

## Development and Validation of a Method for Simultaneous Determination of Bioactive Compounds of *Tanacetum parthenium* (L.) Schultz-Bip

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As lactonas sesquiterpênicas (LS), guaianolídeo (11,13-deidrocompressanolídeo) e partenolídeo (4 $\alpha$ ,5 $\beta$  epóxi-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olideo), encontradas em *Tanacetum parthenium* (L.) Schultz Bip, mostraram significativa atividade *in vitro* contra *Leishmania amazonensis*. Desta forma, um novo método para a determinação simultânea das LS foi desenvolvido e validado. Para a separação, empregou-se cromatografia líquida de alta eficiência acoplada a arranjo de diodos (HPLC Alliance e2695-2998 PDA,  $\lambda$  210 nm), com gradiente linear de fase (A) água acidificada H<sub>3</sub>PO<sub>4</sub> 0,1% (pH 2,8) e fase (B) acetonitrila, em diferentes proporções, na vazão de 1,2 mL min<sup>-1</sup> com coluna XBridge C<sub>8</sub> (4,6  $\times$  100 mm, 3,5  $\mu$ m, 35 °C). O método mostrou resolução (> 1,5), seletividade, linearidade (coeficiente de correlação > 0,99), precisão (desvio padrão relativo de 2,2%) e exatidão (recuperação > 98%); apresentando 52,55  $\mu$ g mg<sup>-1</sup> de guaianolídeo e 566,05  $\mu$ g mg<sup>-1</sup> de partenolídeo na fração diclorometânica do extrato hidroalcoólico de *Tanacetum parthenium*. O método apresenta potencial uso no controle da qualidade e desenvolvimento de novos fitofármacos, uma promissora alternativa no tratamento da leishmaniose.

The sesquiterpene lactones (SL), guaianolide (11,13-dehydrocompressanolide) and parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide), found in *Tanacetum parthenium* (L.) Schultz Bip, showed *in vitro* significant activity against *Leishmania amazonensis*. In this way, a new method for the simultaneous determination of SL was developed and validated. For separation, it was employed high performance liquid chromatography coupled to photodiode array detector (HPLC Alliance e2695-2998PDA,  $\lambda$  210 nm) with linear gradient of phase (A) acidified water 0.1% H<sub>3</sub>PO<sub>4</sub> (pH 2.8) and phase (B) acetonitrile, in different proportions, at a flow rate of 1.2 mL min<sup>-1</sup> with XBridge C<sub>8</sub> column (4.6  $\times$  100 mm, 3.5  $\mu$ m, 35 °C). The method showed resolution (> 1.5); selectivity, linearity (coefficient of correlation > 0.99), precision (relative standard deviation of 2.2%) and accuracy (recovery > 98%); presenting 52.55  $\mu$ g mg<sup>-1</sup> of guaianolide and 566.05  $\mu$ g mg<sup>-1</sup> of parthenolide in the dichloromethane fraction of the hydroalcoholic extract of *Tanacetum parthenium*. The method presents potential use in quality control and development of new phytochemicals, a promising alternative in the treatment of leishmaniasis.

**Keywords:** leishmaniasis, neglected disease, sesquiterpene lactones, guaianolide, parthenolide

## Introduction

Leishmaniasis is an infectious disease that affects mainly the world's poorest regions.<sup>1</sup> Its conventional therapy is normally inefficient, risky and expensive.<sup>1-3</sup> Medicinal plants have a great potential of being novel selective agents for treating important neglected tropical diseases. Alkaloids, terpenes, quinones, flavonoids and especially sesquiterpene lactones present in superior plants such as *Tanacetum parthenium* have shown selective activity against *Leishmania* sp., the major etiologic agent of leishmaniasis.<sup>1,4</sup>

The sesquiterpene lactones 11,13-dehydrocompressanolide a type of guaianolide and the parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide), were found in major proportion in the dichloromethane fraction (DF) of the hydroalcoholic extract produced from the commercial powder of aerial parts of the *T. parthenium* (L.) Schultz Bip. This DF exhibited *in vitro* significant activity against promastigote and amastigote forms of *Leishmania amazonensis*.<sup>1,5</sup>

Medicinal plants contain active complex constituents which require efficient methods for their separation, identification and quantification.<sup>6</sup> The quality control is essential for ensuring safety, effectiveness and quality,<sup>7</sup> consequently, the standardization of phytomedicine and or phytochemical is a prerequisite for quality assurance and to ensure the consistency of therapeutic effects and safety of the user.<sup>8</sup> The selection of chemical markers initially made by phytochemical studies of the secondary metabolites with pharmacological activity is crucial for the quality control of herbal medicines, including the authentication of genuine species, harvesting high-quality raw materials, evaluation of post-harvest handling, and is essential for planning and monitoring actions of processing technology, as well as assessment stability studies of intermediate and final products.<sup>8</sup> Ideal chemical markers should be therapeutic components of herbal medicines.<sup>9</sup>

Some analytical methods have been reported in the literature regarding the separation and determination of the lactone parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide) in *T. parthenium*.<sup>10-13</sup> In our previous paper, the guaianolide was isolated for the first time from *T. parthenium*.<sup>1</sup> No method has been described to simultaneously quantify these two markers.

The aim of the present study was to develop a novel method based on high-performance liquid chromatography coupled with photodiode array detection (HPLC-PDA) to simultaneously quantify bioactive SL 11,13-dehydrocompressanolide and parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide),

in the dichloromethane fraction (DF) of the hydroalcoholic extract from the commercial dried powder of aerial parts of the *T. parthenium*. This method could be used to quantify the chemical markers and to the quality control of this plant, thus contributing to the development of antileishmanial phytomedicines.

## Experimental

### Material and chemicals

Acetonitrile, methanol (both HPLC grade), dichloromethane and formic acid (analytical grade) were purchased from JT Baker (Xalostoc, Mexico). Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) (p.a. plus,  $\geq 85\%$ ) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared with ultrapure water from a Milli-Q<sup>®</sup> system (Millipore, Billerica, MA, USA).

Commercial powder (1000 g) of the *T. parthenium* aerial parts (lot 396253) was purchased from the Herbarium Botanical Laboratory (Colombo, Paraná, Brazil). This material is used for the production of the phytomedicine Tanaceto<sup>®</sup>.

### Equipment and analytical instrumentation

The method was developed using an integrated complex consisted of an UHPLC-PAD, Acquity UPLC H-Class system equipped with an Acquity PDA detector, column manager, solvent selector valves and operated by Empower 3 chromatography data software (all from Waters, Milford, MA, USA) and Fusion QbD-based LC method development software (S-Matrix, Eureka, CA, USA). The purpose of the initial development was to separate complex matrices like DF by the larger number of peaks within resolution better than 1.5. So, the most suitable separation conditions were achieved after testing 4 columns of different selectivity with experimental design. Then, mass spectra were used to confirm identity and purity of the two bioactive markers of interest for leishmaniasis in the obtained chromatograms of DF using ultrahigh performance liquid chromatographic system attached to high-resolution mass spectrometry (UHPLC-MS/MS). This was performed using a quadrupole orthogonal time-of-flight tandem mass spectrometer system (Acquity H-Class UPLC, Xevo G2 QTof, Waters) with MassLynx 4.1 software (Waters) applied for data acquisition and processing. After, an Acquity UPLC column calculator (Waters) was used to obtain equivalent chromatographic conditions for HPLC. These determinations were made in the application laboratory of Waters.

To carry out the validation procedure, the method was transferred to an Alliance e2695 HPLC system connected with a photodiode array detector (2998 PDA) and Empower 2 chromatography data software (all Waters), taken in the laboratories of the Universidade Estadual de Londrina. The initial development of chromatographic method was done in UHPLC-PDA due to its resources. Validation was done only for HPLC-PDA, considering the method greater applicability since the number of HPLC equipments in use is much higher than that of UHPLC.

#### Sample and marker preparations

Sample was prepared according to the following procedure. The *T. parthenium* commercial powder (1000 g) was macerated in 4.0 L flask (maintained ¼ free) under dynamic stirring in shaker with hydroalcoholic solution (ethanol: distilled water 90:10 v/v) at proportion of drug:solvent (1:2 v/v) at room temperature. Every 5 days, the extract was filtered on qualitative paper, being the filtrates collected after 15 days, evaporated in rotavap (R-114, Büchi, Flawil, Switzerland) at 175 mbar pressure at 35-40 °C to eliminate organic solvent, and after then water was removed by lyophilization (Christ®, Alpha 1-2, Osterode, Germany). The produced dry hydroalcoholic extract (85.83 g) was homogenized with 115.7 g of silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany) in porcelain grade, and the adsorbent packed in a preparative glass column (3.0 × 35.0 cm) attached to a Kitasato flask and connected to a vacuum pump. So, the hydroalcoholic extract was fractionated being eluted from the column with 500 mL of increasing polarity solvents (hexane, dichloromethane, dichloromethane ethyl acetate (1:1, v/v), ethyl acetate and methanol). Each of the 5 resulting fractions was collected, the extract evaporated in rotavap to complete dryness and stored at -20 °C. All fractions were analyzed by thin-layer chromatography (TLC) using silica gel chromatoplates (Kieselgel 60 F254, 20 × 20 cm, 2 mm thickness, Merck) eluted with the mobile phase hexane:ethyl acetate (3:2, v/v). Compounds were detected by spraying vanillin-sulfuric acid (2% solution), followed by heating in an oven at 100 °C for 3 min. The obtained dichloromethane fraction (9.63 g of DF) was that provided better chromatographic profile regarding abundance of sesquiterpene lactones and less interfering compounds by TLC, thereby the selected fraction to this study. The dichloromethane fraction of the hydroalcoholic extract obtained from commercial powder of aerial parts of *T. parthenium* (L.) Schultz Bip is called during this article as DF.

The purified and isolated compounds, used as standard to quantify the markers, were prepared as follows. The

guaianolide 11,13-dehydrocompressanolide and the parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide) were isolated from DF according to Silva *et al.*<sup>1</sup> and Tiunan *et al.*<sup>5</sup> The identification was performed using methods of nuclear magnetic resonance (NMR) of one-dimensional <sup>1</sup>H and <sup>13</sup>C, as well as, the bi-dimensional <sup>1</sup>H-<sup>1</sup>H homonuclear (COSY), one-bond (HSQC) and long-range (HMBC) <sup>1</sup>H-<sup>13</sup>C NMR correlation experiments and distortionless enhancement by polarization transfer (DEPT) and mass spectra. The spectral data of the guaianolide 11,13-dehydrocompressanolide are shown in Supplementary Information (SI) section (Figures S1-S7 and Tables S1 and S2). The procedures to obtain DF and the two isolated markers were done in the laboratories of the Universidade Estadual de Maringá.

In order to facilitate, in this article, the two markers the 11,13-dehydrocompressanolide a type of guaianolide and the parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide) is referred only as guaianolide and parthenolide.

The sample and markers were solubilized in water:methanol (1:1, v/v) and filtered through 0.2  $\mu$ m GHP membranes (Pall Co., Port Washington, NY, USA) for UHPLC analysis and 0.45  $\mu$ m PVDF membranes (Vertical Chromatography, Nonhaburi, Thailand) for HPLC analysis.

#### Chromatographic conditions

The UHPLC-PDA analysis for the method development was performed using a UPLC BEH C<sub>8</sub> column (2.1 × 50 mm, 1.7  $\mu$ m, Waters) under the following conditions: run time of 12.4 min, pH 2.8 of the aqueous eluent acidified with 0.1% H<sub>3</sub>PO<sub>4</sub>, flow rate of 0.5 mL min<sup>-1</sup>, injection volume of 4.0  $\mu$ L and oven temperature at 35 °C. The maximum absorption for the markers analyzed in the present study was found to occur at 210 nm, as can be seen from their UV spectra (Figures 1 and 2). Thus, this wavelength was taken for further analysis. The separation was carried out in the linear gradient elution mode using the mobile phase composed of the aqueous eluent acidified with 0.1% H<sub>3</sub>PO<sub>4</sub> (v/v) (A) (pH 2.8) and acetonitrile (B): 5-46.2% B (0-7.5 min), 46.2-95% B (7.6-9.5 min) and 95-5% B (9.6-12.4 min).

The validation process was carried out in the HPLC-PDA system containing an XBridge C<sub>8</sub> column (4.6 × 100 mm, 3.5  $\mu$ m, Waters). The chromatographic conditions for this transfer were as follows: runtime of 50 min, flow rate of 1.2 mL min<sup>-1</sup>, injection volume of 40.0  $\mu$ L, oven temperature at 35 °C and detection wavelength at 210 nm. Like in the previous case, the separation was carried out using linear gradient elution with the same mobile phase

composed of the aqueous eluent acidified with 0.1% H<sub>3</sub>PO<sub>4</sub> (v/v) (A) (pH 2.8) and acetonitrile (B): 5-46.2% B (0-30.4 min), 46.2-95% B (30.5-38.7 min) and 95-5% B (38.8-50 min).

#### Mass spectra conditions

The UHPLC-MS/MS chromatographic analysis for confirming identity and purity of marker peaks were performed using a UPLC BEH C8 column (2.1 × 50 mm, 1.7 μm, Waters) under the following conditions: runtime of 12.4 min, flow rate of 0.5 mL min<sup>-1</sup>, injection volume of 4.0 μL, and oven temperature at 35 °C. The mobile phase was composed of the aqueous eluent acidified with 0.1% formic acid (v/v) and acetonitrile: 5-46.2% B (0-7.5 min), 46.2-95% B (7.6-9.5 min) and 95-5% B (9.6-12.4 min).

Mass spectra were recorded in the positive MS<sup>E</sup>. MS<sup>E</sup> is a mode of data acquisition that permits the seamless collection of a comprehensive catalog of information for both precursor and parents ions in a single analysis and injection.<sup>14</sup> This is achieved by rapidly alternating between two functions, i.e., the first, acquired at low energy, provides exact mass precursor ion spectra; the second, at elevated energy, provides high energy exact mass of the parents ions. In addition to providing increased confidence in identification, fragmentation can help to differentiate between isobaric compounds.

Data were acquired using the following parameters: capillary voltage ± 3.5 kV, cone voltage at 50 V, desolvation temperature at 500 °C; and desolvation gas flow rate of 900 L h<sup>-1</sup>. Argon, used as collision gas, was introduced into the collision cell at 0.4 mL min<sup>-1</sup>. To get fragmentation, a collision energy ramp ranging from 20 to 40 V was applied to the cell.

#### Analytical method validation

The validation of the analytical method was performed in the HPLC-PDA system according to the criteria proposed by International Conference on Harmonization (ICH)<sup>15</sup> and Brazilian Health Surveillance Agency (ANVISA RE 899/03).<sup>16</sup> The following parameters were evaluated: working range, linearity, detection and quantification limits, precision (repeatability and intermediate precision), accuracy (recovery), selectivity and robustness. Statistical data analyses were performed at the 5% significance level using Statistica 8.0 software.

The method linearity for both markers was tested through regression analysis based on three calibration curves constructed using 6.25-100.0 μg mL<sup>-1</sup> solutions for the guaianolide and 125.0-1,000.0 μg mL<sup>-1</sup> solutions

for the parthenolide. The range for the calibration curves was defined based on *in vitro*<sup>1,5</sup> and *in vivo* (data not yet published) antileishmaniasis activities.

The limits of detection and quantification were calculated based on the standard deviation and slope of the regression curves.

The precision was evaluated in two steps: repeatability and intermediate precision. The repeatability was assessed by the same analyst on the same day using six determinations at 100% of the test concentration (25.0 and 250.0 μg mL<sup>-1</sup> for guaianolide and parthenolide, respectively); whereas the intermediate precision was estimated by different analysts on different days.

The accuracy was determined from recovery tests by spiking the sample with solutions of the isolated compounds having low, medium and high concentration levels (25, 50 and 75% (v/v) of the 500.0 μg mL<sup>-1</sup> test concentration).

The selectivity was checked by injecting the sample diluent into the chromatographic system and observing the absence of analytical response at the same retention time for both markers. Besides, the purity of the guaianolide and parthenolide was observed from the spectral profile characteristics of the entire chromatographic peak and from mass spectrometry data.

Some changes in experimental conditions were made to evaluate the method robustness. The effects of varying the flow rate (1.18 and 1.22 mL min<sup>-1</sup>), oven temperature (30 and 40 °C), pH (2.6 and 3.0) of the mobile phase (A) and distinct acetonitrile brand of the mobile phase (B) (JT Baker, Xalostoc, Mexico and Thermo Fisher Scientific, Waltham, MA, USA) were estimated.

#### *Tanacetum parthenium* analysis

The HPLC-PDA quantification of SL was performed for 500.0 μg mL<sup>-1</sup> DF. The amounts of guaianolide and parthenolide were determined using respective regression curves.

The identification of SL was confirmed by the same retention time for the isolated compounds and the extract, proportional increase in peak areas after spiking the isolated standard at different concentrations, and similarity between the UV spectra profiles, molar mass and molecular fragments in mass spectra.

## Results and Discussion

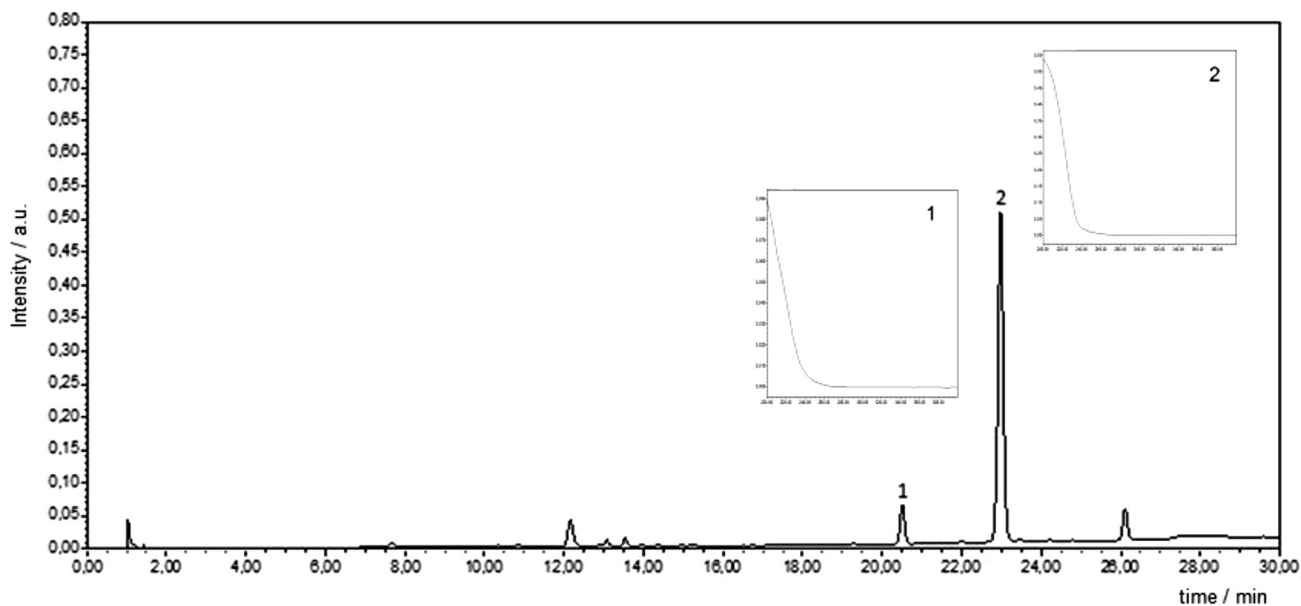
The developed UHPLC-PDA method was successfully transferred in a conventional HPLC-PDA system and validated since the same performance was achieved with both systems. The established conditions to be maintained

were: number of peaks (greater than 63 peaks), number of peaks with resolution  $\geq 1$  ( $> 63$ ), number of peaks with resolution  $\geq 1.5$  ( $> 42$ ), number of peaks with resolution  $\geq 2$  ( $> 28$ ) and asymmetry of peak  $\leq 2$  ( $> 63$ ).

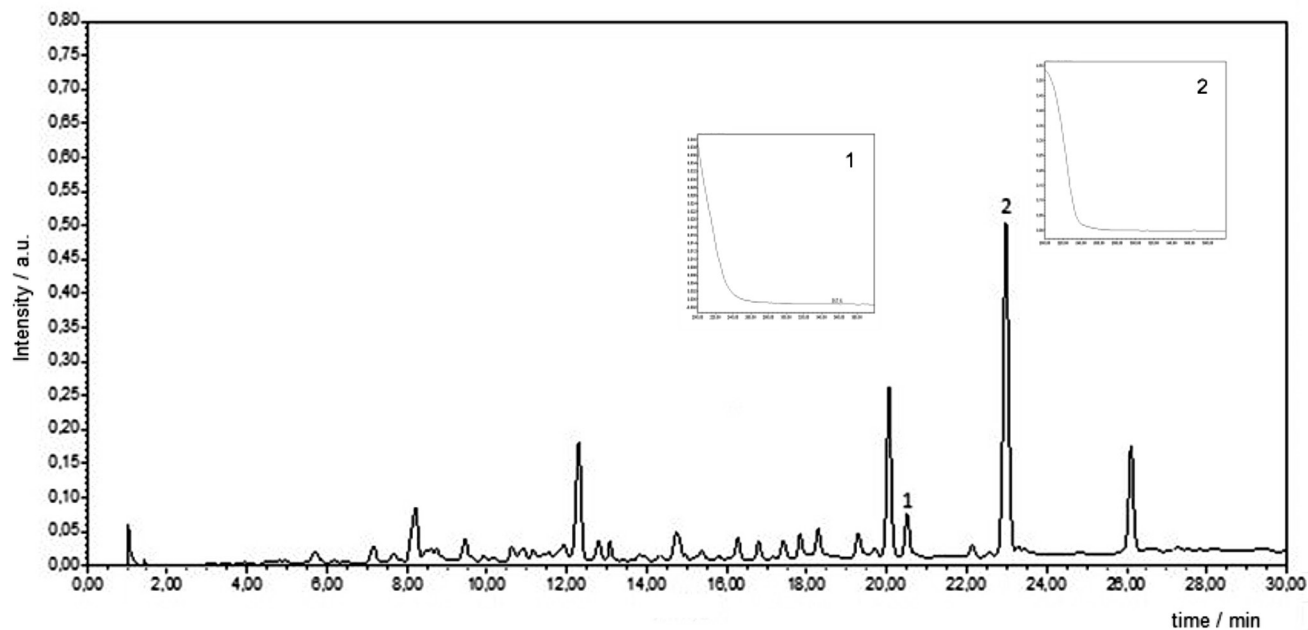
Examining the trends in the analytical columns according to researches performed by Majors,<sup>17</sup> UHPLC systems and small particles ( $< 2\text{-}4\ \mu\text{m}$ ) are the current trends due to faster separation with reduced time and solvent use. However, columns packed with larger particles still remain

the most utilized in many developed and validated HPLC methods.<sup>17</sup> Therefore, the HPLC system was chosen to validate the method aiming at the feasibility of its use for the quality control and also research and development, considering the currently existing and installed number of equipment in industrial and academic laboratories.

So, the method validation was carried out simultaneously for both bioactive compounds for the purified and isolated (Figure 1) and for DF (Figure 2). The retention time was



**Figure 1.** HPLC-PDA chromatogram of purified and isolated sesquiterpene lactone used as standard markers, obtained from fractions of the hydroalcoholic extract of commercial powder of aerial parts of *T. parthenium* (L.) Schultz Bip at 210 nm. Peaks: (1) guaianolide ( $25.0\ \mu\text{g mL}^{-1}$ ) and (2) parthenolide ( $250.0\ \mu\text{g mL}^{-1}$ ).



**Figure 2.** Chromatogram for sample of the dichloromethane fraction of the hydroalcoholic extract obtained of commercial powder of aerial parts of *T. parthenium* (L.) Schultz Bip ( $500.0\ \mu\text{g mL}^{-1}$ ) diluted in water: methanol (1:1, v/v) and detected by HPLC-PDA at 210 nm. Peaks: (1) guaianolide and (2) parthenolide.

found to be 20.4 min for guaianolide and 22.9 min for parthenolide.

As seen from well resolved and integrated peaks of the sample chromatogram generated by the HPLC-PDA system (Figure 2), the chosen conditions allowed the simultaneous determination of both markers. Moreover, the separation provides future perspectives for further studies focused on the identification of other compounds using the UHPLC-MS/MS technique.

The method validation parameters obtained are presented in Table 1.

The regression curves for both markers allowed to appropriately determine coefficients for the lactones guaianolide and parthenolide (coefficient of correlation ( $r^2$ ) of 0.999 and 0.998, respectively). The regression  $F$ -values (10066.73 for guaianolide and 3098.32 for parthenolide) were higher than the critical  $F$ -values (4.38 and 4.49, respectively); in this way, the model followed linear regression in the working range. The lack-of-fit  $F$ -values of the model (1.035 for guaianolide and 0.708 for parthenolide) were lower than the critical  $F$ -value (2.96 and 3.20, respectively), confirming the adjustment of the data to this model.

The precision of the present analytical procedure, expressed by the closeness of agreement (scattering degree) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions,<sup>15</sup> was demonstrated considering low relative standard deviations (RSD) for the lactones guaianolide and parthenolide (RSD of 2.17 and 2.21%, respectively). The intermediate precision showed RSD of 2.27 and 4.20% for guaianolide and parthenolide, respectively, both within the specifications (RSD < 5%).<sup>15,16</sup>

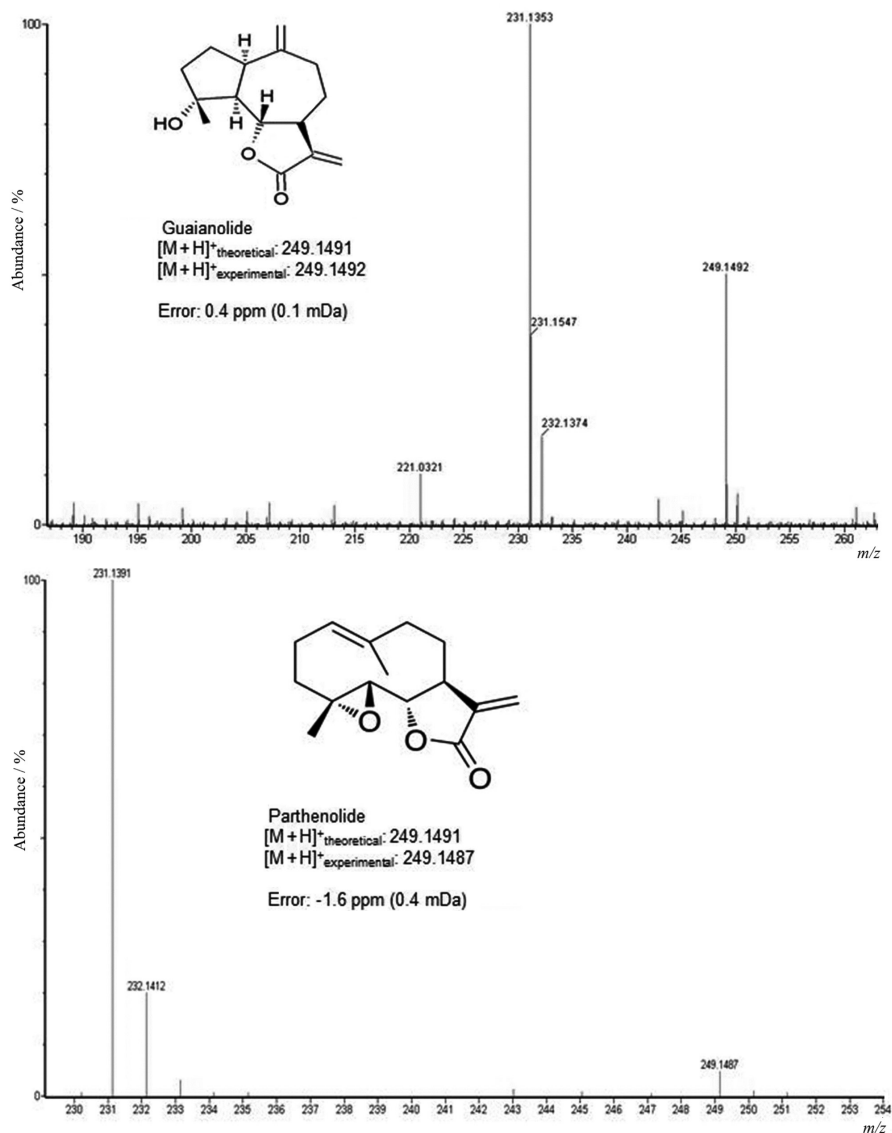
The accuracy, expressed by the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the determined value,<sup>15</sup> was verified by the recovery rates of: 98.56, 98.34, 99.55% for the guaianolide (means of  $98.82 \pm 0.64$ ) and 98.93, 99.80, 100.14% for the parthenolide (means of  $99.63 \pm 0.62$ ), for low, medium and high concentration levels, respectively, being within the range established for complex matrices (80-120%)<sup>17</sup> and even for simple matrices (98-102%).<sup>16</sup> The developed method was robust, since even after small changes in the analytical parameters, no significant differences ( $p > 0.05$ ) were observed between the peak areas.

The selectivity was assessed during the chromatographic run with the methanol:water (1:1, v/v) diluent injected. No interfering signal was observed at the same retention time for the lactones guaianolide and parthenolide. The identity of the markers was confirmed by the same retention time, overlaying the chromatograms of the isolated compounds used as standards and the sample obtained by the Empower 2 and 3 chromatographic softwares. The purity was also confirmed by comparing the similarity of the UV spectra (Figures 1 and 2) profile for SL in their peaks and by calculating compatible recovery rates.

To ensure the peak purity and identity, the mass spectra analysis was also performed for the isolated compounds and the sample of DF (Figure 3). Other peaks were also identified (to be described in our next paper). The accuracy for the mass spectra of the protonated ions was achieved. Since SL are isomers ( $C_{15}H_{22}O_3$ ), the masses obtained ( $m/z$  249.1491) were close to the theoretical value in both cases (error of 0.4 and 1.6 ppm for guaianolide and parthenolide, respectively), but presented different fragmentation patterns.

**Table 1.** Validation parameters of the developed HPLC method for simultaneous determination of sesquiterpene lactones in the dichloromethane fraction of the hydroalcoholic extract obtained from commercial powder of aerial parts of *T. parthenium* (L.) Schultz Bip

Parameter	Guaianolide (11,13-dehydrocompressanolide)	Parthenolide (4 $\alpha$ ,5 $\beta$ -epoxy-germacra-1-(10),11-(13)-dien-12,6 $\alpha$ -olide)
Working range / ( $\mu\text{g mL}^{-1}$ )	6.25-100.0	125.0-1000.0
Number of standards	7	6
Linear equation	$y = 19422.05 x + 8907.69$	$y = 18202.6 x + 276365.9$
Linearity (coefficient of correlation ( $r^2$ ))	0.9981	0.9948
$F$ cal. regression	10066.73 ( $F$ crit. = 4.38)	3098.31 ( $F$ crit. = 4.49)
$F$ cal. residue	1.035 ( $F$ crit. = 2.96)	0.708 ( $F$ crit. = 3.20)
Limit of detection / ( $\mu\text{g mL}^{-1}$ )	1.72	33.57
Limit of quantification / ( $\mu\text{g mL}^{-1}$ )	5.75	111.90
Precision - repeatability / %	2.17	2.21
Intermediate precision / %	2.27	4.20
Average accuracy / %	$98.82 \pm 0.64$	$99.63 \pm 0.62$



**Figure 3.** Mass spectra recorded in the positive MS<sup>E</sup> mode for guaianolide and parthenolide (500.0  $\mu\text{g mL}^{-1}$ ) of the dichloromethane fraction of the hydroalcoholic extract obtained of commercial powder of aerial parts of *T. parthenium* (L.) Schultz Bip.

The amounts of the lactones in the DF samples were evaluated in triplicate, and they were found to be 52.55  $\mu\text{g mg}^{-1}$  (RSD of 2.06%) for guaianolide and 566.05  $\mu\text{g mg}^{-1}$  (RSD of 0.16%) for parthenolide. Moreover, low quantification limits (5.75  $\mu\text{g mL}^{-1}$  for guaianolide and 111.90  $\mu\text{g mL}^{-1}$  for parthenolide) were achieved, thus indicating the effectiveness of the method for quantifying low dosages of these markers.

## Conclusions

The developed chromatographic method herein proved to be efficient and robust, as confirmed by its validated analytical parameters by HPLC-PDA system and by the identity and purity using mass spectra obtained by UHPLC-MS/MS. Besides, it allows reliable simultaneous quantification of

the lactones markers guaianolide and parthenolide, selected by phytochemical isolation, structural elucidation and identification of the secondary metabolites with proven pharmacological activity without toxicity, present in the dichloromethane fraction (DF) of the hydroalcoholic extract obtained from commercial powder of aerial parts of *T. parthenium* (L.) Schultz Bip. Considering these aspects, the present method can be an important tool for assessment quality of plant raw material. The method also represents a promising alternative with great potential for developing and monitoring the technological conversion of medicinal plant into phytomedicine and or phytochemical. Stability studies to preserve chemical composition and pharmacological activity might also benefit from this method, thus ensuring the consistency of therapeutic action and safe use, effective treatment of neglected leishmaniasis.

## Supplementary Information

Supplementary information (Figure S1-S7, Table S1-S2) is available free of charge at <http://jbcs.org.br> as PDF file.

## Acknowledgements

The authors would like to thank the CNPq (project No. 475013/2011-5), CAPES (project No. 23/2008, Nanobio) and Fundação Araucária for their financial support, as well as, Waters Technologies do Brasil for their technical assistance.

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Submitted: October 24, 2013

Published online: April 25, 2014

**FAPESP has sponsored the publication of this article.**