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ORIGINAL ARTICLE

Headspace–Solid-Phase Microextraction Gas Chromatography Method to Quantify *Thymus vulgaris* Essential Oil in Polymeric Nanoparticles

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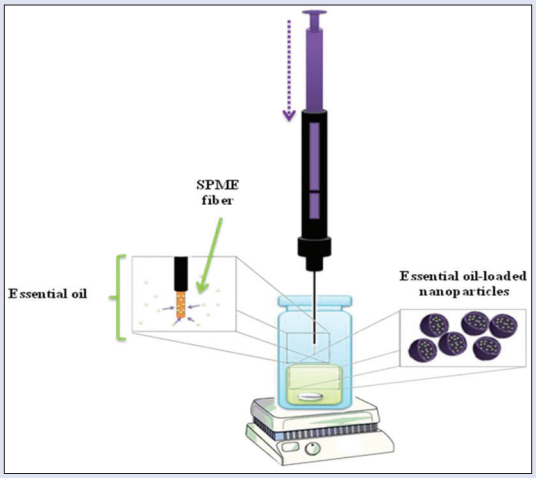
ABSTRACT

Background: *Thymus vulgaris* essential oil (*Tv*-EO) is known to have antibacterial, antifungal, and antioxidant activities. Encapsulation of *Tv*-EO in polymeric nanoparticles (NPs) can prevent volatilization of its components and can provide protection against external agents. Under these circumstances, it is crucial to assure the presence and quantity of the *Tv*-EO components (γ-terpinene, thymol, and carvacrol) in the NPs. **Objective:** To determine the chemical composition and physicochemical characterization of *Tv-EO* as well as develop and validate a HSPM-gas chromatography (GC) method for the analysis of *Tv*-EO components encapsulated in NPs. **Materials and Methods:** *Tv*-EO was characterized by physicochemical analysis for relative density, refractive index, and optical rotation and analyzed by GC flame ionization detector and GC-mass spectrometry. The headspace–solid-phase microextraction-gas chromatography (HS-SPME-CG) validation was assessed, *Tv-EO*-NPs were prepared by nanoprecipitation, and its properties were determined by photon correlation spectroscopy. **Results:** *Tv*-EO was characterized by physicochemical analysis for relative density (0.934 g/cm^3), refraction index (1.559), and optical rotation (−0.084°). Seventeen components were identified in *Tv-EO*; among these, the sesquiterpenes, thymol (34.28%), o-cymene (31.78%) and γ-terpinene (13.22%). The method was validated for linearity ($R^2 \geq 0.99$), precision (intraday 7.02, 10.33, and 8.60 and inter-day 10.60, 10.60, and 10.99), accuracy (99.35, 109.4, and 98.84%) and robustness for γ-terpinene, thymol and carvacrol, respectively. The limit of detection and limit of quantification were calculated as 0.69, 0.40, and 0.39 μg/mL and 2.11, 1.22, and 1.20 μg/mL for γ-terpinene, thymol, and carvacrol, respectively. An encapsulation percentage of 47.51% of total essential oil was obtained. **Conclusion:** The experimental data show that HS-SPME reduces interference of the NP-matrix and concentrates the *Tv*-EO components. HS-SPME-CG can be considered as a good alternative to the already existing methods for analysis of essential oil encapsulated in NPs.

Key words: Essential oil, headspace analysis, polymeric nanoparticles, solid-phase microextraction, *Thymus vulgaris*

SUMMARY

- The essential oil from leaves of *Thymus vulgaris* was extracted by hydrodistillation and characterized
- The headspace–solid-phase microextraction-gas chromatography (HS-SPME-CG) method was validated for linearity; intraday and interday precision; accuracy; robustness for γ-terpinene, thymol, and carvacrol; and the limits of detection and limits of quantification were calculated
- HS-SPME-CG can be considered as a good alternative to the already existing methods for the analysis of essential oil encapsulated in nanoparticles.



Abbreviations used: *Tv*-EO*: Thymus vulgaris* essential oil; NPs: Nanoparticles; HS-SPME: Headspace–solid-phase microextraction; GC: Gas chromatography; PI: Polydispersity index; ZP: Zeta potential; LOD: Limit of detection; LOQ: Limit of quantification; HPLC: High-performance liquid chromatography; *Tv*-EO-NP*: Thymus vulgaris* essential oil-loaded nanoparticles; NIST: National Institute of Standards and Technology; %E: encapsulation percentage; %EE: Encapsulation efficiency percentage.

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INTRODUCTION

Essential oils are secondary metabolites in plants that form part of the plant defense system against predators and infections. Such oils are composed of mixtures of volatile components such as monoterpenes, sesquiterpenes, and phenylpropanes. *Thymus vulgaris* essential oil *(Tv*-EO*)* is known to have antibacterial, antifungal, and antioxidant activities.[1-3] However, although this essential oil has good activities,

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its application is complicated by its unstable nature (highly volatile compounds and degradation and oxidation in the presence of light and air oxygen).[4,5] Encapsulation of the essential oil in nanoparticles (NPs) has been used to protect its components; this can increase the shelf-life of the material and can improve the bioavailability and bioactivity of the active components. Under such circumstances, it is crucial to assure the presence and quantity of the oil in the NPs.

A number of methodologies have been used to quantify essential oils in particles, including ultraviolet-visible spectrophotometric[6] and gravimetric methods.[7] However, in spite of their successful application as quantification methods, these approaches are not specific. This is because the analytical signals used in these methods are really the sum of each analytical signal of the NP components (i.e., the essential oil components, polymer, and surfactant). For this reason, separation techniques such as chromatography can be used as a specific and selective tool to analyze essential oils.

In this context, gas chromatography (GC) is more suited to the analysis of essential oils than other techniques because of the volatile nature and complex composition of the material. Unlike high-performance liquid chromatography, the use of buffers and large quantities of organic solvents can be avoided. GC can be used to separate each component of an essential oil simply by modifying the carrier flow, oven temperature, and rate of temperature change. Furthermore, to improve the performance of GC analysis, the technique can be combined with headspace–solid-phase microextraction (HS-SPME) to deliver a suitable and specific method to analyze and quantify components of the essential oils in complex matrices such as NPs. A major advantage of HS-SPME-GC is that it can avoid matrix effects and contaminants by non-volatile components. Furthermore, because this methodology involves a preconcentration step, very small amounts of sample can be quantified.

Previously, the use of HS-SPME-GC has been reported for the analysis of essential oils and/or their components. Bicchi *et al.*[8] directly evaluated the composition of essential oils of two plants: *Matricaria chamomilla* L. and *Salvia lavandulifolia*, thereby demonstrating the usefulness of HS-SPME-GC for quality control of medicinal plants. Similarly, Adams *et al.* determined the profiles of canola oil enriched with basil, oregano, and thyme, showing that HS-SPME-GC is an efficient and sensitive method for the extraction of volatile components from plants.[9] For this reason, this method could be applied in the quantification of components of thyme encapsulated in polymeric NPs.

HS-SPME-GC has also been used to analyze more complex matrices; for example, Kohlert *et al.* analyzed thymol in human plasma a certain time after intake of a *Tv* preparation, showing that HS-SPME-GC is an effective method for studying the bioavailability of herbal products.[10] On the other hand, Baranauskiene *et al.* used HS-SPME-GC to determine the components of the essential oils of cassia, thyme, and oregano, encapsulated in microparticles.[11] They tested four different fibers, demonstrating that these volatiles can be easily extracted, and that this method is a sensitive and reproducible technique to analyze essential oils in complex matrices. Furthermore, Cavazos-Rodriguez developed a method of HS-SPME-GC for quantification of encapsulated carvacrol in NPs for dermatological application.[12] Their study established that HS-SPME-GC is effective for the quantification of carvacrol (a component of *Tv*-EO) from a vesicular NP system.[13]

Therefore, the aim of this study was to determine the chemical composition and physico-chemical characterization of *Tv*-EO. In addition, since *Tv*-EO has received attention for its antioxidant and antibacterial activities, the development and validation of a HS-SPME-GC method would allow the quantitative analysis of *Tv*-EO encapsulated in NPs for future topical applications.

MATERIALS AND METHODS

Physico-chemical analysis of *Thymus vulgaris* essential oil

The fresh aerial parts of *Tv* were purchased from a local market, the "Mercado Juárez" in Monterrey, Nuevo León. The plant was originally collected from Matehuala, San Luis Potosí, México. The specimen was identified with the number UAN010970. *Tv*-EO was obtained by hydrodistillation for 4 h in a modified Clevenger apparatus. The oil obtained was sealed, and protected from light, and stored at 4°C.

Relative density

Relative density was determined by calculating the ratio between the mass and the volume of the sample at 20°C, used in a previously calibrated 1 mL pycnometer with distilled water at 20°C. The analysis was performed in triplicate.

Refractive index

Refractive index was determined by using a Reichert refractometer (AO-10406, California, USA). *Tv*-EO was analyzed at 25°C. The analysis was performed six times.

Optical rotation

Optical rotation was determined by using a Perkin Elmer Polarimeter (341, Shelton, CT, USA). *Tv*-EO was analyzed at 25°C. The analysis was performed six times.

Qualitative analysis of *Tv*-EO was performed by GC coupled with mass spectrometry (GC-MS) to determine the composition of the oil. A GC-MS (Agilent Technologies, 6890N EM: 5973 INERT, Santa Clara, CA, USA) equipped with HP-5MS (5% phenylmethylpolysiloxane, 30 m × 0.25 mm, 0.25 µm, Agilent J and W) capillary column was used. A linear oven temperature program was used (35°C–270°C at 3°C/min) without split; the carrier gas was helium with a flow rate of 1.0 mL/min, and injector and detector temperatures were fixed at 220°C and 250°C, respectively.

To determine the proportion of each of the components, a quantitative analysis was performed with a GC coupled to a flame ionization detector (Autosystem XL from Perkin Elmer) using the same HP-5MS column. The injector temperature was 270°C, the oven temperature program was 50°C–117°C at 6°C/min with a 3-min hold, then 119°C at 0.5°C/min, and finally 280°C at 45°C/min with a 1-min hold. The detector temperature was 250°C. The carrier gas was helium at 1.0 mL/min and injected in split mode (1:20). This temperature program was used during the validation of the method. Thymol, carvacrol, and γ-terpinene were selected as test compounds due to their biological activities and retention times.

Development and validation of the headspace–solid-phase microextraction-gas chromatography method to quantify essential oil in nanoparticles

The following four fused silica fibers (Supelco, Sigma-Aldrich Corp., St. Louis, MO, USA) with different coating and polarity were chosen for evaluation based on the extraction precision and efficiency of the volatile essential oil compounds: polydimethylsiloxane (PDMS), carboxen/polydimethylsiloxane (CAR/PDMS), polyacrylate (PA), and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fibers were conditioned according to the manufacturer's instructions prior to use and were tested in headspace mode. *Tv*-EO (10 µg/mL) was analyzed in a 7-mL vial with a headspace of 3.5 mL at 55°C. After the equilibration time, the fiber was introduced

into the vial and exposed to the headspace of the sample during the extraction time. A Plackett–Burman experiment design was used to evaluate the extraction parameters. The variables were extraction and equilibration times, agitation, relationship HS/sample, polymer concentration, extraction temperature, and fiber distance from the sample.

Validation was assessed according to regulations set by the Mexican Ministry of Health.[14] Standard calibration samples were prepared with the required proportion of γ-terpinene, thymol, and carvacrol. Linearity was assessed by analyzing five concentrations (2–6 μg/mL) of the selected component mixture in triplicate. Regression analysis was performed according to the lineal equation ($y = mx + b$), where m is the slope, b is the intercept with the Y axis, x is the concentration, and y is the area of the analyte peak. Detection and quantification limits were determined using the values obtained from the regression analysis: the slope (m) and intercept (b). With these data, the random errors were estimated by calculating the standard deviation of the line (Sy/x) and the standard deviation of the intercept (Sa). The limit of detection (LOD) and the limit of quantification (LOQ) were estimated by using Equations 1 and 2, respectively:

$$\mathrm{LOD} = \frac{3.3 \times Sa}{m} \quad (1)$$

$$\mathrm{LOQ} = \frac{10 \times Sa}{m} \quad (2)$$

With the standard solution prepared at low, medium, and high concentrations (2, 4, and 6 μg/ml) in sextuplicate, two levels of precision were evaluated. Intraday precision was evaluated within 1 day, and interday precision was evaluated over 3 nonconsecutive days. The percentages of relative standard deviation (%RSD) were calculated. To determine the accuracy of the method, spiked samples at five concentrations were prepared (2–6 μg/mL) in triplicate. This parameter was evaluated by calculating the recovery percentage (%R). Robustness was determined by following a Plackett–Burman experimental design with small variations of seven parameters at high and low levels: temperature of HS-SPME (A = 71°C, a = 69°C), polymer concentration (B = 12 mg/mL, b = 11 mg/mL), surface-covered HS (C = 100%, c = 75%), sample volume in 7-mL vial (D = 3.005 mL, d = 2.995 mL), fiber distance from the sample (E = 7 mm, e = 5 mm), extraction time (F = 10.2 min, f = 9.4 min), and equilibrium time (G = 10.2 min, g = 9.4 min). Each experiment was performed in duplicate at a concentration of 4 μg/mL.

Preparation and characterization of nanoparticles loaded with the essential oil of *Thymus vulgaris*

The *Tv*-EO loaded NPs (*Tv*-EO-NP) were obtained by nanoprecipitation.[15] An organic phase containing 5 mL of acetone, 15 mg of *Tv*-EO, and 15 mg of ε-polycaprolactone was incorporated into an aqueous phase containing Tween 80® (3% w/v) under constant stirring (200 rpm).[12] NPs without essential oil were also prepared for use as blank control samples in validation parameters. The size, polydispersity index (PI), and zeta potential (ZP) of the NPs were determined by photon correlation spectroscopy with a Zeta sizer Nano (Malvern Instruments, Malvern, UK).

Application of the headspace–solid-phase microextraction-gas chromatography method for analysis of nanoparticles

The *Tv*-EO-NPs were centrifuged at 25,000 rpm for 2 h, and then the pellet was resuspended with water and analyzed by HS-SPME-GC to quantify the components (γ-terpinene, thymol, and carvacrol). Subsequently, encapsulation percentage (%E) and encapsulation efficiency percentages (%EE) were calculated (Equations 3 and 4, respectively), where At is the total amount of essential oil used (grams), An is the amount of essential oil unencapsulated (grams), and P is the amount of polymer used (grams). These calculations were performed with data obtained by using the HS-SPME-GC method validated previously:

$$\%E = \left(\frac{At - An}{P + At}\right) \times 100 \quad (3)$$

$$\%EE = \left(\frac{At - An}{At}\right) \times 100 \quad (4)$$

Tv-EO was chosen as a model mixture for encapsulation into NPs. This oil has been demonstrated to have antibacterial and antifungal activities. These activities are mainly attributed to thymol and carvacrol,[16,17] which interact with the cell surface, leading to leakage of intracellular components of the microorganism.[18,19] *Tv*-EO was obtained by hydrodistillation with a modified Clevenger apparatus; the extraction efficiency was 0.52% (w/w). This percentage is consistent with the findings of de Morais *et al.*[20] who reported that these percentages range from tenths to about 1% (w/w). The full characterization (physicochemical), including chemical and thermal profile as well as the biological activity of the essential oils according to their toxicology or pharmacological properties, contributes to improving safe and effective therapeutic use of the species.[5] The physicochemical properties of *Tv*-EO are summarized in Table 1.

Through the use of GC-MS and GC-FID analyses, 17 components [Table 2] were identified according to Kovats index, arithmetic index, the National Institute of Standards and Technology library, and by comparison with standards and reference data.[21] A typical *Tv*-EO chromatogram is presented in Figure 1.

Table 1: Physicochemical analysis of *Thymus vulgaris* essential oil

Evaluation	Values
Relative density[a]	$0.934 \pm 9.98 \times 10^{-05}$ g/cm^3
Refractive index[a]	$1.559 \pm 4 \times 10^{-03}$
Optical rotation[b]	$-0.084 \pm 5.16 \times 10^{-03}$°

[a]$\bar{x} \pm s$; n=3; [b]$\bar{x} \pm s$; n=6

Table 2: Composition of *Thymus vulgaris* essential oil analyzed by gas chromatography flame ionization detector and gas chromatography-mass spectrometry

n	Component	Retention time (min)	Peak area (%)
1	α-tujene	13.40	0.99
2	α-pinene	15.04	1.07
3	Camphene	19.05	1.45
4	Sabinene	21.56	0.24
5	3-octenol	21.62	0.57
6	Myrcene	22.84	1.75
7	α-terpinene	23.43	1.32
8	*o*-cymene	23.70	31.78
9	γ-terpinene	24.59	13.22
10	Linalool	24.86	4.40
11	Camphor	30.15	1.67
12	Borneol	33.97	3.12
13	4-terpinenol	36.47	1.14
14	Carvacrol methyl ether	38.34	1.04
15	Thymol	38.72	34.28
16	Carvacrol	39.41	1.97
17	ε-caryophyllene	40.33	0.86
Total (%)			100

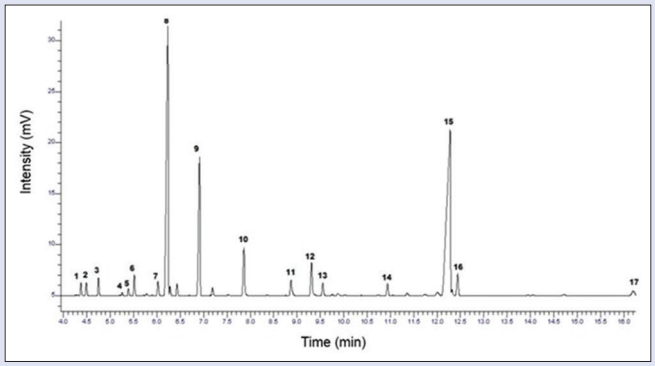

Figure 1: Chromatogram of *Thymus vulgaris* essential oil (20 mg/mL) obtained by gas chromatography with a flame ionization detector. The components are shown in the same order given in Table 2

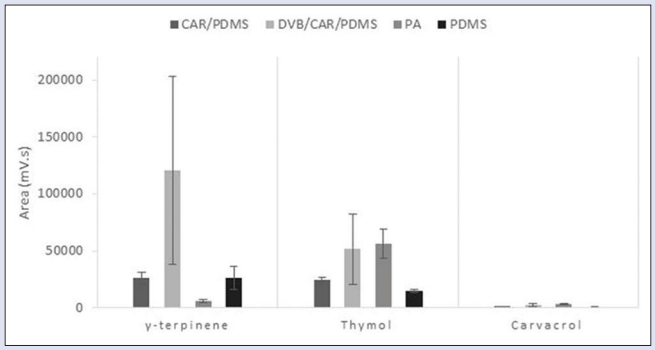

Figure 2: Comparison of the peak areas for the components (total concentration of 10 µg/mL) obtained with solid-phase microextraction fibers by gas chromatography with flame ionization detector ($\bar{x}\pm$ s; n = 3)

Subsequently, the percentages of the components were calculated based on their chromatographic areas [Table 2]. Thymol (34.28%), *o*-cymene (31.78%), and γ-terpinene (13.22%) were the main components. These results were consistent with those reported by Hajimehdipoor *et al.*[22] and Guerra-Boone *et al.* in Mexico,[23] who observed the same main components in *Tv*-EO. The compounds γ-terpinene, thymol, and carvacrol were chosen as analytes for quantification of the essential oil in the NPs because of their biological activities, retention times, and physicochemical properties.

To quantify the selected components in *Tv*-EO-NP, a HS-SPME-GC method was developed. First, the SPME fiber was selected according to the best efficiency of extraction of essential oil with the least variability. The results of extracting the selected compounds with the four SPME fibers are summarized in Figure 2. Each compound presented a different behavior with each fiber because the selectivity of the fiber depends primarily on the volatility, polarity, and molecular weight of each compound.[24]

DVB/CAR/PDMS fiber extracted γ-terpinene better than the other compounds. This compound is the most volatile and hydrophobic of the analyzed compounds (thymol and carvacrol). This fiber has a combination of material of differing polarities, which allows molecules to be adsorbed with different physicochemical characteristics. With PDMS fiber, extraction of thymol and particularly carvacrol was low because its affinity coating contains fewer hydrophobic compounds, such as phenolics. With CAR/PDMS fiber, carvacrol extraction was also low. Mixed fibers such as DVB/CAR/PDMS and CAR/PDMS, as shown in Figure 2, gave better extraction yields than simple fibers such as PDMS or PA because they are composed of two types of phases, liquid (PDMS), which extracts the nonpolar molecules, and solid (DVB, CAR), which extracts polar molecules. However, these fibers were not chosen because they showed high variability in their chromatographic responses. In contrast, PA fiber had a higher extraction yield for thymol and carvacrol than the others and exhibited low variability. These results are consistent with those reported by Bicchi *et al.*, who evaluated the composition of medicinal plants by HS-SPME-GC with eight different fibers.[25] In relation to γ-terpinene, PA fiber showed a low extraction yield due to its physicochemical properties. However, the PA fiber extracts mostly carvacrol and thymol; therefore, fiber coated with PA was selected for quantification of the components of *Tv*-EO.

With the results of the Plackett–Burman design experiments, the variables that influence the HS-SPME system most were established. For the extraction temperature, the greatest influence was observed at low levels. This behavior shows that a temperature of 55°C is not sufficient for the vapor-phase compounds to reach the equilibrium state within the times established, which directly affects mass transfer between the sample and PA fiber.[26]

Regarding the extraction time, the biggest influence was apparent at high levels. Cui *et al.* observed that the extraction time needed to reach the distribution equilibrium, depending on the compound, was 10–20 min for volatile compounds, whereas the equilibrium was not reached in 60 min for semi-volatile compounds.[27] Therefore, the variability increases with longer times. Together with the agitation rate, it was observed that this variable directly affects the mass transfer of the sample to the HS and from the HS to the fiber. Upon increasing the kinetic energy in the system, the transfer of essential oil components from the liquid phase to the gas phase is favored.[28] Related to the distance of the fiber to the sample, the greatest influence was shown at a low level (where the fiber is closest to the sample). Due to the polar nature of the fiber and the aqueous matrix of the sample, the adsorption of water vapor molecules on the fiber occurs readily; this, in turn, modifies the polarity of the fiber. Therefore, the adsorption of essential oil compounds could have higher variability when the fiber is closer to the sample.[24]

The equilibrium time and relationship of HS/sample both had less influence on extraction compared with the other variables. The equilibrium time probably does not have a significant influence because of the volatile nature of the compounds, which, at high temperature, could be quickly transferred into the gas phase; thus, greater variability is reflected in the extraction time. Furthermore, the relationship of HS/sample had only a minor impact.[25] Finally, the polymer concentration in the sample probably exerts a matrix effect, leading either to a release of essential oil components or to adsorption of the NPs. Adsorption could arise because of the nonpolar nature of the compounds, which become caught and accommodated in the linear polymer structure, forming a sphere. This can affect the mass transfer to the SPME fiber. Table 3 shows the optimal conditions for the HS-SPME method.

In accordance with regulations stipulated by the Mexican Ministry of Health,[14] all the parameters of validation were acceptable. The data obtained in the regression analysis [Table 4] show that the standards were plotted with an R^2 value of 0.99. This value indicates a linear behavior and verifies that the correlation between the concentration and the areas of the chromatographic peaks of tested compounds is reliable. The results were as expected, except for γ-terpinene, which had the greatest variability in terms of its calibration curve (R^2 = 0.98). This was possibly caused by the highly volatile nature of this compound, which was the most volatile among the compounds evaluated. The values obtained for LOD and LOQ were lower than the lowest level of linearity concentrations; γ-terpinene

had a LOQ higher than the lowest level in the calibration curve. It was intended that these values were less than the lowest concentration level evaluated to ensure accuracy and precision. Intraday and interday precision values of 15% (%RSD) were acceptable [Table 4], which indicates that our method was precise both on the same day and on non-consecutive days. Accuracy was assessed according to the percentages of recovery (%R) between 98% and 110%. Finally, the robustness of the method was determined with a Plackett–Burman design, with slight variations of the seven parameters evaluated. As shown in Table 5, which shows each of the variables investigated, no significant influence was observed. Thus, the method is robust, and small variations in the analysis did not affect the chromatographic response.

Preparation and characterization of nanoparticles loaded with essential oil of *Thymus vulgaris*

To optimize the production of NPs loaded with *Tv*-EO by the nanoprecipitation technique, the following two parameters were evaluated: the amount of essential oil and the amount of polymer. Our results indicate that the amount of essential oil present in the organic phase has a direct influence on the size of the NP, being inversely proportional to the amount of essential oil. This could possibly arise because increasing the amount of essential oil can lead to more particles but of a smaller size. Furthermore, NPs prepared with different amounts of polymer induced an increase in particle size. This behavior can be explained by considering the mechanism of formation of NP by nanoprecipitation: when the organic and aqueous phases are in contact, the solvent present in the organic phase diffuses to the aqueous phase and induces aggregation of the polymer chains, thus forming the NPs. Therefore, because there are a larger number of polymer chains per unit volume of solvent, the formation of larger NPs is favored.[29] It is noteworthy that none of the formulations presented free essential oil and/or polymer aggregates. The experimental conditions that allowed NPs to be obtained with sizes under 200 nm, with a yield of conversion of polymer to NP of 100% (i.e., without aggregates) and greater encapsulation of the components of the essential oil, were an organic phase composed of 5 mL of acetone, 15 mg of ε-polycaprolactone, 15 mg of *Tv*-EO, and an aqueous phase consisting of 20 mL of Tween 80® (3% w/v). *Tv*-EO-NP size was 180 ± 3 nm and the PI was 0.02 ± 0.142. In particular, the PI is acceptable, considering that this may vary in the range of 0–1, wherein a value closer to 0 indicates a more homogeneous particle size distribution.[29]

Table 3: Optimal extraction conditions for γ-terpinene, thymol, and carvacrol from the essential oil of *Thymus vulgaris* with headspace-solid-phase microextraction by gas chromatography with a flame ionization detector

Parameter	Optimal conditions
HS: Sample	1:1.33
Stirring speed	250 rpm
Distance fiber-sample	6 mm
Equilibrium time	10 min
Extraction temperature	70°C
Extraction time	10 min
Polymer concentration	11.25 µg/mL

HS: Headspace

Table 4: Validation parameters of the headspace-solid-phase microextraction by gas chromatography with flame ionization detector method

Validation parameter	γ-terpinene	Thymol	Carvacrol
R^2	0.98	0.99	0.99
Intercept	92.6	−3132.1	−195.5
Slope	1844.3	6318.4	321.6
LOD (µg/mL)	0.69	0.40	0.39
LOQ (µg/mL)	2.11	1.22	1.20
Accuracy (%R)	99.35	109.4	98.84
Intraday precision (%RSD)	7.02	10.33	8.6
Interday precision (%RSD)	10.6	10.6	10.99

%RSD: Percentages of relative standard deviation; LOQ: Limit of quantification; %R: Recovery percentage

Application of the headspace–solid-phase microextraction-gas chromatography method for nanoparticle analysis

To complete the analysis of NPs, %E and %EE were determined with the HS-SPME-GC method, using Equations 3 and 4, respectively. A %E of 47.51 for γ-terpinene, thymol, and carvacrol content in total essential oil was obtained, indicating that at least 47.51% of the *Tv*-EO added during the preparation of the NPs was encapsulated.

Specifically, the %EE of γ-terpinene, thymol, and carvacrol in the NPs was 25, 68, and 3, respectively (96% of total essential oil). In the case of thymol, these results are consistent with a recent study in which an %EE of 77 for thymol in polymeric nanospheres ethyl cellulose/methylcellulose was reported.[30]

It is well known that *Tv*-EO has important antioxidant and antibacterial activities. Such activities are attributed mainly to the presence of thymol. Thus, the development and validation of a HS-SPME-GC method will allow the quantitative analysis of *Tv*-EO components encapsulated in NPs for future topical applications.

CONCLUSION

A precise, sensitive, lineal, and robust HS-SPME-GC method was developed and validated for the quantitation of *Tv*-EO in NPs. Notably, the method avoids interference by complex matrices. In addition, according to the biodegradable nature and the %E of the essential oil in the NPs, such particles could have great potential as antifungal, antibacterial, or antioxidant agents in the treatment of pathogens that cause skin infections.

Table 5: Values obtained for the robustness of the headspace-solid-phase microextraction by gas chromatography with flame ionization detector methoda

Robustness parameter	γ-terpinene	Thymol	Carvacrol	Statistics
HS-SPME temperature	+	+	+	Differences between high and low levels[b]
Polymer concentration	-	-	+	R<(2) 1/2 SD[c]=(-)
HS surface covered	+	+	+	R>(2) 1/2 SD[c]=(+)
Sample volume in a 7-mL vial	-	-	+	
Distance fiber-sample	+	+	+	
Extraction time	-	-	+	
Equilibrium time	+	+	+	

[a]The + and - marks indicate a significant influence or no significant influence, respectively; [b]Mexican Ministry of Health[15]; [c]SD of the interday precision. SD: Standard deviation; HS-SPME: Headspace-solid-phase microextraction; HS: Headspace

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Conflicts of interest

There are no conflicts of interest.

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