

Optimization of the Loading Process of *Artemisia absinthium* Volatile Oil in a Mesoporous Material as Drug Delivery System

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Abstract: The study aimed to use a mesoporous material as a drug delivery system, loaded in different ways with volatile oil extracted from *Artemisia absinthium*, to evaluate the most effective loading method. A specific design of the mesoporous material was obtained through template/silica source ratio optimization in the synthesis process. Further bioactive compounds from the volatile oil were loaded in the mesopores. The obtained composite material has the potential to be used as low release drug carrier with anthelmintic activity. FT-IR analysis was used to prove the template removal due to the post-synthesis washing steps and the loading efficiency. The final loaded materials were analyzed qualitatively (percentage from the profile of some identified components, with about 90% recovery) and quantitatively (eucalyptol, linalool, β -myrcene, o-cymene, and α , β thujone). Eight different loading methods were compared in this study, and a high correlation factor ($R^2 > 0.73$) was obtained through ANOVA statistical data processing related to the 2-5 °C experiment.

Keywords: mesoporous materials; *Artemisia absinthium* volatile oil; loading process; GC-MS analysis; drug delivery.

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1. Introduction

Due to their specific morphology and characteristics, mesoporous materials are intensively studied as carriers for many types of substances, including volatile oils. These materials have the capacity to entrap, based on their porosity and surface charge, and to encapsulate volatile components, ensuring protection against volatilization and improvement of their efficacy [1-3]. Volatile oils have many therapeutical properties, being used as antibacterial, antiviral agents, antioxidants, as skin penetration enhancers for transdermal drug delivery, even in cancer therapy [4-6]. *Artemisia absinthium* (wormwood) is an important therapeutical plant with significant pharmacological activities, such as antiviral, antimicrobial, hepatoprotective, and wound healing anti-inflammatory, antioxidant, antifungal, anthelmintic, etc. [7-9]. Special attention is given to the anthelmintic activity due to the hepatic toxicity of the pharmaceutical active substances used commonly in treating worm infections [10]. There

have been made numerous tests to prove the effectiveness of wormwood extracts, including the essential oil. Used on mice infected with *Trichinella spiralis* larvae, after 7 days of oral administration, the essential oil at 1 mg/mL showed larvicidal effectiveness up to 99,99 % [11]. Oral administration of essential oils can be challenging because of the possible changes through the gastrointestinal tract related to effective concentration drop or chemical modifications (small intestine and colon) [12]. In this regard, mesoporous materials are feasible options related to their low release capabilities and protective effect, combined with volatile oils' efficacy, including volatile wormwood oil [13].

One of the greatest challenges in this study was related to the mesoporous material's loading process with a proper amount of volatile wormwood oil, considering that most components are slightly volatile and require mild conditions in the loading process. The most common loading techniques found in the literature are represented by two main categories: 1) loading the bioactive compounds using methods without organic solvents like physical mixing, co-milling, melt method, microwave irradiation; 2) by using organic solvents to encapsulate the bioactive compounds through phenomena like adsorption, incipient wetness impregnation, solvent evaporation, supercritical and liquid CO₂ technology, one-pot drug loading and synthesis and chaperone assistance. Some loading techniques are time and money-consuming, while other loading methods' efficiency is significantly affected by the loading capacity and physicochemical properties of the support or by the specific target/support interactions in the loading process. From this perspective, co-milling, solvent evaporation, vapor adsorption, and nitrogen flux adsorption methods can be considered potential techniques for volatile oil loading [14-16].

In the present study, after the mesoporous support synthesis and the essential oil extraction process (neo-Clevenger, hydrodistillation), 8 different loading strategies were compared to optimize the essential oil loading process in the mesoporous matrix. After a qualitative and quantitative approach, an ANOVA statistical evaluation of the obtained data was performed to highlight the best loading method.

2. Materials and Methods

2.1. Reagents and chemicals.

High purity reagents were used: Ethanol absolute, puriss p.a., Acetic acid puriss (99,8-100,5%) and Ammonium hydroxide solution puriss p.a. (~ 25% NH₃ basis) were purchased from Honeywell, Sodium hydroxide pellets from Bernd Kraft, Sodium trisilicate ($\geq 18\%$ Na basis and $\geq 60\%$ Si basis) from Sigma-Aldrich, Linalool from Dr. Ehrenstorfer; p-Cymene from Chromadex; isomers $\alpha+\beta$ Thujone, Myrcene, and Eucalyptol from Sigma-Aldrich, each of them with the certified value of purity, Ethyl acetate from Carlo Erba, Stearic acid from Sigma-Aldrich. During the entire study, water was obtained from the Milli-Q equipment.

2.2. Extraction of volatile oil from *Artemisia absinthium*.

A neo-Clevenger-type hydrodistillation device was used to extract the volatile oil from wormwood (a dried herb with flowers). 300 g of vegetable material was introduced into 2000 mL of water and processed for about 3 hours. The final product was dried and analyzed by GC-MS.

2.3. Synthesis of mesoporous material.

For the mesoporous material synthesis, sodium trisilicate was used as a silica source and stearic acid as a template, in a mildly acidic medium, at room temperature. The silica solution was homogenized under stirring with the template ethanolic solution and precipitated with an acidic solution of acetic acid in the presence of ammonium hydroxide as catalyst. To eliminate the organic components and other residues from the synthesized material, several washing steps with ultrapure water (at 70°C) were performed. The final drying process was performed at 50°C, under vacuum (50 mbar), for 12 hours.

2.4. Loading techniques of the mesoporous materials with *Artemisia absinthium* volatile oil.

To achieve a higher yield of loading for *Artemisia absinthium* volatile oil in the obtained mesoporous material, eight types of loading techniques were approached. The first one was conducted by a gradient concentration method using a rotary evaporator at 30-35 °C, under a high vacuum. The second one consisted of a loading process based on vapor adsorption by mixing the volatile wormwood oil with mesoporous material, in a tightly closed vial, under stirring, at 50°C, for 24 hours. The loaded material was obtained by centrifugation and drying in nitrogen flow. The next two methods used nitrogen flow, at low temperature (2-5°C), one under vacuum and another without vacuum. The same method, in nitrogen flow, at low temperature (2-5°C), without vacuum, was applied using methanol instead of ethanol because it has a lower boiling point, thus the evaporation process of the solvent being faster. The last method was manual homogenization with an agate mortar at low temperature (2-5°C). The mesoporous material was mixed with volatile wormwood oil for 5 minutes. For the process control and to estimate the loading capacity and the molecule competition for the particle pores, the material was loaded with two solutions of α , β -thujone, at different concentrations, one with the concentration level quantified from the volatile oil and the second one with a much higher concentration. The loading process chosen for α , β - thujone standard was under nitrogen flow, at low temperature (2-5°C), without vacuum. All the loaded materials were milled and stored at low temperature (2-8°C) before the GC-MS analysis. The parameters of the eight loading methods are summarized in Table 1.

Table 1. The parameters for loading techniques applied for volatile wormwood oil.

Conditions	Solution	Bath temperature (°C)	Vacuum (Torr)	Flow N ₂ (PSI)	Magnetic stirrer / rotation stirrer (rpm)
Method 1	EtOH	35	50	-	200
Method 2	EtOH	50	-	15	1400
Method 3	EtOH	2-5	-	15	200
Method 4	EtOH	2-5	500	15	200
Method 5	wormwood oil	2-5	-	-	manual milling
Method 6	α , β - thujone standard 1	2-5	-	15	200
Method 7	α , β - thujone standard 2	2-5	-	15	200
Method 8	MeOH	2-5	-	15	200

2.5. B.E.T. and B.J.H. characterization of mesoporous material.

Nitrogen adsorption / desorption isotherms were recorded at 77,35 K at relative pressure range $p / p_0 = 0,005-1,0$, using a Quantachrome NOVA 2200e Gas Sorption Analyzer. Data

processing was performed using NovaWin software version 11.03. Before the adsorption measurements, the samples were degassed at 50°C, in a vacuum, for 5 hours. Using the B.E.T. (Brunauer-Emmett-Teller) theory, the specific surface area, including surface irregularities and pore walls, of particles is determined at the atomic level by adsorption of an inert gas (nitrogen). The total pore volume was estimated from the adsorbed volume at a relative pressure p / p_0 close to unity. The pore size distribution and the mesopore volume were obtained from the isotherm desorption branch. Estimations of total pore volume and pore size distribution were made by applying the Barrett-Joyner-Halenda (B.J.H.) model.

2.6. FT-IR characterization of the mesoporous material and loaded materials.

FT-IR spectra were performed with a Perkin Elmer spectrometer. Using the diamond crystal attenuated total reflective (ATR) mode, 32 spectra were acquired for background and 64 spectra for each sample, at a resolution of 4 cm^{-1} , on the spectral range 550 - 4000 cm^{-1} .

2.7. Gas chromatography (GC-MS) analysis of loaded mesoporous materials.

2.7.1. Preparation of standard solutions for volatile oil quantification.

Approximately 10 mg of each individual compound were weighed in 10 mL graduated flasks and solubilized in ethyl-acetate. The obtained standard solutions were stirred and ultrasonicated for 1 minute. The stock calibration solution was prepared by mixing the accurately measured volumes of each solution and the calibration solutions by diluting the mixed solution in exact proportions with ethyl acetate.

2.7.2. Chromatography analysis of volatile oil.

Chromatographic profile of volatile oil and the quantification of several compounds of interest (linalool, cymene, myrcene, eucalyptol (cineol), α , β thujone) was obtained by using a gas chromatograph Thermo Scientific TRACE 1310 coupled with a triple quadrupole mass spectrometer (TSQ 8000 EVO). The analysis parameters were: column TG-5SilMS, 30 m, $\emptyset = 0.25$ mm, film = 0.25 μm , 5% phenyl methyl polysiloxane, flow rate 1.2 mL/min, inlet temperature 280°C in split mode 50:1, oven program starts from 90°C for 5 min, then with 10°C / min \rightarrow 280°C, transfer line temperature 270°C, source temperature 230°C, the mass spectrometer was set to scan between 40-500 amu.

2.8. Statistical analysis of the loading techniques' efficacy.

The statistical analysis was performed with IBM SPSS Build 1.0.0.1447, 64-bit edition. Experimental data from six runs (N/run= 47, N total = 282) were analyzed using one-way ANOVA. In order to determine which specific group differed from each other, we applied *post hoc* tests (Tukey Test). Tukey Test uses the "Honest Significant Difference," a number representing the distance between groups, to compare every mean with every other mean.

3. Results and Discussion

3.1. B.E.T. and B.J.H. characterization of mesoporous material.

From the B.E.T. results, it was highlighted that the synthesized material has a specific surface area of 536,9 m^2 / g , and from the B.J.H. model, a total pore volume of 0,318 cm^3 / g

and a pore diameter of 2,37 nm were obtained. The obtained adsorption-desorption isotherm is a type IV, specific for mesoporous materials (Figure 1).

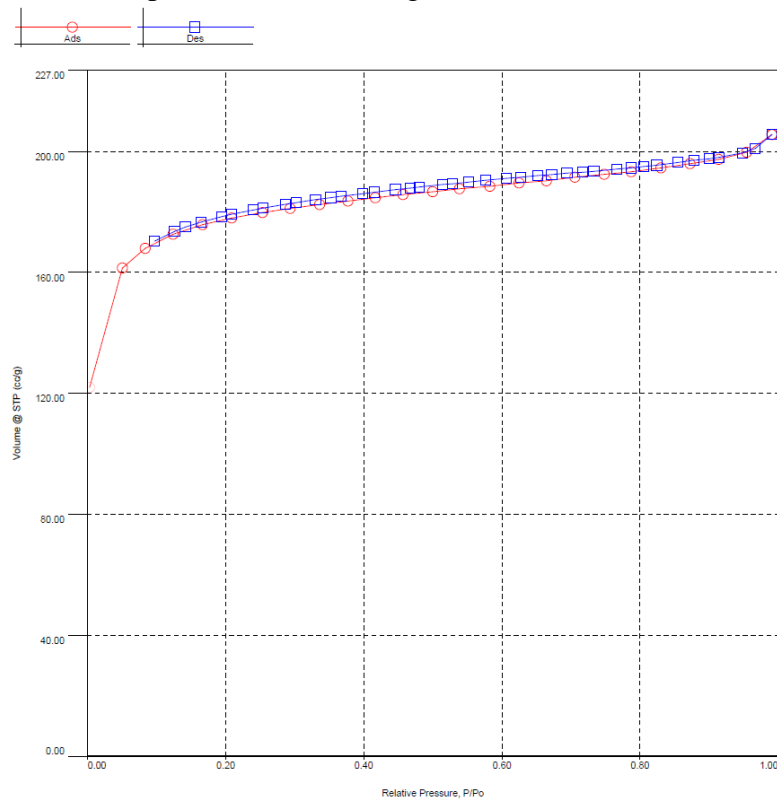


Figure 1. The adsorption-desorption isotherm of mesoporous material.

3.2. FT-IR characterization of the mesoporous material and loaded materials.

FT-IR analysis was performed for those mesoporous materials to confirm the template agent's extraction with ultrapure water and the successful loading with volatile wormwood oil.

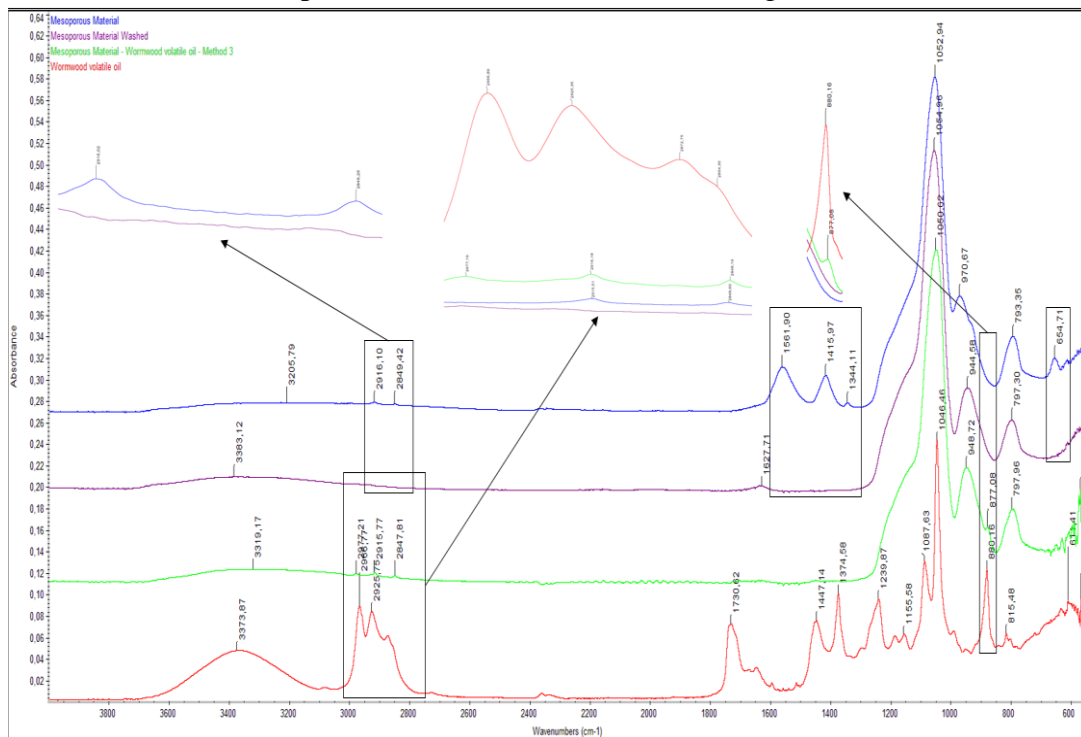


Figure 2. FT-IR spectra of the bulk, processed, loaded mesoporous material and *Artemisia absinthium* volatile oil.

eucalyptol (5.64 %), lavandulyl isovalerate (5.88 %), geranyl isovalerianate (5.99 %), and gerany-p-cymene (6.85 %). In small percentage it can be found components like α -thujene (0.19 %), β -ocimene (0.15 %), trans-2-menthenol (0.19 %), nerol-oxide (0.12 %), sabina ketone (0.09 %), etc.

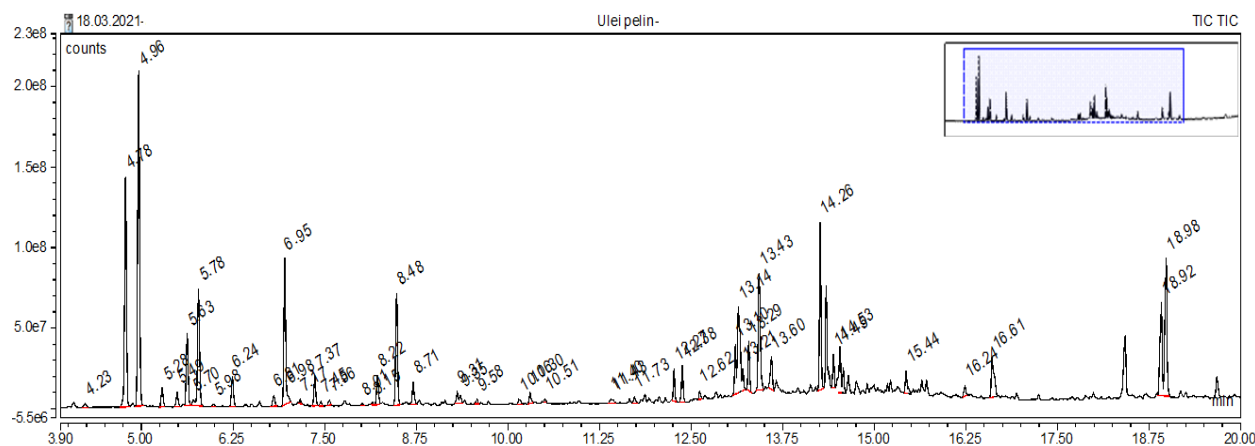


Figure 4. Chromatographic profile of volatile wormwood oil.

Table 2. Total area percentage of components identified volatile wormwood oil.

No.	Peak Name	Retention time (min)	Area %
1	α -Thujene	4.23	0.19
2	Sabinene	4.78	10.45
3	β -Myrcene	4.96	15.41
4	α -Phellandrene	5.28	0.89
5	α -Terpinene	5.49	0.65
6	<i>o</i> -Cymene	5.63	3.32
7	L-Limonene	5.7	0.37
8	Eucalyptol	5.78	5.64
9	β -Ocimene	5.98	0.15
10	γ -Terpinen	6.24	1.39
11	Terpinolene	6.81	0.52
12	Linalool	6.95	6.45
13	Perillen	7	0.33
14	α -Thujone	7.17	0.16
15	β -Thujone	7.37	1.31
16	trans-2-Menthenol	7.45	0.19
17	β -Ocimene epoxide	7.56	0.24
18	Nerol-oxide	8.01	0.12
19	Sabina ketone	8.15	0.09
20	(\pm)-Lavandulol	8.22	1.29
21	Terpinen-4-ol	8.48	5
22	α -Terpineol	8.71	0.98
23	cis-Geraniol	9.31	0.43
24	Myrtanol acetate	9.35	0.33
25	Cumaldehyde	9.58	0.2
26	Perillaldehyde	10.16	0.29
27	Lavandulyl acetate	10.3	0.53
28	Carvacrol	10.51	0.21
29	Eugenol	11.4	0.2
30	Nerol acetate	11.43	0.19
31	α -Copaene	11.73	0.3
32	Lavandulyl butyrate	12.27	1.11
33	Caryophyllene	12.38	1.43
34	β -Sesquiphellandrene	12.62	0.36
35	β -Himachalene	13.1	1.8
36	α -Curcumene	13.14	3.28
37	β -copaene	13.21	0.83
38	beta-Selinene	13.29	1.82
39	Lavandulyl isovalerate	13.43	5.88

No.	Peak Name	Retention time (min)	Area %
40	3,6-Dihydrochamazulene	13.6	1.56
41	Geranyl isovalerianate	14.26	5.99
42	Spathulenol	14.45	1.15
43	Caryophyllene oxide	14.53	2.49
44	Intermedeol	15.44	0.66
45	Chamazulene	16.24	0.47
46	Costol	16.61	1.67
47	geranyl-alfa-terpinene	18.92	4.87
48	Gerany-p-cymene	18.98	6.85

Certain volatile compounds (eucalyptol, linalool, myrcene, cymene, and α,β thujone) were subjected to quantitative determination by using 6 levels of calibration. Linearity ranges: β -myrcene 9.2-462 $\mu\text{g/ml}$, *o*-cymene 2.4-121 $\mu\text{g/ml}$, eucalyptol 1.8-90 $\mu\text{g/ml}$, linalool 7.8-390 $\mu\text{g/ml}$, sum of α,β thujone 1.7-86 $\mu\text{g/ml}$, with the regression factor $R^2 > 0.998$. (Figure 5).

By using the regression equation of each individual compound calibrated, it were quantified 28.8 mg/mL myrcene, 3.5 mg/mL cymene, 7.5 mg/mL eucalyptol, 31.8 mg/mL linalool, and 3.05 mg/mL sum of α,β thujone from volatile wormwood oil, diluted in ethanol.

To evaluate the highest load capacity of the identified volatile components, small amounts (200 mg) of mesoporous material were weighed in 50 mL flasks, for which the loading methods mentioned above have been applied. Following the B.E.T. analysis, where the results show that specific area, volume, and the diameter parameters of the pores it is enough to allow the process, we decided that the synthesized mesoporous material be loaded with volatile oil in a proportion of 30% (mass ratio w / w).

About 50 mg of mesoporous material loaded with volatile oil (30 %) was dispersed in 1 mL of ethyl acetate, which was subjected to ultrasonication, centrifugation, and filtration process, before analysis. According to the data, the most effective loading method is method 8, where the recoveries of the quantified compounds were: 14.1 % myrcene, 25.8 % cymene, 64.4 % eucalyptol, 90.2 % linalool, and the sum of α,β thujone 65.9 %, followed by method 5>method 3> method 4> method 1> method 2. Comparing all the proposed loading methods, the third was the worst option, being found 5.7 % myrcene, 10 % cymene, 17.1 % eucalyptol, 19.5 % linalool, and 20.4 % α, β thujone, from the theoretical amount of loaded compounds (Figure 6).

Due to the relatively low boiling points of volatile compounds, it's possible that in the loading process, some of them may be lost or evaporated together with ethyl alcohol or methanol, used as solvents for the initial dispersion of the components in the pores of materials.

Another control made to evaluate both the loading capacity of the mesoporous material and the competition between oil's components for the available surface was the loading of two different amounts of α,β thujone, one similar to that found in the volatile oil (0.03 mg/mL) and another at a much higher level (30 mg/mL). The results show, in both cases, an average loading of around 55 % (52.8 % for the first solution and 56.9 % for the second one), slightly below the values obtained for the others oil loading methods. This is a confirmation of the assumption that the pores filling process does not interfere with the fact that the oil has a mixed composition, with different behavior and affinity on each component.

Also, it was calculated the recovery % of each component identified in the proposed loading methods by reporting the obtained areas (for each variant) to the initial area's results for volatile wormwood oil diluted in ethanol, used as a reference, at the same concentration.

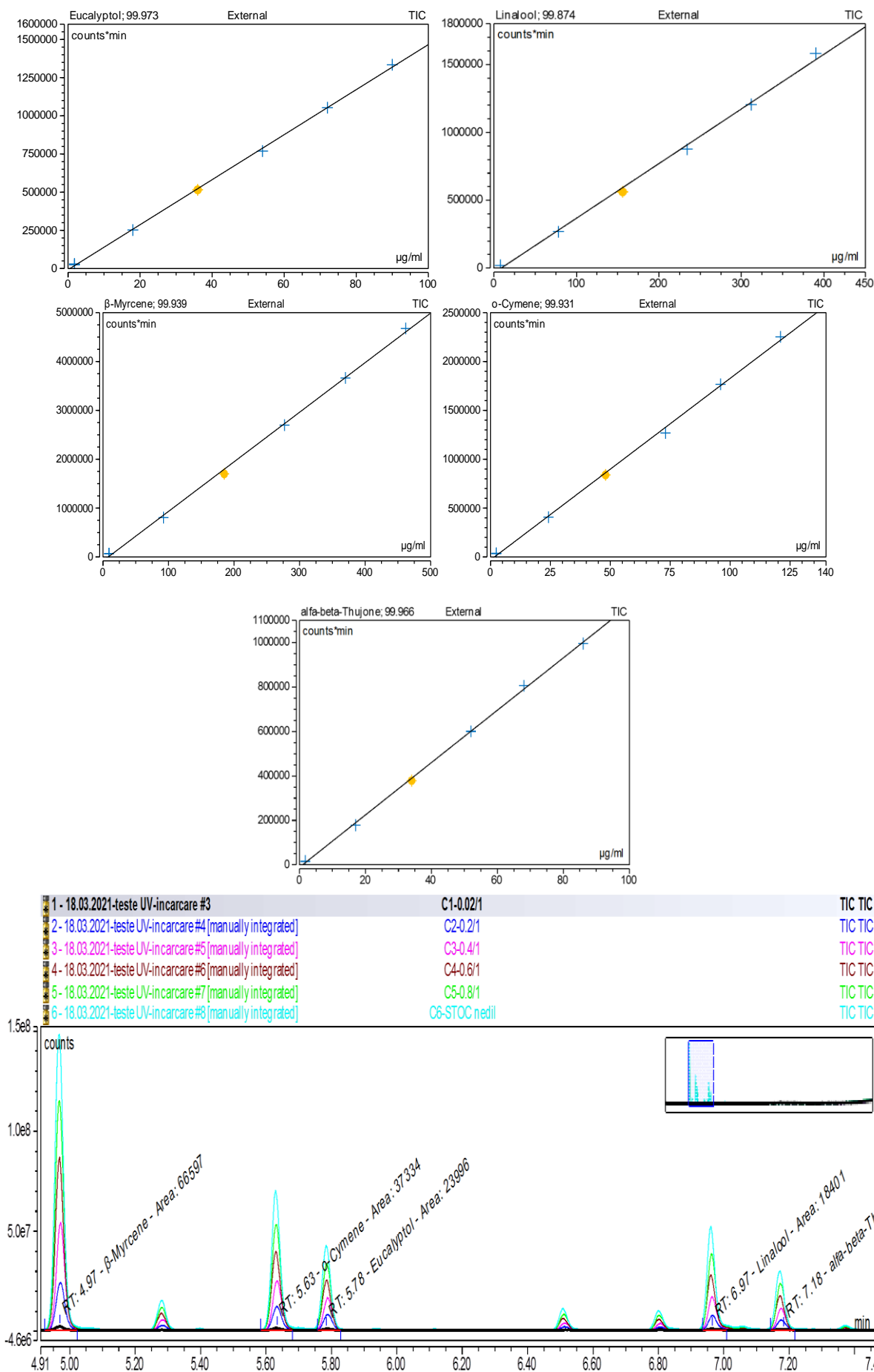


Figure 5. Overlapping chromatograms of the calibration points of β-myrcene, cymene, eucalyptol, linalool, and α,β thujone.

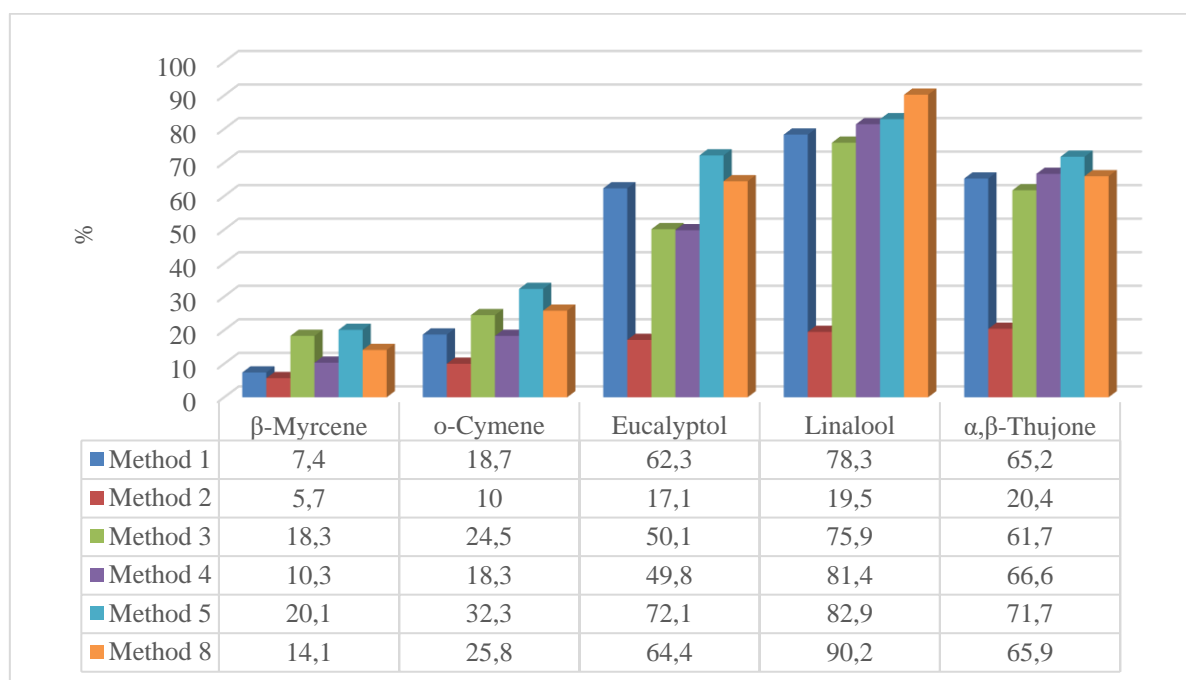


Figure 6. The percentage recovery of calibrated components using different methods of loading.

Table 3. The percentage recovery of total components identified using different methods of loading.

Nr. crt.	Compounds	Method 1	Method 2	Method 3	Method 4	Method 5	Method 8
1	Sabinene	1.61	2.15	8.36	2.52	6.64	2.86
2	β -Myrcene	4.32	3.16	13.18	6.67	14.67	6.51
3	a-Phellandrene	5.64	2.29	16.11	5.34	17.85	6.46
4	alfa-Terpinene	9.51	3.60	17.10	11.44	10.27	6.00
5	o-Cymene	12.99	6.00	17.99	12.64	24.61	14.00
6	L-Limonene	66.92	27.63	44.87	67.47	88.40	74.49
7	Eucalyptol	52.10	14.71	41.78	41.48	60.33	50.62
8	β -Ocimene	47.12	17.08	56.76	47.68	84.78	76.45
9	gamma-Terpinen	14.78	5.47	23.67	17.58	30.15	15.64
10	Terpinolene	41.04	12.07	46.38	42.48	57.11	45.11
11	Linalool	63.06	16.09	64.05	66.91	69.46	70.28
12	Perillen	38.3	38.41	63.78	34.8	50.8	45.3
13	alpha-Thujone	60.06	18.97	53.98	46.12	73.26	45.06
14	beta-Thujone	59.22	15.67	51.60	54.66	60.65	58.96
15	trans-2-Menthenol	15.32	10.99	57.38	39.53	61.08	46.66
16	β -Ocimene epoxide	9.16	24.98	68.56	65.20	77.40	87.96
17	Nerol-oxide	59.09	6.98	55.09	44.31	69.98	56.85
18	Sabina ketone	50.48	33.68	63.22	77.43	63.59	66.95
19	(\pm)-Lavandulol	52.79	14.67	56.99	58.34	63.27	60.99
20	Terpinen-4-ol	66.54	18.07	67.37	69.20	74.65	72.68
21	alpha-Terpineol	62.60	17.45	65.75	74.91	83.54	70.73
22	cis-Geraniol	62.17	14.48	51.68	73.16	79.18	80.91
23	Myrtanol acetate	61.9	20.69	66.27	76.1	61.6	64.1
24	Cumaldehyde	54.72	10.58	48.96	62.82	89.76	70.19
25	Perillaldehyde	76.83	16.72	55.99	63.48	69.00	67.69
26	Lavandulyl acetate	55.65	29.54	46.01	59.99	80.22	66.39
27	Carvacrol	34.01	7.85	48.71	52.45	84.93	83.01
28	Eugenol	28.30	15.70	44.92	31.21	50.53	42.84
29	Nerol acetate	42.06	24.32	48.67	50.68	71.88	47.10
30	alfa-Copaene	72.25	21.69	61.29	62.47	71.01	54.05
31	Lavandulyl butyrate	75.03	23.28	64.15	78.47	78.04	67.14
32	Caryophyllene	35.15	17.86	63.00	58.67	73.65	58.16

Nr. crt.	Compounds	Method 1	Method 2	Method 3	Method 4	Method 5	Method 8
33	β-Sesquiphellandrene	1.72	17.31	67.49	48.08	63.97	49.46
34	β-Himachalene	29.36	9.51	63.46	32.08	36.34	30.02
35	α-Curcumene	74.11	28.49	73.97	54.80	56.77	57.54
36	β-copaene	10.12	29.07	77.48	15.75	41.87	15.09
37	beta-Selinene	53.03	22.60	72.77	67.24	71.90	60.78
38	Lavandulyl isovalerate	73.66	17.34	77.33	76.89	77.40	76.05
39	3,6-Dihydrochamazulene	0.55	3.13	72.85	20.08	40.50	30.04
40	Geranyl isovalerianate	67.78	16.20	71.75	71.88	71.45	71.65
41	Spathulenol	63.82	24.08	69.77	76.48	88.66	87.45
42	Caryophyllene oxide	38.96	59.25	71.45	72.31	74.51	81.84
43	Intermedeol	48.32	24.70	71.67	68.02	67.52	64.22
44	Chamazulene	6.3	11.9	66.96	39.7	84.4	52.02
45	Costol	44.47	14.56	69.61	57.75	56.42	59.29
46	geranyl-alfa-terpinene	46.10	14.68	74.84	62.72	81.56	59.80
47	Gerany-p-cymene	52.09	16.92	78.85	70.66	83.15	63.37

The results show that with the decreasing of the volatility of the components, the recovery is higher, obtaining, in a certain situation, a recovery close to the initial values of the wormwood oil component concentration (linalool 63-70.3 %, terpinen-4-ol 62.6-83.5 %, lavandulyl butyrate 73.6- 77.4 %, spathulenol 63.8- 88.7 %, etc.). The recovery of the compounds sabinene, β-myrcene, α-phellandrene, alfa-terpinene, and o-cymene, located in the first part of the chromatogram, are small and varied due to their low boiling temperatures. α-Thujene, the first compound identified in wormwood oil chromatogram, is poorly detected; therefore, it was not taken into account.

3.4. Statistical analysis of the loading techniques efficacy.

There was a statistically significant difference between groups determined by one-way ANOVA ($F(5,276) = 27.595, p = 0.001$), based on results obtained from SPSS Statistics (Table 4).

Table 4. Results obtained from SPSS Statistics, using one-way ANOVA.

Source	Sum of Squares (SS)	df (degrees of freedom)	Mean Square (MS)	F	Sig. (p value)
Between Groups	62862.374	5	12572.475	27.595	<0.001
Within Groups	125748.932	276	455.612		
Total	188611.306	281			

Analysis of variance revealed significant differences between groups (p value <0.001), and subsequent *post hoc* analysis showed significant differences between certain groups (Table 5).

Table 5. Multiple comparisons with the results of the Tukey *post hoc* test.

(I) Run Number	(J) Run Number		Mean Difference (I- J)	Std. Error	Sig. (p value)	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Method 1	M 2	26.92809*	4.40315	<0.001	14.2917	39.5645
		M 3	-11.47489	4.40315	0.099	-24.1113	1.1615
		M 4	-9.44128	4.40315	0.268	-22.0777	3.1951
		M 5	-19.41723*	4.40315	<0.001	-32.0536	-6.7808
		M 8	-10.46383	4.40315	0.168	-23.1002	2.1726
	Method 2	M 1	-26.92809*	4.40315	<0.001	-39.5645	-14.2917
		M 3	-38.40298*	4.40315	<0.001	-51.0394	-25.7666
		M 4	-36.36936*	4.40315	<0.001	-49.0058	-23.7330

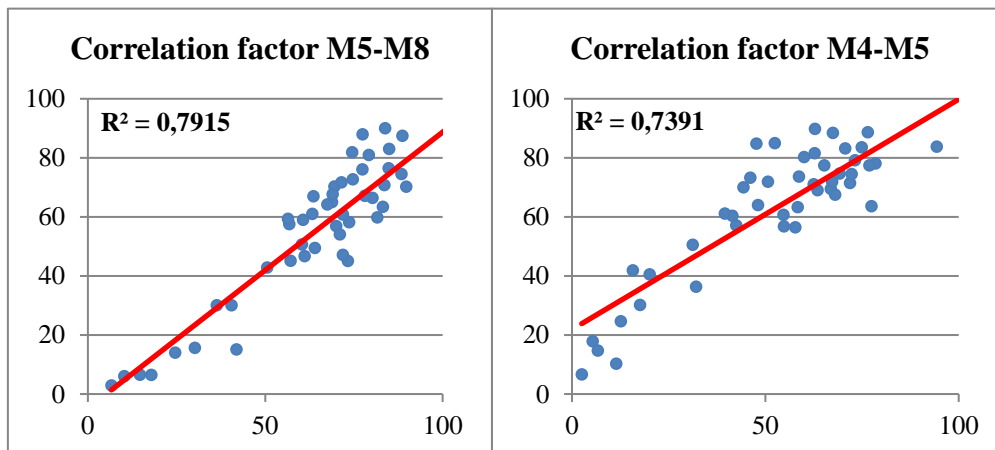
(I) Run Number	(J) Run Number	Mean Difference (I-J)	Std. Error	Sig. (p value)	95% Confidence Interval	
					Lower Bound	Upper Bound
	M 5	-46.34532*	4.40315	<0.001	-58.9817	-33.7089
	M 8	-37.39191*	4.40315	<0.001	-50.0283	-24.7555
Method 3	M 1	11.47489	4.40315	0.099	-1.1615	24.1113
	M 2	38.40298*	4.40315	<0.001	25.7666	51.0394
	M 4	2.03362	4.40315	0.997	-10.6028	14.6700
	M 5	-7.94234	4.40315	0.465	-20.5787	4.6941
	M 8	1.01106	4.40315	1.000	-11.6253	13.6475
	M 1	9.44128	4.40315	0.268	-3.1951	22.0777
Method 4	M 2	36.36936*	4.40315	<0.001	23.7330	49.0058
	M 3	-2.03362	4.40315	0.997	-14.6700	10.6028
	M 5	-9.97596	4.40315	0.212	-22.6124	2.6604
	M 8	-1.02255	4.40315	1.000	-13.6590	11.6138
	M 1	19.41723*	4.40315	<0.001	6.7808	32.0536
Method 5	M 2	46.34532*	4.40315	<0.001	33.7089	58.9817
	M 3	7.94234	4.40315	0.465	-4.6941	20.5787
	M 4	9.97596	4.40315	0.212	-2.6604	22.6124
	M 8	8.95340	4.40315	0.326	-3.6830	21.5898
	M 1	10.46383	4.40315	0.168	-2.1726	23.1002
Method 8	M 2	37.39191*	4.40315	<0.001	24.7555	50.0283
	M 3	-1.01106	4.40315	1.000	-13.6475	11.6253
	M 4	1.02255	4.40315	1.000	-11.6138	13.6590
	M 5	-8.95340	4.40315	0.326	-21.5898	3.6830

*. The mean difference is significant at the 0.05 level.

The obtained statistical data were summarized as follow: method 1 of loading show the significant difference over M 2 & M 5; method 3, 4, and 8 show significant difference only with M 2; method 5 are significant difference over M 1 & M 2; method 2 of loading are different over the all tested methods.

Table 6. Correlation matrix.

	1	2	3	4	5	8
1	1					
2	0.436098	1				
3	0.450397	0.534642	1			
4	0.752597	0.603943	0.68228	1		
5	0.633397	0.48661	0.672227	0.85972	1	
8	0.696927	0.535528	0.655078	0.85933	0.88964	1



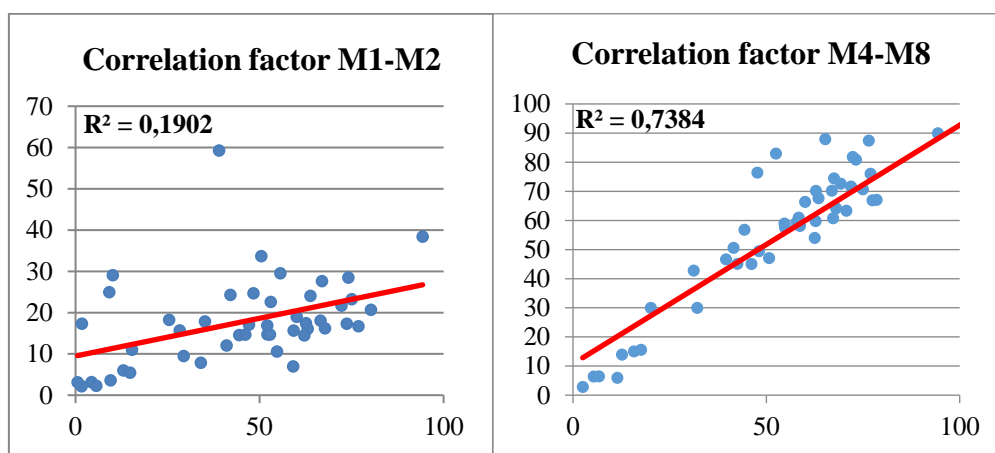


Figure 7. Correlation factors.

According to the correlation matrix, the best correlation factor is between M5-M8 ($R^2=0.7915$), M4-M5 ($R^2=0.7391$), and M4-M8 ($R^2=0.7384$), having a common parameter, the temperature at which the loading process took place. The worst correlation is between M1-M2, M1-M3, and M2-M5 (figure 7).

4. Conclusions

A high surface area mesoporous material was obtained from a trisilicate silica source and stearic acid template by a sol-gel method and used further as a matrix for essential oil loading. The *A. absinthium* essential oil was obtained by hydrodistillation and characterized by GC-MS.

The 8 different loading strategies were compared as total compound recovery after the loading process, and after the qualitative and quantitative analysis, followed by ANOVA statistical evaluation, the best loading method was selected. The loading process proves to be more efficient for the 2-5 °C / N₂ flow method.

For the high concentration components (including α,β thujone, limonene, eucalyptol, terpineol, linalool, terpinen-4-ol, cis- geraniol, carvacrol, caryophyllene oxide, etc.), at least 46% recovery was obtained, excluding method 2, with significantly lower recovery rate.

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Conflicts of Interest

The authors declare no conflict of interest.

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