

Original Research Article

Development and Validation of a Solid-Liquid Extraction with Low Temperature Partitioning (SLE/LTP) Method for Determination of the Herbicide Indaziflam in Brazilian Soils by High Performance Liquid Chromatography (HPLC-UV/Vis)

ABSTRACT

Given the increased use of herbicides in intensive agriculture, it is essential to understand the dynamics of new products in the soil and in the wider environment, in order to avoid problems of contamination. One of the herbicides recently registered in Brazil is indaziflam ($C_{16}H_{20}FN_5$). Although various analytical techniques are available for the determination of herbicides in soils, there have been few studies concerning indaziflam. In the present work, a solid-liquid extraction with low temperature partitioning (SLE/LTP) method was developed, optimized, and validated for the identification and quantification of this herbicide in the soil by high performance liquid chromatography with ultraviolet detection (HPLC-UV/Vis). Samples (4 g) of clayey and sandy Red-Yellow Latosol soils were conditioned with charcoal (1% w/w) obtained from the pyrolysis of sugarcane bagasse, and were fortified with 2 mg L⁻¹ of the herbicide. Extraction was performed using 4 mL of ultrapure water (pH 7.0) and 10 mL of acetonitrile (ACN)/ethyl acetate (EtOAc) in a proportion of 8.1:1.9 mL. The mixture was vortexed and then frozen at -20 °C. The supernatant was filtered, evaporated to dryness, and then recovered in 1.5 mL of ACN, prior to analysis by HPLC-UV/Vis. The recoveries of the herbicide were between 79.56 and 106.41% and the coefficients of variation were between 0.9 and 14.4%, in conformity with the parameters established by national regulatory agencies. The method provided selective, efficient, and accurate determination of indaziflam in the soils studied.

Keywords: Herbicide, method validation, SLE-LTP extraction, HPLC-UV/Vis.

1. INTRODUCTION

The herbicide indaziflam (N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1R)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine) (Fig. 1) is recommended for the pre-emergent control of weeds in sugar cane, coffee, citrus, eucalyptus, and pine crops [27]. Its mechanism of action is related to the inhibition of cellulose biosynthesis [28], which prevents cell wall formation and therefore paralyzes development of the plant [9]. This herbicide has low water solubility (4.4 mg L⁻¹ (pH 4), 2.2 mg L⁻¹ (pH 7), and 2.8 mg L⁻¹ (pH 9), at 20 °C). It is a weak acid with p_{ka} 3.5, K_{OC} <1.000 mL g⁻¹ of organic carbon, and log K_{OW} of 2.8 at pH 4, 7, or 9. It is slightly lipophilic [3] and its half-life in soil (t_{1/2}) is greater than 150 days [15].

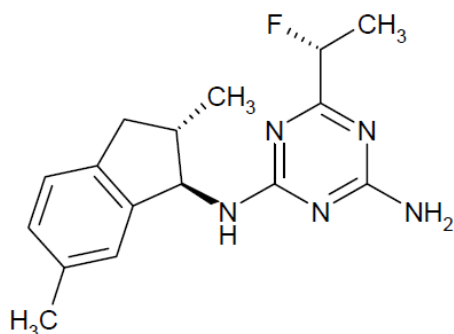


Fig. 1. Structural formula of the indaziflam molecule

Given its lengthy persistence in the environment, the use of this herbicide without knowledge of the dynamics of its dissipation in the soil can lead to serious environmental problems, such as carryover as well as the contamination of surface and subterranean water bodies [25]. Recent studies have indicated that conditioning soils with charcoal obtained from biomass by pyrolysis could help to reduce such problems of herbicide contamination [11, 26, 29].

Analytical methods including high performance liquid chromatography (HPLC) and solid-liquid extraction (SLE) have been employed for the determination of herbicides in soils [4, 5, 16, 23]. However, there have been few studies involving indaziflam. The herbicide has been removed from soil using microwave-assisted extraction with acetonitrile/water (80:20 v/v) [8]. Indaziflam labeled with ^{14}C has been extracted from a liquid medium using a scintillation spectrometry technique [1].

The aim of this work was to adapt, optimize, and validate a solid-liquid extraction with low temperature partitioning (SLE/LTP) method, with analysis using HPLC-UV/Vis, in order to study the behavior of this herbicide in Brazilian soils.

2. MATERIALS AND METHODS

2.1. Equipment and reagents

Dry sugar cane bagasse was pyrolyzed for 1 h in a muffle furnace (Linn Elektro Therm, Eschenfelden, Germany), using a heating rate of $9\text{ }^{\circ}\text{C min}^{-1}$ and a final temperature of $500\text{ }^{\circ}\text{C}$, as described previously [27, 10, 14]. The charcoal was triturated and passed through a 150 mesh sieve, resulting in a particle size $<106\text{ }\mu\text{m}$.

Chromatographic analyses were performed using an HPLC system (Model LC-20AT, Shimadzu) equipped with a UV-Vis detector (Model SPD-20AT, Shimadzu), an auto injector (Shimadzu SIL 10AF) and a stainless steel reverse phase C18 column (VP-ODS, Shimadzu) with a length of 150 mm and internal diameter of 4.6 mm, packed with particles $4.6 \pm 0.3\text{ }\mu\text{m}$ in diameter. The column temperature was $30\text{ }^{\circ}\text{C}$. The reagents used were HPLC-grade acetonitrile (Sigma-Aldrich, Germany), ethyl acetate (99.5% v/v, Vetec, Brazil), acetic acid (99.7% v/v, Isofar, Rio de Janeiro, Brazil), deionized water obtained from a Milli-Q system, and anhydrous sodium sulfate (99% w/w, Sigma-Aldrich, Germany). A stock standard solution of the herbicide was prepared at a concentration of 1000 mg L^{-1} by solubilization of indaziflam BCS-AA10717 (99.5% w/w, Bayer, Brazil) in acetonitrile.

The following equipment and materials were used in the experiments: analytical balances (Model BP 2215, Sartorius, Gottingen, Germany; Model AY 220, Shimadzu, Kyoto, Japan), vortex mixer (Kasvi K45-2810, Econolab, São Paulo, Brazil), freezer (Consul, Santa Catarina, Brazil), rotary evaporator (Model 802, Fisatom, São Paulo, Brazil) operated at 105 rpm, thermostatic bath (Model TE-184, Technal, São Paulo, Brazil) operated at 500 °C, and pH meter (Model DM-22, Digimed, São Paulo, Brazil).

2.2. Sample preparation

Samples of clayey and sandy Red-Yellow Latosol soils were collected from the first 20 cm of the soil profile, in an area free from herbicide applications, in the municipality of Viçosa (Minas Gerais State, Brazil; geographical coordinates: 20° 45' 14"S, 42° 52' 55" W). These samples were used without and with conditioning by addition of 1% (w/w) charcoal. Characterization of the soils was performed in the Viçosa Soil Analysis Laboratory, following the methodology described by Embrapa (1997) (Table 1).

Table 1. Physical and chemical analyses of the soils used to validate the SLE/LTP-HPLC-UV/Vis method.

Soils	pH H ₂ O	P --mg dm ⁻³ --	K --mg dm ⁻³ --	Ca ²⁺ -----	Mg ²⁺ -----	Al ³⁺ -----	H+Al cmol _c dm ⁻³ -----	BS	(t)	(T)	OM dag kg ⁻¹	Clay -----%	Silt -----%	Sand -----%	Textural class
RYL ¹ Viçosa	4.60	1.30	13.00	0.40	0.10	0.00	4.90	0.53	1.73	5.48	1.86	49	12	39	Clayey
RYL ² Viçosa	4.70	2.33	41.00	2.20	0.70	0.20	5.61	3.00	3.20	8.61	2.52	17	7	76	Sandy

Analyses performed in the Viçosa Soil Analysis Laboratory, following the methodology of the Brazilian Agricultural Research Company (EMBRAPA, 1997). BS: base saturation; (t): effective cation exchange capacity; (T): total cation exchange capacity; OM: organic matter; ¹clayey Red-Yellow Latosol; ²sandy Red-Yellow Latosol.

2.3. Chromatographic analyses

The optimized chromatographic conditions were as follows: 20 µL injection volume, mobile phase of acetonitrile/water (60:40), oven temperature of 30 °C, and detector wavelength of 190 nm. Under these conditions, the indaziflam peak appeared at a retention time of 4.6 min. Quantification was performed by the external standardization method, with construction of a standard curve using standards prepared in ACN in the concentration range 0.2-4.5 mg L⁻¹.

2.4. Optimization of the SLE-LTP-HPLC-UV/Vis method

A factorial design with three variables (considering the influence of pH, agitation time, and solvent volume) was employed, with two levels (minimum (-) and maximum (+)). The pH values were 4.0 and 7.0, the agitation times were 1 and 2 min, and the volumes of the acetonitrile/ethyl acetate extractant mixture were 6.5 mL ACN/1.5 mL EtOAc and 8.1 mL ACN/1.9 mL EtOAc). The other experimental conditions were kept constant.

2.5. SLE-LTP-HPLC-UV/Vis method procedure

Samples of 4.0 g of the clayey and sandy RYL soils, without and with conditioning using 1% (w/w) charcoal, were placed in polypropylene tubes and fortified with 1.0 mL of a solution of the herbicide at a concentration of 2 mg L⁻¹, followed by allowing to rest for 48 h. The extraction was then performed by addition of 4 mL of Milli-Q water (at pH 7.0) and 10 mL of

a mixture of ACN (8.1 mL) and EtOAc (1.9 mL). The resulting mixture was vortexed for 2 min and was then cooled at -20 °C for 4 h in a freezer. The supernatant (organic phase) was passed through a filter containing 1 g of Na₂SO₄. The solid phase was then washed with 2 mL of freezing cold acetonitrile, the solvent was evaporated to dryness, and the product was recovered in 1.5 mL of acetonitrile. The HPLC-UV-Vis analysis was performed after filtration of the final extract through a 0.45 µm membrane.

2.5.1. Validation of the SLE-LTP-HPLC-UV/Vis method

The method was validated considering the following parameters: selectivity, linearity, limits of detection and quantification, accuracy, precision (repeatability and intermediate precision), enrichment factor, and matrix effects. The validation was performed according to the protocols of the National Health Surveillance Agency [2] and the National Institute of Metrology, Standardization, and Industrial Quality [12].

2.6. Statistical analysis

The data were analyzed using Statistica v.10 software (StatSoft Corp., Tulsa, USA). Analysis of variance (ANOVA) was performed with Origin software [19, 20].

3. RESULTS AND DISCUSSION

3.1. Optimization of the SLE-LTP-HPLC-UV/Vis method

The extraction percentages obtained for the soils using the SLE/LTP method in the optimization procedure were evaluated by means of Pareto charts [21]. These showed that for the clayey soil without charcoal addition (Figure 2A), only the pH had a significant effect on indaziflam extraction (95% confidence level; $\alpha < 0.05$), with lower solubility of indaziflam at pH 7 than at pH 4. In the case of indaziflam extraction from the same soil with the addition of charcoal (Figure 2B), the agitation time was the variable with the greatest effect, with improved extraction for longer times, which could have been due to the high adsorption capacity of charcoal.

The pH affected the behavior of indaziflam because the herbicide is classified as a weak acid ($pK_a = 3.5$) [7, 13, 22]. As the pH increased, the solubility of indaziflam in water decreased, so a greater amount of the compound was extracted into the organic phase. The addition of organic compounds (such as those in charcoal) to a soil that possesses a highly clayey texture is expected to lead to greater sorption of herbicides [6]. This pronounced reactivity of the organic compounds is mainly related to their high specific surface area and the presence of functional groups such as carboxyls, hydroxyls, and amines, as well as aliphatic and aromatic structures [18]. Overall, the best experimental conditions for application of the SLE/LTP-HPLC-UV/Vis method using the clayey soil without and with charcoal conditioning were as follows: aqueous solution pH 7, agitation time of 2 min, and 10 mL of extractant solution consisting of 8.1 mL of ACN and 1.9 mL of EtOAc.

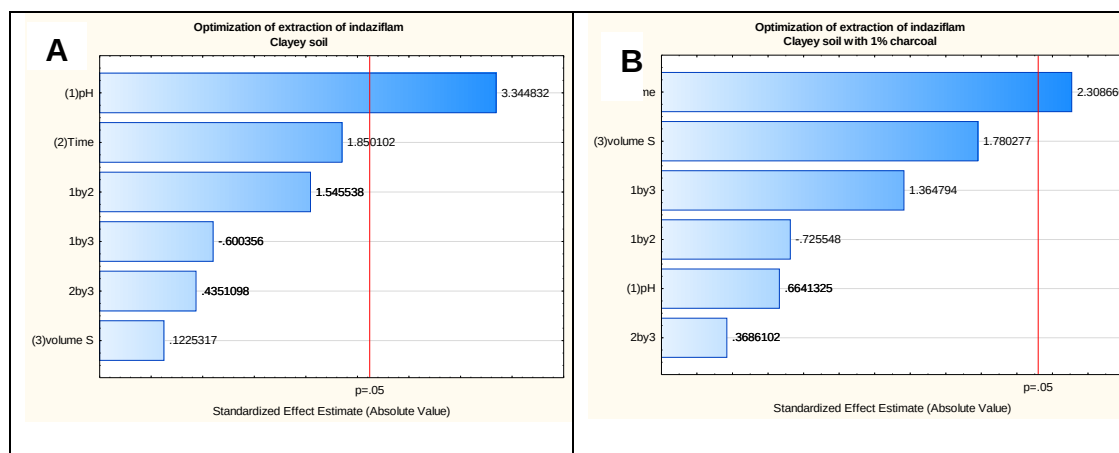


Fig. 2. Pareto charts of the effects of the factors (1) pH, (2) agitation time, and (3) ratio of the extractant solution volume to the mass of the sample on the extraction of indaziflam from the clayey soil without (A) and with (B) addition of charcoal, using the SLE-LTP-HPLC-UV/Vis method.

3.2. Validation of the SLE-LTP-HPLC-UV/Vis method

Use of the optimized solid-liquid extraction with low temperature partitioning method for extraction of indaziflam from the soils resulted in extracts that were clear and free from interferences. The chromatograms obtained for extracts of soil samples that were not spiked with indaziflam showed no peaks corresponding to indaziflam, at a retention time of 4.6 min (Figure 3). These results confirmed the selectivity of the method and demonstrated that it could be used for chromatographic analysis of the herbicide in soils.

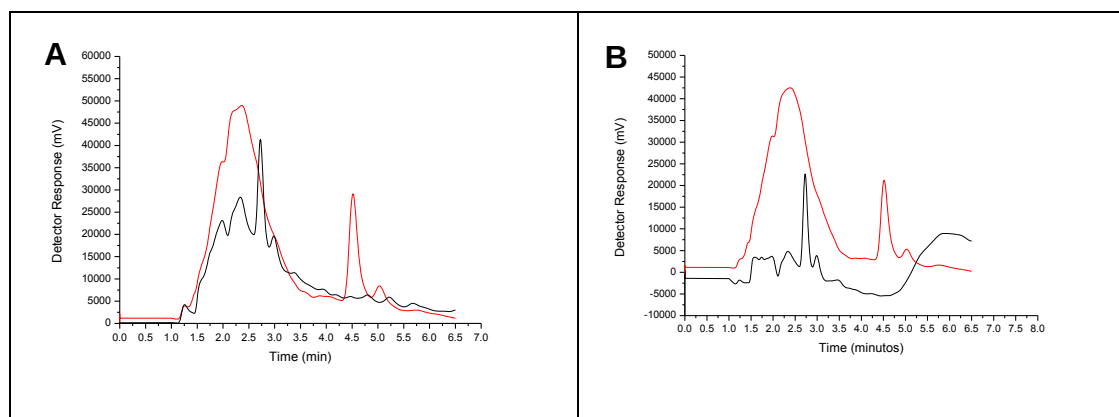
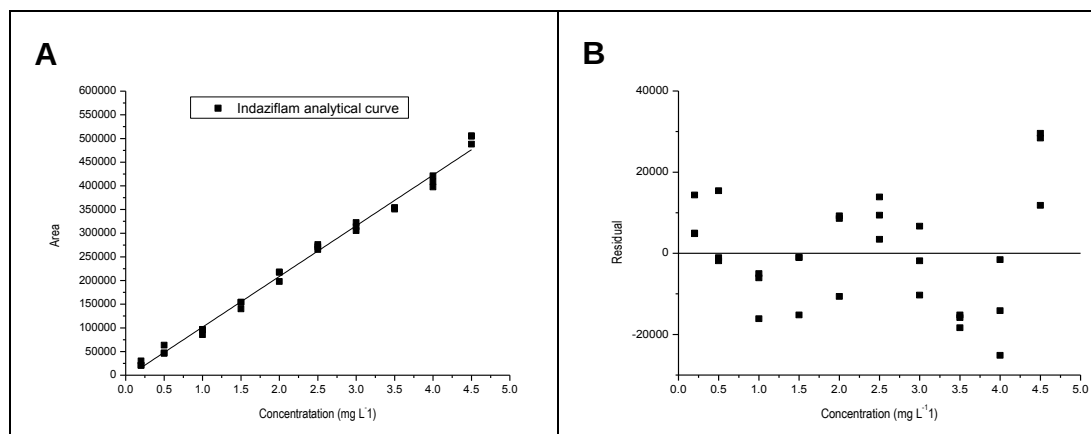


Fig. 3. Chromatograms for the extracts obtained using the SLE/LTP-HPLC-UV/Vis method: Clayey soil without charcoal (A) and with addition of 1% charcoal (B), in the absence of the herbicide (black lines) and fortified with indaziflam at 2.0 mg kg⁻¹ (red lines).

The linearity of the instrumental response was determined using indaziflam standards prepared in acetonitrile, in the concentration range 0.2-4.5 mg L⁻¹ (Figure 4A). The coefficient of determination was 0.99 and the residuals showed a random distribution (Figure 4B), indicative of good linearity and a satisfactory fit. The analytical curve was used to

184 calculate the instrumental limits of detection (LD) and quantification (LQ), with values of 0.15
 185 and 0.44 mg L⁻¹ obtained, respectively.
 186



187
 188 **Fig. 4. Analytical curve for indaziflam in acetonitrile (A), and distribution of residuals**
 189 **of the analytical curve (B)**
 190

191 The linearity of the response of the method was evaluated for each soil substrate spiked with
 192 indaziflam at seven levels (0.2, 0.5, 1.5, 2.0, 3.0, 5.0, and 6.0 mg kg⁻¹). The analyte peak
 193 areas were proportional to the concentrations, with coefficients of determination (R^2) greater
 194 than 0.99. Analysis of the residuals showed a random distribution, demonstrating good
 195 linearity and satisfactory quality of the fit. Hence, the analytical curves could be used for
 196 quantification of the analyte [2, 12, 24]. Table 2 shows the results for the analytical curves
 197 obtained for the determination of indaziflam in the clayey and sandy soils in the absence or
 198 presence of charcoal (at 1% w/w). The limits of detection and quantification were calculated
 199 from the ratio of the standard deviation of the analytical response for the blank and the slope
 200 of the analytical curve.
 201

202 **Table 2. Parameters of the linear regression equations of the curves obtained using**
 203 **the SLE-LTP-HPLC-UV/Vis method applied to samples of clayey and sandy soils**
 204 **without and with conditioning by addition of 1% (w/w) sugar cane bagasse charcoal,**
 205 **fortified with indaziflam.**
 206

Matrix	a	b	R^2	R	LD	LQ
Clayey soil	-24167.63	198822.11	0.9959	0.9979	0.19	0.59
Clayey soil + 1% charcoal	-12536.12	173749.19	0.9954	0.9977	0.21	0.65
Sandy soil	7068.97	164560.59	0.9902	0.9951	0.14	0.43
Sandy soil + 1% charcoal	7504.31	153044.22	0.9944	0.9972	0.16	0.49

207
 208 a: Linear coefficient; b: angular coefficient; R^2 : coefficient of determination; R: coefficient of correlation;
 209 LD: limit of detection (mg kg⁻¹); LQ: limit of quantification (mg kg⁻¹).
 210

211 The method provided accurate and precise determination of indaziflam in samples of soils
 212 without or with charcoal conditioning, fortified with the herbicide at three concentration levels
 213 (0.5, 1.5, and 2.5 mg kg⁻¹) and analyzed using seven replicates (Table 3). The recovery
 214 percentages were between 79.6 and 106.4%, while the coefficients of variation were
 215 between 0.9 and 14.4%. These values were in compliance with established guidelines for
 216 residue analysis [12, 17, 24], which stipulate that recoveries should be in the range 70-
 217 120%, with coefficients of variation below 20%. The experimental enrichment factors (EF)

were close to the theoretical value (2.66), and no trends were observed for the physico-chemical properties of the herbicide and the soils. The matrix effect ratios were higher than 1, indicating a substantial effect of the matrix [5].

Table 3. Repeatability, intermediate precision, and recovery for the proposed method applied to samples of clayey and sandy soils without and with conditioning using 1% (w/w) sugar cane bagasse charcoal.

Matrix	[Indaziflam] (mg kg ⁻¹)	Recovery (%)	CV (%)		EF	ra
			Repeatability	Intermediate precision		
Clayey soil	0.5	84.63	1.4	6.9	2.34	2.9
	1.5	87.63	2.8	8.9		
	2.5	96.29	4.9	12.1		
Clayey soil + 1% charcoal	0.5	85.85	2.5	3.5	2.55	2.5
	1.5	90.28	11.0	9.9		
	2.5	88.53	0.9	13.6		
Sandy soil	0.5	88.97	4.9	5.2	2.80	2.4
	1.5	106.41	3.3	5.6		
	2.5	100.86	14.4	12.8		
Sandy soil + 1% charcoal	0.5	86.83	4.0	5.8	2.66	2.2
	1.5	101.60	8.0	7.7		
	2.5	79.56	14.4	10.4		

EF: Enrichment factor; ra: matrix effect ratio.

4. CONCLUSIONS

The optimized and validated SLE/LTP-HPLC-UV/Vis method provided selective, efficient, precise, and accurate determination of indaziflam in samples of clayey and sandy Red-Yellow Latosol soils without and with conditioning using charcoal obtained from sugar cane bagasse.

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