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Single step determination of fragrances in *Cucurbita* flowers by coupling headspace solid-phase microextraction low-pressure gas chromatography–tandem mass spectrometry

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Abstract

Coupling headspace solid-phase microextraction (HS-SPME) and low-pressure gas chromatography–tandem mass spectrometry (LP–GC–MS–MS) has been used for determining 20 volatile compounds present in flowers. HS-SPME coupled with LP–GC–MS–MS acts in a synergic way allowing a fast extraction and analysis of the target compounds. The method has been optimised studying the influence of the adsorption temperature and adsorption time. The best results were obtained heating the SPME vials at 60 °C for 5 min using 65 μm poly(dimethylsiloxane–divinylbenzene) fibers. The validation of the method ensures the fitness for the purpose of the analytical method, achieving appropriate lower limits, recoveries and precision. The analytical method has been applied to the characterisation of zucchini flowers fragrances in air using passive sampling, in order to improve our knowledge on zucchini fragrances and to better pollination technique in future steps.

Keywords: Fragrances; *Cucurbita pepo*; Volatile organic compounds

1. Introduction

Zucchini (*Cucurbita pepo*, L.) culture in Almeria province, as in many other places of Southern Europe where intensive horticulture is carried out, can be classified as a 'cash-crop' for farmers, mainly at winter time. In spite of that, the zucchini culture requires a lot of manpower, because the production needs the induction of the parthenocarpy by plant growth regulators [1], and this is done by hand. This practice produces frequently abnormalities in the fruits [2], and, moreover, the use of chemicals is not well considered by the consumer, which usually prefers a more natural production, like the use of pollination by insect vectors instead of chemicals.

Nevertheless, the commercial production of zucchini requires a highly effective pollination, because the flower anthesis (time in which the flowers are open) lasts only a few hours. For this reason, the randomness of the pollination by insects must be very well controlled if we want to obtain a similar production compared with chemical growth promoters parthenocarpy induction technique.

Flower attributes such as size, colour, flower organs, and nectar guides on the petals, nectar volume, nectar composition, and amount of pollen are considered to be important factors attracting honeybees and as such can affect visitation frequency [3]. Recent studies indicate that the chemical components contributing to a flower's fragrance also play an important role in the attractiveness of flowers to honeybees [4]. The chemical composition of flower volatiles may also affect bee behaviour. Olfactory signals are rapidly learned, indicating that foraging behaviour results from the association of plant allelochemicals acting as chemosensory cues for the bees [5]. Moreover, the fact that in some cases bees

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are more attracted to flowers with a meagre level of nectar than those with high levels indicates that the olfactory signal(s) may be a more dominant factor controlling bee behaviour [6]. Collectively, these reports indicate that bee behaviour is controlled by the integration of both perceived cues, such as colour and/or fragrance, and the amount of a reward, such as pollen and nectar.

The production of volatile compounds attractive for pollinating insects in flowers of *Cucurbita maxima* and other plant species belonging to the same family (Cucurbits) [7–12] has been studied, founding a series of volatile chemical products, which attracted several species of beetles. There is also a work published on analysis of fragrances of thricomes from the leaves of *C. pepo* [13], focused on plant protection.

Most of analytical methods for flower fragrances use capillary gas chromatography (GC) for analysing volatile or semi-volatile organic compounds, because of its excellent separation efficiency, high speed of analysis, and the wide range of sensitive and selective detectors available [14–16]. At present, GC is mainly focused on speed for the reduction of analysis time. Recently, low-pressure (LP) GC has allowed short analysis times in pesticide residue analysis with the use of a short capillary column and MS–MS detection mode [17,18]. For this reason, we have chosen this technique to analyse volatile compounds.

Only a few papers deal with the application of tandem MS mode detection for determining this kind of compounds [19], which presents a higher selectivity than other detectors. It allows to detect low concentration levels of compounds without loosing identification capability, showing its performance to be used for determining a wide range of semi-volatile compounds in the air [20].

The extraction of the volatile compounds is an essential step of the analytical method. Díaz Maroto et al. [21–25] have applied, solvent distilled extraction (SDE), supercritical carbon dioxide and solid-phase microextraction (SPME) for the analysis of odours from different plants, (mainly spices) and GC–MS detection. SPME has demonstrated its performance for the analysis of volatile compounds in several matrices [26–28]. SPME in mode headspace (HS) presents several advantages over other extracting methods, such as static headspace sampling [29–32] or solvent extraction [33–35]. In addition, this is a cost-effective method, saving in solvents and time being easily automatised. The coupling of several techniques leads often to fast, automatised, repetitive and cheap methods.

This work presents the development and validation of analytical method for determination of 20 volatile compounds in flowers of *C. pepo*, as part of a research project dedicated to study the optimisation of the pollination conditions by insects, in order to increase the natural production of zucchini cultured under greenhouse conditions with the aims of identifying the compounds that increase the activity of bumble bees, to determine the cultivars richer in such compounds and the moment of the anthesis in which the flowers have the highest concentration and thus the activity of pollinators would be higher, improving the production. The proposed analytical method couples HS-SPME–LP–GC–MS–MS, which allows detecting low concentration levels of fragrances, in a fast, accurate and cheap way.

2. Experimental

2.1. Chemicals and reagents

All reference standards and internal standard (IS), *p*-xylene-d10, were of analytical grade and were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Tokyo Kasei (Nihonbashi, Tokyo, Japan). A reference standard solution was prepared for each compound using acetone as solvent at 200 $\mu g\,ml^{-1}$ concentration. A multicompound working standard solution (2 $\mu g\,ml^{-1}$ concentration) was prepared from the above by appropriate dilution with acetone and stored under refrigeration (4 °C). Organic solvents of chromatographic grade were obtained from Panreac (Barcelona, Spain).

The compounds investigated were: benzene, toluene, *o*-xylene, *m*-xylene, *p*-xylene, ethylbenzene, α-(+)-pinene, myrcene, *R*-limonene, eucalyptol, ocymene, linalool, hydroquinone dimethylether, *p*-anisaldehyde, cinnamaldehyde, indole, cinnamyl alcohol, dibutylphtalate, eugenol and 1,2,4-trimethoxybenzene. These compounds were chosen because they were found in the floral odour of several species of Cucurbits [36,37].

The syringe injector of the SPME unit (Supelco, Bellefonte, PA, USA), equipped with 100 μm polydimethylsiloxane (PDMS) and 65 μm poly(dimethylsiloxane–divinylbenzene) (PDMS–DVB) fibres (Supelco), was used for the extraction procedure. Fibres were conditioned prior to use according to supplier's prescriptions.

2.2. Apparatus

GC–MS analysis was performed with a Varian 3800 gas chromatograph with electronic flow control (EFC) and fitted with a Saturn 2000 ion-trap mass spectrometer (Varian Instruments, Sunnyvale, CA, USA). Samples were injected with a Varian 8200 auto sampler with a syringe injector of the SPME unit (Supelco) into an SPI/1079 split/splitless programmed-temperature injector. A fused silica untreated capillary column 2 m × 0.25 mm i.d. from Supelco (Bellefonte) was used as guard column connected to a Rapid-MS [wall-coated open tabular (WCOT) fused silica CP-Sil 8 CB low bleed of 10 m × 0.53 mm i.d., 0.25 μm film thickness] analytical column from Varian Instruments (Sunnyvale, CA, USA) for high speed analysis. The mass spectrometer was operated in the electron impact ionisation mode (EI). The controlling computer system had an EI-MS–MS library specially created for the target analytes under our experimental conditions. Other EI-MS libraries

were also available. The mass spectrometer was calibrated weekly with perfluorotributylamine. Helium (99.999%) at a flow rate of 1 ml min^{-1} was used as carrier and collision gas.

2.3. HS-SPME conditions and figures of merit

Volatilisation of compounds in the vials was achieved by setting them at 60 °C in the thermostatised carrousel of SPME unit. Then the fibre is exposed to the HS during 5 min in order to complete the adsorption of compounds. Fibre is injected into the injection port of the GC, which was set at 250 °C in splitless mode. The desorption of analytes from the fibre took 9 min, which is enough to desorbs and transfer the analytes to the analytical column.

HS-SPME conditions were established for validating the analytical method. The performance of the method was assessed calculating linear range, recovery rates, precision and lower limits. For that purpose, empty HS (10 ml volume) vials were spiked with appropriate volumes of a standard mixture containing all the analytes at a concentration of 0.2 mg l^{-1}, to cover analyte amounts between 10 and 1000 ng for estimating linear ranges; 10 and 100 ng for checking recovery rates (10 replicates each for obtaining precision); and decreasing volumes of standard solution mixture, three replicates, in order to obtain lower limits, as it is explained in the results and discussion section.

2.4. LP-GC–MS–MS conditions

The initial column temperature was set at 35 °C during injection, 9 min hold, then increased at 1 °C min^{-1} to 55 °C, at 3 °C min^{-1} to 65 °C, and finally raised to 300 °C at 100 °C min^{-1} that was held for 5 min.

The ion-trap mass spectrometer was operated in EI-MS–MS. The transfer line, manifold and trap temperatures were 280, 50 and 200 °C, respectively. The automatic gain control (AGC) was activated with an AGC target of 5000 counts. The emission current for the ionisation filament was set at 80 μA generating electrons with energy of 70 eV. The axial modulation amplitude voltage was 4.0 V. The MS–MS process was carried out by collision-induced dissociation (CID) with a non-resonant excitation for all the compounds studied. The electron multiplier voltage was 1700 V (+200 V offset above the auto-tuning process). Scan rate and mass range scanned depended on the number of compounds analysed simultaneously. The specific MS–MS parameters used are shown in Table 1.

2.5. Sampling and analysis procedure

Samples were taken from a commercial zucchini greenhouse. Flowers were selected at random and placed in 10 ml HS vials, previously weighted, and 40 ng of [$^2H_{10}$] *p*-xylene was added as IS. Vials were capped and stored in a portable fridge at 4 °C approximately, being transported to the laboratory and analysed in a period of time within two hours after being taken. Field quality control (QC) samples were also collected by spiking in the field, three empty 10 ml vials with an amount equivalent to the second concentration level of the calibration curve and the internal standard, being stored, transported and analysed in the same batch than flowers sample. The quantification of compounds in the samples was performed using the internal standard calibration method, injecting in the same batch of samples a calibration curve prepared by spiking four empty vials at different concentration levels and the internal standard (covering the linear range obtained), calibration plots obtained

Table 1
Retention time window (RTWs) and GC–MS–MS conditions

Number	Compound	RTW (min)	Parent ion (*m/z*)	CID amplitude (V)	CID rf (*m/z*)	Quantification ion (*m/z*)	Range (*m/z*)
1	Benzene	0.53–0.55	78	37.5	35	59	50–107
2	Toluene	0.75–0.79	91	40	35	65	50–107
3	Ethylbenzene	1.21–1.24	91	40	35	65	50–107
4	*m*-Xylene	1.28–1.35	91	40	35	65	50–107
5	*p*-Xylene	1.30–1.37	91	40	35	65	50–107
6	*o*-Xylene	1.46–1.51	91	40	35	65	50–107
7	α-(+)-Pinene	1.97–2.01	93	40	38	91	90–200
8	Myrcene	3.24–3.29	93	40	38	91	90–200
9	*R*-(+)-Limonene	4.29–4.34	93	40	38	91	90–200
10	Eucalyptol	4.28–4.31	93	40	38	91	90–200
11	Ocimene	4.86–4.91	93	40	38	91	90–200
12	Linalool	8.58–8.63	93	40	38	91	90–200
13	1,4-Dimethoxybenzene	10.85–10.91	138	30	38	123	115–200
14	*p*-Anisaldehyde	17.48–17.52	135	53.5	55	77	70–200
15	Cinnamaldehyde	19.14–19.20	131	56.5	50	102	70–200
16	Indole	21.29–21.34	117	52.5	56.5	89	70–200
17	Cinnamyl alcohol	23.49–23.53	115	55.5	45	89	70–200
18	Dibutyl phthalate	24.56–24.60	149	54	64	91	70–200
19	Eugenol	24.56–24.60	164	58	70.5	131:133	70–200
20	1,2,4-Trimethoxybenzene	25.84–25.89	168	50	65	125	70–200

have to fit to a straight line with a determination coefficient >0.95 and the standard deviation of residuals minor than 30%.

Before the HS procedure, each vial was weighted in order to obtain the weight of each flower sample as the difference with the corresponding empty vial. All samples were analysed under the conditions explained above.

3. Results and discussion

3.1. Optimisation of the HS-SPME conditions

HS-SPME conditions were optimised by trying different temperatures and adsorption times. The temperature of the carrousel of the SPME device was set at 40, 50 and 60 °C in order to achieve the complete volatilisation of compounds. The fiber was exposed to the HS for 5, 10 and 30 min at each temperature tested, in order to test the influence of the adsorption time in the SPME process. Fig. 1 shows the results obtained, it can be seen that the best volatilisation conditions were 60 °C, and 5 min of adsorption time corresponding with a maximum in the curve. At this temperature, the number of volatile compounds adsorbed by the fibre was greater than those obtained at 40 and 50 °C considering the same time of adsorption, so that these conditions were chosen for the method validation experiments. The two different SPME fibers tested 100 μm PDMS and 65 μm PDMS–DVB, were evaluated in the conditions above described yielding similar recovery rate results, although the latter showed slight better precision values for the majority of the compounds.

3.2. Optimisation of chromatographic separation

The optimisation of the LP-GC separation of the target analytes was performed testing several temperature programs and using electronic flow control of the carrier gas. Fig. 2 shows a gas chromatogram of the standard compounds in the selected experimental conditions, containing both the target analytes and the IS. All the compounds were eluted in a reasonably short time of 26 min.

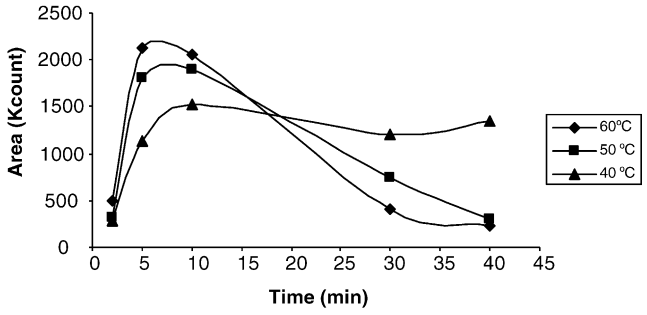

Fig. 1. Effect of adsorption temperature and time on the amount of compound adsorbed in the fiber for linalool.

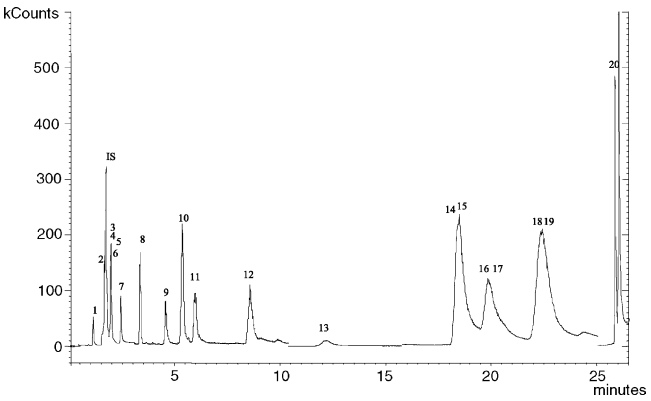

Fig. 2. GC–MS–MS chromatogram of a standard mixture containing the target compounds. Numbers above the peaks correspond with those given in Table 1.

3.3. Optimisation of the MS–MS parameters

The MS–MS detection mode involves the isolation in the trap of a precursor ion or an entire cluster of parent ions and its dissociation through activation by collision with an inert gas. The main parameter to be optimised are: excitation storage level [38], excitation amplitude (or resonance excitation voltage) and the excitation storage level. The parameters set in this study, and the quantification ions are summarised in Table 1, being the excitation time 20 ms. A MS–MS library was created with the MS–MS spectra obtained in such conditions. Finally, in order to assure an appropriate definition of the chromatographic peaks, it was selected 2 micro-scans, adjusting the *m*/*z* range to each compound [17].

3.4. Validation of the method

3.4.1. Identification and confirmation of target analytes

The identification of the target compounds was based on the retention time windows (RTWs), which is defined as the retention time of the analytes obtained from the analysis of 10 spiked samples at the concentration equivalent to the second calibration level, ±3 times their standard deviation (Table 1). Ethylbenzene and the isomers of xylene (*o*, *m*-xylene) co-elute at the same retention time and have similar MS spectra being not possible its quantification as individual compounds, so that they are quantified as sum of isomers. The same occurs with dibutylphtalate and eugenol, but in this case they can be determined individually by selecting in the same segment, a different MS–MS conditions for each compound, being stored in two different channels, in such way the determination of even six co-eluting compounds has been reported [39].

The confirmation of previously identified compounds is performed by matching the MS–MS spectra obtained in the sample with those stored in the MS–MS library created in the same experimental conditions. The library of reference spectra is checked daily by matching it with the results of a spiked sample included in each batch of samples as quality control sample. The results of the comparison (FIT param-

eter) obtained using the match algorithm of the Saturn software are scaled setting 1000 for the best match (identical spectra).

During the validation process, 10 spiked empty HS vials are analysed and 10 spectra are obtained for each compound under the same analytical conditions. One of those spectra is selected as "reference validation spectrum" and the other nine spectra are compared with it. The product of the comparison is nine fit values (from 0 to 1000 for best match) and an average fit value. A threshold fit value defined as the average fit value minus 250 units is considered admissible for identification purposes, the differences in the fit value are due to spectral variations in routine analysis of samples, as consequence of maintenance operations that would slightly affect to the detector response, and therefore the spectra.

3.4.2. Lower limits

Limits of quantification (LOQs) were obtained spiking with decreasing amounts of the target compounds different 10 ml vials ($n = 6$) and calculating the relative standard deviation of the concentration. The LOQ values were established at the concentration that yielded a R.S.D. lower than 25%. Limits of detection (LODs) were established as the amount of analyte that provides a signal-to-noise ratio ≥ 3. Table 2 shows the results obtained for each compound. LOD values ranging from 0.6 to 4 ng were obtained, being the majority minor than 2.0 ng, while LOQs ranging from 2 to 10 ng being the majority lower than 6 ng.

3.4.3. Linearity

Linear ranges were established by spiking empty 10 ml vials with different volumes of the secondary standard solution. We obtained a low linear range between the LOQ and 100 times the LOQ and a second range, which was linear between 100 and 10,000 LOQ of such limits. Chromatographic signals were fitted to linear graphs using least-squares regression. Internal standard calibration was performed plotting amount of analyte versus peaks area ratio (area of analyte/area of IS). Good linearity was found in the mass ranges studied, with determination coefficients >0.97 and the standard deviation of residuals lower than 22%.

3.4.4. Recovery rates and precision

The accuracy of the method was assessed at two mass levels, by spiking empty vials (10 ml) with 10 and 100 ng of each compound respectively. Vials were sealed with a screw-capped top containing Teflon-lined septum. The 65 μm PDMS–DVB coated fibre was exposed at the optimum conditions of analysis. Results show recovery rates in the range of 95–103% (Table 2). It can be noted that recovery rates are calculated from a calibration that includes the likely analyte losses in the adsorption–desorption process of the HS-SPME, nevertheless the good linearity of calibration plots address that the process is not amount dependent in the range of mass studied. The precision (repeatability, $n = 10$) of recovery rates was expressed as R.S.D., being the R.S.D. values lower than 17% for all the compounds (Table 2).

3.5. Application to the analysis of real samples

To assess the applicability of the developed methodology, a set of 20 flowers (10 males and 10 females) of *C. pepo* samples were taken from a commercial greenhouse and analysed. Samples were collected early in the morning,

Table 2
Accuracy and precision at two concentration levels and lower limits of the LP-GC–MS–MS method

Compound	Recovery (%)		Precision (R.S.D., %)		LOD	LOQ
	10 (ng)	100 (ng)	10 (ng)	100 (ng)	(ng)	(ng)
Benzene	95.2	99.3	14	12	2	8
Toluene	96.4	98.8	8	7	2	6
Ethylbezene	100.0	101.1	11	12	2	6
m-Xylene	99.6	97.6	16	17	4	10
p-Xylene	98.3	97.5	17	16	4	10
o-Xylene	95.5	93.3	16	17	4	10
α-(+)-Pinene	97.4	97.4	12	11	1	4
Myrcene	96.4	97.2	14	15	1	4
R-(+)-Limonene	103.1	100.1	9	7	1	4
Eucalyptol	102.4	100.7	8	6	0.8	2
Ocimene	99.3	96.4	7	10	1	4
Linalool	95.5	95.8	13	9	1	4
1,4-Dimethoxybenzene	101.6	103.0	6	6	0.6	2
p-Anisaldehyde	102.0	97.6	7	6	0.6	4
Cinnamaldehyde	95.4	102.4	10	8	4	8
Indole	102.1	96.3	12	12	0.8	2
Cinnamyl alcohol	96.4	97.2	15	11	4	8
Dibutyl phthalate	97.1	101.4	17	14	2	6
Eugenol	101.3	96.4	17	15	1	4
1,2,4-Trimethoxybenzene	98.1	102.5	13	11	1	4

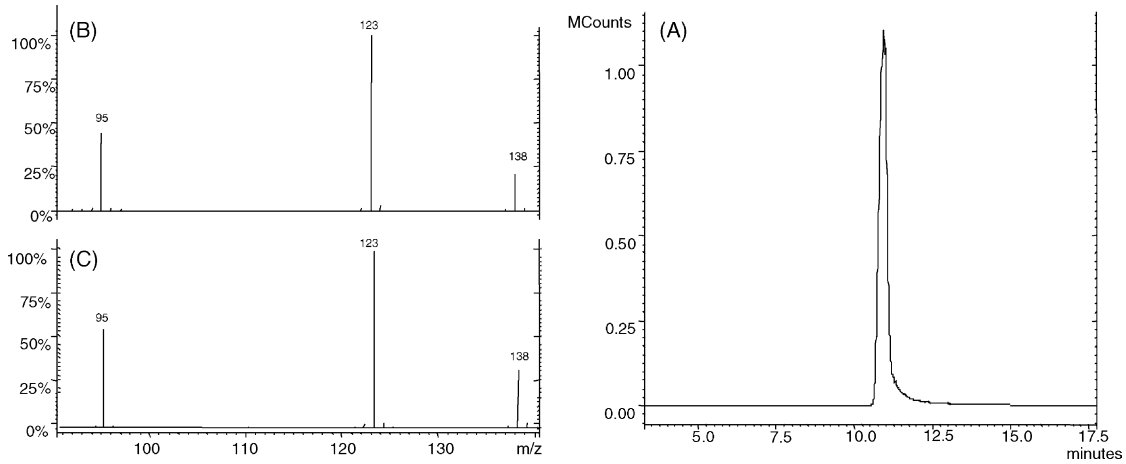

Fig. 3. (A) GC–MS–MS chromatogram of 1,4- dimethoxybenzene found in a male flower. (B) and (C) Matching MS–MS spectra for the target compound in the library and in a current sample, respectively.

from developed plants in spring, being the air temperature 29 °C and the relative humidity 84%. Samples were placed in empty SPME vials, as it is explained in Section 2. Vials containing samples were placed in the carrousel and the temperature was set at 60 °C. In order to identify and quantify a calibration curve was also included and analysed in the same batch as the flower samples. In addition, the field QC samples were analysed in the same batch for checking likely analyte looses and field recoveries.

The QC measures showed that the target compounds are properly identified, calibrations fit to linear curves with determination coefficients in agreement with those found in the validation experiments and the relative standard deviation of residuals minor than 19% in all cases. Recovery rates of the field spikes were also in a range between 93 and 110%, being consequent with those obtained in the validation in addition the signals of the internal standard added to flower samples did not differ from those found in the empty vials used for the calibration.

All the target compounds were quantified in the samples, being the main compounds found in the flowers 1,4-dimethoxybenzene (Fig. 3), and 1,2,4-trimethoxybenzene that were present in all the male flowers at concentrations ranging between 6 and 7.2 $\mu g\,g^{-1}$ (expressed as mass of volatile per gram of flower). It can be noticed that these compounds were also the most abundant in female flowers, but at amounts about five times minor than in males, between 1.2 and 1.5 $\mu g\,g^{-1}$ (Fig. 4). The rest of the compounds were also found, being the most abundant, eucalyptol, indole, linalool, ocimene, myrcene, α-pinene, *o*, *m*, *p*-xylene in amounts about 0.5 $\mu g\,g^{-1}$.

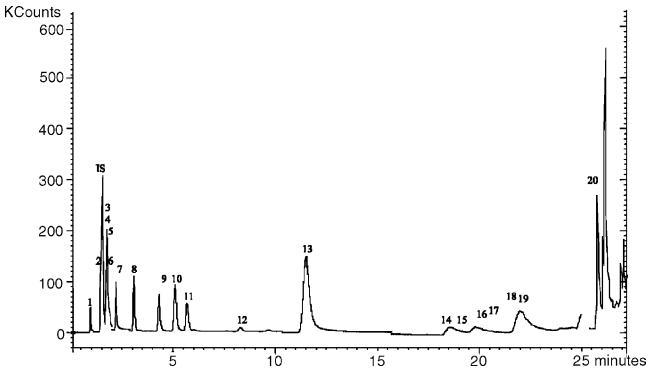

Fig. 4. GC–MS–MS chromatogram corresponding to a female flower sample.

4. Conclusions

HS-SPME allows to analyse samples with a high degree of automatisation, which translates in precise results, fast analysis and a reduction of costs, since solvents consumption and manpower is reduced to minimum. The time of analysis is further reduced in the chromatographic step by coupling the LP mode. The performance parameters obtained show an analytical method fit for the purpose of determining the target compounds. The calibration step includes likely analyte looses, because it reproduces the same volatilisation, adsorption–desorption process than current samples. The field QC procedure ensures that results are provided under statistical control. Finally, the most abundant compound found in flower samples was 1,4-dimethoxybenzene, at concentrations higher than 6 $\mu g\,g^{-1}$ in male flowers, noticeably greater than the same compound found in female flowers. The rest of the target compounds were found as traces compared with the above.

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References

[1] R.W. Robinson, S. Reiners, Hort. Sci. 34 (1999) 715.
[2] I. Rylski, B. Aloni, Acta Hort. 287 (1991) 117.
[3] H.E.M. Dobson, G. Bergström, Plant Syst. E 222 (2000) 63.
[4] M.H. Pham-Delegue, C. Masson, P. Etievant, M.P. Azar, J. Chem. Ecol.12 (1986) 781.
[5] M.H. Pham-Delegue, P. Etievant, E. Guichard, R. Marilleau, P. Douault, J. Chauffaille, C. Masson, J. Chem. Ecol. 16 (1990) 3053.
[6] H. Dafni, Y. Lensky, A. Fahn, J. Apicult. Res. 27 (1988) 103.
[7] R.L. Metcalf, R.L. Lampman, Proc. Natl. Acad. Sci. U.S.A. 88 (1990) 1869.
[8] R.L. Metcalf, R.L. Lampman, L.J. Deem-Dickson, J. Chem. Ecol. 21 (1995) 1149.
[9] R.L. Metcalf, R.L. Lampman, P.A. Lewis, J. Econ. Entomol. 91 (1998) 881.
[10] G. Flamini, P.L. Luigi, I. Moreli, J. Chromatogr. A 998 (2003) 229.
[11] L.N. Fernando, I.U. Grun, Flavour Frag. J. 16 (2001) 289.
[12] G. Flamini, P.L. Luigi, I. Moreli, J. Agric. Food Chem. 50 (2002) 4647.
[13] J.K. Peterson, R.J. Horvat, K.D. Elsey, J. Chem. Ecol. 20 (1994) 2099.
[14] J. Fillion, F. Sauvé, J. Selwyn, J. AOAC Int. 83 (2000) 698.
[15] S.J. Lehotay, K.I. Eller, J. AOAC Int. 78 (1995) 821.
[16] J. Fillion, R. Hindle, M. Lacroix, J. Selwyn, J. AOAC Int. 78 (1995) 1252.
[17] M.J. González Rodríguez, A. Garrido Frenich, F.J. Arrebola Liébanas, J.L. Martínez Vidal, Rapid Commun. Mass Spectrom. 16 (2002) 1216.
[18] P.E. Joos, A.F.L. Gosoi, R. De Jong, J. De Zeeuw, R. Van Grieken, J. Chromatogr. A 985 (2003) 191.
[19] R. Schneider, A. Razungles, C. Augier, R. Baumes, J. Chromatogr. A 936 (2001) 145.
[20] F.J. Egea González, A. Mena Granero, C.R. Glass, A. Garrido Frenich, J.L. Martínez Vidal, Rapid Commun. Mass Spectrom. 18 (2004) 537.
[21] M.C. Díaz-Maroto, M. Soledad Pérez-Coello, M.D. Cabezudo, J. Chromatogr. A 947 (2001) 23.
[22] M.C. Díaz-Maroto, M. Soledad Pérez-Coello, M.D. Cabezudo, Chromatographia 55 (2002) 723.
[23] M.C. Díaz-Maroto, M. Soledad Pérez-Coello, M.D. Cabezudo, Eur. Food Res. Technol. 215 (2002) 227.
[24] M.C. Díaz-Maroto, M. Soledad Pérez-Coello, M.D. Cabezudo, J. Agric. Food Chem. 50 (2002) 4520.
[25] M.C. Díaz-Maroto, M. Soledad Pérez-Coello, M.D. Cabezudo, Res. Adv. Food Sci. 3 (2002) 101.
[26] T. Krämer Alkalde, M.C. Ruaro Peralba, C. Alcaraz Zini, E. Bastos Caramão, J. Chromatogr. A 1027 (2004) 37.
[27] N. Fidalgo-Used, G. Centineo, E. Blanco-González, A. Sanz-Medel, J. Chromatogr. A 1017 (2003) 35.
[28] S. Frías, M.A. Rodríguez, J.E. Conde, J.P. Pérez-Trujillo, J. Chromatogr. A 1007 (2003) 127.
[29] A. Sides, K. Robards, S. Helliwell, Trends Anal. Chem. 19 (2000) 322.
[30] N.S. Kim, D.S. Lee, J. Chromatogr. A 982 (2002) 31.
[31] H. Prosen, L. Zupancic-Kralj, Trends Anal. Chem. 18 (1999) 272.
[32] F. Augusto, A. Leite e Lopes, C. Alcaraz Zini, Trends Anal. Chem. 22 (2003) 160.
[33] J.L. Martínez Vidal, F.J. Egea González, C.R. Glass, M. Martínez Galera, M.L. Castro Cano, J. Chromatogr. A 765 (1997) 99.
[34] F.J. Egea González, M.L. Castro Cano, J.L. Martínez Vidal, M. Martínez Galera, J. AOAC Int. 80 (1997) 1091.
[35] A. Garrido Frenich, F.J. Egea González, J.L. Martínez Vidal, P.P. Vázquez, M.M. Sánchez, J. Chromatogr. A 869 (2000) 497.
[36] L.R. Metcalf, R.L. Lampman, Appl. Biol. Sci. 88 (1991) 1869.
[37] L.R. Metcalf, R.L. Lampman, P.L. Lewis, Ecol. Behav. 91 (1998) 881.
[38] S. Schachterle, C. Feigel, J. Chromatogr. A 754 (1996) 411.
[39] J.L. Martínez Vidal, M.J. González Rodríguez, F.J. Arrebola, A. Garrido Frenich, F.J. Sánchez López, J. AOAC Int. 86 (2003) 856.