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Short communication

# Analysis of diterpenoids from *Sideritis* species by reversed-phase high-performance liquid chromatography

P. Gómez-Serranillos[a], O.M. Palomino[a], A.I. Villarrubia[a], M.A. Cases[b], E. Carretero[a,*], A. Villar[a]

[a]*Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[b]*CIT-INIA, Carretera de La Coruña, Km 7,300, 28040 Madrid, Spain*

**Abstract**

The qualitative and quantitative determination of diterpenoids in the hexane and methanolic extracts of four species of the genus *Sideritis* (*Lamiaceae*) has been carried out for the first time using a new reversed-phase high-performance liquid chromatography method. Knowledge of the content of diterpenoids allows their relationship to pharmacological activity to be determined and validates their use in folk medicine as anti-inflammatory agents. © 1997 Elsevier Science B.V.

*Keywords:* *Sideritis*; Diterpenoids

## 1. Introduction

Several *Sideritis* species are used in Spain as anti-inflammatory and anti-ulcer agents [1,2]. Extracts of the aerial parts of *Sideritis* species contain flavonol glycosides, flavones and diterpenes [3,4].

High-performance liquid chromatography (HPLC) has been applied to the analysis of flavonoids from *Sideritis* species [5], while the qualitative and quantitative determination of terpenes by HPLC is still problematic and limited. The complex matrix of *Sideritis* extracts, containing waxes, chlorophylls and polyphenolic compounds as the major impurities, as well as the lack of standards, are the main problems.

This paper is part of our on-going studies on this genus in which we describe a new HPLC isocratic method for the determination of diterpenoids in the hexane and methanolic extracts from four species (*Sideritis biflora, Sideritis cillensis, Sideritis almeriensis and Sideritis luteola*); our aim is to contribute to their chemotaxonomic determination as well as to find a relationship between their content of terpenoids and their use in folk medicine.

## 2. Experimental

### 2.1. Reference substances

The terpenoid standards, serradiol, linearol, conchitriol, foliol, isofoliol, andalusol, lagascatriol, tobarrol, sidol and siderol were isolated from different species of *Sideritis* [6–9] and were purchased from the Institute of Organic Chemistry, CSIC (Madrid, Spain).

### 2.2. Vegetal material

*S. biflora, S. almeriensis* and *S. luteola* were collected in Almeria (Spain) in May, 1990; *S.*

*Corresponding author.

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*cillensis* was collected in Guadalajara (Spain) in August, 1988. An identified and classified specimen of each species is on deposit in the Botany Department Herbarium (M.A.F.) of the Faculty of Pharmacy, UCM, Madrid, Spain.

After collection, all samples were dried at room temperature, powdered and stored under suitable conditions until their use.

### *2.3. Extraction of diterpenoids*

Extraction of the diterpenoids was performed using a maceration and percolation extraction process at room temperature. Solvents of increasing polarity, *n*-hexane (3×150 ml, 72 h) and methanol (3×150 ml, 72 h), were used. The extracts obtained were concentrated to dryness below 35°C.

### *2.4. Chromatographic system*

HPLC analyses were carried out on a Varian 2510 pump with a Varian DS 604 data processor and a Varian polychrom 9065 photodiode-array detector, operating at $\lambda$=220 nm (at which waxes, chlorophylls and phenolic acids do not interfere). Chromatographic runs were performed on a Hypersil ODS 15 cm×4.6 mm I.D., 5 μm, column (Shandon Science, Astmoon, UK). Working solutions contained an exactly weighed quantity of 0.5–1 mg/ml of the *n*-hexane and methanol extracts (HPLC grade, Scharlau, Barcelona, Spain) and were filtered through 0.22 μm filters. The mobile phase was water–methanol (30:70. v/v) and elution was isocratic. All of the solvents were of HPLC grade (Scharlau and Milli-Q system) and were filtered through 0.45 μm filters and degassed before use. The flow-rate was 1 ml/min. The volume of sample injected was 10 μl.

A single analysis was used for detection of the ten standards.

### *2.5. Calibration graphs*

Several aliquots of the solution of each standard were diluted in the eluent to obtain reference solutions of decreasing concentration. These solutions were analyzed and the corresponding peak areas were compared against the concentration of ginsenosides injected. The concentrations of the components were calculated from the peak areas of the chromatogram, areas, using the normalization method.

Identification of the different compounds was achieved by comparing both the retention times ($t_R$) and the absorption spectra obtained for each eluted peak with those obtained for the standards.

### *2.6. Evaluation of peak purity*

To check peak purity, the eluates were monitored with a photodiode array detector ($\lambda$=190–390 nm). The three spectra corresponding to the up-slope, apex and down-slope of each peak were computer-normalized and superimposed. Peaks were considered pure when there was exact coincidence between the three spectra (match factor≥99.5).

## 3. Results and discussion

The retention time±S.D., the capacity factor and the selectivity of each standard ($t_0$=1.30 min; flow-rate=1 ml/min) are shown in Table 1. The diterpenoid content in the hexane and methanolic extracts, expressed in per cent of plant dry mass, is shown in Table 2 Table 3, respectively. Table 4 shows the final diterpenoid content for the species studied.

Table 1
Retention times, capacity factors and selectivity of diterpenoids

| Diterpenoid | $t_R$ (min) (±S.D.) | $k'$ | $\alpha$ |
|---|---|---|---|
| Serradiol | 1.681 (±0.041) | 0.27 | |
| Linearol (I) | 6.205 (±0.427) | 4.11 | 15.22 |
| Conchitriol (II) | 7.736 (±0.609) | 5.13 | 1.24 |
| Foliol | 8.138 (±0.216) | 5.37 | 1.04 |
| Isofoliol (III) | 8.409 (±0.659) | 5.68 | 1.05 |
| Andalusol | 10.415 (±0.487) | 6.48 | 1.14 |
| Lagascatriol | 11.906 (±0.944) | 7.29 | 1.12 |
| Tobarrol | 23.360 (±1.086) | 15.74 | 2.16 |
| Sidol | 26.388 (±0.630) | 19.30 | 1.23 |
| Siderol | 31.755 (±0.289) | 23.37 | 1.23 |

Hypersil ODS column: 15 cm×4.6 mm, 5 μm film thickness.
Solvent system: water–methanol (30:70, v/v), isocratic elution.
Total elution time: 35 min.
Flow-rate: 1.4 ml/min.
$t_0$: 1.10 min.

Table 2
Content of diterpenoids in the hexane extracts, expressed as a percentage of dry plant mass

| Diterpenoids | *Sideritis* species | | | |
|---|---|---|---|---|
| | *S. almeriensis* | *S. biflora* | *S. cillensis* | *S. luteola* |
| Serradiol | | 0.016 | 0.025 | 0.029 |
| Linearol | 0.010 | 0.001 | | |
| Conchitriol | 0.007 | 0.009 | | |
| Foliol | | 0.019 | 0.090 | |
| Isofoliol | 0.005 | 0.002 | | 0.009 |
| Andalusol | | 0.185 | | |
| Lagascatriol | | 0.007 | | |
| Tobarrol | | | | |
| Sidol | | 0.016 | | |
| Siderol | | | 0.006 | |

Table 3
Content of diterpenoids in the methanolic extracts, expressed as a percentage of dry plant mass

| Diterpenoids | *Sideritis* species | | | |
|---|---|---|---|---|
| | *S. almeriensis* | *S. biflora* | *S. cillensis* | *S. luteola* |
| Serradiol | | | 0.011 | 0.012 |
| Linearol | 0.023 | 0.008 | 0.036 | |
| Conchitriol | 0.005 | 0.003 | | |
| Foliol | | 0.005 | | |
| Isofoliol | 0.007 | | | 0.001 |
| Andalusol | | | 0.098 | 0.075 |
| Lagascatriol | 0.002 | | | 0.004 |
| Tobarrol | | | | |
| Sidol | | | | |
| Siderol | | | | |

Fig. 1 shows the structures of the standards that were studied. The calibration graphs obtained for each standard are shown in Fig. 2.

Fig. 3 show the chromatogram of the hexane extract of *Sideritis almeriensis*, under the analytical conditions previously described in Section 2.

On reviewing literature regarding the analysis of terpenoids, we found a lack of HPLC systems for studying these compounds, and this induced us to develop a new HPLC method to be applied to the study of the Genus *Sideritis*.

Successive assays were carried out to determine the optimum mobile phase. The composition of the mobile phase was varied from water–methanol (10:90, v/v) to 100% water and a water–methanol ratio of 30:70 (v/v) was found to be optimal, with isocratic elution. We obtained good chromatographic resolution within 35 min, as well as high repro-

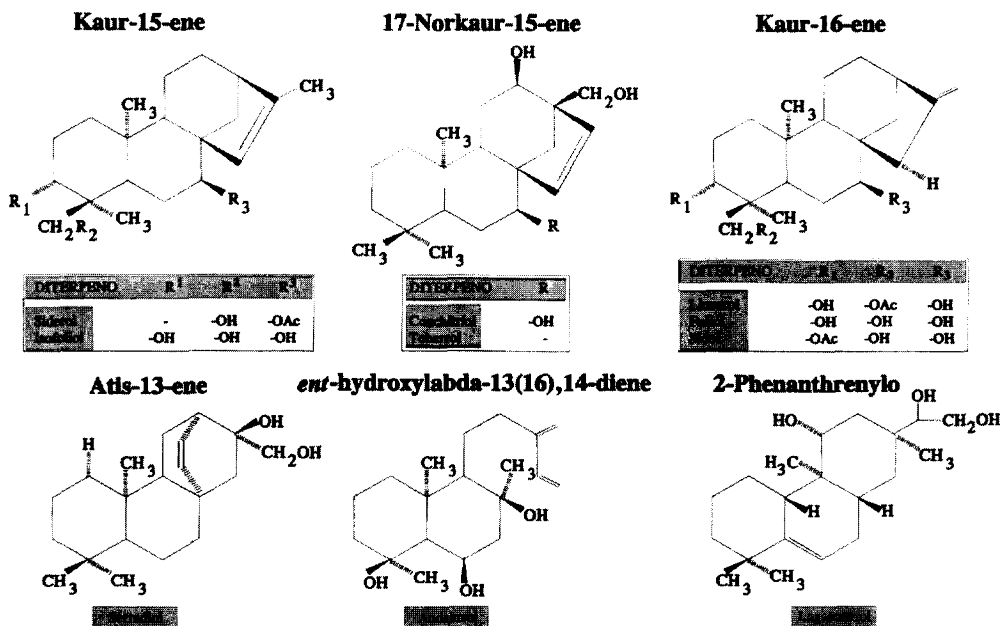



Fig. 1. Structure of terpenoids.

Table 4
Final content of diterpenoids in *Sideritis almeriensis*, *S. biflora*, *S. cillensis* and *S. luteola*, expressed as a percentage of dry plant mass

| Diterpenoids | *S. almeriensis* | *S. biflora* | *S. cillensis* | *S. luteola* |
|---|---|---|---|---|
| Serradiol | | 0.016 | 0.036 | 0.041 |
| Linearol | 0.033 | 0.009 | 0.036 | |
| Conchitriol | 0.012 | 0.012 | | |
| Foliol | | 0.024 | 0.090 | |
| Isofoliol | 0.012 | 0.002 | | 0.010 |
| Andalusol | | 0.185 | 0.098 | 0.075 |
| Lagascatriol | 0.002 | 0.007 | 0.007 | 0.004 |
| Tobarrol | | | | |
| Sidol | | 0.016 | | |
| Siderol | | | 0.006 | |

ducibility (S.D. for the retention times was lower than 0.5%, indicating highly reproducible separation). All of these characteristics make this method suitable for the analysis of *Sideritis* extracts and, in general, for the study of vegetal extracts.

In general, the diterpenoid content is higher in the

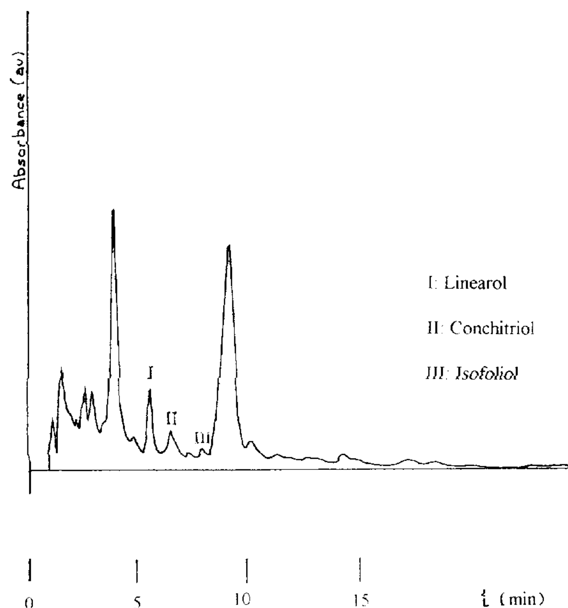
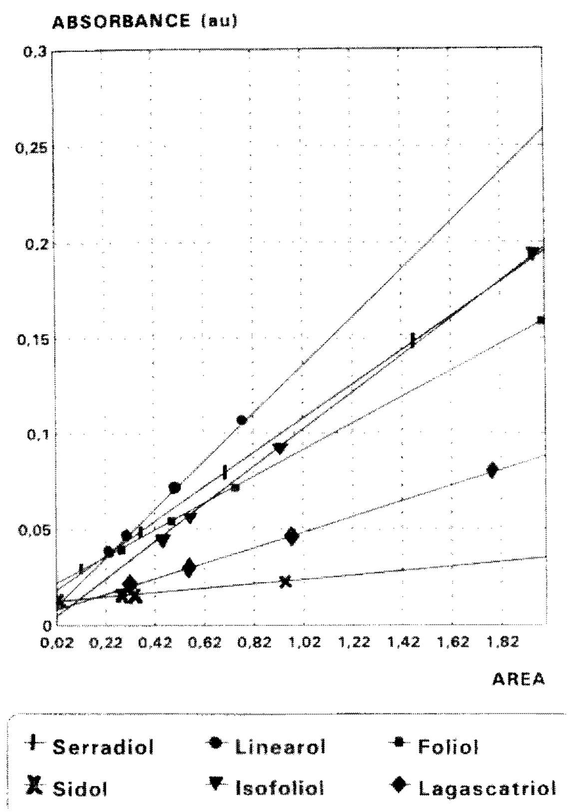



Fig. 3. Chromatogram of the hexane extract of *Sideritis almeriensis* under the analytical conditions described previously in Section 2.



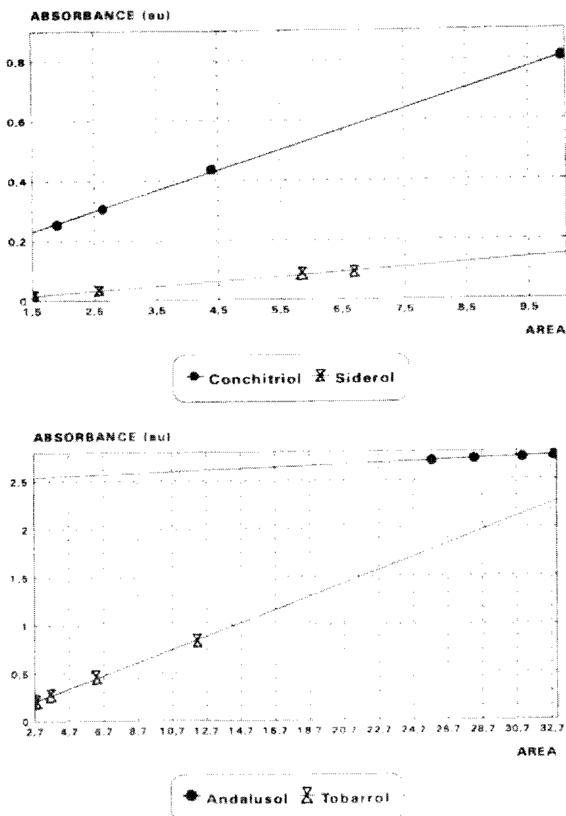




Fig. 2. Calibration graphs of the analyzed terpenoid standards.

hexane extracts than in the methanol extracts; andalusol was the most abundant terpenoid in both the hexane and methanol extracts.

Linearol is the most abundant terpenoid in *S. almeriensis*, while andalusol is the main terpenoid in *S. biflora, S. cillensis* and *S. luteola*. The anti-inflammatory activity of andalusol that was previously described [10] justifies the popular use of these species, especially *S. biflora* as anti-inflammatory agents.

The diterpenoids serve as taxonomic markers, therefore, we also intend to improve the taxonomy of this genus, along with the classification obtained after analysis of the flavonic compounds of several species of *Sideritis* [11,12].

## References

[1] A. Villar, J. Esplugues, M.J. Alcaraz, Planta Med. Phytother. 16 (1982) 157.
[2] A. Villar, M.A. Gascó, M.J. Alcaraz, J. Pharm. Pharmacol. 36 (1984) 820.
[3] C.V. Carstenn-Lichterfelde, S. Valverde, B. Rodríguez, Aust. J. Chem. 27 (1974) 517.
[4] A. Villar, R. Salom, M.J. Alcaraz, Pharmazie 38 (1984) 566.
[5] O.M. Palomino, P. Gómez-Serranillos, E. Carretero, A. Villar, J. Chromatogr. A 731 (1996) 103.
[6] T.G. De Quesada, B. Rodríguez, S. Valverde, Tetrahedron Lett. 22 (1972) 2187.
[7] T.G. De Quesada, B. Rodríguez, S. Valverde, Phytochemistry 14 (1975) 517.
[8] M.A. López, C. Von Carstenn-Lichterfelde, B. Rodríguez, J. Org. Chem. 42(14) (1977) 2517.
[9] F. Martín Panizo, B. Rodríguez, S. Valverde, An. Quim. 70 (1974) 164.
[10] A. Navarro, A.J. Godoy, B. de las Heras and A. Villar, 10th Int. Conference on Prostaglandins and Related Compounds, Vienna, September 1995.
[11] D. Rivera, C. Obón, F. Tomás-Lorente, F. Ferreres, F.A. Tomás-Barberán, Bot. J. Linnean Soc. 103 (1990) 325.
[12] F. Ferreres, F. Tomás-Lorente, F.A. Tomás-Barberán, Z. Naturforsch. C44 (1989) 568.