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Determination of nepetalactone in *Nepeta cataria* by reversed phase high performance liquid chromatography

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Catnip, *Nepeta cataria* L. (Lamiaceae) is well known for its stimulating effect on cats, the so called "catnip response" [1]. Iridoids in the essential oil of this up to 1 m high, white flowering perennial are responsible for this behavior. The plant is native to southeastern Europe and North America (with commercial cultivations in North Carolina) and has a substantial folkloric reputation as a mildly sedative and antispasmodic herb, pharmacological effects which are ascribed to 4aα,7α,7aα-nepetalactone (**1**, see Fig. 1), the main iridoid in the essential oil [2–4]. Compound **1** is characteristic for the species and is therefore used as a marker compound.

Like other volatile compounds, **1** is usually determined by GC after steam distillation of the fresh flowering tops [4–6]. Not only is this procedure time consuming but it also requires a rather large amount of sample, if commercial products (usually the dried, aerial parts) are to be analyzed. HPLC seems to be a suitable alternative, as **1** shows a sufficient UV absorption and polarity to be analyzed by this technique.

After the isolation and identification of **1**, optimum HPLC conditions were determined (Fig. 1). The best results were obtained with a Synergi MAX-RP 80Å column from Phenomenex, a mobile phase consisting of water and acetonitrile and the detection wavelength set to 225 nm, the UV maximum of **1**. This setup enables the baseline separation of **1** from compounds of similar polarity within 12 min, without the necessity to use buffers, acids or modifiers. By performing the analysis at 40 °C the required separation time could be significantly reduced without a decrease of the peak symmetry. Each run was followed by a 5 min washing procedure with acetonitrile/methyl t-butyl ether = 3 : 1, ensuring the run-to-run reproducibility.

The analysis of a volatile compound like **1** by HPLC was only possible with an internal standard. Due to the low content of **1** in the products, 2.0 g of plant material had to

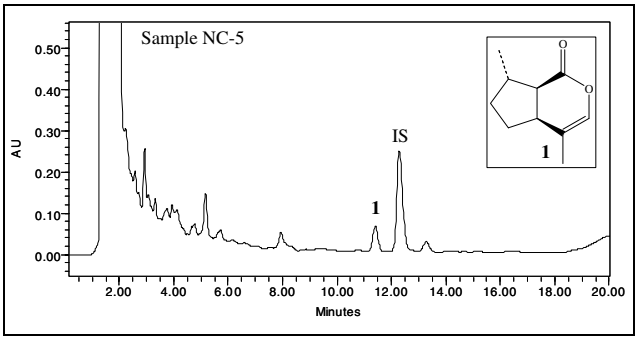

Fig. 1: Separation of a methanolic *N. cataria* extract (sample NC-5) under optimized conditions. Assignment of peaks: nepetalactone (**1**), geraniol (IS, internal standard)

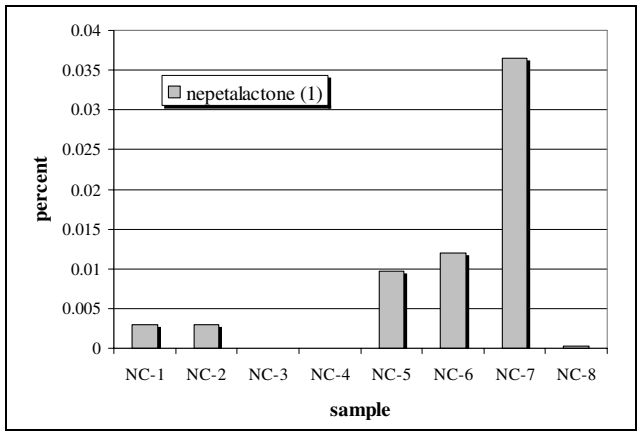

Fig. 2: Percentage (g/100g) of **1** in different samples (NC-1 to NC-5 are commercial products, others specific *N. cataria* plant parts)

be extracted in order to obtain reliable results. Methanol, compared to hexane, dichloromethane or ethyl acetate, was found to be the most effective solvent (94.9% of the total **1** were in solution after a 3-fold extraction of sample NC-5). The extract was dried under vacuum and re-dissolved in methanol; thus, an internal standard had to have very similar physical properties (polarity, boiling point) as **1**. Geraniol fulfills these requirements and is not present in *N. cataria*; therefore it was used as internal standard. The essential oil of one catnip variety (*N. cataria* var. citriodora, dragon grass catnip) contains large amounts of geraniol but no **1** [7, 8]. If the presence of this variety, which essential oil (and smell) is totally different to *N. cataria* is suspected, the analysis has to be repeated without the use of the internal standard. This procedure will show if geraniol is natively present or not.

Several analytical parameters were determined to validate the new HPLC method, and all requirements could be fulfilled. The method is precise (maximum standard deviations of 1.67% were observed while analyzing the samples) and accurate (by the use of an internal standard a recovery rate of 100.7% was obtained). Within a range of 1000.0 to 4.1 μg/ml the detector response for **1** was linear, with a limit of detection at 0.5 μg/ml. Finally, peak purity was confirmed by analyzing the PDA data of each peak of interest.

By analyzing different catnip samples the practical use of our method could be demonstrated. In conformity with literature, flowering stems (NC-7) showed the highest percentage of **1** (0.036%); leaves (NC-6) and especially stems (NC-8) contained much less. In liquid commercial products (NC-3 and NC-4) no nepetalactone was found, while the percentages in products consisting of powdered plant material were similar or lower than those of the leaves.The results of this study show that reversed phase HPLC can be readily used for the analysis of essential oil compounds. By doing so, sample preparation is easier and feasible with a much smaller amount of specimen compared to GC; the only essential requirement is a suitable internal standard.

Experimental

1. Isolation of 4aα,7α,7aα-nepetalactone (1)

Fresh, aerial parts of *N. cataria* (3 kg) grown in the greenhouse of the NCNPR, University of Mississippi, were subjected to steam-distillation, yielding 2.41 g of essential oil. The oil was separated by column chroma-

tography (silica gel with hexane/ethyl acetate as eluent), to afford 0.35 g pure **1**. Identity of the compound was verified by TLC, HPLC and comparison of NMR-data with those in the literature [9].

2. High performance liquid chromatography

Commercial samples (NC-1 to NC-5) were bought from Nutrimart (Diamond Bar, CA, USA), additional plant material (NC-6 to NC-8) was grown in the greenhouse. Voucher specimens of all samples are deposited at the NCNPR. Solvents and reagents were of HPLC grade and purchased from Fisher (Fair Lawn, NJ, USA) and Sigma (St. Louis, MO, USA). All samples were analyzed in triplicate (max. standard deviation 1.67%).

Each sample (2.00 g; dried and powdered) was spiked with 1.00 ml of internal standard solution (10 mg geraniol/ml in methanol) and extracted 3-times with 10 ml methanol by sonication for 10 min. The combined extract was dried in vacuum and re-dissolved in 1.50 ml methanol. Prior to use, all samples were filtered through a 0.45 μm Nylaflo filter (Gelman, Ann Arbor, MI, USA). Liquid samples (NC-3 and NC-4) were injected directly after filtration.

Standard solutions were prepared by dissolving 10.0 mg of **1** in 10.0 ml methanol; further calibration levels (concentrations from 1000.0 to 4.1 μg/ml) were prepared by diluting the stock with methanol (resulting regression equation: $y = 89300\ x$; correlation coefficient: $R^2 = 0.9999$).

All HPLC experiments were performed on a Waters 2690 Alliance system, equipped with a 996 PDA detector, using a Synergy MAX-RP 80 Å (150 × 4.6 mm; 4 μm particle size) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of water (A) and acetonitrile (B), which were applied in the following gradient elution: from 60A/40B to 55A/45B in 20 min. Each run was followed by a 5 min wash with an acetonitrile/methyl t-butyl ether-mixture (3:1) and an equilibration period of 10 min. The flow rate, column temperature and detection wavelength were set to 1.0 ml/min, 40 °C and 225 nm, respectively; the sample volume injected was 10 μl. Compound **1** was assigned by comparison with the retention time and UV-spectra of a standard.

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Micro-quantitative determination of quercetin by perturbation of a non-equilibrium stationary state in the Bray-Liebhafsky reaction system

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The aim of the present work is to acquaint a broader readership with a novel method for micro-quantitative determination of quercetin. It uses a general approach to micro-quantitative analysis [1, 2] based on specific features of nonlinear chemical systems far from thermodynamic equilibrium [3]. The method described in this particular work relies on disturbing a delicate balance between the species existing in the matrix reaction system, that is established in a stable non-equilibrium stationary state in the vicinity of a bifurcation point[1]. By introducing the analytes, this balance may be disturbed. The response of the system following the applied perturbation is then monitored. The species examined need not be intrinsic to the matrix reaction system, but it is sufficient that it reacts with it. The choice of the matrix reaction system is optional [1, 2] and, for practical purposes, it is not necessary to know the actual mechanism by which the analyte reacts with the matrix.

For the micro-quantitative determination of quercetin, we have chosen the Bray-Liebhafsky (BL) oscillatory reaction [4, 5], i.e. the reaction of hydrogen peroxide decomposition catalyzed by H^+ and IO_3^-, as the matrix system. This deceptively simple reaction proceeds through a complex mechanism comprising a number of intermediates: I^-, I_2, HOI, HIO_2 etc. [2, 4–14], which makes it suitable for such analysis.

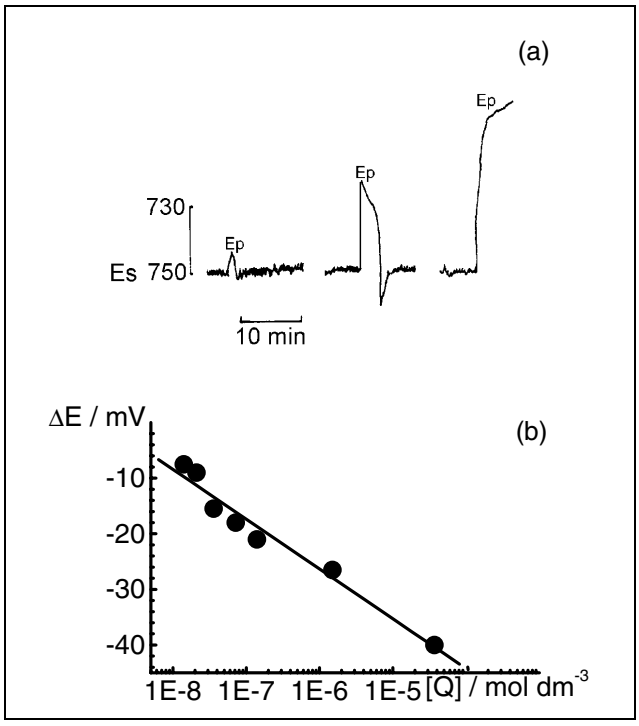

Fig. 1: (a) Typical response curves obtained after perturbing a stationary state in the BL reaction by addition of microvolumes of ethanolic solution of qercetin. The quercetin concentrations, from left to right, are: $[Q] = 1.4 \times 10^{-8}$ mol dm^{-3}; $[Q] = 1.5 \times 10^{-7}$ mol dm^{-3}; $[Q] = 3.7 \times 10^{-5}$ mol dm^{-3}. (b) A standard series calibration curve for quercetin