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Validation of a GC-MS method for the simultaneous determination of five coumarins derivatives in natural soil samples

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Abstract

1,2-Benzopyrones are secondary metabolites are widely distributed in plants. As these compounds potentially migrate into the soil, their mineralization kinetics, chemical transformation and concentration are important to define their toxic potential. However, despite the various analytical techniques available for the determination of xenobiotics in soil, there are no validated methods for the extraction and determination of coumarins in soil.It was therefore deemed of interest to develop a method for their extraction and quantification. The GC-MS-based method reported herein was validated with external calibration for the simultaneous determination of 5 coumarins in soil samples. After testing various solvent systems, ethanol: acetone (1:1, v/v) was found to be the most effective. Recoveries of coumarin ranged from 77% to 97%, with a variation coefficient smaller than 3.55 and limits of detection at 0.6, 0.5, 2.7, 1.2 and 1.2 mg kg-1 soil for compounds 1-5, respectively, were obtained. Matrix interferences do not show effects in the determinations.

Keywords: Coumarin, GC-MS, soil, extraction, validation.

Validación de un método por CG-EM para la determinación simultanea de cinco derivados de cumarina en muestras de suelo natural

Resumen

1,2-Benzopironas son metabolitos secundarios extensamente distribuidos entre las plantas. Estos metabolitos potencialmente pueden migrar al suelo, donde

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su cinética de mineralización, transformación química y niveles de concentración en el suelo definen su potencial toxico. Sin embargo, a pesar de los diferentes métodos que existen para la determinación de xenobioticos en suelo, no existen métodos validados para la extracción y determinación de derivados de cumarinas en suelo. Es por tanto de interés desarrollar un método para su extracción y cuantificación. El método basado en CG-EM reportado aquí fue validado mediante calibración con estándar externo para la determinación simultánea de 5 cumarinas en muestras de suelo. Luego de evaluar varios sistemas de solventes, etanol: acetona (1:1, v/v) fue el más afectivo. Recuperación de cumarina se encuentra entre un 77% a 97%, con un coeficiente de variación menor a 3.55 y límites de detección de 0,6; 0,5; 2,7; 1,2 and 1,2 mg kg-1 suelo para los compuestos 1-5 respectivamente fueron obtenidos.

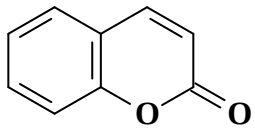
Palabras clave: Cumarina, Cromatografía de Gas –Espectrometría de Masas (CG-EM), suelo, extracción, validación.

Introduction

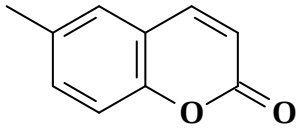
The use of plant species with weed-suppressing ability has been considered for biological weed management in crop production (1-6). Plant growth suppression activities have been reported for numerous secondary plant products including phenolics, flavonoids, and terpenoids (7-11).1,2-Benzopyrone (coumarin) is ubiquitous in plants (12-14), and occurs in every plant part. Due to its pleasant fragrance, it was frequently used as a flavoring ingredient in food products and drugs, despite its adverse effects (15-17) such as coma and death in animals (18), as well as liver degeneration, necrosis and blood vessel changes, dilation of the capillaries, secondary thrombosis of the interlobular veins and narcolepsy in smaller doses. Nonetheless, coumarin still remains in use as food additive in some countries. On the other hand, the potential of coumarin derivatives as weed control agents has been cited (5).Their translocation to the soil by the ways of shed foliage and root exudates leads to significant levels of the wild compounds or derivatives thereof (16).These levels depend on many factors, biotic and abiotic, acting upon the producing plant, mineralization kinetics and chemical species into which these products are transformed. Difficulty stemming from variations of coumarin type and phenology-dependent biosynthesis /accumulation in plants have limited systematic phytotoxic and herbicide potential studies to simple coumarins.

Additionally, the geochemistry of coumarins is not well understood, despite its importance to predict herbicidal activity, environmental fate, and potential limitations in its use.

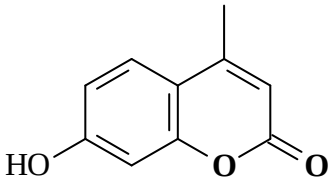
Many different methods have been developed for the analysis of coumarin derivates in different matrixes by means of thin layer chromatography, GC–MS, HPLC-UV, and LC–MS (19-26).However, to the best of our knowledge there is a paucity of data as regards to methods of extraction and quantitative analysis of coumarin derivatives in soil, which it is today necessary to characterize the fate of these secondary metabolites and their environmental impact.The main goal of this work was to develop one such practical method based on GC-MS using five coumarins 1-5 (Figure 1) in natural soil extracts that could also allow the identification of microbial degradation products and their fate in the environment.



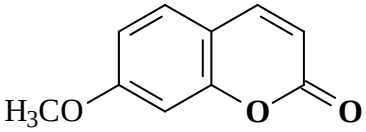
(1) coumarin



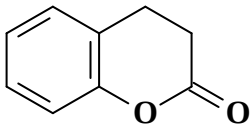
(2) 6-methyl-coumarin



(3) 7-hydroxy-4-methyl-coumarin



(4) 7-methoxy-coumarin



(5) Dihydro-coumarin

Figure 1. Molecular structures of the five coumarins studied

Materials and Methods

Soil Sampling Sites

Soil was collected from the Experimental Station, Universidad Federal de Viçosa (Viçosa, Minas Gerais; Brasil).The experimental station is located in a dry tropical climate zone (Köppen climate classification: Aw), with a mean annual temperature of 19 °C (range: 10-23 °C) and a mean annual rainfall of 200 mm.

The evergreen broadleaf plant community is secondary forest as a result of anthropogenic activity. The more representative plant families were Lauraceae (11spp), Euphorbiaceae (8 spp), Annonaceae (8spp), Mimosaceae (8 spp), Myrtaceae (7spp), Rubiaceae (6 spp), Flacourtiaceae (6spp), Caesalpiniaceae (5spp) and Fabaceae (5 spp) (27). Some herbaceous species were also observed. The soil samples were collected during the rainy season (February to May). Monthly rainfall average 39 mm, with a 23-32 ºC temperature range. The general setup comprised extraction of 5 gof fresh weight (FW) A-horizons of soils at 5-20 and 30-50 cm depth. The samples were takenin a radius of 20 cm around the plants.

Before the studies, dry vegetable material was removed from the samples as well as calcareous stones by a sieve that allows a distribution of soil particles smaller than 1mm, and preserved at -2 °C until its study.

Soil chemical and physical analysis

The characterization reported for different soil depths were as follows (27):2-20 cm depth: Bulk density (1.305, t m-3); coarse sand (230 g kg-1); fine sand (150 g kg-1); silt (80 g kg-1); clay (540 g kg-1); pH (5.5); potassium (134 mg dm-3); phosphorous (14.3 mg dm-3), magnesium (0.5 cmolc dm-3).Organic carbon 22.6 mg dm-3.30-50 cm depth: Bulk density (1.249, t m-3); coarse sand (150 g kg-1); fine sand (120 g kg-1); silt (90 g kg-1); clay (640 g kg-1); pH (5.7); potassium (50 mg dm-3); phosphorous (1.1 mg dm-3), magnesium (0.6 cmolc dm-3).Organic carbon 22.3 mg dm-3.

Chemicals and reagents

Pure coumarin (1, >98%), 6-methyl-coumarin (2, >99%), 7-hydroxy-4-methyl-coumarin (3, >97%), 7-metoxycoumarin (4, >98%), and dihydrocoumarin (5, >99%) were purchased from Sigma Aldrich Corp. (St. Louis, MO, USA) and used without further purification.

HPLC-grade methanol (MeOH), acetone (Ac), ethanol (EtOH) were obtained from VETEC QUÍMICA FINA LTDA., Brazil, Rio do Janeiro and Sigma-Aldrich Corp.

Standard preparation

The five standards were weighed (0,1000 g) and dissolved in methanol to prepare stock solutions at 1000 mg L-1.Methanol was used to prepare intermediate standards with 4, 15.56, 31.12, 62.25, 124.4, 249.0 and 500 mg x L-1. All solutions were stored at 4°C in the dark before analysis.

Natural soil sample

Three forest soils of various types were obtained after removal of plant residues and pebbles, sieving through a 1 mm sieve. Soil samples were taken at two depths: Soil A) at 2-20 cm depth and Soil B) at 30-50 cm depth, placed in plastic bags, and preserved at -5 °C until their study was performed.

Soil extraction

Extractions were performed with three solvents: methanol (MeOH), acetone (Ac), and a 1:1 v/v mixture of ethanol and water (EtOH:H_2O). A subsample of FW soil (4.0 g) solvent was added (25 mL), solids were suspended with shakingand placed in an ultrasound bath (15 min). Solids were filtered througha 0.44 μm glass fiber filter and a fresh batch of solvent (25 ml) added. The procedure was repeated thrice. Washings were pooled, solvents evaporated at reduced pressure and the residue was redissolved in methanol in preparation for the GC-MS analysis.

Coumarin recovery

Analyte recovery was assessed by spiking a soil sample (4mg of coumarin kg-1 FW soil). Soil samples were air-dried overnight prior to spiking, and wetted to their previous wáter content during spiking, and

stored at 5 °C until extraction (in triplicate, 1 h later). Two replicates per spiking were studied. The background concentration (instrumental responses) of the coumarin in the sample matrix, if present, is determined in a separate aliquot so that the values in the laboratory matrix spike are corrected for their presence, and the percentage recovery calculated. The natural content of coumarins in the soil extracts was subtracted before the matrix effect was calculated (apparent recovery %). The calculations of the percent apparent recovery (%RA) for each selected compound was as follows:

$$\%R_A = \frac{Xa\,(exp)}{Xa\,(theo)} \text{ x100} \quad \text{Ec.1}$$

Were

Xa*(exp)*=experiential quantity (mass) derived from the regression curve for each coumarin derivative.
***Xa*(*theo*)**= mass reference quantity or theoretical value.

Calibration and Validation

The limit of detection (LOD) was defined as the concentration of a standard that corresponded to three times the signal-to-noise ratio (S/N=3) and the limit of quantification (LOQ) was defined as the concentration of a standard that corresponded to 10 times the signal-to-noise ratio (S/N = 10), from the mean calibration curve using the following equations (28):

$$LOQ = \frac{10 \text{ x} Sb}{m\sqrt{n}} \quad \text{Ec.2}$$

$$LOD = \frac{3 \text{ x} Sb}{m\sqrt{n}} \quad \text{Ec.3}$$

Where Sb is the intercept standard deviation, m is the slope of the mean calibration curve and n is the number of calibration curves.

Six solutions at different concentrations of the five coumarins were prepared in soil, and each soil solution was extracted and chromatographically (GC) resolved to obtain calibration curves. The determination coefficients (r2) were obtained and the calibration curves were plotted for all the compounds. The intra-day precision (coefficient of variation, CV) was estimated by analyzing the five standard solutions coumarin derivatives six concentrations on the same day. The inter-day precision (CV) were determined by analyzing three replicate solutions at six different concentrations on three successive days.

Assessment of matrix effect

The matrix effect on ionization in the GC-MS method was studied by adding equal concentrations of the analytes to soil samples. A calibration curve was prepared and compared to a standard curve using external calibration. For each soil sample, spiking was performed in triplicate, after which the samples were analyzed by GC-MS. This analysis is carried out in the best chromatographic conditions achieved validation, and treatment of soil samples. Because the soil samples had a natural content of coumarin derivatives, they were analyzed and their coumarin content subtracted before the matrix effect was calculated. By comparing the peak areas of the analyte standards, standards spiked before and after soil

extraction were assessed. To investigate the influences of matrix substances on the MS responses originated from soil, a calibration with an external standard and additional standard curves (matrix matched standards) were prepared. The standard calibration curve was prepared by adding a standard of five coumarins in MeOH, whereas the additional standard curve was prepared by adding the same standard solution. The slopes of standard curves constructed in MeOH and in the soil extracts may serve as an indicator of the absolute matrix effect.

Instrumentation

GC-MS analyses were performed on a SHIMADZU model PQ5050A equipped with a SHIMADZU AOC-5000 on-column auto injector and a fused silica capillary column (DB-5, 30m×0.25mm ID, 0.25µm film thickness). Operating conditions were as follows: Helium as carrier gas with a flow rate of 1.6 mLmin-1; column temperature between 40 °C - 80 °C; heating rate of 24.4 °C - 80 °C min-1 from 80 °C to 285 °C. The injector temperature was set at 290 °C, injected volume, 1µL in split-less mode. Total flow: 8.1 mL min-1, column flow 1.9 mL min-1.Volume injected, 1 µL at 4.65 µg g-1 soil, in split-less mode.MS were recorded in electron ionization (EI) mode full-scan, with energy of 70 eV. The ion source temperature was 290 °C; with 5.00 min solvent cut time.

Conditions: A.-Initial column temperature: 40 °C for 5 min, then increasing at 20 °C min-1 from 40 to 200 °C, then increasing at 30 °C min-1 from 200 to 300 °C, 41 min. Total analysis time: 57.33 min. Injector temperature, 290 °C;. Cutting times: 7 min. B.- GC-MS of soil sample. Initial column temperature 40 °C for 5 min, then increasing at 24.4 °C min-1 to 200 °C, then increasing at 30 °C min-1 to 300 °C. Total analysis time: 57 min. Injector temperature, 290 °C. Cutting times: 5 min. C.- GC-MS Chromatograms of the five coumarins under optimized chromatographic conditions. Initial column temperature: 40 °C for 5 min, then was increasing at 24.4 °C min-1 from 80 to 285 °C. Total analysis time: 50 min. Injector temperature, 290 °C. Solvent cut times: 5 min.

Statistical methods

An analysis of variance (ANOVA) was used to evaluate the within- and between-day variation of the analytical method. Results were analyzed using a one-way ANOVA, between groups design, and a Tukey's test (STATISTICA, version 5.0). Analytic validation calculations (LOQ and LOD), were performed using the Chemometric software ALAMIN (29).

Repeatability of the GC-MS method

The repeatability of the method was assessed by determining the relative standard deviation (RSD) of the GC-MS-peak area, using the equation:

$$RSD = \frac{SD}{mean} \times 100 \quad \text{Ec. 4}$$

Where SD is the standard deviation mean is average for n= 3.

Results and discussion

Development of analytical methods

The study of initial conditions of temperature and heating rate analysis shows that the five coumarins can be separated satisfactorily in a total time of 16 min (Figure 2A). Gas chromatograms of the five coumarins were obtained after testing several temperature programming gradients which were critical for soil extract analysis. Figure 2B illustrates chromatograms of the soil sample.

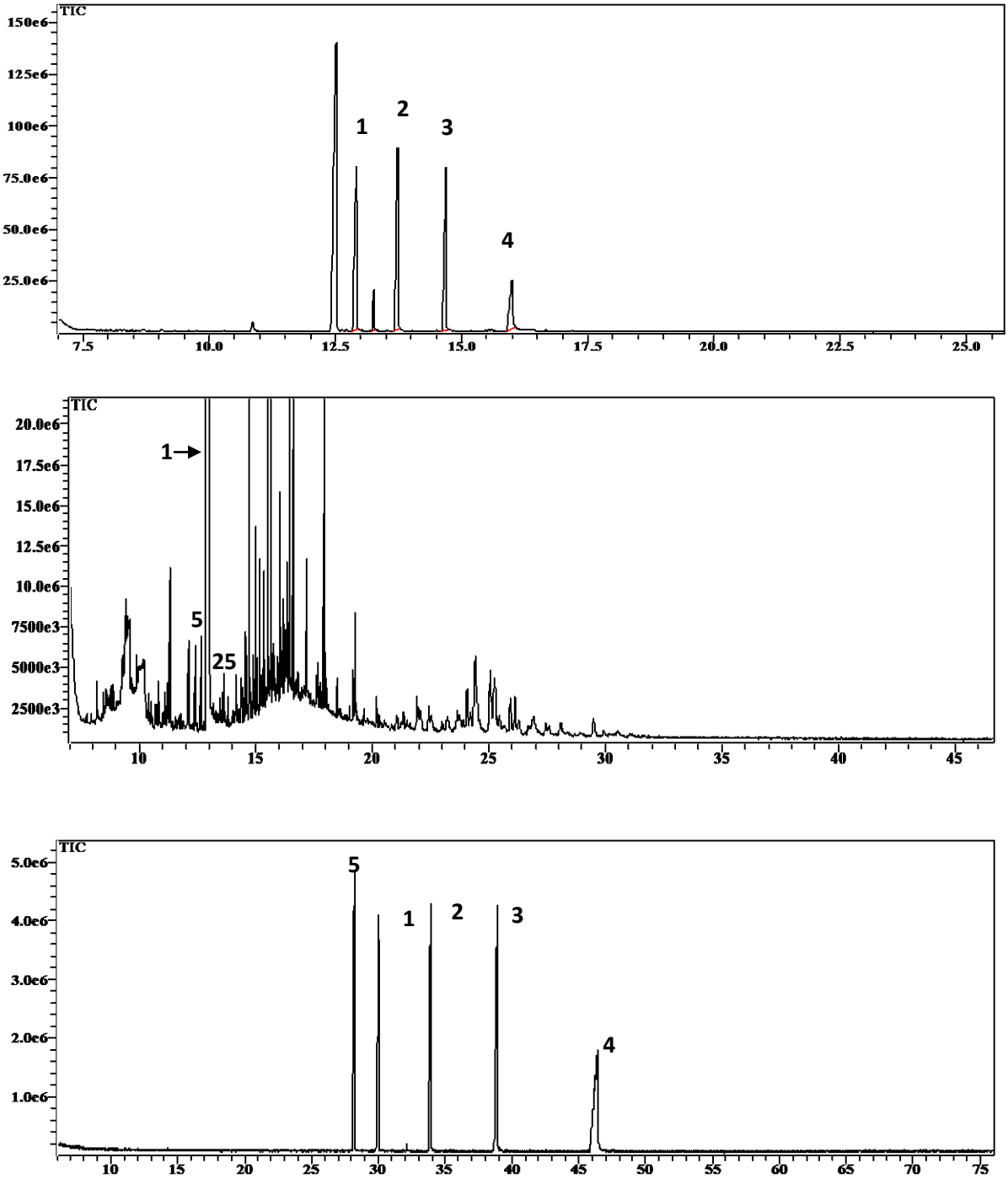


Figure 2.**A.**-GC-MS Chromatograms of the five coumarins (Conditions A). **B.-**GC-MS of soil sample (Conditions B). **C.-**GC-MS Chromatograms of the five coumarins under optimized chromatographic conditions (C). See text for details of the conditions A, B and C

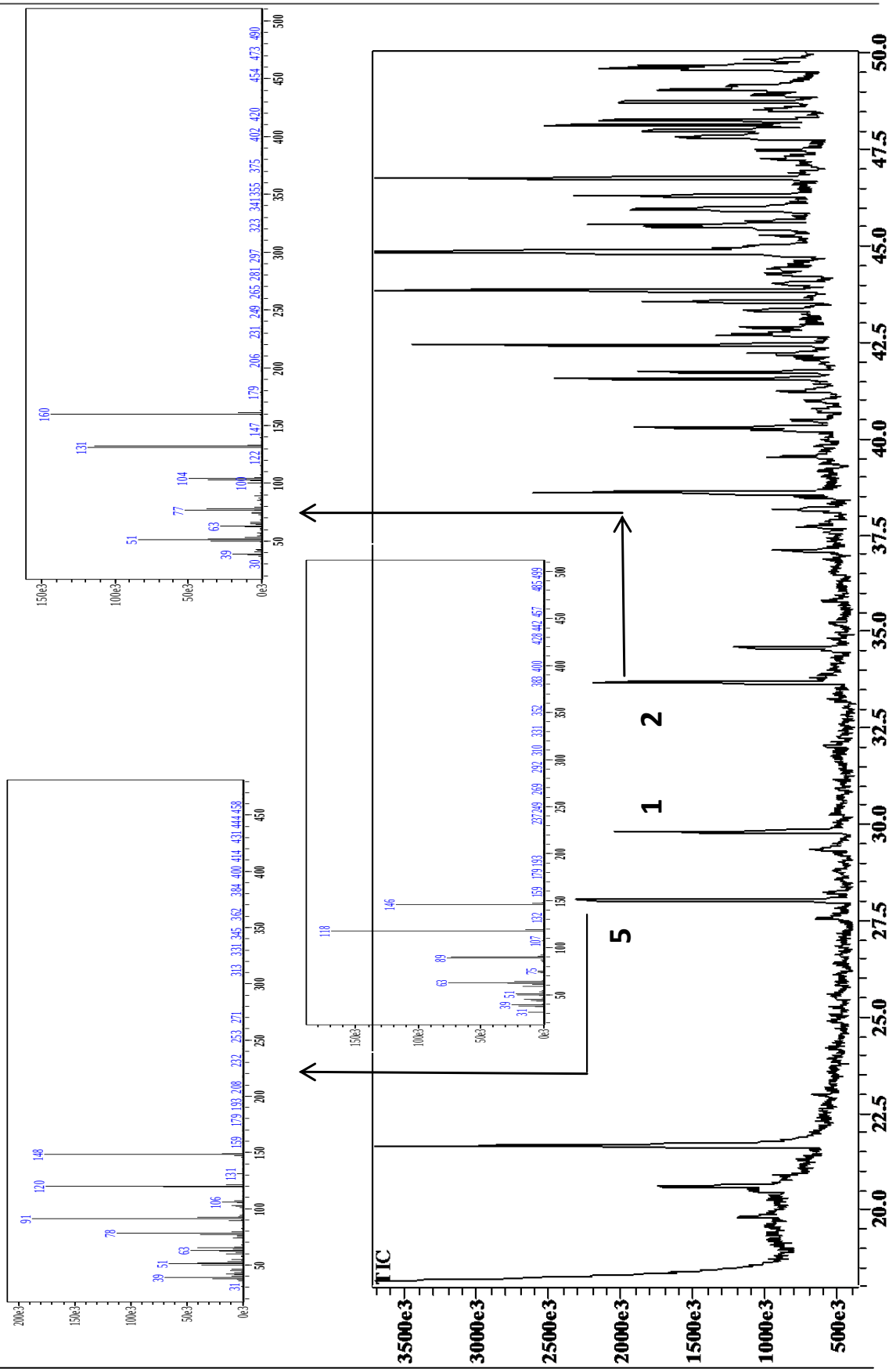


Figure 3.- GC-MS Chromatograms of soil sample under optimized chromatographic conditions. Initial column temperature: 40 °C for 5 min, then was increasing at 24.4 °C min-1 from 80 to 285 °C. Total analysis time: 50 min. Injector temperature, 290 °C. Solvent cut times: 5 min.

However, some considerations for optimization were studied. First, because of the potential application of the method to optimize the study of target coumarins in the environment, cutting times, initial column temperature and heating rate were selected, so as to allow the detection and separation of compounds of different molecular weights.

Coumarin biotransformations are known to yield a number of products, this increasing the analytical complexity of the samples at a large extent.

Figure 2B shows the complexity and the need for change in separation conditions. Thus different conditions were studied to improve the separation of standards and natural soil samples. (Figure 2C and 3) shows a typical chromatogram obtained under the best conditions achieved, according to the above mentioned characteristics.

The main ions of their mass spectra were used as characteristic ions for qualitative and quantitative analysis (Table 1, Figure 4).

Soil extraction

Recoveries from spiked soil

Coumarins are usually isolated from plants by extraction with solvents such as ethanol, methanol, benzene, chloroform, diethyl and petroleum ethers, or their combinations. The most exhaustive extraction of coumarins (in free form and as glycosides) is achieved with ethanol and its aqueous solutions, either cold or heated (33-35).

For the isolation of the 7-hydroxylated coumarins, it was suggested to use sequential extraction with acetone and acetone-methanol (1:1) mixture (36). In the present study, extraction solvents were selected according to this background.

Table 1. GC-MS analysis of coumarins

Coumarin simple	Principal ions and relative abundance, m/z (% base peak)
Coumarin (**1**)	146 (63), 118 (100), 90 (47), 63 (44)
6-methyl-coumarin (**2**)	160 (100), 132 (85), 103 (25), 104 (40), 51 (68)
7-hydroxy-4-methyl-coumarin (**3**)	176 (83), 148 (100), 120 (20), 91 (34), 39 (24)
7-metoxycoumarin (**4**)	176 (86), 148 (81), 133 (100), 105 (18), 77 (34), 51 (44)
Dihydrocoumarin (**5**)	148 (89), 91 (100), 78 (63), 77 (21), 39 (36)

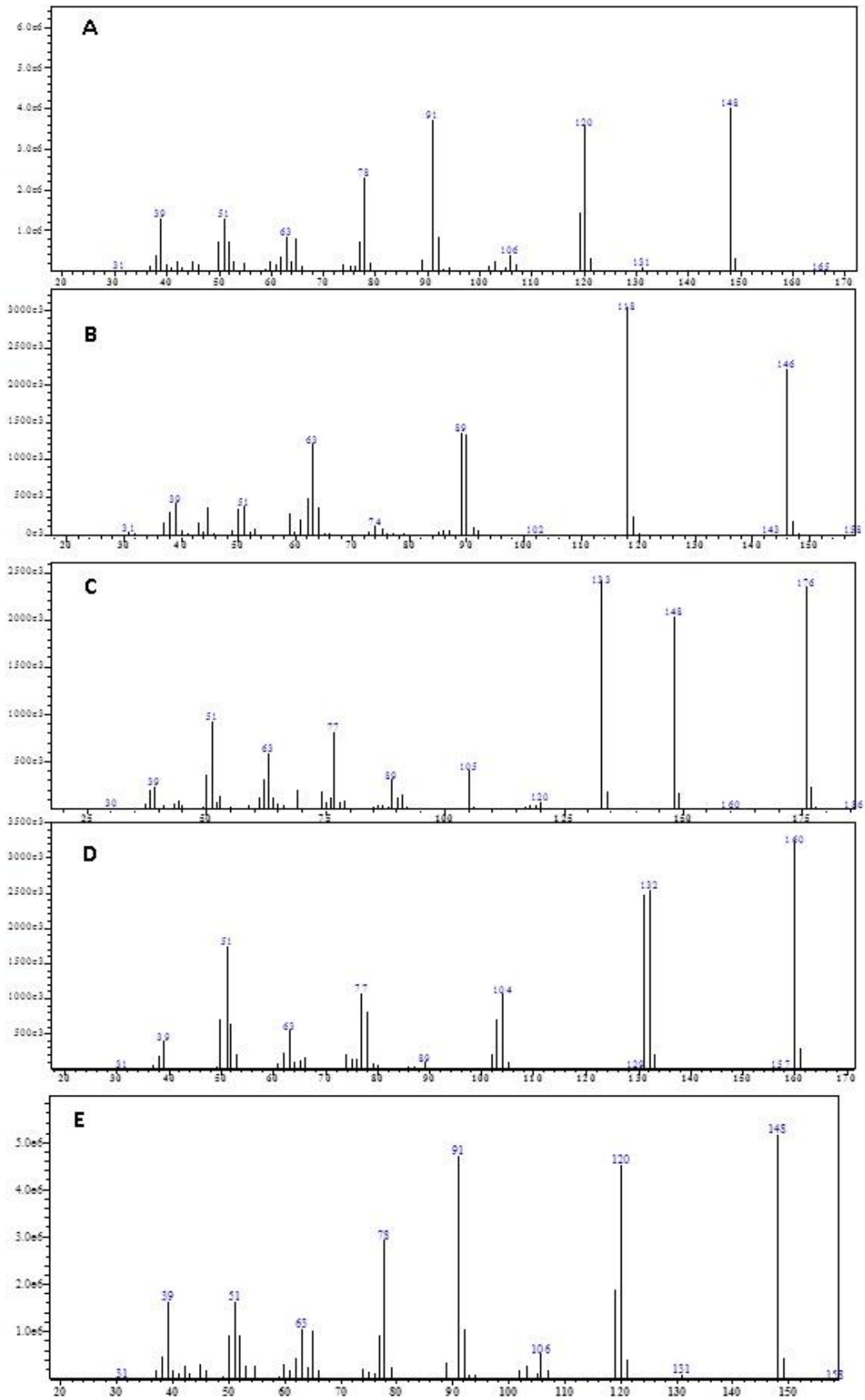


Figure 4. Mass spectra of (A) coumarin (1), (B) 6-methyl-coumarin (2), (C) 7-hydroxy-4-methyl-coumarin (3), (D) 7-metoxycoumarin (4), and (E) dihydrocoumarin (5)

Thus, mixtures of EtOH: water (1:1,v/v), MeOH:AcOEt (1:1, v/v) and MeOH were evaluated for their effectiveness to extract the five coumarins under study (Figure 5).

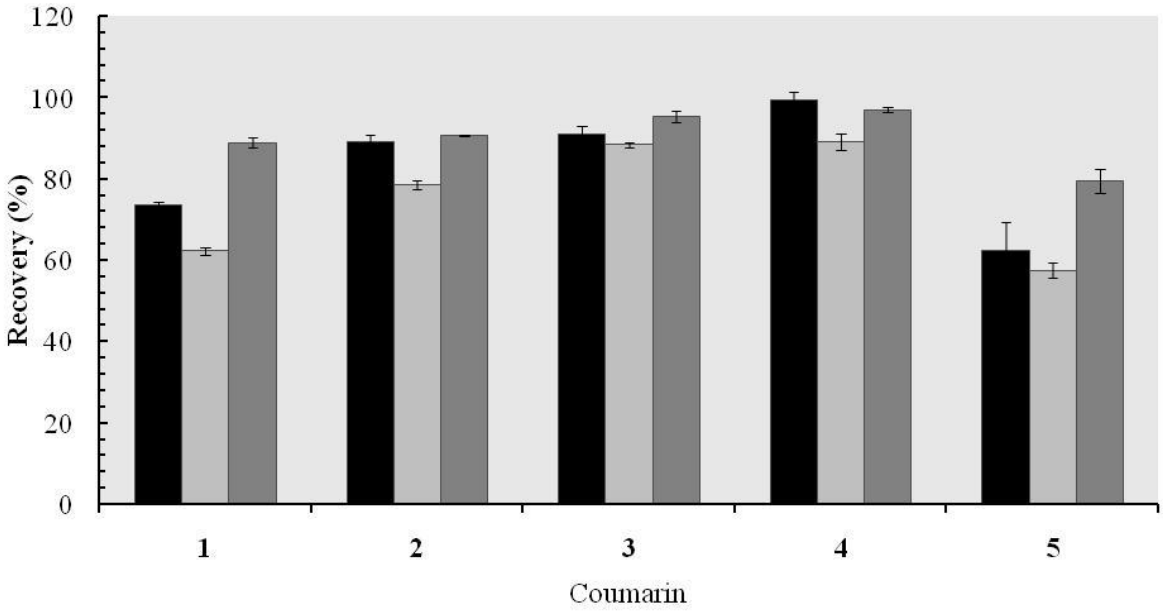


Figure 5. Recoveries for the Soil Extraction Procedure of five coumarin with different solvents using spiked soil samples. Doses at 4.0 mg kg^{-1} FW of soil. Bars represent an and standard deviation; *n=3*. FW= fresh weight.: MeOH. : $EtOH:H_2O$ (1:1, v/v). MeOH + Ac (1:1, v/v)

The results of Figure 3 show that the MeOH + AcOEt possessed the best extraction capacity for all coumarins examined with a minimum of 80% recovery for compound 5.

Absolute matrix effect

Some reports state that the extent of matrix effect may be dependent on the interface employed in a given method (37,38). The ionization mechanism is different in the presence of some matrix compounds, which may affect the efficiency of formation of the appropriate ions in the presence of other compounds, especially in complex matrixes such as natural soil.

While these species do not appear at the chromatographic trace when selected ions of the analyte are monitored, they may, however, significantly affect the efficiency and reproducibility of the ionization process (37,39), stated that co-eluting matrix components may reduce the ionization efficiency of the analytes and cause poor reproducibility and accuracy.

The soil matrix effect, which may result from the interface employed in the extraction, and the potential alterations in the ionization of the desired analytes owing to the presence of other components in the mixture (37-39), was studied in the concentration range used for the validation of the analytical method.

The absolute matrix effect was calculated by comparing the slope of matrix-matched standard curves with the slope of the standard calibration curve (Table 2). The amounts of coumarin derivatives added to the soil extract solutionswere unaffectedby the presence of the soil matrix (an approximate slope ratio of 1).Thus, under the extraction process and conditions optimized for chromatographic analysis, no significant interference from the matrix in the analysis of coumarin in this soil was observed. Based on these results, the GC-MS method developed in this study was proven to be acceptable for the simple and simultaneous determination of the five coumarins.

Extraction of natural soil sample

Qualitative analyses of soil extracts revealed the presence of coumarin (1), dihydrocoumarin (5) and 6-methylcoumarin (2), with 1 and 2 as major components (Table 3). The identity of all coumarin peaks were confirmed by spiking and comparison with the data in the Wiley 7.0 and NIST libraries. We anticipated this result in view of the known occurrence of various coumarins in several species of the plant community where soil samples were obtained (13,14).Nevertheless, they do not necessarily originate from the existing plants in the sampling site because it has long been known that a

Table 2. Absolute matrix effect (AME) calculated comparing the slope (*m*) of matrix-matched standard curve with the slope of the standard calibration curve (expressed in %).

	CV(%) *m*		
Coumarin	m_s	m_m	AME (%)
1	0.38	0.47	-0.16
2	0.47	0.47	0.00
3	0.80	0.80	0.00
4	0.56	0.56	0.01
5	1.16	1.18	-0.05

ms= Slope regression with standard, *mm*= Slope regression with matrix. ***CV***= Variation coefficient (%). m_s= Slope regression with standard, m_m= Slope regression with matrix.

Table 3. Soil coumarin concentration in natural soil sample

Coumarin Depth (cm)	Concentration (mg kg^{-1})	
	5 - 20	30 - 50
1	6.62 ± 0.01^a	9.23 ± 0.19^a
2	14.29 ± 3.48^a	9.58 ± 1.51^a
3	< LOQ	<LOQ
4	Absent	Absent
5	1.62 ± 0.05^a	0.63 ± 0.19^b

The averages followed by the same letter do not differ statistically between them. Tukey´s test for n = 3. *LOQ*= Limit of Quantization

Calibration and validation

Table 4 shows the regression equations for the coumarins studied. All five compounds showed good linearity (r2>0.999) with wide linear ranges (0.7 - 23 mg kg-1). The LODs and LOQs are showed in table 4. Precision (by analyzing three replicates at six concentrations) in one day or in three days were 98.7 ±3.8 and 98.3 ± 3.4 %, respectively.

Intra-day and inter-day precision were 1.1 ± 1.2 and 2.4 ± 1.9 respectively (Table 5). CV values show deviations in subsequent determinations between days; however, these values are generally considered acceptable in the process of validation of analytical methods.41 The results revealed good precision of the method.

Table 4. Regression analysis of GC-MS method on calibration curves

Coumarins	Regression equation	Coefficient of determination (r^2)	Linear range (mg kg^{-1} soil)	LOQ (mg kg^{-1} soil)	LOD (mg kg^{-1} soil)
1	y = 320882x – 2E+06	0.9999	0.33-23.20	0.58	0.18
2	y = 435292x – 1E+06	0.9997	0.33-23.20	0.48	0.14
3	y = 222535x – 4E+06	0.9999	0.33-23.20	2.72	0.81
4	y = 104142x – 2E+06	0.9992	0.33-23.20	1.16	0.35
5	y = 806071x - 174342	0.9992	1.44-23.20	1.21	0.36

In the regression equation, $\boldsymbol{y = ax + b}$, $\boldsymbol{y}$ refers to the peak area, $\boldsymbol{x}$ refers to concentration of the reference compound. ***LOQ*** is the limit of quantification. ***LOD*** is the limit of detection

Table 5. Intra- and inter day precision and recovery for the five simple coumarin performance

Coumarin	Nominal (mg kg^{-1} soil)	Mean	Intra day RSD	Recovery (%)	Mean	Inter day RSD	Recovery (%)
	0.73	0.67	0.76	92.1	0.67	0.76	92.1
	1.44	1.42	1.58	92.4	1.42	3.71	97.9
1	2.91	2.68	0.07	92.3	2.79	3.29	95.9
	5.81	5.74	3.16	98.8	5.67	1.04	97.4
	11.62	11.18	0.02	96.2	10.82	3.25	93.2
	23.25	23.22	0.03	99.9	23.44	0.81	100.8
	0.73	0.76	0.01	105.3	0.76	0.97	104.1
	1.44	1.46	0.59	100.5	1.37	5.76	94.2
2	2.91	2.94	0.17	101.0	2.90	1.29	99.7
	5.81	5.19	2.22	89.3	5.56	7.70	95.6
	11.62	11.12	2.50	95.6	10.95	1.02	94.2
	23.25	24.56	3.78	105.6	24.20	3.80	104.1
	0.73	0.72	0.11	99.5	0.72	0.27	99.6
	1.44	1.47	1.14	101.1	1.47	1.14	101.1
3	2.91	2.81	0.96	96.8	2.93	3.81	100.8
	5.81	5.72	0.47	98.4	5.66	0.53	97.3
	11.62	11.11	2.22	95.6	11.34	3.67	97.5
	23.25	23.17	0.64	99.6	23.33	1.08	100.3
	0.73	0.75	3.93	103.7	0.75	3.94	103.7
	1.44	1.45	0.82	100.1	1.45	0.82	100.1
4	2.91	2.88	0.23	99.1	2.86	1.22	98.5
	5.81	5.92	0.23	101.9	5.92	0.23	101.1
	11.62	11.79	0.70	101.4	11.62	0.69	99.9
	23.25	23.18	0.73	99.7	23.02	0.92	99.9
	1.44	1.44	0.50	98.5	1.33	6.22	91.9
5	2.91	2.82	0.47	97.2	2.90	1.88	99.9
	5.81	5.75	0.25	98.9	5.81	3.01	100.0
	11.62	11.51	0.06	99.0	11.16	3.13	96.0
	23.25	23.12	2.24	99.4	21.95	2.94	94.4

Conclusion

The results presented in this study indicate that the proposed method is useful for extraction and five coumarin derivatives using GC-MS. Moreover, the analysis time can be decreased (from 45 to 16 min) without significantly altering the resolution of the compounds, but this would significantly increase interference by various analytes present in the soil matrix, until it renders the method useless, which must be resolved by chromatography of the compounds of interest, to enable both the detection and resolution of potential degradation products. Experimental variables and analytical criteria, including optimization with calibration curves, extraction and recovery studies, which are essential in studies of natural compounds (allelochemicals) in soil concerning methods development have been considered in the analytical methodology developed in this work.

The results shown indicate that the proposed method for extracting soil coumarin and its derivatives and their subsequent detection and quantification is applicable for the analysis of coumarin, 6-methyl-coumarin, 7-hydroxy-4-methyl-coumarin, 7-metoxycoumarin, and dihydrocoumarin in soil. The extraction of soil coumarin derivatives is carried out only using a fast procedure, which not involves more instrumentation. Ethanol and acetone are used in a mixture for the extraction of the five coumarins. The extraction method is relatively quick and simple and can be easily implemented in routine analysis. The method is reproducible with high precision for the analysis of these compounds in soil at low concentration levels, and can be used in the study of coumarin derivatives mobility in the environment. Natural soil samples were studied with this method, detecting and quantifying these three coumarins. Thus, the whole method can be applied in the analysis, not only of coumarin in soil, but in the determination of the kinetics of dissipation and environmental fate of these compounds. In fact, due to the specificity of the detection system, the method can be applied in structural analysis of potential transformation products in soil, with mass spectrometry detection in scan mode. However, using the SIM mode (single ion monitoring), it is possible to lower the detection limit by one order of magnitude. However, this method allows the structural determination of possible degradation products.

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