Application of a Dynamic Extraction Model to the Supercritical Fluid Extraction of the Limonoid Cedrelone from *Cedrela toona*

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Abstract

Dynamic supercritical fluid extraction of the limonoid cedrelone from the ground wood of *Cedrela toona* (*Meliaceae*) with pure and modified CO_2 is studied. Extracts are collected at regular intervals to investigate the extraction kinetics of this compound. A theoretically derived model that involves extrapolating data obtained from shorter extractions to estimate the amount of cedrelone in ground wood is employed. The estimated quantitative results are found to be similar to exhaustive extractions obtained with liquid hexane and supercritical carbon dioxide modified with methanol. Furthermore, data from the supercritical fluid extractions are fitted to the model to produce a characteristic kinetic curve.

Introduction

Limonoids are a group of compounds obtained from plants belonging to the families *Meliaceae*, *Cneoraceae*, and *Rutaceae*. Structurally, the limonoids are derived from tetracyclic triterpenoids similar to euphol and tirucallol by a series of oxidative changes interspersed with molecular rearrangements. In this process, the eight-carbon side chain is converted to a C_{17} α-substituted furan ring and four carbon atoms are lost. Many limonoids are potentially available in large quantities. The timber of some species may yield 1% of an isolated crystalline limonoid, and a single tree of *Entandrophragma angolense* may contain more than 100 kg gedunin; much of it is easily recoverable from timber mill offcuts. The biological advantage of producing such large amounts of material is not well understood, but it has been shown that many limonoids are active as insect antifeedants (1), although most are not directly insecticidal (2). The effect may be so powerful that insects will starve rather than eat leaves treated with limonoids, and it is probable that this is the biological advantage of the limonoids to the *Meliaceae*. Some limonoids have also been found to be active against some types of cancer (3); activity is confined to certain compounds of the prieurianin and havanensin groups that contain an epoxide ring between the carbons in positions 14 and 15. This is the family to which the model compound used in this study belongs.

Limonoids have traditionally been extracted with liquid solvents in a Soxhlet apparatus. However, as long extraction times are typically used and many natural products are unstable, alternative extraction methods have been investigated. Supercritical fluid extraction (SFE) is one method that is attractive. Its usefulness for the extraction of natural products is due to the combination of gas-like mass transfer properties and liquid-like solvating characteristics with intermediate solute diffusion coefficients (4). This makes SFE often faster and more efficient than Soxhlet extraction. Furthermore, through control of fluid density, the solvating power of CO_2 can be tailored to extract the particular target compounds of interest. CO_2 also evaporates on decompression at the restrictor outlet, which facilitates off-line collection of extracts in a solvent for subsequent analysis or on-line combination with methods such as gas chromatography (GC). Other advantages of CO_2 are that it is inexpensive, nontoxic, and not harmful to the environment. As a result, SFE has been widely applied to the extraction of plant materials. This is a subject of a recent review (5).

One of the characteristics of SFE is that the majority of the compounds of interest are removed from in and around the surface of the matrix during a short period at the beginning of the extraction, but subsequently, the extraction rate tails off dramatically. This is thought to be caused by solute adsorption on the matrix surface and because some of the solute remains held in the structure of the matrix and is only extracted with time. Thus, complete extraction is rarely accomplished. This behavior is independent of the manner in which the solutes are distributed, and it results in quantitation problems in analytical extractions. However, if a suitable extraction model could reliably predict the amount of solute present without exhaustively extracting the compound, such problems could be resolved. Furthermore, such a model should be able to predict the controlling factors in the extraction of a particular solute, that is, whether the extraction is controlled by diffusion processes, solubility limitations, or a combination thereof. Various

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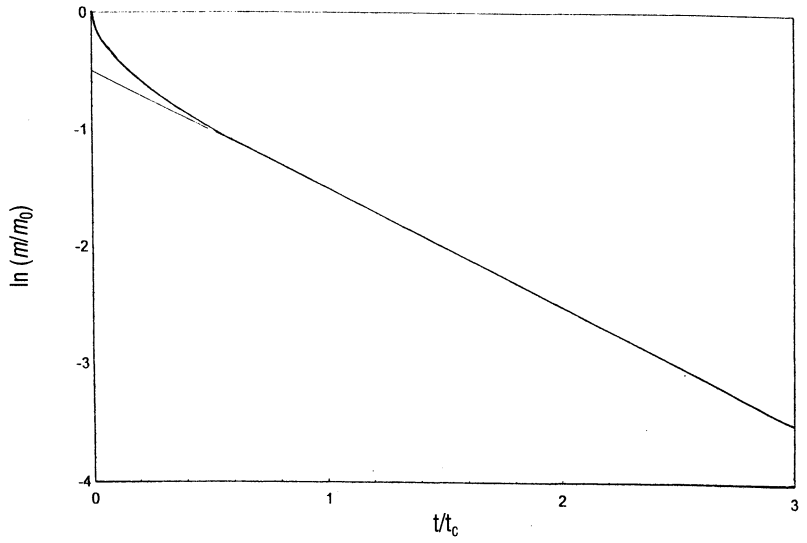

Figure 1. Theoretically derived extraction curve for a perfect sphere.

models for SFE, mostly based on mass balance, have been proposed (6–9). However, as most models require a knowledge of the matrix characteristics, which in the case of natural products is difficult to understand (because samples are never spherical and vary in particle size), it is difficult to model an extraction.

In many cases, the simple first-order extraction rate law or "hot ball" model applies (6). The model describes a solid sphere of radius r with a uniform initial concentration of a material that is immersed in a fluid in which the concentration of extracted material is 0. It is also assumed that the flow rate of the fluid is so high that the fluid remains infinitely dilute and the solute is uniformly distributed throughout the matrix. If the mass of the solute is m_0 initially and m after a given time period of extraction, then a plot of ln (m/m_0) versus time has the form shown in Figure 1. The initial steep fall represents the extraction of the majority of the material. The exponential behavior of the extraction after this initial period means that extrapolation may be used to obtain the actual mass of analyte present without exhaustively extracting the sample. The intercept of the linear portion of the kinetic plot indicates particle size and distribution as well as the nature of the extraction process. The linear portion of the curve is indicative of extraction from deeper within the matrix. It is the most time consuming and difficult part of the extraction.

If the extraction is carried out to obtain an extracted mass m_1, followed by two successive extractions to obtain extracted masses m_2 and m_3, then the model can be used to predict the mass of solute m_0 in the sample:

$$m_0 = m_1 + \frac{m_2^2}{m_2 - m_3} \qquad \text{Eq 1}$$

A number of studies have been undertaken to evaluate this model. Walker and co-workers (10) investigated the model for the SFE of pinene, camphene, camphor, and borneol from rosemary (10) and found that the results compared well with the mean of three exhaustive extractions to give m_0. Liu and co-workers (11) also used the model to determine the amounts of polycyclic aromatic hydrocarbons from a spiked soil sample, and Subra and co-workers (12) tested whether this method could be used to model the supercritical fluid extraction of two major carotenes present in carrots.

In this paper, we report the extraction and subsequent off-line GC analysis of the limonoid cedrelone from the wood of *Cedrela toona* (*Meliaceae*) with supercritical CO_2, methanol-modified CO_2, and liquid hexane. The "hot ball" model was used to estimate the value of m_0 by extrapolating data obtained from shorter CO_2 extractions with the use of Equation 1. The calculated value of m_0 was then compared with those from exhaustive extractions to see whether the model was indeed applicable.

Experimental

Sample preparation

The test sample consisted of ground wood of *Cedrela toona* taken from a branch that had been dried under solar conditions for 6 weeks. The wood was separated from the bark, ground into a powder (for which the particle size distribution ranged from 250 to 700 µm using mesh sieves), and extracted with various fluids.

Extractions

Pure CO_2 extractions

Dynamic extractions of 3.0 g of the ground wood were performed on a homemade SFE unit that was constructed with a Lee Scientific series 501 pump (Dionex, CA) for fluid delivery and a Perkin-Elmer 3B gas chromatographic oven (Norwalk, CT) for temperature control. The extraction vessel was a 24-mL stainless steel vessel (Keystone Scientific; Bellefonte, PA). Supercritical fluid extractions with the use of SFC/SFE-grade CO_2 (Air Products and Chemicals; Allentown, PA) were conducted at pressures of 300 and 350 atm at 40°C. Deactivated fused-silica capillary columns (30 cm × 50 or 75-µm i.d.) (SGE; Australia) were used as the extraction cell outlet restrictors. The restrictors were kept from plugging with the use of hot air blown from a hand-held drier. The extracted compounds were collected for several time intervals by purging the extraction cell effluent into about 5.0 mL analytical-grade dichloromethane.

Methanol-modified CO_2 extractions

Methanol-modified CO_2 extractions were performed with an ISCO SFE system (Lincoln, NE) that consisted of a 260D syringe pump and an SFX 220 oven unit that housed two 10-mL stainless steel extraction vessels. Methanol (40 µL) was spiked into a 0.6-g sample that was in the vessel prior to extraction. A 30-cm × 50-µm i.d. deactivated fused-silica capillary (SGE) was used as the extraction cell outlet restrictor. A 30-min static

extraction time at 400 atm and 40°C followed by a 40-min dynamic extraction under the same conditions was performed. Extracts were collected as described previously.

Soxhlet extraction

The ground wood sample (3.0 g) was put in a 100- × 30-mm i.d. cellulose thimble and extracted exhaustively with 150 mL refluxing hexane in a Soxhlet apparatus for 5.5 h.

Quantitation of extracted analyte

An internal standard method was used for quantitation. Standard solutions containing known concentrations of a standard sample of cedrelone and a fixed concentration of methyl palmitate internal standard were prepared in dichloromethane and used to generate a five-point calibration curve of the form $y = 0.84x + 0.028$. The correlation coefficient was 0.997. Methyl palmitate was added to sample extracts prior to analysis by capillary GC, and the response factor (slope = 0.84) obtained from the calibration curve was used to quantitate the concentration of cedrelone in the extracts.

Capillary GC analysis

Capillary GC analysis of the extracts was performed on a Model 8160 GC (Fisons Instruments; Milan, Italy) with flame-ionization detection using a 25-m × 250-µm i.d. fused-silica capillary column coated with a 0.25-µm film of PTE-5 (Supelco; Bellefonte, PA). After sample dilution and addition of methyl palmitate (internal standard), 0.5-µL aliquots were injected manually into the column (which was held in the oven at 60°C) via an on-column injector. The oven temperature was maintained at 60°C for 1 min after injection and then increased at 18°C/min to 150°C, held 1 min, and increased to 320°C at 7°C/min. The oven was held at 320°C for 10 min prior to cooling for the next analysis. The flame-ionization detector was heated to 320°C and operated using hydrogen (30 mL/min), air (300 mL/min), and nitrogen (25 mL/min) makeup gases. The carrier gas was helium at an initial flow rate of 1.2 mL/min (a linear velocity of about 40.7 cm/s).

Results and Discussion

Preliminary GC analysis

The GC separation efficiency was first optimized with initial mobile phase flow rates of 0.5–1.2 mL/min (approximately 17.0–40.7 cm/s). An initial mobile phase flow rate of 1.2 mL/min (and an injection volume of 0.5 µL) was used to eliminate band broadening of the later eluting peaks. Figure 2 shows a typical GC chromatogram of the wood extract at 350 atm and 40°C. It is apparent from the chromatogram that two major compounds are present in the wood. From our previous study (13), the compound labeled B on the chromatogram was identified as geranyl geraniol, and the one labeled C was cedrelone. Cedrelone was chosen as a model compound for this study since it occurs in a reasonable amount.

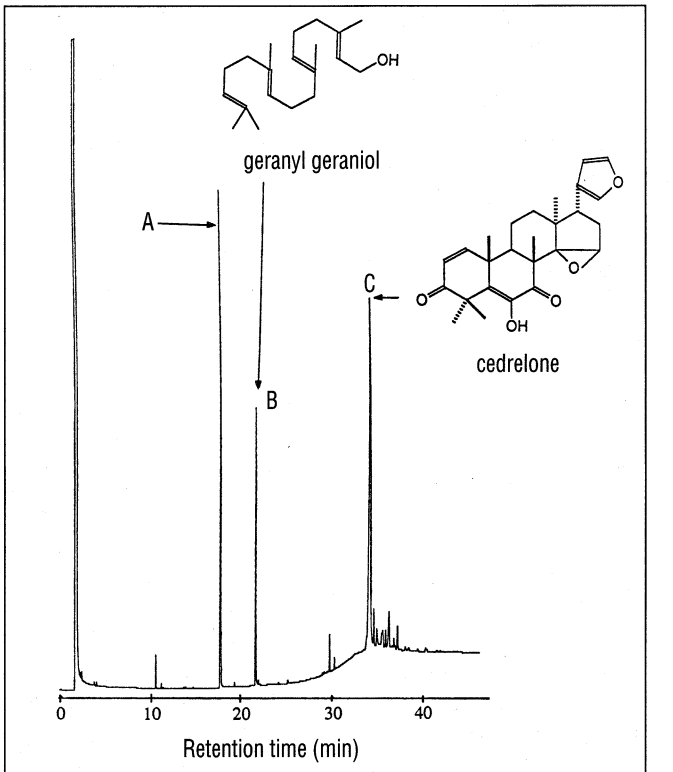

Figure 2. Capillary gas chromatogram of a supercritical CO_2 extract from the wood of *Cedrela toona*. SFE was performed at 350 atm and 40°C. Peaks: A, internal standard; B, geranyl geraniol; and C, cedrelone.

Kinetic study

The exponential behavior of the extraction after the initial steep period allows the use of extrapolation to obtain quantitative analytical information in a shorter time than would be required for exhaustive extraction. Three successive extractions were performed on three 3.0-g samples using CO_2 at 300 atm and 40°C (with a 30-cm × 75-µm i.d. fused-silica capillary restrictor) for 10 min to obtain m_1 followed by two successive 5-min dynamic extractions on each sample to obtain m_2 and m_3, respectively. Equation 1 was then used to determine m_0 for cedrelone in the wood. The results are shown in Table I. The relative standard deviation of 11.9% for the measurement of m_0 represents the total reproducibility of the extraction process. Variations in measuring m_1, m_2, and m_3 are higher than that obtained for m_0. This is caused by variations in the extraction efficiency between runs. Also, since the major portion of the

Table I. Data for Three Successive Extractions in the Determination of m_0

Sample	m_1 (mg/g)	m_2 (mg/g)	m_3 (mg/g)	m_0 (mg/g)
1	2.49	0.15	0.017	2.67
2	3.37	0.054	0.040	3.59
3	3.21	0.035	0.013	3.27
Mean	3 .02 ± 0.38	0.080 ± 0.050	0.023 ± 0.012	3.18 ± 0.38
RSD (%)	12.6	63.7	51.7	11.9

sample is extracted during the m_1 period, the variation in estimating m_1 becomes smaller than in the tailing sections of the extraction (m_2 and m_3) where extractions are very difficult to perform and therefore reproducibilities are low. Unfortunately, Walker and co-workers (10), who used this model in a quantitative study of the SFE of flavor and fragrance compounds from lavender and rosemary, did not show the variations in the measurement of the m_1, m_2, m_3, and m_0 values for comparison. However, the results by Liu and co-workers (11) showed a trend similar to that reported in Table I, where variations in measuring m_1, m_2, and m_3 were higher than that obtained in measuring m_0. Similarly, variations in m_1 were lower than in m_2 and m_3.

After the value of m_0 was determined, extractions were performed at different lengths of time using CO_2 at 350 atm and 40°C with a fresh 3.0 g wood sample each time. A 30-cm × 50-µm i.d. fused-silica capillary was used as the restrictor at the extraction cell outlet. Amounts of cedrelone extracted (m_e) were calculated and used to plot a bar graph of the percentage yield verus extraction time, as shown in Figure 3. This figure shows the general trend typical of SFE extractions, that is, an initial steep rise and subsequent tailing off of the amount of cedrelone extracted. Table II shows the m/m_0 values that were fitted into the kinetic model. Figure 4 shows the kinetic plot of ln (m/m_0) versus extraction time. Characteristically, the curve initially falls steeply, with the rate of fall finally assuming linearity. Although the initial steep fall appears as a relatively small feature, in reality, it represents the loss or extraction of the majority of the material from the matrix. On computing the percentage cedrelone extracted from the point where linearity is attained (at the eighth minute of extraction) about 66% of the cedrelone is extracted within the initial portion of the curve.

The particle size has a major influence on the slope of this nonlinear portion of the curve. In our study, matrix particle sizes that ranged from 0.25 to 0.70 mm gave a kinetic plot with an initial nonlinear portion followed by a linear portion with an intercept on the ln m/m_0 axis at approximately –0.53. These results compare favorably with those of Subra and co-workers (12) who investigated the extraction of β-carotene from carrot matrix; particle sizes ranged from 0.16 to 1.0 mm. They obtained a graph similar to that in Figure 4 with an intercept of the linear portion on the ln m/m_0 axis at approximately –0.5. However, on studying particle sizes greater than 1.0 mm, a straight-line graph was obtained with an intercept on the ln m/m_0 axis at 0.0. With particle sizes less than 0.16 mm, an extensive and steeply sloping nonlinear portion of the curve was obtained followed by a linear portion with an intercept on the ln m/m_0 axis at approximately –2.4.

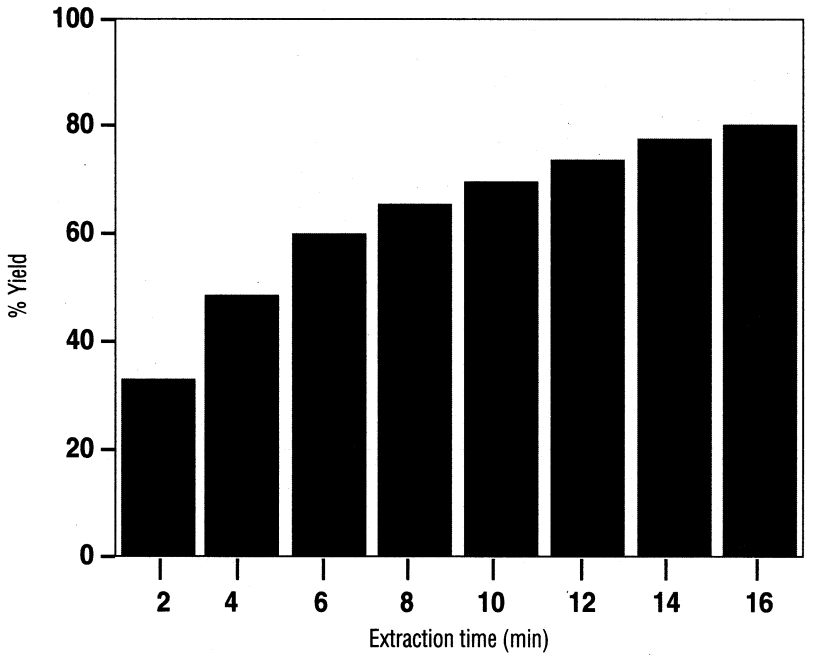

Figure 3. Graph of percent yield versus time for cedrelone.

Table II. Data for the Kinetic Plot

Time (min)	m_e (mg/g)	RSD (%)	m (mg/g)*	m/m_0	ln(m/m_0)
2	1.05	1.73	2.13	0.67	–0.40
4	1.55	5.05	1.63	0.51	–0.68
6	1.91	2.53	1.27	0.40	–0.82
8	2.09	2.59	1.09	0.34	–1.08
10	2.23	1.65	0.95	0.30	–1.22
12	2.35	4.67	0.83	0.26	–1.36
14	2.48	1.72	0.70	0.22	–1.50
16	2.57	2.29	0.61	0.19	–1.64

* $m = m_0 - m_e$

It can be concluded that different mass transfer mechanisms are involved depending on particle size. For the larger particles, diffusion out of the matrix plays a significant role and becomes the limiting step. In contrast, as the matrix particle size decreases, the analyte molecules become more accessible to the supercritical fluid. Hence, a large proportion of the analyte is removed by a simple washing process. As m (amount of analyte remaining in the matrix at any time) decreases rapidly, ln m/m_0 becomes more negative, which leads to an increase in the steepness of the nonlinear portion of the kinetic plot. Thus the steepness of the initial portion of the graph could be used as a practical guideline to select an optimum matrix particle size to maximize extraction yield in the shortest possible time.

Another interesting feature of this plot is the intercept of the linear portion of the kinetic curve on the ln (m/m_0) axis. The theoretical model predicts a value of –0.5 assuming that the particles are spherical and have a uniform size and that the solutes are uniformly distributed. It also predicts that the extraction is a diffusion process. The effects of solubility limitation are to reduce

the high rate of extraction at the beginning of the run, to reduce the slope of the linear portion, and to move the linear portion upwards on the graph. The extrapolation of the linear portion of the kinetic plot in Figure 4 intercepts the ln (m/m_0) axis at just below the theoretical value. Within limits of experimental error, it could be predicted that the rate of extraction was not limited by solubility; rather, it was controlled by a diffusion process. There is also the possibility of a uniform distribution of solute in accordance with the model, but in reality this is not likely to be the case because of the large variations in the data shown in Table I. Walker and co-workers (10) showed similar results for the SFE of camphene from lavender where the intercept of the linear portion of the curve was at –0.48. The intercepts of the linear portions of the curves for other real systems, however, may be different from the theoretical value. For example, in the SFE of camphor from rosemary, the intercept was –2.36 (10).

Other than the effect of solubility limitation described above, deviations from the theoretical intercept of –0.5 may also be explained in terms of (*a*) the irregular shapes of the matrix particles, which lower the intercept, and (*b*) the nonuniform extractable solute distribution, which can either lower or raise the intercept. Table III shows the amounts of cedrelone obtained by the different extraction techniques; the results indicate a significant correlation between the various methods. Although a 4-h extraction time for hexane resulted in a lower yield for the target compound as compared with a 5.50-h extraction time, extractions of the wood using hexane over 5.5, 6, and 8-h periods produced similar extraction yields of cedrelone. The 5.5-h extraction time of the 3.0 g wood sample was therefore considered to be exhaustive. The amount of cedrelone extracted in the 5.5-h period with hexane compared favorably with that obtained using the kinetic model. Further, since our preliminary study had shown that small concentrations of methanol improved the extraction yield of the target analyte, an exhaustive extraction with 0.4 mol % methanol-modified supercritical CO_2 was performed. The results compared well with those obtained using the kinetic model as well as with those obtained with the 5.5-h Soxhlet extraction method that used hexane.

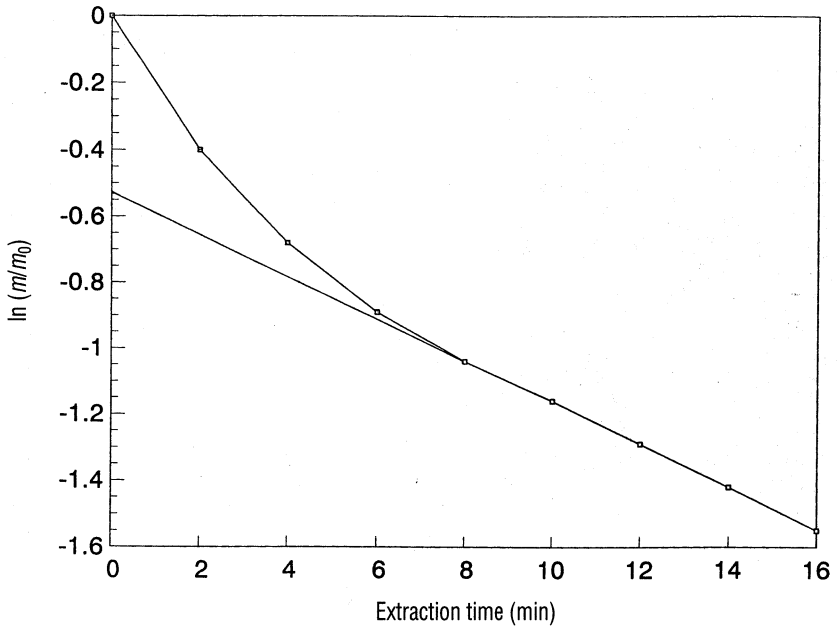

Figure 4. Graph of ln(m/m_0) versus extraction time for cedrelone.

Table III. Comparative Data for the Amounts of Cedrelone (in Milligrams per Gram) by Various Extraction Techniques

	Soxhlet extraction (hexane, 4 h)	Soxhlet extraction (hexane, 5½ h)	Model	CO_2 with methanol
	2.65	2.88	2.67	3.41
	3.06	3.18	3.59	3.18
	2.61	3.63	3.27	3.14
Mean ± SD	2.77 ± 0.20	3.23 ± 0.31	3.18 ± 0.38	3.24 ± 0.19
RSD (%)	7.34	9.54	11.9	3.64

Conclusion

From the results obtained in our work, the extrapolation approach derived from the "hot ball" model predicts, to a very high degree, the amounts of natural product compounds present in plant materials. Thus it is possible to study the factors controlling the extraction of certain naturally occurring compounds from plant matrices with the possibility of improving extraction yields for a particular target analyte.

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