Tartrazine	1934-21-0	E102	azo

Table II: List of artificial food colorants analysed in this work: names, corresponding monoisotopic mass, relevant MS peaks (calculated) and molecular ion formulas.

Colorant	Monoisotopic mass / g/Mol	Relevant MS peak / m/z	Molecular ion
Allura Red AC	496.0	451.0	$[M\text{-}2Na^{+}+H+]^{-}$
Amaranth	603.9	537.0	$[M\text{-}3Na^{+}+2H+]^{-}$
Cochineal Red A	603.9	537.0 268.0	$[M\text{-}3Na^{+}+2H^{+}]^{-}$ $[M\text{-}3Na^{+}+H^{+}]^{2-}$
Quinoline Yellow WS	375.0 (monosodium) 477.0 (disodium)	352.0 432.0	$[M\text{-}Na+]^{-}$ $[M\text{-}2Na^{+}+H^{+}]^{-}$
Sunset Yellow FCF	452.0	407.0	$[M\text{-}2Na^{+}+H^{+}]^{-}$
Tartrazine	534.0	467.0	$[M\text{-}3Na^{+}+2H^{+}]^{-}$

alcoholic drinks towards teenagers. As some colorants (e.g., E102, E110 and E129) are suspected of influencing activity and attentiveness of children, or to cause allergies, all food in the EU containing specific dyes nowadays has to be labelled accordingly.

Three beverages and one snack were chosen as samples for the analysis of artificial food colorants utilising hyphenated LC-MS technique. Prior to analysis sample preparation procedures kept as fast and simple as possible were conducted. This step was mainly used to remove undissolved matter. Here, the robust monolithic silica column technology applied makes more tedious methods unnecessary, as column clogging is minimised by the intrinsic column feature of large eluent transport pores. Target analytes were separated from the complex, hydrophilic matrix (sugar and additives such as artificial sweeteners, preservatives and acidifiers) via short HPLC gradient runs and detected utilising mass spectrometry.

In this work several simple methods are presented for the analysis of various food samples. Utilising robust monolithic silica column technology enables a fast detection of dyes without the need for complex sample preparation.

Experimental

Materials and Methods

The HPLC system used was a Dionex Ultimate 3000 (Thermo Scientific Dionex Corporation, Sunnyvale, California, USA) including Chromolith® FastGradient RP-18 endcapped 50 x 2mm analytical monolithic silica column and Chromolith® RP-18 endcapped 5 x 2mm guard column (both Merck Millipore, Darmstadt, Germany). Depending on the analytes, a UV detector was operated at 416, 480 or 500 nm. The data acquisition was performed with Chromeleon software.

A Bruker Esquire 6000 mass spectrometer with an ion trap and an on-line electrospray ionisation (ESI) source operated in negative mode was utilised in the m/z range scan from 140-850 (depending on the type of colorant). Flow and temperature of the drying gas was set to 12 L/min and 365 °C respectively, nebuliser gas pressure was 2.8*105 Pa. Trap conditions: Max. acquistion time 100 ms, target 200.000, averages: 2.

Sample preparation

All food samples were purchased in supermarkets. Detailed information about sample preparation or fortification can be found in the different sections below.

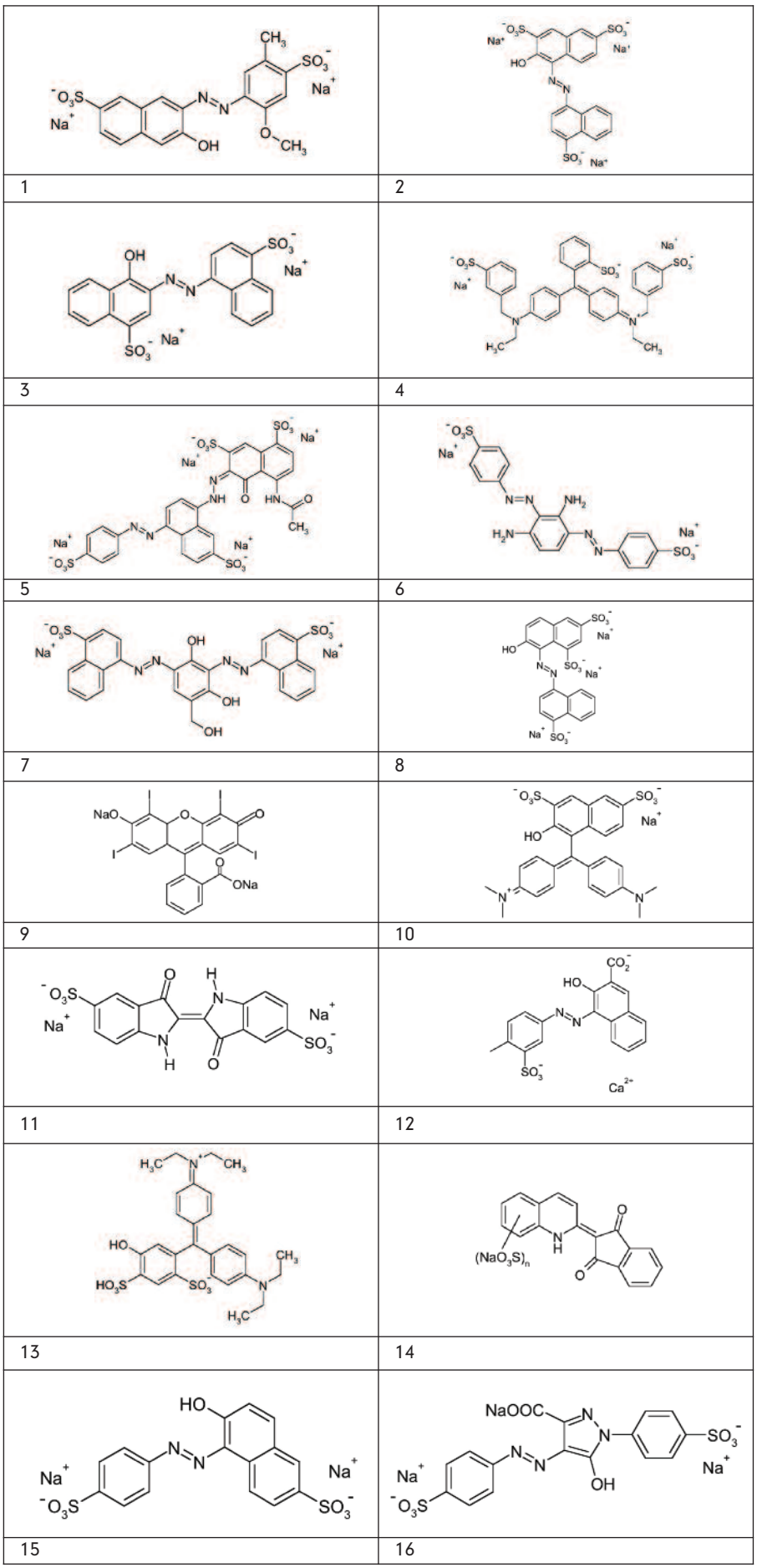

Figure 1: Chemical structures of all artificial food colorants permitted for use in the EU (without pigments and natural dyes). 1: Allura Red AC, 2: Amaranth, 3: Azorubine, 4: Brilliant blue FCF, 5: Brilliant black BN, 6: Brown FK (exemplary structure), 7: Brown HT, 8: Cochineal Red A, 9: Erythrosine, 10: Green S, 11: Indigocarmine, 12: Lithol Rubine BK, 13: Patent Blue V, 14: Quinoline Yellow WS, 15: Sunset Yellow FCF, 16: Tartrazine.

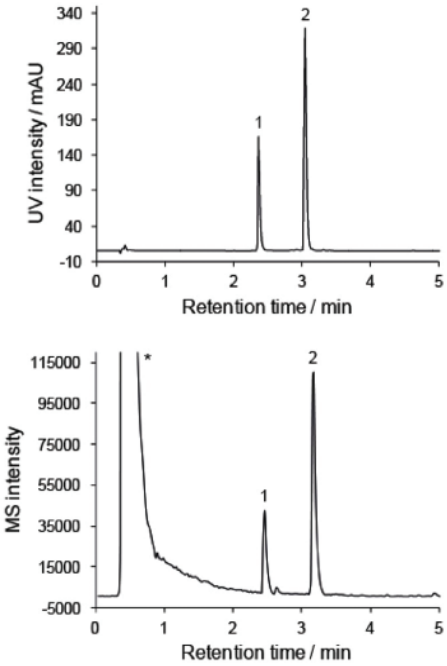

Figure 2: LC-UV and LC-MS chromatograms of a rum-watermelon alcopop sample separated on Chromolith® FastGradient® RP-18 endcapped 50-2mm analytical monolithic silica column. Top: UV, 500nm; bottom: neg. ESI-MS (m/z 140 – 850), base peak chromatogram (BPC). Mobile phase A: acetonitrile, mobile phase B: 20 mM ammonium acetate pH 4.70; gradient: 0' 100% B, 0.5' 100% B, 4.5' 50% B, 5.5' 50% B. 1: Amaranth, 2: Allura Red AC. Asterisk: matrix components (saccharose, citric acid).

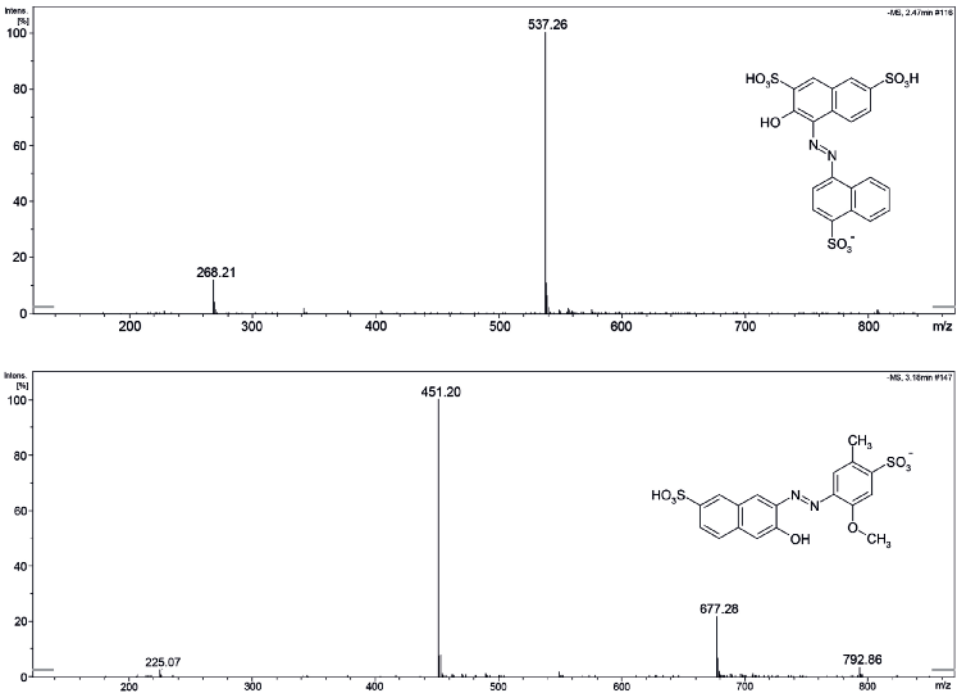

Figure 3: MS spectra of the two colorants Amaranth ([M-3Na⁺+2H⁺], m/z = 537.3, top) and Allura Red AC ([M-2Na⁺+H⁺], m/z = 451.2, bottom) eluting at 2.47 and 3.18 minutes, respectively. For MS conditions see Figure 2.

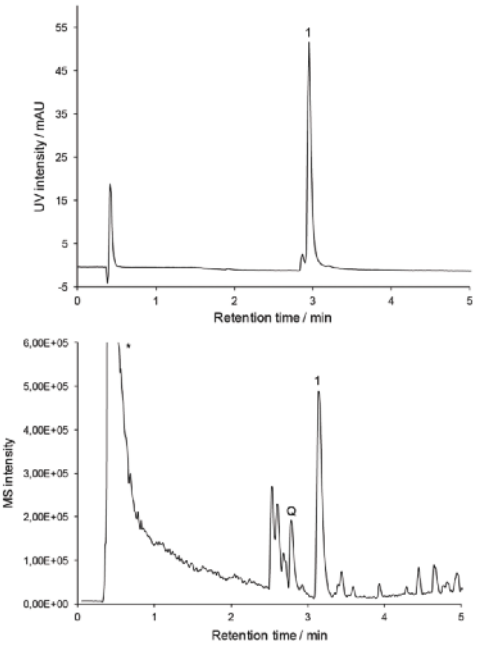

Figure 4: LC-UV and LC-MS chromatograms of an aperitif sample separated on Chromolith® FastGradient® RP-18 endcapped 50 x 2mm analytical monolithic silica column. Top: UV, 500 nm; bottom: neg. ESI-MS (m/z 100 – 850), BPC. Mobile phase A: acetonitrile, mobile phase B: 20mM ammonium acetate pH 4.70; gradient: 0' 100% B, 0.5' 100% B, 4.5' 50% B, 6.5' 50% B. 1: Sunset Yellow FCF, Q: quinine. Asterisk: matrix component (saccharose) eluting in void volume.

Colorant stock solutions

Several samples were spiked using Cochineal Red A (E124) or Quinoline Yellow WS (E104) as single components. Concentrations of the dyes in the stock solutions using water as a solvent were 79.3 mg/L (E104) and 1,388.8 mg/L (E124) and were used to ensure adequate sensitivity for the ESI/MS method employed.

Rum-watermelon alcopop

This alcopop beverage is an aqueous mixture of rum and ingredients such as sugar, flavouring, acidifiers, preservatives and the two red colorants Amaranth (E123) and Allura Red AC (E129). Prior to analysis the only sample preparation was a filtration step using a 0.45 µm syringe micro filter (Merck Millipore).

Italian aperitif

A volume of 10 mL of the clear orange aperitif containing Sunset Yellow FCF (E 110) was heated for 120 min at 40 °C under nitrogen stream (1 bar) using a TurboVap II (Biotage, Uppsala, Sweden) for complete removal of ethanol. The residue was made up with water to 10 mL and filtered using a 0.45 µm syringe micro filter prior to injection.

Sugar coated chickpeas

Initially the sample consisted of chickpeas coated with sugar in four different colours. Out of these, red chickpeas (10 g) containing Cochineal Red A (E124), Sunset Yellow FCF (E110) and Tartrazine (E102) were picked and crushed using a pestle and mortar. All soluble compounds were then dissolved in 20 mL water by means of an ultrasonic bath. Finally the sample was filtered in two steps utilising a paper filter and a syringe filter disc (0.45 µm). Spiking of the resulting red solution was performed by mixing with E124 stock solution in a 2:1 ratio (v/v).

Lemonade

This light yellow and cloudy sample consisted of sugar, lemon juice, lemon flavouring, potassium sorbate, sodium benzoate and ascorbic acid. Quinoline Yellow WS (E104) serves as a food dye. Sample preparation was performed via filtration using a 0.45 µm syringe micro filter. Spiking was done by mixing lemonade and E104 stock solution in a 20:1 ratio (v/v).

Results and discussion

The rum-watermelon alcopop sample was purchased in a Spanish supermarket, in which various alcoholic beverages in all possible colours ranging from red to pink, yellow, green and blue were available. After a brief check of the list of ingredients the author picked a red bottle - because in contrast to the other drinks this alcopop contained not only one but two artificial red colorants (Amaranth and Allura Red AC).

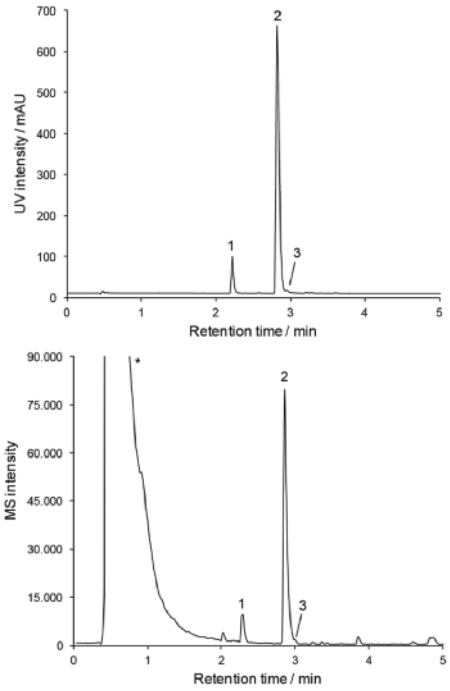

Figure 5: LC-UV and LC-MS chromatograms of an extract of sugar coated chickpeas spiked with Cochineal Red A and separated on Chromolith® FastGradient® RP-18 endcapped 50 x 2mm analytical monolithic silica column and Chromolith® RP-18 endcapped 5 x 2mm guard column. Top: UV, 480nm; bottom: neg. ESI-MS (m/z 100 – 600), BPC. Mobile phase A: acetonitrile, mobile phase B: 20mM ammonium acetate pH 4.70; gradient: 0' 100% B, 0.5' 100% B, 5' 50% B, 7' 50% B. 1: Tartrazine, 2: Cochineal Red A, 3. Sunset Yellow FCF. Asterisk: matrix component (saccharose).

Another look at the ingredients revealed that the only thing the drink had in common with a watermelon was its name printed on the bottle. Figure 2 shows the LC-UV and LC-MS data for this sample. An acquisition wavelength of 500nm was chosen as a compromise in order to detect both colorants near their absorption maxima, while matrix components did not show UV absorption. MS spectra of the two colorants (see Figure 3) reveal that an analysis time of roughly three minutes is sufficient to separate both compounds from the matrix components (saccharose, citric acid), which mainly elute in the void volume. The high robustness of the column was later shown by conducting a long-term stability test (data not shown). After more than 8,000 injections the resulting separation was still excellent and in accordance with the chromatographic data displayed in Figure 2.

The second sample was a famous Italian aperitif coloured in deep orange by utilising the azo compound Sunset Yellow FCF as a dye (see chromatograms in Figure 4). The dye peak is well separated from all matrix components by applying a fast gradient. In addition a peak for quinine, used as a taste additive, was identified via MS. In contrast to the alcopop drink the MS data displays additional unassigned peaks in the range from approximately 2.5 to 5 minutes, which

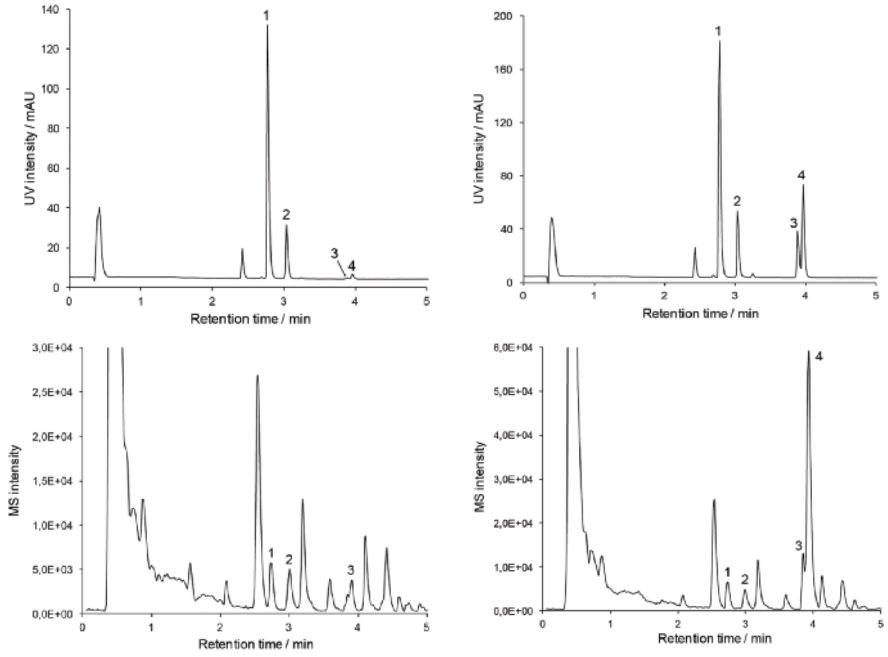

Figure 6: LC-UV and LC-MS chromatograms of a lemonade separated on Chromolith® FastGradient® RP-18 endcapped 50 x 2mm analytical monolithic silica. Top: UV, 416nm; bottom: neg. ESI-MS (m/z 140 – 850), BPC; left column: unspiked sample, right column: spiked with Quinoline Yellow WS monosodium salt. Mobile phase A: acetonitrile, mobile phase B: 20 mM ammonium acetate pH 4.70; gradient: 0' 100% B, 0.5' 100% B, 5' 50% B, 8' 50% B. 1/2: Quinoline Yellow WS, disodium salt, 3/4: Quinoline Yellow WS, monsodium salt. MS peak at 0.6 min: matrix component (saccharose).

are presumably caused by compounds from herb digests used for flavouring.

The chromatograms resulting from the analysis of sugar coated chickpeas are displayed in Figure 5. This candy comes in four different colours (red, orange, yellow and white), of which the chickpeas covered with red sugar were chosen for analysis. Cochineal Red A was used for colouring by the manufacturer and was also spiked, nevertheless low amounts of the two yellow dyes Tartrazine and Sunset Yellow FCF deriving from orange and yellow sugar coatings were also visible in both UV and MS detection as 'contaminants'. Like in the examples shown previously, MS allows for the identification of huge amounts of matrix components, while these are invisible in UV detection. In order to improve lifetime of the analytical column, a 5 x2mm monolithic silica guard column was employed in this separation. As was shown in the beverage analyses, the sample preparation procedure for the chickpeas was kept as short and simple as possible. Though the sample was complex and contained fat, proteins and carbohydrates, the target analytes were well resolved from the matrix and clearly identified.

The last sample to be analysed was lemonade. According to the manufacturer, the lemonade contained 10 percent natural lemon juice. However, the other 90% of constituents didn't have anything in common with natural lemonade. These included: sugar, lemon flavour, potassium sorbate, sodium benzoate, ascorbic acid and Quinoline Yellow WS. Figure 6 shows the UV and MS data for the unspiked and colorant spiked samples. The manufacturer utilised the disodium salt of the dye containing low amounts of the monosodium form, and for the former a baseline separation of both isomers was achieved. The spiking solution mainly consisted of the monosodium salt of Quinoline Yellow WS and exhibits a double peak to the chromatogram at a retention time of ca. four minutes. Even though matrix load was again high, all components were identified after four minutes using fast gradient runs.

Conclusion

This work shows that monolithic silica technology combined with fast and simple sample preparation methods is a robust system ideally suited for the analysis of complex foodstuff. Matrix components such as carbohydrates, fat, proteins or preservatives and acidifiers were removed via the sample preparation process or separated from the colorants applying a suitable and fast gradient run. Artificial colorants were then easily identified applying UV or MS. Depending on the matrix load a guard column was utilised to protect the analytical column.

References

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