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Autor(en): **Rooselaar, J. / Liem, D.H.**

Objektyp: **Article**

Zeitschrift: **Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène**

Band (Jahr): **73 (1982)**

Heft 4

PDF erstellt am: **22.07.2024**

Persistenter Link: <https://doi.org/10.5169/seals-983468>

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HPLC/GC Determination of AETT in Cosmetics

J. Rooselaar and D. H. Liem
Keuringsdienst van Waren, Enschede

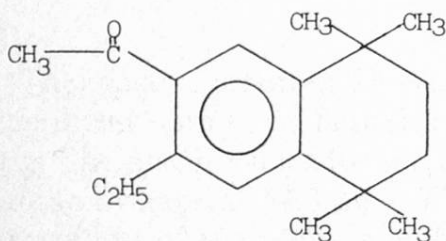
Introduction

AETT (formula I) is a versatile synthetic musk fragrance which was extensively used in cosmetics before its voluntary discontinuance by the chemical producer in 1977 because of its neurotoxic properties. Recently AETT has officially been banned in the EEC and it was put as compound no 362 of its negative list (document 82/162/EEC of February 11, 1982). This report concerns the analysis of several Dutch market cosmetics in 1981. Though four years after the recognition of its toxic properties, AETT containing cosmetic products can still be purchased in the Dutch market.

The method studied in this work is a reversed-phase HPLC cleanup step followed by GC determination of AETT. Confirmation of the identity of AETT has been performed by a GC/MS check in the final extract. *Wisneski et al.* (1) have also published a HPLC/GC-MS analysis of fragrances recently. Their use of straight-phase HPLC, however, is cumbersome and not very convenient in practice.

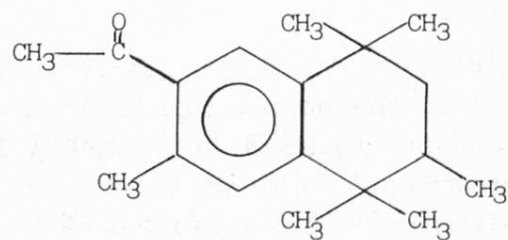
The proposed method is suitable for all kinds of cosmetic products down to a level of 25 ppm. Special attention in our work was made to the possibility of confusion with an isomeric compound AHMT (formula II), with the same musk fragrance and a possible replacement for AETT. The resemblance is so near (mass spectra are almost similar) that both compounds are included in the standard solutions used in our method.

Formula I



AETT "Versalide"®
7-Acetyl-6-ethyl-1,1,4,4-tetramethyl tetralin
($C_{18}H_{26}O = 258.41$)

Formula II



AHMT "Tonalide"®
7-Acetyl-1,1,3,4,4,6-hexamethyl tetralin
($C_{18}H_{26}O = 258.41$)

Experimental

General Remarks

Fragrance analysis is extremely complex. One should be aware when analysing a fragrance compound such as AETT in cosmetics by HPLC or GC that interference on or nearby the AETT peak is highly probable and therefore confirmation of the peak identity mandatory. This is done in our method in the same way as published by *Wisneski et al.* (1), namely by the coupling of HPLC/GC-MS. Their method to analyse fragrances, however, used straight-phase HPLC. This method, which is suitable for all kinds of cosmetic products, uses a reversed-phase HPLC which is much more convenient than straight-phase HPLC. Our proposed method is as follows:

The product is mixed or diluted with methanol (or acetone in case of a soap), homogenized and filtered. An aliquot of the filtrate is analysed by HPLC using a C-18 column, aqueous acetonitrile as mobile phase and UV detection at 254 nm. The AETT fraction (ca. 2 ml) is collected. By a carefully standardized evaporation procedure the volume is reduced and the polar solvents (water and acetonitrile) replaced by a suitable non-polar solvent butylacetate. Quantitation of AETT by GC, using a SE-52 capillary column and an on-column injection system. Final confirmation can be made by GC with MS detection of the butylacetate extract.

Method

Reagents

AETT (K & K chemicals, no 2373; N. Y.)
AHMT (Tonalid I, Polak Fruital Works, Holland)
n-Butyl acetate
Methanol
Acetone
Acetonitrile

Standard solutions

Stock solution AETT/AHMT: Weigh accurately 100 mg AETT and 120 mg AHMT and dissolve in ethanol to a volume of 100 ml. Storage: protected from light.

Standard solution AETT/AHMT: Dilute 10 ml of the stock solution with ethanol to a volume of 100 ml. Further dilute 5 ml of this solution with ethanol to a volume of 25 ml. Prepare this standard solution freshly. Strength: 1 μ l contains 20 ng AETT and 24 ng AHMT.

Internal standard solution: Weigh 100 mg acenaphtene and dissolve in iso-octane to a volume of 100 ml. Dilute 5 ml of this solution with butyl acetate to a volume of 50 ml. Strength: 1 μ l contains 100 ng acenaphtene.

HPLC conditions

Apparatus Waters Associates (Pump M6000A and detector model 450 with variable wavelength).

Column Zorbax ODS (Dupont; a reversed-phase column with octadecylsilane coating) 25 x 4.6 mm.
Mobile phase Water-acetonitrile (15 + 85, by volume).
Flow 1 ml/min.
Detection UV 254 nm.

N. B. With these conditions the following retention volumes were obtained: AETT = 10.4 ml, AHMT = 11.0 ml.

GC conditions

Apparatus Carlo Erba 4160 with on-column injector.
Column Capillary, 15 meter x 0.3 mm intern diam. coated with SE-52, 0.1 μ film thickness.
Carrier gas Helium 0.3 atm.
Detection FID
Temperature Oven Programmed 100 °C–200 °C (8 °C/min) Hold 5 min at 200 °C.
Injector: 100 °C

N. B. The following retention times were obtained:

Acenaphtene (internal standard): 333 s

AETT: 591 s

AHMT: 637 s

Procedure

Weigh accurately a suitable amount (m gram) of sample containing 200–500 μ g AETT ($m = 2$ gram for samples at 100 ppm level, and $m = 0.2$ gram for samples of 1000 ppm AETT) in a 10 ml volumetric flask. Add ca. 0.3 g NaCl and ca. 5 ml methanol (for soaps use acetone). Extract for 10 minutes in an ultrasonic bath. Fill to the mark with methanol (or acetone in case of soaps). Mix thoroughly. Filter. Use clear filtrate for HPLC.

Inject 100 μ l of extract into HPLC. Collect 2 ml fraction containing AETT and AHMT (starting at 10 ml and ending at 12 ml eluted volumes) in a small glass vial of 5 ml.

Add 1 ml butyl acetate and a small magnetic stirrer. Blow softly with nitrogen on the surface at room temperature and while stirring with the magnetic stirrer. Stop blowing when ca. 1 ml fluid is left (after approx. 25 min). Keep stirring and add 10 μ l of the internal standard solution. Close vial and stop stirring. Do not shake and wait few minutes for phase separation. A little water remains at lower phase. Use clear upper phase of butyl acetate for GC.

NB. The blowing operation should remove the acetonitrile and most of the water. If blowing time is too short and acetonitrile removal is not complete, appreciable tailing occurs at GC stage.

Inject 1 μ l of the butylacetate fraction into GC, using the on-column injector.

Inject also 1 μ l of the following reference standard mixture: 100 μ l of standard solution AETT/AHMT + 10 μ l of internal standard solution + 1 ml butylacetate.

Calculation

The following formulas can be used to calculate the contents (in ppm or μ g/gram) AETT or AHMT from the GC data of sample and standard.

$$\text{ppm AETT} = \frac{A_{\text{sample}}^{\text{AETT}} \cdot A_{\text{standard}}^{\text{IS}} \cdot 200}{A_{\text{sample}}^{\text{IS}} \cdot A_{\text{standard}}^{\text{AETT}} \cdot m}$$

$$\text{ppm AHMT} = \frac{A_{\text{sample}}^{\text{AHMT}} \cdot A_{\text{standard}}^{\text{IS}} \cdot 240}{A_{\text{sample}}^{\text{IS}} \cdot A_{\text{standard}}^{\text{AHMT}} \cdot m}$$

in which: m = mass in grams of test portion.

$A_{\text{sample}}^{\text{AETT}}$ = peak area of AETT of GC chromatogram of sample.

$A_{\text{sample}}^{\text{IS}}$ = peak area of Internal Standard (acenaphtene) in GC chromatogram of sample.

$A_{\text{standard}}^{\text{AETT}}$ = peak area of AETT of GC chromatogram of standard.

$A_{\text{standard}}^{\text{IS}}$ = peak area of Internal Standard (acenaphtene) in GC chromatogram of standard.

(same for AHMT)

GC/MS check

For some sample identification checks in the final GC extracts were made by GC/MS. Aparatus: Finnigan 1020, quadrupole. Operating conditions: Full scan 35–350 M/E in 0.5 s. Separator 230 °C. Injector 230 °C. Column: capillary, fused silica CP-Sil 5 (Chrompack), 23 meter. Inject 0.5 μl with open cover; split/sweep time 30 s; then close cover. Oven temperature programmed: Initial 170 °C, increase to 230 °C (6 °C per min).

Results and discussion

The reliability of the GC determination has been checked by the repeatability of successive injections. The linearity of the calibration curve was checked within the GC working range from 0.5 to 10.0 μg AETT or AHMT per ml butylacetate solution. The results are in table 1.

Recovery trials with three different cosmetic emulsions for the whole HPLC/GC method gave satisfactory results (table 2; average 96%).

We further found that the losses of AETT or AHMT were mainly due to the evaporation step of the HPLC fraction. Standard solutions of AETT/AHMT treated by HPLC and the collected fraction evaporated by the proposed procedure and analysed by GC gave average yields of 94.5 % for AETT and 95.0% for AHMT. When AETT/AHMT were added to 85% acetonitrile and evaporated as proposed, the average yields are approximately the same (94.9% for AETT and 96.5% for AHMT).

Table 1. Reliability of the GC method

| Compound | Variation coefficient of n successive injections of the same standard solution | Linearity of the calibration curve in the GC working range c , expressed in the correlation coefficient r |
|----------|--|---|
| AETT | $n = 17$ 3.0% | $c = 0.5$ to $10.0 \mu\text{g/ml}$ final solution for GC $r = 0.9996$ |
| AHMT | $n = 17$ 3.3% | $c = 0.5$ to $10.0 \mu\text{g/ml}$ final solution for GC $r = 0.9994$ |

Table 2. Recovery trials with three different cosmetic emulsions

| Matrix | Added to 1 gram matrix | Recovery (separate trials) | |
|----------------|------------------------|----------------------------|-------|
| Facial cream | 200 μg AETT | 94.9% | 99.0% |
| | 240 μg AHMT | 98.4% | 98.5% |
| Cleansing milk | 200 μg AETT | 97.6% | 97.0% |
| | 240 μg AHMT | 97.5% | 96.7% |
| Body lotion | 200 μg AETT | 97.5% | 96.7% |
| | 240 μg AHMT | 96.7% | 98.7% |

Table 3. Analysis of 8 commercial samples purchased in 1981 in the Netherlands

| No | Brand | Product Type | AETT in μg per g (ppm) | AHMT in μg per g (ppm) |
|----|---------|----------------------------------|---|---|
| 1 | Brand A | Soap | 1885 | — |
| | | | 1832 | — |
| 2 | Brand B | Aftershave | 200 | — |
| 3 | Brand C | Aftershave | — | 274 |
| 4 | Brand A | Foam bath | 112 | 80 |
| | | | 116 | 75 |
| 5 | Brand A | Body milk | — | 12 |
| 6 | Brand A | Hand and body lotion | 38 | — |
| 7 | Brand A | Perfume oil | 645 | — |
| 8 | Brand A | as no 7, but a more recent batch | 701 | — |

The method has been applied to the analysis of 8 selected market samples of the Dutch market in 1981. The results in table 3 indicate that in particular one brand (A) is still using AETT in their product line. In one sample (no 4) both musk compounds (AETT and AHMT) were found.

Acknowledgement

We wish to express our thanks to Dr. *A. M. de Roos*, director of the Food Control Station of Enschede for his encouragement of this study.

Summary

AETT (7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin), a toxic synthetic fragrance compound (musk type) was determined in several market cosmetics (soap, aftershave, cream, lotion, foam bath, perfume oil) purchased from the Dutch market in 1981. Five samples (two brands) were positive and contained 40 to 2000 ppm ($\mu\text{g/g}$) AETT. Attention has been made to prevent confusion with an isomeric (also musk type) compound AHMT (7-Acetyl-1,1,3,4,4,6-hexamethyltetralin), which has nearly the same mass spectra.

The method consists of a cleanup step by means of reversed-phase HPLC followed by GC determination of AETT. Confirmation has been made by GC/MS in the purified extract.

Zusammenfassung

Eine Methode für die Bestimmung von AETT (7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin), einem toxischen synthetischen Riechstoff (Musk-Typus), in verschiedenen kosmetischen Handelsprodukten, z. B. Seifen, Rasierwasser, Cremes, Lotionen, Schaumbädern und Parfümölen, welche in den Niederlanden 1981 auf dem Markt waren, wird beschrieben. In fünf Produkten (zwei Marken) ist AETT in einer Konzentration von 40–2000 ppm ($\mu\text{g/g}$) nachgewiesen worden. Man achtete darauf, eine Verwechslung mit dem Isomer AHMT (7-Acetyl-1,1,3,4,4,6-hexamethyltetralin), einem Riechstoff (ebenfalls vom Musk-Typus), welcher ein ähnliches Massenspektrum aufweist wie AETT, zu vermeiden.

Die Methode besteht aus einer vorangehenden Reinigung durch HPLC (Umkehrphase), gefolgt von einer GC-Bestimmung. Bei Anwesenheit von AETT geschieht eine weitere Identifizierung im gereinigten Extrakt durch GC/MS.

Résumé

Une méthode basée sur la chromatographie en phase gazeuse est décrite pour le dosage de l'AETT (acétyl-7 éthyl-6 tétraméthyl-1,1,4,4 tétraline), une substance aromatique synthétique toxique (du type musc) entrant dans la composition de produits cosmétiques, tels des savons, des produits après-rasage, des crèmes, des lotions, des produits moussants pour le bain et des parfums obtenus sur le marché en 1981 aux Pays-Bas. Dans cinq produits (de

deux marques différentes), des teneurs en AETT de 40–2000 ppm ($\mu\text{g/g}$) ont été déterminées. On a pris soin d'éviter toute confusion avec un isomère, l'AHMT (acétyl-7 1,1,3,4,4,6-hexaéthyl tétraline), un composé du type musc également, qui présente un spectre de masse quasi identique à celui de l'AETT.

La méthode comporte une purification préalable par HPLC en phase inversée, suivie d'un dosage de l'AETT par GC. Confirmation est obtenue par la méthode GC/MS dans l'extrait purifié.

Literature

1. Wisneski, H. H., Yates, R. L. and Davis, H. M.: Gaschromatographic determination of synthetic musk (7-acetyl-6-ethyl, 1,1,4,4-tetramethyltetralin) in fragrances. J. Assoc. Offic. Analyt. Chemists 65, 598–601 (1982)

J. Rooselaar
Dr. D. H. Liem
Keuringsdienst van Waren
P. O. Box 777
7500 AT Enschede
The Netherlands