Food Anal. Methods (2016) 9:1807–1813
DOI 10.1007/s12161-015-0358-5

HPLC Determination of the Alkamide Affinin in Fresh and Dry Roots of *Heliopsis longipes* (Asteraceae) and HS-SPME-GC-MS-TOF Analysis of Volatile Components

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Received: 22 July 2015 / Accepted: 9 November 2015 / Published online: 20 November 2015

Abstract *Heliopsis longipes* (Asteraceae) is a plant whose roots are commonly used in Mexican cuisine as a substitute for hot pepper. This plant is also used in traditional medicine for its therapeutical properties. Previous reports detected the presence of affinin—an alkamide with analgesic properties—in extracts of *H. longipes*. Here, we describe the development of a high-performance liquid chromatography-UV detector method for the determination of the alkamide affinin (*1*) as the major component in fresh and dry roots of *H. longipes*. Compound 1 was quantitatively determined employing a Kromasil 100 C_{18} 3 μm particle size column, using the isocratic mobile phase acetonitrile-water (55+45). The flow rate was 0.8 mL/min and the UV detection was at 213 nm. The limits of detection and quantitation were 0.0009 and 7.6 μg/mL, respectively. Compound 1 showed good linearity in the 75 to 150 μg/mL range; recovery was within 99.3 and 101.9 %. Quantities of affinin measured by this method ranged between 6.9 and 8.5 mg per gram of the dry and fresh roots; thus 1 could be used as marker for *H. longipes*. The LC method described here proved to be reliable, reproducible, accurate, and could be used for quality control of *H. longipes* medicinal materials. In addition, its volatile chemical composition is described and was assessed via GC-MS using headspace solid-phase microextraction; compounds were identified by matching against the standard mass spectral database of the National Institute of Standards and Technology (NIST). Twenty-eight recognized compounds represent 99.9 % of the total relative content of constituents from *H. longipes* roots.

Keywords RP-HPLC · Quantification · Affinin · HS-SPME-GC-MS-TOF · *Heliopsis*

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Introduction

The roots of *Heliopsis longipes* (A. Gray) S. F. Blake (Asteraceae) provide a crude plant drug commonly used in Mexican cuisine as a substitute for hot pepper due to the production of similar organoleptic stimulus (Molina-Torres et al. 2004). These roots—known in Nahuatl as "chilcuán", "chilcuage" (Martínez 1989), or "raíz de oro" and "raíz azteca" in Spanish—have also been employed in traditional medicine. Its uses include buccal analgesic and anesthetic (Correa et al. 1971; Molina-Torres et al. 2004; Cilia-López et al. 2010; Carîno-Cortés 2010), antibacterial (Molina-Torres et al. 1999; Gutiérrez-Lugo 1996), and as antiparasitic (Martínez, 1989; Cilia-López et al. 2008). Furthermore, its effectiveness in eliminating intestinal parasites upon addition of dried roots to food has been tested (Molina-Torres et al. 2004). The distribution of the genus *Heliopsis* is restricted to the occidental hemisphere and particularly 10 of the 14 known species are found in Mexico; 8 of them are endemic in this country.

H. longipes roots are well-known not only as an edible root but in Mexican herbolaria as its popular Nahuatl denominations indicate. Chemical studies of this plant have identified

aliphatic, olefinic, and acetylenic alkamides as major components (Acree 1945; López-Martínez et al. 2011); however, terpenoids, lignans, and sterols have also been isolated (López-Martínez et al. 2011; Molina-Torres 1995). Among the olefinic components, affinin (*N*-isobutyl-2*E*,6*Z*,8*E*-decatrienamide) (Fig. 1) was shown to be the major component in the chloroform extract of the roots (Ríos and Aguilar 2007). In analgesic in vivo assays, it possesses antinociceptive effect (Déciga-Campos et al. 2010), modification of anxiety behavior, and prolonged the time of sodium pentobarbital-induced hypnosis in mice (Déciga-Campos et al. 2012).

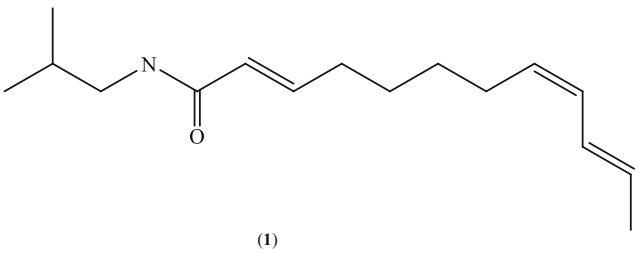
The use of column high-performance chromatography (LC) in the quality control of plant extracts is well-documented and consists of an appropriate method for the identification and quantification of active marker principles (ICH-Q2A 1995 and ICH-Q2B 1997) that eventually could be used for a future standardization and/or quality control of herbal preparations. Therefore, we developed and validated a liquid chromatographic technique to quantify affinin (*1*) as the major compound of *H. longipes*. In the analytical method, the validation was carried out according to the International Conference on Harmonization (ICH) guidelines (ICH-Q2A 1995 and ICH-Q2B 1997), which requires the evaluation of linearity, precision, accuracy, and stability parameters.

In order to compare the contents of 1 in dry and fresh samples of roots of *H. longipes*, we developed and validated a HPLC method. It is a well-known the fact that consumers have a preference for the dry roots. The root of *chilcuague* is economically and culturally important in Mexico and is sold all year long in the traditional markets in the Central part of the country.

Materials and Methods

Chemicals and Materials

The fresh roots of *H. longipes* were wild collected on 28 July 2007 at "Real de Xichu" in humid regions of Guanajuato State. Some of the roots were air dried in the shadow at room temperature for 15 days. The plants were identified by one of the authors (R. Ríos). A voucher specimen was deposited in FEZA Herbarium, UNAM (number 5904). In this study, we used a fresh sample and a dried sample at room temperature.



(1)

Fig. 1 Structure of affinin (*1*)

Acetonitrile (EMD Chemicals Inc., NJ), water (Caledon Laboratories LTD; Ont., Canada) and methanol (EMD Chemicals Inc., NJ) were of HPLC grade. Analytical-grade dichloromethane was used to extract compound 1. Highly-pure affinin (*1*) (>98 % determined by HPLC) was isolated from *Heliopsis longipes* in our laboratory and used for the calibration (Déciga-Campos et al. 2010). Its identity was confirmed on the basis of spectral data (IR, UV, NMR, and MS) and by comparison with reported values in the literature (Correa et al. 1971).

Equipment and Chromatographic Conditions

The LC system consisted of Waters (Waters Corp., Milford MA, USA) Model 515 pumps, a Jasco automated injector, a Waters model 680 automated gradient controller, a Waters Model 2487 2-channel UV/VIS Waters detector, and a computerized data station equipped with Waters Millenium software. Separation was achieved on a Kromasil 100 C_{18} column (150×4.6 mm I.D.; 3 μm particle size) operating at 30 °C. The mobile phase consisted of acetonitrile (55 %), water (45 %); it was applied in an isocratic form. The flow rate was adjusted to 0.8 mL/min, and affinin (*1*) was monitored at 213 nm. Each run was followed by a 5-min wash with 100 % acetonitrile and an equilibration period of 15 min.

Preparation of Stock and Working Solutions

A stock solution of affinin (*1*) containing 400 μg/mL in acetonitrile was prepared.

Fresh (25 mg) and dry (50 mg) roots of *Heliopsis longipes* were ground in a mortar (200 mg, particle size <2000 μm, mesh 2 mm), and independently sonicated with acetonitrile (2.5 mL) at room temperature for 15 min, and centrifuged (10 min×3500 rpm); this operation was repeated three times. All supernatants were collected in a 10-mL volumetric flask and diluted to volume with acetonitrile. Prior to injection (10 μL), an adequate volume (ca 2 mL) of the solution was passed through a 0.45 μm PVDF membrane syringe filter. The first 1 mL was discarded, and the remaining liquid was collected in a LC sample vial.

HPLC Analytical Method

In preliminary liquid chromatographic work, we detected *1* in a sample of *H. longipes* was detected as a single signal at a retention time (R_T) of 21.4 min (Fig. 2a). The latter sample was then spiked with a known amount of the standard, in order to verify the mentioned retention times of *1*. The validation method included a pre-validation phase where the optimal chromatographic conditions were developed for the sample

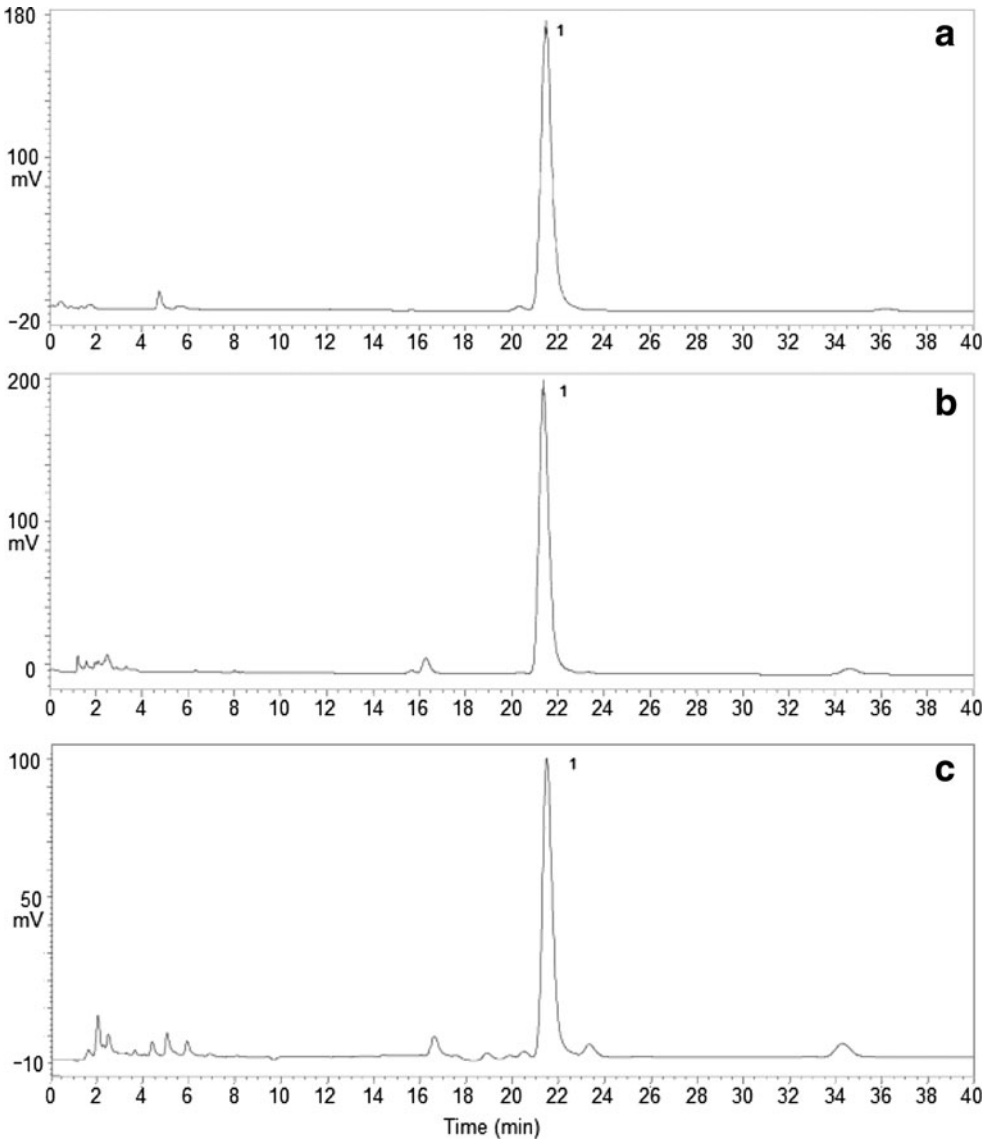


Fig. 2 Typical column liquid chromatograms of pure affinin standard (*1*) **A** plant extracts (**B–C**), with detection at 213 nm

solution of *H. longipes* and for the determination of the response. Analyses of one fresh and one dry sample of *H. longipes* were performed using the methodology described in Preparation of Stock and Working Solutions section.

Headspace Solid-Phase Microextraction

Five-hundred milligrams of dried fragmented plant material, sodium chloride (75 mg), and distilled water (10 mL) were hermetically sealed in a 25-mL vial with a polypropylene hole-cap and PTFE/coated silicone septa. The SPME fiber (2 cm, 50/30 μm DVB/Carboxen/PDMS fiber, Supelco Bellefonte, PA, USA) was conditioned for 40 min at 300 °C, placed and exposed to the vapor phase to the plant material. Experimental conditions for the analysis were set as follows: extraction temperature, 25 °C; equilibrium time, 5 min; extraction time, 10 min. After sampling, the SPME fiber was directly inserted into the GC inlet and the fiber thermally desorbed. Desorption time was 2 min at 300 °C. All samples were analyzed in triplicate, and the relative proportions of individual components adsorbed to the fiber under these conditions was calculated based on the total ion chromatogram (TIC) peak areas as a percentage of the sum of all peak areas.

GC-MS

Analyses by GC-MS were carried out in an Agilent 6890 N series gas chromatograph (Agilent Technology, Palo Alto, CA, USA) coupled with a LECO time of flight mass spectrometer (MS-TOF, Leco Corporation, St. Joseph, MI, USA). Compounds were separated on a 5 % diphenyl-

95 % dimethyl polysiloxane (10 m×0.18 mm, film thickness 0.18 μm) capillary column, using the following GC oven temperature program: 3 min at 40 °C up to 300 °C during 15 min, at 20 °C/min, splitless mode. Helium was used as the carrier gas at a flow rate of 1 mL/min. The electronic ionization energy was 70 eV, and the mass range scanned was 33–500 uma. Scan rate was 20 spec/s. Transfer line and ionization chamber temperatures were 250 °C and 200 °C, respectively. Data acquisition and processing were performed with LECO ChromaTOF^{TM} software system. The volatile compounds were identified based on their MS fragmentation patterns and Kovats Index, calculated in relation to the retention times of a series of alkanes (C_8–C_{20}). Both were compared with chemical compound data gathered by Adams (2007) and the spectral database of the National Institute of Standards and Technology (NIST) (Linstrom and Mallard 2015). Conclusive identification was made by comparing the MS and Kovats Index of compounds with available authentic standards purchased from Sigma-Aldrich (≥98 % purity).

Results and Discussion

Validation of the LC Method

The following analytical parameters were determined for the validation of the LC method.

System Linearity

Five solutions at concentrations of 50, 75, 100, 150 and 200 μg/mL in acetonitrile from the stock solution of affinin (*1*) were prepared. A standard curve was drawn by plotting area versus compound *1* concentrations. The 100 % or work solution was considered as the central solution for the calibration graph (100 μg/mL^{-1}). Aliquots (10.0 μL) were analyzed by HPLC, and the areas under the peaks were determined. Table 1 shows the calibration data, correlation coefficient (r^2), and calculated limit of detection (LOD). The calibration graph allowed the calculation of the concentration levels of *1* in each plant sample (fresh and dry). The LOD and LOQ values obtained for a signal-to-noise (S/N) ratio of 3 and 10, respectively, using the calibration graphs, were 0.0009 and 7.6 μg/mL for affinin (*1*).

Method Linearity

It was tested by recovery, assaying independently and by triplicate, three concentrations (75, 100, and 150 μg/mL) of affinin (*1*). A calibration graph correlating the area (y) versus concentration (x) of *1* was drawn. Accordingly, the regression equation for affinin (*1*) was found to fit: $y=18.19x+324.05$ ($r^2=0.9993$); the coefficient of variation was less than 2 % at each concentration level.

Precision

The precision of the experiments was expressed by coefficient of variation. System Precision was based on sextuplicate assay of the "work solution" at concentration of 80 μg/mL for compound *1*. The assessment of method precision involved two parts: repeatability (within-run precision) and reproducibility (between-run precision). Repeatability was ascertained for compound *1* by analyzing the two plant materials within a day; three levels of concentration (same as for method linearity) for *1* were used, and each sample was run three times. Standard deviation values were <3 %, and the coefficient of variation was <2 % in all experiments. Reproducibility was determined by assaying the mentioned solutions of the sample and compound *1* on two different days and by two different analysts. For this assay, the corresponding statistical analyses were performed to process the results. The calculated relative standard deviations were <3 %, and the coefficient of variation was <2 % in all experiments.

Method Accuracy

Method Accuracy was assessed by analyzing three different concentrations of compound *1*: 75, 100, and 150 μg/mL, in triplicate. Compound *1* was independently added to each sample of the plant material, processed as indicated above in the Sample Preparation section, and analyzed using the proposed

Table 1 Calibration data (regression equation and correlation coefficient [r^2]), limits of detection (LOD) and contents of affinin (*1*) in roots of *H. longipes*

Analyte	Regression equation	r^2	LOD, g/mL	mg/g root (%, RSD)	
				Fresh	Dry
1	$y=69.46x+584.91$	0.9978	0.000931	8.51 (0.85, 1.6)[a]	6.94 (0.69, 0.97)[a]

[a] Mean values ($n=3$); RSD=Relative standard deviation

Table 2 Volatile components identified by HS-SPME and GC-MS technique in *Heliopsis longipes* roots

Compound[a]	Area (%)	R. T. (s)	RI exp[b]	RI lit[c]	Method of identification[d]
β-pinene	1.56	277	980	979	a, b, c
α-pinene[e]	7.92	286	991	–	c
δ-3-carene	25.72	300	1011	1011	a, b, c
(E)-ocimene	5.18	319	1046	1050	a, b, c
1,3,8-p-menthatriene	8.06	352	1108	1110	a, b, c
α-terpinolene	8.06	354	1110	1099	a, b, c
Allo-neo-ocimene	0.18	371	1142	1144	a, b, c
(+)−neomenthol	0.27	384	1166	1165	a, b, c
Cis-pinocarveol	1.52	392	1181	1183	a, b, c
(Z)-3-hexenyl-2-methylbutanoate	0.47	410	1217	1224	a, b, c
Citral	0.47	420	1241	1240	a, b, c
Trans-myrtanol	1.59	429	1262	1259	a, b, c
Endo-2-methylnorbornane[e]	1.56	435	1275	–	c
6-isopropylidene-bicyclo[3.1.0]hexane[e]	0.90	437	1281	–	c
Cis-α-terpineol[e]	0.53	439	1284	–	c
Decanal[e]	0.25	448	1306	–	c
O-methylthymol[e]	5.26	458	1327	–	c
Methyl-decyl-ketone	0.75	486	1393	1389	a, b, c
(E)-5,6-epoxy-β-ionone	0.73	507	1449	1455	a, b, c
α-vatirenene[e]	0.73	509	1453	–	c
Dehydroaromadendrene	1.80	514	1466	1466	a, b, c
β-chamigrene	10.66	516	1474	1475	a, b, c
γ-cadinene	10.85	531	1513	1513	a, b, c
Aromadendrene	1.34	564	1601	1600	a, b, c
Cadina-1(10),4-diene[e]	0.77	573	1630	–	c
Cedr-8-en-13-ol	0.46	586	1670	1668	a, b, c
Isopropyl palmitate[e]	0.14	733	2163	–	c
Verticilol[e]	2.26	740	2190	–	c
Monoterpene hydrocarbons	32.1	–	–	–	–
Oxygenated monoterpenes	21.4	–	–	–	–
Sesquiterpene hydrocarbons	21.4	–	–	–	–
Oxygenated sesquiterpenes	3.57	–	–	–	–
Oxygenated ditepenes	7.14	–	–	–	–
Oxygenated hidrocarbons	7.14	–	–	–	–
Others	7.14	–	–	–	–
Total identified	99.89	–	–	–	–

[a] Compounds are listed in order of elution on the DB-5 column

[b] Retention indices experimentally determined on the DB-5 column

[c] Retention indices of literature on non polar columns reported from NIST (Linstrom and Mallard 2015) and Adams (2007)

[d] a, retention time; b, retention index; c, mass spectrum

[e] Tentatively identified by mass spectral library search only

–, not identified

HPLC method described. Recovery was within 98.7 and 101.1 %; standard deviation for each level of concentration was below 3 % in all experiments, and the coefficient of variation was below 2 %.

Stability

The stability of the sample was assayed by subjecting work samples independently to different conditions (room

temperature, darkness, refrigeration, refrigeration and darkness), while analyzing samples at different times, from zero time, 20, 45 h, up to 8 and 15 days. Results showed that affinin (*1*) was stable up to 8 days.

Analysis of Samples

Figure 2 shows the variations of *1* in two different samples of *.H longipes* (Fresh and dry). The extracts were analyzed using HPLC, and contents of analyte *1* were calculated (Table 1) in each sample. To accomplish this, the following equation was chosen using the weighted least squares linear regression (Almeida et al. 2002):

$$\text{Affinin}\left(\text{mg}\Big/\text{g plant}\right) = \left[A(\text{Sample})\Big/A(\text{Compd.})\right] \times [\text{Conc.Compd.}] \times \left[F_{\text{dil}}\Big/g(\text{plant})\right] \times \left[1\Big/1000\right].$$

where A(Sample) is the affinin area obtained from the chromatograms; A(*Compd.*) is the affinin area corresponding to the central solution of the calibration graph; *Conc.Compd.* is the real concentration of the affinin obtained from the calibration curve, considering the weighing factor $W_i = 1/x^2$ (11); F_{dil} is the dilution factor of sample, and g (plant) is the amount in grams of plant assayed.

Affinin (*1*) is a useful chemical marker for *H. longipes* since this compound was found in a very high proportion in both plant samples analyzed.

Highest amount of *1* was detected in the fresh sample (Fig. 2b) (8.5 mg/g of plant) while 6.9 mg/g of plant were found in the dry sample (Fig. 2c). The low variation in the high contents in both samples assayed is important, considering the extent of use of *H. longipes* in the Mexican traditional medicine, and as food; furthermore, our study provides a method of quality control for plant extracts containing the alkamide *1*. Our results are in agreement with quantities of affinin obtained in a GC/MS determination by García-Chávez et al (2004) on dry samples of *H. longipes*. Our LC method provides a reliable and quantitative determination of this constituent.

Volatile Chemical Composition Analysis

The analyses and identification pointed by mass fragmentation pattern and retention index obtained by HS-SPME and TOF-GC/MS analyses of dried roots of *H. longipes* revealed the presence of 28 compounds (Table 2, Fig. 3), representing 99.9 % of the total contents of volatiles, with 53.6 % of monoterpenes, 25 % of sesquiterpenes, 7.1 % of diterpenes, 7.1 % of oxygenated hydrocarbons, and 7.1 % of esters. The qualitative and quantitative composition of roots volatile compounds, determined after GC-MS analysis, is shown in

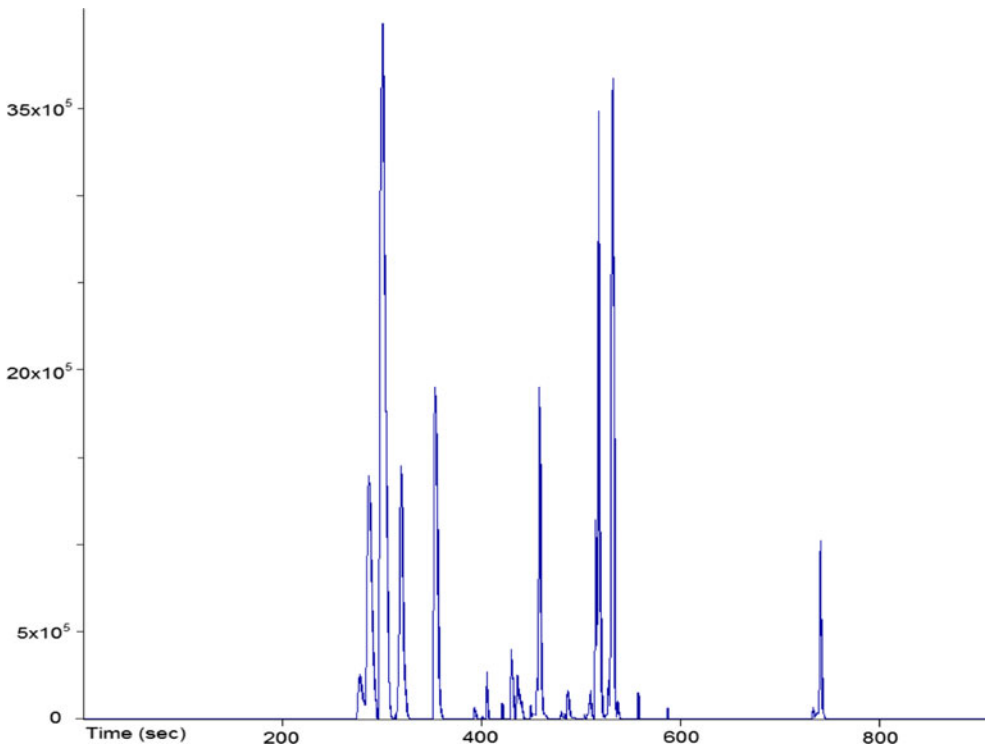


Fig. 3 Total ion current chromatogram of the volatile components from *H. longipes* extracted using HS-SPME-GC

Table 2, listed in order of their elution on a DB-5 column together with their retention indexes. The major volatile compounds were δ-3-carene (25.7 %), α-Terpinolene and 1, 3,8-p-menthatriene (8.06 % both), γ-cadinene (10.8 %), β-chamigrene (10.6 %), (E)-ocimene (5.1 %), *O*-methylthymol (5.2 %), and verticilol (2.26 %).

Conclusions

The LC method described herein allows the quantitative analysis of affinin (*1*) in fresh and dry *H. longipes* samples. Our method constitutes a useful analytical tool for determining the quality of commercial plant material and eventual medicinal preparations. The HS-SPME-GC-TOF-MS analysis using a PDMS fiber is a useful method for the analysis of volatile compounds in *H. longipes* roots.

Acknowledgments This work had the financial support of Dirección General de Asuntos del Personal Académico (DGAPA- IN-217914). The authors wish to thank Alejandrina Acosta, for running the NMR spectra, and to Ramiro del Carmen and Roberto Díaz for technical assistance. Dr. Manuel Gutiérrez critically read the manuscript.

Compliance with Ethical Standards

Conflict of Interest María Isabel Aguilar declares that she has no conflict of interest. Nancy E. Castillo declares that she has no conflict of interest. Cristian Alvarado-López declares that he has no conflict of interest. Georgina Duarte-Lisci declares that she has no conflict of interest. Ramiro Ríos-Gómez declares that he has no conflict of interest. María Yolanda Rios declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed on by any of the authors.

Informed Consent Not applicable.

References

Acree F, Jacobson M, Haller L (1945) The structure of affinin, the insecticidal amide from *Erigeron affinis* DC. J Org Chem 10:449–451

Adams RP (2007) Identification of essential oil components by gas chromatography and mass spectrometry. Allured Publishing Corporation, Chicago

Almeida AM, Castel-Branco MM, Falcao AC (2002) Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods. J Chromatogr B 774:215–222

Carĩno-Cortés R, Gayosso-De-Lucio JA, Ortiz MI, Sánchez-Gutiérrez M, García-Reyna PB, Cilia-López VG, Pérez-Hernández N, Moreno E, Ponce-Monter H (2010) Antinociceptive, genotoxic and histopathological study of *Heliopsis longipes* S.F. Blake in mice. J Ethnopharmacol 130:216–221

Cilia-López VG, Aguirre-Rivera JR, Reyes-Agüero JA, Juárez-Flores BI (2008) Etnobotánica de *Heliopsis longipes* (Asteraceae: Heliantheae). Bol Soc Bot Méx 83:81–87

Cilia-López VG, Juárez-Flores BI, Aguirre-Rivera JR, Reyes-Agüero JA (2010) Analgesic activity of *Heliopsis longipes* and its effect on the nervous system. Pharm Biol 48:195–200

Correa J, Roquet S, Díaz E (1971) Multiple NMR analysis of the affinin. Org Magn Reson 3:1–5

Déciga-Campos M, Rios MY, Aguilar-Guadarrama B (2010) Antinociceptive effect of *Heliopsis longipes* extract and affinin in mice. Planta Med 76:665–670

Déciga-Campos M, Arriaga-Alba M, Ventura-Martínez R, Aguilar-Guadarrama B, Rios MY (2012) Pharmacological and toxicological profile of extract from *Heliopsis longipes* and affinin. Drug Dev Res 73:130–137

García-Chávez A, Ramírez-Chávez E, Molina-Torres J (2004) El género *Heliopsis* (Heliantheae; Asteraceae) en México y las alcamidas presentes en sus raíces. Acta Bot Méx 69:115–131

Gutiérrez-Lugo MT, Barrientos-Benítez T, Luna B, Ramírez-Gama RM, Bye R, Linares E, Mata R (1996) Antimicrobial and cytotoxic activities of some crude drug extracts from Mexican medicinal plants. Phytomedicine 2:341–347

ICH harmonized tripartite guideline, validation of analytical procedures: methodology ICH-Q2A. (1995) International Conference on Harmonization, Geneva, Switzerland

ICH harmonized tripartite guideline, validation of analytical procedures: methodology ICH-Q2B (1997) International Conference on Harmonization, Geneva, Switzerland

Linstrom PJ, Mallard, WG (eds) (2015) NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg MD, 20899, http://webbook.nist.gov (09 may 2015)

López-Martínez S, Aguilar B, Rios MY (2011) Minor Alkamides from *Heliopsis longipes* S.F. Blake (Asteraceae) fresh roots. Phytochem Lett 4:275–279

Martínez M (1989) Las plantas medicinales de México. Ed. Ediciones Botas, México DF

Molina-Torres J, Salgado-Garciglia R, Ramírez-Chávez E, Del-Río R (1995) Presence of the bornyl ester of deca-2E,6Z,8E-trienoic acid in *Heliopsis longipes* roots. J Nat Prod 58:1590–1591

Molina-Torres J, García-Chávez A, Ramírez-Chávez E (1999) Antimicrobial properties of natural alkamides traditionally used in Mesoamerica: affinin and capsaicin. J Ethnopharmacol 64:241–248

Molina-Torres J, Salazar-Cabrera CJ, Armenta-Salinas C, Ramírez-Chávez E (2004) Fungistatic and bacteriostatic activity of alkamides from *Heliopsis longipes* roots: affinin and reduced amides. J Agric Food Chem 52:4700–4704

Ríos MY, Aguilar B, Gutiérrez M (2007) Analgesic activity of affinin, an alkamide from *Heliopsis longipes* (Compositae). J Ethnopharmacol 110:364–367

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