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GC–MS analysis of incenses for possible presence of allergenic nitromusks

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Abstract

A Gas chromatographic method with mass detector was developed to identify and determine nitromusks in incense sticks of different origin (India, China, Tibet). The proposed method was found useful to correlate dermatological allergic reactions with the use and composition of commercial incense sticks. The incense sticks were powdered, extracted with methanol and after the addition of 1-eicosanol as internal standard, injected into the GC–MS, using 25 m bonded phase fused capillary column methyl, 5% phenyl silicone (0.32 mm I.D., 0.25 μm film thickness). Musk ambrette was identified and determined in one kind of chinese incense together with musk ketone and musk xylene. This latter compound was also found alone in another kind of chinese incense. © 1998 Elsevier Science B.V.

Keywords: Gas chromatography; Mass detector; Incense sticks; Nitromusks; Contact dermatitis

1. Introduction

Incense is an oleoresin that oozes from incisions in the trunks and leaves of trees of the genus Boswellia (*B. carterii* and *B.papyrifera*, native of India, Arabia and Somalia). Incense is burnt in churches and private homes for religious and domestic purposes. Airborne pigmented and depigmented contact dermatitis have been reported due to incense [1,2].

When incense is burnt, on the exposed skin surface, airborne particles may dissolve in sebum and allergic contact dermatitis accompanied by depigmentation or pigmentation might arise.

Patch test positive reactions to incense were accompanied by positive reactions to musk ambrette, a nitromusk which has been reported as photosensitizer [3]. Moreover peroxides produced by the decomposition of nitromusk may be involved in skin cancer promotion.

Therefore, analytical methods suitable for the detection and determination of nitromusks in commercial incenses are of interest.

This paper deals with the application of gas-chromatography–mass spectrometry to the analysis of some commercially available incense sticks of different sources (China, India, Tibet).

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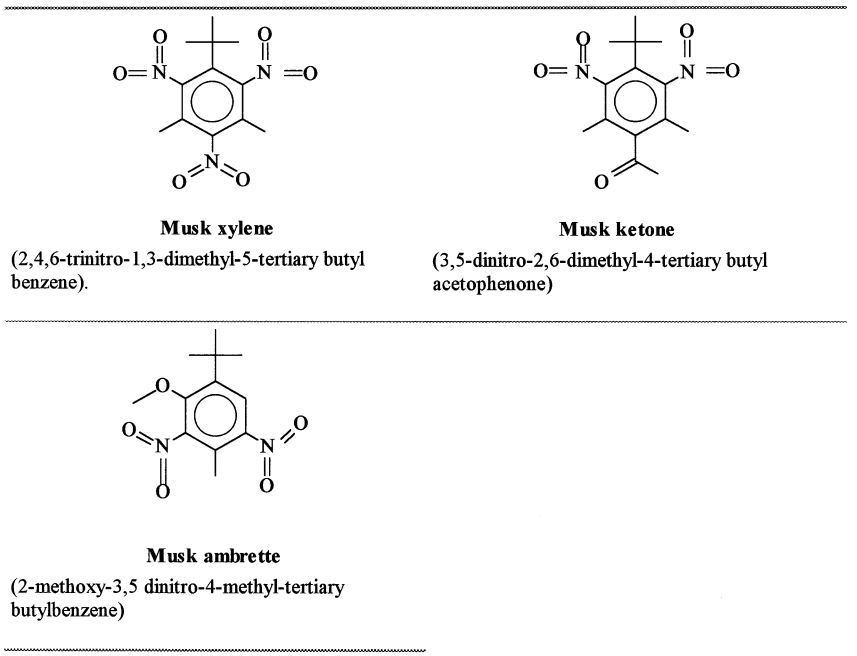


Fig. 1. Structures of nitromusks.

2. Experimental

2.1. Materials

Incense samples and musk ambrette standard (5% in white petrolatum) were kindly supplied by Clinica Dermatologica (Ospedale S. Orsola, Bologna, Italy). 1-Eicosanol (standard for gas chromatography) was obtained from Carlo Erba Reagent (Italy). All solvents were of analytical grade.

2.2. Apparatus and chromatography

A gas-chromatograph Hewlett–Packard model HP 5890 series II with a mass selective detector HP 5971 was used with a 25 m bonded phase fused capillary column methyl, 5% phenyl silicone (0.32 mm I.D., 0.25 μm film thickness). The chromatographic conditions used were: carrier gas: helium at 0.6 kg cm^{-2}, injection port at 280°C; oven temperature 50°C for 1 min, 50–250°C at 10°C min^{-1}.

2.3. Calibration graph

A calibration curve was obtained by analysing standard musk ambrette methanolic solutions (concentration range 20–190 μg ml^{-1}), containing 1-eicosanol as internal standard (90 μg ml^{-1}). The peak area ratios of musk ambrette to internal standard were plotted against the corresponding analyte concentrations to obtain the calibration graph.

2.4. Analysis of commercial samples

Approximately 1 g of incense stick was powdered, extracted with 80 ml of methanol at 65°C for 20 min. The methanolic extracts were filtered and evaporated to dryness. The residue dissolved in 10 ml of methanol and injected into the GC–MS.

When the musk ambrette quantitative analysis had to be performed the residue was reconstituted by addition of 10 ml of 1-eicosanol methanolic solution (90 μg ml^{-1}), as internal standard, and injected in triplicate into the gas chromatograph.

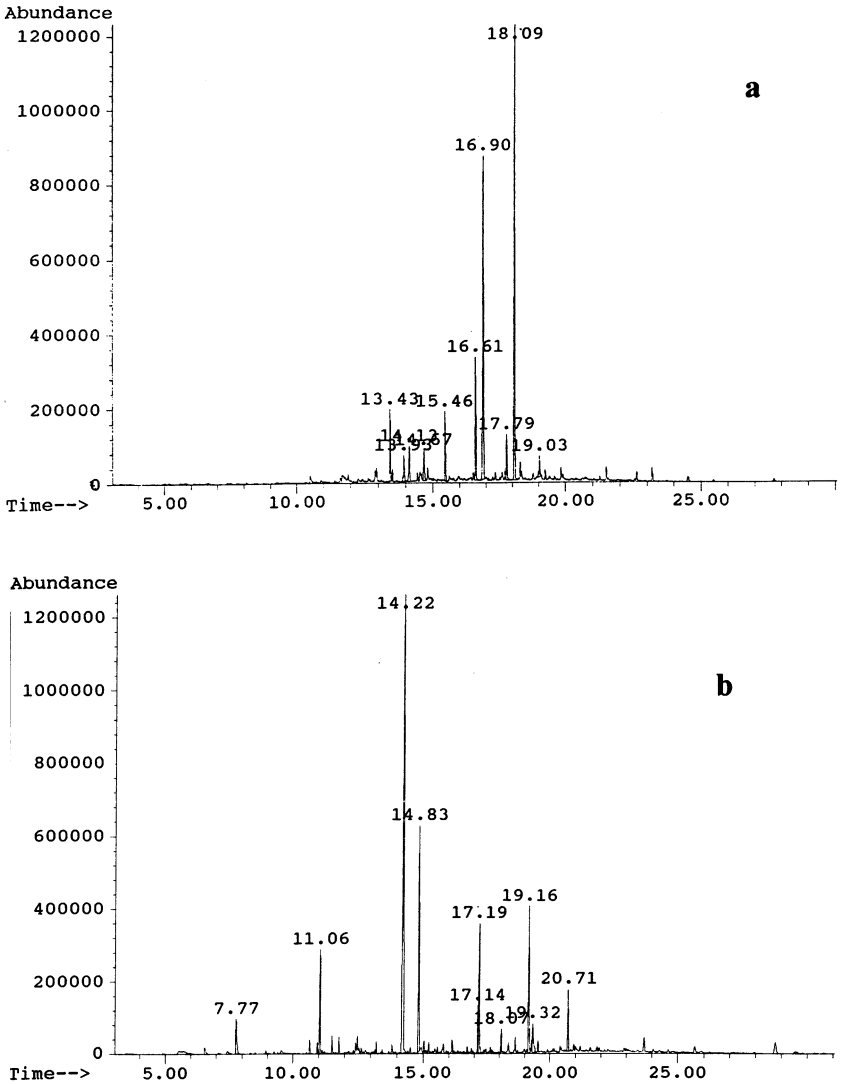


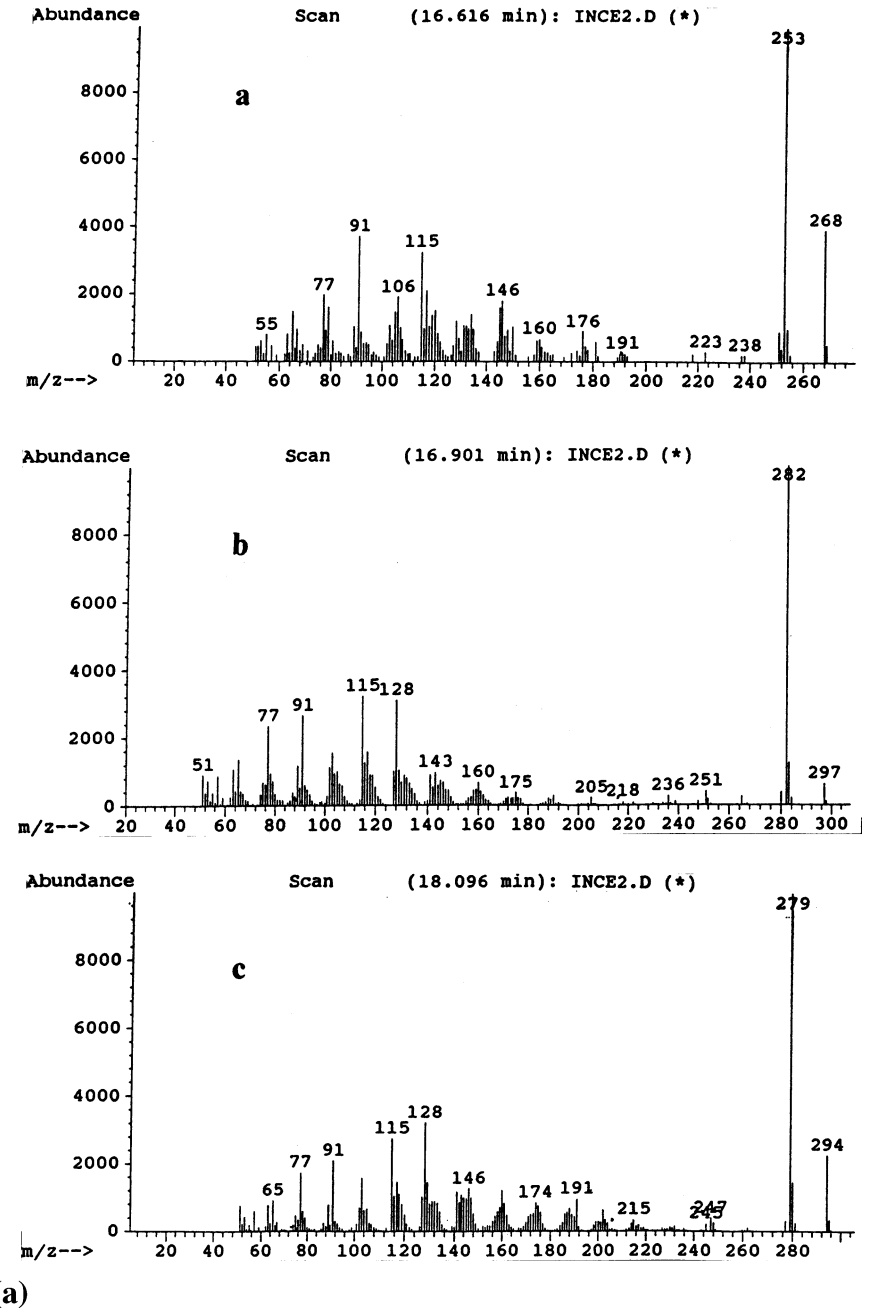
Fig. 2. (a) Gas chromatogram of a methanolic extract of a chinese incense (product a) containing three nitromusks: musk ambrette (Rt = 16.61), musk xylene (Rt = 16.90) musk ketone (Rt = 18.09); (b) gas chromatogram of a methanolic extract of a chinese incense (product b) containing musk xylene (Rt = 17.19). Other identified components were: phenylethyl alcohol (Rt = 7.77), benzoic acid 2-amino-methyl esther (Rt = 11.06), diethyl phtalate (Rt = 14.22), 2-(phenylmethylene)-heptanal (Rt = 14.83), 1-nonadecanol (Rt = 19.16), [4-(dimethylamino)phenyl]phenyl-methanone (Rt = 20.71).

The musk ambrette content was calculated by comparison with an appropriate standard solution.

3. Results and discussion

Five different kinds of incense sticks of various origin (India, China, Tibet) were analysed by GC–MS for possible presence of nitromusks such as musk ambrette (2-methoxy-3,5 dinitro-4-methyl-tertiary butylbenzene); musk ketone (3,5-dinitro-2,6-dimethyl-4-tertiary butyl acetophenone); musk xylene (2,4,6-trinitro-1,3-dimethyl-5-tertiary butyl benzene) (Fig. 1).

Gas chromatography with electron capture de-



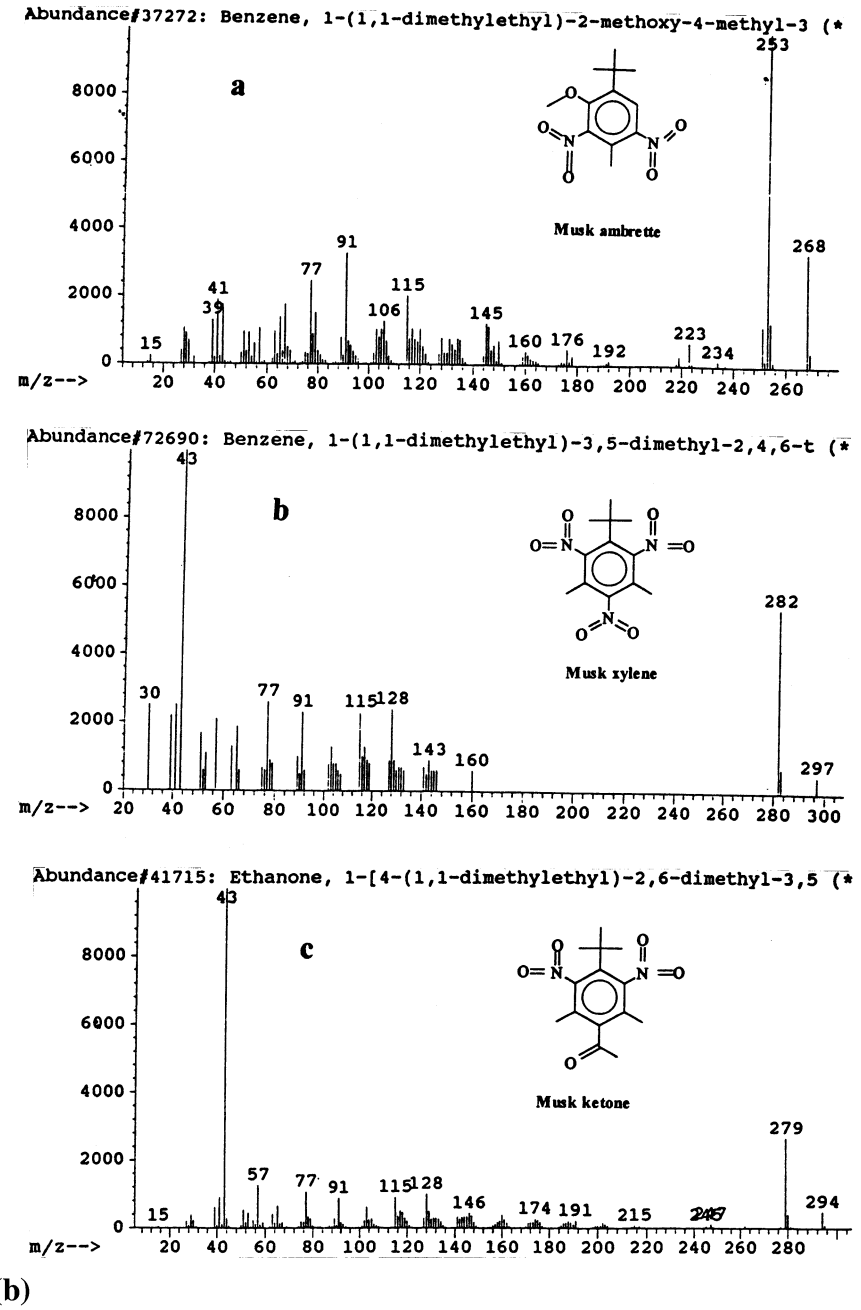
(a)

Fig. 3. (a) Peak apex mass spectra of the three nitromusks in the analysed incense (product a; Fig. 2a). Key: a, musk ambrette; b, musk xylene; c, musk ketone; (b) corresponding library mass spectra.

tector has been previously applied to the analysis of musks in simpler matrices (toiletries) [4,5].

In this application the sample preparation was performed using methanol as extraction solvent; dyes present in some stick were also extracted, but did not interfere with the gas chromatographic analysis.

The reported chromatographic conditions were



(b)

Fig. 3. (*Continued*)

suitable for the separation of the three nitromusks in the methanolic extracts of incenses (Fig. 2a, b), which were found to contain a variety of volatile compounds.

Musk ambrette was identified in one kind of chinese incense (product a, Fig. 2a) together with musk ketone and musk xylene by registration of the peak apex mass spectra (Fig. 3). Other iden-

tified components in the same sticks were: phenethyl alcohol, 2,6 dimethoxy phenol, diethyl phtalate, alfa farnesene (Fig. 2a).

Musk xylene was also found alone in another kind of chinese incense (product b, Fig. 2b). Other identified components in this sample were: phenylethyl alcohol, benzoic acid 2-amino-methyl esther, diethyl phtalate, vanillin, 1,3-benzodioxole-5-methanol, 2-(phenylmethylene)-heptanal, 1-nonadecanol, [4-(dimethylamino)phenyl]phenylmethanone.

The analyte identification was done by matching the peak apex mass spectra with mass spectra in library (quality of matching over 92%) (Fig. 3b); musk ambrette and diethyl phtalate identifications were confirmed also by standard sample injections.

The GC–MS analysis of incense stick from India and Tibet did not reveal any nitromusk content.

Using the described chromatographic conditions for quantitative application, a linear regression curve was obtained ($r = 0.9972$, slope $= 0.013979 \pm 0.000233$, intercept $= -0.025995 \pm 0.02624$, $n = 6$) with good precision, by plotting musk ambrette peak area ratios to internal standard peak area against the corresponding analyte concentration ($\mu g\ ml^{-1}$).

The method was applied to the determination of musk ambrette content in the chinese incense (product a): 0.601 mg per 1 g of incense was found with good precision (RSD% = 1.59, $n = 5$) of the whole method.

4. Conclusion

Incense sticks of different origin exhibit significantly different composition; GC–MS constitutes an effective analytical tool for ascertaining the presence of nitromusks, whose photosensitising action is recognised.

The method could be of utility to correlate dermatological allergic reactions with the use and composition of commercial incense sticks.

Acknowledgements

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