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***ARTICLE***

# Derivative Spectrophotometry allows Quantitative Determination of Essential Oils in Dispersible Powders of Spray-Dried Nanocapsules

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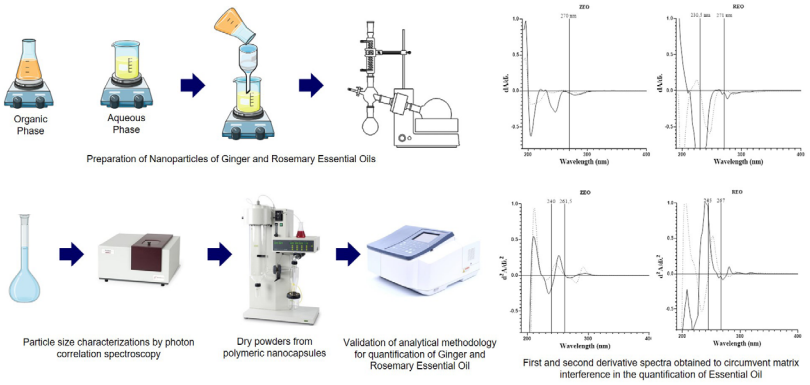


This study aimed to develop and validate an analytical method based on derivative spectrophotometry to determine the true concentration of ginger (ZEO) and rosemary (REO) essential oils in nanocapsule redispersible powder formulations. Second-order derivative spectrophotometry allowed the interference of nanocapsule components to be cancelled out at wavelengths of 240 and 245 nm for ZEO and REO, respectively. The validated method was linear in the range 0.01-0.04 mg $mL^{-1}$ for ZEO and 0.6-0.96 mg $mL^{-1}$ for REO. It is also accurate with RSDs of 1.82% and 1.44% for ZEO and REO respectively, and shows recovery rates close to 100% for both essential oils when assessing accuracy. The method allowed the determination of the essential oils in the presence of the formulation components in a simple and rapid way, showing that it is an option to be used for the quantification of essential oils in these nanometric systems.

**Keywords:** essential oils, nanocapsules, one pot, derivative spectrophotometry, analytical validation

## INTRODUCTION

Essential oils (EOs) are volatile aromatic liquids extracted from plant materials such as flowers, roots, bark, leaves, among others. These compounds have broad biological activity attributed to their composition as terpenes, mainly mono and sesquiterpenes.[1–4] Ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*) are well-known plants throughout the world. Its EOs contain compounds to which a wide range of biological activities are attributed, justifying their multiple uses as medicines and foods.[4–6]

The diverse bioactive potential of EOs, coupled with the great commercial interest that these compounds present, makes them the focus of several studies aimed at their potential application in different industrial

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sectors, mainly food, pharmaceutical and cosmetic.[3,7] Nevertheless, their applicability is still limited as these compounds are generally sensitive to oxygen, light and heat, as well as low water solubility, volatility and strong flavor.[8,9] Taking these factors into consideration, the use of technologies that aim to protect these compounds from possible degradation or allow new forms of application becomes highly interesting.

Among the possible innovative technologies that enable the application of bioactive compounds, encapsulation at the nanometric or micrometric level has been applied as a promising way to increase their stability. Key encapsulation advantages include protection against negative external conditions during compound processing and storage.[10,11] There is an increasing number of studies evaluating the production of nanocapsules to encapsulate essential oils. Their objectives may be to increase protection against volatilisation,[12] temperature and light variations,[8] as well as to control release.[10] Also included is the possibility of producing dry systems for nano-encapsulation of essential oils as an alternative to the disadvantages of conventional nanometric systems,[12–14] representing one of the first steps for the production of more stable nanometric systems, enabling the production of carriers for these oils in medium and large scale.

In general, all compound encapsulation methods have generated loss or degradation of the encapsulated substance during the process of obtaining. In the case of nanocapsules, the influence of phase partition and component interactions can make it difficult to know the actual concentration of the compound after its production.[15] So far, studies using techniques such as gravimetry,[12] spectrophotometry,[10,16] chromatography GC-MS[17] and HPLC (UV and DAD)[18] for quantification of the encapsulated essential oil by the developed nanometric systems.

The use of gravimetry requires prior extraction by heating with solvents (Clevenger apparatus), which can lead to chemical conversion of the components;[19] methods using GC and HPLC for quantification, despite their advantages, are expensive and time consuming, making them less suitable, especially when a large number of measurements are required.[20] Spectrophotometry (UV-Vis), in turn, is often used for quantification of essential oils in nanometric systems because it is a fast, simple and appropriate technique for routine analysis.[15,21] However, most studies have indirectly quantified essential oils through the non-encapsulated oil fraction, assuming a non-existent loss of OEs content during the process of obtaining the system.[22] This could raise doubts as to the validity of the values obtained by these methods. In addition, many studies that quantify the concentration of essential oils in particulate systems do not mention performing analytical validation of the methods used.

Based on this, the present work proposes the development and validation of analytical methods based on derivative spectrophotometry to determine the concentration of rosemary and ginger essential oils in dry powder of poly(ε-caprolactone) (PCL) nanocapsules eliminating spectral overlap of components with high sensitivity, selectivity and accuracy according to International Council for Harmonisation (ICH) Q2 (R1).[23]

## MATERIAL AND METHODS

### *Reagents*

Poly(ε-caprolactone) (PCL) (Mw = 80,000), Sorbitan Monostearate and Polysorbate 80 were purchased from Sigma-Aldrich (São Paulo, Brazil). Acetone was obtained from Nuclear (São Paulo, Brazil). Acetonitrile (ACN) and isopropyl alcohol were purchased from Tedia (Goiás, Brazil). Lactose and L-phenylalanine were purchased from Vetec (São Paulo, Brazil). *Rosmarinus officinalis* and *Zingiber officinale* essential oils were purchased from Quinari (Ponta Grossa, Brazil).

### *Equipment*

UV-Visible Spectrophotometer Shimadzu® (UV-1800PC, Shimadzu, JP) and quartz cuvettes (1 cm), equipped with UV-Probe software (version 2.42, Shimadzu, Japan), being used for all spectrum readings and data processing.

***Preparation of dry powders from polymeric nanocapsules***

To obtain dry powders from polymeric nanocapsules, we employ the single step drying methodology as previously described.[24] Thus, the nanocapsule suspension was initially developed, where an organic phase consisting of acetone (37 mL), polymer poli(ε-caprolactone) (PCL) (0.15 g), sorbitan monostearate (0.12 g) and the essential oil (ZEO or REO) (300 µL) was poured into an aqueous phase containing ultrapure water (145 mL) and polysorbate 80 (0.12 g) at 40 °C under magnetic stirring. After 30 min, the suspension was dried in the presence of lactose and *L*-phenylalanine (90:10 w/w) at 5% (w/v). For this step, we use the equipment Mini Spray Dryer B-290 (Buchi, Flawil, China) with the following parameters: inlet temperature: 120 °C; feed flow: 10% (4 mL $min^{-1}$); atomizer diameter: 1 mm; drying air pressure: 40 Kgf $cm^{-2}$; outlet temperature: 70 ± 2 °C. Similarly, formulations without the presence of OE (placebo) were prepared (PNC). All formulations were made in duplicate, and after preparation, the powders were stored at room temperature (25 °C) in amber vials, protected from light and moisture.

***Determination of resuspension index of dry powder particles after redispersion in water***

The particle resuspension index was determined following a previously described methodology,[25] which represents the recovery of the average particle size according to the parent formulations. The dried products were redispersed in ultrapure water and subjected to magnetic stirring for 5 min (maintaining the same ratio as the original nanocapsule components) and after that they were filtered (0.45 µm). For the calculation basis, the average diameters obtained by the dynamic light scattering technique (DLS) were used (NanoBrook 90Plus Zeta®, Brookhaven Instruments Corportation, USA), according to Equation 1. The DLS technique relies on evaluating fluctuations in the intensity of scattered light at a specific angle. When a particle is illuminated by a light source, such as a laser, it scatters light in varying intensities and directions due to the random motion of particles in the sample. This approach allows for the determination of the particle's translational diffusion coefficient. Finally, by applying the Stokes-Einstein equation, the hydrodynamic diameter of the particles can be obtained.

$$\text{Resuspension index} = Sf / Si \qquad \text{Equation 1}$$

where *Sf* is the average particle diameter of the dried products after redispersion in water, and *Si* is the average particle diameter of the formulations prior to the drying process.

***Standard solutions and sample preparation***

Initially a stock solution of *Zingiber officinale* essential oil (ZEO) (2 mg $mL^{-1}$) was prepared by dissolving 20 mg with a binary mixture of ACN and isopropanol (90:10 v/v) in a 10 mL volumetric flask. Then it was placed in an ultrasonic bath for 2 min, aiming the complete dissolution of the oil. In parallel, *Rosmarinus officinalis* essential oil (REO) (2 mg $mL^{-1}$) was prepared by dissolving 20 mg in ACN.

In order to extract the EO from the dry nanocapsule powders, we adapted a previously proposed methodology.[16] Thus, in a 5 mL volumetric flask, a fraction of the dry powder of the ZEO nanocapsule formulations at the theoretical concentration of 0.025 mg $mL^{-1}$ and REO 0.78 mg $mL^{-1}$ were dissolved in a binary mixture of ACN:isopropanol (90:10 v/v) and ACN, respectively. After that, the solutions containing the samples were homogenized in an ultrasonic bath for 45 min, followed by centrifugation (10.20 g for 10 min). At the end, the supernatant was filtered under 0.45 µm membrane before all analyzes.

***Validation of analytical methodology for ZEO and REO quantification***

The validation of the analytical method was performed according to ICH,[26] following the parameters: selectivity, linearity, precision and accuracy.

*Selectivity*

The selectivity of the method was evaluated by the use of placebo solutions, which were prepared with the formulation matrix (PNC), without the presence of OE, in its usual concentration.

*Linearity*

Linearity was assessed by constructing three different calibration curves for each essential oil, with seven points each, in the range 0.01 to 0.04 mg $mL^{-1}$ for ZEO and 0.6 to 1.0 mg $mL^{-1}$ for REO. Linearity was assessed by linear regression analysis, calculated by the least squares regression method.

*Precision*

Repeatability was assessed by six different EO determinations in the formulations, all on the same day (n = 6). Intermediate precision was analyzed by repeating the procedure by determinations on three different days (day 1, n = 3; day 2, n = 3; day 3, n = 3). Data were expressed as a function of the relative standard deviation (RSD) of a series of measurements.

*Accuracy*

Accuracy was assessed by recovery in matrix samples (PNC) enriched with known concentrations of essential oil. Samples of essential oil solutions were added to the matrix to obtain concentrations at three different levels (low, medium and high). All samples were prepared in triplicate. The recovery percentage was evaluated.

## RESULTS AND DISCUSSION

### *Resuspension index*

The production of spray-dried nanometric systems has been studied by several authors,[13,25,27] however, studies that have encapsulated essential oils using an innovative technique in the production of redispersible dry powders containing nanocapsules are not yet described. It is important to note that for the success of the nanocapsule dry powder technique, a complete assessment of the recovery of its size after resuspension is important. In this work, the average particle size obtained by the dynamic light scattering technique (NanoBrook 90Plus Zeta®, Brookhaven Instruments Corportation, USA) and the resuspension index were calculated, where values close to 1 represent a good recovery from the original formulations.[25]

After redispersion of PNC-REO and PNC-ZEO, the average particle size was 248 ± 08 nm (*Sf* / *Si* = 1.0) e 286 ± 03 nm (*Sf* / *Si* = 1.2), respectively. Other studies using spray dried nanocapsule suspensions had similar *Sf* / *Si* values to the present study.[25,28,29] The recoverability of the mean particle size within the nanometer range after redispersion in water for PNC-REO and PNC-ZEO was evident.

### *Derivative spectrophotometry*

Quantification of the essential oils in nanocapsule powder is necessary due to losses that may occur during the process. Ultracentrifugation, a method widely used for this purpose, is an indirect method, which allows the quantification of unencapsulated EO in the NC suspension. However, it is not a method applicable to a solid formulation, due to the fact that it considers the theoretical value of added EO, which may lead to erroneous values, considering the expected losses, due to interactions with the polymer and system stability, together of the influence of partition between aqueous and organic phases in the process of obtaining nanocapsules.[15]

Extraction and quantification of the real OE content is necessary, and studies that quantify the real OE content by spectrophotometry do not consider the possible interferences of nanocapsule components, since their studies do not cite the validation of the method used.[22] On this premise, the present proposal to use derivative spectrophotometry, to determine the real OE value in nanocapsule powder, arises from the observation of the interference that the matrix exerted on the absorbance spectra in OE solutions (Figure 1), which makes it impossible direct application of spectrophotometry. Thus, in order to circumvent

the matrix interference for the quantification of the EO, the first and second order derivative spectra were obtained (Figure 2).

*Selection of Δλ and Scale Factor*

During the optimization of the experimental conditions for derivative spectrophotometry, the effect of Δλ and scale factor (SF) were examined to find the best derived spectra. The most appropriate value of Δλ was 5 for the first derivative ($^1$D) and 10 for the second derivative ($^2$D) methods, allowing smooth spectra and favoring signal clarity, being important for such measurements.[30] The scale factor values varied according to the derivative order of 10 and 100 for the first order ($^1$D) and second ($^2$D) spectrophotometric methods, respectively.

*Selecting appropriate wavelengths*

First and second order derived spectra of OE and PNC are shown in Figure 2. As noted, the zero crossing points of the PNC where the essential oils were not interfered with were (270 nm) and (240 and 261.5 nm) for ZEO and (230.3 and 370.8 nm) and (245 and 267 nm) for REO, for first and second order, respectively. The selection of optimal wavelengths was based on recovery tests, where EOs were added to the PNC. The mean recoveries and corresponding relative standard deviations of each EO are expressed in Table I. Thus, the wavelengths of 240 nm and 245 nm at the $^2$D level were chosen as working wavelengths for ZEO and REO, respectively.

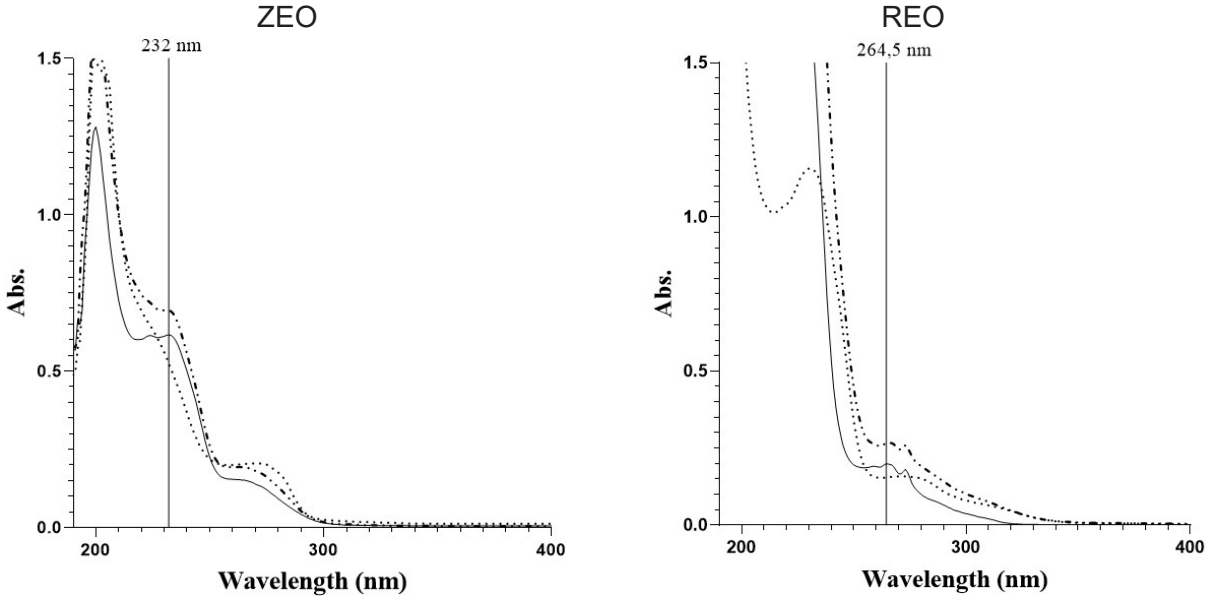


**Figure 1.** Comparison of zero-order absorption spectra for the following solutions: ZEO (0.025 mg mL$^{-1}$) and REO (0.78 mg mL$^{-1}$) (—), PNC (·····), and PNC + EO (– ·).

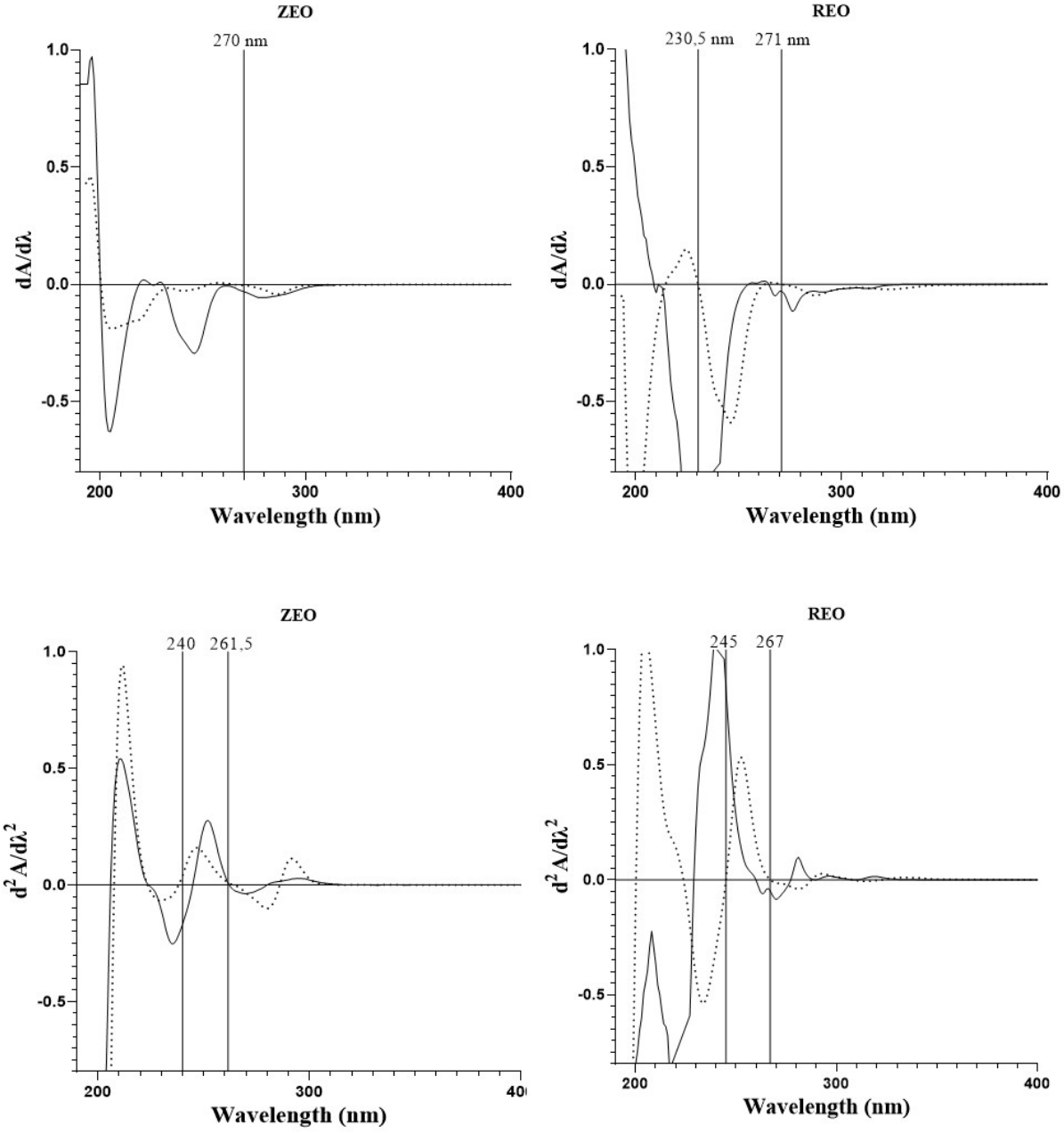


**Figure 2.** First (dA/dλ) and second ($d^2A/d\lambda^2$) order spectrum for: ZEO (0.025 mg $mL^{-1}$) and REO (0.78 mg $mL^{-1}$) (——) and PNC (·····).

**Table I.** Statistical parameters of calibration graphs for ZEO and REO by derivative spectrophotometry

| Parameters | ZEO | | | REO | | | |
|---|---|---|---|---|---|---|---|
| | First Derivative | Second Derivative | | First Derivative | | Second Derivative | |
| | $^1D_{270nm}$ | $^2D_{240nm}$ | $^2D_{261.5nm}$ | $^1D_{230.5nm}$ | $^1D_{271nm}$ | $^2D_{245nm}$ | $^2D_{267nm}$ |
| Linearity Range (mg $mL^{-1}$) | 0.01 - 0.04 | 0.01 - 0.04 | 0.01 - 0.04 | 0.6 - 0.96 | 0.6 - 0.96 | 0.6 - 0.96 | 0.6 - 0.96 |
| Coefficient of determination ($r^2$)[a] | 0.9955 | 0.9981 | 0.9924 | 0.9759 | 0.9975 | 0.9973 | 0.996 |
| Intercept | -1.2071 | -6.775 | 0.3304 | -1.1326 | -0.036 | 0.9223 | -0.0574 |
| Inclination | -0.0003 | -0.0013 | 0.0013 | -0.6248 | -0.0016 | 0.1068 | -0.0032 |
| Average Recovery (%) | 90.68 | 89.1 | 11.0 | 57.3 | 65.5 | 92.1 | 66.5 |
| DPR (%) | 8.43 | 1.91 | 107.12 | 8.62 | 9.41 | 1.80 | 6.71 |

[a]Equation of the line: y = ax + b, where “y” is the amplitude of the peak, “a” is the slope, “b” is the intercept, and ”x” is the concentration.

### *Validation of the analytical method*

*Selectivity*

Selectivity is the ability to unambiguously evaluate the analyte in the presence of components that may be present where its inability to detect or differentiate them would lead to an increased trend in analysis.[31,32] In this principle, the zero-crossing method allowed the nulling of the matrix spectra of the formulation, which in turn presented null contractions in the tested PNC samples.

*Linearity*

The linearity of the method was evaluated by plotting calibration curves for both EOs. The Lambert-Beer law was obeyed in seven-point concentration ranges ranging from 0.01 to 0.04 mg $mL^{-1}$ for ZEO and 0.6 to 0.96 mg $mL^{-1}$ for REO in triplicate. This confirms that the response of the instrument is indeed proportional to the concentrations of the EOs.[23] Statistical analyzes of these calibration curves were performed using the least squares method, represented in Table I.

*Precision*

Table II shows the results for intermediate precision (inter-day) and repeatability (intra-day), expressed as relative standard deviation (RSD). Repeatability was assessed by analyzing six different samples on the same day and under the same experimental conditions. Intermediate accuracy was assessed by a total of twelve analyzes for three consecutive days. The RSD values for intermediate precision (inter-day) and repeatability (intra-day) were less than 2% for both ZEO and REO, demonstrating the high reproducibility of the results and accuracy of the proposed methods within the established limits.[23] The EO contents recovered in the formulations were 87% and 28% for PNC-ZEO and PNC-REO, respectively. Where it is believed that the variation in the amount of encapsulated oil is justified by factors such as: the partition between the aqueous and organic phases during system formation; interactions between nanocapsule components and the protection provided by the drying aid.[15,28]

**Table II.** Intermediate precision and repeatability for the analytical validation of the ZEO and REO essential oils determination method

| | **ZEO** | | | | **REO** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Day 1 (n=6)** | **Day 2 (n=3)** | **Day 3 (n=3)** | **Inter-day[a]** | **Day 1 (n=6)** | **Day 2 (n=3)** | **Day 3 (n=3)** | **Inter-day[a]** |
| Intra-day[a] | 1.83 | 1.59 | 1.23 | 1.82 | 1.96 | 0.48 | 1.65 | 1.44 |

[a]RSD: Relative standard deviation (%).

*Accuracy*

The accuracy of the method was determined by investigating the percentage of recoveries at three concentration levels. Recovery and accuracy values were calculated using the data presented in Table III. The recovery rate values ranged from 87% to 93% for ZEO and 90 to 103% for REO, with low standard deviations, indicating high accuracy of the proposed analytical method. Acceptable recovery rates depend on the purpose of the analysis, where acceptable ranges are usually between 80% to 120%, depending on sample preparation and the proposed analytical procedure.[32,33]

**Table III.** Accuracy study for the analytical validation of the ZEO and REO essential oils determination method

| Theoretical concentration of ZEO (mg $mL^{-1}$) | Concentration found (mg $mL^{-1}$) | Variation of concentration found (mg $mL^{-1}$)* | Average Recovery (%) | Theoretical concentration of REO (mg $mL^{-1}$) | Concentration found (mg $mL^{-1}$) | Variation of concentration found (mg $mL^{-1}$)* | Average Recovery (%) |
|---|---|---|---|---|---|---|---|
| 0.040 | 0.036 | 0.036 ± 1.38% | 92.14 | 0.96 | 0.927 | 0.92 ± 0.51% | 96.25 |
| | 0.036 | | | | 0.918 | | |
| | 0.037 | | | | 0.926 | | |
| 0.025 | 0.021 | 0.022 ± 1.91% | 89.17 | 0.78 | 0.710 | 0.718 ± 1.80% | 92.09 |
| | 0.022 | | | | 0.712 | | |
| | 0.022 | | | | 0.733 | | |
| 0.010 | 0.009 | 0.009 ± 0.92% | 92.05 | 0.60 | 0.621 | 0.616 ± 0.73% | 102.68 |
| | 0.009 | | | | 0.610 | | |
| | 0.009 | | | | 0.616 | | |

*Mean ± RSD

## CONCLUSIONS

The method used, based on derivative spectrophotometry, allowed the determination of the essential oils of ginger and rosemary in the formulation of PNC in a simple and rapid way. The results showed that the method is selective for each EO used, in addition to being linear, precise and accurate, considering the international standards. Therefore, the proposed method is suitable and can be conveniently used for the quantification of their OEs in dry nanocapsule powder. In addition, the findings may enable further studies using OE in nanometric systems to quantify the actual content, allowing the cancellation of the inherent components present in the formulation, using a fast and simple method with reliable results.

### Conflicts of interest

The authors declare that they have no conflict(s) of interest.

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