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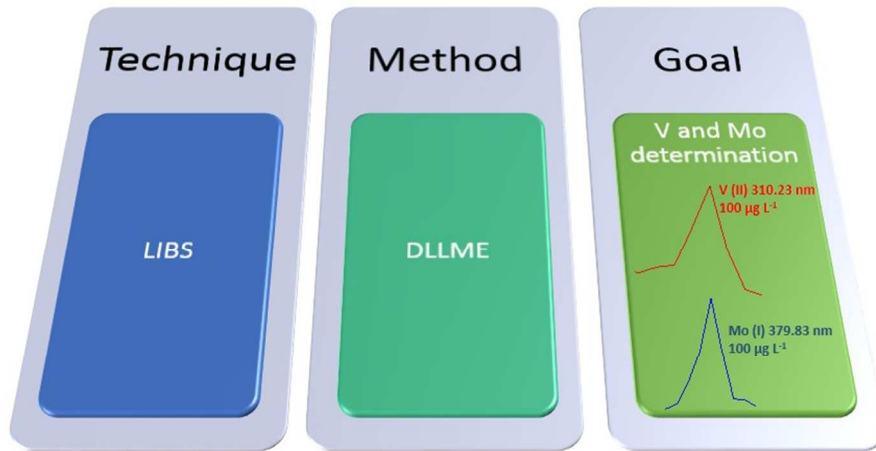


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Graphical Abstract  
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3 **The determination of V and Mo by dispersive liquid-liquid microextraction**  
4 **(DLLME) combined with laser-induced breakdown spectroscopy (LIBS)**  
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3 Laser-induced breakdown spectrometry (LIBS) is a promising analytical technique with  
4 well-known advantages and limitations. However, despite its growing popularity, this  
5 technique has been applied mainly to solid samples and there have been a smaller  
6 number of studies devoted to liquid samples. This lack of studies is mainly due to  
7 experimental difficulties in the analysis of liquid matrices. Sensitivity can be improved  
8 and matrix effects minimized in the LIBS analysis of aqueous samples by using a  
9 dispersive liquid–liquid microextraction (DLLME) procedure followed by drying the  
10 extract on a suitable surface prior to laser irradiation. The combination of DLLME-  
11 LIBS is fast, easy to use, and inexpensive. The small volume of the final extract is  
12 sufficient for LIBS analysis, and the procedure generates little waste. It is likely that this  
13 combination could be automated during future work. The Limits of detection (LOD)  
14 and quantification (LOQ) achieved with the proposed method were 30 and 70  $\mu\text{g L}^{-1}$  for  
15 Mo and 5 and 20  $\mu\text{g L}^{-1}$  for V, respectively. Using this method, we analyzed samples of  
16 pharmaceutical, multimineral formulation, soil, mineral water and a reference material  
17 NCS ZC 85005 (Beef Liver). In the latter, the concentration of V was below the LOQ,  
18 and the recovery of Mo was 103%.

## 1 Introduction

Laser-induced breakdown spectroscopy (LIBS) technique in analytical chemistry has become popular due to its versatility and simplicity when applied to the multi-element analysis of solid, liquid or gas samples, as minimizes or eliminates sample pretreatment. In addition, LIBS is a portable technique, permitting field analysis and remote measurements. These factors allow the technique to be safely used in dangerous environments.<sup>1</sup>

The LIBS technique has been successfully used for the determination of elements in different types of samples. These include biological materials<sup>2,3</sup>, metal alloys<sup>4,5</sup>, polymers<sup>6,7</sup>, soil and minerals<sup>8,9</sup>, geological samples<sup>10</sup>, among others<sup>11,12</sup>. LIBS is applied mainly to solid samples, primarily because the samples can be analyzed directly without further preparation if standards are available.

The determination of V and Mo is generally difficult. This is especially true in the case of aqueous samples. The most common experimental difficulties when using LIBS are the formation of plasma and the generation of bubbles that affect the characteristics of subsequent plasmas<sup>13,14</sup>. These drawbacks result in poor sensitivity and reproducibility in aqueous samples<sup>13-16</sup>.

One practical way to circumvent the limitations of LIBS with aqueous samples is to dry the sample on a suitable surface. We present the use of a microextraction technique followed by the evaporation of the organic phase as one reliable example. Liquid-liquid extraction has been widely used to eliminate interferences and increase the sensitivity of analytical procedures. There has been an increase in the use of miniaturized liquid-liquid extractions since the year 2000. Among these techniques is

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3 dispersive liquid-liquid microextraction (DLLME), that is in accordance with the  
4 principles of green chemistry: it is a simple, fast and inexpensive procedure <sup>17</sup>.  
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7 The use of a single drop of DLLME solvent dried on an aluminum surface  
8 combines the benefits of preconcentration by microextraction with the advantages of  
9 LIBS, such as multi-element determination. The goal of this study was to combine the  
10 DLLME technique with LIBS in the determination of V and Mo.  
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## 18 **2 Experimental**

### 19 *2.1 Reagents*

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22 All reagents used were analytical grade. Solutions were prepared using ultrapure  
23 water obtained from a Milli-Q<sup>®</sup> purification system (Millipak-40 Filter Unit 0.22  $\mu\text{m}$   
24 NPT, Bedford, MA, USA) with a resistivity greater than 18.2  $\text{M}\Omega\text{ cm}$ .  
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32 Analytical reference solutions were prepared by diluting stock standard solutions  
33 containing 1000  $\text{mg L}^{-1}$  of V and Mo High-Purity Mono Element Standard Solutions  
34 (Charleston, USA) with ultrapure water.  
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38 The solution of chelating agent, 8-hydroxyquinoline (8-HQ) (Vetec, Rio de  
39 Janeiro, RJ, Brazil) was prepared daily by dissolving the appropriate amounts of 8-HQ  
40 in 10 mL of ethanol and storing these solutions in brown glass flasks. Nitric acid 65%  
41 (w/w),  $\text{H}_2\text{O}_2$  30% (w/w) and  $\text{HClO}_4$  65% (w/w) (Merck, Darmstadt, Germany) were  
42 used for microwave sample preparation.  
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### 51 *2.2 Instrumentation*

52 The LIBS system was composed of a Nd:YAG laser (model HYL-101 Handy-  
53 YAG, Q-switched, Quanta System S.P.A., Varese, Italy). We used the fundamental  
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3 wavelength of the laser (1064 nm) with a pulse energy of 180 mJ (pulse width 6 ns  
4 FWHM), operated in single-pulse mode. The laser beam was focused on the sample by  
5 a biconvex lens with a focal length of 100 mm. The emitted radiation was collected by a  
6 five-furcated optical fiber (5x400  $\mu\text{m}$  fibers, model FC5-UV400-2, Avantes, Eerbeek,  
7 The Netherlands) and detected by a five-channel spectrometer (model AvaSpec-2048-  
8 SPU Avantes) covering the wavelengths from 197.146 to 852.190 nm.  
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16 A delay system consisting of two pulse generators (delay generator / digital  
17 pulse, Model DG 535, Stanford Research Systems, Inc. and 1 Hz to 50 MHz pulse  
18 generator, model PM-5715, Philips) was used for synchronizing the firing of the laser  
19 and data acquisition. An LG laptop (Intel Core 2, 1.00 GB of RAM and Windows Vista)  
20 equipped with the AvaSoft© complete software (v. 7.6.1., Avantes) was used for data  
21 acquisition.  
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29 In order to compare the results obtained, an ICP OES spectrometer (Perkin  
30 Elmer, model Optima 4300DV, Norwalk, CT, USA) with dual view capacity but that  
31 was operated in the axially viewed plasma mode (radiofrequency power of 1400 W)  
32 was used.  
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### 40 *2.3 Samples and samples preparation*

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42 To demonstrate the applicability of the proposed method, different samples were  
43 tested: (1) water, (2) pharmaceutical, (3) multiminerall formulation, (4) soil and (5) food  
44 samples. Water samples were used without further preparation. The pharmaceutical  
45 sample and multiminerall formulation were ground manually using an agate mortar and  
46 pestle to obtain a homogeneous material. Before the dispersive liquid-liquid  
47 microextraction procedure, 500 mg of each samples were weighed and digested using  
48 7.0 mL of  $\text{HNO}_3$  65% (w/w) and 1 mL of  $\text{H}_2\text{O}_2$  30% (w/w). For the soil sample, 250 mg  
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3 of the sample were weighed and digested using 6 mL of HNO<sub>3</sub> 65% (w/w), 1 mL of  
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5 H<sub>2</sub>O<sub>2</sub> 30% (w/w) and 1 mL of HClO<sub>4</sub> 65% (w/w). The digestion procedure was  
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7 conducted in a microwave (MW) oven (Ethos, Milestone, Italy). The MW digestion  
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9 program used for the pharmaceutical, multimineral formulation and soil samples was  
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11 composed of only one step: 30 min at 200 °C (in the first 10 min the temperature was  
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13 increased from room temperature up to 200°C).  
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16 A beef liver certified reference material (NCS ZC 85005) was also used. A  
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18 sample mass of 100 mg was weighed and MW-digested using 10 mL of HNO<sub>3</sub> 65%  
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20 (w/w). The digestion program was configured as follow: 20 min at 180 °C (in the first  
21  
22 10 min the temperature was increased from room temperature up to 180°C). In all cases  
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24 the microwave power was 1000 W.  
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#### 29 30 *2.4 Dispersive liquid–liquid microextraction procedure*

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32 The microextraction procedure is summarized in 3 steps: (1) in a glass tube, 15  
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34 mL of sample, 166 µL of a 8-hydroxyquinoline complexing agent (8-HQ) solution, was  
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36 added (0.05 or 0.1% w/v) and the pH value was adjusted to 2 or 5 with HNO<sub>3</sub> or  
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38 NH<sub>4</sub>OH solutions. Then, either 30 or 60 µL of the extraction solvent (1-undecanol) was  
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40 added, and the mixture was shaken using a vortex shaker for a specified time (2 or 4  
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42 min). (2) The solution was centrifuged (2000 or 4000 rpm) for either 4 or 8 min to  
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44 separate the two phases, with the organic phase containing the analytes at the top. (3)  
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46 Ten microliters of the organic phase was collected using a microsyringe. During the  
47  
48 optimization, a solution containing 500 µg L<sup>-1</sup> of both V and Mo was used.  
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### 2.5 Analysis of extracts from DLLME by LIBS

For LIBS analysis, 10  $\mu\text{L}$  of the solvent containing the analyte was placed on a suitable sample holder. This holder consisted of a piece of thin Al foil in which several cells had been previously molded with a micropipette tip to contain and prevent spreading of the drop. The Al foil was placed on a plate, heated for 5 min on a hot plate to evaporate the organic phase from the microdroplet, and then allowed to cool.<sup>18</sup> Once the support was at room temperature the LIBS measurements were carried out. Figure 1 shows a pictorial diagram of the DLLME and LIBS analysis steps.

## 3 Results and discussion

### 3.1 Optimization of dispersive liquid–liquid microextraction procedure

The optimization of the DLLME procedure was divided in two complementary parts. In the first part, a Plackett-Burman design was used to identify the most significant among the 7 variables. In this case, a solution containing both V and Mo at a concentration of  $500 \mu\text{g L}^{-1}$  was used. The DLLME variables investigated were (a) the concentration (0.05 or 0.1 w/v) of complexing agent (8-HQ), (b) volume (30 or  $60 \mu\text{L}$ ) of the extractant solvent (1-undecanol), (c) centrifugation time (4 or 8 min), (d) vortexing time (2 or 4 min), (e) pH (2 or 5), (f) presence or absence of NaCl and (g) centrifuge speed (2000 or 4000 rpm). The variables were studied in two levels (-1 and +1), and 12 experiments were performed.

The two variables pH value and volume of extractant solvent showed a significant effect in the Plackett-Burman experiment. Microsoft Excel was used in these calculations.

Therefore, a central composite design (CCD) was performed to optimize these two variables. Here the variables were investigated at five levels and the coded values ranged from  $-\sqrt{2}$  to  $\sqrt{2}$  and Microsoft Excel was also used. Table 1 shows the values established in the CCD to investigate the behavior of pH and extractant solvent (SE) volume and the predictive ability of the emission signals obtained for V and Mo. While carrying out the CCD, 12 additional experiments were performed with the V and Mo concentrations fixed again at  $500 \mu\text{g L}^{-1}$ . Four experiments were performed at the central point (variables coded in 0, see experiments 9 – 12 at Table 1) to calculate the sum of the squares for the pure error and to evaluate the significance of the coefficients models proposed for V and Mo.

The regression models (only the significant coefficients) proposed for V and Mo are presented as Equations 1 and 2, respectively:

$$\text{V (emission intensity)} = 38328 - 12886\text{pH} - 10985(\text{pH}^2) \quad \text{Equation 1}$$

$$\text{Mo (emission intensity)} = 15823 - 4125\text{pH} - 3270\text{SE} - 3527(\text{pH}^2) - 3614(\text{SE}^2)$$

Equation 2

In the case of V, only the linear and quadratic coefficients for pH presented significant values at a confidence level of 95%. In this case, any extractant solvent volume between evaluated range (32 and 88  $\mu\text{L}$ ) can be used. For Mo both linear and quadratic coefficients of pH and extractant solvent volume were significant. Figure 2 show the overlapped contour plots for the models obtained for V and Mo. As observed for V (see vertical lines), high signals are obtained when the pH is in the range of 3.0 to 3.8, but the signal is indifferent to the extraction solvent volume in the evaluated range (32 – 88  $\mu\text{L}$ ). For Mo, an optimal condition exists when the pH value lies between 3.0 and 3.8 and the extraction solvent volume is between 48 and 56  $\mu\text{L}$  (see ellipses). For this reason, a compromise condition is necessary to determine both analytes in the same

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3 microextraction procedure. Observing the practical operational conditions, a pH of 3.6  
4 and an extraction volume of 50  $\mu\text{L}$  were chosen as optimal conditions for both of the  
5 variables studied and both of the analytes. The other final optimized conditions for the  
6 DLLME procedure were: concentration of 8-HQ of 0.1(%) w/v, vortex time of 2 (min),  
7 centrifugation time of 8 (min) and centrifugation speed of 4000 (rpm).  
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14 As mentioned in the experimental section (section 2.5), after the microextraction  
15 procedure, a droplet of the organic layer with a volume of 10  $\mu\text{L}$  was dried on an  
16 aluminum plate (see details in Figure 1) and was subsequently analyzed by LIBS.  
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### 20 21 22 23 *3.2 Figures of merit*

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25 The figures of merit of the developed procedure were evaluated by calculating  
26 the limits of detection (LOD) and quantification (LOQ), defined as:  $\text{LOD} = 3\sigma/s$  and  
27  $\text{LOQ} = 10\sigma/s$ , where  $s$  is the slope (sensitivity) of the analytical curve and  $\sigma$  is the  
28 standard deviation of 10 consecutive measurements of the blank.  
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34 Figure 3 shows some emission signals obtained for V (Figure 3a) and Mo  
35 (Figure 3b) when 10  $\mu\text{L}$  aqueous standard solutions were analyzed by only LIBS (40 mg  
36  $\text{L}^{-1}$ ), i.e., without the prior DLLME procedure and by DLLME-LIBS (100  $\mu\text{g L}^{-1}$ ). As  
37 can be observed when 100  $\mu\text{g L}^{-1}$  of V and Mo was determined combining DLLME-  
38 LIBS it was possible to obtain analytical signals in the same order of magnitude when  
39 40 mg  $\text{L}^{-1}$  was determined using only LIBS. The combined method of DLLME-LIBS  
40 was linear from 20 to 750  $\mu\text{g L}^{-1}$  for V and from 70 to 750  $\mu\text{g L}^{-1}$  for Mo.  
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50 A comparison of the figures of merit obtained with the proposed method  
51 (DLLME-LIBS) and using only LIBS analysis is shown in Table 2. By using two  
52 standard calibration curves with microextraction (DLLME-LIBS) and without  
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3 microextraction (LIBS), it was possible to estimate the preconcentration factors as 12-  
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5 fold for V and 9-fold for Mo.  
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### 8 9 10 3.3 Application to samples

11 The recovery of both V and Mo in a sample of mineral water was evaluated by  
12 using spiked/recovery assays. The added concentrations of analyte varied from 506 to  
13 240  $\mu\text{g L}^{-1}$ , and the recoveries ranged from 94 to 105%. The basal concentrations of V  
14 and Mo in the sample were below the LOD (see Table 2) for DLLME-LIBS method.  
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20 All the digested samples (pharmaceutical, multimineral formulation and soil),  
21 including the reference material (food), were analyzed using only the proposed  
22 DLLME-LIBS procedure in order to prove experimentally the feasibility of this  
23 combination.  
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29 Table 3 shows the results obtained for the pharmaceutical, multimineral  
30 formulation and soil samples. These results were compared with those obtained from  
31 ICP OES analysis. Using these ICP OES results as reference values, the recovery  
32 obtained with DLLME-LIBS methodology ranges from 92 to 104%. As observed from  
33 this Table, pharmaceutical (vanadium chelate) and multimineral formulation samples  
34 were tested. The first has been suggested for the treatment of diabetes, and the second is  
35 a multimineral and multivitamin supplement. The V concentration in the chelate was  
36 high (3352  $\text{mg kg}^{-1}$ ), whereas a much lower concentration was found in the  
37 multivitamin sample (9.9  $\text{mg kg}^{-1}$ ). Only Mo was observed in the multimineral at a  
38 concentration of 13.2  $\text{mg kg}^{-1}$ . In the case of the soil sample, only V was detected with a  
39 concentration of 12.0  $\text{mg kg}^{-1}$ .  
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53 The analysis of solid samples by digestion + microextraction + LIBS has been  
54 made to demonstrate experimentally the feasibility of this combination. In addition, the  
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3 solid samples digestion makes feasible the comparison with aqueous calibration  
4 standards.  
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7 The accuracy of the proposed procedure was evaluated from the analysis of a  
8 certified reference material (CRM), NCS ZC 85005 (Beef Liver). Vanadium and Mo  
9 certified values are 0.267 (reference value) and  $3.97 \pm 0.28 \text{ mg kg}^{-1}$ , respectively. The V  
10 concentration found was below the LOQ of the proposed method and the Mo recovery  
11 was 103%.  
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#### 18 19 20 21 **4 Conclusions**

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25 LIBS can be successfully used in combination with the technique of dispersive  
26 liquid-liquid microextraction for the analysis of V and Mo in different types of samples  
27 (i.e., solid and liquid). When solid samples are analyzed aqueous standard calibration  
28 solutions can be used after the digestion of solid samples.  
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34 The sensitivity obtained with DLLME-LIBS is approximately 11 and 7 times  
35 greater for V and Mo, respectively, than that obtained without DLLME, and the LOD is  
36 approximately 12 and 9 times lower for V and Mo, respectively.  
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41 This study presents a new step forward in the applicability of LIBS to the  
42 analysis of liquid samples. Obviously, further work is mandatory and this is under  
43 investigation in our laboratories.  
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**Table 1** Variables and levels studied in the central composite design for the DLLME procedure optimization and the emission intensities obtained for V and Mo.

Experiment	pH		Extractant solvent volume (SE)		Emission intensity	
	Coded	Real	Coded	Real value	V	Mo
	value	value	value	( $\mu\text{L}$ )		
1	-1	3.1	-1	40.0	53314	12758
2	1	5.1	-1	40.0	22019	11299
3	-1	3.1	1	80.0	40304	9812
4	1	5.1	1	80.0	3456	2140
5	$-\sqrt{2}$	2.6	0	60.0	25022	16888
6	0	4.1	$-\sqrt{2}$	31.7	28557	13247
7	$\sqrt{2}$	5.5	0	60.0	314	10
8	0	4.1	$\sqrt{2}$	88.2	35677	3305
9	0	4.1	0	60.0	32244	18073
10	0	4.1	0	60.0	38734	14505
11	0	4.1	0	60.0	37493	15008
12	0	4.1	0	60.0	44844	15707



**Table 2** Figures of merit obtained with the LIBS and DLLME-LIBS methods.

Parameters	VII 310.23 nm		MoI 379.83 nm	
	LIBS	DLLME-LIBS	LIBS	DLLME-LIBS
Linear range (number of calibration points = 5)	0.2 to 40 mg L <sup>-1</sup>	20 to 750 µg L <sup>-1</sup>	0.5 to 40 mg L <sup>-1</sup>	70 to 750 µg L <sup>-1</sup>
Correlation coefficient (number of calibration points = 5)	0.995	0.994	0.966	0.966
Sensitivity (counts/L mg <sup>-1</sup> )	7575	82901	1407	9810
LOD (µg kg <sup>-1</sup> )	60	5	300	30
LOQ (µg kg <sup>-1</sup> )	200	20	500	70
Blank signal (mean ± Standard Deviation)	145 ± 24	158 ± 39	387 ± 213	245 ± 73
Repeatability (500 µg L <sup>-1</sup> ) (RSD %) <sup>(a)</sup>	-	6	-	9
Relative sensitivity <sup>(b)</sup>		11		7.0
Relative LOD <sup>(c)</sup>		12		9

<sup>a</sup> n=10<sup>b</sup> Sensitivity DLLME-LIBS / Sensitivity LIBS<sup>c</sup> LOD LIBS / LOD DLLME-LIBS

**Table 3** V and Mo concentrations ( $\text{mg kg}^{-1}$ ) obtained in the pharmaceutical, multiminerall formulation and soil samples using DLLME-LIBS and ICP OES.

Samples	Analyte concentration ( $\text{mg kg}^{-1}$ )			
	ICP OES		DLLME-LIBS (recovery, %)	
	V	Mo	V	Mo
Pharmaceutical (Vanadium chelate)	$3210 \pm 92$	<LOD	$3352 \pm 748$ (104)	<LOD
Multimineral formulation	$10.7 \pm 2.4$	$13.7 \pm 2.7$	$9.9 \pm 2.7$ (92)	$13.2 \pm 4.9$ (96)
Soil	$12.3 \pm 3.0$	<LOQ	$12.0 \pm 5.0$ (97)	<LOQ

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7 **Fig. 1** Pictorial description of the steps related to: (a) the microextraction procedure (1 –  
8 mixture of sample and 8-HQ solution, 2 – addition of the organic extractant solvent, 3 –  
9 vortex shaking and 4 – phase separation), (b) organic microdroplet collection and  
10 deposition in the cell, (c) drying process and (d) LIBS analysis of the dried  
11 microdroplets deposited on the aluminum support.  
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20 **Fig. 2** Contour plots overlapped for the regression models proposed for V (vertical  
21 lines) and Mo (ellipses). The star shows the optimal conditions.  
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27 **Fig. 3** Emission signals of VII (310.23 nm) (a) and MoI (379.83 nm) (b) using LIBS  
28 and DLLME-LIBS methodologies.  
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Figure 1

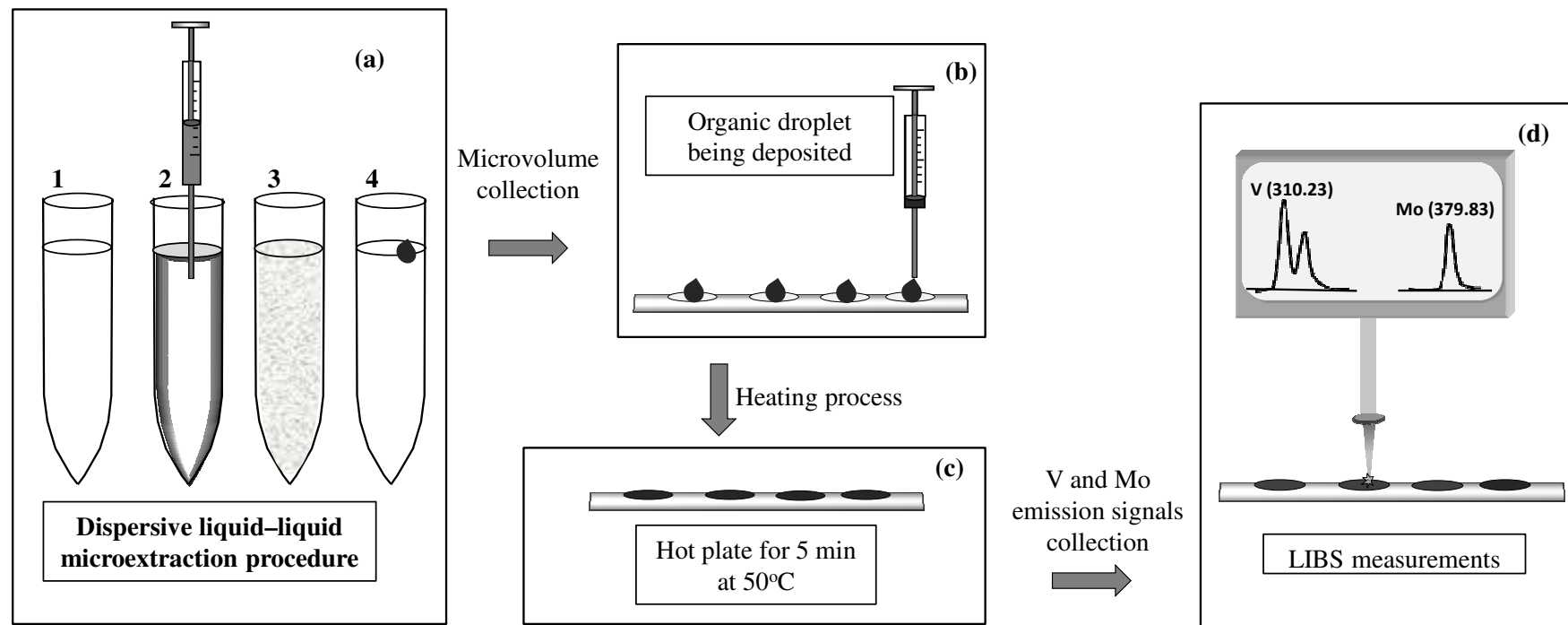


Figure 2

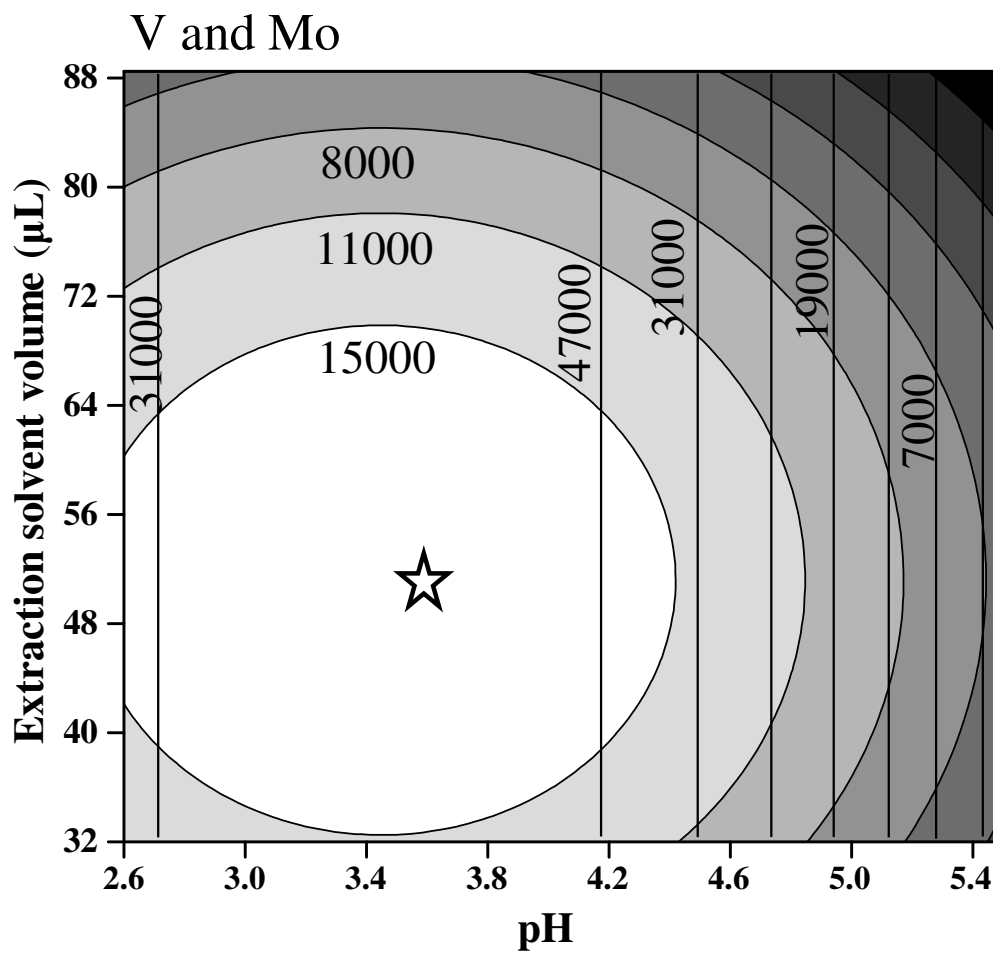


Figure 3a

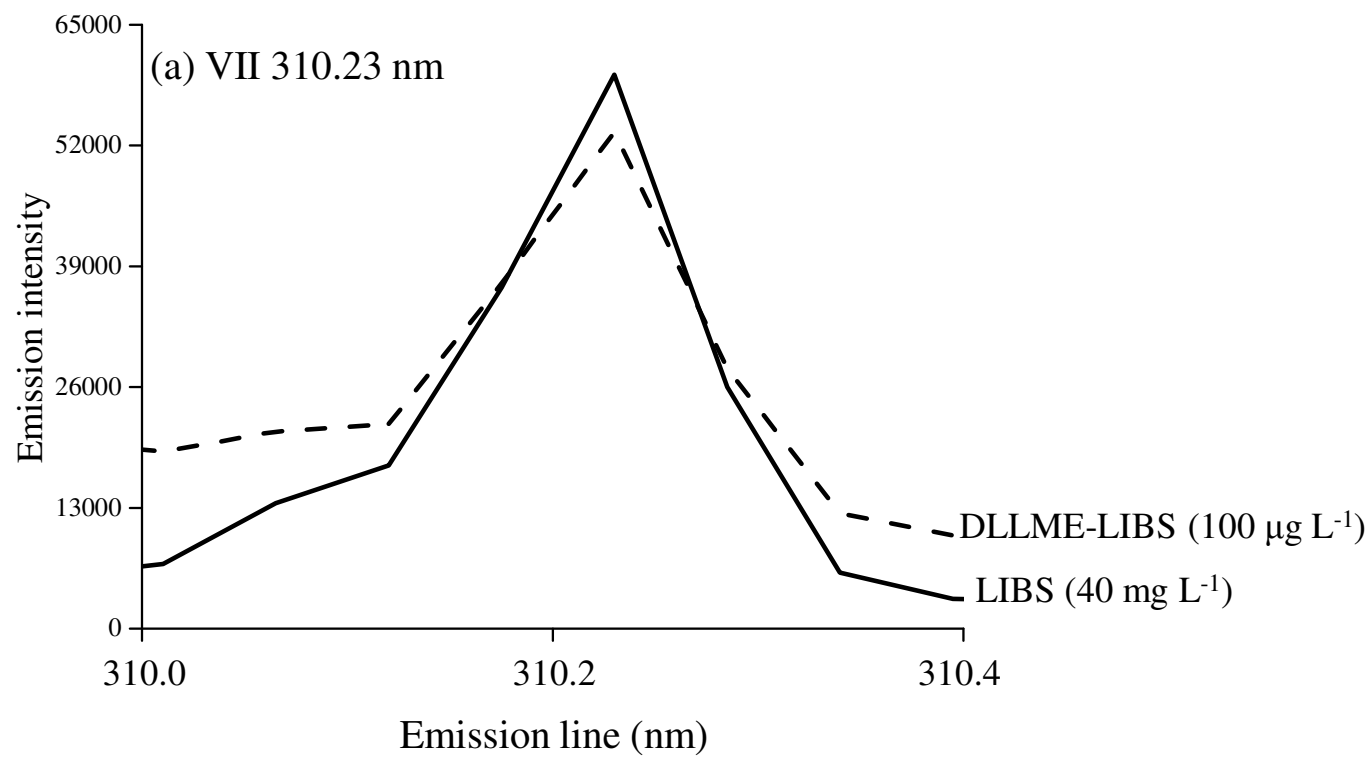


Figure 3b

