# Analysis of Macacaporanga (*Aniba parviflora*) Leaf Essential Oil by Using Comprehensive Two-dimensional Gas Chromatography Combined with Rapid-Scanning Quadrupole Mass Spectrometry

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**The present research is focused on the use of a comprehensive 2D GC methodology, in the analysis of a Brazilian essential oil, *viz.*, Macacaporanga leaf oil. A rapid scanning quadrupole mass spectrometer (qMS), employed as detection system and operated at a 20 Hz scanning frequency, supplied high quality mass spectra. The effectiveness of the three-dimensional GC x GC-qMS experiment was compared to that of GC-qMS analysis on the same sample. Peak identification, in both applications, was achieved through MS library matching with the support of linear retention index (LRI) data.**

Keywords: comprehensive two-dimensional gas chromatography; GC x GC; GC x GC-MS; rapid-scanning quadrupole mass spectrometry; LRI; Macacaporanga leaf essential oil.

## Introduction

Single column gas chromatography in combination with a flame ionization detector (FID) and/or a mass spectrometer is commonly employed in the elucidation of essential oil profiles. The latter are to be considered medium to highly complex matrices and, hence, can only be partially resolved even using a conventional column. Inevitably, several essential oil peaks are the result of two or more overlapping compounds, often complicating or hindering reliable identification and quantitation. Consequently, the employment of more powerful analytical techniques in this specific research field is desirable.

A great increase in separation power, with respect to 1D GC, is the main characteristic of comprehensive 2D GC (GC x GC). The latter is a multidimensional technique, introduced at the beginning of the 90's [1]. GC x GC applications are generally achieved on two capillaries connected in series, with a transfer device (the modulator) located at the head of the second column. The modulator, which operates continuously throughout each experiment, isolates, re-concentrates and then re-injects sequential heart-cuts of the first dimension effluent, onto the secondary column (commonly a micro-bore column segment). A GC x GC chromatogram is nothing more than a high number of fast sequential second-dimension chromatograms with a duration normally between three and eight seconds (defined as the modulation period). By using dedicated programs, GC x GC data are visualized in a 2D format: each compound is represented as an oval-shaped peak, with a dimension and colour related to its specific concentration. It is obvious that there is a considerable amount of separation space in a twin-axes chromatogram. The enhanced resolving power of GC x GC, as well as the greater sensitivity (due to modulator re-concentration) are ideal characteristics for essential oil analysis.

Modulation compression produces very rapid 2D peaks (normally in the 50-100 ms range), altogether comparable to those observed in

very fast GC [2]. Detection systems, as a consequence, must be characterized by small internal volumes and high acquisition frequencies for correct peak reconstruction. FID systems present both of these features and are the most commonly used.

The combination of a mass spectrometer to a comprehensive GC system generates a third separation dimension. The use of two types of MS instrumentation is usually reported, viz., time-of-flight (ToF MS) and quadrupole mass spectrometers. The former systems can easily achieve the required spectra acquisition rates (50-100 Hz) for reliable 2D peak identification and quantitation [3]. However, the rather high cost of such instrumentation is the main reason behind its limited laboratory use. The quadrupole mass spectrometer, on the contrary, is much cheaper and is the most commonly employed instrument in hyphenated GC [4]. Additionally, ultimate generation rapid-scanning qMS instruments present 10,000 amu/s full-scan capacity. Although such qMS systems cannot reach the ToF MS performance, precious qualitative information can be obtained in GC x GC-MS experiments. Moreover, high quality mass spectra are generated in which scan-to-scan mass bias (peak skewing) is negligible. Comprehensive two-dimensional gas chromatography in combination with mass spectrometry has been the subject of a recent review [5].

The present investigation is focused on the 3D GC x GC-qMS analysis of Macacaporanga leaf essential oil volatiles. *Aniba parviflora*, commonly known as Macacaporanga, is from a botanical viewpoint, quite similar to *Aniba rosaeodora*. Rosewood essential oil, a highly valuable product, is extracted from the latter species. *A. parviflora* oil is obtained from its leaves, is characterized by a strong, pleasant and persistent aroma, and could potentially be a product of value. However, the literature related to the chemical composition of *A. parviflora* oil is rather scarce.

## Experimental

### Sample and sample preparation

The Brazilian hydrodistilled Macacaporanga *(Aniba parviflora)* leaf essential oil was stored at +4°C and diluted 1:10 in *n*-hexane prior to GC analysis. A $C_7$-$C_{30}$ linear alkane series was provided by Supelco (Milan, Italy).

### GC-MS analysis

GC-MS analyses were carried out on a Shimadzu GCMS QP2010 gas chromatograph-quadrupole mass spectrometer, equipped with an AOC20i autoinjector and split/splitless injector (300°C) (Shimadzu, Milan, Italy). Column: SLB-5ms [silphenylene polymer virtually equivalent in polarity to poly(5% diphenyl/95% methyl siloxane)] 30 m x 0.25 mm ID, 0.25 µm film thickness (Supelco, Bellefonte, PA, USA); constant helium head pressure: 37.1 kPa; initial linear velocity: 31.6 cm/sec; temperature program: from 50°C to 300°C (5 min) at 3°C/min; injection volume and mode: 1 µL, split (100:1); MS conditions: acquisition mode: scan; scan speed: 1666 amu/s; mass range: 40-400 *m/z*. Data were collected by the GCMS® solution software (Shimadzu).

### GC x GC-MS analysis

Comprehensive two-dimensional GC applications were carried out on a Shimadzu GC x GC-MS system consisting of two independent GC 2010 gas chromatographs and a QP2010 Plus quadrupole mass spectrometer. The two GC ovens were linked through a heated (300°C) transfer line (Shimadzu). The primary GC was equipped with an AOC-20i auto-injector, a split-splitless injector (300°C), a cable extension for the MS connection (due to the presence of the second GC oven) and a programmed temperature vaporizer injector. The final part of the primary column was passed through the heated transfer line and connected to the secondary column by using an SGE SilTite mini-union (Ringwood, Victoria, Australia). The first column was an Equity-1 30 m x 0.25 mm ID, 0.25 µm film thickness one, while the secondary column was an Omegawax [100% poly(ethylene glycol)], 1.5 m x 0.1 mm ID, 0.1 µm film thickness (Supelco) one. Both capillaries were temperature-programmed as follows: 50°C to 300°C (5 min) at 3°C/min; injection volume and mode: 0.2 µL; split: 100:1. Carrier gas: Helium was delivered at an initial pressure of 319.2 kPa (constant linear velocity). Cryogenic modulation was carried out in the second GC and was applied every 6 s by using a dual-stage loop-type modulator. MS parameters: the sample was analyzed in the full scan mode with a scan speed of 10000 amu/sec, a mass range of 40-400 *m/z* and a sampling frequency of 20 spectra/s; interface and ion source temperatures were 280 and 250°C, respectively. MS ionization mode: electron ionization; detector voltage: 1.0 kV. Data were collected by the GCMS® solution software and directly opened by using the Comprehensive Chromatography Manager v. 1.0 software (Chromaleont, Messina, Italy).

In GC-MS and GC x GC-MS applications, mass spectral identification was carried out by using the Shimadzu FFNSC 1.2 library, as well as the Adams library (4th edition).

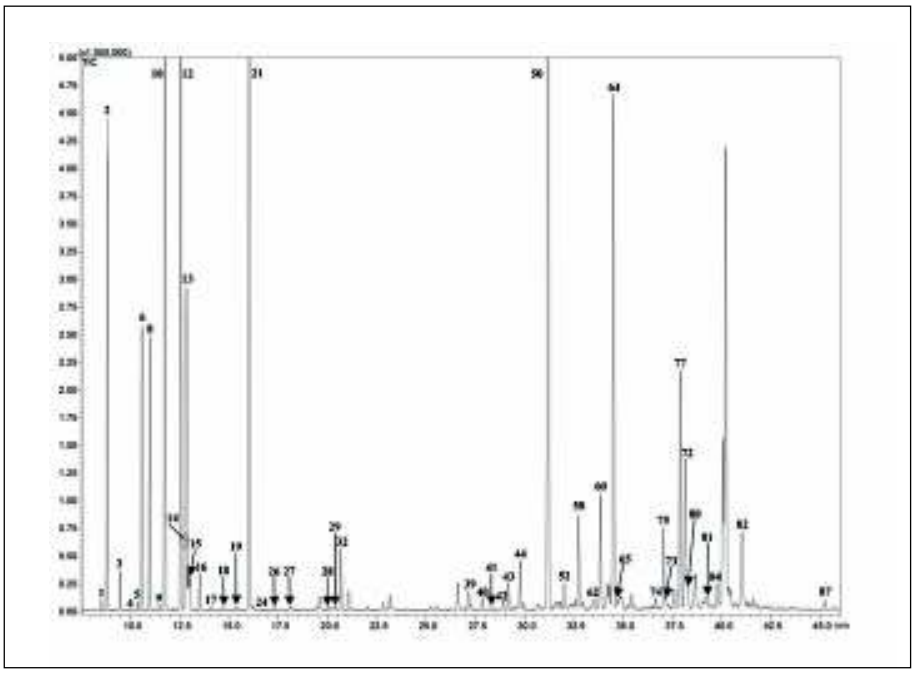

*Figure 1: TIC GC-qMS chromatogram of Macacaporanga leaf essential oil (refer to Table I for peak identification).*

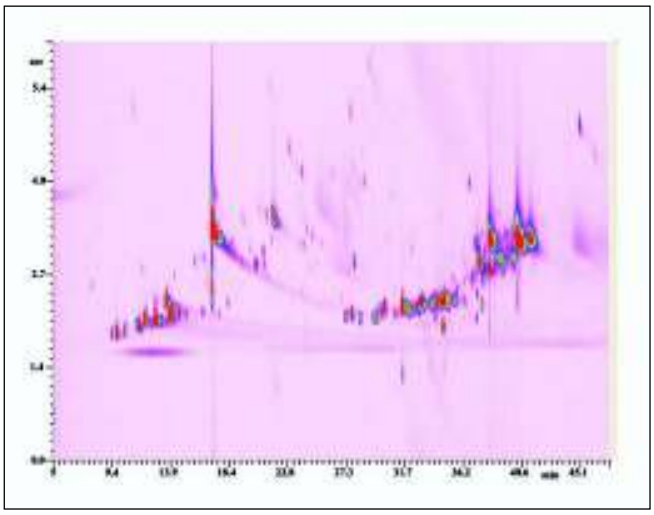

*Figure 2: TIC GC x GC-qMS chromatogram of Macacaporanga leaf essential oil (refer to Table I for peak identification).*

## Results and discussion

The Macacaporanga leaf essential oil was initially subjected to GC-qMS analysis, using an apolar column. The single axis total ion current (TIC) GC-qMS chromatogram relative to this application is reported in Figure 1. Peak assignment was carried out through mass spectra probability matching using a commercial library and the interactive use of LRI. The search procedure is as follows: the LRI relative to an unknown peak is calculated prior to assignment. During spectra library matching, the GC-MS software automatically deletes library hits with lower than 90% probability (filter 1) and with a LRI, in respect to the calculated unknown peak value, outside an acceptable retention index window (filter 2). The latter, selected by the analyst, is typically +/- 5 units for a single apolar column analysis. Altogether, 46 essential oil analytes were assigned (numbered peaks in Figure 1) with library similarities, in 41 cases (approx. 89%), within the 94-99% range (Table I). The GC-qMS result, both in terms of separation and mass spectra quality, can be considered as good.

A GC x GC-qMS application was carried out on the Brazilian essential oil; Helium linear velocities of 18.7 cm/s and 196.3 cm/s were calculated in the first and second dimension, respectively. A relatively "slow" analysis in the first dimension is necessary in order to attain a sufficient number of modulations per peak (at least three); a very fast high-resolution secondary separation is required in order to ultimate each second dimension analysis within the modulation period (in this case 6 s). The double axes TIC GC x GC-qMS chromatogram is illustrated in Figure 2: two factors are evident after a brief observation of the chromatogram, *viz.*, the number of resolved peaks is higher than that observed in the single column application and the bidimensional peaks are spread over a substantial part of the space plane. This last aspect is due to the high degree of column-set orthogonality: 1D solutes are differentiated solely on their boiling-point values while specific interactions on the 2D polar stationary phase enable the separation of analytes characterized by the same or similar vapour pressures. Peak

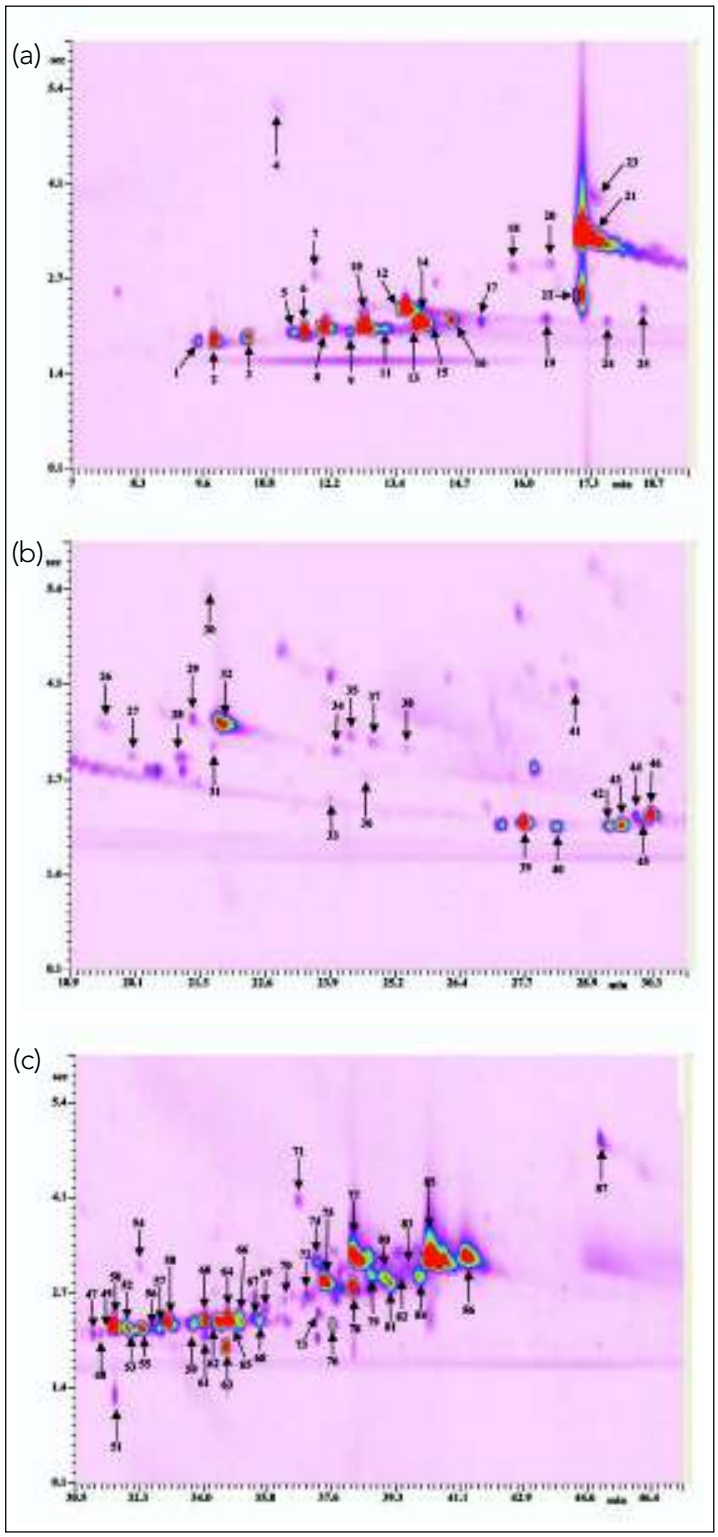


*Figure 3 a,b,c: TIC GC x GC-qMS chromatogram expansions derived from Figure 2.*

identification was carried out in the same manner as in the GC-qMS analysis. LRI were calculated through data derived from untransformed GC x GC chromatograms and was carried out as follows: the retention time of the central component was considered in the case of an odd number of modulated analyte peaks, while in the case of an even number of modulated peaks the central retention time between the two most internal peaks was considered. In this

case, linear retention indices were calculated for a 31.5 m column (30 m apolar plus 1.5 m polar); the values obtained were matched with those reported in the MS libraries employed. It must be added, that the influence of a 1.5 m polar capillary, in terms of retention, is negligible for apolar analytes (e.g. hydrocarbons), while the more polar components (e.g. oxygenated compounds), on the contrary, have more intense interactions and are strongly retained. Hence, a large retention index window of 50 units was applied, thus reducing the effectiveness of the LRI filter (see Table I). The qMS instrumentation, operated at a wide mass scan range (40-400 *m/z*), supplied a good performance in terms of data acquisition rate (20 Hz). The attainment of 3-4 high quality MS spectra, for the narrower GC x GC peaks, proved to be sufficient for peak assignment in many cases. The good mass spectral quality attained was related to the separation of pure components in the second dimension and to the great reduction of chemical background interference (stationary phase bleed is also focused and resolved from the sample compounds). Furthermore, the observed differences of mass spectra relative to different points across a given peak were negligible demonstrating a low qMS skewing effect. The 87 peaks identified, with a similarity of at least 90%, can be observed in the three chromatogram expansions illustrated in Figure 3a, b, and c. Library matches, in terms of similarity, were in 53 cases (approx. 61%) within the 94-99% range (Table I).

## Conclusions

The GC x GC-qMS method, developed in the present research, proved to be a very suitable alternative in this type of application, with a great improvement achieved in terms of separation and number of identified peaks. Although more than double the number of unknowns were identified, the authors are of the opinion that co-elutions still occur. In fact, a series of mass spectra were characterized by insufficient quality denoting the presence of interfering analytes. It must be observed, though, that this factor could be linked to the lack of the relative mass spectra in the commercial libraries. Future research will be dedicated to the application of the three-dimensional method to other complex essential oils and to the development of dedicated MS libraries.

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*Table I. Peak assignment, MS similarity values (%), and difference between experimental and theoretical LRI values (Δ LRI exp.-theor.) in the GC-MS and GC x GC-MS experiments.*

| Peak | Compound | GC x GC-MS% | Δ LRI exp.-theor. | GC-MS% | Δ LRI exp.-theor. |
|---|---|---|---|---|---|
| 1 | α-thujene | 97 | -5 | 99 | +2 |
| 2 | α-pinene | 99 | -4 | 98 | 0 |
| 3 | camphene | 98 | -2 | 98 | +3 |
| 4 | benzaldehyde | 96 | -17 | 97 | -5 |
| 5 | sabinene | 98 | -3 | 98 | 0 |
| 6 | β-pinene | 97 | -6 | 97 | 0 |
| 7 | 6-methyl 5-Hepten-2-one | 96 | -7 | | |
| 8 | myrcene | 98 | -4 | 97 | +3 |
| 9 | δ-2-carene | 96 | -4 | 97 | -1 |
| 10 | α-phellandrene | 98 | -7 | 97 | 0 |
| 11 | δ-3- carene | 94 | -6 | | |
| 12 | p-cymene | 98 | -13 | 98 | 0 |
| 13 | β-phellandrene | 93 | -6 | 96 | 0 |
| 14 | limonene | 96 | -10 | 95 | 0 |
| 15 | (Z)-β-ocimene | 93 | -7 | 95 | -3 |
| 16 | (E)-β-ocimene | 98 | -7 | 99 | +1 |
| 17 | γ-terpinene | 95 | -7 | 96 | -4 |
| 18 | (Z)-linalool oxide | 95 | -11 | 91 | -3 |
| 19 | terpinolene | 94 | -5 | 95 | 0 |
| 20 | (E)-linalool oxide | 91 | -5 | | |
| 21 | linalool | 95 | -20 | 97 | 0 |
| 22 | perillen | 94 | 0 | | |
| 23 | hotrienol | 91 | -30 | | |
| 24 | 4,8-dimethylnona-1,3,7-triene | 95 | -2 | 94 | 0 |
| 25 | neo-allo-ocimene | 98 | -7 | | |
| 26 | (E)-p-menth-2-en-1-ol | 92 | -21 | 96 | -3 |
| 27 | pinocarvone | 95 | -5 | 94 | -3 |
| 28 | terpinen-4-ol | 93 | -10 | 93 | -2 |
| 29 | cryptone | 93 | -10 | 90 | -5 |
| 30 | p-cymen-8-ol | 94 | -25 | | |
| 31 | myrtenal | 96 | -9 | | |
| 32 | α-terpineol | 94 | -10 | 96 | -2 |
| 33 | linalyl acetate | 96 | +1 | | |
| 34 | carvotanacetone | 92 | -9 | | |
| 35 | piperitone | 92 | +2 | | |
| 36 | (E)-2-decenal | 95 | -11 | | |
| 37 | ascaridol (iso) | 92 | -27 | | |
| 38 | phellandral | 91 | -12 | | |
| 39 | δ-elemene | 93 | -8 | 91 | 0 |
| 40 | α-cubebene | 95 | 0 | 98 | 0 |
| 41 | benzylidenacetone | 93 | -19 | 94 | -4 |
| 42 | α-ylangene | 95 | -7 | 96 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| 43 | α-copaene | 94 | -10 | 97 | -5 |
| 44 | β-elemene | 94 | -2 | 97 | -3 |
| 45 | β-bourbonene | 93 | -14 | | |
| 46 | longipinene | 92 | +3 | | |
| 47 | α-gurjunene | 92 | -12 | | |
| 48 | β-maaliene | 93 | -9 | | |
| 49 | isogermacrene D | 92 | -22 | | |
| 50 | caryophyllene E | 97 | -12 | 98 | 0 |
| 51 | β-caryophyllene | 98 | -23 | | |
| 52 | aromadendrene | 93 | +1 | 96 | -5 |
| 53 | β-funebrene | 93 | -30 | | |
| 54 | isopentyl-benzoate | 91 | -11 | | |
| 55 | β-sesquifenchene | 91 | -23 | | |
| 56 | cubeb-11-ene | 94 | -12 | | |
| 57 | (Z)-β-farnesene | 92 | -20 | | |
| 58 | humulene | 98 | -28 | 98 | -5 |
| 59 | 4,5-di-epi-aristolochene | 96 | -12 | | |
| 60 | germacrene D | 94 | -2 | 95 | +5 |
| 61 | γ-amorphene | 91 | +4 | | |
| 62 | α-muurolene | 95 | +3 | 92 | 0 |
| 63 | isolepidozene | 95 | -23 | | |
| 64 | bicyclogermacrene | 93 | -6 | 97 | -3 |
| 65 | (E,E) α-farnesene | 93 | -9 | 94 | -1 |
| 66 | β-bisabolene | 92 | -25 | | |
| 67 | ε-amorphene | 92 | -29 | | |
| 68 | δ-amorphene | 94 | -18 | | |
| 69 | 10-epi-italicene-ether | 91 | -22 | | |
| 70 | cadina-1,4-diene | 91 | +4 | | |
| 71 | elemicine | 96 | -1 | | |
| 72 | caryophyllene oxide | 96 | -10 | 98 | -1 |
| 73 | germacrene B | 94 | -12 | 97 | -5 |
| 74 | elemol | 97 | -29 | 95 | -4 |
| 75 | nerolidol (3E,7E)-4,8, 12-trimethyltrideca-1,3,7,11-tetraene | 98 | -15 | 98 | -1 |
| 76 | | 96 | -18 | | |
| 77 | spathulenol | 97 | -20 | 96 | -5 |
| 78 | caryophyllene oxide | 97 | -15 | | |
| 79 | ledol | 91 | -7 | | |
| 80 | viridiflorol | 93 | -27 | 95 | +3 |
| 81 | humulene epoxide 2 | 92 | -17 | 96 | -3 |
| 82 | β-eudesmol | 94 | -2 | | |
| 83 | intermedeol | 94 | -15 | | |
| 84 | 10-epi-γ-eudesmol | 92 | -23 | 96 | +3 |
| 85 | agarospirol | 94 | -13 | | |
| 86 | α-eudesmol | 94 | -26 | | |
| 87 | benzyl benzoate | 95 | -15 | 97 | 0 |