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HPLC/GC Determination of AETT in Cosmetics

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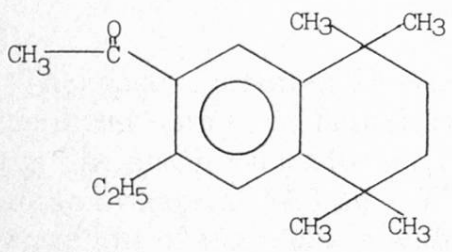
Introduction

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 recognation 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.

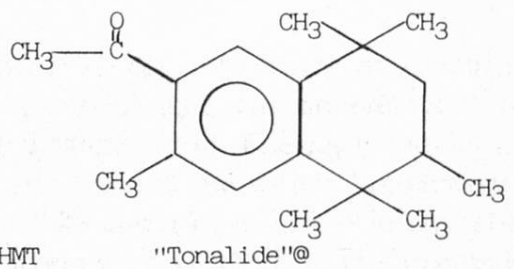
The proposed method is suitable for all kinds of cosmetic products down to a level of 25 ppm. Special attention in our work was made to the possibility of confusion with an isomeric compound AHMT (formula II), with the same musk fragrance and a possible replacement for AETT. The resemblance is so near (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 μl 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. Strenght: 1 μl 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 μg 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 μl 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 μl of the internal standard solution. Close vial and stop stirring. Do not shake and wait few minutes for phase separation. A little water remains al 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 μl of the butylacetate fraction into GC, using the on-column injector.

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

Calculation

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

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

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

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

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

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

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

$A^{\text{IS}}_{\text{standard}}$ = 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 succesive 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 µg/ml final solution for GC $r = 0.9996$
AHMT	$n = 17$ 3.3%	$c = 0.5$ to 10.0 µg/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 enouragement 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 (μg/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, Cremen, 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 (μg/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 ähnlisches 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 (μg/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)

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