## B049 Quantitative analysis of hyoscyamine in *Hyoscyamus reticulatus* L. by GC-MS

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*Hyoscyamus reticulatus* is used as a allucinogenic drug in East part of Turkey. Hyoscyamine content of leaves and root samples of *H. reticulatus* L. from Bulanık-Mus were investigated by capillary GC-MS. Gas chromatography-mass spectrometry was carried out on a Varian-Chrompack 3800 gas chromatograph coupled to a Saturn 2000 mass detector. Mass spectrometer with ion trap detector in full scan (80-325 amu) under electron impact ionization (70 eV) was used. The chromatographic column for the analysis was Chrompack WCOT-Fused Silica CP-Sil 5CB capillary column (30 m x 0.25 mm i.d, film thickness 0.25 µm). The carrier gas used was helium at a flow rate of 1 ml/min. Dried and powdered roots and leaves of *H. reticulatus* samples were extracted with methanol in a Soxhlet apparatus for 2 hours. The methanol was evaporated in vacuo at 50 ºC and the crude alkaloid fractions were obtained using the alkaloid extraction procedure (1). 1 µl crude alkaloid fractions were injected and analysed with the column held initially at 125 ºC for 1 min and then increased to 250 ºC with a 10 ºC/min heating ramp and then kept at 250 ºC for 5 min. The injection was performed in splitless mode at 280 ºC. Hyoscyamine, the predominant compound, reached 0.036 ± 0.004 % in the leaves and 0.056 ± 0.011 % in the root. These findings are in accordance with the reports on hyoscyamine content in other *Hyoscyamus* species (2,3). The limit of detection was calculated to be 3.125 µg/mL and the limit of quantification was calculated to be 6.25 µg/mL for hyoscyamine. The method has been shown to be linear and sensitive.

**References: 1.** Kartal, M et al. (2001) Turk J. Med. Sci. 31: 487-492. **2.** Robbers J.E. et al. (1996) Pharmacognosy and Pharmacobiotechnology, William & Wilkins, Baltimore. **3.** Evans, W.C. (1989) Trease and Evans' Pharmacognosy, ELBS, London.

## B050 *Physalis angulata* L. – MPLC as an improved technique to obtain physalins

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The ergostane derivatives, physalins, with twenty eight carbon atoms are produce by several species of *Physalis* genus. These compounds possess a 13,14–seco–16,24-cycloergostane skeleton with a carbonyl group at C–15. Physalins have shown to be active against neoplasic tumors, inflammatory and tropical endemic diseases, as well as, in some immunological disorders (1). The main aim of this work is to develop a new methodology for optimizing the yield in the obtention of physalins. From the stems ethanolic extract a "pool" of those derivatives were obtained according to Mabry's modified technique (2). The "pool" was separated by a single medium performance liquid chromatography MPLC, (Büchi apparatus) using Lichroprep Si 60 column (50 cm height x 2.5 cm diameter), as stationary phase and cyclohexane–chloroform gradient system, as eluting phase (flux 20 mL/min). The pure substances percentages are pointed the out in Table 1, side by side, with the results of open column (CC) having Si gel 60 (30.0 cm height x 2.0 cm internal diameter), as stationary phase. Physalins were detected in hexane–chloroform (3:7) solvent system. That methodology allows obtention of four pure compounds in an overall yield ranging from 16% to 20%, a much better result than those described, so far, in the literature (3).

| Physalins | Yield % CC | Yield % MPLC |
|---|---|---|
| B | 5,0 | 5,5 |
| F | - | 8,2 |
| G | 3,5 | 4,6 |
| D | 4,3 | 8,0 |

**Table 1:** Yields of physalins.

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**References: 1.** Purushothaman KK et al. (1988) J. Scient. Ind. Res. 47: 326. **2.** Tomassini TCB et al. (1999) USA Patent 09/417.779 **3.** Kawai M et al. (1996), Phytochemistry 47, (3): 661.