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Journal of Chromatography A, 1064 (2005) 129–134
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Towards gas chromatography–mass spectrometry coupling protocols for both identifying and quantification essential oils of *Thymus capitatus* Hoff et Link

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Received 7 October 2004; accepted 29 November 2004
Available online 24 December 2004

Abstract

Essential oil of *Thymus capitatus* Hoff et Link is analysed by using four techniques: GC/pyrolyse/MS, GC/FID, electronic impact GC/MS (quadripole), and GC/MS (ion trap). Both major and trace components are analysed. The GC/pyrolyse/MS coupling provides reference to the exact mass compositions without any need of the previously purified references, neither for major or trace components. The comparison between this reference analysis and GC/FID shows that the FID response coefficients may vary by a mean 7% from one component to another. As it was expected, quadripole or ion trap response coefficients vary to a much greater extent (a mean 25%), although the two MS techniques response coefficients are first order consistent. We conclude that GC/MS coupling could be used not only as it is usual for reliable identifications, but also for a complete quantitative routine analysis of essential oils. Expected precision could be very similar to GC/FID precision provided correcting species by species the MS analysis by a mean value of the response coefficient measured for the MS 70 eV electronic impact ionisation technologies. The GC/pyrolyse/MS coupling is proposed as a relevant tool for analysing reference samples containing trace natural species that could not be purified.

Keywords: *Thymus capitatus* Hoff et Link; CG/pyrolyse/MS; Essential oil quantification; Response coefficients; Quadripole ion trap mass spectrometry; Flame ionisation detection; Quadripole mass spectrometry

1. Introduction

In the genus *Thymus*, the attention has been mainly directed towards the composition of oils of the Mediterranean species. The thyme is a shrub, which is native of the Mediterranean basin. It contains an essential oil endowed with a therapeutic action thanks to aromatic alcohols (thymol, carvacrol, ...) which have an antiseptic, antibacterial and analgesic action [1]. Among origanum oils, thyme is both the most important market and the most expensive oil.

The analysis of essential oils is generally performed by gas chromatography, using FID [2] detection for quantification. Qualitative identifications [2,3] are obtained by crossing retention indices and the mass spectra is obtained by 70 eV electronic impact MS_1, more scarcely MS_n detection. Both reliable quantification and identification would thus need at least two data acquisitions of each essential oil sample. Our concern is the feasibility of protocols where only one data acquisition both combines the reliability of MS identification with the reliability of FID quantification. A possibility would be to split the gas fluxes between the two kinds of detectors when just eluted out of the chromatography column. But this is not expected to be a good solution for at least two

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0021-9673/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.chroma.2004.11.095

reasons. First, segregation artefacts are often observed when using splits. The second reason is that MS detection allows to discriminate the signature of co-eluted trace components, whereas FID is a restricted one-channel detector. As a consequence, the level of trace details observed with a MS detector is generally much more important than using a FID. We thus study the feasibility of reliable complete mixture quantification and identification by using only MS detection.

FID detection is expected to give reliable mass abundance because it is supposed to be an absolute mass detector, with first order the same response coefficient whatever the molecular nature of an essential oil component. MS detectors are well known of providing quite dispersed response coefficients. Replacing FID by MS for complete quantification would thus require the response coefficient of each component first to have been identified. This puts some questions. What about the dependence of the response coefficient on the very MS detector technology can they be used? Are these response coefficient consistent enough to justify the creation of a database? How to access the response coefficient of those natural molecular metabolites that are too scarce to be purified as a pure reference authentic sample? The purpose of this paper is to find answers to these question.

2. Essential oils extraction

Experiments reported here have been made by using Tunisian *Thymus capitatus* Hoff et Link essential oils (*carvacrol* chemotype) [4], at the flowering stage (August 1998). Essential oils have been obtained by steam distillation at atmospheric pressure.

3. First analytical methods: gas chromatography–mass spectrometry (GC–MS)

In the first analytic stage of this work the analysis of essential oil has been achieved by two type of gas chromatography–mass spectrometry (GC–MS) who differs mainly by their MS detection technologies.

3.1. Gas chromatography–mass spectrometry (GC–MS) (Agilent GC: HP 5890 series II/HP5972)

The capillary column is a HP-5 MS (length 30 m, 0.25 mm i.d., 0.25 μm phase). Oven temperature increases from 50 to 240 °C with a 2 °C/min slope, carrier gas helium (1.2 ml/min), splitless injection. Before injection, samples were diluted according to 1% into methanol. The injected volume was 0.2 μl.

3.2. Gas chromatography–mass spectrometry (GC–MS) (Varian GC: Star3400s/Saturn 4D)

The capillary columns is a RESTEK MTX1 (length 100 m, 0.25 mm i.d., 0.25 μm phase). Oven temperature increases from 40 to 250 °C with a 2 °C/min slope, carrier gas helium (1 ml/min). Same injection as the former apparatus.

3.3. MS detection technologies

The Agilent HP5972 mass spectrometer is used. It is a quadripole. The Varian Saturn 4D is used. It is an ion trap. Both detectors have been used in 70 eV electronic impact ionisation, scanning between 20 and 400 Da, with 1 scan/s. In the case of the ion trap, the automatic gain control is activated. This procedure allows to store a constant number of ions in the trap before counting them, and thus to better control the collision rate between ions and the residual molecular gas. This procedure allows to get more stable mass spectra. This facilitates molecular identifications of the mass spectra databases. This also enhances the MS response linearity in order to be at least as good as the one obtained with the quadripole.

3.4. Identification of components

The oils components are identified by comparison of their retention indices either with those of authentic compounds or with data published in the literature [2,3]. Each identified molecule is confirmed by a comparison of its mass spectra with those stored in the HP Chemstation database: HP NBS 75K.L.

4. Second analytical methods: GC/pyrolyse(Pyr)/MS calibration tool

Coupling pyrolyse and GC/MS is more often performed by pyrolysing organic matter in an injection headspace. This is called Pyr/GC/MS. The principle is quite different in a GC/pyrolyse/MS coupling. The essential oil sample (0.2 μl diluted at 1% in methanol) is splitless injected way, than eluted in the gas chromatography column. Here, we used a 25 m capillary column, with a DB5 stationary phase.

The eluted out organic components are on-line burned into H_2O, CO and CO_2. On-line means that the eluted components do not mix during this chemical transformation. Each initial organic peak mass is thus replaced by its mass balance equivalent to a $H_2O + CO + CO_2$ peak mass. These peak masses finally enter the MS where they are detected and quantified. Thus whatever the initial nature of the organic compound, only three kinds of final molecular components are detected.

Provided the signal default to linearity and the response coefficient have been identified for both CO and CO_2, provided the raw chemical formula (CHONS mass balance) of each initial organic mixture molecular component is known, it becomes straightforward to deduce the exact mass fraction of each initial organics in the injected oil from the integration of the different CO and CO_2 peaks.

In practice, oxidation is performed by transferring the just eluted gases in a glass capillary tube (1 mm internal diameter) filled with 40 mm copper oxide (CuO) powder (simple

gravity packing). The glass tube is placed in an oven. While flowing in the copper oxide powder, the organic chemicals partly reduce the copper oxide into complex non stoichiometric red copper oxides. Downstream combustion gases are directly transferred to the mass detector. The chemical reaction of course implies organics to adsorb onto the copper oxide, then further desorb as combustion gases. This adsorption induces a chromatographic effect; say first order a global shift of the retention times when compared to those obtained in absence of pyrolyse equipment. Oven temperatures can be selected to fit the chemical nature of the molecular species to be burned. For aromatics, 450 °C can be sufficient for completing combustion, whereas for light saturate hydrocarbons (paraffin) a temperature about 550 °C is necessary. Adjusting oven temperature allows to validate the attribution of any given combustion peak to a definite molecular species. If temperature is too low, the initial mass spectra of this molecular species spreads in the relevant combustion gas peak background.

What is mostly difficult is to optimise the gas hydrodynamics in the combustion reactor. If the specific surface of the copper oxide is too small, combustion remains little efficient even at 550 °C. If the "CuO" powder granulometry is too small, tight packing occurs and no more gas can transfer in the column.

When injected masses of organics are too large, or in the presence on iron traces in the powder, combustion peaks broaden and tail. We also noticed the necessity of an activation of the copper oxide powder. It becomes more efficient after several cycles than when new.

But once the helium carrier gas speed, the oven temperature and hydrodynamic constraints in the glass tube were optimised, we obtained full combustion without significant enlarging of any initial chromatographic peak. Using the downstream MS detector, the shape of the total ion current (TIC) signal versus time is qualitatively very similar to that of an usual GC/MS coupling, with of course a retention time shift first order related to the oxidation reactor hold up. The electronic impact mass spectrum in each peak is replaced by only two major contributions of carbon bearing molecules CO_2 ($m/z = 44$) and CO ($m/z = 28$). The abundance ratio between these two ions mainly depends on the oxidation oven temperature, but we did not observe any significant dependence on the chemical nature of the organics to be burnt. While the oxidation reactor temperature has been defined, only the response linearity on the corresponding CO_2/CO mixture is to be identified.

This can easily be done by injecting in the GC/Pyr/MS coupling a calibration mixture (C) of weighted pure organics. Let the formula of one of these chemicals be $C_nH_pO_q$, and "M_{gas}" the molar mass of the mean CO_2/CO mixture obtained after combustion. A mass "m" (nanograms) injected in the GC/Pyr/MS coupling generates a mass:

$$m_{gas} = \frac{m M_{gas} n}{12n + p + q16} \quad (1)$$

of the CO_2/CO mixture. This mass "m_{gas}" gives a TIC signal peak which surface is "S". If the mass detector was perfectly linear, plotting "S" versus "m_{gas}" would give a straight line. Linearity calibration with mixture (C) gave us a linearity default about 10% by decade on abundances. Abundances in the calibration mixture (C) should be calculated so that they scan both majors and trace abundances in the essential oils to be analysed.

Once this calibration has been done, considering any component "i" of an essential oil, whether a trace or a major, whether a known molecular species or a non elucidated one, provided its CHO atomic mass balance is known, let "S_i" be the TIC surface of the corresponding peak on the GC/pyr/MS coupling, the corresponding absolute mass of combustion gas "$m_{gas}(i)$" is obtained from the combustion gas CO_2/CO calibration table, and the effective mass "$m(i)$" of that molecular species in the essential oil is obtained by inversion of Eq. (1).

The reproducibility of the response coefficients relative to the reference GC/Pyr/MS analysis is consistent with the peak integration precision. This means generally within 1% for numerically well conditioned peaks (height/width >10). The response coefficient "R_i" of species "i" is defined by:

$$R_i = \frac{S_i}{m_i} \quad (2)$$

where "S_i" is a TIC signal or a FID peak signal surface, and "m_i" is the true mass of species "i" injected in the coupling. The GC/pyr/MS coupling can thus be considered as an absolute carbon mass detector. For the restricted detection of carbon atoms, it can be considered as a concurrent to the atomic emission detection (AED) [5,6].

The major advantage of this class of detectors is the possibility to quantify reference natural mixtures containing components which are much too scarce to be purified within realistic economic constraints. These mixtures can then be used as primary reference for calibrating the response of the routine analysis GC detectors.

5. Results and discussion

Reference compositions have been obtained by using the GC/pyr/MS coupling for our thyme essential oils. In this section, this exact composition is compared with that obtained on the same oil either by FID detection (GC/FID), Agilent MS quadripolar detection (GC/MS quad), or by Varian MS ion trap (GC/MS trap). For each detection, response coefficients have been normalised with the choice of a natural internal standard: *para*-cymene, as the reference value $R_i = 1$.

Table 1, contains results of these normalised response coefficients. The experimental retention indices on the MTX1 column have been calculated from the retention times by mixing *n*-paraffin spikes in essential oils. Response coefficients reproducibility has been estimated by the comparison of at least three experiments for each molecular species. When

Table 1
Mass percent, MTX1 retention indices and normalised response coefficients for different CG detection technologies; essential oils of *Thymus capitatus* Hoff et Link

Compound	Mass percent (%)	Retention indice MTX1	Molecular weight	Normalized response coefficients		
				FID	MS quad	MS trap
Monoterpene hydrocarbons						
α-Thujene	1.74	924	136	1.05	NA	1.16
α-Pinene	0.93	930	136	1	NA	1.12
Camphene	0.35	938	136	1.08	0.84	1.05
β-Pinene	0.2	963	136	1.03	0.8	0.78
β-Myrcene	1.87	975	136	0.98	0.68	0.74
α-Phellandrene	0.39	995	136	NA	0.56	0.48
α-Terpinene	1.64	1000	136	1.07	1.1	0.91
p-Cymene	10	1003	134	1	1	1
E-β-Ocimene	1.16	1012	136	0.94	NA	1.26
γ-Terpinene	11.66	1035	136	1.01	0.97	1.07
Monoterpene alcohols						
Linalool	2.55	1074	154	0.96	0.83	0.86
Phenols						
Carvacrol	53.71	1286	150	0.99	1.04	0.89
Sesquiterpene						
Isocaryophyllene	0.49	1398	204	NA	NA	0.97
β-Caryophyllene	9.13	1414	204	0.88	1.05	1.06
Aromadendrene	0.33	1450	204	NA	1.08	1.1
Germacrene D	0.35	1480	204	NA	NA	1.16
β-Bisabolene	0.39	1495	204	NA	1.06	0.98
δ-Cadinene	0.13	1509	204	NA	0.96	1.19
γ-Cadinene	0.33	1519	204	NA	0.96	1.19
Caryophyllene oxide	0.53	1561	220	1.01	NA	1.25

NA: non available (worse than 2% reproducibility).

this reproducibility is worse than 2%, the response coefficient measure is rejected (so called non available). Fig. 1 gives the normalised response coefficients as a function of the retention indices. This mainly visualises the response coefficient dependence on the chemical nature of the components. Lower indices mainly define the monoterpene region, higher ones the sesquiterpene region, with the major phenol (carvacrol) around indice 1300. This figure illustrates the fact that the MS response coefficients are statistically much more sensitive to the chemical nature of each component than the FID response coefficients. It also suggests that the response coefficient dispersion is higher in the same family. For example, here the monoterpenes than when comparing family to family; monoterpenes, aromatics, phenol, oxygenated terpenes and sesquiterpenes.

One may ask whether response coefficients are sensitive to the molecular species abundance in the mixture. Fig. 2 points out that the dilution of each component is not correlated to

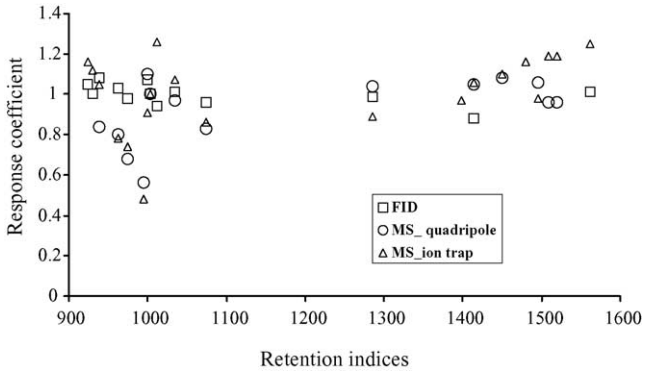

Fig. 1. Response coefficient for different CG detection.

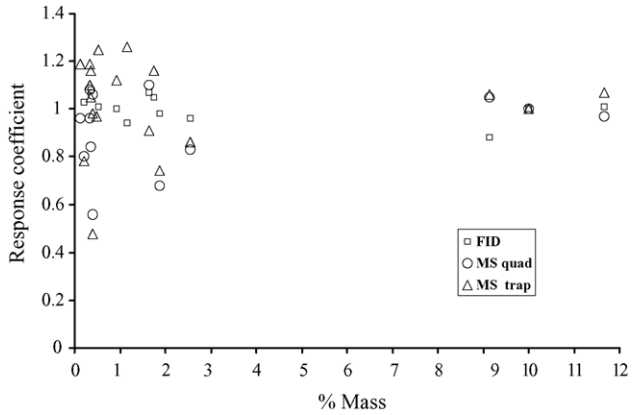

Fig. 2. Response coefficient vs. percent mass for different compound (except carvacrol 53.71%) in the essential oil of *Thymus capitatus* Hoff et Link.

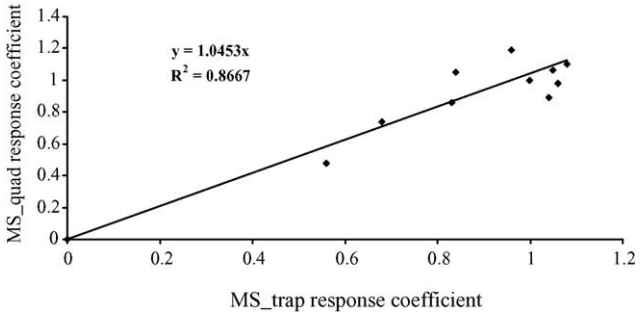

Fig. 3. MS quad vs. MS trap response coefficient.

its response coefficient, here at least for traces representing more than 1000 ppm.

Fig. 3 compares the MS quadripole response coefficients and the MS ion trap response coefficients. They range around the first diagonal within a mean statistical fluctuation of 8%. As a consequence, within the criterion of the response coefficient dispersion, there is no advantage for any of the two MS technologies. Furthermore, remaining of Fig. 1 where the statistical fluctuation of the FID response also is about 7%, this means that after correcting the MS detected analyses by the mean value of the response coefficients obtained from quadripolar or trap technology, the residual statistical incertitude on abundances is typically the same as when using a FID.

Concretely, this means that provided that a database would be published, giving a mean value of the response coefficient for each species as measured with the current market 70 eV electronic impact technologies, the quality of a GC/MS quantitative analysis will be at least equal to that of a GC/FID analysis. The current trend in flavour analysis is to provide databases both containing mass spectra and retention indices. We suggest it also is a highly valuable and feasible task to include response coefficients. The two MS technologies mainly differ by the method for detecting ions, although ionisation procedures are quite similar. Fig. 3 suggests that the first order universality of the MS response is related to the existence of that common electronic impact ionisation process, and more precisely the fact that only cations are detected. The ability of molecules to make cation residues in electronic impact fragmentation is probably the leading factor that determines the response coefficient dispersion of 70 eV electronic impact MS technologies.

The MS signal linearity may influence the response factor. Quadripolar detection is generally supposed to give a better signal linearity than ion trap detection. With the AGC option on the Varian ion trap, we have not observed such an advantage of quadripoles. Figs. 2 and 3 show that if any such correlation existed, it would be second ordered in front of the intrinsic response of each molecular species.

In the ion trap technology, exactly half of all the cations that have been formed will be detected, whatever their mass provided it ranges between 20 and 650 Da. In the quadripole technology, only some thousands of the total ions will be detected, and this proportion depends on the range of ions to be scanned, but also in practice on the quality of the ion beams alignments. This alignment may change because the detector is dirty, but also just because the tune of the electronic optics was changed. As a consequence of these alignment slight defaults, the abundance of each ion in the mass spectra of a given molecular species should be multiplied by a function of each ion mass. This function typically varies as an exponential of the *m*/*z* value.

In other words, for molecules which differ to a large extent in the shape and the mass range of ions, one may fear quadripoles to provide poorer reproducibility than ion traps on the response coefficients, just because day after day, it is difficult to ensure exactly the same tune of the detection electronics. Here, integrating on the total ion current (TIC) a set of chromatograms acquired during several months, we did not observe significant date dependant correlation of the response coefficients. The day after day electronic tune reproducibility default would thus also be second ordered in front of the intrinsic response of each molecular species.

As a final conclusion, for flavour *quantitative* analysis, it will be possible to prefer GC/MS to GC/FID as soon as a response coefficient database will be available. The concept of such a database is realistic because there exist a sufficient response coefficient universality level provided similar ionisation technologies are involved; say 70 eV electronic impact technologies.

The evolution to GC/MS protocols may deeply influence essential oil professionals. At this time, a flavour is finally evaluated by a human nose, because minor traces are often at least as much important as major components to define the final flavour. With a GC/FID, this quantification of traces is quite poor. For example, a geranium oil analysed by GC/FID hardly will quantify more than 100–120 components, whereas the same oil analysed by GC/MS points out more than 600 components. This also may influence the pharmacological approach to natural extracts or essential oils. With current GC/FID, attention is focused on the major components activity. GC/MS will allow to better appreciate the synergy effects between these major components and the surrounding bunch of traces.

Building the response coefficients data base does not require the purification of each molecular components. The specific response coefficient of each component, even when chemically non elucidated, can be obtained by quantification of a natural mixture where it exists, even as a trace, using GC/AED or GC/Pyr/MS. The only key requirement is the possibility of that trace component to be solely eluted in at least one specific essential oil. The GC/Pyr/MS clearly needs some know how to be operated, but it is cheaper to access than the AED technology.

References

[1] J. Cabo, J.M. Solar, A. El Ghouth, M.E. Wisniewski, Plant Dis. 81 (1997) 204.

[2] T. Adzet, R. Granger, J. Passet, R. San Martin, Biochem. Sys. Ecol. 5 (1997) 269.
[3] P. Sandra, C. Bicchi, Capillary Gas Chromatography in Essential Oil, Dr. Alfred Huethig Verlag, Heidelberg, 1987.
[4] L. Hedhili, M. Romdhane, A. Abderrabba, H. planche, I. Cherif, Flavour Fragr. J. 17 (2002) 26.
[5] J.J. Sullivan, B.D. Quimby, J. High Res. Chromatogr. 12 (1989) 282.
[6] J.J. Sullivan, B.D. Quimby, Anal Chem. 62 (1990) 1034.