THE INTERNATIONAL FRAGRANCE ASSOCIATION

Quantification of Free Formaldehyde in Fragrance Ingredients and Fragrance Oils (mixtures)

THE INTERNATIONAL FRAGRANCE ASSOCIATION

Contents

1. Background and purpose of the method

1.1. Background

Formaldehyde is a naturally occurring organic compound having the formula CH_2O (H−CHO) and CAS Number 50-00-0. It is the simplest of the aldehyde family (R−CHO).

Formaldehyde is an important precursor to many other materials and chemical compounds. It was historically used in the production of industrial resins, e.g., for particle board and coatings. However, another of its key properties is in the preservation of products where the presence of even low concentrations can provide a significant increase in shelf life. This can be undertaken by the addition of formaldehyde itself or, increasingly, using Formaldehyde Releaser compound's which, as the name suggests, slowly release low dosages of formaldehyde from another chemical structure into the product into which they are placed, enabling a longer shelf life to be maintained.

Most recent regulatory activities in Europe have strongly restricted the presence of formaldehyde in cosmetic products due to its classification as CMR 1B. According to the Regulation (EU) 2019/831 of May 22, 2019 amending Annexes II, III and V to Cosmetic Regulation, i.e., Regulation (EC) No 1223/2009, formaldehyde as such is banned for use in cosmetic products (Annex II, entry 1577). All finished products containing substances in Annex V of the Cosmetic Regulation which release formaldehyde must be labelled with the warning 'contains formaldehyde' where the concentration of formaldehyde in the finished product exceeds 0.05 %. To ensure that cosmetic products are in line with the regulatory requirements, analytical methods are performed to both identify and quantify free and bound formaldehyde. However, when applied to certain fragrance materials, a number of those analytical methods that can be used to determine the free and bound formaldehyde can produce unwanted false positive results.

1.2. Purpose

This protocol is designed to be targeted on the analysis and quantification of Free Formaldehyde only in Fragrance Ingredients and Fragrance Oils (mixtures) by HPLC with post-column derivatization and UV detection.

2. Principle of the method

Formaldehyde reacts with 2,4-pentadione in the presence of ammonium acetate (the Hantzsch reaction – figure 1) to form the product 3,5-diacetyl-1,4-dihydrolutidine. This product absorbs in the visible wavelength at λmax = 410nm.

The reaction occurs once the formaldehyde has passed through an HPLC (High Pressure Liquid Chromatography) column and takes place in a post-column reactor held at 95°C. Formaldehyde is not retained by the column in the HPLC system and is therefore not influenced by other potentially closely eluting materials. The general schematic for the post column reactor is shown in figure 2.

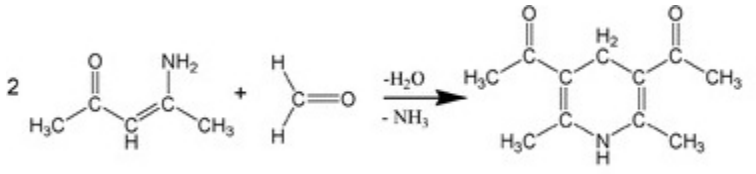

Figure 1. The Hantzsch Reaction between formaldehyde and 2,4-penatdione

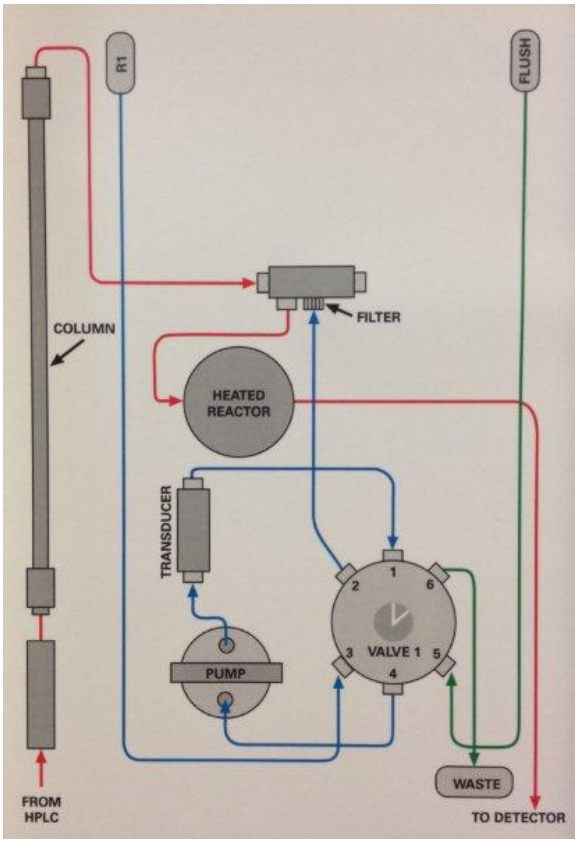

Figure 2. Schematic showing configuration of the HPLC Column and Post-Column Derivatization System

3. Scope

This method is applied for the quantification of free formaldehyde in Fragrance Ingredients and Fragrance Oils (mixtures).

4. Specification

The concentration range of the method is between 1 and 100mg/kg. For concentrations found above 100mg/kg, the sample must be diluted

5. Definitions

HPLC – High Pressure Liquid Chromatography

PCR – Post Column Reactor

DAD – multi-wavelength Diode Array Detector

6. Health and safety

The use of personal protective equipment must comply with safety procedures. Figure 3 displays examples of hazards of each of the active materials used in this method (determined at the time of writing of the present document).

	Hazard pictograms								
Chemicals Used									
	GSH 02 Flammable	GSH 09 Hazardous to the environment	GSH 06 Acute toxicity	GSH 05 Corrosive	GSH 07 Health hazard	GSH 03 Oxidising	GSH 04 Gas under pressure	GSH 08 Serious health hazard	GSH 01 Explosive
Formaldehyde			X	X				X	
2,4 Pentane-dione	X		X						
Acetic acid	X			X					
Methanol	X		X					X	
Acetonitrile	X				X				

Figure 3. Recognized hazards for the reagents used in this protocol

7. Precautions

There are a number of precautions that must be undertaken to ensure the correct and on-going effective use of the analytical system:

- The buffer line of the post-column system should be entirely rinsed with water after each set of analysis in order to prevent the build-up of residue which may lead to a blockage in the buffer line.
- The HPLC column should be kept in an Acetonitrile / Water (90/10) mixture to prevent the column from drying out and affecting overall performance.
- The Post Column Derivatization system should be entirely rinsed both initially and post-analysis sequence with a solution of H2O/MeOH 80/20 v/v. Before starting the heating cycle of the PCR, ensure that the pressure is stable, and the flow is constant (ideally at 0.5 mL/min).

Failure to ensure these are followed can lead to system blockages and/or variable results being produced.

8. Equipment and chemicals

8.1. Specific equipment

HPLC Agilent 1260 (or equivalent) equipped with a quaternary pump and DAD UV detector

Column (Cartridge): type - LiChrospher 100 RP-18 endcapped 250mm x 4 mm (id) (5 µm) (1.50838.0001) Merck or equivalent

Guard Column: type - LiChrospher 100 RP-18 (5 µm) (LichroCART 4-4) (1.50957.0001) (Merck) or equivalent

Post-column derivatization: Pinnacle PCX, model Sigma 1153-1022 (Pickering Laboratories) or equivalent

Vortex: type - Scentific Industries – Vortex Genie 2 or equivalent

Magnetic stirrer: type - Ikamag EOA 9 or equivalent

Balance: Mettler XS204 or equivalent

Centrifuge

Pasteur pipette; plastic 5ml

Pipettes: Gilson with adjustable volume (from 50 to 250 mL and 100 to 1000 mL) or equivalent

Syringe Filters: Nylon 0.45µm 30mm

Volumetric flasks: 10ml

Vials: 15g with screw cap

Data acquisition is made on an appropriate LC system (Type Agilent 1260 with quaternary pump, detector DAD VL+) or equivalent

8.2. Chemicals

Formaldehyde 37% - (Merck 1.04003.1000 or equivalent)

Solution of phosphate buffer pH7 – (Merck 1.09439.1000 or equivalent)

2,4-Pentanedione - (Sigma-Aldrich P7754-250ML or equivalent)

Ammonium acetate - (Merck 1.01115.1000 or equivalent)

Acetic acid Merck - (Merck 1.04003.1000 or equivalent)

Distilled water - (Sartorius Stedim Arium 611 or equivalent)

Methanol - (Merck 1.06007.2500 or equivalent)

Acetonitrile - (Merck 1.00030.2500 or equivalent)

9. Analytical method for fragrance oils

9.1. Sampling

The sampling of the material to be analysed is a key factor in obtaining reliable and reproducible results. The homogeneity of the sample should be ensured prior to any sub-sample being taken in order to prevent over- or under-estimations of the free formaldehyde present. This can be achieved through thorough

mixing, though it should also be noted that mixing in an open vessel could lead to loss of the target material through evaporation etc.

9.2. Storage of samples

The Analysis should be performed as soon as is practically possible after sample preparation.

Standard solutions A and B can be kept for 1 month in a freezer.

9.3. Operating conditions

9.3.1. Initialization of the system

The initialization of the full system should follow the manufacturers recommended procedure. This would, typically, be as follows:

- Turn on equipment
- Open helium valve (solvent degassing for HPLC)
- Create a suitable sequence for formaldehyde quantification (using suitable Chromatography data acquisition system)
- Open software of the Pinnacle system from the acquisition system
- Use the correct sequence for the acquisition
- Start the sequence of the “Pinnacle” / Post Column Derivatization system at the 2nd injection of the HPLC

9.3.2. HPLC operating conditions

DAD: The wavelength used to measure the response is 410 nm

Flow: 0.5ml / min

Injection volume: 25 µL

HPLC Eluent table:

Time (min)	H_2O %	ACN %	Diluted Buffer (pH = 7) %
0	0	15	85
6	0	15	85
9	15	85	0
12	50	15	35
13	0	15	85

9.3.3.Pinnacle operating conditions

Method Pinnacle: Running time 15 min

Equilibration time: 5 min

Reactor temperature: 95°C

Reactor volume: 0.5ml

Pump flow rate: 0.3ml/min

9.3.4.Preparation of buffer for LC eluent and analysed samples

The initial buffer is diluted by 50-fold for the LC eluent and for samples to be analysed with double-distilled water or water proven to be formaldehyde free (i.e., from an Ultra – Pure water generation system).

9.3.5.Preparation of samples

Weigh 0.25g of the sample (Fragrance Ingredient or Fragrance Oil Composition) into a 10 mL volumetric flask and make up to the mark with the diluted buffer solution at pH=7.

Stir with a vortex mixer then transfer immediately into a 15g sample bottle with a screw cap including a magnetic stirring bar.

Stir again for 30 min, then centrifuge for 15 min.

Remove the upper surfactant layer into a vial then inject. A filtration step is optional depending on the nature of the original sample and the final extract.

9.3.6.Preparation of standards

The standard solution is prepared using a solution standard of formaldehyde, made from a solution of formaldehyde at 37% (370000 mg/L) Merck N° 1.04003.1000.

Solution A in distilled H_2O: 62.90 mg formaldehyde

To prepare Solution A, weigh accurately about 175mg of formaldehyde 37% (65 mg) in 25mL => equivalent to **2600 mg/L**

Solution B in distilled H_2O:

To prepare Solution B, take 200µL of solution A and dilute to 10mL => equivalent to **52 mg/L**

Calibration Standards Preparation

Standard 1 / 0.052 mg/L in diluted buffer pH 7 :

Take 50µL of solution B and dilute to 10mL in diluted buffer pH 7

Standard 2 / 0.208 mg/L in diluted buffer pH 7 :

Take 40µLof Solution B and dilute to 10mL in diluted buffer pH 7

Standard 3 / 0.520 mg/L in diluted buffer pH 7 :

Take 100µL of Solution B and dilute to 10mL in diluted buffer pH 7

Standard 4 / 1.040 mg/L in diluted buffer pH 7 :

Take 200µL of Solution B and dilute to 10mL in diluted buffer pH 7

Standard 5 / 2.600 mg/L in diluted buffer pH 7 :

Take 0.5 mL of Solution B and dilute to 10mL in diluted buffer pH 7

The Quality Control (QC) sample is prepared in the same manner as Standard 3. However, note that it must be made in addition to standard 3.

9.3.7. Preparation of spiked solution and spiked sample(s)

A spiked solution of Formaldehyde in Acetonitrile is prepared at about 2 mg/mL (About 100 mg of 37% Formaldehyde in 20 mL Acetonitrile)

A spiked sample is obtained by adding 100µL to 2g of the studied sample (Fragrance Ingredient or Composition). The spiked sample is then prepared as described in section 9.3.5.

9.3.8. Preparation of the reagent

Weigh 31.25 g of ammonium acetate into a beaker of 250 ml. Dissolve the ammonium acetate in 500ml of distilled water by the addition of several volumes of water followed by gentle agitation, then transfer into a brown glass bottle dedicated for the reagent. Continue this until all the ammonium acetate is dissolved and the container used has been thoroughly rinsed with the water.

Add 3.75 ml acetic acid (Gilson) and 2.5 ml of 2,4-pentanedione (Gilson). Stir for 1 hour.

9.4. Data acquisition and calculation

An external calibration is made before each set of measurements by determining a calibration curve that represents the area of the formaldehyde peak versus mg/L formaldehyde.

Two injections of the QC sample should be carried out: the first after the standards are analysed, the second after the last sample of the sequence.

10. Conformity of results

Results are considered to conform to the method if they are within the range defined in the above document.

If the result is out of specification, then further investigations are required.

11. Analytical method for commercial formulations

This method can also be adapted to deal with the analysis of commercial Consumer Product samples where there is considered to be a risk of formaldehyde being present, or where formaldehyde releasers are used but where their breakdown is not to be encouraged.

The sample preparation step involves the portioning of the formaldehyde (if present) into a water phase based on the change in polarity and it's affinity to a more polar medium. Once in the water phase then the sample can be analysed as normal.

All steps in the process are as mentioned above with the exception of the sample preparation step which should be as follows.

Preparation of samples

Weigh 0.25g of the sample (Consumer Product etc.) into a 10 mL volumetric flask and make up to the mark with the diluted buffer solution at pH=7.

Stir with a vortex mixer then transfer immediately into a 15g sample bottle with a screw cap including a magnetic stirring bar.

Stir again for 30 min, then centrifuge for 15 min.

Remove the upper surfactant layer into a vial then inject. If there is evidence of any particulate matter or precipitation present in the upper phase then a filtration step is recommended to reduce the risk of column blockage or interference in the analysis sequence.

12. Reference

Méthode standard AFNOR: ASTM D 5910/ January 2005.