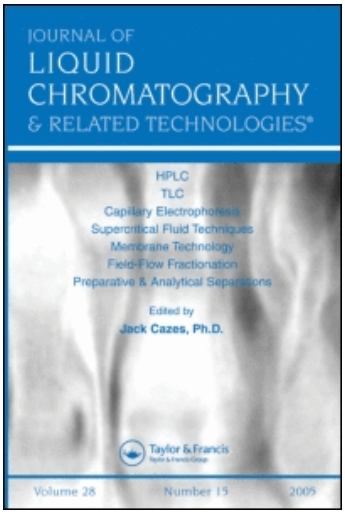

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HPLC/DAD ANALYSIS OF PHENOLIC COMPOUNDS FROM LAVENDER AND ITS APPLICATION TO QUALITY CONTROL

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ABSTRACT

A reversed phase HPLC procedure is proposed for the determination of eight phenolic compounds (2-O-glucosilcoumaric acid, *o*-coumaric acid, rosmarinic acid, apigenin-7-O-glucoside, coumarin, herniarin, luteolin, and apigenin) in lavender. The chromatographic separation was achieved using a reversed–phase Spherisorb ODS 2 (5 μm particle size: 25.0 x 0.46 cm) column. From the several extractive solvents assayed, only ethanol was able to extract all the mentioned compounds. Best resolution was obtained using a gradient of water-formic acid (19:1) and acetonitrile. Ten samples were subjected to quantification, all of them showing a common composition pattern.

INTRODUCTION

Lavender (*Lavandula officinalis* Chaix, *L. spica* Lois, *L. vera* D. C., *L. angustifolia* Mill. ssp. *angustifolia*, or *L. officinalis* Chaicah) is a species cultivated as a garden ornamental, as a source of essential oil largely used in perfumery, or as a condiment (non-alcoholic beverages, ice-creams, candies, chewing-gums).[1] The flowers are also present in several mixtures of medici-

nal plants used in phytotherapy, where they act as an aromatizing or for its own medicinal properties. In fact, lavender has been used to treat flatulent dyspepsia[2] or as a light sedative.[3] In folk medicine, its infusions are used as a diuretic and spasmolytic.[3]

The quality control of this species is made official, e. g. in the French pharmacopoeia,[4] where the chemical control is made by the TLC search of several constituents from the volatile oil. HPLC is now looked at as a more reliable and simple technique for routine analysis, and has the advantage of being easily adapted to quantification of individual compounds.

Since phenolic compounds are widespread in nature and have been successfully applied to quality control of several foodstuffs,[5] we tried to put at work, a methodology based on HPLC of the phenolic compounds of lavender that can also be used on a quantitative level.

Several extracts were prepared and analysed by HPLC using the eluents more usually described for the analysis of phenolics.[6] Subsequently, HPLC conditions were adjusted in order to obtain better resolution and applied to the analysis of 10 samples.

EXPERIMENTAL

Plant Samples and Standards

Plant samples were commercially available or collected in the North littoral of Portugal (Table 1). Authentic standards were obtained from Sigma Chemical Co. or from Extrasynthése. 2-O-Glucosylcoumaric acid was identified by comparing its RT and UV spectrum with 2-O-glucosylcoumaric acid present in a purified fraction of *Melilotus officinalis,* and by identification of hydrolysis products.

Extraction of Phenolic Compounds from Plants

For analytical purposes, 3g of lavender flowers were ground and extracted twice, with 50 mL of ethanol, 15 min each time, at room temperature, with agitation. The extract obtained was taken to dryness under reduced pressure at 30°C. The residue was dissolved in 1 mL of methanol and 20 μL were analysed by HPLC. The entire protocol was repeated using petroleum ether, chloroform, ethyl ether, ethyl acetate, acetone, methanol, ethanol 80% and 30%, and boiling water.

For quantification purposes, 3g of each sample were ground to pass through a 910 μm sieve and extracted, at room temperature, with agitation,

Table 1

Phenolic Contents of Lavender Samples

	Phenolic Compounds[a] (mg/Kg, Dry Basis)							
Sample	2-O-Glucosilcoumaric Acid	o-Coumaric Acid	Rosmarinic Acid	Apigenin 7-Glucoside	Coumarin	Herniarin	Luteolin	Apigenin
Ancede 30/07/98	107.90±5.60	10.90±1.60	110.20±0.06	33.20±1.10	26.30±1.10	48.30±1.20	14.40±0.60	46.60±1.20
Serralves 23/06/99	73.70±2.10	3.30±0.50	57.60±2.50	23.80±3.00	6.80±0.60	16.30±1.90	2.40±0.30	23.80±0.70
Penacova 25/06/99	97.50±8.70	4.00±0.50	60.50±3.50	15.20±0.60	12.40±1.70	14.90±0.60	3.50±0.20	25.00±7.00
Arcos de Valdevez 25/06/99	52.20±6.60	8.30±0.60	53.00±1.00	20.40±3.40	16.70±1.10	24.30±1.70	2.60±0.00	18.30±2.70
Arouca 30/06/99	40.30±1.70	3.90±0.30	58.10±1.90	18.70±0.90	14.30±0.10	20.40±0.90	5.80±0.40	31.70±0.20
Ovar 15/7/99	68.40±2.50	10.70±0.80	42.00±1.20	16.10±1.30	8.40±0.70	16.40±2.30	3.90±0.50	21.80±2.20
A*	121.00±2.10	35.60±3.60	118.40±6.90	142.80±3.40	37.00±2.00	172.50±7.20	26.20±1.60	117.10±4.20
B*	57.90±0.10	17.50±1.90	157.90±4.30	28.00±1.10	26.10±0.40	52.40±0.02	14.00±2.50	39.00±0.40
C*	37.50±0.60	10.00±0.60	126.70±2.70	27.00±3.00	34.30±3.10	54.60±3.30	28.00±0.20	39.00±3.50
D*	69.50±1.00	23.00±0.60	180.60±6.50	42.20±1.80	78.50±2.00	169.00±6.30	16.10±1.00	45.60±1.20

[a] Values are expressed as mean ± standard deviation of two assays for each sample. * Commercially available samples.

using ethanol: 50 mL for 15 min, followed by 50 mL (10 min) and, finally, 25 mL for 5 min. The three extracts were gathered, filtered, and taken to dryness under reduced pressure at 30°C. The residue was dissolved in 2 mL of methanol and 20 µL was analysed by HPLC.

HPLC Analysis of Phenolic Compounds

Separation of phenolic compounds was achieved with an analytical HPLC unit (Gilson), using a reversed-phase Spherisorb ODS 2 (5 µm particle size; 25.0 x 0.46 cm) column. For analytical purposes, the solvent system used (gradient nº 1) was a gradient of water/formic acid (19:1) (A) and methanol (B). The gradient was as follows: 0 min- 30% B; 15 min - 30% B; 20 min – 40% B; 30 min – 45% B; 50 min – 60% B; 51 min – 80% B; 52 min – 80% B. Elution was performed at a solvent flow rate of 1 mL/min. Detection was accomplished with a diode-array detector and chromatograms were recorded at 280, 320, and 350 nm. The different phenolic compounds were identified by comparing their retention times and UV-vis spectra in the 200-400 nm range with authentic standards.

For quantitative purposes, the solvent system (gradient nº2) used was a gradient of water/formic acid (19:1) (A) and acetonitrile (B). The gradient was as follows: 0 min – 17% B; 35 min – 23% B; 52 min – 49% B. Elution was performed at a solvent flow rate of 1 mL/min. Quantification of phenolics was achieved by the absorbance recorded in the chromatograms relative to external standards at 320 nm. 2-O-Glucosylcoumaric acid was quantified as *o*-coumaric acid.

RESULTS AND DISCUSSION

Bearing in mind that lavender has no characteristic chemical marker, we must consider that a species is, at best, characterised as having a higher number of compounds than can be identified in it. So, the samples were initially subjected to extraction by several solvents, in order to choose what solvent was able to extract a higher number of compounds, preferably identified by standards commercially available. All the extracts were analysed using gradient nº 1. The extract obtained with petroleum ether was characterised by the presence of coumarin and herniarin (Figure 1-A) and, on increasing the polarity of the solvents, other compounds, namely phenolic acids and flavonoids, began to be detected on the chromatograms (Figure 1-B and 2). On the first part of the chromatograms of the more polar extracts, a set of signals are observed that exhibited the UV characteristics of the glycosyl derivatives of coumaric acid; two of them already described in this species (2-O-glucosylcoumaric acid and 2-O-glucosyl-4-methoxycoumaric acid).[7]

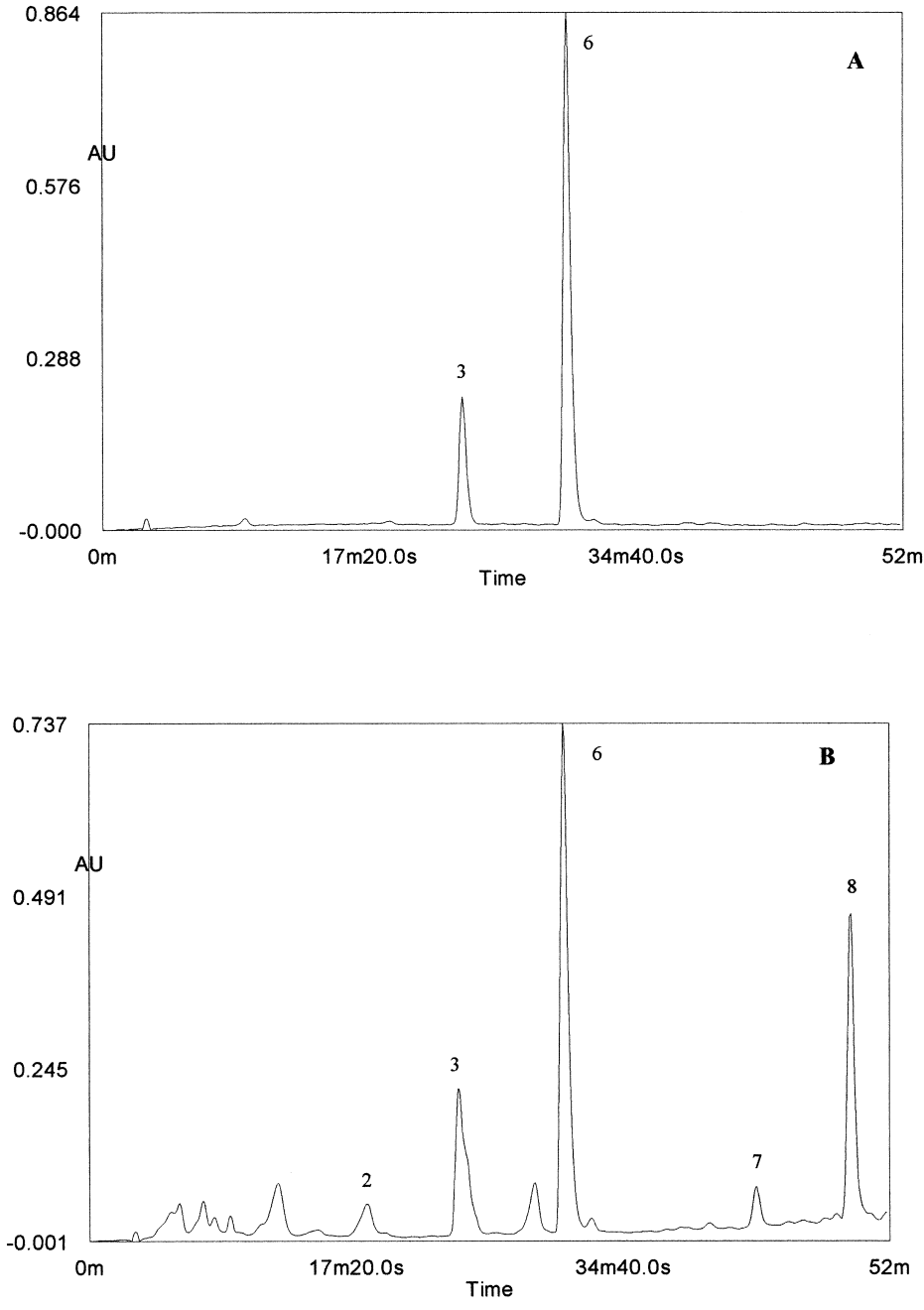
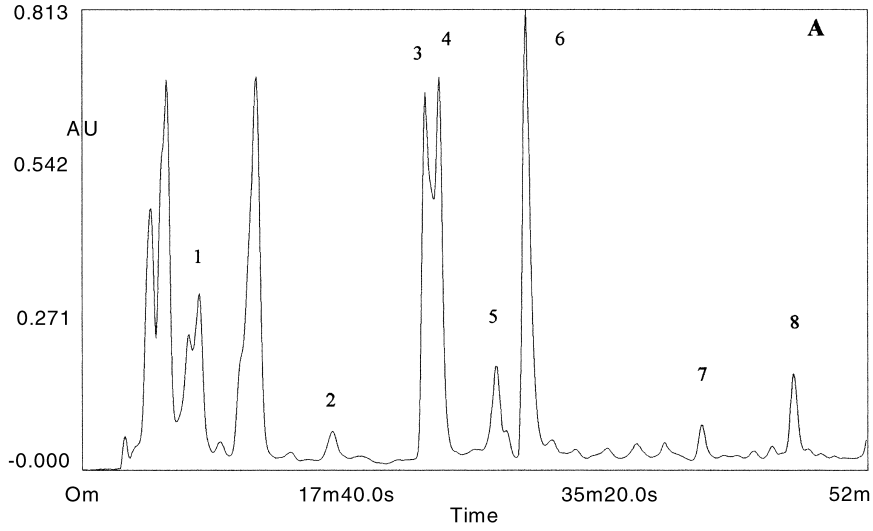


Figure 1. HPLC profile of a lavender sample (gradient nº 1), extracted with petroleum ether (A) and ethyl ether (B). Detection at 320 nm. Peaks: (2) *o*-coumaric acid; (3) coumarin; (6) herniarin; (7) luteolin and (8) apigenin.



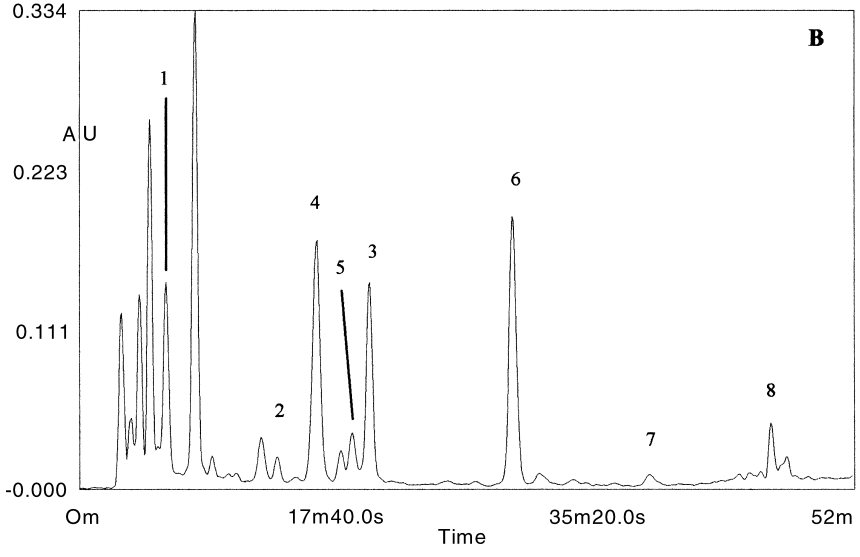


Figure 2. HPLC profile of a lavender sample extracted with ethanol with gradient nº 1 (A) and gradient nº 2 (B). Detection at 320 nm. Peaks: (1) 2-*O*-glucosilcoumaric acid; (2) *o*-coumaric acid; (3) coumarin; (4) rosmarinic acid; (5) apigenin 7-O-glucoside; (6) herniarin; (7) luteolin and (8) apigenin.

Table 2

Lavender Compounds Separated by HPLC

No.	Compound	RT Gradient No. 1	RT Gradient No. 2
1	2-O-Glucosilcoumaric Acid	8m23s	6m02s
2	*o*-Coumaric Acid	18m59s	15m31s
3	Coumarin	24m00s	23m12s
4	Rosmarinic Acid	25m22s	18m10s
5	Apigenin 7-O-Glucoside	26m34s	20m14s
6	Herniarin	29m36s	34m08s
7	Luteolin	41m30s	41m50s
8	Apigenin	47m26s	48m56s

The solvent that exhibited the higher number of compounds was ethanol (Figure 2). On using this solvent, it was possible to identify almost all the phenolic compounds so far described to exist in the species (2-O-glucosyl-coumaric acid, *o*-coumaric acid, coumarin, rosmarinic acid, herniarin, luteolin)[3,8,9] and, still, apigenin and an apigenin derivative. The signal due to the apigenin derivative can be atributed either to apigenin 7-glucoside or to apigenin 7-rutinoside, since both compounds have the same UV spectra and the same RT in gradient nº 1. The resolution obtained with this gradient is not very good and, therefore, not quite suited to qualitative analysis. Besides, the signals of the glycosyl derivatives of coumaric acid are split by the *cis/trans* isomerism, which adds an unnecessary complexity to the chromatogram. So, several other eluent systems were tried and best results were obtained employing acetonitrile and gradient nº2. (Figure 2-B). On using this gradient, coumarin and rosmarinic acid are well resolved; the coumaric acid derivatives are not split and it is possible to guarantee that compound 5 is apigenin 7-glucoside, since the two possible apigenin derivatives have different RT with this gradient (Table 2). A mixture of the seven quantified compounds was run with this gradient (Figure 3).

For quantification purposes, and in order to guarantee a full extraction of the phenolic compounds and the repeatability of the method, one sample was subjected to a set of six extraction conditions, using different times and volumes of solvent (data not shown). Best results were obtained using a three-fold extraction (see EXPERIMENTAL). The tested analytical conditions were applied to several samples collected during 1998 and 1999 in the littoral North of Portugal and to four commercially available samples (Table 1). Quantification was performed at 320 nm, a wavelength at which all the com-

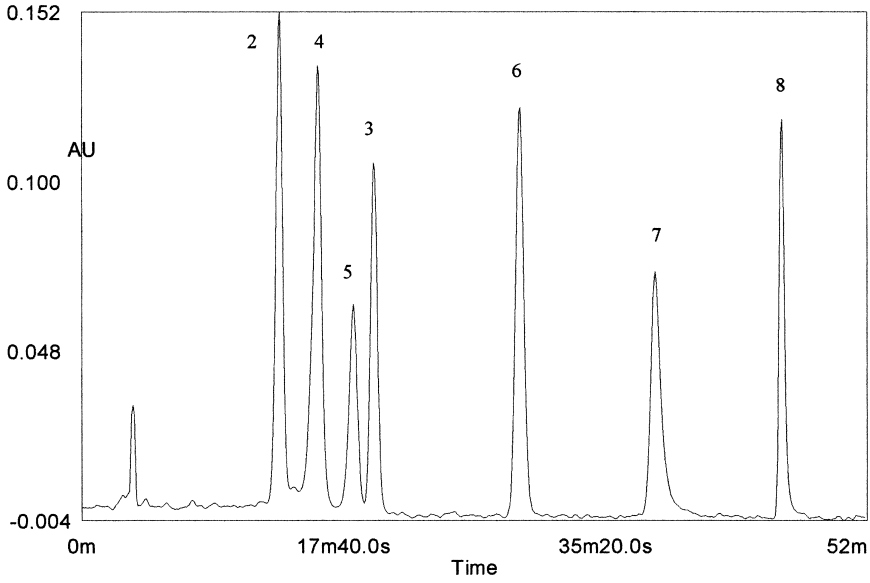


Figure 3. HPLC of a solution of standards commercially available (gradient nº 2). Detection at 320 nm. Peaks: (2) *o*-coumaric acid; (3) coumarin; (4) rosmarinic acid; (5) apigenin 7-O-glucoside; (6) herniarin; (7) luteolin and (8) apigenin.

pounds are detected and can be quantified by the software available. The use of only one wavelength makes routine analysis more feasible, allowing the quantification of all the compounds in one run, even when a diode-array is not available.

All the samples showed a common pattern of compounds, where the most abundant phenolics are 2-O-glucosilcoumaric acid and rosmarinic acid, while *o*-coumaric acid and luteolin are the minor ones (Figure 4). As can be seen, a reasonable proportion is observed among compounds 2, 3, 4, 5, 7, and 8, both in samples collected by us, or in samples from the market. With this last set of samples, and probably due to natural hydrolysis occurring during storage, a relative decrease is observed on 2-O-glucosylcoumaric acid, which is transformed to coumarin, and an increase of herniarin is also observed that can be due to the hydrolysis of compounds observed in the first part of the chromatogram, namely to the referred 2-O-glucosyl-4-methoxycoumaric acid.

Another striking fact, is that samples commercially obtained showed higher amounts of phenolics. Although, the geographical origin of the commercial samples is unknown, they are probably cultivated in the South of

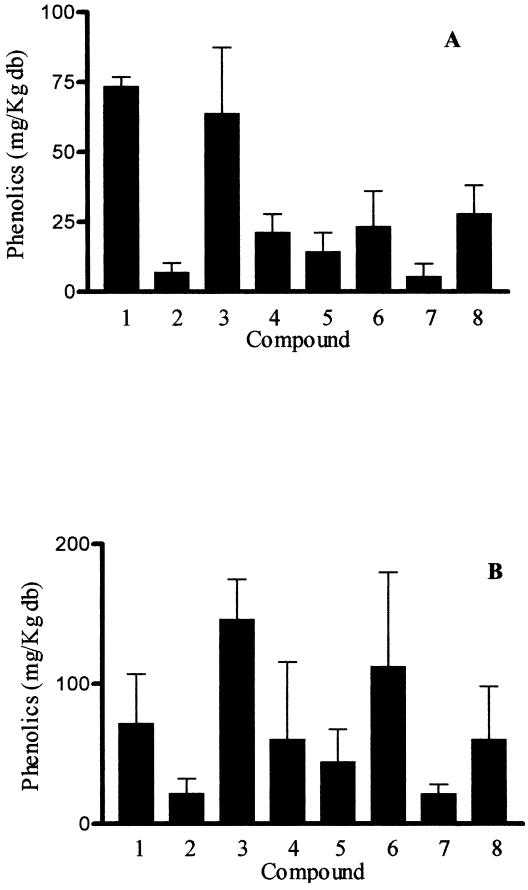


Figure 4. Phenolic amounts in samples of known origin (A) and commercially available (B): (1) 2-*O*-glucosilcoumaric acid; (2) *o*-coumaric acid; (3) coumarin; (4) rosmarinic acid; (5) apigenin 7-O-glucoside; (6) herniarin; (7) luteolin and (8) apigenin. Results are means of 6 (A) and 4 (B) replicates, and standard error bars are on top of each column.

Portugal or even imported from other countries, since it is a known fact that lavender is a shrub of Mediterranean climates.

In conclusion, this study suggests that the technique herein is quite useful for the analysis of phenolic compounds in lavender, allowing its quality control.

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REFERENCES

1. M. Vincenzi, F. Maialetti, M. Dessi, Fitoterapia, **62**, 39-63 (1991).

2. W. C. Evans, **Trease and Evans's Pharmacognosy,** 13 ed., Baillière Tindall, London, 1989, pp. 433-434.

3. J. van Helemont. **Compendium de Phytotherapie**, Association Pharmaceutique, Belgue, 1986, pp. 226-227.

4. **Pharmacopée Française**, X^{e} Édition, 1989.

5. P. B. Andrade, A. R. F. Carvalho, R. M. Seabra, M. A. Ferreira, J. Agric. Food Chem., **46**, 968-972 (1998).

6. P. B. Andrade, R. M. Seabra, P. Valentão, F. Areias, J. Liquid Chrom. & Rel. Technol., **21(18)**, 2813-2820 (1998).

7. S. Brown, Science, **137**, 977-978 (1962).

8. R. Paris, H. Moyse, **Matiére Médicale, Vol. 3**, Masson & Cie., Paris, 1971, pp. 256-264.

9. S. Brown, Phytochemistry, **2**, 137-144 (1963).

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