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Optimization of the Extraction of Flavonoids Compounds from Herbal Material using Experimental Design and Multi-response Analysis

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SUMMARY. Statistical analysis such as experimental design, regression and multi-response analyses are powerful tools for the characterization and optimization of the pharmaceutical process by studying the effects of variables affecting them and their possible interactions. In this paper, these analyses were detailed presented during the development and optimization of a process for the extraction of flavonoid compounds (active markers) from *Bauhinia forficata* Link leaves by hydro-ethanolic solutions. The results permitted the determination of the variables affecting the extraction process and allowed the determination of the best conditions for the extraction of active compounds from the leaves of *Bauhinia forficata* Link.

RESUMEN. "Optimización de la Extracción de Flavonoides de Hierbas empleando Diseño Experimental y Análisis Multi-respuesta". El análisis estadístico, así como el diseño experimental, el análisis de regresión y el análisis multi-respuesta son potentes herramientas para la caracterización y la optimización de los procesos farmacéuticos, permitindo el estudio de los efectos de variables que afectan el proceso y sus posibles interacciones. En este trabajo, los métodos estadísticos fueron utilizados en el desarrollo y la optimizacion del proceso de extracción de los compuestos flavonoides (marcadores activos) de hojas de *Bauhinia forficata* Link por soluciones etanol:agua. Los resultados permitieron la determinación de las variables que afectan el proceso de extracción y de las mejores condiciones para la extracción de compuestos activos de las hojas de *Bauhinia forficata* Link.

INTRODUCTION

During the development of herbal medicinal products like standardized fluid and dried extracts preparations several decisions need be done, in order to obtain a product with acceptable level of active substances. The aim is to determine the set of conditions, which will result in a product with an acceptable combination of physical and chemical properties. The definition of the production course involves the evaluation of the effects of several parameters on the desired responses by studying its effects on the process and their possible interactions. This is a problem involving the simultaneous optimization of several response variables (the desirable combination of properties), which depend of a number of independent variables or sets of conditions. Statistical methods, such as experimental planning, analysis of variance and multi-response analysis can be powerful tools in order to aid the selection of the optimum set of processing conditions [1-5].

In this paper, it is presented how to use the statistical methodology for the development and optimization of a process for extraction of flavonoid compounds (active markers) from *Bauhinia forficata* Link leaves using hydro-ethanolic solutions. Flavonoids, a large group of plant polyphenols are normally present in plant tissues at relatively high concentrations, either

KEY WORDS: *Bauhinia forficata* Link, Box-Behnken design, Extraction process, Flavonoid, Multi-response analysis.
PALABRAS CLAVE: Análisis multi-respuesta, *Bauhinia forficata* Link, diseño de Box-Behnken, Flavonoides, Proceso de extracción.

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as sugar or as aglycones [6]. Some of them serve as markers in chemotaxonomic studies and reference substances for the quality control of phytopharmaceutical products [7,8].

Preliminary extraction trials were performed to compare the conventional extraction methods (maceration) with the sonication method and with an extraction system composed by a jacketed agitated vessel connected to a circulation batch (heated stirred reactor). Selected the extraction method, statistical methods were applied to examine the effect of the different parameters namely, the extraction temperature (30 to 70 °C), the concentration of the ethanol in extractive solvent $|m_w/m_{et}|$ (0 to 1) and the ratio between plant to solvent mass (0.1 to 0.2), aiming the optimization of the extraction of active compounds from *Bauhinia forficata* Link.

The *Bauhinia forficata* Link ("pata-de-vaca") was chosen as model material due to its large use in the herbal medicine, mainly as hypoglycemic, depurative and diuretic. In Brazilian herbal medicine, the *Bauhinia* species have been referred to as *vegetable insulin* [9]. Antioxidant properties [10,11], and anticoagulant and antifibrino-genolytic activities against *Bothrops jaracussu* venom and its isolated thrombin-like-serine protease enzyme induced by aqueous extract from this plant have been also reported [12]. The genus *Bauhinia* is a member of the family *Leguminosae,* subfamily *Caesalpiniaceae*. The tree *Bauhinia forficata* is commonly known as pata de vaca and unha de vaca (Brazil), *Brazilian orchid tree* (English), and pezuña de vaca (Spanish). *Bauhinia forficata* is an arboreal plant (height 5 a 9 m) native from south of Brazil, Paraguay, Argentina and Uruguay and adapted well to the Brazilian climate [13-15].

MATERIAL AND METHODS

Materials

The extracts were prepared with dried and powdered leaves of *Bauhinia forficata* Link, with a mean diameter of 0.3 mm, acquired from Ely Martins S.A. (Ribeirão Preto, SP, Brazil) and stored in dark bags to protect them from the humidity and from the light. Ethyl acetate, methanol, acetone, aluminum chloride, ethanol, chloridric acid (Synth-Brazil), hexametilenetetramine (Vetec-Brazil), dehydrated quercetin (Sigma-Aldrich) were used as reagents and standard materials. All chemicals were at least of analytical grade.

Physicochemical characteristics of the plant material

The vegetal drug was characterized by the determination of the loss on drying, extractable matter and total flavonoids content, used as chemical marker in the extraction studies.

Loss on drying (gravimetric determination)

Samples of the vegetal drug (5 g) were maintained in an oven at 102 ± 1 °C until constant mass. The result was expressed in ponderal percentage, through the average of the three determinations [16].

Extractable matter [17]

Solutions 1:100 grams of the vegetal material (dry basis) in distilled water and in water: ethanol (70 % v/v) were heated until boiling being filtered after cooling. Five samples of 20.0 g of the filtrate were withdrawn and put in oven to 102 ± 1 °C until constant mass. The extractive matter was calculated by the percentage ratio between the dry residues to the vegetal material masses (average of three determinations). The extractive content was determined in triplicate, according to Eq. **1**:

$$TE = \frac{m.500}{p} \qquad [1]$$

where TE = extractable matter (%, w/w), m = mass of dry residue (g), and, p = mass (dry base) of vegetal drug (g).

Total flavonoid content

It was evaluated by spectrophotometry (UV-vis), being used predefined masses of samples. The procedure includes hydrolyze of the glycosides, followed by the flavonoids extraction with ethyl acetate and colour development with $AlCl_3$ [2,8,18-20]. The detailed description of the method was presented elsewhere [21]. The absorbance is measured at 425 nm after 30 min after the addition of a 0.5 % $AlCl_3$ solution (w/v), using a spectrophotometer UV-VIS HP 8453 running the software HP Chem-Station. The percentage of total flavonoid (TF), expressed as quercetin, was calculated by the Eq. **2**:

$$TF = \frac{A.Fd}{611.1 \cdot C_s} \qquad [2]$$

where TF is the total flavonoid contents express as quercetin (%, w/w), A is the absorbance measured (UA), C_S is the solid contents (g/g), and Fd is the dilution factor.

Preliminary evaluation of extractive methods

In order to select the method to be submitted to optimization studies using the Box-Behnken design and multi-response analysis, preliminary extraction assays were performed to compare the conventional extraction method (maceration) with the sonication method and with an extraction system composed by a jacketed stirred vessel connected to a circulation batch (heated stirred reactor). The strategies used to obtain of the extractives solutions were based on previous studies presented in literature [22-25].

Conventional extraction method (maceration)

Maceration was performed with 50 g of leaves and 250 mL of the extraction solvent (water/ethanol - 1:2). The mixture was left at room temperature for seven days with sporadic shaking. At the end of the test, the extract was filtered through a vacuum filtration system using filter paper (grade 80 g/m^2).

Sonication method

Fi g of sample with water/ethanol (1:2) were placed in ultrasound bath during 0.5, 1.0, 1.5 and 2.0 hours, into cycles of 20 min on and 10 min off. Sonication was carried out at room temperature. At end, the extracts were filtered through a vacuum filtration system.

Heated stirred reactor

The dried and powdered leaves were placed in contact with an extraction solvent (water/ethanol at 1:2) with preset temperatures (30 and 60 °C), being maintained under agitation during all extraction time (1 to 6 h) in a heated stirred reactor. At the end of the experiment, the extracts were filtered through a vacuum filtration system.

Samples of the extracts were obtained at distinct extraction times and subsequently analyzed in relation to the solid contents, density, total flavonoid contents and extraction yield. The methods and experimental conditions studied are presented in the Table 1. Based on the evaluation of the results it was possible to select the heated stirred reactor as the method to be submitted to the optimization studies using the Box-Behnken design, analysis of variance (ANOVA), and multiresponse analysis.

Extraction method	**Extraction time (hours)**	**m_{pl}/m_{sol} (w:w)**
Heated stirred reactor	1	1:5.8
(30 °C)	2	1:5.8
	4	1:5.8
	6	1:5.8
Heated stirred reactor	1	1:5.8
(60 °C)	2	1:5.8
	4	1:5.8
	6	1:5.8
Maceration	24	1:5
with sporadic shaker	72	1:5
	120	1:5
	168	1:5
Sonication	0.5*	1:5
with frequency of 40 kHz	1.0 *	1:5
	1.5*	1:5
	2.0*	1:5

Table 1. Extraction methods and experimental conditions studied. * In cycles of 20 min of vibrations and 10 min of rest.

Study of the extraction process using Box-Behnken design and variance analysis

Three-factor and three-level Box-Behnken design was used to evaluate the effect of the extraction parameters on the extraction efficiency [1,26]. The Box-Behnken design is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the parameters combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require 3 levels of each factor. For three factors, the Box-Behnken design offers advantages over the Box-Wilson Central Composite Designs in requiring a fewer number of runs [27]. This design allows the construction of a second-order polynomial model, which can be used to characterize or optimize a process. The resulting model has the following form:

$$Y_i = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_2X_3 + a_6X_1X_3 + a_7X_1^2 + a_8X_2^2 + a_9X_3^2 + E,$$

where, a_0 to a_9 are the regression coefficients, X_1 a X_3 denotes the factors, Y is the relative average or expected response associate with the combination factors and E represent the experimental error.

The studied factors include the extraction temperature (30 to 70 °C), the ratio between plant to solvent mass, m_{pl}/m_{sol} (0.1 to 0.2) and the concentration of the ethanol in extraction solvent (0 to 100 %), parameterized by Eq. **3**:

$$\left|\frac{m_w}{m_{et}}\right| = \left(\frac{m_{et}}{2 \cdot m_w + m_{et}}\right) \qquad [3]$$

Equation **3** gives the value 0, for the aqueous extract, 1 for the ethanolic extract and 0.5 for the hydroalcoholic extract (water/ethanol 1:2 w/w). The factor levels, equally spaced, were coded for low, medium and high settings according to equation **4**:

$$V_c = \frac{V - \overline{V}}{\Delta V / 2} \qquad [4]$$

where V_c is the coded variable, $\overline{V}$ is the mean value of the variable and ΔV is the interval of variation. Table 1 shows the factors and levels investigated.

The extracts were prepared with dried and powdered leaves of *Bauhinia forficata* Link with a mean diameter of 0.3 mm, acquired from Ely Martins S.A, Brazil. The extraction was carried out in an extraction system composed by a jacketed stirred vessel connected to a heating circulating batch with temperature control (Brookfield TC-500). After extraction, the crude extracts were filtered in a vacuum system using filter paper (grade 80 G). Fig. 1 presents a schematic diagram of the extraction process optimized. The resulting extracts were characterized by the determination of the density, solids concentration and total flavonoid contents. Density was determinate by picnometry, solids concentration by the gravimetric method [16] and the total flavonoids contents by spectrophotometry.

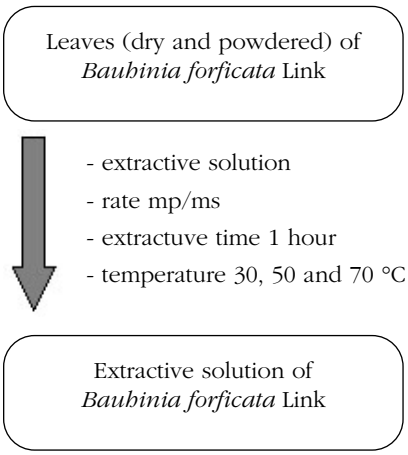

Figure 1. Schematic diagram of the extraction process in the heated stirred reactor.

RESULTS AND DISCUSSION

Physicochemical characterization of the plant material

The raw plant material was characterized by the determination of the loss on drying (moisture content), of the extractable material and total flavonoid content. The moisture content of the plant material is a parameter directly related with the stability of the actives compounds present in the vegetal material during storage. Excessive moisture content in raw material favors the microbial growth and the active compounds hydrolysis [28-30]. The loss on drying may supply data about the yield of the extractive processes, since the drying has effect on the state of the cell structures that can became more or less exposed to the extracting solvent. In this work, a value of 7.8 ± 0.6 (%, w/w) was found for the residual moisture (dry base) of the vegetal drug. This value is inside the range of 6 to 14 %, established for products stored in non-hermetic conditions [29-31].

The extractable material was determinate using water and water:ethanol (70% v/v) as extractive solvent, being obtained the values of 20.1 and 27.1%, respectively. The experimental value of the total flavonoid contents present in the leaves of the *Bauhinia forficata* Link was of 1.88 ± 0.02 (%, w/w).

Preliminary evaluation of the extractive methods

Table 2 shows the experimental results of the total flavonoids, of the solids concentration and of the extraction yield obtained for the three extractive methods and experimental conditions used. From the data presented, it can be concluded that the heated stirred reactor presented a better performance, giving solids concentration and total flavonoid contents 50 to 100 % higher than the values obtained by the other extractive methods. Due to, the heated stirred reactor was the selected method for performing the optimization studies.

Extraction methods	Time (hour)	Flavonoid content (F, % $g_{flav}/g_{.ext}$)		Solids concentration (C_s, % g/g_{ext})		Yield (%)	
		theor.	exp.	theor.	exp.	TF	Cs
Heated stirred reactor (30 °C)	1	0.28	0.20	4.11	4.07	71.43	99.03
	2	0.28	0.23	4.11	4.91	82.14	119.05
	4	0.28	0.20	4.11	3.11	71.43	75.67
	6	0.28	0.20	4.11	3.34	71.43	81.27
Heated stirred reactor (60 °C)	1	0.28	0.21	4.11	3.21	75.00	78.10
	2	0.28	0.21	4.11	2.47	75.00	60.10
	4	0.28	0.22	4.11	2.10	78.57	51.10
	6	0.28	0.22	4.11	3.04	78.57	73.97
Maceration with sporadic shaker	24	0.33	0.10	4.80	1.22	30.30	25.42
	72	0.33	0.08	4.80	2.40	24.24	50.00
	120	0.33	0.09	4.80	1.70	27.27	35.42
	168	0.33	0.10	4.80	1.19	30.30	24.79
Sonication with frequency of 40 kHz	0.5*	0.33	0.07	4.80	1.33	21.21	27.71
	1.0 *	0.33	0.08	4.80	2.35	24.24	48.96
	1.5*	0.33	0.09	4.80	2.01	27.27	41.67
	2.0*	0.33	0.09	4.80	1.72	27.27	35.83

Table 2. Experimental and theoretical results of the extraction methods evaluation. * In cycles of 20 min of sonication and 10 min of rest.

Exp.	Variables				Results	
	T (°C)	$\lvert m_w/m_{et} \rvert$ (g/g)	m_{pl}/m_{sol} (g/g)	ρ (g/cm^3)	TF (% $g_{flav}/g_{.sol}$)	C_s (g/g_{ext})
1	-1	-1	0	1.00	1.33 ± 0.07	0.017 ± 0.001
2	1	-1	0	1.00	2.55 ± 0.15	0.021 ± 0.005
3	-1	1	0	0.80	7.71 ± 0.17	0.012 ± 0.004
4	1	1	0	0.81	6.92 ± 0.88	0.018 ± 0.006
5	-1	0	1	0.89	5.19 ± 0.06	0.036 ± 0.012
6	1	0	1	0.89	6.39 ± 0.77	0.033 ± 0.006
7	-1	0	-1	0.89	5.38 ± 0.12	0.018 ± 0.004
8	1	0	-1	0.88	5.64 ± 0.06	0.022 ± 0.006
9	0	-1	1	1.01	1.67 ± 0.10	0.027 ± 0.009
10	0	1	1	0.80	9.32 ± 0.40	0.016 ± 0.005
11	0	-1	-1	1.00	2.37 ± 0.15	0.011 ± 0.006
12	0	1	-1	0.81	6.17 ± 0.37	0.013 ± 0.007
13	0	0	0	0.89	5.29 ± 0.04	0.029 ± 0.006
14	0	0	0	0.89	5.58 ± 0.04	0.032 ± 0.007
15	0	0	0	0.89	5.86 ± 0.18	0.025 ± 0.004

Table 3. Box and Behnken design and experimental results of the extractive tests.

Study of the extraction process using Box-Behnken design and variance analysis

According to the Box and Benkhen design, several extraction experiments were carried out in order to evaluate the effect of the extraction variables on the extraction yield (solids concentration and total flavonoid content). Table 3 presents the experimental design used and the experimental results obtained. Variance analyses were performed for the experimental data of solids concentration and total flavonoids content in order to identify the variables having significant effect on the extraction process. Tables 4 and 5 show the variance analysis results.

Table 4 shows that the variables mpl/msol (linear effect) and $\lvert m_w/m_{et} \rvert$ (linear and quadratic effects) presented significant effect on total flavonoid content in the extract at $\alpha \leq 0.01$. Interaction effects between the linear effects of the variables m_{pl}/m_{sol} and $\lvert m_w/m_{et} \rvert$ ($\alpha \leq 0.05$)

Variable	Sum of squares	Degrees of freedom	Mean square	F_{Calc}
T	0.477	2	0.238	1.489
$T_{(L)}$	0.447	1	0.447	2.781
$T_{(Q)}$	0.030	1	0.030	0.190
m_{pl}/m_{sol}	1.232	2	0.616	3.836
$m_{pl}/m_{sol\ (L)}$	1.133	1	1.133	7.053+
$m_{pl}/m_{sol\ (Q)}$	0.100	1	0.100	0.620
$\lvert m_w/m_{et}\rvert$	64.325	2	32.163	200.305*
$\lvert m_w/m_{et}\rvert_{(L)}$	61.605	1	61.605	383.668*
$\lvert m_w/m_{et}\rvert_{(Q)}$	2.720	1	2.720	16.941*
$(T \times \lvert m_w/m_{et}\rvert)_{(L)}$	1.010	1	1.010	6.290++
$(T \times m_{pl}/m_{sol})_{(L)}$	0.221	1	0.221	1.376
$(\lvert m_w/m_{et}\rvert \times m_{pl}/m_{sol})_{(L)}$	3.706	1	3.706	23.078*
Error	0.803	5	0.161	-

Table 4. Analysis of variance (ANOVA) for the total flavonoid contents. * Term is significant (α= 0.01); + term is significant (α= 0.05); ++ term is significant (α= 0.10); (Q) quadratic effect; (L) linear effect.

Variable	Sum of squares	Degrees of freedom	Mean square	F_{Calc}
T	0.000015	2	0.000008	0.737
$T_{(L)}$	0.000014	1	0.000014	1.394
T (Q)	0.000001	1	0.000001	0.079
m_{pl}/m_{sol}	0.000300	2	0.000150	14.608*
$m_{pl}/m_{sol\ (L)}$	0.000295	1	0.000295	28.773*
m_{pl}/m_{sol} (Q)	0.000005	1	0.000005	0.442
$\lvert m_w/m_{et}\rvert$	0.000480	2	0.000240	23.420*
$\lvert m_w/m_{et}\rvert_{(L)}$	0.000032	1	0.000032	3.167
$\lvert m_w/m_{et}\rvert$(Q)	0.000448	1	0.000448	43.672*
$(T \times \lvert m_w/m_{et}\rvert)_{(L)}$	0.000000	1	0.000000	0.038
$(T \times m_{pl}/m_{sol})_{(L)}$	0.000014	1	0.000014	1.368
$(\lvert m_w/m_{et}\rvert \times m_{pl}/m_{sol})_{(L)}$	0.000038	1	0.000038	3.720
Error	0.000051	5	0.000010	-

Table 5. Analysis of variance (ANOVA) for the solids concentration. * Term is significant (α = 0.01); (Q) quadratic effect; (L) linear effect.

and, between the extraction temperature and $\lvert m_w/m_{et}\rvert$ ($\alpha \leq 0.10$) were also observed. From Table 5, it can be concluded the solids concentration in the extract is dependent on the parameters m_{pl}/m_{sol} (linear effect) and $\lvert m_w/m_{et}\rvert$ (quadratic effect) at significant level, $\alpha \leq 0.01$, while the extraction temperature presented non-significant effect on the extractive process. Figs. 2 and 3 show the Pareto plots presenting the effect of the parameters investigated on the responses investigated. Negative values indicate that optimized conditions are obtained for lower levels of the parameters.

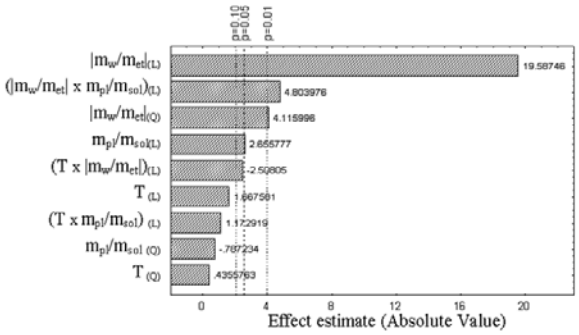

Figure 2. Pareto plots showing the effect of the parameters studied on the total flavonoid content of the extractive solution.

A second order polynomial model relating the total flavonoid contents with the extraction variables was fitted by regression analysis, using only the parameters showing statistical significance at $\alpha \leq 0.05$. Equation **5** presents the fitted model, which presented a correlation coefficient higher than 0.98 (coded variables):

$$TF = 5.62 + 2.77 \cdot \left|\frac{m_w}{m_{et}}\right| - 0.86 \cdot \left|\frac{m_w}{m_{et}}\right|^2 + 0.38 \cdot \frac{m_{pl}}{m_{sol}} + 0.96 \cdot \left|\frac{m_w}{m_{et}}\right| \cdot \frac{m_{pl}}{m_{sol}} \quad [5]$$

Figure 4 showed the response surface generated by Eq. **5**, together with the experimental data, showing the good agreement between the experimental results and the fitted model. Increasing the polarity of the solvent system reduces the flavonoid content in the extract. Higher flavonoid content was obtained for the ethanolic extract with a ratio plant to solvent mass (m_{pl}/m_{sol}) of 1:5.

The same calculation procedure was performed for the experimental results of the solids concentration, considering only the significant terms at $\alpha \leq 0.01$). The fitted model, presenting a correlation coefficient higher than 0.90, is the following:

$$Cs = 0.028 - 0.002 \cdot \left|\frac{m_w}{m_{et}}\right| - 0.011 \cdot \left|\frac{m_w}{m_{et}}\right|^2 + 0.006 \cdot \frac{m_{pl}}{m_{sol}} \quad [6]$$

Figure 5 shows the comparison between the response surface generated by Eq. **6** with the experimental data, showing the good agreement between the experimental and calculated results. From Figure 5, it can be observed that the maximum solids concentration is obtained for an intermediary value of the relation |mw/met| for a ratio plant to solvent mass (m_{pl}/m_{sol}) of 1:5.

Eqs. **5** and **6** are useful used for performing optimization studies aimed at determining the set of conditions leading to the best extraction yield (total flavonoid contents and solids concentration).

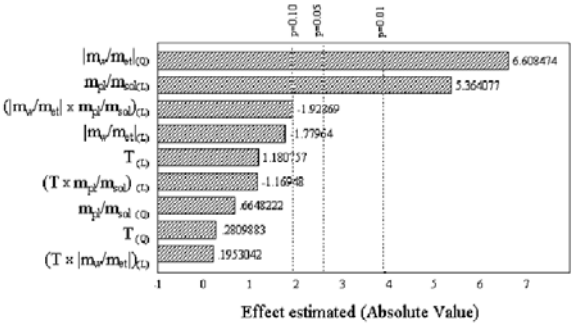

Figure 3. Pareto plots showing the effect of the parameters studied on the solids concentration of the extractive solution.

Optimization studies using the multi-response analysis (The desirability approach)

The selection of the best conditions for the extract preparation was carried out using the

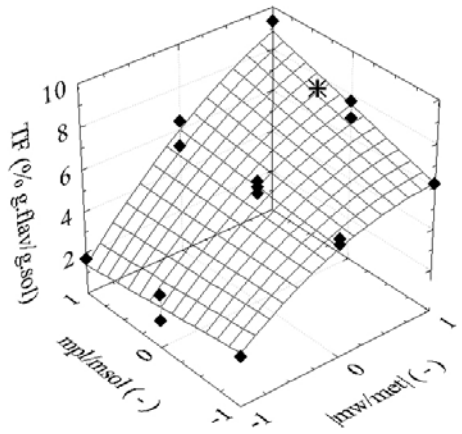

Figure 4. Response surface of the total flavonoid content, TF, as a function of (m_{pl}/m_{sol}) and of the concentration of ethanol in the extracting solvent, $|m_w/m_{et}|$, showing the optimized condition.

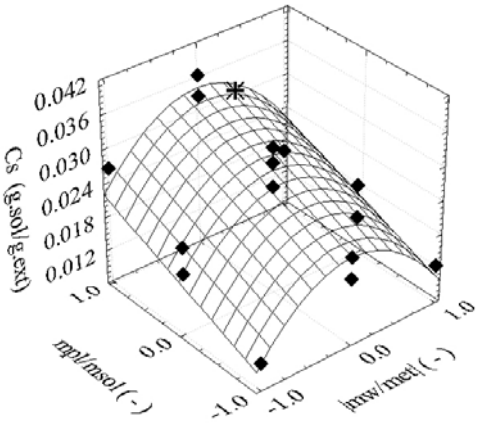

Figure 5. Response surface of the solids concentration, Cs, as a function of (m_{pl}/m_{sol}) and of the concentration of ethanol in the extracting solvent, $|m_w/m_{et}|$, showing the optimized condition.

multi-response analysis, following the methodology presented by Derringer & Suich [3]. According to these authors, each response function, Yi, should be transformed in a "desirability" function, gi, having values in the range 0 to 1. This is a popular method that assigns a "score" to a set of responses and chooses factor settings that maximize that score [27].

In the case where the response functions are unknown, the typical procedure is the obtaining of estimates of the functions by experimental design and regression analysis. This procedure was presented in the previous section, giving the Eqs. **5** and **6**. These estimates should be transformed in the desirability functions, included in the range [0,1]. In the present study the following transformation was used (Eq. **7**) [3,27]:

$$g_i = \begin{cases} 0 & \hat{Y}_i \le Y_{i*} \\ \left[\dfrac{\hat{Y}_i - Y_{i*}}{Y_i^* - Y_{i*}}\right]^R & Y_{i*} < \hat{Y}_i < Y_i^* \\ 1 & \hat{Y}_i \ge Y_i^* \end{cases} \qquad [7]$$

The Y_{i*}, value is the minimum accepted value of $\hat{Y}_i$, and should be defined by the experimenter. Each $\hat{Y}_i$ value lower than Y_{i*}, produce an unacceptable result. The value is the maximum value of $\hat{Y}_i$. Higher Y_i values cause small effect on the process. The experimenter should specify the R-value used in the transformation. If the desired values of $\hat{Y}_i$ are considerably higher than Y_{i*}, a high R-value should be used. Otherwise, small R-value can be used [3].

The global desirability of the combined responses, G, is also included in the [0,1] interval, being generated through the geometric mean of the individual functions g_i.

$$G = (g_1 \cdot g_1 \ldots g_k)^{1/k} \qquad [8]$$

In essence, the global desirability function summarizes a multi-response optimization in a problem with one response function (G), which can be minimize or maximized by univariated analysis.

The minimum and maximum values adopted for the flavonoids content were 7.0 (Y_{1*}) and 9.7 (Y_i^*). For the solids concentration, 0.030 (Y_{2*}) and 0.037 (Y_i^*) were used. After the transformations, the following global desirability function was obtained:

$$f(x,y) = \sqrt{\left(\frac{0.028 - 0.002x - 0.011x^2 + 0.006y - 0.03}{0.007}\right)^R \left(\frac{5.619 + 2.775x - 0.864x^2 + 0.376y + 0.963xy - 7.0}{2.7}\right)^R} \qquad [9]$$

In Eq. **9**, x and y are the response variables $|m_w/m_{et}|$ and m_{pl}/m_{sol}, respectively. The maximization of the G function was performed with the aid of the software MathCAD 8.0. An evaluation of the effect of the R-value on the optimized responses was performed aiming the obtaining the best results. Varying the R-value from 1 to 100 produce non-significant effect on the optimization results. Due to, a R-value of 1 was selected, leading to the determination of the optimum conditions for the extract preparation: ratio $|m_w/m_{et}|$ of 0.410 (≈ ethanol:water at 83 %, w/w); plant to solvent mass, m_{pl}/m_{sol}, of 0.2; extraction time of 1 hour at extraction temperature of 50 °C.

Fig. 6 shows the response surface generate by the multiplication of the flavonoids contents (TF) and solids concentration (C_s) as a function of the relation between plant to solvent mass (m_{pl}/m_{sol}) and of the ratio $|m_w/m_{et}|$, together with the plot of the optimized result obtained by the methodology presented. Fig. 6 shows clearly the existence of a maximum region (ideal value), presenting the highest combination of the flavonoids and solids concentration, in close agreement with the optimized results.

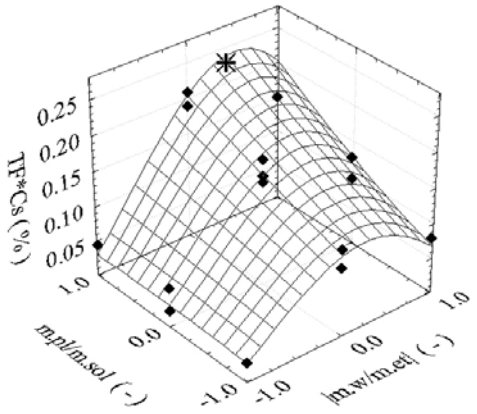

Figure 6. Response surface generate by multiplying the flavonoids contents (TF) and solids concentration (C_s) as a function of the ratios plant to solvent mass (m_{pl}/m_{sol}) and $|m_w/m_{et}|$, together with optimized result.

FINAL REMARKS

This paper summarizes the main steps performed for the development and optimization of an extraction process of active compounds from leaves of *Bauhinia forficata* Link using experimental planning *(Box-Behnken design),* regression and variance analyses and multi-response optimization. Here, it was used the Box and Behnken design in the evaluation of the effect of process variables on the extraction yield but, depending of the number of variables, different experimental planning should be used.

In this work, this analysis permitted the determination of variables affecting the extraction process and allowed the determination of the best extraction conditions of active compounds from the leaves of *Bauhinia forficata* Link namely: ethanol concentration in the extractive solvent, parameterized by the ratio $|m_w/m_{et}|$ of 0.410 (≈ ethanol:water at 83%, w/w); plant to solvent mass, m_{pl}/m_{sol}, of 0.2; extraction time of 1 hour and extraction temperature of 50°. However, the procedure can be promptly extended to the study of several others pharmaceutical processes like purification of bioactive substances, drying of extracts and development of the pharmaceutical dosage forms.

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