Revista Brasileira de Ciências Farmacêuticas
*Brazilian Journal of Pharmaceutical Sciences*
vol. 39, n. 3, jul./set., 2003

RBCF

# Quantification of furanoheliangolides by HPLC and GC

**Pierre Alexandre dos Santos, Izabel Cristina Casanova Turati, José Carlos Tomaz, Norberto Peporine Lopes***

*Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto*

*Correspondence:
N.P. Lopes
Departamento de Física e Química
Faculdade de Ciências Farmacêuticas - USP/Ribeirão Preto
Av. do Café, s/n
14040-903 - Ribeirão Preto - SP
E-mail: npelopes@fcfrp.usp.br

*The development and comparison of two analytical methods (HPLC and GC) for the quantification of the most common furanoheliangolides from Lychnophora is reported in this paper. Both methods are sensitive and suitable for quantification of these metabolites.*

**Uniterms**
- Furanoheliangolide
- *Lychnophora*
- HPLC
- GC

## INTRODUCTION

The genus *Lychnophora* is endemic to Brazil (Semir, 1991) and the hydro-alcoholic extract of leaves and the inflorescence of some species are used as analgesic and anti-inflammatory agents (Cerquiera *et al.*, 1987; De Oliveira *et al.*, 1996). Furanoheliangolides are the major secondary metabolites of *Lychnophora* (Bohlmann *et al.*, 1982), and exhibit several biological activities. A recent study has revealed that these compounds modulate the inflammatory process by inhibition of the transcription of the factor NF-kB by selective alkylating the p65 sub-unit (Rüngeler *et al.*, 1999). Despite its use in Brazilian folk medicine and the commercial value of *Lychnophora* species as analgesic and anti-inflammatory agents, some of them do not accumulate furanoheliangolides and preliminary results demonstrate seasonal and circadian variation in this metabolism. The literature reports the determination of lactones using HPLC and GC and both systems have been used for studying lactones on an analytical scale (Leven, Willuhn, 1987; Dolman *et al.*, 1992), presenting some limitations concerning separation time or peak resolution. The aim of this work was to develop an analytical methodology in HPLC and GC to monitor differences in furanoheliangolide metabolism. In this study the most common sesquiterpene lactones that occur in the genus *Lychnophora*: goyazensolide centratherin, lychnopholide, eremantholide C, 15-deoxygoyazensolide and 15α-4,5-dihydroeremantholide C (Figure 1) were analyzed.

## MATERIAL AND METHODS

### Reagents and standard solutions preparation

All solvents used were either of analytical or HPLC grade. The sesquiterpene lactones analyzed (Figure 1) were previously isolated from species of *Lychnophora* in our laboratory (Vichnewski *et al.*, 1989; Borella *et al.*, 1992; Lunardello *et al.*, 1995) .

Standard solutions of each sesquiterpene lactone under study (Figure 1) were prepared in methanol for HPLC analysis and in dichloromethane for GC analysis.

Coumarin was added as an internal standard both for the HPLC and GC analysis.

### High-performance liquid chromatography

In this study, a Shimadzu LC-6A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a Rheodyne injector fitted with a 20 mL loop, CR-6A integrator, SCL-6B system controller and an SPD-6AV UV detector set at 265 nm was used. The analytical column was a reversed-phase Shim-Pack ODS, 250 × 4.6 mm I.D.,

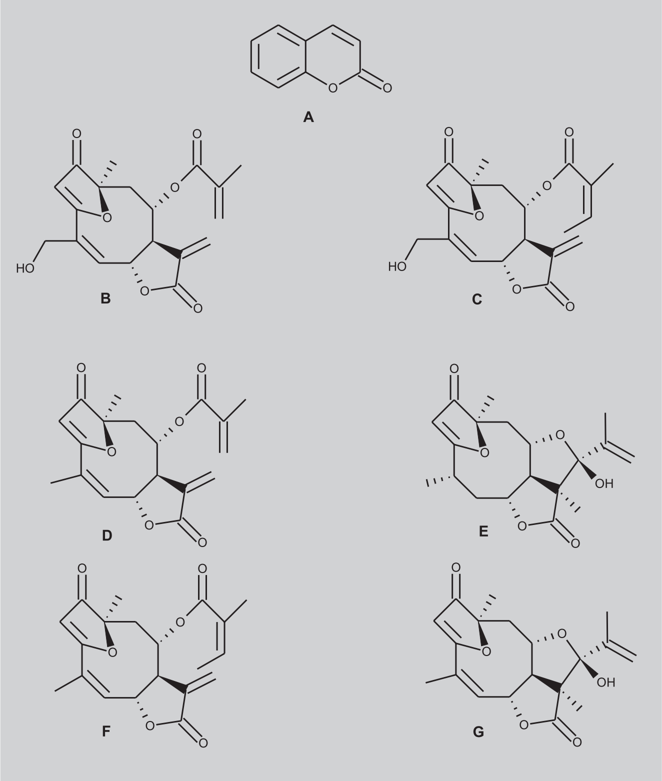

**FIGURE 1** - Structures of (**A**) coumarin (internal standard); (**B**) goyazensolide; (**C**) centratherin; (**D**) 15-deoxygoyazensolide; (**E**) 15a-4,5-dihydroeremantholide C; (**F**) lychnopholide; (**G**) eremantholide C

5 µm, from Shimadzu, fitted with a guard column (10 × 4.6 mm I.D.) packed with similar material (Merck, Darmstadt, Germany). The mobile phase used was a mixture of MeOH:$H_2O$. The mobile phase gradient program was as follows: star at 30% MeOH, increasing to 60% MeOH in 15 min, held constant until 40 min, then return to 30% MeOH in 5 min. The next sample was injected after a further 5 min. The flow-rate was 1.0 mL min$^{-1}$, at room temperature. The injection volume was 20 µL.

### Gas chromatography

Gas chromatography was performed on a Hewlett Packard GC 5890 Series II (Wilmington, DE., USA) using a HP-1 column (30 m × 0.25 mm × 0.25 µm), FID detector at 300 ºC, manual injector at 250 ºC and hydrogen as carrier gas (linear velocity 39 cm/s). Column oven temperature conditions as follows: initial temperature 100 ºC, held for 1min then increased at 9 ºC/min to 225 ºC, then held constant for 5 min and increased at 3 ºC/min to 280 ºC, where it was held constant for 15 min, cool down to 100 ºC in 2.2 min, and finally held constant for 4 min. 2 µL of the samples were injected on the column, at a split ratio of 1:90.

## RESULTS AND DISCUSSION

The HPLC separation is shown in Figure 2 and the corresponding structures can be seen in Figure 1. All six lactones could be well separated within less then 40 min. According to the method of Passreiter (1998), an isocratic system showed better results for the quantitative determination of furanoheliangolides in *Neurolaena lobata*, but in the present study a gradient was used for better resolution of the peaks.

Furanoheliangolides could be detected at very low concentrations, considering that only 20 µL of the sample were injected. All compounds were easily detectable from concentrations of 1.5 µg mL$^{-1}$ upwards, and the results are highly reproducible. Earlier a quantitative method by HPLC was used to quantify sesquiterpene lactones containing a α-methylenebutyrolactone group in *Tanacetum parthenium.* However, prior derivatization was

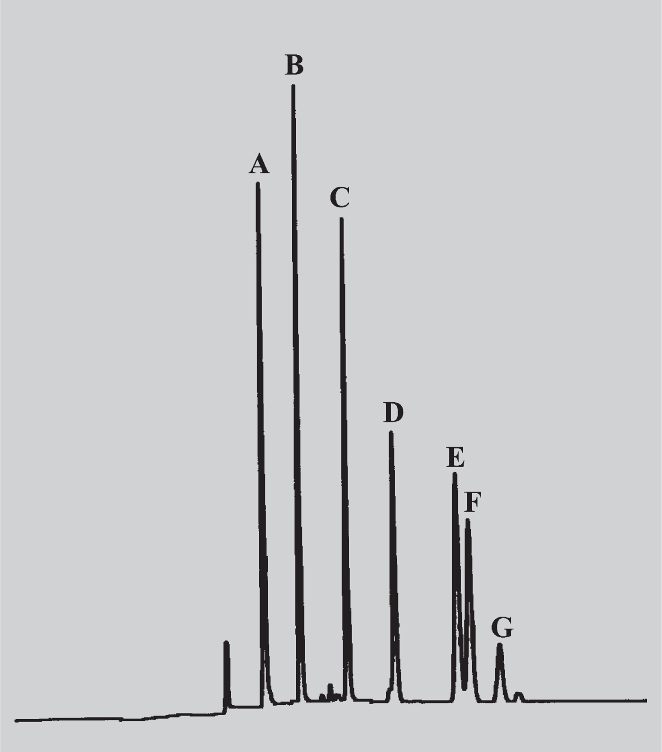

**FIGURE 2 -** HPLC chromatogram of foranoheliangolides from *Lychnophora* with coumarin as internal standard. (**A**) coumarin; (**B**) goyazensolide; (**C**) centratherin; (**D**) lychnopholide; (**E**) 15α-4,5-dihydroeremantholide C; (**F**) 15-deoxygoyazensolide; (**G**) eremantholide C. Chromatographic conditions in the text.

necessary to introduce alkylthiols containing a large chromophore by Michael addition to the α-methylene-butyrolactone function (Leven, Willhun, 1987; Dolman *et al.*, 1992). The determinations developed in this study do not require this treatment, and good resolution is obtained at room temperature.

A GC method for quantification of furanoheliangolides was also developed. With our procedure, a good separation of the lactones is possible within 28 min (Figure 3), using coumarin as the internal standard. Temperature gradient optimization was necessary.

Peak area linearity for the RP-HPLC and GC methods was checked by analyzing the samples at various concentrations. Linear relationships were found between the peak areas and the analyte concentrations. For the HPLC method developed, good linearity was obtained for all lactones between 1.5 and 150 μg mL$^{-1}$. Calibration plots and regression data for all analytes were linear ($r$>0.997) at the tested concentrations. For the GC analysis between 0.15 and 1.5 μg mL$^{-1}$, calibration plots and regression data for all lactones were linear ($r$ > 0.996) at the tested concentrations. Each calibration level was run in triplicate. The average slopes and $y$-intercepts of the regression line, and correlation coefficients ($r$) are shown in Table I.

## CONCLUSIONS

The data demonstrated that the analytical methodologies using RP-HPLC and GC developed in this study were suitable for the separation and quantification of the furanoheliangolides most common in the genus *Lychnophora*. These systems are applicable for detection and quantification of these metabolites without any prior derivatization. Both systems showed a good resolution for all tested lactones and all peaks were resolved in GC, and in RP-HPLC at room temperature.

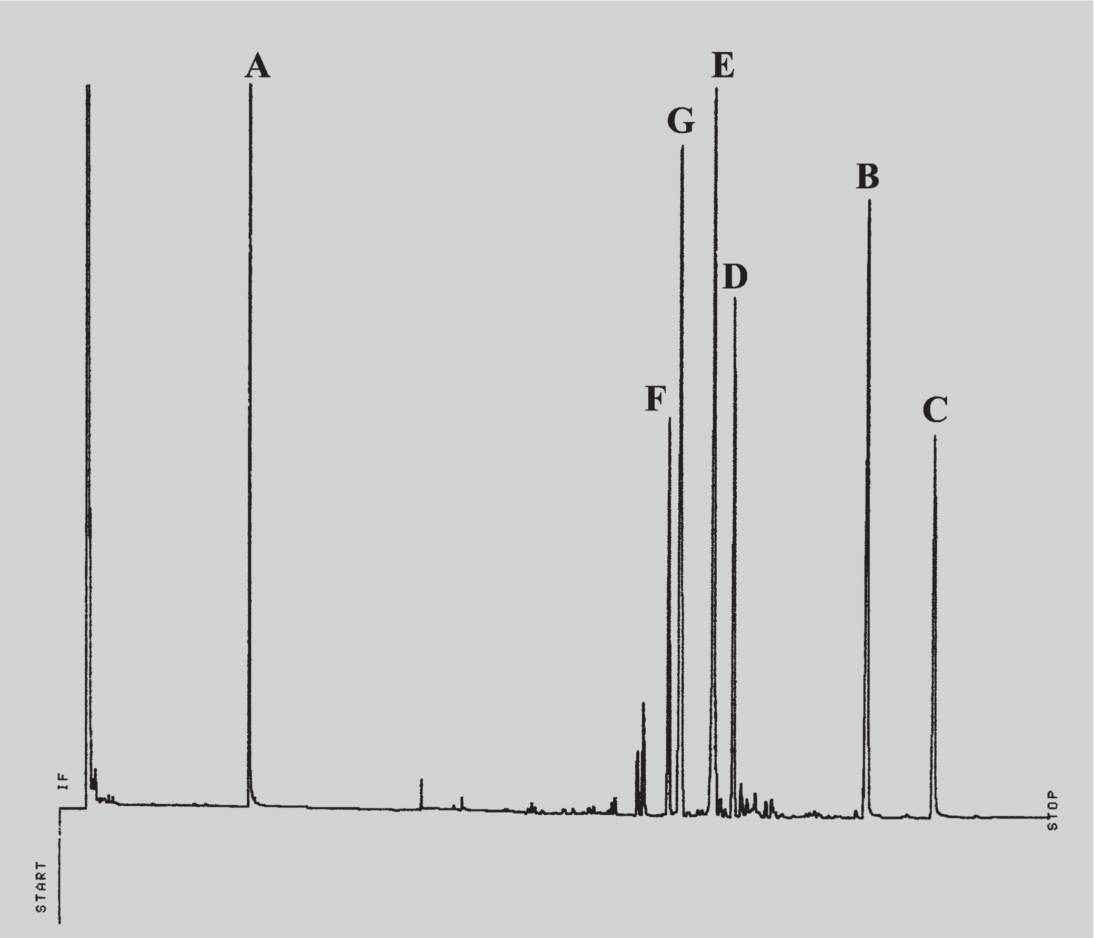

**FIGURE 3** - GC chromatogram of furanoheliangolides from *Lychnophora* with coumarin as the internal standard. (**A**) coumarin; (**B**) goyazensolide; (**C**) centratherin; (**D**) lychnopholide; (**E**) 15α-4,5-dihydroeremantholide C; (**F**) 15-deoxygoyazensolide; (**G**) eremantholide C. Chromatographic conditions in the text.

**TABLE I -** Linear regression equations for quantitative analysis of sesquiterpene lactones by HPLC and GC

| | HPLC | | | GC | | |
|---|---|---|---|---|---|---|
| Analyte | Regression equation | *r* | Concentration (mg/mL) | Regression equation | *r* | Concentration (mg/mL) |
| Goyazensolide | $Y = 0.10155 + 1.8808x$ | 0.9984 | 1.5 – 150 | $y = -0.60223 + 45.4981x$ | 0.9993 | 0.15 - 1.5 |
| Centratherin | $Y = 0.08961 + 1.6156x$ | 0.9976 | 1.5 – 150 | $y = -0.45632 + 41.6328x$ | 0.9996 | 0.15 - 1.5 |
| Lychnopholide | $Y = 0.01921 + 1.1836x$ | 0.9984 | 1.5 – 150 | $y = 0.01031 + 46.7642x$ | 0.9977 | 0.15 - 1.5 |
| Eremantholide C | $Y = 0.01921 + 0.4206x$ | 0.9986 | 1.5 – 150 | $y = -0.10789 + 13.7212x$ | 0.9997 | 0.15 - 1.5 |
| 15-Deoxygoyazensolide | $Y = 0.06008 + 1.2065x$ | 0.9987 | 1.5 – 150 | $y = 0.05166 + 42.3369x$ | 0.9960 | 0.15 - 1.5 |
| 15a-4,5-Dihydroeremantholide C | $Y = 0.05586 + 1.3475x$ | 0.9980 | 1.5 – 150 | $y = -0.36854 + 33.0132x$ | 0.9991 | 0.15 - 1.5 |

$y$ = peak area of lactone/peak area internal standard, $x$ = calculated mass, $r$ = correlation coeficient.

## RESUMO

### Quantificação dos furanoeliangolidos por HPLC e CG

*Neste trabalho são descritos o desenvolvimento e comparação de dois métodos analíticos (CLAE e CG) para quantificação dos furanoeliangolidos mais comuns em* Lychnophora*. Ambos os métodos são sensíveis e adequados para a quantificação desses metabólitos.*

*UNITERMOS: Furanoeliangolidos.* Lychnophora. *CLAE. CG*

## ACKNOWLEDGEMENTS

The authors are grateful to FAPESP for financial support and to Tatiana Fonseca for language review.

## REFERENCES

BOHLMANN, F.; ZDERO, C.; ROBINSON, H.; KING, R. M. Naturally-occurring terpene derivatives. 393. a-humulene derivatives including a sesquiterpene acid with a rearranged carbon skeleton from *Lychophora columnaris*. *Phytochemistry*, Oxford, v.21, n.3, p.685-689, 1982.

BORELLA, J. C.; LOPES, J. L. C.; LEITÃO-FILHO, H. D.; SEMIR, J.; DIAZ, J. G.; HERZ, W. Eudesmanolides and 15-deoxygoyazensolide from *Lychnophora pseudovilasossima*. *Phytochemistry*, Oxford, v.31, n.2, p.692-695, 1992.

CERQUEIRA, M. B. S.; SOUZA, J. T.; AMADO-JÚNIOR, R.; PEIXOTO, A. B. F. Ação analgésica do extrato bruto aquoso do caule e das folhas da *Lychnophora ericoides* Mart. (arnica). *Cienc. Cult.*, São Paulo, v.39, n.5/6, p.551-553, 1987.

DE OLIVEIRA, A. B.; SAÚDE, D. A.; PERRY, K. S.; DUARTE, D. S.; RASLAN, D. S.; BOAVENTURA, M. A. D.;. CHIARI, E. Trypanocidal sesquiterpene lactones from *Lychnophora* species. *Phytother. Res.*, Bognor Regis, v.10, n.4, p.292-295, 1996.

DOLMAN, D. M.; KNIGHT, D. W.; SALAN, U.; TOPLIS, D. A quantitative method for the estimation if partenolide and other sesquiterpene lactones containing a-methylenebutyrolactone functions present in feverfew, *Tanacetum partenium*. *Phytochem. Anal.*, Bognor Regis, v.3, n.1, p.2631, 1992.

LEVEN, W.; WILLUHN, G. Sesquiterpene lactones from *Arnica chamissonis* Less. 6. Identification and quantitative determination by high performance liquid and gas chromatography. *J. Chromatogr.*, Amsterdam, v.410, n.2, p.329-342, 1987.

LUNARDELLO, M. A.; TOMAZ, J. C.; VICHNEWSKI, W.; LOPES, J. L. C.; GUTIERREZ, A. B.; DIAZ, J. G.; HERZ, W. Sesquiterpene lactones and flavonoids from *Eremanthus mattogrossensis* and *Eremanthus eriopus*. *J. Braz. Chem. Soc.*, São Paulo, v.6, n.3, p.307-311, 1995.

PASSREITER, C. M. Quantification of sesquiterpene lactones in leaves of *Neurolaena lobata*. *Phytochem. Anal.*, Bognor Regis, v.9, n.2, p.67-70, 1998.

RÜNGELER, P.; CASTRO, V.; MORA, G.; GÖREN, N.; VICHNEWSKI, W.; PAHL, H. L.; MERFORT, I.; SCHMIDT, T. J. Inhibition of transcription factor NF-kB by sesquiterpene lactones: a proposed molecular mechanism of action. *Bioorg. Med. Chem.*, Oxford, v.7, n.11, p.2343-2352, 1999.

SEMIR, J. Revisão taxonômica de *Lychnophora* Mart. (Vernoniae: Compositae). Campinas, 1991. 515 f. (Tese de Doutorado – Instituto de Biologia - Universidade Estadual de Campinas).

VICHNEWSKI, W.; TAKAHASHI, A. M.; NASI, A. M. T.; GONÇALVES, D. C. R. G; DIAS, D. A.; LOPES, J. N. C.; GOEDKEN, V. L.; GUTIÉRREZ, A. B.; HERZ, W. Sesquiterpene lactones and other constituents from *Eremanthus seidelii, E. goyazensis* and *Vanillosmopsis erythropappa*. *Phytochemistry*, Oxford, v.28, n.5, p.1441-1451, 1989.

Recebido para publicação em 02 de dezembro de 2002.