ELSEVIER

Journal of Chromatography A, 663 (1994) 119–122

JOURNAL OF CHROMATOGRAPHY A

Short Communication

High-performance liquid chromatographic determination of atractyloside and carboxyatractyloside from *Atractylis gummifera* L.

M. Calmes, F. Crespin, C. Maillard, E. Ollivier, G. Balansard*

Laboratoire de Pharmacognosie, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille cédex 5, France

(First received July 27th, 1993; revised manuscript received November 23rd, 1993)

Abstract

A reversed-phase high-performance liquid chromatographic method is described for the identification and determination of two toxic compounds, atractyloside and carboxyatractyloside, in a 60% methanolic extract of *Atractylis gummifera* L. roots. Separation was achieved in 55 min with a water–acetonitile gradient in the presence of trifluoroacetic acid. Detection was effected with a light-scattering detector. The detection limit was determined and was *ca.* 9 μg of atractyloside standard injected. The method of quantification was validated.

1. Introduction

Atractylis gummifera L. (Asteraceae) is a toxic plant widely distributed in mediterranean countries. In the north of Africa many intoxications from *Atractylis gummifera* have been reported, mainly accidental but also criminal or suicidal. Intoxications are fatal in most cases because there is no effective treatment. Symptoms are respiratory, vascular and nervous difficulties and disturbance of glycaemia and anuria. Intoxications are induced by oral, respiratory and cutaneous routes [1–3]. This toxicity is related mainly to two heterosides: atractyloside (or atractyline) and carboxyatractyloside (or gummiferine) (Fig. 1) [4]. Atractyloside and carboxyatractyloside are both specific inhibitors of mitochondrial oxidative phosphorylation; this inhibition is more important from carboxyatractyloside [5,6].

Several quantitative assays but no specific methods for these compounds have been described. All used spectrophotometric measurement after a chromogenic reaction [7–10]. The

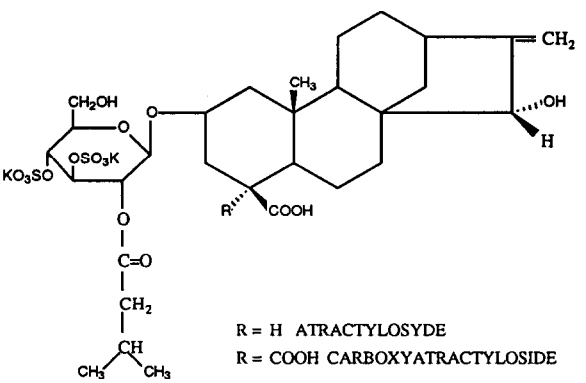

Fig. 1. Structure of atractyloside and carboxyatractyloside.

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1255-X

limit of detection is *ca.* 1 μg. No HPLC method has been reported because these compounds are not detectable by UV spectrophotometry.

In this paper we describe a procedure for the determination of these two toxic compounds by HPLC with light-scattering detection.

2. Experimental

2.1. Sample preparation

Fresh roots of *Atractylis gummifera* (5 g), collected in Tunisia (Zaghouan), were lyophilized and extracted with 40 ml of water–methanol (40:60, v/v) under reflux for 30 min and filtered without cooling. The volume was completed to 50 ml with the same solvent. A 20-ml volume of this solution was evaporated to dryness under vacuum. The residue was dissolved in 10 ml of water–methanol (40:60, v/v).

Standard (atractyloside) was dissolved in water–methanol (60:40, v/v) to give a concentration of 1 mg/ml. All samples were filtered on a 0.2-μm Dynagard filter (Merck, Montluçon, France).

2.2. Apparatus and conditions

The liquid chromatograph consisted of a U6K universal injector, two M 501 solvent-delivery systems (Waters, St. Quentin en Yveline, France) and a Cunow DDL 21 light-scattering detector (Waters) connected to an NEC computer to monitor chromatographic parameters and process data.

The column was LiChrosorb RP-8 (7 μm) (250 × 4 mm I.D.) from Merck and a μBondapak C_8 (15–20 μm) guard column (25 × 4 mm I.D.) from Waters.

The eluent was water containing 0.05% trifluoroacetic acid (TFA). (Merck) (solvent A) and acetonitrile (pesticide residue analysis grade; Prolabo, Paris, France) (solvent B), with a linear gradient from 10 to 30% of solvent B over 40 min. The solvents were filtered through a 0.45-μm Millipore filter. A re-equilibration period of 10 min was necessary. The flow-rate was 1 ml/min and the injection volume was 20 μl.

For evaporative light-scattering detection, nebulization of the eluent was provided by a stream of pressurized air at 2.2 bar. The nebulized solvent was evapored at 64 ± 2°C. The pressurized air was filtered through a Millex FG_{50} filter (0.2 μm) (Millipore).

3. Results and discussion

3.1. Optimization of detection conditions

The optimum operatory conditions were established by studying the temperature of evaporation and the pressure of nebulization. At low temperature the detector response was maximum, but the noise level was too high. The signal-to-noise ratio was better at 64 ± 2°C. On the other hand, at low pressures the noise level was high. To obtain an acceptable noise level it was necessary to work at an evaporation temperature of about 64 ± 2°C and a nebulization pressure of 2.2 bar.

3.2. Chromatography

A chromatogram of *Atractylis gummifera* L. root extract was obtained with a water–acetonitrile gradient in the presence of TFA in 55 min, as shown in Fig. 2.

Two compounds were identified by means of authentic standards: carboxyatractyloside (Fluka, St. Quentin Fallavier, France) and atractyloside (Extrasynthèse, Lyon, France), the retention times of which were 22.77 and 27.21 min, respectively. Determination of carboxyatracthyloside and atractyloside in this extract was achieved by the external standard method with the atractyloside standard. The contents of carboxyatractyloside and atractyloside were expressed as atractyloside.

Extract of *Atractylis gummifera* L. roots was analysed using the above procedure. Roots were extracted with water–methanol (40:60, v/v) as described previously. The contents of atrac-

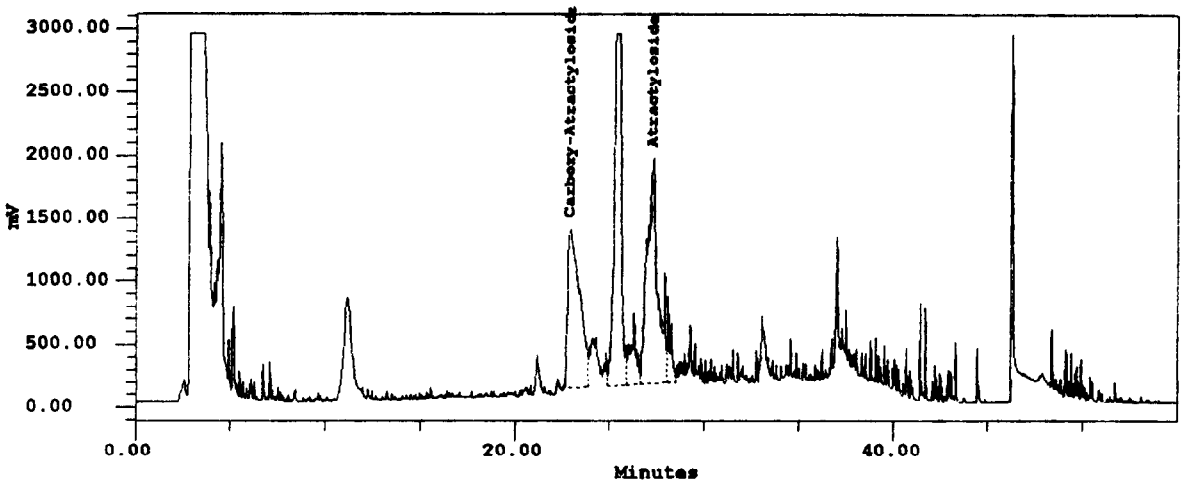

Fig. 2. Chromatogram of *Atractylis gummifera* extract.

tyloside and carboxyatractyloside in the roots were 0.981% and 0.63%, respectively, or 1.45% expressed as atractyloside.

3.3. Validation

The method was tested on atractyloside as the external standard. The regression curve calculated by assaying standard solutions three times consecutively showed a sigmoidal response of the detector in the concentration range 0.6–2.4 mg/ml; a plot of peak area *versus* sample concentration in double logarithmic coordinates was linear. The calibration graph is shown in Fig. 3. The results of the regression analysis and the correlation coefficient (r) were $\log C = 0.610 \log A - 4.386$ ($r = 0.9990$), where C is the concentration in mg/ml and A is the peak area.

Repeatability at 0.95 mg/ml ($n = 10$) gave a relative standard deviation (R.S.D.) of 1.064%. The reproducibility for a standard preparation tested by assaying four solutions at a concentration of 1.25 mg/ml showed an R.S.D. of 1.51%. The reproducibility of the extraction method displayed an R.S.D. of 2.89% ($n = 8$).

The detection limit was determined and was *ca.* 9 μg of atractyloside standard injected, corresponding to a methanolic solution of 0.450 mg/ml.

4. Conclusions

This method is not as sensitive as spectrophotometric methods [7–10], but it is more specific. The complete separation and the simultaneous determination of the two toxic compounds in *Atractylis gummifera* L., atractyloside and carboxyatractyloside, can be achieved in 55 min. This is the first HPLC method to be described for these compounds using light-scattering detection.

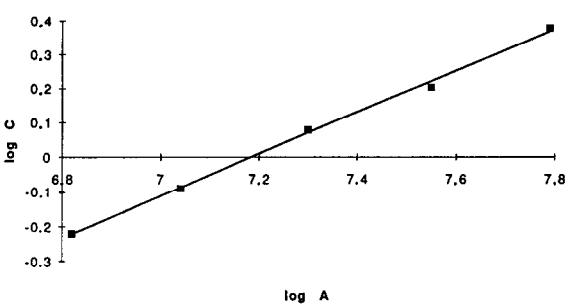

Fig. 3. Calibration graph for atractyloside.

5. References

[1] G. Chardon, A. Viala, P. Vignais and E. Stanislas, *Therapie*, 19 (1964) 1313.
[2] M. Georgiou, D. Biol, L. Sianidou, T. Hatzis, J. Papadatos and A. Koutselinis, *Clin. Toxicol.*, 26 (1988) 487.
[3] M.L. Martin, A. Moran and L. San-Roman, *An. R. Acad. Farm.*, 51 (1985) 751.
[4] F. Piozzi and A. Quilico, *Tetrahedron Lett.*, 23 (1965) 1829.
[5] A.C. Passam and J.O.D. Coleman, *J. Exp. Bot.*, 26 (1975) 536.
[6] A. Hedili, J.M. Warnet, M. Thevenin, C. Martin, M. Yacoub and J.R. Claude, *Arch. Toxicol., Suppl.*, 13 (1989) 312.
[7] E. Stanislas, A. Viala and P. Tourte, *Toulouse Pharm.*, 7 (1960) 1.
[8] A. Viala and J.P. Cano, *Bull. Soc. Pharm. Marseille*, 59 (1966) 161.
[9] A. Viala and J.P. Cano, *Bull. Soc. Pharm. Marseille*, 59 (1966) 167.
[10] A. Viala and J.P. Cano, *Bull. Soc. Pharm. Marseille*, 59 (1966) 175.