

ORIGINAL PAPER

HPLC-DAD METHOD FOR DETECTION OF SOME PESTICIDE RESIDUES IN SOIL AND CROPS CULTIVATED IN BANAT COUNTY

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Abstract. *High productivities and economic benefits in modern agriculture have been accomplished only by the use of pesticides. The introduction of these compounds in the food chain can be considered a risk for human health due to their toxicity. So, pesticides residues and their degradation products resulted by hydrolytic, photochemical and microbial processes in soil and cereals must be monitored and controlled. The aim of this research was to develop rapid and sensitive HPLC-DAD methods for residues detection of several pesticides, imidacloprid, amidosulfuron, bromoxynil and deltamethrin, from soil and some crops, maize, wheat and rape seeds, cultivated in Banat County-Moravita Zone. In order to perform the pesticides extraction, the samples were subjected to an ultrasonic technique, at 59 kHz and 30°C, during 30-45 min., using acetonitrile and acetonitrile-water mixture as solvents. Acetonitrile alone proved to be more appropriate for this purpose. The HPLC analyses were conducted on HPLC-DAD apparatus, Dionex Ultimate 3000 (Dionex Corp., USA), equipped with quaternary pump LPG 3400A, thermostat of columns TCC-3000 and a C-18 Acclaim® 120 Silica-reversed-phase (4.6x150 mm, 5 µm) column, using different compositions of acetonitrile-water as mobile phase. The presented HPLC - Dionex system proved to be very suitable for determination of pesticides residues in soil and crops.*

Keywords: *HPLC-DAD method, pesticide detection, soils and crops.*

1. INTRODUCTION

The European Directive 491/2014 established the maximum residues limits (MRLs) for imidacloprid in maize and wheat: 0.1 mg/kg [1, 2]. Imidacloprid is an insecticide approved for use in the EU with certain restrictions for flowering crops. This is a systemic insecticide of new generation of chloro-nicotinic insecticides, with applications for soil, seeds and foliage. Imidacloprid is used to control sucking insects such as rice leaf hoppers, aphids, mites, white-flies, termites and the insects from grass. The herbicides, amidosulfuron and bromoxynil

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exhibit a systemic and contact action with high efficiency including advanced weeds in vegetation and perennial plants, even at low temperature (4-5°C) [3].

The researchers used different methods for detection and quantification of pesticides [4-16]. Imidacloprid was the most studied compound and was detected by HPLC-UV and HPLC-MS, UFLC, gas chromatography and voltammetry [6-12]. Some researchers consider that imidacloprid toxicity is relatively low, but recent demonstrations showed its toxicity to bees and the penetration into organisms by the water-cycle (soil degradation DTS50) in 191 days [6].

This work aims to develop/to perfect a sensitive and rapid method HPLC-DAD for detection and quantification of four pesticides in soil and crops cultivated in Banat County - Moravita Zone. From the pesticides in use, we focused on: Nuprid AL 600 FS (insecticide, imidacloprid as active compound), Sekator Progress OD (herbicide, amidosulfuron as active compound), Buctril Universal (herbicide, bromoxynil as active compound) and Decis Mega 50 EW (insecticide, deltamethrin as active compound).

2. MATERIALS AND METHODS

2.1. MATERIALS

Standards of imidacloprid-Pestanal (99.9%, M=255.66), amidosulfuron-Pestanal (99.6%, M=369.37), bromoxynil-Pestanal (99.9%, M=276.91) and deltamethrin-Pestanal (99.9%, M=505.21), methanol, acetonitrile, water (HPLC grade) were purchased from Sigma-Aldrich. Samples of maize, wheat and rape seeds cultivated in Moravița zone, in 2017, were obtained from local farmers.

2.2. METHODS

2.2.1. Sample preparation

Soil and crop samples from zones close to the borders with Serbia, Moravița (around Railway station and Dealu Mare) and Gherman village were studied. Each soil sample was collected from a depth of 15-25 cm, near the plants. The upper part of the soil was removed and the rest was grinded, dried, sieved to remove the remains of roots, pebbles. The particles with dimensions $\leq 1\text{mm}$ (size that ensures better contact with the solvent) were then subjected to extraction.

Samples of wheat, maize and rape seeds cultivated in western part of Romania (i.e., Moravița Area) and harvested in 2017, July and October, respectively, were grinded, and dried.

The humidity of samples (weight percentage) was determined by gravimetric method. The dried samples were then submitted to extraction procedures.

For the determination of pesticides, the extraction was carried out in two steps, in an ultrasonic bath (Falc Instruments, Italy), at 59 kHz and $30\pm 2^\circ\text{C}$, in acetonitrile and acetonitrile-water mixture (50:50 v/v). The ratio of liquid: solid was 2:1 (v/w). In the first step, the samples were ultrasonated for 30 min., then the extracted solid was filtered and resubjected to ultrasonication for another 15 min. (second step). The reunited extracts were

centrifuged during 30 min. at 3000g (EBA20 Hattech Zentrifugen, Germany), then evaporated under vacuum (40–45 mbar) at 30–35°C in a rotary evaporator (Laborota 4000 Efficient-Heidolph, Germany) and brought to 10 mL with acetonitrile.

2.2.2. HPLC analysis

HPLC analyses were conducted on HPLC-DAD system, Dionex Ultimate 3000 (USA), with quaternary pump LPG 3400A and thermostat of columns TCC-3000. A C-18 Acclaim® 120 Silica-reversed-phase (4.6x150 mm, 5 µm) column was used. Analyses conditions were: column temperature 30°C, sampling volume 20 µL, mobile phase A: water acidulated with H₃PO₄ (water HPLC grade with 0.1% v/v H₃PO₄ p.a., ≥85%) and B: acetonitrile in different ratios, isocratic mode, 1 or 0.5 mL/min. flow rate, six sample injections (n=6) and analysis time, t=10 min.

Chromatograms were recorded and processed with Chromeleon 6.8 Software.

The work conditions were:

1. Imidacloprid: A: B, 50:50 (v/v), 0.5 mL/min. flow rate, concentration range 0.04-3.00 µg/mL, λ=270 nm
2. Amidosulfuron: A: B, 30:70 (v/v), 0.5 mL/min flow rate, concentration range 0.07-3.00 µg/mL, λ=240 nm.
3. Bromoxynil A: B, 10:90 (v/v), 1 mL/min flow rate, concentration range 0.035-0.350 µg/mL, λ=254 nm
4. Deltamethrin: A: B, 10:90 (v/v), 1 mL/min flow rate, concentration range (0.100-0.375 µg/mL, λ=235 nm)

The standards solutions in acetonitrile and extracts were filtered through 0.45 µm Phenex PTFE filter before injection and were analyzed using the presented methods.

Limit of detection, LOD and limit of quantitation, LOQ were established using the root mean squared error, RMSE method [17].

$$\text{LOD} = 3.3 \cdot \sigma / S \quad (1)$$

$$\text{LOQ} = 10 \cdot \sigma / S \quad (2)$$

where σ is the standard deviation of the residuals taken from the regression line and S is the slope of the calibration curve.

Intra-assay precision (repeatability) and inter-assay accuracy (intermediate precision) were established for imidacloprid, in triplicate. One-point ($c=2.76$ µg/mL) was evaluated 6 times for intra-assay (intra-day) test and 12 times for inter-assay (on two consecutive days), using the same equipment and the same operator.

$$\% \text{ RSD} = (\text{SD}/x) \cdot 100 \quad (3)$$

where SD is the standard deviation of the y intercept of three curves and x is the mean of the peak area [18].

In order to determine the degree of analyte recovery, the samples of Gherman soil, wheat (Dealul Mare) and maize were first infested with a known amount of imidacloprid (0.27 µg) and then subjected to the extraction with acetonitrile. The extracts, after filtration, were evaporated to the same volume (10 mL) and analysed using the HPLC-DAD method. The accuracy based on the analyte recovery was established by contamination of samples with imidacloprid (0.27 µg). Theoretical concentration (TC) of imidacloprid represents the sum of the concentration of imidacloprid detected in samples and the concentration of imidacloprid

added as contaminant. Experimental concentration (EC) is the concentration of imidacloprid experimentally determined.

$$\% \text{ Recovery degree} = (\text{TC}/\text{EC}) * 100 \quad (4)$$

3. RESULTS AND DISCUSSION

The half life of pesticides depends on the soil moisture, the lower the water content, the lower the pesticide degradation [19-21]. The humidity of the samples ranged between 9.00-11.00 % for crops and 14-16.00 % for soil.

The samples were extracted with acetonitrile and acetonitrile-water mixture. When using acetonitrile-water as extraction solvent, along pesticides, other components of plant were extracted, so, the extraction was further carried out in acetonitrile. For example, in Fig. 1, the chromatographic profile of imidacloprid in soil, in the both studied solvents, is presented.

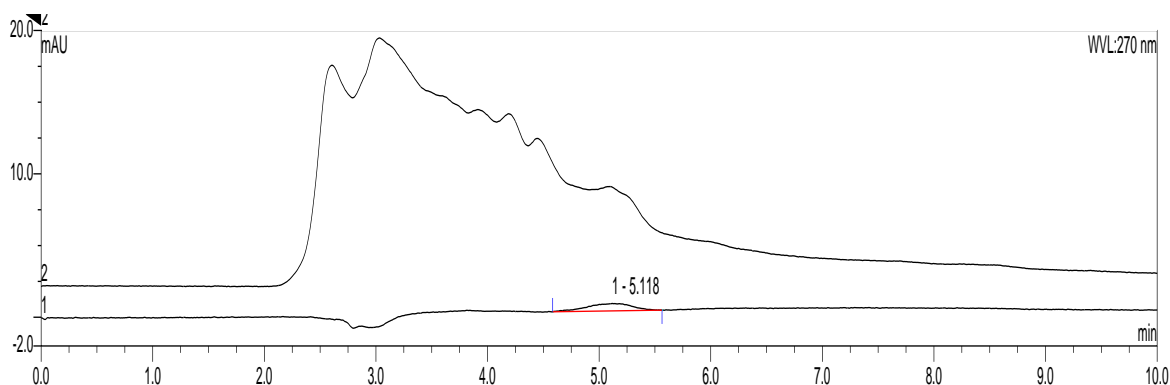
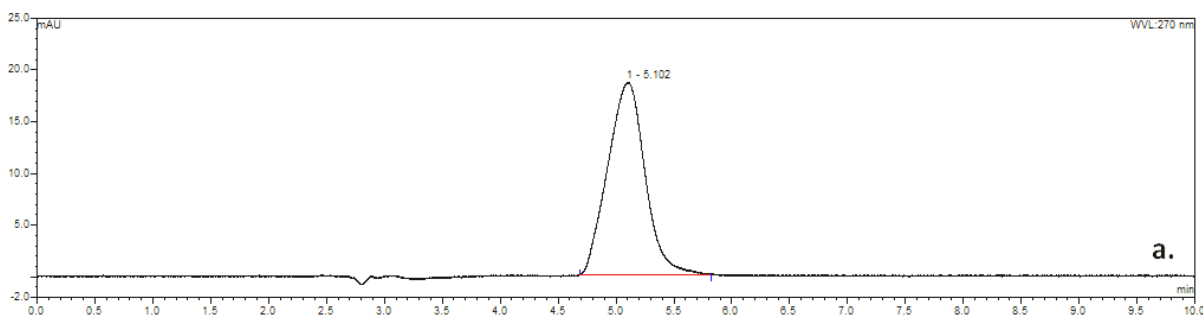


Figure 1. Chromatograms for imidacloprid in soil: 1- ultrasonic extraction with acetonitrile; 2- ultrasonic extraction with water-acetonitrile mixture (50:50).

In Fig. 2, chromatographic profile of each pesticide standard are presented. For each contaminant, a calibration curve was plotted (Fig. 3) and each point was obtained as an average value of 6 injections. Subsequently, these standard curves were used for quantification of pesticide residues in crop and soil samples.



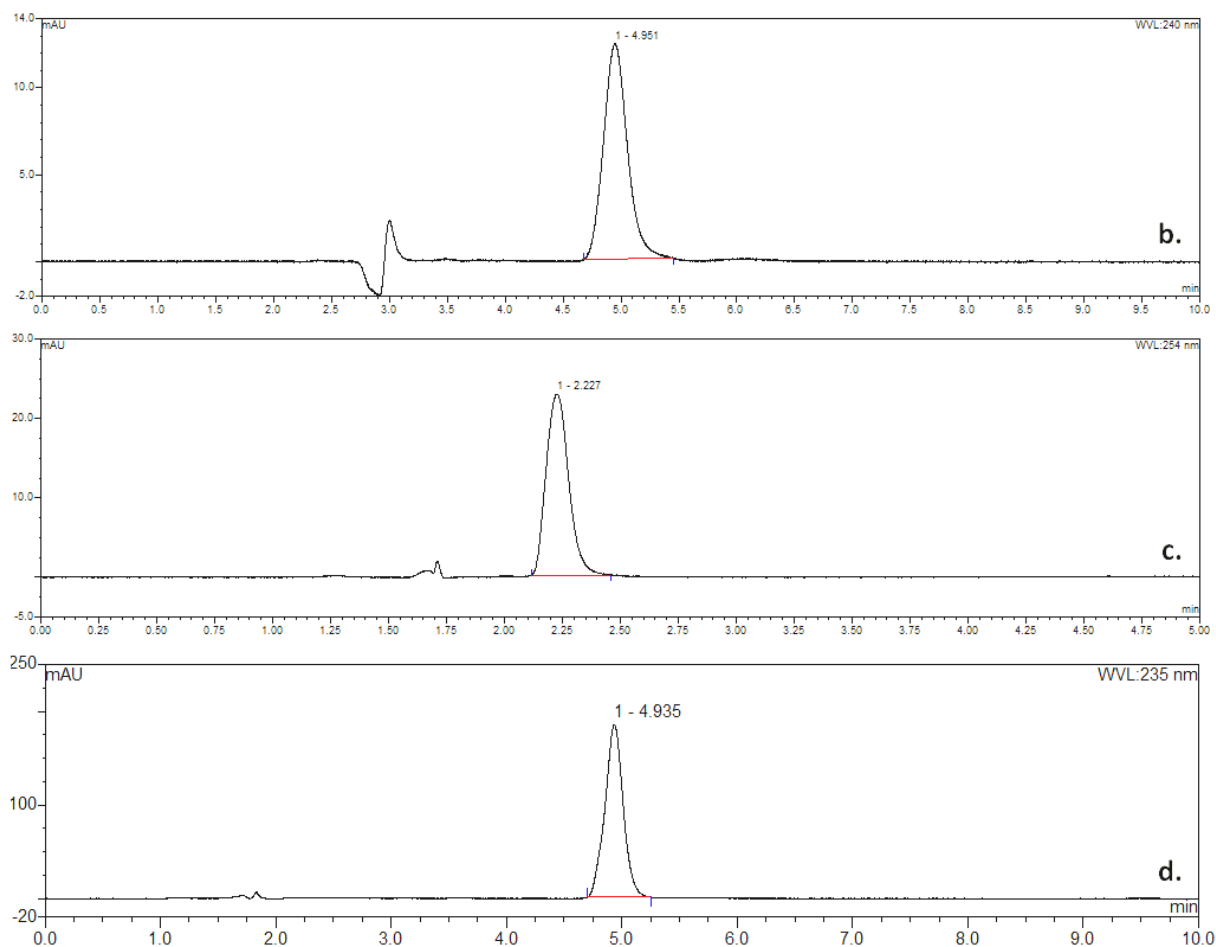
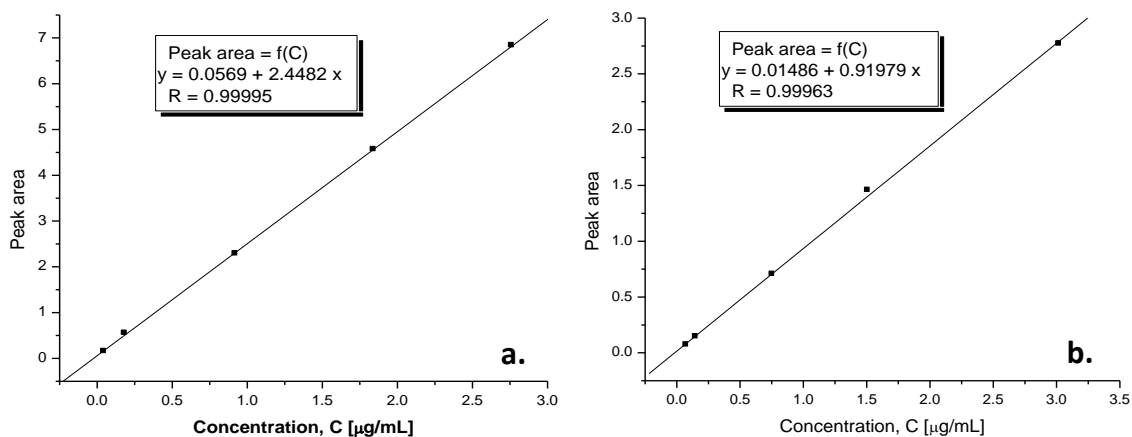


Figure 2. Chromatograms of standard pesticides: a. imidacloprid; b. amidosulfuron; c. bromoxynil; d. deltamethrin.

Imidacloprid (Fig. 2a) showed an intense signal at 270 nm ($y=0.0569 + 2.4482 x$; $R=0.99995$; $SD=0.03132$; $n=5$; $p<0.0001$) at retention time $t_R=5.102$ min. Amidosulfuron (Fig. 2b) appeared in chromatogram as an intense signal at 240 nm ($y=0.01486 + 0.91979 x$; $R=0.99963$; $SD=0.03496$; $n=5$; $p<0.0001$), at $t_R=4.951$ min. Bromoxynil (Fig. 2c) showed an intense signal at 254 nm ($y=0.0008 + 0.69801 x$; $R=0.9999$; $SD=0.00171$; $n=4$; $p<0.00001$) and deltamethrin (Fig. 2d) showed a specific signal at $\lambda=235$ nm ($y = -0.01274 + 0.6537 x$; $R = 0.9992$; $n=5$; $SD=0.00314$; $p<0.0001$). Retention times were $t_R=2.227$ min and $t_R=4.935$ min, respectively. The values obtained for the correlation coefficients (>0.9992) prove an excellent linearity.



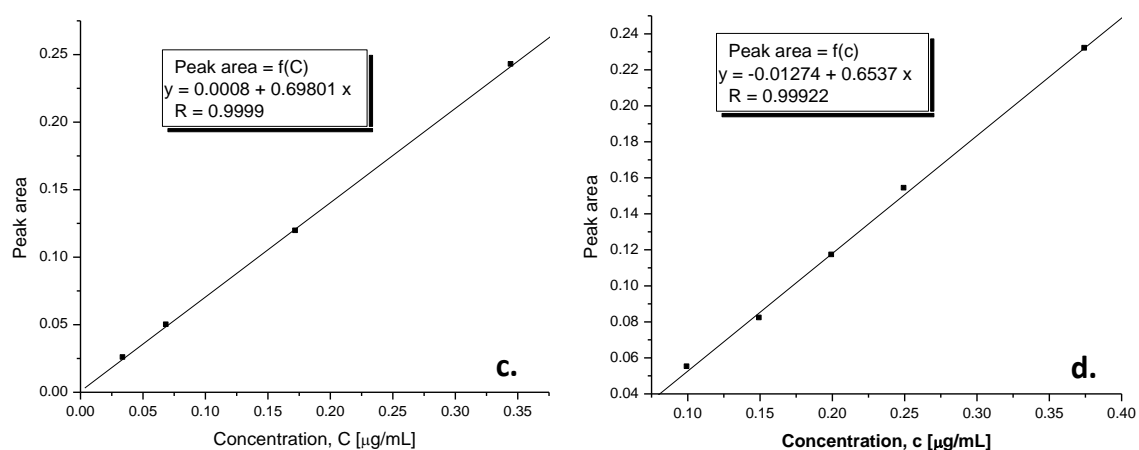


Figure 3. Calibration curves of standard pesticides: a. imidacloprid; b. amidosulfuron; c. bromoxynil; d. deltamethrin

In order to evaluate the precision, accuracy, repetability of the HPLC method, limit of detection (LOD), limit of quantitation (LOQ), intra-assay precision, inter-assay accuracy and recovery degree were established. The results for LOD and LOQ were presented in Table 1.

Table 1. LOD and LOQ of pesticides.

Pesticide	σ	Slope	LOD	LOQ
Imidacloprid	0.0053	6.6276	0.0026	0.0080
Deltamethrin	0.0036	0.7093	0.0166	0.0503
Bromoxynil	0.0026	0.7234	0.0119	0.0361
Amidosulfuron	0.0049	1.2717	0.0128	0.0388

***LOD-Limit of detection; LOQ- Limit of quantitation*

Table 2. Precision and accuracy parameters for imidacloprid.

No.	Parameters	Values
1	Intra-assay precision (repeatability, n = 6 determinations)	*RSD = 3.41%
	Theoretical concentration, µg/mL	2.76
	Concentration obtained, µg/mL	2.77
	Intra-assay accuracy (n = 6 determinations)	3.43%
2	Inter-assay precision (n = 12 determinations)	*RSD = 3.94%
	Theoretical concentration, µg/mL	2.76
	Concentration obtained, µg/mL	2.80
	Inter-assay accuracy (n = 12 determinations)	3.99%

**RSD-Relative standard deviation;*

The lower values of LOD and LOQ indicated a better fit of data. σ value is a good measure of how accurately the model predicted the response. The results obtained for the intra-assay precision (repeatability) and for inter-assay precision were great since, RSD values were lower than 5% (Table 2) [18]. The recovery degree (Table 3) ranged between 96-132%. For a proper analysis, the accepted range for recovery degree of pesticides is 80-120%. The values obtained for soil (Gherman) and wheat (Dealul Mare) respect these limits.

Table 3. Values of contaminated samples.

No.	Samples	*A _{med}	SD	**RSD [%]	Recovery degree [%]
1	Soil Gherman	0.3158	0.005	1.7	
2	Contaminated soil Gherman (0.27µg imidacloprid)	0.6348	0.047	7.43	96.50
3	Wheat - Dealu Mare	0.6541	0.047	7.11	
4	Contaminated wheat Dealu Mare (0.27µg imidacloprid)	1.0314	0.005	0.46	114.14
5	Maize	0.2236	0.012	5.22	
6	Contaminated maize (0.27µg imidacloprid)	0.6573	0.042	6.34	131.27

*A_{med}-mean peak area; **RSD-Relative standard deviation

The detected values of imidacloprid in soils and maize, wheat and rape seeds are presented in Table 4.

Table 4. Concentrations of pesticides (µg/g dried weight) in soils and crops.

No.	Sample	Imidacloprid concentration [µg/g]	Amidosulfuron concentration [µg/g]	Bromoxynil concentration [µg/g]	Deltamethrin concentration [µg/g]
1	Soil Gherman	0.21	*	*	*
2	Soil Rail Station	0.13	*	*	*
3	Soil Dealu Mare	0.16	*	*	*
4	Maize	0.14	*	*	*
5	Wheat Apache	0.02	*	*	*
6	Wheat Exotic	0.10	*	*	*
7	Wheat Dealu Mare	0.46	*	*	*
8	Rape seeds	0.08	*	0.25	*
9	Rape strains	0.50	*	1.29	*

*under LOQ

The presence of imidacloprid was observed in the studied soils, and the residual amounts determined were ranged between 0.13-0.21 µg/g. Amidosulfuron, bromoxynil and deltamethrin were not detected in soil and maize and wheat samples. The quantity of bromoxynil found in rape seeds and strains was 0.25 and 1.29 µg/g, respectively. In maize and wheat (Dealu Mare) the values of imidacloprid concentration exceeded MRLs.

4. CONCLUSIONS

This work has demonstrated that HPLC analysis is a powerful tool for pesticides determination in complex matrices. The pesticides extraction was performed in ultrasonic field. Between the two tested solvents, acetonitrile was more appropriate, in order to minimize the matrix interferences, so the pesticides could be reliably identified and quantified. Sensitive and rapid HPLC-DAD methods were developed specifically for identification and quantification of imidacloprid, amidosulfuron, bromoxynil and deltamethrin.

The results obtained for linearity, precision and accuracy prove that the developed methods are suitable for residual pesticides determination. Imidacloprid was found in all studied soil and crop samples, in maize and wheat (Dealu Mare) the quantity of imidacloprid exceeded MRLs. The high sensibility and the short time of analyses recommends this work for pesticides monitoring at farmer's demand: sampling and analysis of cereals immediately after

harvest and sampling and analysis of cereals after a period of storage. The precise results of the implemented HPLC methods may be useful for intermediaries, supermarkets, and especially for end-consumers.

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