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Application of headspace solid phase microextraction to qualitative and quantitative analysis of tobacco additives in cigarettes

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Abstract

Cigarettes may contain up to 10% by weight additives which are intended to make them more attractive. A fast and rugged method for a cigarette-screening for additives with medium volatility was developed using automatic headspace solid phase microextraction (HS-SPME) with a 65 μm carbowax-divinylbenzene fiber and gas chromatography–mass spectrometry (GC–MS) with standard electron impact ionisation. In three runs, each cigarette sample was extracted in closed headspace vials using basic, acidic and neutral medium containing 0.5 g NaCl or Na_2SO_4. Furthermore, the method was optimized for quantitative determination of 17 frequently occurring additives. The practical applicability of the method was demonstrated for cigarettes from 32 brands.

Keywords: Cigarettes; Gas chromatography–mass spectrometry; Headspace solid phase microextraction; Tobacco additives

1. Introduction

During the manufacturing of cigarettes, up to 10% by weight additives are added to tobacco, water not included. The main additives are sugars (glucose, fructose, sucrose, corn syrup), cocoa and humectants (glycerin, propylene glycol) but there are also many volatile and semi-volatile additives. Such additives affect the smoking behavior and are intended to increase the attractiveness of cigarettes. The compounds influence taste, moisture, burn rate and smoke pH but also such sensory properties like harshness, smoothness and impact [1]. More than 600 substances are published in different toxicological studies of the tobacco industry [2–14], composite lists of tobacco ingredients of the tobacco industry [15–17] and in lists of the legislators [18]. Many scientists argue that some tobacco additives increase the toxic potential of the cigarette by a direct influence on the nicotine-uptake and reinforcement of addiction. Furthermore, it is known that few aroma compounds form toxic components during combustion. Therefore, as a consequence of newer developments in the tobacco legislation in several countries, an analytical control of the additives is required.

The traditional isolation procedures of aroma and flavor compounds from tobacco used in these procedures such as steam distillation, solvent extraction, trapping on adsorbents, or combination of these methods are very labor intensive and are sometimes lacking in sensitivity due to the very low compound concentrations in a rather complex matrix [19–25].

In the last decade, headspace solid phase microextraction (HS-SPME) has found a broad usage for analysis of volatile and semi-volatile substances in a large variety of matrices [26,27]. SPME was developed by Pawliszyn [26] in 1989 and proved to be a very convenient and solvent saving way of sample preparation for gas chromatography–mass spectrometry (GC–MS) in numerous applications. It integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. The analytes are directly extracted and concentrated into the extraction fiber. The headspace mode of SPME (HS-SPME) appeared to be particularly advantageous for volatile and "semi-volatile" substances because of the very low chromatographic background. The method was especially useful for the detection

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of volatile and flavor compounds. As an example, Yang and Peppard [28] described the analysis of fruit juice beverage, espresso-roast ground coffee and butter flavor in vegetable oil. A comprehensive review on SPME was recently published by Vas and Vekey [29].

HS-SPME should be particularly suitable for analysis of cigarette ingredients and additives since the majority of these substances are evaporable and can be absorbed or adsorbed at SPME fiber materials. Therefore, this technique was already used several times for analysis of tobacco and of cigarette smoke [30–36]. The methods were in most cases limited to a few substances such as organic acids [30,31], alkenylbenzens, coumarin, piperonal and pulegone [32–34], acetates of C_1 to C_4 alcohols [35] or flavor additives [36]. The determination of polyphenols in tobacco was performed by HPLC coupled with electrospray ionisation–mass spectrometry after solid phase extraction [37]. In this study a fast and simple screening procedure for cigarette additives and the quantitation of some of them by HS-SPME is described.

2. Experimental

2.1. Reference substances and reagents

γ-Nonalactone was obtained from Aroma Chemicals (Holzminden, Germany). *trans*-Anethol and phenylacetaldehyde were purchased from ICN Biochemicals (Eschwege, Germany). All other reference substances were purchased from Merck (Darmstadt, Germany) or Sigma–Aldrich (Taufkirchen, Germany). The substances used as internal standards (2,6-dichlorotoluene, acetophenone-d3, benzophenone-d5, benzylalcohol-d5, and pyridine-d5) were obtained from Sigma–Aldrich (Taufkirchen, Germany). The chemicals and reagents used in the analytical procedures were obtained in analytical grade purity from Merck (Darmstadt, Germany).

2.2. Tobacco and cigarette samples

Packs of 32 different common cigarette brands were bought in a tobacco shop (Bern, Switzerland). For comparison, altogether 23 further packs of four of these brands were bought in several other countries. The Kentucky Reference Cigarette 2R4F was purchased from University of Kentucky (Lexington, USA), the additive-free cigarette Yesmoke was purchased from Yesmoke Tobacco SA (Switzerland), four raw tobacco samples were obtained from PLANTA Tobacco Manufacture (Berlin, Germany). Deutersheimer Korso is a self-grown tobacco, which was outdoor cultivated in a garden in Germany. The cigarettes were stored at −15 °C, the raw tobaccos at room temperature in tightly closed glass bottles. A raw tobacco mixture was made in percentage 45% Virginia (in equal shares from Zimbabwe and Germany), 35% Burley (Malawi) and 20% Oriental (Greece).

2.3. Instruments and accessories for GC–MS and HS-SPME

A gas chromatograph 6890 N coupled to a mass selective detector 5973 N MSD (Agilent Technologies, Waldbronn, Germany) was used for the GC–MS measurements. This was combined with a multipurpose sampler MPS2 (Gerstel, Mühlheim/Ruhr, Germany) for automatic performance of the HS-SPME measurements. The sampler was controlled by the software PAL Cycle Composer 1.5.2 (Gerstel, Mühlheim/Ruhr, Germany).

A PTA-5 capillary column (30 m × 0.25 mm × 0.5 μm, Supelco, Bellefonte, USA) with helium as carrier gas (flow rate 1.0 ml/min) was used for the gas chromatographic separation. For optimization, also a capillary column HP-5-MS (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Waldbronn, Germany) was tested.

For optimization four fibers were tested: a 65 μm CW-DVB fiber, a 85 μm PA-fiber, a 65 μm PDMS-DVB-copolymer fiber and a 100 μm PDMS fiber (all from Supelco, Bellefonte, USA). The optimized analyses were performed with the CW-DVB fiber.

All GC/MS measurements were performed using the Enhanced ChemStation G1701 DA, Version D.00.01.27 (Agilent Technologies, 2002).

2.4. Sample preparation for qualitative screening method and HS-SPME

In the optimized qualitative screening method, each cigarette sample was analyzed under three different conditions. An aliquote of the cigarette (50 mg including the cigarette paper) was separated and exactly weighted in a 10 ml headspace vial. To this, 2 μg 2,6-dichlorotoluene (20 μl of an 0.1 mg/ml solution in methanol) as internal standard, 0.5 g Na_2SO_4 and 1 ml 1 M NaOH were added for the basic extraction. 0.5 g NaCl + 1 ml distilled water was added for the neutral extraction, and 0.5 g Na_2SO_4 + 1 ml 1 M H_3PO_4 for the acidic extraction. Then the vials were tightly closed with R 20-L/Sil-HS septa caps (CS GmbH, Langerwehe, Germany) and placed into the vial rack of the sampler. The samples were incubated 3 min at 95 °C, the headspace analytes were extracted for 15 min at 95 °C and 250 rpm interval agitation (5 s agitation, 2 s rest on) followed by desorption in the GC-injector for 5 min at 250 °C.

2.5. Quantitation

For calibration, five different amounts of each component (concentration range given in Table 1) and the internal standard were added to 50 mg of the reference cigarette 2R4F or to an additive-free cigarette (Yesmoke) followed by basic or neutral sample preparation as described in Section 2.4. Each concentration was measured five times. For the determination of benzaldehyde, 2-ethyl-1-hexanol, menthol, indole, acetophenone and 3-phenylpropanol the neutral sample preparation was conducted with 0.5 μg acetophenone-d3 as internal standard (50 μl of a 0.01 mg/ml solution in methanol). Pyridine, furfurylamine, benzyl alcohol and propylene glycol were quantified after the basic sample preparation. The internal standards were 0.25 μg benzylalcohol-d5 in 25 μl methanol for benzylalcohol, 0.25 μg pyridine-d5 in 25 μl methanol for pyrdidine and furfurylamine, and 5 μg pyridine-d5 in 50 μl methanol for the determination of propylene glycol.