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Title of the Manuscript:	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)
Type of the Article	Original Research Article

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	REMARKS R1: This work is not original .Similar has been published 2016 Validation of a liquid chromatography ultraviolet method for determination of herbicide diuron and its metabolites in soil samples An. Acad. Bras. Ciênc. vol.88 no.3 Rio de Janeiro Sept. 2016 Epub Aug 25, 2016 Abstract There have been few studies concerning indaziflam R2; It is not true In the present work, a solid-liquid extraction with low temperature partitioning R3: From which media you do the extraction Were conditioned with charcoal R3: for which objective Were fortified with 2 mg L-1 herbicide R4: which herbicide In a proportion of 8.1: 1.9 R5: if you change to 8:2 what can be happen The mixture was vortexed and then frozrn at -20 0C R6: why this low temperature <i>Keywords: Herbicide, method validation, SLE-LTP extraction, HPLC-UV/Vis.</i> R7: At the place of herbicide you should put indaziflam 1.INTRODUCTION R8: the references are cited in disorder because you start by 28 not by 1	R1. We consider this research is original. Because there are different chemical techniques to extract compounds in soils, but each technique must be developed, tested and validated to define it as suitable for extracting the each compound (this case the indaziflam herbicide) efficiently, selectively and accurately. At the paper cited by the Reviewer, the authors made a validation of method for extract diuron of soil with methanol, agitation and analysis by HPLC. In our paper we described other method of extraction that was performed, modified and validated for the first time to adapt it to the characteristics of indaziflam. R2. The term "determination" is used to describe the method, for example: the extraction and quantification of the analyte. In this case we found few publications showing the extraction and analysis of indaziflam. R3 The extraction was made of the soil. R3 – Experiments were carried out with the purpose of evaluating the effect of the addition of charcoal to the soil in the movement, the leaching and persistence of the herbicide in the soil and half- life. For this reason We needed a method of extraction that applied to the pure soil and the soil plus charcoal. R4 – A word was changed. R5 - We believe that it should not generate large variations in response since the method is sufficiently robust. R6 –By decreasing the temperature (-20 °C) the equilibrium of the homogeneous mixture (water / ethyl acetate / acetonitrile) is broken down into two phases: water / ethyl acetate and acetonitrile. At this temperature the aqueous phase is frozen and the acetonitrile remains in the upper layer in the liquid state (Melting Point = -45 °C) containing the analyte. This makes it possible to extract the indaziflam in the organic solvent phase (mixture of acetonitrile with ethyl acetate). R7 – A word was changed. 1.INTRODUCTION R8 – The references were organized.



	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, 28 such as carryover as well as the contamination of surface and subterranean water bodies R9: How you can avoid the toxicity of this herbicide by knowing the dynamics 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, 31 29]. R10 : please describe how 2. MATERIALS AND METHODS Dry sugar cane bagasse was pyrolyzed for 1 h in a muffle furnace (Linn Elektro Therm, Eschenfelden, Germany), using a heating rate of 9 oC min⁻¹ and a final temperature of 500 51 oC, as described previously [27, 10, 14]. The charcoal was triturated and passed through a 52 150 mesh sieve, resulting in a particle size <106 µm. R11: How avoidind the combustion of the bagass 2.2. Sample preparation Table 1. Physical and chemical analyses of the soils used to validate the SLE/LTP-84 HPLC-UV/Vis method. R12: Table 1 is badly presented R13: How you determined all the parameters in this table 2.3. Chromatographic analyses and detector wavelength of 190 nm. Under these conditions, the indaziflam peak appeared at a retention time of 4.6 min. R14; How is the resolution. should be two compounds appeared at this wavelength. 2.4. Optimization of the SLE-LTP-HPLC-UV/Vis method 2.5. SLE-LTP-HPLC-UV/Vis method procedure allowing to rest for 48 h. R15: why you selected 48 h 2.5.1 Validation of the SLE-LTP-HPLC-UV/Vis method 3. RESULTS AND DISCUSSION 3.1. Optimization of the SLE-LTP-HPLC-UV/Vis method	R9 – The great mobility of the herbicide can cause environmental problems because it can be carried to the bodies of water or groundwater. The knowledge of its dynamics in the soil can give information that leads to its use (doses and season of application according each weather and soil.) R10 –It was described. 2. MATERIALS AND METHODS R11: When biomass is burnt in an oxygen atmosphere, the main reaction is combustion and the final products will be CO₂, water and ashes. This process can reach temperature as high as 800 to 950 °C. In this research o process used was pyrolysis with temperature 500 °C and using hermetically sealed cylinders in absence of oxygen (muffle (Linn Elektro Therm). Thus we avoidind the combustion of the bagass. The products of this process of pyrolysis result in a charcoal with high ash content. “Pyrolysis is described by Bridgwater (2003; 2006) as thermal decomposition in absence of oxygen, and is always the first step in the processes of gasification and combustion. Production of charcoal is favored by low temperatures and very long residence time conditions (Bridgwater, 1992). R12. A table was organized. R13. A determination of all parameters was described below the table in the paper. 2.3. Chromatographic analyses R14. The chromatographic conditions presented in this study were optimized, for this it was used highly pure indaziflam (purity 99.5% w/w). Thus the retention time in which indaziflam appears is determined (4.6 min). When the herbicide is extracted from the soil using the same chromatographic conditions defined in the optimization of the method, the soil chromatograms (without indaziflam and with indaziflam) are compared (figure 3). Being the method selective for this herbicide, it is possible to determine indaziflam exactly in the presence of interferers or other compounds such as impurities, degradation products and coextractives of the soil. As can be seen from the comparison of the chromatograms, there is no presence of any compound peak in the same retention time defined for indaziflam, demonstrating that the method is selective for indaziflam analysis. R15. 48 h it was the time which the indaziflam was evaporaded from soil at natural air.
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	method in the 138 optimization procedure were evaluated by means of Pareto charts R16: It should be better if you describe briefly Pareto charts method These showed that 139 for the clayey soil without charcoal addition (Figure 2A), only the pH had a significant effect 140 on indaziflam extraction (95% confidence level; $\alpha < 0.05$), with lower solubility of indaziflam 141 at pH 7 than at pH 4. In the case of indaziflam extraction from the same soil with the addition 142 of charcoal (Figure 2B), the agitation time was the variable with the greatest effect, with 143 improved extraction for longer times, which could have been due to the high adsorption 144 capacity of charcoal. R17; this section not clear and figures 2a And 2B are badly presented. They do not make sense The pH affected the behavior of indaziflam because the herbicide is classified as a weak 147 acid ($pK_a = 3.5$) [7, 13, 22]. R18; The word behavior is usually used in the psychology not in scientific research As the pH increased, the solubility of indaziflam in water 148 decreased, R19; Can you explain why 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 R20; it is not clear This pronounced 151 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 154 application of the SLE/LTP-HPLC-UV/Vis method using the clayey soil without and with 155 charcoal conditioning were as follows: aqueous solution pH 7, agitation time of 2 min, and 10 156 mL of extractant solution consisting of 8.1 mL of ACN and 1.9 mL of EtOAc. R21: Rewrite this section more clear 3.2. Validation of the SLE-LTP-HPLC-UV/Vis method 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 176 absence of the herbicide (black lines) and fortified with indaziflam at 2.0 mg kg⁻¹ (red 177 lines). R22: this figure is badlt presented 4. CONCLUSIONS 229 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. R23: the conclusion is poor and does make sense	R16. A description of Pareto Chart Method was including in the paper. R17. We considered that explication is clear, if it understand o pareto charts explication it should understand this explication about pH was a significant variable (Figure 2A) and the agitation time variable in the figure 2B. We consider the figures are good presented. They make sense because it is an optimization of the method which there were tested different factors. R18. We consider the word behavior is used in scientific research of herbicides (Mendes, k.f. et al. 2017. methodologies to study the behavior of herbicides on plants and the soil using radioisotopes, Behavior of Dinitroaniline Herbicides in Soils, 2017, and many others research studies). R19. In the introduction there are as chemicals conditions of herbicide, such as Pka, solubility in water, according this characteristics a solubility in water decrease with pH increase. R20. It was rewriting more clear. R21. It was rewriting more clear R22. These figure is good presented, are the chromatograms that exactly showed the HPLC. R 23. When validating methods for analysis of chemical compounds, it is determined if the method was able to identify and quantify the compound within the established regulations. At the end, applying the statistical parameters, we conclude if the method was selective, efficient, precise and accurate to extract that compound in the matrix studied in this case the soil.
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Minor REVISION comments		
Optional/General comments		