Characterization of some extracts for therapeutic use by GC/MS

Andreea Maria Iordache, Monica Culea and Onuc Cozar
Babes-Bolyai University, Faculty of Physics, 1 Mihail Kogalniceanu, 400084 Cluj-Napoca, Romania
E-mail: mculea@phys.ubbcluj.ro

Abstract. A liquid-liquid extraction method (LLE), compared with a solid-phase extraction method (SPE), was used for characterizing some plant extracts for therapeutic use. Precision of the extraction methods gave relative standard deviation lower than 3%. The extracts were analyzed by GC-FID and GC-MS. A HP-5 capillary column, 30 m x 0.32 mm, 0.25 µm film thickness was used. By using an appropriate mixture of solvents, good recovery mean values were obtained by LLE (81%) comparable with the recoveries of other extraction methods as SPE (92%). The study was applied to characterize the compounds of therapeutic value extracted from herb plants.

1. Introduction
In the last years the interest for the study of the organic compounds from plants and their activity has increased. A lot of extraction methods and analytical methods as spectrophotometry, high performance liquid chromatography, gas chromatography-mass spectrometry (GC/MS) were developed for plant active compounds study [1-7].

The aim of the present work is the characterization of the compounds extracted from some herb plants.

The methods were applied to characterize “Floratonic”, a “swedish” tincture, used internal or external, in traditional medicine in our country. It is prepared from some herbs growing in Romania as hydroalcoholic extract which contains 12 plant species: Aloe (folium), Angelica arhangelica (radix), Juniperus communis, Fraxinus excelsior (fructus) Gentiana lutea (radix), Iris palida (radix), Robinia pseudacacia (flos), Polygonum aviculare (herba), Arctium lappa (radix), Laurus nobilis (folium), Abies alba.

2. Experimental
2.1. Apparatus
A Hewlett Packard GC 5890 coupled with a MS engine 5989B in the EI mode was used for compounds identification. The GC was equipped with a HP-5MS capillary column 30 m x 0.25 mm diameter, 0.25 µm film thickness, in the temperature program: 50°C for 2 min, to 250°C with a rate of 8°C/min, then increased with 30°C/min to 310°C and kept 10 min. The GC/MS interface line and the ion source were maintained at 280°C and respective 200°C, and quadrupole analyser at 100°C. Electron energy was 70 eV and electron emission 300 µA.
2.2. **LLE extraction**

A mixture of three solvents, named solvent A, was prepared as follow: ethyl acetate: hexane: methylene chloride (5:1:1, v/v/v). The LLE extraction procedure was: 3 ml hydroalcoholic extract (or 10 µl mixture (stock solution) in 3 ml solution distilled water: ethanol (1:1, v/v)), 3 ml distilled water and 1 ml solvent A (3:3:1, v/v/v), 3 g NaCl were mixed 5 min and then centrifuged 2 min. For recovery study 1 µl methyl myristate was added to the supernatant and then 1 µl was injected two times by using the autosampler injector. For method validation, 20 ng/ml or 2 µg/g methyl myristate, the internal standard (IS), was added before extraction.

2.3. **SPE extraction**

The solid phase was conditioned with 3 ml methanol and 3 ml distilled water. After sample application, washing and drying 10 min at vacuum, the sample was eluted with 3 x 0.3 ml solvent A. After the addition of 1 µl external standard to the extract, 1 µl was injected by using the autosampler injector. Each sample was injected twice.

2.4. **Standard solutions**

A stock solution was obtained by diluting 100 µl of each compound.

2.5. **Method validation**

Linearity was studied in the range 0-24 ng. The regression curves obtained for the standards gave good correlation coefficients higher than 0.99 for each standard: 1) 3-hepten-2-one \( y = 0.03 x - 0.03, r = 0.994 \); 2) fenchone \( y = 0.04 x - 0.01, r = 0.998 \); 3) phenyl ethyl alcohol \( y = 0.05 x - 0.04, r = 0.995 \); 4) terpinen-4-ol \( y = 0.05 x - 0.002, r = 0.997 \); 5) citronellol \( y = 0.04 x - 0.03, r = 0.997 \); 6) carvone \( y = 0.05 x - 0.01, r = 0.997 \); 7) anisaldehyde \( y = 0.04 x - 0.01, r = 0.998 \).

Precision for LLE extraction gave relative standard deviation (RSD) lower than 3% (n = 4) and for SPE, RSD was lower than 3% (n = 5). Recovery for LLE was 81% (n = 3) and for SPE 92% (n = 4). Accuracy for 20 ng shows a mean value for RSD of 5% and for 24 ng of 6%. The sensitivity was lower than 100 pg at a ratio S/N = 10 and LOD was 10 pg, S/N = 10 for each standard.

3. **Results and discussion**

The separation chromatogram of the standards studied for the two extraction modes is presented in figure 1. Figure 2 shows the regression curves obtained for the standards studied.

![Figure 1. Mixture A separation chromatogram after extraction.](image-url)
Absolute recoveries were determined by using external calibration with the standard methyl myristate. The results of the recovery studies are presented in table 1. The mean values are resulted from four (LLE) and five (SPE) extraction procedures and two injections of each extract. The relative standard deviation values were below 6% for LLE and 3% for SPE extraction procedure. The methods were applied for qualitative and quantitative determination of the organic compounds from the bitter studied.

**Table 1.** Comparative recovery mean values between two extraction procedures.

| Compound                  | t\(_R\) (min) | LLE (%) | SPE (%) |
|---------------------------|---------------|---------|---------|
| 1. 3-hepten-2-one         | 8.33          | 76.03   | 85.19   |
| 2. fenchone               | 11.40         | 79.12   | 87.55   |
| 3. phenyl ethyl alcohol   | 12.00         | 76.13   | 92.85   |
| 4. terpinen-4-ol          | 12.80         | 80.97   | 90.76   |
| 5. citronellol            | 13.60         | 82.62   | 89.84   |
| 6. carvone                | 14.00         | 83.96   | 94.01   |
| 7. anisaldehyde           | 14.40         | 87.50   | 102.45  |
| 8. methyl myristate (ES)  | 21.00         | -       | -       |
| Mean values               | -             | 80.91   | 91.81   |

\( t_R = \) retention time

**Figure 2.** The regression curves of the studied standards.

Table 2 presents the compounds identified in the LLE extract from the bitter. Minor differences were observed between the two extraction methods in the analyzed standard mixture. The two extraction methods were applied for qualitative and quantitative determination of the major organic compounds in “Floratonic” bitter. Bitter shows sedative, antiseptic, anti-inflammatory, carminative, antirheumatic, antidepressive and tissue regenerating effects.

The high levels of lignan flavonoids lariciresinol and matairesinol (figure 3) in “Floratonic” bitter may contribute to the protective effect on coronary heart diseases and arteriosclerosis. The plant lignans, as well as their mammalian metabolites enterolactone and enterodiol, have antioxidative properties [5, 8].
| Compound                     | \( t_R \) | ng/ml |
|------------------------------|-----------|-------|
| beta-myrcene                 | 7.20      | 8.11  |
| ethyl caproate               | 7.40      | 9.70  |
| 1,8 cineole                  | 8.09      | 106.53|
| benzyl alcohol               | 8.28      | 2.32  |
| phenyl acetaldehyde         | 8.35      | 2.32  |
| fenchone                     | 9.22      | 8.35  |
| linalool                     | 9.40      | 1.24  |
| phenyl ethyl alcohol         | 9.80      | 1.59  |
| isopinocarveol               | 10.20     | 9.57  |
| camphor                      | 10.30     | 9.26  |
| pinocarvone                  | 10.60     | 1.59  |
| p-ethyl phenol               | 10.77     | 1.59  |
| 4-terpineol                  | 11.03     | 20.04 |
| p-cymen-8-ol                 | 11.07     | 0.83  |
| alpha-terpineol              | 11.15     | 36.96 |
| estragol                     | 11.24     | 0.83  |
| verbenone                    | 11.53     | 0.83  |
| 2,3-dihydrobenzofuran        | 11.74     | 22.57 |
| Z-citral                     | 12.10     | 0.83  |
| carvone                      | 12.20     | 0.99  |
| p-anisaldehyde               | 12.34     | 0.74  |
| ethyl salicylate             | 12.57     | 0.74  |
| dimethylhexylnedio           | 12.60     | 0.74  |
| p-ethyl guaiacol             | 12.97     | 0.74  |
| trans-anethole               | 12.80     | 16.45 |
| 4-vinyl 2-methoxyphenol      | 13.33     | 0.74  |
| alpha-terpinyl acetate       | 13.88     | 4.30  |
| eugenol                      | 14.05     | 0.74  |
| M=164                        | 14.50     | 0.74  |
| methyl eugenol               | 14.78     | 0.74  |
| vanillin                     | 14.80     | 0.74  |
| M=152                        | 15.80     | 0.74  |
| M=152                        | 16.35     | 2.71  |
| ethyl p-hydroxybenzoate M=166| 16.77     | 5.98  |
| p-hydroxybenzoic acid +propyl p-hydroxybenzoate M=180 | 16.80 | 9.43 |
| ethyl vanillate M=196        | 17.20     | 3.41  |
| alpha-m-cadinol M=222        | 18.60     | 0.74  |
| methyl myristate             | 19.50     | 24.75 |
| ethyl m-hydroxycinnamate(Z) M=192 | 19.53 | 8.94 |
| M=180                        | 19.55     | 9.73  |
| erythrocentaurine M=176      | 19.80     | 9.83  |
| 2-acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane M=238 | 20.30 | 8.35 |
| furocoumarin M=186           | 20.40     | 9.53  |
| M=198                        | 21.00     | 78.50 |
| ethyl m-hydroxycinnamate(E) M=192 | 21.40 | 5.98 |
| M=220                        | 22.00     | 3.41  |
| palmitic acid M=256          | 22.50     | 3.01  |
| dehydrocostuslactone M=230   | 23.05     | 18.62 |
| vanillosmin M=230            | 23.23     | 14.08 |
| (12R,13R)-8,12-epoxy-14-labden M=306 | 23.38 | 4.99 |
| osthol M=244                 | 24.68     | 9.83  |
| M=278                        | 24.80     | 18.52 |
| M=272                        | 25.20     | 7.06  |
### 4. Conclusions

The methods presented are suitable for the determination of trace amounts of organic compounds in hydroalcoholic plant extract. Good recovery mean values were obtained by LLE (81%) using an appropriate mixture of solvents comparable with the recoveries of the other extraction method, SPE (92%). Precision gave RSD lower than 3%. Each of the extraction methods is simple, rapid, accurate and inexpensive with reduced solvent and time consuming. The methods presented are suitable for medicinal herb organic compounds determination. No major differences were observed between LLE and SPE in the plant extracts studied. Terpenic compounds, polyunsaturated fatty acids and flavonoids are some of the compounds responsible for anti-inflammatory, antioxidant, anticarcinogenic activity of plants used in traditional medicine.

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