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Analytical Methods

On-line RPLC–GC analysis of terpenes using polydimethylsiloxane as a packing material

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Abstract

The effectiveness of absorbent polymers as packing materials alternative to adsorbents in the interface of the on-line coupling of RPLC to GC via PTV for the analysis of terpenes in orange juice was evaluated. To that aim, a comparative study of an absorbent (polydimethylsiloxane, PDMS), and an adsorbent (Tenax TA) was carried out. As a result, satisfactory repeatability was achieved from both packing materials obtaining relative standard deviation values lower than 10% in most cases. Regarding sensitivity, higher recoveries and far lower detection limits were however attained by using PDMS as the packing material inside the PTV injector. Specifically for PDMS the recoveries varied from 52% to 63% whereas in the case of Tenax TA values ranging from 10% to 22% were obtained. Detection limits varied from 1.5 to 1.9 ppb for PDMS and from 30 to 1900 ppb with Tenax TA. In addition to the sensitivity enhancement, PDMS proved to be more effective in the elimination of the solvent coming from the RPLC-pre-separation. Besides, PDMS is more thermally stable and, as a consequence, it results in lesser degradation products.

Keywords: RPLC–GC; Absorbent; PDMS; Adsorbent; Tenax TA

1. Introduction

The analysis of complex mixtures such as foodstuffs is generally rather difficult and tedious because of the great number of constituents occurring in them. Time-consuming and laborious analytical procedures are normally required prior to the chromatographic analysis to effectively isolate the compounds of interest from the matrix. The analyte isolation is even more complicated when chiral compounds are intended to be determined since each chromatographic peak can split at least into two signals increasing considerably the probability of overlapping.

In this context, the use of multidimensional systems can be especially worthwhile since the fraction of interest can be selectively separated from the rest of components in the first dimension of the system and transferred to the second one, increasing notably the analysis reliability. Specifically, the on-line coupling between high performance liquid chromatography (HPLC) and gas chromatography (GC) has proven to be a powerful technique for the analysis of samples formed by chemically different compounds. LC–GC coupling combines the effectiveness in the pre-separation provided by LC with the high chromatographic efficiency and sensitivity obtained by GC. The employment of LC as a replacement of the conventional sample preparation procedures offers various advantages such as the avoidance of a source of error, the lower overall analysis time and the no need for large organic solvent amounts (Grob, 1991; Grob, 1995; Vreuls, de Jong, Ghijsen, & Brinkman, 1994).

The great challenge of on-line LC–GC is encountered in the development of interfaces that enable two chromatographic techniques using incompatible mobile phases to successfully be coupled. This coupling becomes particularly tricky when working in the reversed phase mode, as the high polarity of the eluents commonly used may result in

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the irreversible damage of the gas chromatographic gas column. A few interfaces allowing the coupling LC–GC can be found in the literature. Most of them are however aimed for the use of normal phase in the pre-separation because of the lesser difficulty that it involves compared to the use of reversed phase (Cortes, Pfeiffer, & Richter, 1985; Goosens, de Jong, de Jong, & Brinkman, 1994; Grob & Li, 1989; Mol et al., 1993; Noroozian et al., 1987; Vreuls, Goudriaan, Brinkman, & de Jong, 1991).

In this respect, we have already proposed an interface for the direct coupling RPLC–GC based on the utilization of a programmed temperature vaporizer (PTV), which acts, in turn, as the injector of the gas chromatograph. As earlier discussed (Blanch, Ruiz del Castillo, & Herraiz, 1998), the performance of this interface is based on the effective elimination of the eluent coming from the RPLC-pre-separation by both the evaporative and non-evaporative modes, while simultaneously the solutes are retained in a packing material placed inside the PTV injector. In former works, adsorbent polymers, mainly Tenax TA, have mostly been utilized as packing materials. Nonetheless, although the use of these materials is in general advisable, they also possess some limitations, such as their thermal degradation and the low recoveries obtained in some cases. For this reason packing materials alternative to adsorbent polymers overcoming these drawbacks are still sought. On the basis of the usefulness of certain absorbents in a number of different sample preparation techniques (Baltussen, Cramers, & Sandra, 2002), we have recently reported for the first time the use of an absorbent polymer, i.e. polydimethylsiloxane, as a packing material inside the PTV injector for both the introduction of large sample volumes in capillary gas chromatography (Flores, Herraiz, Blanch, & Ruiz del Castillo, 2007) and on-line coupling RPLC–GC via PTV (Flores, Ruiz del Castillo, & Herraiz, 2007). As a result of these studies, the viability of this material has been demonstrated by using model solutions. However, the application of polydimethylsiloxane to the analysis of real-life samples by RPLC–GC via PTV and, therefore, the matrix effect on the retention of the compounds of interest has not been studied thus far.

The aim of this work was to develop a method based on the employment of absorbents as packing materials inside the interface of on-line coupling RPLC–GC via PTV to analyze complex matrices. A further purpose was to improve the recoveries obtained to date by using adsorbent materials instead. To that end, a comparative study of an absorbent material, i.e. PDMS, and an adsorbent material, i.e. Tenax TA, to determine certain terpenes in orange juice was accomplished.

2. Materials and methods

2.1. Samples and solutions

A standard solution containing a mixture of myrcene and γ-terpinene, as non-chiral terpenes, and limonene and α-pinene, as chiral terpenes, was employed for identification purposes. This solution was prepared by adding 4 μg of myrcene and γ-terpinene and 2 μg of each enantiomer in the case of limonene and α-pinene to 10 ml of methanol. All the standards were acquired from Fluka (Buchs, Switzerland). Methanol (HPLC grade) was provided by Lab Scan (Dublin, Ireland) and the water used was obtained from a Milli-Q water purification system (Millipore, Milford, MA).

Orange juice was purchased in the commercial market. Prior to its RPLC–GC analysis, it was only centrifuged (10^4 rpm, 10 min at 10 °C) and then directly introduced into the RPLC–GC system.

2.2. RPLC-pre-separation

The pre-separation of the investigated compounds was performed using a liquid chromatograph (Hewlett–Packard model 1050, Wilmington, DE). The HPLC system was composed of a manual injection valve (model 7125, Rheodyne, Cotati, CA) having a 20-μl sample loop, an ultraviolet (UV) detector operated at 205 nm and a 100 mm × 4.6 mm I.D., 5-μm-ODS2 column (Waters, Madrid, Spain) operated at 26 °C. Methanol/water was used as the mobile phase. Different eluent flow rates were set depending on whether one packing material or another was employed. Specifically the flows used were 0.5 ml/min for PDMS and 0.7 ml/min for Tenax TA. The initial eluent composition (methanol/water, 35:65, v/v) was maintained for 10 min and subsequently a linear gradient was applied within 5 min up to 100% methanol which was kept during the transfer of the selected cut from LC into GC. The LC equipment was adequately washed by passing methanol through the equipment between consecutive runs.

2.3. RPLC–GC transfer

The transfer of the terpenes of interest was performed through a 75-cm × 0.25-mm i.d. fused silica tube inserted into the septum of the PTV injector, which acted as the interface of the RPLC–GC system, filled with a packing material as detailed below. The transfer was carried out by switching from the waste position to the transfer position a multiport valve model 7060 (Rheodyne), placed immediately after the UV detector. As a consequence of the different eluent flow rates set according to the packing material used, different volumes were equally transferred. Specifically, the volumes of the transferred fractions were 630 μl for PDMS and 700 μl in the case of Tenax TA. Based on our previous experience (Flores et al., 2007), during transfer the injector was maintained at a fixed temperature (20 °C for PDMS and 5 °C for Tenax TA) to facilitate the retention of the studied compounds. As already reported (Blanch et al., 1998), the solvent elimination was promoted during the transfer step by removing the column end from the injector body while passing a helium flow through the PTV. As later explained in Section 3, the

applied helium flow was optimized separately for each material being 20 ml/min and 400 ml/min the selected values for PDMS and Tenax TA, respectively. Once the transfer is finished, the helium flow is still maintained during an additional time (the so-called purge time, 5 min), to complete the removal of the remaining solvent. After that, the column is reconnected and the solutes retained in the packing material of the PTV are transferred to the GC column by increasing the interface temperature at 200 °C/min from the initial temperature up to 220 °C (kept for 20 min) for both PDMS and Tenax TA.

2.4. Packing materials

Polydimethylsiloxane (PDMS, Sigma–Aldrich, Madrid, Spain) coated (50/50, w/w) on the synthetic silica-based support Volaspher A-2 (80–100 mesh, Merck, Darmstadt, Germany) and Tenax TA (80–100 mesh, Supelco, Madrid, Spain) were used as packing materials. On the basis of the equipment design, the possible maximum amount of each one, which corresponds to a 3-cm length, was respectively placed in the glass-liner (a silylated insert of 55 mm × 3.4 mm i.d. × 6.3 mm o.d. purchased from Varian, CA, USA) of the GC-injector and maintained there with two plugs of glass wool. The 3-cm length was equivalent to a 230-mg weight for PDMS on Volaspher A-2 and to 76 mg for Tenax TA. Since Tenax TA is a porous solid, it could be directly introduced into the glass-liner with no prior handling. On the contrary, the PDMS packing had to be previously prepared because of its viscosity. This preparation was carried out by following the experimental procedure described elsewhere (Flores et al., 2007). Prior to its use, PDMS and Tenax TA traps were properly conditioned under a helium stream at 210 °C for 30 min and 350 °C for 2 h, respectively.

2.5. GC analysis

A gas chromatograph (Varian model CP-3800, Palo Alto, CA, USA) was employed to accomplish the analyses. This equipment was fitted with both a split/splitless and a PTV injectors as well as a flame ionization detector (FID) set at 250 °C. The GC analyses were performed on a 25-m × 0.25-mm i.d. capillary column coated with a 0.25-μm layer of permethylated β-cyclodextrin (Chirasil-β-Dex, Chrompack). Helium was used as the carrier gas in the constant pressure mode setting the initial flow at 1 ml/min. The oven temperature was increased from 40 °C (2 min) at 2 °C/min up to 75 °C (5 min), subsequently at 2 °C/min up to 180 °C. The split mode (split ratio; 5:1) was used in all instances. In fact, the splitless mode would have been more recommendable in terms of the sensitivity achievable in the overall analysis. However, we meant to establish more unfavourable conditions to study the potential of the employment of an absorbent material into the glass-liner of the PTV to eventually perform the direct analysis of minor aroma compounds in orange juice. Satisfactory blanks between runs were obtained.

3. Results and discussion

As a starting point, the RPLC–GC analysis of the orange juice was mostly accomplished by applying the experimental conditions elected on the basis of our previous experience (Caja, Blanch, Herraiz, & Ruiz del Castillo, 2004; Ruiz del Castillo, Caja, Blanch, & Herraiz, 2003). Specifically, the eluent composition and interface temperature during transfer were individually selected for PDMS and Tenax TA from a careful optimization process performed in a recent work (Flores et al., 2007). For that reason, the values of these variables used in this study were respectively 0.5 ml/min and 20 °C for PDMS and 0.7 ml/min and 5 °C for Tenax TA.

One exception was the helium flow passing through the PTV during the transfer step, which was evaluated in the present work to make the analysis feasible. It is important to keep in mind that this flow is aimed to facilitate the solvent removal and, therefore, the higher helium flow upon transfer, the lesser remaining solvent amount in the gas chromatographic column. The values of helium flow considered for each packing material were: 20, 100, 200 and 400 ml/min. As a result of this experiment, we selected 20 ml/min for PDMS since the employment of higher flows resulted not only in the elimination of the solvent but also in the loss of the analytes. A helium flow of 400 ml/min was regarded as the most convenient for Tenax TA since flows lower than this value resulted in the overlapping of the peak solvent with the most volatile analytes.

At this point it is interesting to note that the possibility of working at low helium flows made the analysis more economic, which denotes an advantage of the use of PDMS.

The fact that the effect of helium flow during transfer of the analytes depends on the packing material used is due most likely to the different mechanism (absorption vs adsorption) by which the solutes are retained in both materials (i.e. PDMS and Tenax TA). This involves that in absorption the analyte molecules are dissolved into the bulk of the polymer and, thus, no real chemical bond between the packing material and the solutes occurs. Consequently, the use of high helium flows when working with PDMS may bring about the target compounds to be more easily swept by the solvent. In contrast, in adsorption the molecules bind directly to the surface of the polymeric material through an actual chemical interaction. For this reason, with Tenax TA the target compounds are more strongly retained on the polymer, even when high helium flows are applied, in such a way that their loss along with the solvent is more unlikely.

Table 1 indicates the repeatability (expressed as relative standard deviation, RSD), recovery and detection limit obtained for the target compounds from the RPLC–GC analysis via PTV by using PDMS and Tenax TA. The

Table 1
Relative standard deviation (RSD, $n = 3$), recoveries and detection limits obtained for the target compounds from the RPLC–GC analysis via PTV by using PDMS and Tenax TA, respectively, as the packing materials inside the interface of the system

Compounds	RSD[a] (%)		Recovery[b] (%)		Detection limit[c] (ppb)	
	PDMS	Tenax TA	PDMS	Tenax TA	PDMS	Tenax TA
Myrcene	16.1	7.2	58.7	22.3	1.6	30
R-α-Pinene	5.6	12.7	63.5	10.1	1.9	190
S-α-Pinene	8.9	16.5	62.7	10.3	1.8	140
R-Limonene	8.9	–	52.4	10.5	1.5	1660
S-Limonene	9.1	9.1	56.7	10.0	1.5	1900
γ-Terpinene	14.0	9.1	60.5	17.8	1.5	80

[a] Data estimated from orange juice.
[b] Data estimated from the standard solution.
[c] Data estimated from the standard solution.

repeatability was estimated by calculating the RSD from a minimum of three replicates of the orange juice under the experimental conditions described in Section 2. The recovery and detection limit (signal/noise = 5) were estimated from the standard solution. The direct injection in the splitless mode of 0.2 μl into the gas chromatograph by using a glass-liner with no packing material was utilized as a reference in the recovery estimation. As seen in Table 1, the RSD values were always lower than 17% and in most cases they were not higher than 10%. Generally speaking, significant differences between the tested materials were not encountered as PDMS and Tenax TA appeared to provide acceptable repeatability. On the contrary, the recovery and detection limit improved considerably by using PDMS. Specifically, data on the recoveries varied between 52.4% and 63.5% for PDMS whereas the obtained values for Tenax TA ranged from 10.0% to 22.3%. These results were supported by the detection limit data, which varied from 1.5 to 1.9 ppb in the case of PDMS and from 30 to 1900 ppb in the case of Tenax TA. The reason of this general enhancement in the RPLC–GC analysis sensitivity is the non-polar nature of the studied compounds and, thus, their higher affinity for the PDMS material. The striking improvement of the detection limits was due to the lesser background noise obtained from PDMS as a consequence of its lower thermal degradation, which represents other additional advantage of PDMS over Tenax TA. This aspect is apparent from Fig. 1, which depicts the enlargement of the baseline provided by both packing materials, i.e. PDMS (a) and Tenax TA (b), and recorded at the same full range (0.1 mV). From Fig. 1 it is clear that, as just mentioned, the background noise is considerably higher (around 200 times) when Tenax TA was used as the packing material, which, results in higher detection limit.

Fig. 2 illustrates the chromatograms obtained from the RPLC–GC analysis of an orange juice by using either (a) PDMS or (b) Tenax TA in the interface of the system. Both chromatograms were recorded at the same full range. The above mentioned observation on the influence of helium flow on the solvent removal is clearly reflected in Fig. 2. As observed, the remaining solvent amount from the RPLC-pre-separation reaching the gas chromatographic column was greater when PDMS was employed instead of Tenax TA because of the lower gas flow applied during transfer (20 ml/min over 400 ml/min). Nevertheless, even so, the chromatographic signals corresponding to the studied terpenes could be properly separated from the peak solvent obtained when using PDMS, thus enabling, in short, their positive identification. This is owing, on the one hand, to the remarkable efficiency of this polymeric material in

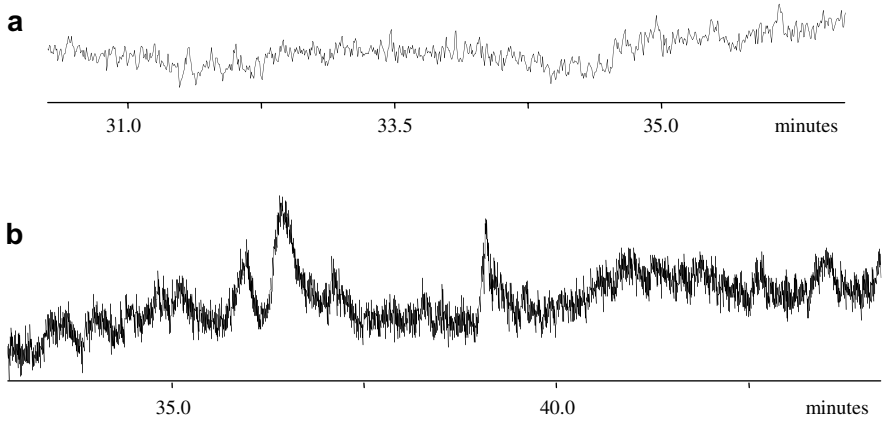

Fig. 1. Enlargement of the baseline obtained from either PDMS (a) or Tenax TA (b), which represents the background noise provided by both packing materials. See text for further details.

removing the eluent coming from the RPLC system, which permits working under low helium flows upon transfer to eventually introduce acceptable solvent amounts into the chromatographic column and, on the other hand, to the narrower chromatographic signals due to the faster desorption of the previously retained analytes. Also included in Fig. 2 are the chromatograms obtained when working at helium flows of 400 ml/min for PDMS and 20 ml/min for Tenax TA (Fig. 2c and d). In both cases the RPLC–GC analyses of the investigated compounds under these experimental conditions was, as above commented, unviable.

From Fig. 2 it is also obvious that the sensitivity achievable in the RPLC–GC analysis via PTV was substantially improved by using PDMS instead of Tenax TA. As a matter of fact, whereas *R*-limonene and γ-terpinene were practically negligible when Tenax TA was used, their occurrence could be certainly established through the employment of PDMS. As also can be seen in Fig. 2, the enhancement in the sensitivity attained by the use of PDMS enabled the detection of *S*-limonene in orange juice to be accomplished. This enantiomer, however, could not be detected when Tenax TA was utilized.

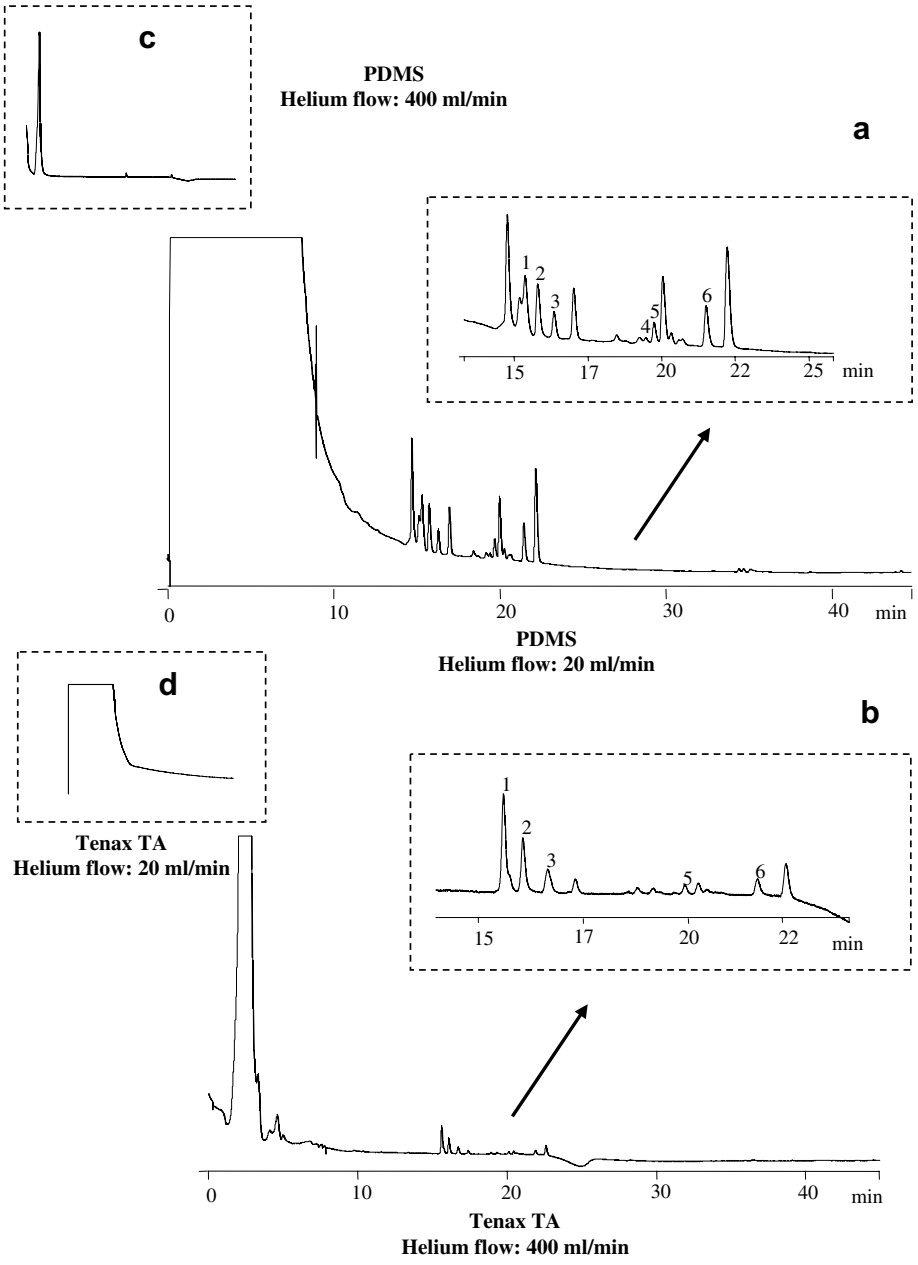

Fig. 2. Chromatograms resulting from the RPLC–GC analysis of orange juice using either (a) PDMS and 20 ml/min or (b) Tenax TA and 400 ml/min as the packing material and helium flow, respectively. The chromatograms (c) and (d) represent the RPLC–GC analysis of the target terpenes in orange juice when working at 400 ml/min with PDMS and at 20 ml/min with Tenax TA, respectively. Peak identification: 1. myrcene, 2. (*S*)-α-pinene, 3. (*R*)-α-pinene, 4. (*S*)-limonene, 5. (*R*)-limonene, 6. γ-terpinene. See text for more details.

Concerning the enantiomeric excess of the chiral terpenes studied, i.e. α-pinene and limonene, slight differences were found for α-pinene according to the packing material employed. While 33% was the value obtained from PDMS, the RPLC–GC analysis with Tenax TA provided a value of 22%, the *S*-enantiomer prevailing in both cases. This minor variation can be simply due to the experimental error inherent to the analytical method as well as to the measurement of the peak areas. Regarding limonene, the enantiomeric composition obtained from the RPLC–GC analysis with PDMS was of 60%, the *R*-enantiomer being predominant. These values suggest the addition of artificial aromas to the sample since enantiomeric excesses close to 100% have been formerly reported for *R*-α-pinene and *R*-limonene in natural products by other authors (König, 1998) and in our own laboratory (Caja et al., 2004; Ruiz et al., 2003). The addition of synthetic aromas is very common in the industry to enhance the natural aroma of foodstuffs. As already mentioned, this information could not be however confirmed by Tenax TA since the lower sensitivity provided by this material did not allow to detect the *R*-enantiomer of limonene. In all cases, the enantiomeric excesses were calculated from peak areas obtained from the FID signals and excess of predominant enantiomer was expressed as a percent, i.e.: [(predominant enantiomer − minor enantiomer)/(predominant enantiomer + minor enantiomer)] × 100.

4. Conclusions

As a conclusion of this work we can state that PDMS may be a valuable packing material in the analysis of terpenes in orange juice by on-line RPLC–GC via PTV. In comparison with Tenax TA, PDMS allows to considerably enhance the analysis sensitivity, i.e. higher recoveries and lower detection limits. This improvement may enable, in turn, very minor compounds such as some enantiomers to be reliably identified. Further advantages of this polymeric material over Tenax TA are its lesser degradation and, consequently, the lesser background noise obtained as well as its higher effectiveness in the solvent removal and the possibility of obtaining narrower chromatographic signals due to the faster desorption of the retained analytes.

On-line coupling RPLC–GC via PTV using PDMS as a packing material inside the interface offers numerous advantages over other analytical techniques for the analysis of aroma compounds in orange juice: sensitivity, selectivity, reliability and rapidity since the analysis is carried out in just one step, the overall analysis time being 45 min, with no demand for the previous elimination of carbohydrates.

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