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# Orientation to determine quality attributes of flavoring excipients containing volatile molecules

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ABSTRACT

Pharmaceutical excipients containing volatile odor-active molecules can be used in pharmaceutical development to increase patients' compliance. However, capturing the molecular composition of these odor-active substances is challenging. Therefore, guidance for the analytical investigation of these excipients should be developed. Using a model flavor, lead molecules were chosen and a gas chromatographic method was validated according to pharmaceutical guidelines. Changes during storage as well as batch homogeneity and conformity were investigated. The knowledge gained could be used to understand molecular differences between batches caused by aging. A suitable attempt to capture the volatile molecular composition of flavoring substance was presented and the found results could be used for the determination and interpretation of quality attributes.



## 1. Introduction

Handling and processing of pharmaceutical excipients is essential in development and manufacturing of medicinal products. Excipients are defined as substances that are included in medicinal products apart from the active pharmaceutical ingredient (API) [1,2]. Due to their distinct properties they are used to define the behavior of medicinal products in the patient (e.g., rapid disintegration, API protection by coating, sustained release of the API), to facilitate manufacturing steps (e.g., solvents for granulation, lubricants for tablet compression), and/or to increase patients' compliance (e.g., pleasant color, acceptable taste/odor) [3].

According to the current state of knowledge, most excipients are neither thermodynamically inert nor pharmacologically/toxicologically inactive [4,5]. Furthermore, researchers have demonstrated that excipients of the same group and same pharmaceutical grade (e.g., the disintegrants croscarmellose sodium and sodium starch glycolate) do not necessarily provoke their functional characteristics in the same manner or magnitude (e.g., disintegration mechanism/time) [6,7]. Therefore, regulatory authorities require that the safety and quality of the excipient be established in addition to justification for its usage in drug formulations [2]. Toxicological testing can ensure safety of pharmaceutical excipients. Quality must be proven by appropriate analytical methods capturing the functional properties [1].

With respect to drug therapy adherence and patient compliance, flavoring substances have gained increasing interest in pharmaceutical industry. They are either of chemical (e.g., maltitol, saccharin or aspartame), or natural origin (e.g., orange concentrate, vanilla, chocolate) and provoke an acceptable, pleasant organoleptic impression [3]. So far, the requirement is "to describe the general qualitative composition" of the chemical and natural products [2,8]. New regulatory guidelines, such as the "Guideline on pharmaceutical development of medicines for pediatric use", increased the requirements by demanding the "qualitative and quantitative composition. . .of the flavoring agents that are known to have a recognized action or effect" [9].

In the case of chemical products, quality and quantity can be investigated by well-described analytical methods, such as HPLC, GC, or UV–vis spectroscopy [10,11]. In contrast, the composition of flavors of natural origin is, difficult to determine, due to their complexity. Furthermore, natural products often provoke organoleptic impressions by numerous substances, which make a contribution to the sensory impression in a different manner. The most reliable investigation method for natural products is a human perception panel, since both the qualitative and quantitative impression can be evaluated [12]. Additionally, analytical instrument-based approaches, such as the use of electronic tongues or noses can

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be applied. These systems gain signals from multisensory arrays, which can be correlated with information from human perception panels [13,14]. However, neither approach meets the aforementioned regulatory requirements, since neither determines the molecular composition. Besides the critical point of an appropriate evaluation method, odor-active compounds, either of chemical or natural origin, deal with evaporation, leading to thermodynamically unstable formulations.

The olfactory impression of a substances flavor can be correlated with volatile molecules in its vapor [15]. Following this idea, the aim of this work was to develop a versatile gas chromatographic analytical method for the analyses of volatile molecules in a flavoring substance with respect to the quality of the pharmaceutical excipients. With this method, the content of distinct volatiles in a flavoring substance should be assessed during storage and their distribution within a storage container should be analyzed. Finally, the feasibility of the system should be proven by comparing two different batches with respect to manufacturing date.

## 2. Experimental

### 2.1. Chemicals and Reagents

Gas to operate the gas chromatograph (GC), helium, nitrogen, and hydrogen, were purchased from Air Liquide (Duesseldorf, Germany) and pressurized air was obtained from the supply network in the building. 2-heptanone, 2-n-pentylfurane, and 3-methylbutanal were received from Alfa Aesar GmbH (Karlsruhe, Germany), 2-methylpropanal and toluol as well as the internal standard chloroform from Merck KGa.A. (Darmstadt, Germany). Sigma-Aldrich (Saint Louis, USA) delivered 2-methylpyrazin and methanol was purchased from VWR International (Darmstadt, Germany). Lithium chloride was bought from Carl Roth GmbH (Karlsruhe, Germany) and distilled water was obtained by in-house distillation. The batches 61A30199-I and 61A90552-I of the flavoring substance "spray dried pork liver powder EHT" (American Laboratories Inc., Omaha, NE, USA) were analyzed.

This solid brown powder is slightly soluble in water (~35%) and is manufactured from pork livers by spray drying. In the pharmaceutical industry, it is used as a flavoring substance in oral dosage forms for dogs. So far, no information has been given on the molecular composition.

### 2.2. Gas Chromatographic Method

#### 2.2.1. HS-GC–FID System

Volatile compounds were analyzed using a headspace sampler (HS) Agilent 7694 (Agilent, Böblingen, Germany) combined with a gas chromatograph Hewlett Packard 6890 (Agilent, Böblingen, Germany) equipped with a flame ionization detector (FID). A DB-624 capillary column (Agilent, Böblingen, Germany) with a length of 60 m, inner diameter of 0.25 mm, and film thickness of 1.4 μm was used. Helium served as carrier gas at an initial flow rate of 3 mL/min and was decreased after 18 min runtime with a rate of 0.04 –2 mL/min. The initial oven temperature was set to 35 °C increased with 0.5 °C/min up to 40 °C, further increased with 5 °C/min up 200 °C and kept for 8 min. Further GC-parameters are listed in Table 1.

**Table 1**
Detailed parameters of the gas chromatograph and the headspace sampler for the analysis of the sample substances.

| Device | Parameter | Value |
|---|---|---|
| Detector | Temperature | 250 °C |
| | Hydrogen | 40 mL/min |
| | Synt. air | 450 mL/min |
| | Nitrogen (make up) | 40 mL/min |
| Inlet | Mode | Split |
| | Split ratio | 1:1 |
| | Temperature | 210 °C |
| Headspace sampler | Temperature oven | 85 °C |
| | Temperature loop | 170 °C |
| | Temperature transferline | 185 °C |
| | Vial pressure | 110 kPa |
| | Vial equilibration time | 300 min |
| | Pressurization time | 0.10 min |
| | Loop fill time | 0.10 min |
| | Loop equilibration time | 0.05 min |
| | Inject time | 1.0 min |

#### 2.2.2. Identification of Lead Molecules

Structural information of the lead molecules was gained by combining an Agilent 7890A GC, an Agilent 5975C mass spectrometer (Agilent, Böblingen, Germany), the NIST-database 5.0 (NIST, USA), and the developed method (section 6.2.1).A total of 200 mg of pork liver powder (batch 61A90552-I) were analyzed, while the temperature of the source and the quadrupole was set to 250 °C.

#### 2.2.3. Preparation of Standard and Sample Solution

Defined amounts of 2-heptanone and toluol were weighed into 77 g of methanol (stock solution I).A total of 1.0 g of stock solution I and a defined amount of 2-methylpropanal, 3-methylbutanal, 2-methylpyrazin, and 2-n-pentylfuran were added to 76 methanol (stock solution II). The standard solution was prepared by adding 200 mg of stock solution II to 11 g of an aqueous solution containing 20% lithium chloride (reduction of relative humidity).A total of 100 mg of chloroform was dissolved in 77 g of methanol and diluted 1–50 in an aqueous solution of 20% lithium chloride to form the internal standard solution. Liquid samples of 1.0 mL and solid samples of 150 mg were transferred into headspace vials (VWR International, Darmstadt, Germany), 1.0 mL of the internal standard solution were poured into an HPLC-vial (VWR International, Darmstadt, Germany), which was inserted in the headspace vial. Finally, the headspace vial was closed by a Butyl–PTFE-Plug (VWR International, Darmstadt, Germany).

#### 2.2.4. Method Validation

Method validation was performed for the six chosen lead molecules according to ICH guideline Q2(R1) [16] using pork liver powder batch 61A90552-I. Specificity of the chromatographic method was shown by calculating the resolution ($r$) of the lead molecules to the peak at the front and at the back. Concentration dependent signals for each lead molecule were measured and defined according to linear ranges. To evaluate the correlation between peak area and concentration, slope, y-axis intercept and coefficient of determination ($R^2$) were calculated. Repeatability was evaluated using six repetitive injections of the same standard solution. Precision of the analytical method was investigated with six samples of the same pork liver powder mixture. In both cases, the relative standard deviation was calculated. Six standard solutions of known concentrations were investigated to estimate the accuracy of the method. Limit of detection and limit of quantitation were calculated on the basis of the slope of the calibration and the standard deviation of the responses (ICH Q2(R1) section 6.3 and 7.3) [16].

#### 2.2.5. Stability Studies

Investigations of content changes of the mixture pork liver powder during storage were performed at 4 different conditions of 25 °C and 11% RH (LiCl), 25 °C and 57% RH (NaBr), 30 °C and 75% RH (NaCl)