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Application of low-pressure gas chromatography–ion-trap mass spectrometry to the analysis of the essential oil of *Turnera diffusa* (Ward.) Urb.

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Abstract

Turnera diffusa Willd. var. *afrodisiaca* (Ward) Urb. (syn. *T. aphrodisiaca*) belongs to the family of Turneraceae and is an aromatic plant growing wild in the subtropical regions of America and Africa. It is widely used in the traditional medicine as e.g. anti-cough, diuretic, and aphrodisiac agent. This work presents a 3 min chromatographic analysis using low-pressure (LP) gas chromatography (GC)–ion-trap (IT) mass spectrometry (MS). The combination of a deactivated 0.6 m × 0.10 mm i.d., restrictor with a wide-bore CP-Wax 52 capillary column (10 m × 0.53 mm i.d., 1 μm) reduces the analysis time by a factor of 3–7 in comparison to the use of a conventional narrow bore column. Chromatographic conditions have been optimized to achieve the fastest separation with the highest signal/noise ratio in MS detection. These results allow fast and reliable quality control of the essential oil to be achieved.

Keywords: *Turnera diffusa*; Essential oils; Gas chromatography, fast; Quality control; Thymol; Cineole

1. Introduction

Many species of the genus *Turnera* (Turneraceae) are widely used in the traditional medicine as, e.g. anti-cough, diuretic and aphrodisiac agent [1]. Nowadays, phytopharmaceutical formulations containing plants of this genus are commercialized not only in Brazil but also in Europe. The species most commonly used is *Turnera diffusa* Willd. var. *afrodisiaca* (Ward.) Urb. (syn. *T. aphrodisiaca*), because of its alleged aphrodisiac properties. *T. diffusa* is an aromatic plant popularly known as "damiana" that grows wild in the subtropical regions of America and Africa. Its aphrodisiac activity is related to the irritating properties on the urinary tract caused by the substances of the essential oil [2].

The essential oil of *T. diffusa* can be easily obtained from steam distillation of the fresh or dried leaves. The analysis of this oil is of great interest because of the utilization of *T. diffusa* in many phytopharmaceutical formulations. Since the pharmacological properties of the essential oils are closely related to their chemical composition, it is important to develop methods that can warrant the quality of these types of materials.

Recently, Bicchi et al. [3] have investigated the composition of the essential oil of *T. diffusa* by gas chromatography (GC)–mass spectrometry (MS) and have found 1,8-cineole to be one of the main constituents (11.4%). About 50 components were identified using a 40 min conventional GC–MS chromatographic analysis. Camargo [4] has shown that 1,8-cineole and thymol can be used as chemical markers for quality control of the essential oils. Though these two monoterpenes elute quickly from the GC-system, the high retention of the other essential oil components hampers the application of GC–MS. Therefore, fast and more efficient methods of analysis are required to perform the quality control of complex mixtures containing *T. diffusa* as well as other natural products. Successful alternative techniques for the fast analytical and/or preparative separation as well as for identification of natural products have already been reported [5–7]. However,

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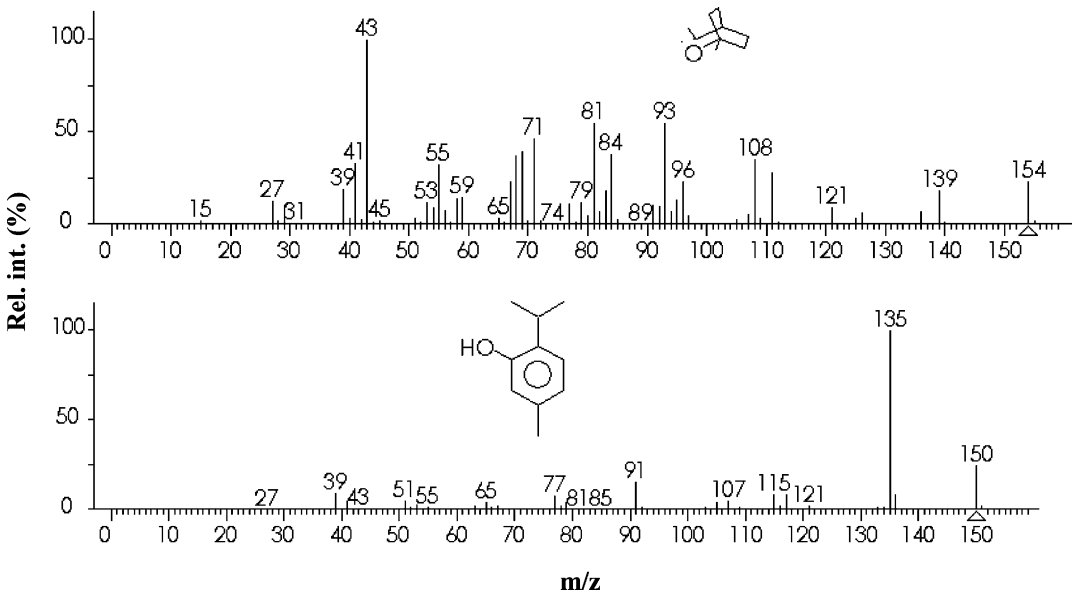

Fig. 1. Positive ion mass spectra of 1,8-cineole (top) and thymol (bottom).

these separations involved compounds of medium or high polarity.

Reduction in analysis time has been the subject of many scientific publications in the last decade. Shorter and narrower columns (0.15 mm i.d. or less) have acquired growing popularity [8–14]. However, the decreased peak widths make the use of a fast scanning MS, such as time-of-flight, mandatory to get a sufficient number of data points for each signal. A significant gain in speed can also be obtained with a column operating at vacuum-outlet conditions [15–20]. De Zeeuw et al. [21] demonstrated that the use of short wide-bore capillary columns (i.e. 10 m × 0.53 mm i.d.) operating under the reduced pressure of the MS allowed higher gas velocities to be applied, resulting in analysis up to 10 times shorter. In spite of the reduced analysis time, wide-bore columns produce peaks of sufficient width to be adequately detected by a conventional MS analyzer, with the only expense of a small loss in the number of theoretical plates.

In this work we report the use of such approach, here called as low-pressure (LP) GC–ion-trap (IT) MS and previously optimized for volatile alkyl benzenes and polycyclic aromatic hydrocarbons (unpublished research) [22], for the analysis of the essential oil of *T. diffusa*. In particular, attention has been focused to the target compounds thymol and 1,8-cineole (see structures in Fig. 1) to be used as quality control markers.

2. Materials and methods

2.1. Plant material

Aerial parts of *T. diffusa* Willd. var. *afrodisiaca* (Ward.) Urb. were collected at Quintana Roo (Mexico) in July 2002 and authenticated by Dr. Cástulo Chan. A voucher specimen was kept at Centro de Investigación Científica de Yucatan (C. Chan, No. 3773—CICY). The dried aerial parts (500 g) were submitted to hydrodistillation at July 2002 using Clevenger apparatus for 4 h. Subsequently, the oil (approximately 1 ml) was extracted from the water layer with diethyl ether. The diethyl ether was evaporated overnight and the oil was stored at −18 °C. The oil was dissolved in n-hexane and the extract was analysed by LP-GC–IT–MS.

2.2. LP-GC-IT–MS analyses

For the chromatographic experiments, a Varian Saturn 2000 ion trap MS system was used in combination with

Table 1
Chromatographic and mass spectrometric parameters studied for optimization of *T. diffusa* essential oil analysis using LP-GC–IT–MS recorded on a CP-Wax 52 CB column

Parameter	Studied range	Optimized value
Rate (°C min^{-1})	5–80	60[a]
Injector temperature (°C)	220–270	270
He flow rate (ml min^{-1})	0.8–1.5	1.5
Transfer line temperature (°C)	200–250	220
Trap temperature (°C)	200–230	200
Scan time (scan s^{-1})	2–5	3.7
Multiplier offset (V)	−100–(+100)	+100
Emission current (μA)	5–100	50
Target total ion current (TIC; counts)	5000–25000	20000
Maximum ionization time (μs)	10000–50000	40000
Prescan ionization time (μs)	100–1000	500

[a] Column oven temperature programmed from 80 to 230 °C at a rate of 60 °C min^{-1}. The final temperature was kept for 0.87 min to complete elution.

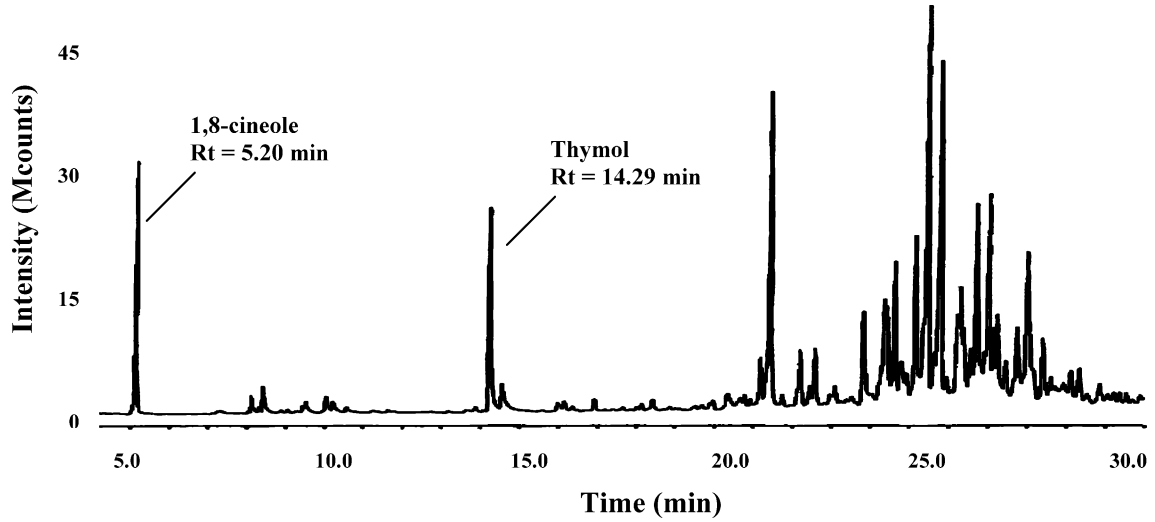

Fig. 2. Conventional GC–MS chromatogram of *T. diffusa* extract using a LM-5 column (5% phenyl −95% methylpolysiloxane, 15 m × 0.2 mm; $d_f = 0.2\,\mu m$) [4]. R_t: retention time.

a Varian 3800 gas chromatograph (Walnut Creek, CA, USA). The GC system had a universal injector (Varian 1079), used in the split mode. Samples were introduced using an 8200 Varian autosampler. A polyethylene glycol column CP Wax 52 (10 m × 0.53 mm i.d.; $d_f = 1\,\mu m$, Varian-Chrompack, Middelburg, The Netherlands) was coupled to a non-coated restriction column of 60 cm × 100 μm i.d. (Varian-Chrompack) by a single ferrule column connector and a vespel ferrule 0.53–0.25 mm i.d. and used for separation. Helium (Air Liquide, Liège, Belgium) was used as carrier gas.

The different gas chromatographic and mass spectral parameters were varied as described in Table 1 to achieve fast analysis together with reliable separation.

3. Results and discussion

Current quality control of the essential oil of *T. diffusa* by conventional GC [3,4] implies a total time of 40 min in order to elute all compounds. The temperature programming was thus compulsorily low enough to achieve complete separation of the several compounds. To avoid the time-consuming chromatographic step in quality assessment routines, low-pressure GC becomes attractive to shorten the analysis time and increase the capacity. The comparison of Figs. 2 and 3 shows indeed that chromatograms of similar quality could be obtained by LP-GC in only a fraction of the time required for conventional GC.

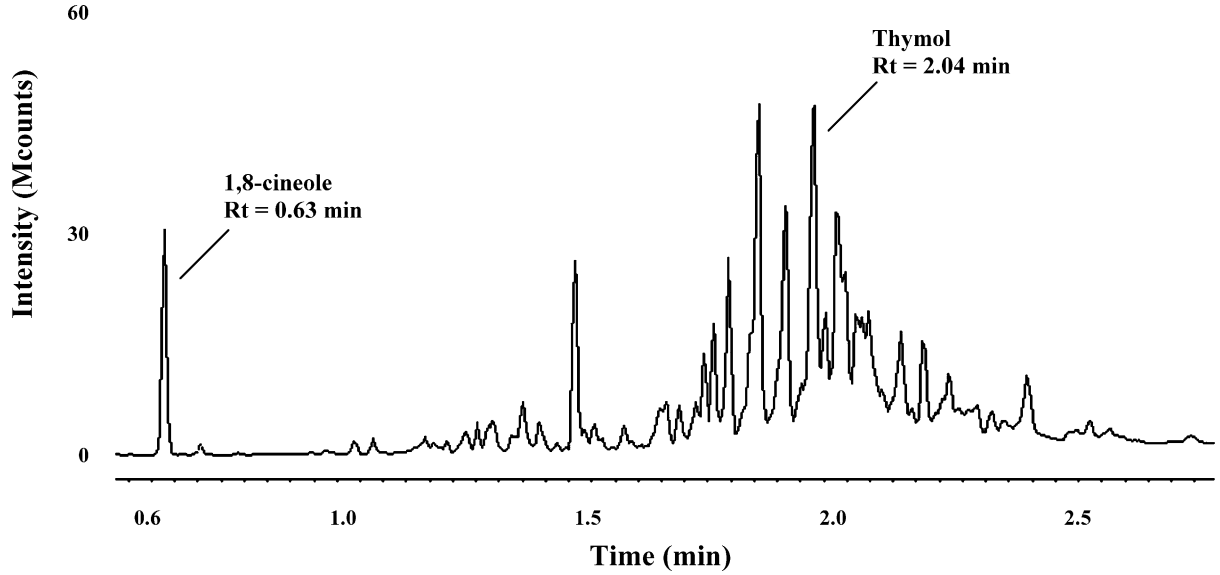

Fig. 3. LP-GC–IT–MS chromatogram of *T. diffusa* extract using a CP Wax 52 column (10 m × 0.53 mm, 10 m; $d_f = 1\,\mu m$).

The best separation was achieved with the CP Wax 52 column using a very fast program rate of 60 °C min^{-1}, between 80 and 230 °C. The complex mixture of compounds could be separated within only 3 min. Whereas conventional GC offered the advantage of 1,8-cineole and thymol eluting long before the other oil constituents, thymol became now part of the complex pattern at about 2 min in LP-GC. Nevertheless, its identification was still possible due to mass spectra detection.

Use of the fast temperature-programming rate made the peaks higher and sharper, while elution temperatures were lower. This resulted from the high carrier gas velocity provided by the vacuum condition. Furthermore, it was possible to start the chromatographic program at 80 °C, making the total time much shorter. As an additional parameter, the repeatability of the technique in the analysis of cineol and thymol was evaluated using a series of repeated injections. Relative standard deviations were around 7.1% for 1,8-cineole and 5.1% for thymol.

Concerning the mass spectral parameters, the most critical one was the scan time, which had to be adjusted to 3.7 scans s^{-1} in order to provide enough points for a good peak definition. The target compounds were identified by GC retention times, comparison with authentic standards, and from their recorded mass spectra by comparison with the US National Institute of Standards and Technology (NIST) library (100 000 compounds). The mass spectra produced with the Rapid-MS column agreed well with those previously reported using conventional GC–MS [3], in that fit-factors were similar. Despite the high number of components, our approach led to the similar identification capabilities with the additional advantages of lower time- and gas-consumption. These are decisive parameters to take into account for analysis not only at industrial scale, but also in the research laboratory.

4. Conclusions

The potential use of LP-GC–IT–MS for the rapid and reliable quality control of essential oil components of *T. diffusa* has been demonstrated. In comparison to the currently applied method using conventional capillary GC, rapid GC triggers particular interest by the decrease of the retention time by a factor of about 7 for cineol (0.626 min) and for thymol (2.04 min). This short period required for this analysis makes GC–IT–MS the method of choice for quality control of *T. diffusa* essential oil. In addition, the identification capabilities for the other components of the essential oil remain at the same level as in conventional GC.

The applicability of GC–IT–MS will be checked in a near future for the analysis of other natural products from plant origin. Specifically, the reduced decomposition of substances by the lower temperature conditions that can be used in LP-GC–IT–MS relative to conventional GC must be investigated.

Acknowledgements

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