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Possible interest of various sample transfer techniques for fast gas chromatography–mass spectrometric analysis of true onion volatiles

I. Arnault[a], N. Mondy[a], F. Cadoux[b], J. Auger[a,*]

[a]*IRBI UPRES A CNRS 6035, University F. Rabelais, Parc de Grandmont, 37200 Tours, France*

[b]*CRITT Innophyt, University F. Rabelais, Parc de Grandmont, 37200 Tours, France*

Abstract

We improved GC–MS analysis of onion volatiles by comparing organic solvent partition with solid-phase microextraction (SPME) following cryo-trapping isolation and by comparing the same extraction methods on direct onion juice. Cryo-trapping produces very small quantities of volatiles and therefore is not a suitable extraction method for GC–MS analysis. We confirm that SPME accelerates the degradation of labile thiosulfinates but the lacrymatory factor remains intact. The identification of *Allium* thiosulfinates is only obtained on juice extracted by diethyl ether using a fast GC–MS analysis on a 10 m X 0.3 mm column of 4 μm coating, with routine splitless injection. The lacrymatory factor is best analysed directly on fresh onion juice by SPME with the same chromatographic conditions. To characterise and to quantify all the true onion volatiles, we propose to analyse the same sample by successive SPME–GC–MS and solvent extraction–GC–MS.

Keywords: *Allium* spp.; Extraction methods; Cryogenic trapping; Thiosulfinates; Thiopropanal-S-oxide

1. Introduction

Characteristic flavours of *Allium* species are associated with compounds formed enzymatically from odourless precursors when the plants are cut or crushed. Intermediate alk(en)ylsulfenic acids rearrange rapidly to thermally unstable thiosulfinates (Ti). In the case of onion (*Allium cepa*), 1-propenylsulfenic acids rearrange to give mostly (Z,E)-thiopropanal-S-oxide, the lacrymatory factor (LF), and zwiebelanes, isomers of di(1-propenyl)Ti [1,2] (Table 1). Qualitative and quantitative analyses of *Allium* volatiles take an added interest because of reported health benefits from the consumption of fresh members of the genus *Allium*. For the isolation of these reactive compounds, methods such as solvent and supercritical carbon dioxide extraction [3,4], room temperature steam distillation and cryo-trapping were recommended [5]. Direct methods, like static head-space [6] and headspace solid-phase microextraction (SPME) in a water solution of sample or in a cryo-trapped headspace concentrate, were reported with no success for Ti analysis [2,7].

Gas chromatography (GC), liquid chromatography (LC) and supercritical fluid chromatography (SFC), as well as GC–mass spectrometry (MS), LC–MS and SFC–MS have been used for the separation and identification of LF, Ti and their breakdown products. Each method has its strengths and weaknesses [2].

The identification of those compounds primarily responsible for the characteristic flavour of freshly

*Corresponding author. Tel.: +33-02-4736-6970; fax: +33-02-4736-6911.

E-mail address: auger@univ-tours.fr (J. Auger).

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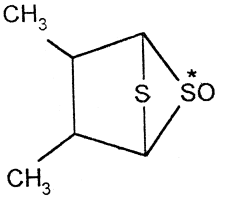
Table 1
Abbreviations and structures of compounds analysed

Compound	Abbreviation	Structure
1	LF (Z)	CH_3–CH=SO
2	LF(E)	CH_3–CH=SO
3	$TiMe_2$	CH_3–SO–S– CH_3
4	TiMePr	CH_3–SO–S–CH_2–CH_2–CH_3
5	TiMePe	CH_3–SO–S–CH=CH–CH_3
6	TiPrMe	CH_3–CH_2–CH_2–SO–S–CH_3
7	TiPeMe	CH_3–CH=CH–SO–S–CH_3
8	$TiPr_2$	CH_3–CH_2–CH_2–SO–S–CH_2–CH_2–CH_3
10	TiPrPe	CH_3–CH_2–CH_2–SO–S–CH=CH–CH_3
9	Zwiebelane 1	
11	Zwiebelane 2	
12	Zwiebelane isomer	
13	TiPePr	CH_3–CH=CH–SO–S–CH_2–CH_2–CH_3

cut *Allium* spp. was claimed impossible to do with traditional GC because of thermal decomposition or artefact formation and needed on-column cold injection [1,8]. Particularly in onion, the low levels of LF usually found, were probably due to its extreme volatility and inefficient trapping. LF is lost during other minor compounds analysis which need a drastic sample concentration step [1] and GC conditions allowing the simultaneous analysis of LF and Ti (maximum 15 m for a wide-bore column) which are hardly compatible with MS coupling. Total LF and Ti quantification are therefore only described with flame ionization detection (FID) [9]. A new method, using a 5 m wide-bore column was applied to onion juice extracted by solvent after 10 min allowed for the LF to be formed and followed by a concentration step [10]. The FID profile shows LF, minor Ti and zwiebelanes poorly separated and not directly identified. This last method cannot be used to compare varieties and *Allium* species with various profiles.

Our purpose, in the present study, is to determine a possible new GC–MS method with routine thermal conditions and column diameter to observe on the same sample all the compounds emitted by onions by improving sampling, transfer to GC and column length. Consequently, we compared SPME and organic solvent extraction without concentration directly on onion juice or on cryo-trapped onion volatiles using a benchtop GC–MS equipment with classical hot splitless injection. We paid also a special attention on the thickness of the column coating.

2. Experimental

2.1. Gas chromatography–mass spectrometry

GC–MS analysis were performed using a benchtop Perkin-Elmer Turbomass system (Perkin-Elmer, Norwalk, CT, USA) with a split–splitless PSS injector and a fused-silica capillary column (10 m or 30 m×0.32 mm I.D.) with a thick methylsilicone coating (4 μm). The carrier gas was 99.999% helium at 1.5 ml/min or 3.5 ml/min flow for the 30 m or 10 m column length respectively. The column temperature program was 5°C/min from 70°C to 200°C, according to our previous GC–FID results on Ti [8].

Total ion chromatograms (TICs) and mass spectra were recorded in the electron impact ionisation mode

at 70 eV. The transfer line and the source temperature were maintained at 150°C.

2.2. Sample preparation, extraction and transfer

2.2.1. Sample preparation

Onion samples were obtained from local market. Fifty g of bulbs were rapidly crushed using a manual onion crusher. The screen with holes of 8 mm diameter in the bottom of the crusher separate the juice (approximately 20 ml) leading to the sample (S). The reaction time (*t*) of between 15 and 80 min, allowed for allinase to react with the precursors at room temperature (20°C).

2.2.2. Sample odour cryo-trapping

A flask containing S was fitted to another immersed in liquid nitrogen and connected to a vacuum pump. Volatiles and water emitted at room temperature were trapped for 20 min. This trapped odour (O), usually used for direct HPLC analysis [2], was submitted to solvent partition or headspace SPME.

2.2.3. Sample and trapped odour solvent partition

S or O, saturated with sodium chloride salt, were extracted by 10% of their volume of diethyl ether. The sample extract (SE) or odour extract (OE) were dried on sodium sulfate, filtered on 0.4 μm membrane and injected (1 μl) immediately onto the GC without concentration, or, if it was not possible, extracts were kept at −80°C prior to analysis.

2.2.4. Isolation and transfer of volatiles by headspace SPME

The headspace of S or O were isolated and transfered to the injector by headspace SPME. During the transfer, the fused-silica fibre coated with various polymeric film coatings was introduced via a septum into the headspace of a closed 5 ml vial containing 2 ml of S or O. The fibre remained above the aqueous phase for 2 min to equilibrate. Then, the fibre was immediately inserted in the GC injector for desorption.

3. Results and discussion

Firstly, we mainly found, in SE analysed (*t* 15 min) on the 30 m length column, the LF, thiopropanal-S-oxide (Z) (1) and its isomer E (95:5) (2), with degradation sulfur compounds and no Ti at all (Fig. 1a). Similar chromatograms were got with SPME on the same column, but with relatively lower quantities of LF and apparition of degradation compounds (disulfides), (Fig. 1b). OE and O transfered by SPME gave the same profiles but with a much lower abundance of LF (Fig. 1c).

Beforehand, GC–MS analysis of O on the 10 m column transferred by SPME or direct SPME of S showed mainly the presence of LF when the time allowed for allinase to react with precursors was no more than 15 min. Formerly, the two isomers of LF were reported as a single peak [3,10]. We note also that SPME does not lead to the detection of Ti. The profiles (*t* 15 min) show only, besides LF, many disulfides and other degradation products (trisulfides), (Fig. 2). As expected, our experimental results, even with *t* 80 min, proved the degradation of Ti on the SPME fibre during the process. Similar results have been obtained with a very low and surprising desorption temperature (35°C) [7]. In our experiment, these degradations occur with the poly(dimethylsiloxane) fibre as well as with the polyacrylate fibre.

In comparison to direct sample analysis, trapping of volatiles at low temperature does not present special advantage for GC analysis and is therefore not recommended.

The profile recorded with SE on the same column and with *t* 80 min, the most favourable for Ti, is presented in Fig. 3. These results, compared with our previous work [5] and those of Block et al. [1], allowed us to detect all the suspected Ti in less than 13 min: $TiMe_2$ (3), TiMePr (4), TiMePe (5), TiPrMe (6), TiPeMe (7), $TiPr_2$ (8) and TiPrPe (10).

Moreover, we tentatively identified TiPePr (13) which never appeared before, the mass spectrum of which is presented in Fig. 4.

Interestingly, three zwiebelane isomers were present and identified in SE. In agreement with the work of Block et al. [1] and our previous results [5], the compounds (9) and (11) are the *trans* zwiebelane

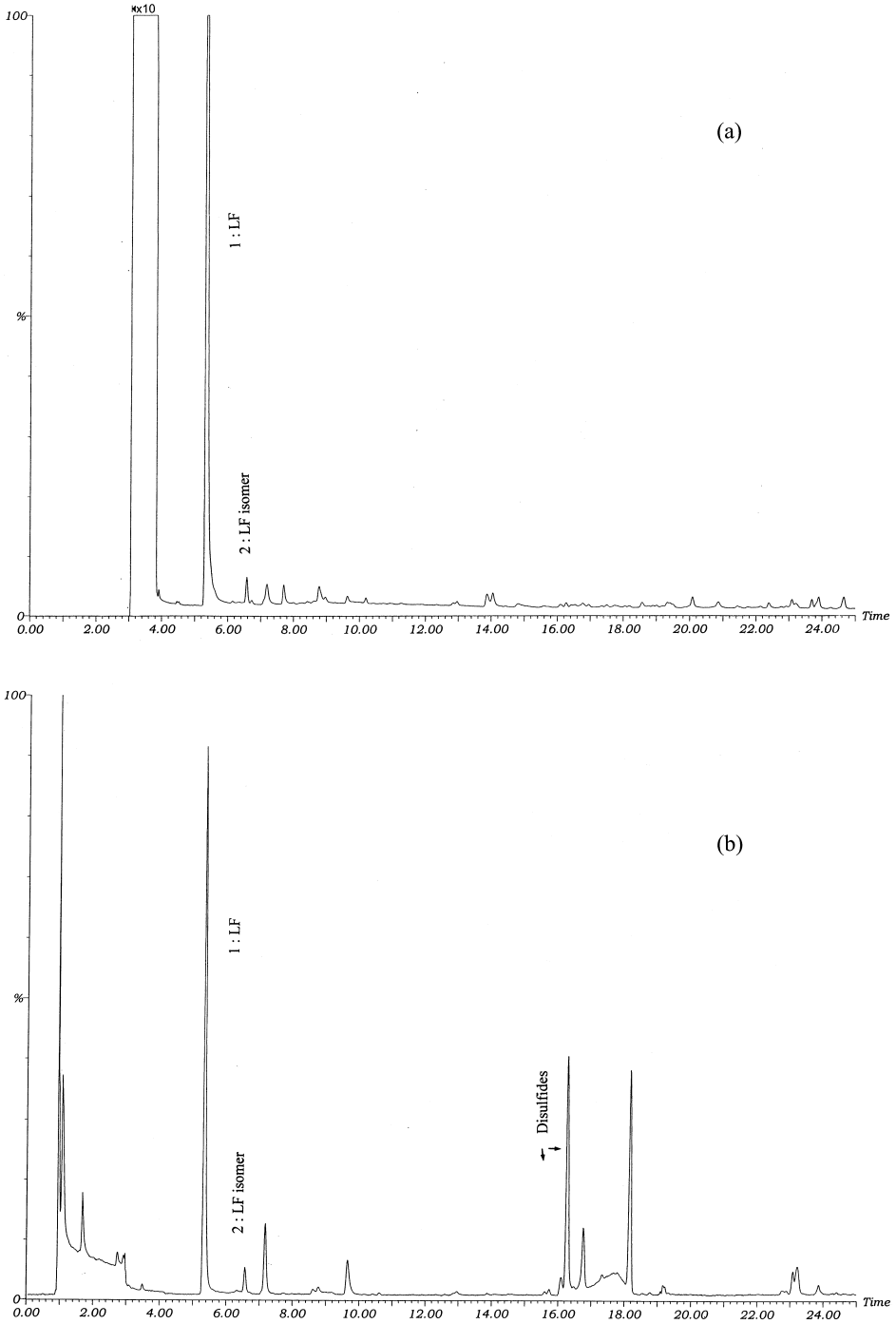


Fig. 1. TICs of fresh onion juice (a) extracted by solvent and analysed on the 30 m length column (*t* 15 min), (b) transferred by SPME and analysed on the 30 m length column (*t* 15 min) and (c) cryo-trapped volatiles transferred by SPME and analysed on the 30 m length column (*t* 15 min). Time scale in min.

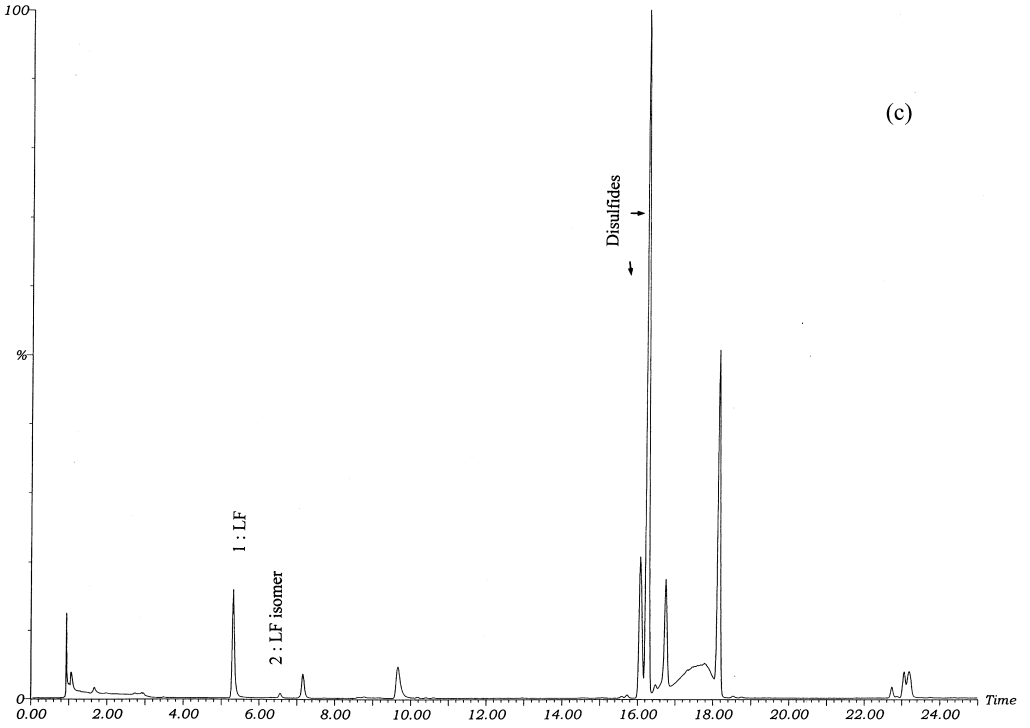


Fig. 1. (*continued*)

and the *cis* zwiebelane respectively [11,12]. Moreover, we detected a third isomer (12), the mass spectrum of which is presented in Fig. 5.

Block et al. discovered that a narrow bore column rapidly lost resolution and concluded that this was due to deposition of non volatiles [1]. The origin of wide-bore column success was probably their usual important thickness of stationary phase and we claim to obtain now similar results with a narrow bore column of 4 μm film thickness. According to our discussions with column suppliers, thin films present frequent interruptions of the coating or imperfections that thick films never present, allowing interactions of reactive sulfoxides with free silanols of the column wall. Moreover, we succeeded in TI analysis with a hot injector which was claimed to be impossible by Block et al.

In our mind it is more important to realise a rapid separation than to proceed with a low injection temperature. However, it seems unreasonable to use columns of lengths less than 10 m if we want to keep an acceptable peak resolution and to couple GC to MS.

In addition, the maximum Ti abundance corresponds to *t* 80 min and the LF is easily quantified only by SPME as it appears in the solvent peak by SE (preferentially for a reaction time less than 15 min). Therefore, we suggest using the same sample for (i) an analysis of the LF by SPME before *t* 15 min and (ii) a complementary analysis by solvent partition at *t* 80 min. Results in absolute and relative concentrations of LF, Ti and related compounds get by this method will be used to evaluate flavour differences between onion varieties and hybrids and during their storage.

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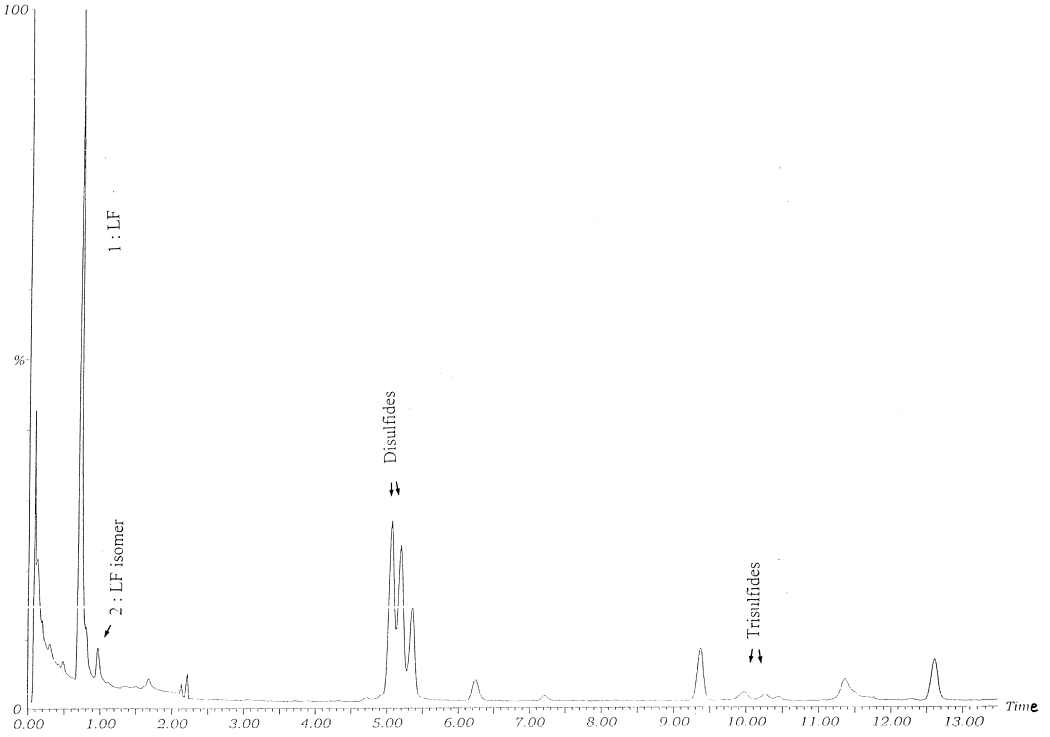

Fig. 2. TIC of fresh onion juice transferred by SPME and analysed on the 10 m length column (*t* 15 min). Time scale in min.

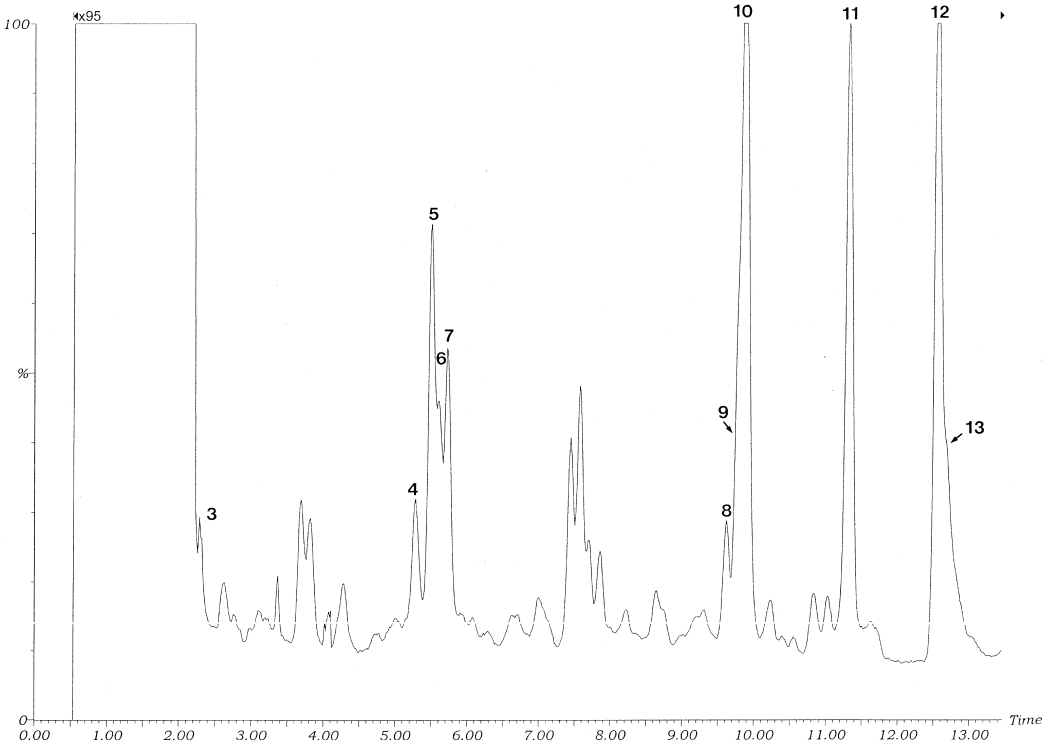

Fig. 3. T.I.C. of fresh onion juice extracted by solvent and analysed on the 10 m length column (t 80 min). Time scale in min.

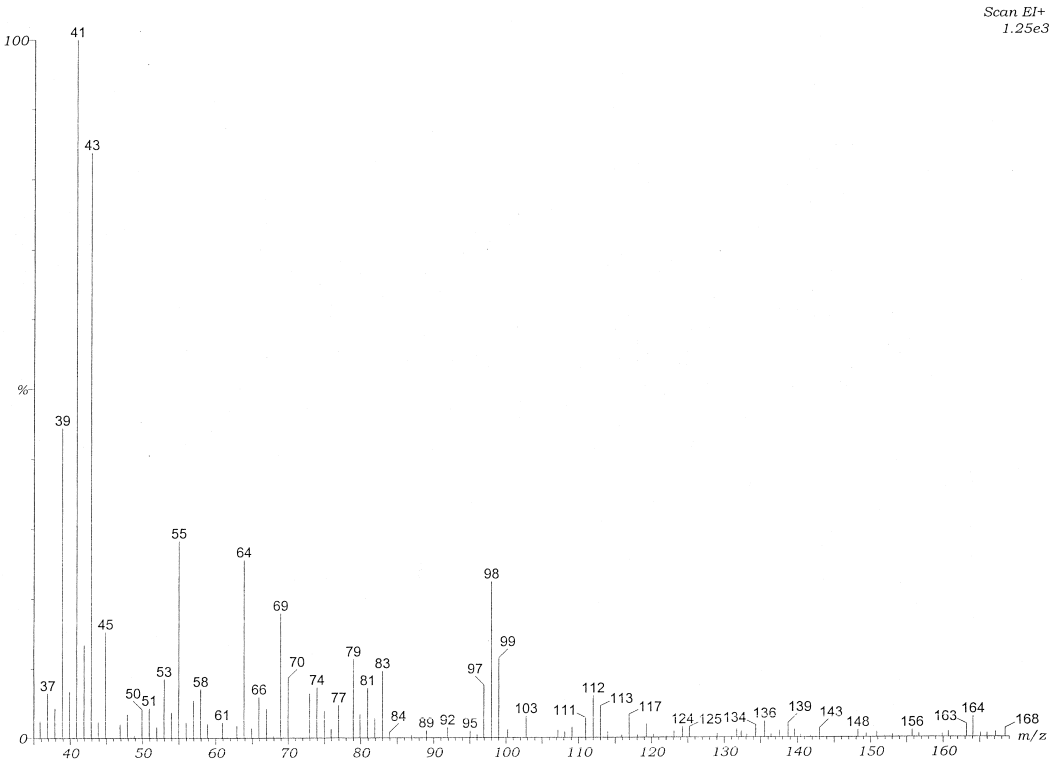

Fig. 4. Mass spectrum of TiPePr.

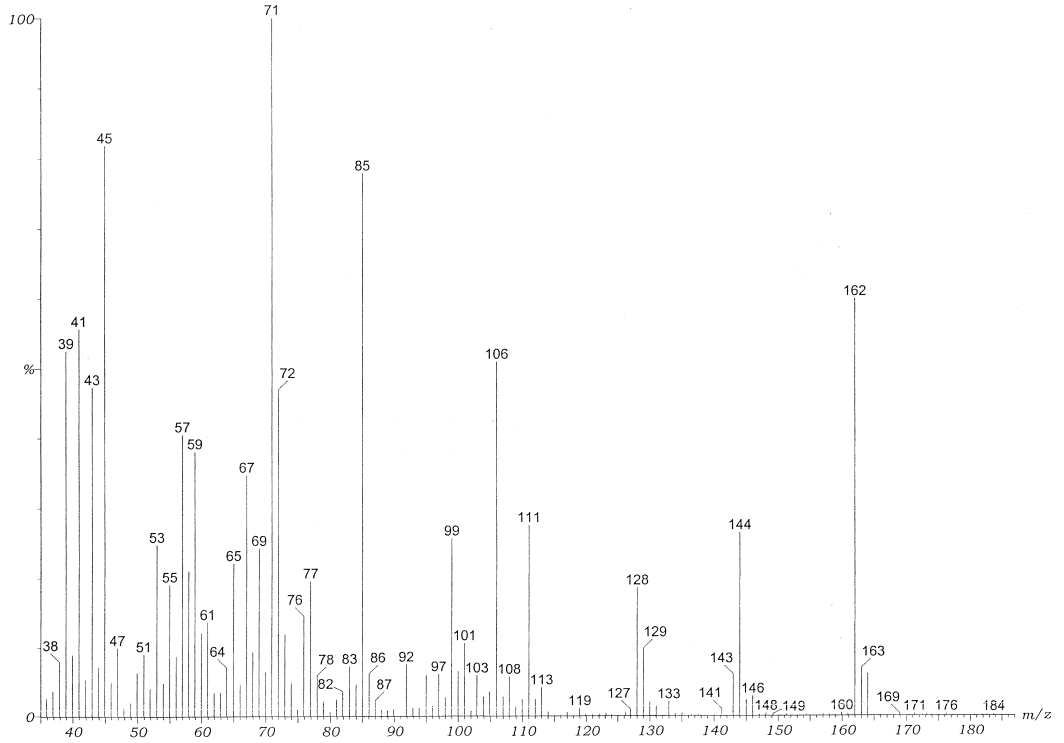

Fig. 5. Mass spectrum of zwiebelane isomer.

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