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Novel spectrofluorimetric method for boldine alkaloid determination in herbal drugs and phytopharmaceuticals

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ABSTRACT

A new green on-line method for Boldine determination (BOL) in herbal drugs and phytopharmaceuticals, using its native fluorescence in acid media ($\lambda_{ex} = 282$ nm; $\lambda_{em} = 373$ nm) has been developed. The presented methodology involves for the first time, a flow injection (FI) strategy using a mini-column of multiwalled carbon nanotubes as retention agent coupled with molecular fluorescence. Different parameters influence as sample pH and flow rate, eluent flow rate and composition; on BOL sensitivity and elution time was investigated by multifactorial techniques.

Adequate dynamic calibration range ($r^2 = 0.9993$) was obtained over a concentration interval of 0.029–27.0 μg mL^{-1} BOL. The limits of detection (LOD) and quantification (LOQ) were 0.008 and 0.029 μg mL^{-1}, respectively. The average recoveries in explored samples ranged from 95% to 103%. Under optimized conditions, the throughput sample as high as 30 h^{-1} was achieved with high repeatability performance (99%). The proposed development represents a useful and valuable tool emulating the analytical efficiency of the official methodologies for quality control of herbal and phytopharmaceutical drugs containing BOL. Moreover, this approach shows advantages respect to low cost, simplicity and environmental and analyst friendly.

1. Introduction

Infusions tree leaf and bark of *Peumus boldus* contain high levels of an alkaloid of the aporphine class called Boldine (BOL, Fig. 1) [1]. It is associated to antioxidant properties and free radicals diminishing [2,3]. Additionally, it is an α-adrenergic antagonist and has been reported to have hepatoprotective, cytoprotective, antipyretic and anti-inflammatory effects [4]. Boldo is not only used in the form of infusion, but also as tincture and extracts as digestive, diuretics, relaxants and in the treatment of hepatic disorders [1,5–9].

BOL determination currently carried out through different analytical techniques such as spectrofluorimetry, paper zone electrophoresis (PZE), thin layer chromatography (TLC), gas chromatography (GC) and more recently voltammetry, high performance liquid chromatography (HPLC) and ultra-high pressure liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) [1,10–16]. An HPLC method with UV detection has been developed by Pietta et al. [17] and recommended in different pharmacopoeias for quality control of a number of boldo preparations. These methods involve using organic solvents and time consuming sample clean up steps.

Molecular fluorescence spectroscopy (MF) is a very popular analytical technique as a consequence of its low cost and wide availability in most quality control laboratories. When an analyte is fluorescent, direct fluorometric detection is possible using a spectrofluorometer [18]. On-line methods advantage batch approaches in terms of precision and sampling times, amount of waste generated and also consumption of analytical reagents and solvents [19–21]. On-line approaches such as flow injection analysis (FIA) involve several experimental conditions that must be studied in the optimization process. This is a critical point considering difficulty to find out the compromise optimal conditions, but also in terms of the time and reagents consumption after a huge number of experimental observations. This last reveals the need of studying different aspects of the analysis simultaneously, and it may be carried out through multifactor optimization [22].

Experimental designs are useful approaches to do this, and specifically response surface analysis, [23,24] of the type of Box-Behnken matrix, are independent quadratic models in that they do not contain embedded factorial or fractional factorial matrixes. In these designs,

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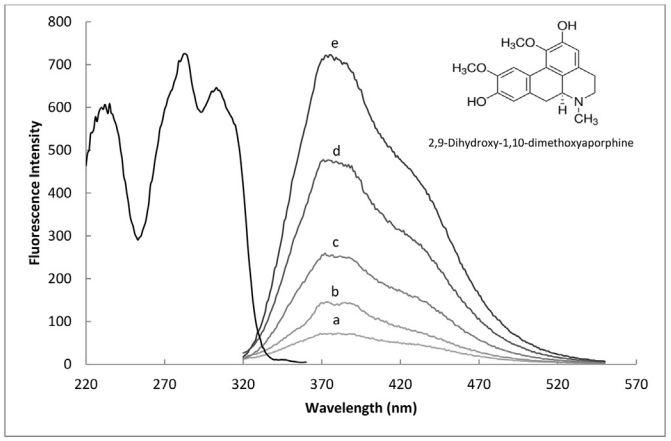

Fig. 1. Excitation and emission BOL spectra and chemical structure of BOL. C_{BOL} (μg mL^{-1}) = (a) 0.05, (b) 0.1, (c) 0.2, (d) 0.4, (e) 0.6; Slits = 3/5 nm.

the treatment combinations are at the midpoints of edges of the process space and at the center. They are rotatable (or near rotatable) and require 3 levels of each factor [25].

In this work, a new green on-line method with fluorescence detection has been developed for determination of BOL using its native fluorescence in acid media. A multivariate study including a screening phase and a response surface method was used to optimize the overall method aiming to achieve adequate sensitivity and low elution time.

2. Experimental

2.1. Instrumentation and Apparatus

A Shimadzu RF-5301PC spectrofluorometer (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp was used for the fluorescent measurements. A 1.0 cm quartz cell was employed for the batch assays and a 120 μL flow cell unit (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan) for the flow measurements. The BOL fluorescence measurements were carried out operating the spectrofluorometer in the time-course mode (transient signals; $\lambda_{ex} = 282$ nm; $\lambda_{em} = 373$ nm, slits = 3/5 nm).

The flow injection analysis (FIA) manifold consisted of a Rheodyne (Rohnert Park, CA) model-5020 six-port two-way rotary valve. The reagent and sample solutions were pumped through the FIA system using two Gilson (Villiers, France) Minipuls 3 peristaltic pumps connected to 1.52 and 1.65 mm i.d. Tygon tubing (Middleton, WI, USA). In order to preconcentrate the analyte, a mini-column of multiwalled carbon nanotubes previously activated following the procedure described by Acosta et al. [26] was used as adsorbent material.

A Model EA 940 pH-meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) equipped with a glass combination electrode was employed for all pH measurements.

A TDCM model horizontal blade/high speed grinder (Dalvo Instruments, Argentina) was used for pulverizing dried boldo leafs.

2.2. Materials

BOL ($C_{19}H_{21}NO_4$, MW 327.37) was kindly supplied by Laboratorios Bagó S.A., (Bs. As., Argentina, batch 35960). In the optimization study, the pH was adjusted using NaOH (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA) and HCl (Merck, Darmstadt, Germany) solutions until the desired pH value. Sodium dodecylsulfate (SDS) was purchased from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). Multiwalled carbon nanotubes (MWCNTs) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents employed in this study were of analytical grade.

2.3. Stock Solutions

BOL stock solutions containing 1 mg mL^{-1} were weekly prepared by dissolution of suitable amounts of the drug in absolute ethanol (Merck Darmstadt, Germany). The standard working solution of 10 μg mL^{-1} was prepared daily by step-wise dilutions with doubly distilled water.

2.4. Sample Preparation

2.4.1. Tablets

Six tablets of Bil 13® (Bagó S.A., Bs. As., Argentina) labeled to contain 1 mg BOL per tablet, were weighted and finely powdered. A portion of the powder, equivalent to one tablet (0.65 g), was accurately weighted, dissolved in 30 mL of ethanol 40% *v*/v and filtered to remove insoluble material. The filtered solution was transferred to a 50 mL volumetric flask and taken to volume with the same solvent. The BOL final concentration was about 20 μg mL^{-1}. This solution was further diluted with doubly distilled water and was adjusted to pH 1.3 with HCl (5 mol L^{-1}) to obtain suitable experimental conditions for BOL quantification.

2.4.2. Digestive Drops

Three aliquots (1 mL each) of Chofitol plus® drops (Gramon Millet S.A., Bs. As., Argentina) were transferred to 10 mL volumetric flasks, adjusted to pH 1.3 and diluted with doubly distilled water to volume. The same procedure was applied to Palatrobil® drops (Monserrat & Eclair S.A., Bs. As., Argentina).

2.4.3. Herbal Tea Bags

The tea preparation was carried out following the recommendation described in the pack. With the aim to obtain a representative sample, five tea bags were randomly selected from one box of tea, their contents were mixed and a portion of the powder, equivalent to tea bag content (1.5 g) La Virginia® or tisane Saint Gottard® (Lab. Pharmamerican, Mar del Plata, Argentina) was used to infusion preparation. The infusion was prepared adding 250 mL of boiling distilled water and covered with a watchglass and let to infuse for 5 min; then it was filtered through paper filter Whatman number 2, adjusted to pH 1.3 and made to final volume with double distilled water.

2.4.4. Boldo Leaves

Boldo dried leaves manufactured by "El Ceibo" herbal shops (San Luis, Argentina) were pulverized using a lab grinder. The infusion was prepared following the same procedure described in 2.4.3. Herbal Tea Bags.

2.5. General Procedure

Preliminary tests were carried out with the aid of different flow schemes to select the optimal on-line configuration. The FIA scheme used in previous reported works [27] was used here aiming to produce the highest peak area and adequate peak shape. Specific FIA protocol was as follows: a MWCNTs minicolumn (mC), was loaded with BOL standard (or sample) solutions prepared as just noted, with valve V in load position. Simultaneously, the carrier (HCl solution) flowed to detector for record the baseline fluorescence (experimental background). After this loading step, valve V was switched to the injection position, so that the carrier flowed through the mC in counter current (in order to minimize analyte dispersion effects) to the flow cell of fluorescence detector. Afterwards, valve V was switched to load position and the sequence started again for next standard/sample.

2.6. Software

Experimental design, data analysis and desirability function calculations were performed by using the software Stat-Ease Design-Expert trial Version 8.0.

2.7. Multivariate Optimization

2.7.1. Screening Phase: Full Factorial Design (FFD)

Experimental factorial design is a factorial analysis involving 2^k experiments (with k = number of studied variables). This type of analysis allows defining important variables affecting the analytical response(s), and its possible interactions. Moreover, a good experimental design provides a simple, efficient, and systematic approach to optimize designs for performance, quality, and cost. Consequently, a FFD was built for the evaluation of the main factors affecting the sensitivity and elution time that may be quantified through Eqs. (1) and (2), respectively:

$$Sensitivity\ (S) = \frac{peak\ area}{BOL\ standard\ concentration} \quad (1)$$

$$Elution\ time\ (t) = baseline\ time - injection\ time \quad (2)$$

A two-level full factorial design with $2^{(5)} = 32$ experiments (Table 1) is described here for the variables: Sample pH (A); HCl concentration, mol L^{-1} (B); SDS concentration, mol L^{-1} (C); eluent flow-rate, rpm (D); and sample flow rate, rpm (E); while sensitivity, cps L mol^{-1} (S) and elution time, s (t) were regarded as the dependent variables. Maximum and minimum levels of the five factors were determined based on preliminary studies. All experiments were performed in random order to minimize the effects of uncontrolled factors that may introduce bias on the measurements.

Table 1
Full factorial design (FFD) build for the responses Elution time (t) and Sensitivity (S).

Experiment (run)	Sample pH (A)	HCl conc (B)	SDS conc (C)	Sample flow rate (D)	Eluent flow rate (E)	Elution time (s)	Sensitivity (cps L mol^{-1})
18	1	0.1	0.01	7.5	12	54[a]	36.28
3	1	0.1	0.01	7.5	7.5	100	58.01
9	1	0.1	0.006	7.5	12	55	37.99
24	1	0.1	0.006	7.5	7.5	78	59.33
30	1	0.01	0.01	7.5	12	77	37.07
28	1	0.01	0.01	7.5	7.5	87	57.43
8	1	0.01	0.006	7.5	12	80	33.33
16	1	0.01	0.006	7.5	7.5	90	52.55
19	1	0.1	0.01	12	12	90	39.57
23	1	0.1	0.01	12	7.5	96	59.51
15	1	0.1	0.006	12	12	81	33.88
1	1	0.1	0.006	12	7.5	88	55.30
25	1	0.01	0.01	12	12	90	47.40
27	1	0.01	0.01	12	7.5	98	84.46
26	1	0.01	0.006	12	12	86	50.01
21	1	0.01	0.006	12	7.5	107	74.40
22	3	0.1	0.01	7.5	12	92	81.33
29	3	0.1	0.01	7.5	7.5	109	145.52
14	3	0.1	0.006	7.5	12	90	90.63
5	3	0.1	0.006	7.5	7.5	100	139.09
10	3	0.01	0.01	7.5	12	102	104.11
31	3	0.01	0.01	7.5	7.5	108	166.34
11	3	0.01	0.006	7.5	12	106	92.73
4	3	0.01	0.006	7.5	7.5	117	149.70
13	3	0.1	0.01	12	12	90	113.81
12	3	0.1	0.01	12	7.5	104	182.63
6	3	0.1	0.006	12	12	88	107.51
2	3	0.1	0.006	12	7.5	116	174.52
20	3	0.01	0.01	12	12	95	126.80
32	3	0.01	0.01	12	7.5	111	203.11
7	3	0.01	0.006	12	12	108	115.96
17	3	0.01	0.006	12	7.5	150[a]	148.68

[a] Outlier eliminated after Cook's distance test.

2.7.2. Optimization Phase: Box Behnken Design (BBD)

Once influencing factors were established with the factorial analysis, a RSM was used to find the best conditions for Sensitivity and Elution time at once. The non-significant factors were fixed at the central levels (i.e. HCl = 0.05 mol L^{-1}, SDS = 0.008 mol L^{-1}). To do this, the Derringer's desirability function (D) was used [28]. The desirability approach is a widespread method that gives scores (Y) to a set of responses and chooses factor settings that maximize that score (Eq. (3)) with *di*, as the individual desirability and *k* denoting the number of responses.

$$Desirability\ (D) = \frac{d_1(Y_1)d_2(Y_2)\ldots d_k(Y_k)}{k} \quad (3)$$

The response surface was built with the matrix of data acquired according to a Box-Behnken design that consisted in 17 experiments. Experiments were combinations of the independent variables in the following ranges: sample pH 1–3, sample flow rate 7.5–12 rpm and eluent flow-rate 7.5–12 rpm (Table 2). The levels studied were selected considering the results of the FFD. Sensitivity and Elution time were thus fitted to polynomial models and the maximum in the experimental domain was then assigned according to the desirability function maximum obtained through simplex optimization.

All experiments were performed in random order to minimize the effects of uncontrolled factors that may introduce bias in the measurements.

3. Results and Discussion

3.1. Fluorescence Characteristics of BOL in Aqueous Medium

BOL was reported to exhibit native fluorescence that is intensified significantly in the presence of acid medium. BOL showed a maximum emission at 373 nm when was excited at 282 nm (Fig. 1). These wavelengths were selected to measure the fluorescence intensity for the subsequent assays.

Flow injection analysis (FIA) is a well-established on-line technique with numerous and widespread applications in quantitative chemical analysis. Compared to batch methods, FI offers an increased sampling rate, lower reagents consumption, better precision and high versatility. These advantages have led to a continuously increasing interest for

Table 2
Box Behnken with three replicates of the central point built for Sensitivity (S) and Elution time (t).

Run	Sample pH	SFR (rpm)	EFR (rpm)	Elution time (s)	Sensitivity (cps L mol^{-1})
7	1	12	9.75	101	55.34
12	1	9.75	12	100	45.21
14	1	7.5	9.75	105	58.10
15	1	12	7.5	112	72.33
1	2	7.5	9.75	110	66.75
2	2	9.75	9.75	114	69.01
3	2	9.75	9.75	119	68.49
5	2	7.5	12	115	52.88
6	2	12	12	112	52.73
9	2	9.75	7.5	122	83.93
11	2	12	7.5	128	88.38
13	2	12	9.75	123	69.56
16	2	12	12	120	67.72
4	3	9.75	9.75	131	91.76
8	3	7.5	7.5	149[a]	138.76[a]
10	3	7.5	12	67[a]	81.33
17	3	12	9.75	77[a]	105.23

[a] Outlier removed after Cook's distance analysis.

pharmaceutical analysis and quality control applications [21]. In view of the above, it is proposed to make the automatization of the developed methodology.

3.2. Optimization of FIA System and Flow Conditions

3.2.1. Factorial Analysis

The factors selected during optimization process were sample pH (factor A); HCl concentration (factor B, [HCl]); SDS concentration (factor C, [SDS]); eluent (factor D, EFR) and sample (factor E, SFR) flow rates. These factors were evaluated at two levels each as discussed above (Table 1). The evaluation consisted in the analysis of stock standard solutions of BOL in ways to miming the analytical sample concentrations, and in all cited conditions. ANOVA tests (data not shown) were applied to experimental data in order to establish the significance of the factors studied ($p < 0.05$) upon the analytical responses. In order to get deeper insights, percentage normal probability plots for analyzed responses were plotted (Fig. 2).

3.2.2. Box-Behnken Design (BBD)

Factorial design enabled the discrimination amongst the most significant factors, though an accurate interpretation of those effects over the analytical response was not feasible. To this aim, a multilevel design was then applied following the outcomes of the former.

As it was discussed, RSMs allow defining empirically how responses behave at all values of the studied variables in the experimental domain [23]. In order to obtain optimal conditions, a Box-Behnken design was built, in which each design variable was assayed in the ranges mentioned in Section 2.7.2., and with 3 replicates of a central point. The experimental matrix defined for the factors previously selected in the FFD is shown in Table 2.

Polynomial models were used to fit the responses for all the 17 experiments (see Table 2) [23]. The model coefficients were calculated by multiple regressions, and validated by the analysis of variance (ANOVA). In all cases, irrelevant terms were eliminated (alpha > 0.1), although some of them were maintained to ensure hierarchy. As can be observed, most model terms are significant ($p < 0.05$) and the lack of fit was not significant.

The optimization procedure was thus carried out and response surfaces were obtained. The BBD models yield polynomial equations in which the model terms are preceded by the adjusted coefficients, including the intercept. The responses in terms of studied factors effects were obtained (Eqs. (4) and (5)):

$$S = 95.11 + 19.24\ \text{Sample pH} - 6.17\ \text{EFR} \quad (4)$$

$$t = 98.63 + 13.82\ \text{Sample pH} + 1.07\ \text{SFR} - 1.93\ \text{EFR} \quad (5)$$

Fig. 3 shows the desirability function for each individual factor under study, while maintaining the others at their optimal values.

The experimental conditions corresponding to one maximum (Elution time = 110.27; Sensitivity = 74.04) are sample pH = 1.3, SFR = 7.5 and EFR = 7.5 rpm.

These conditions were determined through a simple optimization using design points as starting conditions. Twenty solutions were suggested, predicting values between 109.7 and 115.2 s for elution time, and 73.16–80.90 cps L mol^{-1} for sensitivity.

3.3. Analytical Performance

3.3.1. Green Parameters

According to P. Anastas [29], Green Chemistry practices can be summarized in the idea to replace hazardous substances with less polluting ones or, if possible, innocuous products, and prevention of wastes products in origin together to restrictive use of the prime matters and energy. Since the analytical work involves a use of reagents and solvents, employs energy, and it generates wastes, some of the Anastas principles can be easily translated to this field. The main tools available today for greening analytical methods concern chemometrics, automation, and miniaturization from those, with consequent reduction in reagents consumption and waste generation, improving also the analytical parameters. Taking into consideration the objectives of green analytical chemistry, it could be enough adding to the afore mentioned figures of

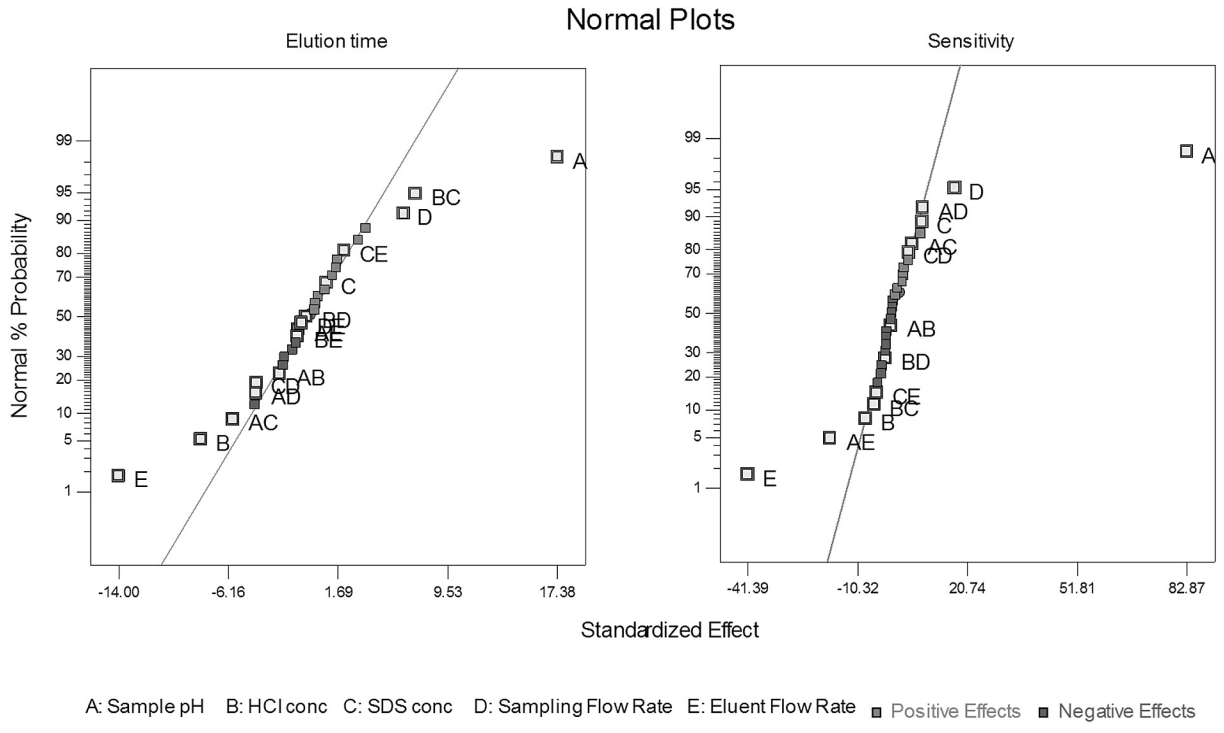

Fig. 2. Half-normal plots of the FFD.

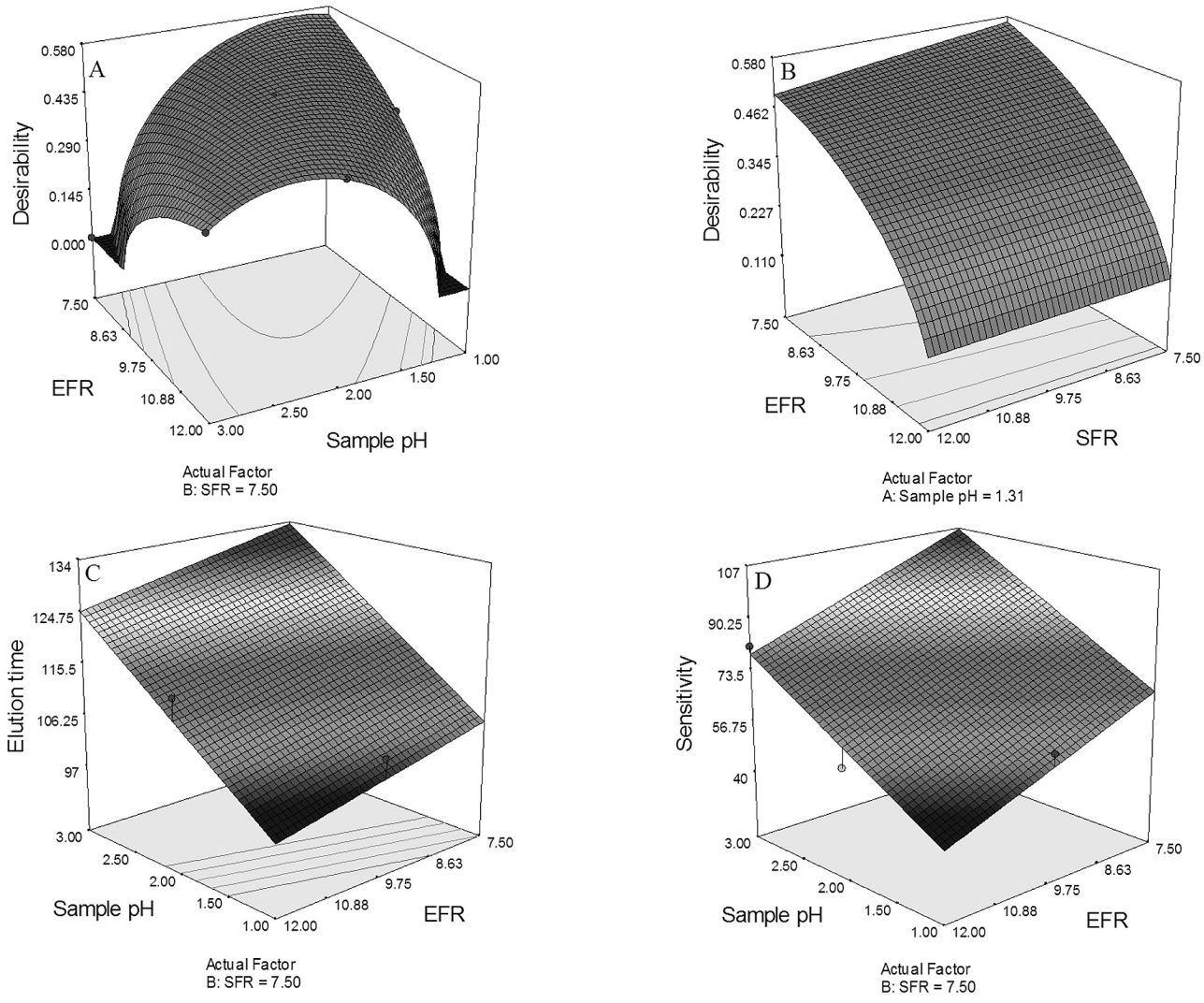

Fig. 3. Response surface plots for the desirability function (A and B), and for the individual analytical responses (C, Elution time; and D, Sensitivity).

merits, the so called green parameters; i.e. the analysis of 1) the toxicity or dangerous nature of reagents and solvents implied, 2) the volume of reagents and solvents employed, 3) the energy consumed and 4) the amount of waste generated. Table 3 summarizes the green parameters of this method, and compares them with those of other published methods [29–32].

3.3.2. Analytical Figures of Merit

Fig. 4 shows the transient responses (fiagram) obtained for the BOL standards and sample analyzed using the recommended general procedure. The quadruplicate signals put in evidence good repeatability. The calibration plot of FIA fluorescence peak area vs. BOL concentration was linear over the concentration range of 0.029 to 27 μg mL^{-1} (r^2 =

Table 3
Green analytical parameters of the FIA method compared with conventional approaches. 1) the toxicity or dangerous nature of reagents and solvents implied, 2) the volume of reagents and solvents employed, 3) the energy consumed and 4) the amount of waste generated.

Operation mode	Toxicity or dangerous nature of reagents[a]	Volume of reagents and solvents employed (mL run^{-1})	Energy consumption (KJ run^{-1})	Amount of waste generated (mL run^{-1})	Ref.
Official EuPh Method	Acetonitrile (health, 2; inflammability, 4; reactivity, 1).	1.497	~1900	6	[8]
	Diethylamine (health, 3; inflammability, 3; reactivity, 0).	0.018			
	Water (health, 0; inflammability, 0; reactivity, 0).	7.485			
UPLC-MS/MS Method	Aetonitrile (health, 2; inflammability, 4; reactivity, 1).	0.88	~360	2.25	[16]
	Formic Acid (health, 3; inflammability, 2; reactivity, 0; Corrosive).	0.136			
	Water (health, 0; inflammability, 0; reactivity, 0).	1.233			
FI-SPE-MF	Concentrated hydrochloric acid (health, 0; inflammability, 0; reactivity, 0).	0.013	~20	3	[This work]
	Water (health, 0; inflammability, 0; reactivity, 0).	2.8			
	Sodium dodecylsulfate solution 0.02 mol L^{-1} (health, 0; inflammability, 0; reactivity, 0).	0.012			

Note: all parameters were evaluated per analysis unit (a blank, standard, or sample measurement).
[a] National Fire Protection Association (NFPA) classification [33].

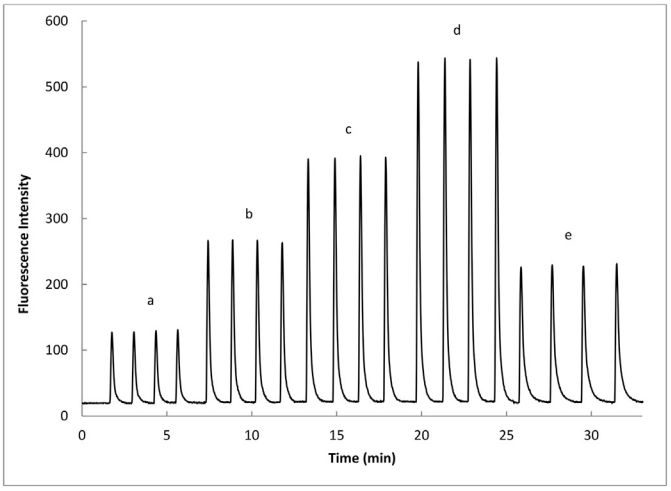

Fig. 4. BOL fiagram. Standard aqueous solutions of BOL at pH 1.3 (μg mL^{-1}) = (a) 2.5, (b) 5, (c) 7.5, (d) 10 and real sample (boldo leaves (e)); λ_{ex} = 282 nm and λ_{em} = 373 nm; slits = 3/5 nm.

0.9993) [34]. Table 4 summarizes the analytical figures of merit for this and other reported methods for determination of BOL, highlighting the advantages of the new proposal. The assay of BOL and related alkaloids contained in crude boldo samples, in extracts, or in pharmaceutical preparations is based mainly on the initial separation of the alkaloids by high performance liquid chromatography.

As can be seen, analytical parameters of this method are similar of others available, with the exception of the method presented by Han et al. [15] that employs UHPLC - MS/MS allowing reaching lowers LODs. On the contrary, as a disadvantage of that method (apart from prohibitive apparatus price), pretreatment samples involves tedious time-consuming steps. This proposal has a high sampling rate, beyond 30 samples h^{-1}, in comparison with others reported (Table 4); avoiding sample pretreatments. Additionally, precision and accuracy are adequate. Besides, the Solid Phase Extraction (SPE) device (a mini-column of carbon nanotubes in a FI scheme) enables preconcentration and selective determination of BOL in complex samples of the type of pharmaceutical products. This is an important advantage of the method, since it allows the determination of BOL without a prior (off-line) separation step and simple operating mechanisms. The main features of this method are the following: inexpensive equipment, low consumption of reagents, ease of operation, and no side effects, thus avoiding environmental pollution through toxic waste.

3.4. Applications

In order to evaluate the applicability of the proposed methodology, BOL was determined in different types of real samples. The results from the analyzed samples are summarized in Table 5. As can be seen, very satisfactory recoveries were obtained for all real samples, indicating that no interference was observed from concomitants usually present in pharmaceutical dosage forms.

The intra-day and inter-day precisions of the method, based on repeatability, were determined by replicating injections (n = 6) on

Table 4
Methods for BOL determination.

Instrumental methodology	Comments	LOD	LOL	%RSD	Samples	Ref.
CV at liquid/liquid interfaces	The analysis was performed using two kinds of liquid/liquid interfaces: water/1,2-dicholoroethane and water/PVC (polyvinyl chloride)–gelled 1,2-dichloroethane.	19.97 μg mL^{-1}	3.4–169.9 μg mL^{-1}	10.71	Extracts of boldo leaves	[10]
HPLC	A reversed-phase C18, Phenomenex® (150 × 4.6 mm, 4 μm) column was employed. The mobile phase consisted of 0.1% trifluoroacetic acid and acetonitrile (78:22, *v*/v) at a flow rate of 0.8 mL min^{-1}. The column was maintained at 30 °C and the BOL peak detection was performed at a wavelength of 281 nm.	2.41 μg mL^{-1}	5–25 μg mL^{-1}	1.73	Fluid extract of boldo (*Peumus boldus* Mol.)	[11]
HPLC fluorescence	It includes liquid–liquid extraction/back-extraction of BOL, its chromatographic separation and fluorescence detection ($\lambda_{ex/em}$ = 320/370 nm). Separation was carried out on a pentafluorophenyl core–shell column in gradient elution mode with solvent system consisting of an acetonitrile–ammonium formate buffer (5 mM, pH = 3.8).	WD	0.033–16.37 μg mL^{-1}	6.10	Plasma, bile and urine of rats	[12]
HPLC-SPE-NMR	Use of a 0.1 M NaOH solution as the post-column dilution enhanced trapping of alkaloids on the commonly used cartridge containing divinylbenzene polymer (GP resin).	WD	WD	WD	*Huperzia selago, Triclisia patens*	[13]
HPLC-UV-MS	The HPLC mobile phase consisted of a mixture of water containing 10 mM ammonium acetate ajusted at pH 3 with acetic acid-acetonitrile (90:10, *v*/v) (A) and acetonitrile (B) used in a gradient mode (0–40%).	13 μg mL^{-1}	20–510 μg mL^{-1}	3.90	*Cassytha filiformis*	[14]
UHPLC-MS/MS	The analysis was performed on an Acquity UPLC BEH C18 column utilizing a gradient elution profile and a mobile phase consisting of (A) water containing 10 mM ammonium acetate adjusted to pH 3 with acetic acid and (B) acetonitrile. An electrospray ionization (ESI)–tandem interface in the positive mode was employed prior to mass spectrometric detection.	2×10^{-6} μg mL^{-1}	0.0171–0.856 μg mL^{-1}	2.70	*Lindera aggregata*	[15]
UHPLC-MS/MS	BOL in plasma was recovered by liquid–liquid extraction using 1 mL of methyl*tert*-butyl ether. Chromatographic separation was performed on a C18 column at 45 °C, with a gradient elution consisting of acetonitrile and water containing 0.1% (*v*/v) formic acid at a flow rate of 0.3 mL min^{-1}.	WD	2.555×10^{-3}–2.555 μg mL^{-1}	7.40	Plasma	[16]
This work	Flow injection spectrofluorimetric method is used for BOL determinations. $\Lambda_{ex/em}$ = 282/373 nm; pH 1.3; sampling rate: 30 samples h^{-1}.	8×10^{-3} μg mL^{-1}	0.029–27 μg mL^{-1}	2.99	Pharmaceuticals, phytopharmaceuticals and herbal preparations	–

LOD: Limit of detection, LOL: Limits of linearity, RSD: Relative standard deviation, HPLC: High-performance liquid chromatography, UHPLC: ultra-high-pressure liquid chromatography, MS: mass spectrometry, SPE: solid phase extraction, NMR: nuclear magnetic resonance, UV: ultraviolet, CV: cyclic voltammetry, WD: without datum.

Table 5
Analysis of BOL in pharmaceutical samples and teas by the spectrofluorimetric developed method.

Sample	BOL added (μg mL^{-1})	BOL found[a] (μg mL^{-1})	Recovery[b] (%)	% RSD intraday	% RSD interday
1	–	0.92	–	–	–
	0.75	1.69	102.67	1.21	2.01
	1.50	2.36	96.27	1.52	2.05
2	–	2.10	–	–	–
	1.50	3.63	102.14	1.97	2.37
	3.00	5.09	99.67	0.78	1.18
3	–	2.79	–	–	–
	1.50	4.32	102.16	0.59	0.91
	3.00	5.78	99.46	1.98	2.28
4	–	2.05	–	–	–
	1.00	3.00	95.37	2.32	2.99
	3.00	5.14	102.99	0.96	1.89
5	–	0.96	–	–	–
	1.00	1.93	97.04	1.06	2.23
	3.00	3.98	98.94	1.39	2.57
6	–	3.81	–	–	–
	5.00	8.65	96.85	0.60	0.89
	10.0	13.89	100.79	0.74	0.90

Samples: 1—Bil 13®, 2—Chofitol plus®, 3—Palatrobil®, 4—Boldo La Virginia®, 5—Saint Gottard®, 6—Boldo hojas.
RSD: Relative standard deviation.
[a] Mean value, $n = 6$.
[b] Recovery = [(found − base) / added] × 100.

three sample solutions of different commercial formulations prepared by the standard addition method at different concentration levels. A relative standard deviation (RSD) ≤ 3% was obtained in all cases. Each tablet of sample 1 contains: Choline Orotate: 100 mg; Dehydrocholic acid: 100 mg; Deoxycholic acid: 50 mg; Casantranol: 35 mg; Boldine: 1 mg. The results obtained in sample 1 were in good agreement with the labeled content of BOL, calculated considering that the preparation contain the amount reported by the manufacturing laboratory. The samples 2 and 3 (digestive drops) contains each 100 mL: Artichoke alcoholate (1:1.75) 46.228 mL, Concentrated Boldo Tincture (2:1) 7.544 mL, *Daucus carota* Alcoholate (1:1) 46.222 mL, Menthol 0.152 g. In the drops analyzed the values for the BOL content was found in both samples in comparable levels. They satisfy the specifications established in FA (Farmacopea Argentina) [35] for boldo tincture. Sample 5 is an herbal blend containing every 10 g: Boldo (*Boldea boldus*) leaves 3.5 g; Manzanilla (*Matricaria recutita*) flowers 3.0 g; Poleo (*Lippia turbinata*) aerial parts 2.0 g; Carqueja (*Baccharis articulata*) aerial parts 0.8 g; Mint (*Mentha piperita*) leaves 0.5 g; Artichoke (*Cynara scolymus*) leaves 0.2 g. With regard to sample 4 (boldo La Virginia®), sample 5 (Tisane Saint Gottard®) and sample 6 (boldo leaf), BOL concentrations were in accordance with those established in EP [8].

4. Conclusions

The development of a simple FI-spectrofluorimetric method in acid medium has been successfully applied to quality control of pharmaceutical preparations and herbal products containing BOL. Experimental conditions were optimized under a multivariate design approach; adequate precision and wide dynamic linear range (over three orders of magnitude) were achieved. This work presents many advantages such as simplicity, high speed sampling, accuracy and the use of inexpensive equipment and reagents. Moreover, the methodology represents an eco-friendly alternative due to less consume of reactive without using toxic additives. This alternative to conventional methods for BOL quantification shows suitability to be utilized as a helpful tool for the routine quality control analysis.

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