Determination of flavors and their metabolites in e-liquids and biological fluids Identification of biomarkers of exposure to prominent e-cigarette flavors

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Abstract
Almost all e-cigarette liquids (e-liquids) contain flavor chemicals. Although the majority of the flavors used are ‘generally regarded as safe’ (GRAS) food additives, very little is known about their metabolic fate after inhalation. Thus, concerns are raised by regulatory authorities on the potential health risks caused by flavor additives in e-cigarettes. As a pre-requisite for further toxicological evaluation of this new product category, novel analytical methods for the determination of the most prominent flavor compounds are needed to investigate the uptake into the human body via the route of inhalation.

We developed and validated bioanalytical methods for the quantification of menthol, a frequently used flavor additive, and its major metabolite, para-menthane-3,8-diol (M-I) in various human body fluids by means of Headspace (HS)-SPME-GC-MS and LC-MS/MS, respectively. Moreover, a multi-analyte method based on HS-SPME-GC-MS was developed for the simultaneous determination of 12 most abundant flavors in e-liquids. The straightforward sample preparation procedure allows a time- and cost-efficient screening of those compounds in clinical studies. Here, the analytical methods for the quantification of flavor additives are presented and their potential as biomarkers of exposure to e-cigarettes are discussed.

1. HS-SPME-GC-MS analysis of menthol

Validation according to FDA guidelines
Robust method for menthol determination in various body fluids validated according to FDA guidelines
Calibration range: 7 – 14,000 ng/ml (urine); 100 – 2,500 ng/ml (plasma)
Applicable for various matrices: Plasma, urine, cell medium
Discrimination of the 4 stereoisomers of menthol

2. Determination of the primary menthol metabolite M-I using LC-MS/MS

Validation according to FDA guidelines
Robust, precise and accurate
Range: S – 1,000 ng/ml
Matrices: Plasma, cell medium
Baseline separation between cis- and trans-form of M-I
High throughput

3. Quantification of flavors by means of a HS-SPME-GC-MS (Flavoromics)

• Screening of various e-liquids from the German market by means of the flavoromics approach
• Implementation of further relevant flavors and expansion of ABF’s Flavoromics library
• Determination of flavors in different body fluids
• Detailed investigation of their metabolic fate

References:

Outlook
• HS-SPME-GC-MS analysis for quantification of 12 most abundant flavor agents in e-liquids, e.g. vanillin, ethyl maltol, menthol, piperonal, linalool, eugenol, citral, citronellal, benzyl benzoate, and a number of terpenes
• Extraction procedure developed based on Merckel et al.
• Acidic extraction of 3 further analytes (Fig. 4B)
• 4 different flavors quantified in the liquids: 3-methyl-1,2-cyclopentanedi-2one, 3-methyl, ethyl maltol, 3-methyl, ethyl maltol, and 3-methyl, ethyl maltol

• Automated SPME for high-throughput screening of e-liquids
• Low background and high selectivity due to Headspace injection
• Flavoromics approach will be extended to human body fluids, esp. urine
• Constant expansion of flavor library