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Quantification of Nepetalactones in Catnip (*Nepeta cataria* L.) by HPLC Coupled with Ultraviolet and Mass Spectrometric Detection

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Abstract: Nepetalactones, the major chemical components of catnip (*Nepeta cataria* L.), were analysed by reversed-phase HPLC coupled with UV and MS detection. Two major nepetalactones, *Z,E*-nepetalactone and *E,Z*-nepetalactone, were successfully identified and quantified. The linearity range for *Z,E*-nepetalactone was determined as 0.00655–0.655 mg/mL with a correlation coefficient of 0.9999, and the linearity range of *E,Z*-nepetalactone was found to be 0.00228–0.456 mg/mL with a correlation coefficient of 0.9999, under UV detection at 228 nm. The linearity ranges were from 0.00164 to 0.0328 mg/mL, with a correlation coefficient of 0.9999, for *Z,E*-nepetalactone and 0.00114–0.0228 mg/mL, with a correlation coefficient of 0.9999, for *E,Z*-nepetalactone by MS detection with selected ion monitoring of ion peak *m/z* 167. The MS detection was found to be more sensitive than UV detection and this method was validated as simple, reliable and sensitive for catnip nepetalactone analysis. This method can be used for identification and fingerprinting of catnip products. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: HPLC-UV; HPLC-MS; nepetalactones; catnip; *Nepeta cataria* L.

INTRODUCTION

Catnip (*Nepeta cataria* L.) and related *Nepeta* spp., collectively referred to as catmints, have been used for ornamental and culinary purposes and as folk medicines with mildly sedative and antispasmodic effects for a long time (Ganzera *et al.*, 2001). Catnip is a member of the Lamiaceae family and has a reputation of stimulating and attracting cats. More recently, catnip has become of interest for its insect repellent activities. The steam distillate of catnip was found to be as good, and in some cases better, at repelling house flies, *Musca domestica* L., and American cockroaches, *Periplaneta americana* L., than widely used commercial products such as *N,N*-diethyl-*m*-toluamide or citronellal (Schultz *et al.*, 2004).

Among the chemicals identified in catnip, nepetalactones are of great interest owing to their potential application as aphid sex pheromones and insect repellents (Birkett and Pickett, 2003; Schultz *et al.*, 2004). Two major nepetalactones, *Z,E*-nepetalactone and *E,Z*-nepetalactone, are found in the essential oil of catnip. Previous studies have demonstrated the *E,Z*-nepetalactone isomer to possess a stronger repellent activity against cockroaches than the dominant isomer, *Z,E*-nepetalactone, whilst both nepetalactones exhibited mosquito repellent activity (Coats *et al.*, 2003; Schultz *et al.*, 2004). In addition, the nepetalactones are characteristic for species of *Nepeta* and can be used as marker compounds for identification purposes and botanical standardisation applications (Ganzera *et al.*, 2001). For this reason it is important to develop analytical methods by which to analyse the nepetalactones.

As for most volatile compounds, nepetalactones are generally analysed by GC and GC-MS (Handjieva *et al.*, 1996; Tropnikova *et al.*, 1999; Baranauskiene *et al.*, 2003; Morteza-Semnani and Saeedi, 2004; Schultz *et al.*, 2004), but GC analytical procedures are somewhat time-consuming. Moreover, GC analysis requires extensive preparation time involving extraction of the oil by the hydro and/or steam distillation of a large amount of plant material, and subsequent dissolution of the essential oil in organic solvent prior to analysis. HPLC offers a suitable alternative for the analysis of nepetalactones as the compounds are UV-detectable, and HPLC has been shown to be a reliable method for the analysis of the dominant isomer, *Z,E*-nepetalactone, in catnip oil (Ganzera *et al.*, 2001). However, this published HPLC method was used for the determination of just one nepetalactone, and the sample preparation procedure described is complex and time-intensive, involving three extractions, an evaporation and a final dissolution of the residue in solvent.

The aim of the present study was to develop and validate simple, fast and reliable HPLC-UV and HPLC-MS methods for the routine measurement of the major

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nepetalactones in catnip, which could be used to rapidly scan large numbers of samples in a quantitative manner.

EXPERIMENTAL

Materials and instrumentations. HPLC-grade acetonitrile, methanol and formic acid, anhydrous sodium sulphate, hexane and deuterochloroform were obtained from Fisher Scientific (Springfield, NJ, USA). Water was purified using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA) and was used to prepare all solutions. Catnip (*Nepeta cataria* L.) samples were collected from field-grown plants in NJ, USA, and voucher samples (reference number NUANPP20040801) were deposited at the New Use of Agriculture and Natural Products Program of Rutgers University (New Brunswick, NJ, USA). HPLC was conducted on an Agilent Technologies (Santa Clara, CA, USA) 1100 system coupled with an auto-sampler, a quaternary pump system, a photodiode array detector (PAD), and an HP Chemstation data system. ESI-MS were measured on an Agilent 1100 LC-MSD system equipped with an electrospray source, Bruker Daltonics 4.1, and data analysis 4.1 software. ^{1}H- and ^{13}C-NMR spectra were obtained on an INOVA-400 NMR instrument (Varian Inc., Palo Alto, CA, USA). Semi-preparative HPLC was carried on a Waters Delta 600 system (Milford, MA, USA).

Purification and structural elucidation of nepetalactones. Dried aerial parts of catnip (200 g) were subjected to hydro-distillation for 2.5 h using a Clevenger-type apparatus to extract the essential oil. The essential oil, which was heavier than water, was collected, dissolved in 3 mL of methanol and loaded onto the semi-preparative HPLC system. The separation was performed on a Waters Pre Nova-Pak HR C_{18} column (300 × 19 mm i.d.; 6 μm) eluted with acetonitrile:water (40:60) at a flow rate of 10 mL/min and monitored by UV detection at 228 nm. Fractions (10 mL) were collected and subjected to HPLC-MS analysis. The fractions containing individual nepetalactones were separately combined and evaporated at 35°C to remove the acetonitrile. The remaining water phase was extracted with hexane (3 × 25 mL) and the hexane phase dried over anhydrous sodium sulphate and evaporated to dryness at 35°C under reduced pressure to afford purified individual compounds. Three separate compounds, **1**–**3**, were obtained in amounts of 1, 120 and 30 mg, respectively. The purified samples were dissolved in deuterochloroform and subjected to NMR analysis for structural identification.

Preparation of standard solutions for linearity and calibration test. About 15 mg of each nepetalactone were accurately weighed and introduced into a 25 mL volumetric flask; 20 mL of pure methanol were added, the mixture sonicated for 15 min, cooled to room temperature and diluted to 25 mL using methanol (stock solution). Calibration standards were prepared by diluting the stock solution with methanol 2, 5, 25, 100, 200 and 400 times, respectively. An aliquot (10 μL) of each solution was injected onto the HPLC in order to construct the calibration curves.

Preparation of plant samples for HPLC-UV and HPLC-MS analysis. About 1 g of powdered flowering aerial part of catnip was accurately weighed into a 50 mL volumetric flask and 35 mL of methanol were added. The flask was closed with a stopper and sonicated for 45 min. The volume was made up to the mark with methanol, the solution transferred to a centrifuge tube and centrifuged at 12,000 rpm for 2 min in order to obtain a clear solution for HPLC analysis (Fisher accu Spin* Micro Bench top Centrifuge (Thermo-Fisher Scientific Inc. Waltham, MA, USA)).

HPLC and MS conditions for the analysis of nepetalactone. HPLC separation was performed on a YMC-Pack Pro C_{18} column (150 × 4.6 mm i.d.; 3 μm) (Waters Inc. Milford, MA, USA) with a gradient mobile phase system of water containing 0.1% (v/v) formic acid (mobile phase A) and acetonitrile (mobile phase B). The linear gradient profile was from 36 to 54% B in 15 min, then from 54 to 96% B in 5 min; finally the column was washed by 96% B for 5 min with a post running time of 12 min. The wavelength of UV detection was 228 nm and the flow rate was 1 mL/min. The effluent from the column passed through the UV detector directly into the MSD detector. The ESI-MS was operated in the positive ion mode with an optimised collision energy level of 80%; spectra were scanned from m/z 50 to 600 for identification purposes and from m/z 100 to 200 for quantification purposes. ESI were measured using a needle voltage of 3.5 kV. High-purity nitrogen (99.999%) was used as the drying gas at a flow rate of 12 mL/min; the capillary temperature was 350°C; helium was used as the nebuliser gas at 60 psi. Selected ion monitoring (SIM) was performed at m/z 167, representing the protonated molecular ion peak, $[M+H]^+$, of *E*,*Z*-nepetalactone and *Z*,*E*-nepetalactone.

RESULTS AND DISCUSSION

Using an YMC-Pack Pro C_{18} column with a water (containing 0.1% formic acid) and acetonitrile gradient solvent system, it was possible to achieve baseline separation for the major components in catnip samples. Chromatograms of a representative catnip sample detected by UV (at 228 nm) and by MS (total ion

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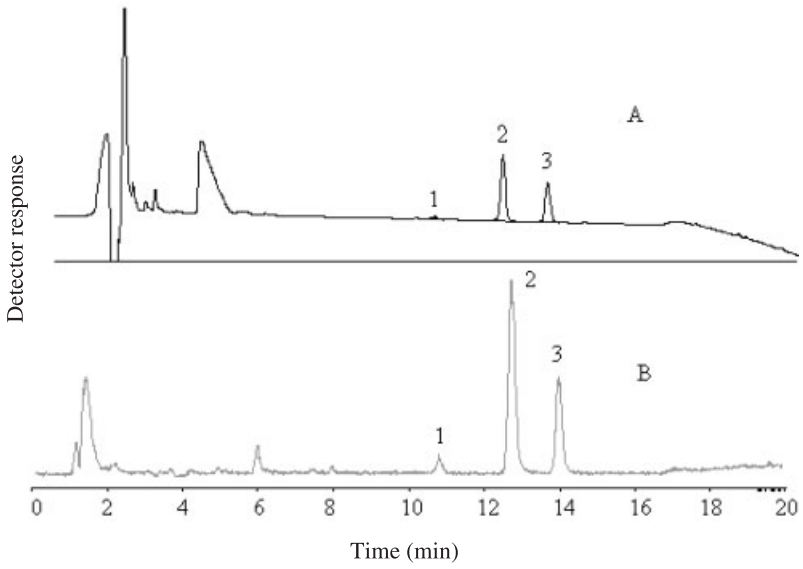
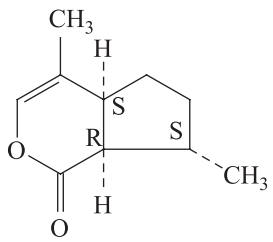
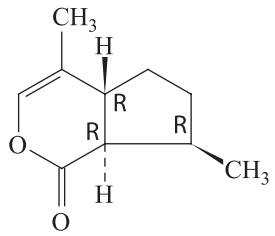


Figure 1 HPLC chromatograms of a representative sample of catnip detected simultaneously by (A) UV at 228 nm, and (B) MS (total ion current). Key to peak identities: **1**, minor nepetalactone (structure undefined); **2**, *Z*,*E*-nepetalactone; and **3**, *E*,*Z*-nepetalactone. (For extraction and analytical protocols see Experimental section.)



Z, *E*-nepetalactone (**2**)



E, *Z*-nepetalactone (**3**)

current) are shown in Fig. 1. In both chromatograms, only a few peaks were observed and the peaks labelled **1–3** all showed MS with dominant ions at *m*/*z* 167 and their UV maximum absorptions were at 227, 228 and 223 nm, respectively, indicating the presence of a nepetalactone skeleton. In order to confirm the structures of components **1–3**, and to obtain pure standards, hydro-distilled catnip oil with a high content of these compounds was subjected to semi-preparative separation by HPLC. The purified compounds **1–3** were then subjected to NMR analysis in deuterochloroform. The ^{13}C-NMR of **2** showed signals at δ 171.4 (s), 133.7 (d), 115.7 (s), 49.5 (d), 40.8 (d), 40.0 (d), 33.2 (t), 31.1 (t), 20.4 (q), 15.6 (q) ppm, whilst that of **3** showed signals at δ 170.5 (s), 136.0 (d), 120.6 (s), 49.2 (d), 37.5 (d), 32.2 (d), 30.1 (t), 26.3 (t), 17.8 (q) and 14.5 (q) ppm (the ^{1}H-NMR data are not shown here). Compounds **2** and **3** could be differentiated from their ^{13}C-NMR spectra and were found to be *Z*,*E*-nepetalactone (4aα,7α,7aα-nepetalactone; *cis*, *trans*-nepetalactone) and *E*,*Z*-nepetalactone (4aα,7α,7aβ-nepetalactone; *trans*, *cis*-nepetalactone), respectively, by comparison of their ^{1}H- and ^{13}C-NMR spectra with literature data (Eisenbraun *et al.*, 1980). The minor component **1** (1 mg) of catnip oil was not present in sufficient quantity in order to obtain NMR data appropriate for structural elucidation.

For the accurate quantification of bio-active/marker compounds in plant samples, the extraction solvent is of critical importance. Fortunately the extractive ability of different solvents for nepetalactones in catnip has been studied, and methanol was observed to be the most effective solvent amongst those tested, namely, hexane, dichloromethane, ethyl acetate and methanol (Ganzera *et al.*, 2001). In the present study, methanol was thus selected as the extraction solvent. However, the extraction procedure described herein was much simpler than the previously reported method (Ganzera *et al.*, 2001) and involved sonication of 1 g of powdered plant sample in 50 mL methanol for 45 min. The resulting extract was directly injected into the HPLC for analysis. In the present study, two major nepetalactones in catnip were quantified using an external standard method with pure reference marker compounds. The described method is superior to the HPLC method published earlier, which involved the qualitative and quantitative analysis of only one nepetalactone.

Validation data indicated that the described procedures were satisfactory and reliable. Initially, the reproducibility, linearity and precision of the HPLC method with UV detection at 228 nm were evaluated. Method reproducibility was evaluated by performing 10 replicate analyses of nepetalactones in one catnip sample. The percent relative standard deviations (%RSD) of the retention times of *Z*,*E*- and *E*,*Z*-nepetalactone were

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<1%, and the %RSDs for the peak areas of *Z,E*- and *E,Z*-nepetalactone were 3.56 and 2.56%, respectively. The linearity was tested in the concentration range between 0.00164 and 0.655 mg/mL for *Z,E*-nepetalactone and between 0.00114 and 0.456 mg/mL for *E,Z*-nepetalactone, respectively. The linearity range for *Z,E*-nepetalactone was determined as 0.00655–0.655 mg/mL with a correlation coefficient of 0.9999, and the linearity range of *E,Z*-nepetalactone was found to be 0.00228–0.456 mg/mL with a correlation coefficient of 0.9999. The precision of the extraction procedure was then validated by using one powdered catnip sample. A total of six extractions (each of 1 g of catnip powder) were prepared using the procedure described above. An aliquot of each extraction was then subjected to HPLC analysis. The %RSDs of the contents of *Z,E*- and *E,Z*-nepetalactone were 2.33 and 2.51%, respectively, demonstrating the excellent precision of this sample preparation. The contents of nepetalactones present in the various samples tested were each over 1.2% in agreement with data reported in literature (Duppong *et al.*, 2004) but contrasting with the results produced by the previously reported HPLC method in which the highest content was only 0.036% (Ganzera *et al.*, 2001).

In the present method, the effluent from the HPLC passed through the UV detector directly into the MS detector for analysis. In the positive ESI mode at different energy levels, the protonated molecular ion peak of the nepetalactones ($[M+H]^+$ at m/z 167) was always the dominant ion peak and so it was employed for monitoring these components. The reproducibility and linearity of SIM at m/z 167 were also evaluated. As described above, the method reproducibility was evaluated by comparing the %RSD of 10 replicate analysis of one catnip sample. For SIM at m/z 167, the percent %RSDs of the retention times of *Z,E*- and *E,Z*-nepetalactones were less than 1%, and the %RSDs of the peak areas of *Z,E*- and *E,Z*-nepetalactones were 3.45 and 3.66%, respectively, suggesting that SIM is suitable for the analysis of nepetalactones. The calibration curve was constructed in the same concentration range as described above. The linearity range was found to be 0.00164–0.0328 mg/mL with a correlation coefficient of 0.9999 for *Z,E*-nepetalactone, and 0.00114–0.0228 mg/mL with a correlation coefficient of 0.9999 for *E,Z*-nepetalactone. The sensitivity of the MS detector was much higher than the UV detector, but the linear range was narrower. For catnips containing more than 1% nepetalactones, the extraction of 1 g of plant material with 50 mL of methanol would produce an extract that was already out of the linearity range of the MS detector. In order to remain within the MS linearity range, 50 mg of catnip sample should be extracted with 50 mL of methanol.

In the present study, HPLC with either UV detection or with MS (selected ion monitoring) were found to be suitable for the qualitative and quantitative determination of nepetalactones. MS/SIM was found to be more sensitive than UV detection, and capable of analysing samples with low nepetalactone contents such as catnip stems and intermediary genetic materials. The method was shown to be simple, reliable and sensitive and could also be used to identify and fingerprint catnip products.

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