Association for Medicinal and Aromatic Plants
of South-East European Countries

4th Conference
on
Medicinal and Aromatic Plants of South-East European Countries

PROCEEDINGS

Iaşi – România
28th – 31st of May 2006

Organisers:
University of Medicine and Pharmacy 'Gr. T. Popa' Iaşi
University of Bacău
'Al. I. Cuza' University of Iaşi

ALMA MATER PUBLISHING HOUSE

4th Conference on Medicinal and Aromatic Plants of South-East European Countries - Iaşi, România

9th National Symposium 'Medicinal Plants – Present and Perspectives'

3rd National Conference of Phytotherapy

Editors:
Prof. dr. Ghiorghiță Gogu
Prof. dr. Stănescu Ursula
Prof. dr. Toma Constantin

ISBN 973-8392-32-2

BUILDING OF DATABASE FOR THE FAST SCREENING OF FLAVOURS AND FRAGRANCES BY LS/MS TECHNIQUE

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Summary

The usage of LS/MS technique for the fast screening of commercial flavours and fragrances was tested. For this purpose, 49 samples of flavours and fragrances of different origin were analysed by LS/MS. Acquired mass spectra were processed and stored in MS library, whose usability for differentiation of samples was tested by common search engines. Further, MS data from created library were transformed, imported, and statistically processed to get better insight into differentiation power of LS/MS technique applied to selected samples.

Keywords: *LS/MS technique, flavours, fragrances, HCA.*

Introduction

In our first contribution in this field, the concept of development of a new analytical technique for characterisation of essential oils has been described. The concept of liquid sampling mass spectrometry (LS/MS), developed in the first instance for the characterisation of essential oils, consisted of following main items: selection of hardware components for LS/MS analytical system (a), selection and LS/MS working conditions (b), selection of software for essential oil mass spectra processing (c), testing and optimisation of items a to c (d), acquisition of essential oil mass spectra and preparation of appropriate MS libraries (e). At that time LS/MS technique was tested on a wide range of essential oil samples and promising results opened wide range of possibilities in the application of LS/MS technique in the field of characterisation of these products [1].

In the next phase, our efforts were focussed to comparison of similarity of LS/MS spectra of pure samples, to those which could be obtained from the analysis of diluted samples and/or already acquired appropriate standard GC/MS data files, as well as enlargement of initially prepared spectral libraries for available essential oil samples. It was concluded that similarity between the native, composite and extracted LS/MS spectra were enormous, which could elevate expansion of newly created spectral libraries with already acquired data by common GC/MS [2,3].

Furthermore, LS/MS technique was tested for the fast chemical screening of essential oils of several *Thymus* species [4], as well as for fast quantification of certain major constituents in selected essential oils [5].

Although compositions of flavours and fragrances are used for quite different purposes, there are few details where these could be compared from the point of view of analysts dealing with their characterisation. The most important one is that both mentioned group of product are in essence formulations, containing often very complex mixtures of several ingredients. In the case of flavours, for example, natural, nature-identical, or synthetic active (and other) ingredients could be used in formulation of finished product, where named origin can be detrimental for its price and acceptability on the market [6,7].

The aim of the present work was to check usability of LS/MS technique for the fast screening of compositions of different flavours and fragrances used in pharmaceutical, cosmetic and food industry.

Material and methods

Samples selection

Among 21 samples supplied by company Drom (Germany), 19 were belonging to the group of fragrance compositions [d_459730 (vita-jogurt), d_459729 (jogurt), d_460650 (bambi baby), d_460652 (babes), d_460651 (lola), d_460653 (baby balance), d_460654 (baby care), d_414259 (od italie), d_460656 (hand & cream), d_460657 (hand & nail), d_459731 (jogurt & vanila), d_459732 (cerealy jogurt), d_459733 (jogi-balance), d_414492 (naturella), d_414493 (gino), d_414494 (foot spray), d_460785 (colorviva), d_460786 (repair), d_460782 (voodoo lounge), and remaining 2 to the group of flavours [d_0030.139 (strawberry flavour), and d_0035.061 (mint flavour)]. Further 6 aroma compositions, supplied by company Firmenich (Germany) and designated as dentifrice series (for dentistry), were f_52.723, f_52.722T, f_52.721T, f_52.720T, f_52.739T, and f_52.737T. Next 10 mainly fruit aroma compositions (orange, mandarin, walnut, almond, strawberry, caramel, chocolate, vanilla, lemon and coconut), were products of Lachema aromatica Co. (Belgrade). Milk aroma was produced by Quest (The Netherlands), lemon, strawberry, and raspberry flavours from Eterika (Serbia and Montenegro), and latest 5 fruit aroma compositions [wild strawberry (02-601 PiA, 011897), bilberry (18350/279), raspberry (186040031), black currant (01300122) and red currant (186490002)] from other suppliers. Three additional perfume compositions [s_102480 (musk S), s_108046 (carat), and s_ 108032 (birch)] were obtained by Symrise Co. (UK). Approximately a half of selected samples (49) were fragrance compositions (24), and other half – flavour compositions (25).

LS/MS analytical system and working conditions

Apparatus has been built on the platform of HP G-1800C GCD analytical system, whose mainframe, split-splitless injector equipped with automatic liquid sampler (ALS), detector and data station were used without any modification as the backbone of the new system. Standard capillary GC column has been replaced with fused silica capillary (i.d.=100 μm, l=5 m), which was used as the transfer line between injector and MS detector. Injector, transfer line (oven) and detector were heated at 250 °C, 260 °C and 260 °C, respectively. To enable normal ALS operation duration of analytical runs was extended to 3 minutes. Pure samples of selected flavour and fragrance compositions (200 nl) were injected in split mode (1:60) by ALS equipped with nanoliter adapter in 3 repetitions. Carrier gas was helium. Electron impact (EI) mass spectra of samples were acquired in the m/z range 40-400.

Selection of software for processing of essential oil mass spectra

For processing of the acquired mass spectra, two types of software were used. Probability merge search (PBM), revision B0.01, and NIST MS Search 2.0, as well as additional software for conversion of data between two mentioned data formats. Further analysis of normalised mass spectral data was achieved by hierarchical cluster analysis (HCA), where SPSS software (ver. 10.0.1) was efficiently used.

Results and discussion

The average retention time of peak appearing in virtual "columnless chromatograms" obtained by LS/MS analysis was very short (about 30 seconds). However, peaks mainly were of irregular shape, asymmetric, and far away of those expected theoretically. The example of such a chromatogram is shown in Figure 1.

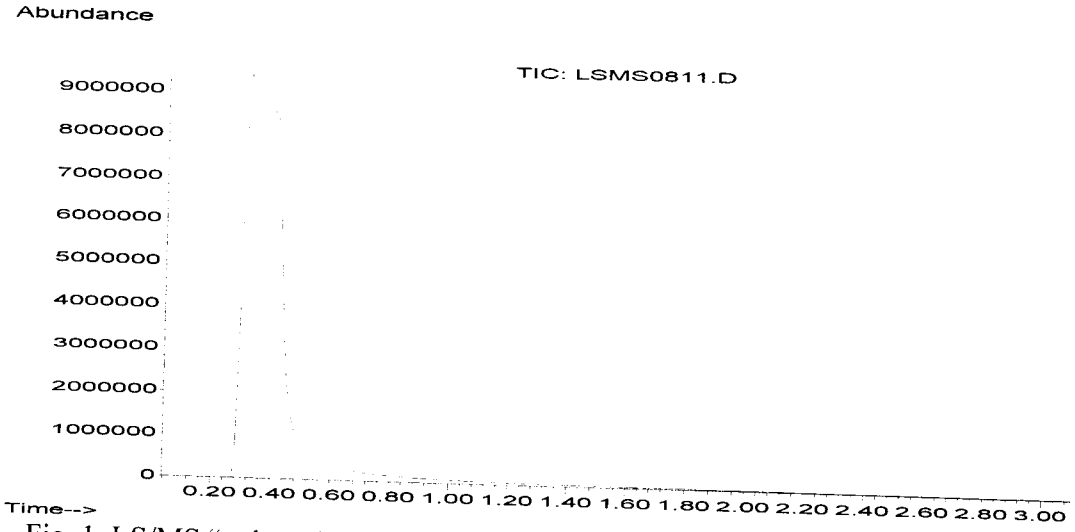

Fig. 1. LS/MS "columnless" chromatogram of Dentifrice 52.723 flavour (Firmenich)

Response of detector is obviously too high, although capillary with higher restriction was used, as wall as injection volume of only 200 nl. In spite of this, the structure of corresponding mass spectrum appeared acceptable (Figure 2).

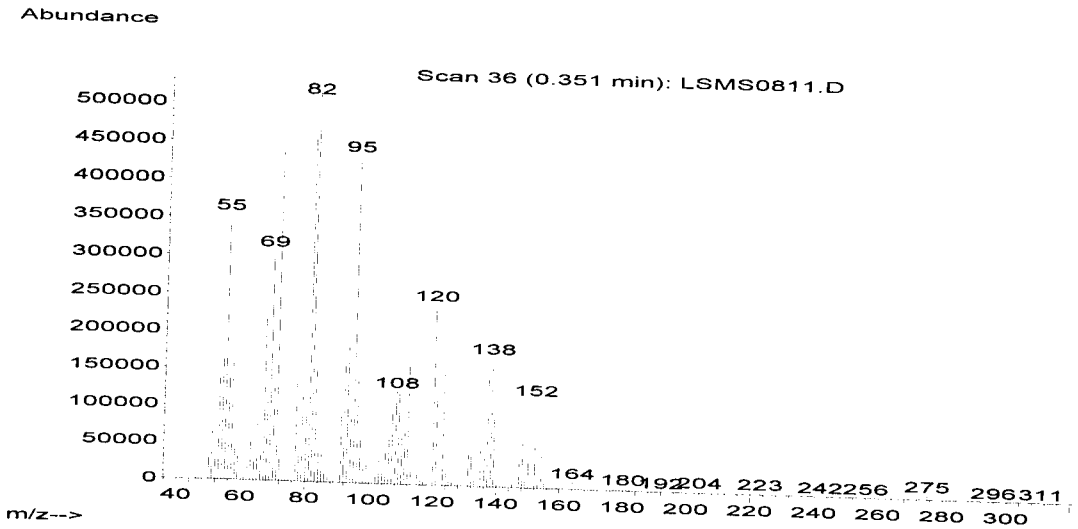

Fig. 2. MS of the peak presented in Figure 1 [Dentifrice 52.723 flavour (Firmenich)]

From data files obtained by multiple injections of all samples, the library of composition of flavour and fragrance mass spectra (ff_2005.l), consisting of 49 records has been created. Evaluation of possibilities of its use for differentiation of flavour and fragrance compositions, according their mass spectra was conducted using two search engines. PBM search was applied directly, giving excellent differentiation between examined samples in the majority of cases. It should be noticed that it was expected from the search engine to recognise analysed sample in the library, and to put that sample as the first on the list of possible hits. One such result is given in Figure 3, where hit list obtained from created database for example mass spectrum (Figure 2) is presented.

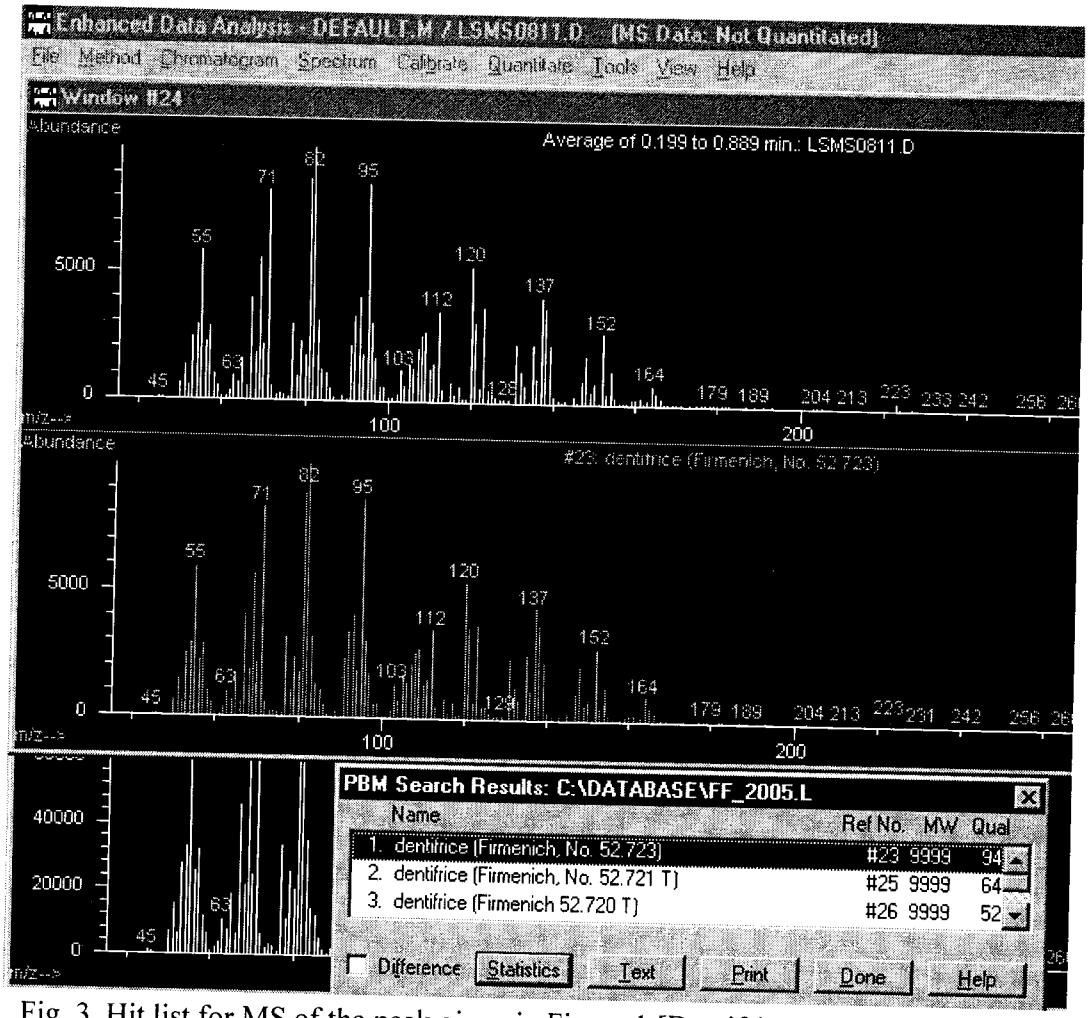

Fig. 3. Hit list for MS of the peak given in Figure 1 [Dentifrice 52.723 flavour (Firmenich)]

It could be seen that PBM search result for all three records from the MS library, which appears in the appropriate window presented in Figure 3, point at the better match quality (94) for the first record. The first suggested result is the right one, other two results are numerically far from that, indicating clear differentiation between these spectra.

Knowing that PBM search algorithm uses selected number of peaks from mass spectrum of library record, with the biggest value of specificity, and compares them with spectra of unknown, we have decided to evaluate NIST search engine, in order to achieve better differentiation between mass spectra. Subsequently, library of the mass spectra of flavour and fragrance compositions was translated from HP to NIST format, necessary for putting NIST MS Search 2.0 software into operation. From the difference of PBM search algorithm, NIST search algorithm takes into account and operates with all peaks from the mass spectrum of the sample.

In the case of comparison of mass spectra of different commercial composition of flavours and fragrances, NIST search engine gave better results than the PBM one.

At the end, hierarchical cluster analysis (HCA) was implemented on mass spectra from the library ff_2005. It showed grouping of clusters, which approves usage of total mass spectra of composition of flavours and fragrances for their characterisation and differentiation (Figure 4).

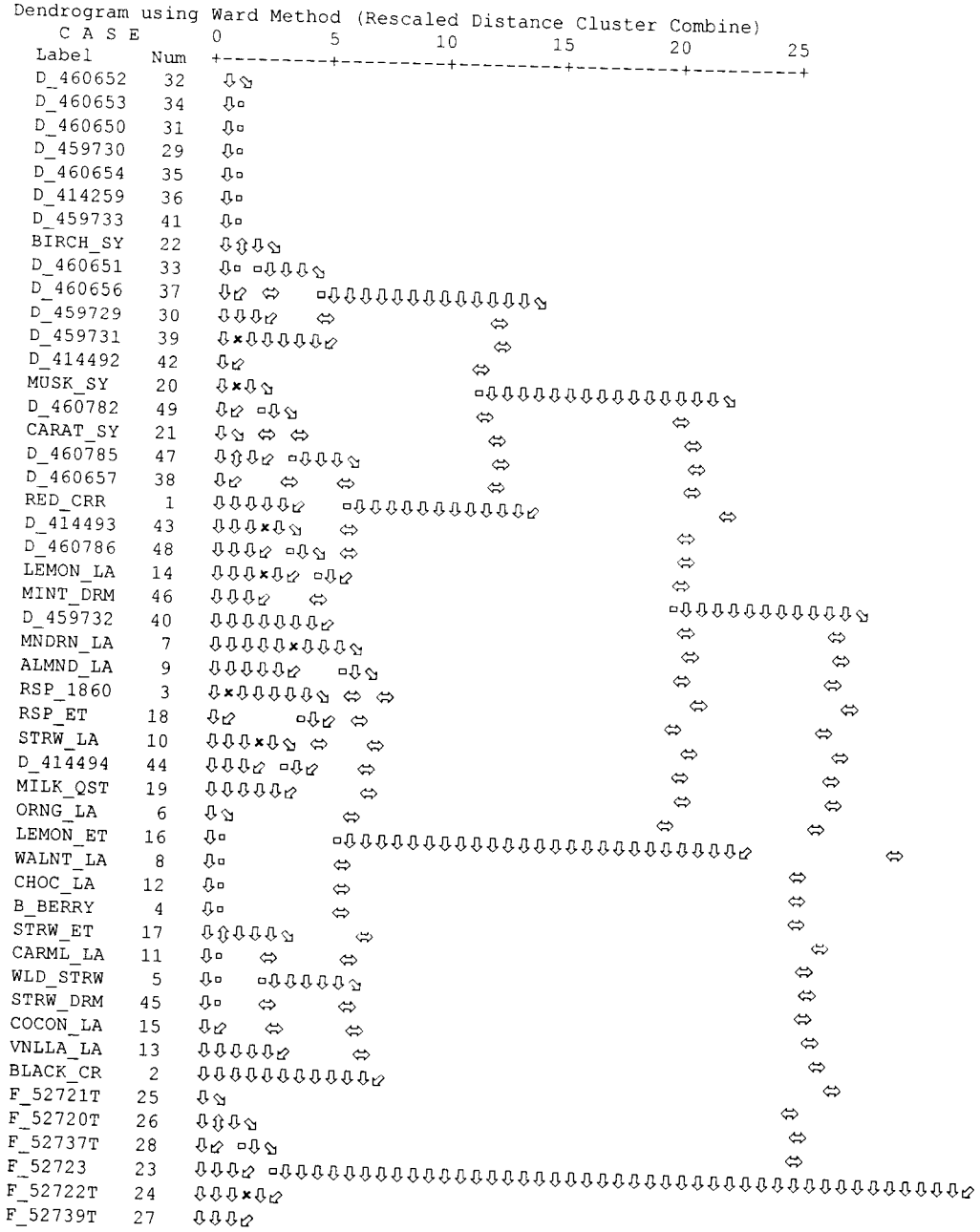

Fig. 4. HCA of total mass spectra from the library ff_2005 (49 records)

It should be noticed that sample, whose basic characterisation has been given as an example in Fig. 1-3 [Dentifrice 52.723 flavour (Firmenich)=F_52723], at the end of cluster presented in Fig. 4, belongs to the sub-cluster containing six similar flavour compositions of the same origin. Although distinction, which could be estimated from mentioned sub-cluster (visually) seemed foggy, results obtained by LS/MS were perfect and quite clean prove of certain differences, approving huge differentiation potential of LS/MS technique.

Conclusions

Clear differentiation of samples belonging to different groups of products (fruit flavours, flavours used in dentistry, and fragrances), as well as those within the same group was

achieved by the use of LS/MS technique. However, screening of different compositions of flavours and fragrances such the way, requires work with large MS-libraries, covering whole spectrum of these products. Moreover, data obtained by LS/MS should be correlated with those obtained by GC/FID, GC/MS, and others, actually required. Subsequently, our further efforts in the application of LS/MS in the field of flavours and fragrances will be focussed in this direction.

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