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Original Article

Development and Validation of UV-spectrophotometric Method for the Estimation of Wintergreen Oil in Pharmaceutical Formulation

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ABSTRACT

Objectives: The study aimed to develop and validate a simple UV-spectrophotometric method for quantifying Wintergreen oil in micro/nanosponges. **Materials and Methods:** The method was developed using acetonitrile as a solvent system and validated for various parameters such as linearity, precision, repeatability, the Limit of Detection (LOD), and the Limit of Quantification (LOQ), and accuracy according to ICH guidelines. **Results:** The oil showed an absorption maximum at 237nm. Linearity between concentration and absorbance was established within a concentration range of 2 to 28 mcg/mL and showed a regression coefficient of 0.9746. The recovery of 97.14-107.82% and % RSD of less than 2% for repeatability, intraday, inter-day, and ruggedness revealed that the method is accurate and precise. The LOD and LOQ were determined as 0.103 mcg/mL and 0.312 mcg/mL respectively. Insignificant changes in absorption values with deliberate variation in absorption maximum indicate the method's robustness. The entrapment efficiency and loading capacity ranged between 88-97.82% and 3.085-24.35%. **Conclusion:** The validation results conclude that the method is simple, sensitive, precise, accurate, and robust. Hence, the method was used for quantifying wintergreen oil in micro/nanosponges. The procedure can further be adapted for analysing wintergreen oil in other pharmaceutical preparations and commercial products.

Keywords: Wintergreen oil, UV-spectrophotometry, Validation, Micro/nanosponges, Pharmaceutical formulation.

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INTRODUCTION

Scientific articles in previous reports have indicated the inherent biological activity, favourable safety profile, ephemeral and biodegradable nature of most essential oils. Some of the essential oils are classified as Generally Recognized as Safe (GRAS) substances by US FDA.[1,2] Even though plant essential oils exhibit promising therapeutic and preservative potential, they have some technical drawbacks such as high volatility, reactivity, poor water solubility, uneven dispersal, and instability.[3] In addition, essential oils are also sensitive to ambient oxygen, temperature, and light which may change their activity.[4] These limitations need to be circumvented for their application in the food and drug delivery systems. In this connection, one of the approaches is loading essential oils in microparticles/microsponges/nanosponges for improved bio-efficacy and stability.

Wintergreen Oil (WO) is one of the essential oils explored for antiseptic, antipyretic, analgesic, anti-inflammatory, antirheumatic, antioxidant, antimicrobial, insecticidal, astringent, and antispasmodic activities.[5-7] It is also been reported for hepatic regeneration and platelet aggregation inhibitor properties. WO is extracted from *Gaultheria procumbens* L., and belongs to the family *Ericacea*.[8] Remarkably, the anti-inflammatory effect of Gaultheria species is believed to be due to the presence of 96.9–98% of methyl salicylate as the main active ingredient.[9] It also contains tannin, resin, α-pinene, myrcene, delta-3-carene, 3, 7-guaiadiene, and delta cadinene which gives the plant a distinct medicinal tannins odour.[10] The salicylates act by inhibiting the enzyme Cyclooxygenase (COX), which is a key enzyme in the biosynthesis of pro-inflammatory cytokines and prostaglandins from arachidonic acid.[11] It has been found that WO is unsafe orally and direct application of high doses of WO can be toxic,[12] which demands the need for micro/nanoencapsulation of WO in the polymeric matrix. At the same time, estimation of WO in micro/nanosponges is of utmost importance to determine encapsulation efficiency, loading capacity, and release pattern. To do so, the choice of an analytical method is of prime importance. Analysis of

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essential oils in pharmaceutical dosage forms is challenging due to the complexity of the composition of the oil. A literature survey described a few analytical methods for the estimation of methyl salicylate and WO, which include a liquid chromatographic method for simultaneous estimation of aceclofenac, methyl salicylate, and benzyl alcohol in pharmaceuticals;[13] headspace chromatography for estimation of essential oils in the ointment;[14] GC for estimation of menthol and methyl salicylate in a topical cream and gel.[15,16] There are no simple methods available for the routine estimation of WO. Hence, there is a need for the development of a simple analytical method for determining the amount of WO in micro/nanosponges. Thus an attempt was made to develop and validate the UV-spectrophotometric method for the estimation of WO in micro/nanosponges.

MATERIALS AND METHODS

Materials

WO was obtained from Falcon, Exports of 100% pure and essential oils, Bangalore, India. HPLC grade acetonitrile was procured from Fisher scientific chemicals.

Instrumentation

A Shimadzu UV-visible-spectrophotometer (UV-1800) and Shimadzu electronic weighing balance were used for spectrophotometric analysis of WO.

Method development

Selection of solvent tannins

The basis for the selection of solvent was the solubility of WO. In the current study, preliminary trials were carried out to check the solubility of WO in various solvents such as ethanol, dichloromethane, a mixture of ethanol-dichloromethane, and acetonitrile. Since the oil is completely soluble in acetonitrile and produced a stable solution, the UV-spectrophotometric method was developed using acetonitrile as a solvent system for the estimation of WO.

Preparation of Standard Stock Solutions

Accurately weighed 500mg of WO and dissolved in 100mL of acetonitrile to obtain a concentration of 5mg/mL (stock solution-I). 1mL of the stock solution-I was further diluted to 100mL using acetonitrile to obtain a concentration of 50µg/mL (stock solution-II). Dilute solutions of concentrations ranging between 2-28µg/ml were prepared by transferring aliquots of 0.4mL, 0.8mL, 1.2mL, 1.6mL, 2.0mL, 2.4mL, 2.8mL, 3.2mL, and 3.6mL from stock solution-II and diluting to 10mL individually with acetonitrile.

Determination of wavelength of maximum absorption (λ_{max})

The determination of λ_{max} was done by scanning the solution of 18µg/mL within a wavelength range of 200-400nm. The λ_{max} was found to be 237nm. The absorbance of the rest of the dilutions was measured at 237nm against acetonitrile as a blank.

Method validation

The developed method was validated according to ICH guidelines for parameters viz; specificity, linearity, precision, ruggedness, LOD, LOQ, accuracy, and robustness.[17]

Specificity

The specificity of the method was demonstrated by UV-spectrophotometric scanning of each concentration of 2-28µg/mL within a range of 200-400nm against acetonitrile as blank.

Linearity

The linearity between the concentration and absorbance was determined by constructing the calibration curve with a series of concentrations from 2 to 28 µg/mL on the X-axis and respective absorbance values on the Y-axis.

Precision

The precision of the method was assessed in terms of repeatability, ruggedness, intra-day and inter-day precision. Repeatability was determined by measuring the absorbance of each concentration in triplicate. Intermediate precision was established by intra-day and inter-day variation studies to determine the effect of random incidents during the study. Intraday precision was determined by analysing dilution of low, middle, and high concentrations; 2µg/mL, 18µg/mL, and 28µg/mL at three-time points within the same day and for inter-day determination with the same concentrations on three consecutive days. The ruggedness of the method was determined by measuring the absorbance of 2µg/mL, 18µg/mL, and 22µg/mL by different analysts. All the measurements were done in triplicate and %RSD was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is defined as the lowest concentration of an analyte that can be identified by an analytical method. LOQ is defined as the least concentration of analyte that can be quantified consistently with acceptable accuracy, precision, and variability. LOD and LOQ were calculated for the proposed method based on the standard deviation of the response and the slope of the calibration curve using the following equation.

$$LOD=3.3\sigma/S$$

$$LOQ=10.0\sigma/S$$

Where σ=the standard deviation of response

S=the slope of the calibration curve

Accuracy

Accuracy is described as the percentage recovery of the known or spiked amount of analyte in the sample. As per ICH guidelines, accuracy should be evaluated by performing recovery studies in triplicates at 3 concentration levels as 80%, 100%, and 120%. In this method, the recovery was determined by spiking the concentration of 18µg/mL at 3 different levels such as 80%, 100%, and 120%.

Robustness

The robustness of the proposed method is the ability to endure variation in method parameters. The influence of change in wavelength of measurements is one of the variations made to establish the robustness of an analytical method. Thus the robustness was measured (18µg/mL) at a different wavelength (237±2nm).

Quantification of WO in pharmaceutical formulation

The prepared pharmaceutical formulation was WO loaded micro/nanosponges. The loaded WO in micro/nanosponges was quantified in terms of entrapment efficiency and loading capacity. A quantity of micro/nanosponge equivalent to 10 mg of WO was treated with 10 mL acetonitrile and subjected to sonication for 5min to dissolve the unentraped oil. The dispersion was filtered and 1mL of the filtrate was diluted with acetonitrile to 10 mL. 2mL was further diluted to 10mL with the same to get the concentration within the established linearity range and the absorbance was measured. The amount of free WO was determined using the regression equation. The amount of oil entrapped was calculated by subtracting the free WO from the total WO initially added. The entrapment efficiency and loading capacity were calculated using the below formulae.

$$\%EE = \frac{\text{Actual WO content in the micro/nanosponges obtained in (mg)}}{\text{Theoretical WO added (mg)}} X100$$

$$\%LC = \frac{\text{Actual WO content in the micro/nanosponges obtained in (mg)}}{\text{Total product weight (mg)}} X100$$

RESULTS

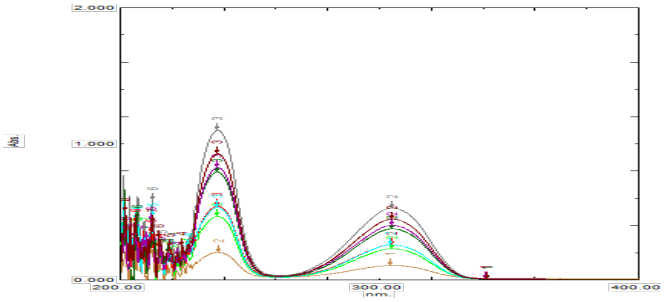

Figure 1: Linearity.

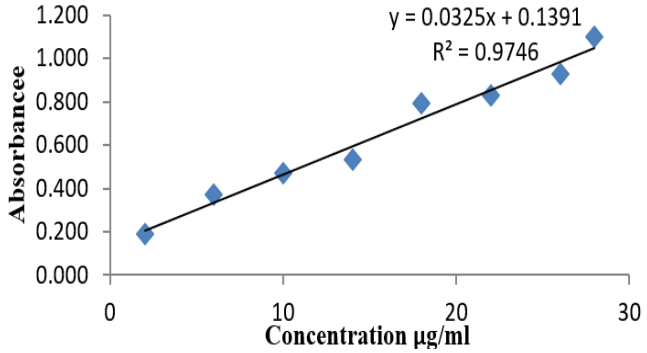

Figure 2: Calibration curve.

DISCUSSION

The objective of this method was to develop a simple, economical, precise, and accurate method for routine estimation of WO in micro/nanosponges. Thus a new UV-spectrophotometric method was developed for estimation of WO and validated as per ICH guidelines considering, specificity, precision, LOD, LOQ, robustness, and accuracy.

The process of method development started with a selection of a common solvent for dissolving WO to enable estimation of oil in the formulation easier. The solvents tried were ethanol, dichloromethane, the mixture of ethanol-dichloromethane, and acetonitrile but the solutions prepared using acetonitrile were found to be stable in comparison with other solvents mentioned. Therefore, acetonitrile was chosen as a solvent for preparing the stock solutions and dilutions. The wavelength of maximum absorption (λ_{max}) was found at 237nm (Figure 1 and 2), thus the same wavelength was used for the analysis throughout the validation procedure.

Specificity

The maximum absorption of WO was found at 237nm. Retention of the same λ_{max} at all concentrations for WO is an indicative of the specificity of the method. Further, the λ_{max} obtained for WO of 237nm was compared with reported λ_{max} of methyl salicylate,[18-20] close agreement between λ_{max} of WO and methyl salicylate affirms the maximum absorption of WO, which is solely due to the content of methyl salicylate of 96.9–98%.

Linearity

The linearity of WO was established within concentration range of 2-28µg/mL. The results of regression analysis gave regression equation and R^2 values of 0.9746 (Tables 1, and 2) which demonstrated a good correlation between absorbance and concentration of oil (Figures 1 and 2).

Precision

The precision measurements were expressed in % RSD. The % RSD of repeatability, inter-day, intra-day, and ruggedness was

found to be 0.052-0.565%, 0.052-0.333%, 0.073-0.637%, and 0.183-1.093 % respectively, which indicated precision of the method as it complies with acceptable limits of <2% (Tables 3, 4 and 5).

Accuracy

Accuracy indicates the recovery efficiency of the method, which was determined by the standard addition method and ranged between 97.14% -107.82%, demonstrating that the method is accurate (Table 6).

Robustness

The robustness of the proposed method shows a non-significant influence on the absorption level through the analysis by altering the λ_{max} 237±2nm. The percentage relative standard deviation (%RSD) values of 0.188-1.088% with deliberate changes in λ_{max} of 237±2, described the robustness of the method (Table 7).

Quantification of WO in pharmaceutical formulation

The entrapment efficiency and loading capacity of prepared micro/nanosponges were found to be 88-97.82% and 3.085-24.35% respectively.

Table 1: Validation parameters.

Parameters	WO
Absorption maxima (nm)	237nm
Linearity range (µg/mL)	2-28µg /mL
Regression coefficient (R^2)	R^2=0.974
Standard regression equation	Y=0.0325x + 0.139
LOD	0.103 µg/mL
LOQ	0.312µg/mL

Table 2: Data of calibration curve.

Concentration µg/mL	Absorbance		
	Average	SD	%RSD
2	0.189	0.001	0.285
6	0.369	0.002	0.414
10	0.468	0.003	0.565
14	0.532	0.001	0.109
18	0.794	0.003	0.333
22	0.827	0.002	0.185
26	0.927	0.002	0.165
28	1.101	0.001	0.052

Table 3: Inter-day precision.

Concentration µg/mL	Absorbance at 237 nm (Mean ±SD)		
	Day 1	Day 2	Day 3
2 (Lowest concentration)	0.189	0.181	0.181
	0.188	0.182	0.182
	0.189	0.182	0.182
Average	0.189	0.182	0.182
SD	0.001	0.001	0.001
%RSD	0.306	0.318	0.318
18 (Middle concentration)	0.793	0.786	0.786
	0.792	0.785	0.785
	0.797	0.787	0.787
Average	0.794	0.786	0.786
SD	0.003	0.001	0.001
%RSD	0.333	0.127	0.127
28 (Highest concentration)	1.101	1.093	1.087
	1.102	1.093	1.085
	1.101	1.095	1.092
Average	1.101	1.094	1.088
SD	0.001	0.001	0.004
%RSD	0.052	0.106	0.331

Table 4: Intra-day precision.

At 9.00am						
Concentration µg/mL	Absorbance at 237 nm					
	Trial 1	Trial 2	Trial 3	Average	SD	%RSD
2	0.18	0.182	0.182	0.181	0.001	0.637
18	0.782	0.781	0.783	0.782	0.001	0.128
28	1.088	1.093	1.095	1.092	0.004	0.330
At 2.00pm						
Concentration µg/mL	Absorbance at 237nm					
	Trial 1	Trial 2	Trial 3	Average	SD	%RSD
2	0.182	0.183	0.182	0.182	0.000	0.317
18	0.786	0.785	0.787	0.786	0.001	0.127
28	1.084	1.082	1.089	1.085	0.003	0.332
At 6.00 pm						
Concentration µg/mL	Absorbance at 237 nm					
	Trial 1	Trial 2	Trial 3	Average	SD	%RSD
2	0.182	0.183	0.182	0.182	0.001	0.317
18	0.793	0.793	0.794	0.793	0.0005	0.073
28	1.087	1.085	1.092	1.088	0.003	0.331

Table 5: Ruggedness data.

Concen-tration µg /mL	Analyst 1						Analyst 2					
	Trial 1	Trial 2	Trial 3	Average	SD	% RSD	Trial 1	Trial 2	Trial 3	Average	SD	% RSD
2	0.182	0.184	0.182	0.183	0.001	0.632	0.201	0.203	0.202	0.202	0.001	0.495
18	0.784	0.781	0.783	0.783	0.002	0.195	0.791	0.795	0.795	0.794	0.002	0.291
28	1.091	1.093	1.095	1.093	0.002	0.183	1.087	1.089	1.089	1.088	0.001	0.106

Table 6: Accuracy.

Level	Amount of WO taken (µg/mL)	Amount of WO added (µg/mL)	Total amount of WO added (µg/mL)	Quantity of WO recovered (µg/mL) (*n*=3)	Mean % recovery (*n*=3)
80%	18	14.4	32.4	34.6	106.79
100%	18	18	36	38.47	97.14
120%	18	21.6	39.6	42.70	107.82

Table 7: Robustness data.

Concentration µg /mL	Absorbance at 235 nm					
	Trial 1	Trial 2	Trial 3	Average	SD	%RSD
2	0.193	0.192	0.193	0.193	0.001	0.300
18	0.771	0.775	0.772	0.772	0.002	0.269
28	1.046	1.051	1.044	1.047	0.003	0.344
Concentration µg /mL	Absorbance at 237 nm					
	Trial 1	Trial 2	Trial 3	Average	SD	%RSD
2	0.202	0.203	0.202	0.202	0.001	0.285
18	0.793	0.792	0.797	0.794	0.003	0.333
28	1.087	1.089	1.089	1.088	0.001	0.106
Concentration µg /mL	Absorbance at 239 nm					
	Trial 1	Trial 2	Trial 3	Average	SD	%RSD
2	0.188	0.189	0.188	0.188	0.001	0.307
18	0.785	0.811	0.813	0.803	0.016	1.945
28	1.083	1.086	1.084	1.084	0.002	0.141

CONCLUSION

UV-spectrophotometric method was successfully developed for WO and validated in terms of validation parameters as per ICH guidelines. The method was found to be simple as it involves a single solvent, specific, precise, robust, and accurate. Hence, the method was used for the determination of WO in micro/nanosponges during the process of formulation development. Further, the method can also be adopted for routine estimation of WO in other pharmaceutical dosage forms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

WO: Wintergreen oil; **ICH:** International Conference on Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard Deviation.

SUMMARY

In pharmaceutical formulation research, quantification of essential oil is based on the amount of oil encapsulated in micro/nanosponges. Hence there was an exigency for the development of a simple, sensitive, precise, accurate, and robust UV-spectrophotometric method to quantify the amount of essential oil in pharmaceutical formulations. The developed method was validated as per ICH guidelines the results confirm that the developed method can be used to quantify the same in pharmaceutical formulations.

REFERENCES

1. Kiran S, Prakash B. Assessment of toxicity, anti-feedant activity, and biochemical responses in stored-grain insects exposed to lethal and sublethal doses of *Gaultheria procumbens* l. Essential oil. J Agric Food Chem. 2015;63(48):10518-24. doi: 10.1021/acs.jafc.5b03797, PMID 26558484.
2. US FDA. Title-Fragrances https://www.fda.gov/cosmetics/cosmetic-ingredients/fragrances-cosmetics#essential (Assessed on 16/06/2022).
3. Shikha T, Bijendra S, Nawal D. Encapsulation of essential oils–a booster to enhance their bio-efficacy as botanical preservatives. J Sci Res. 2020;64:175-78.
4. Claudia T, Florian S. Stability of essential oils: A review. 2013;12:41-53.
5. Michel P, Dobrowolska A, Kicel A, Owczarek A, Bazylko A, Granica S, *et al*. Polyphenolic profile, antioxidant and anti-inflammatory activity of eastern teaberry (*Gaultheria procumbens* L.) leaf extracts. Molecules. 2014;19(12):20498-520. doi: 10.3390/molecules191220498, PMID 25493634.
6. Zhang D, Liu R, Sun L, Huang C, Wang C, Zhang DM, *et al*. Anti-inflammatory activity of methyl salicylate glycosides isolated from *Gaultheria yunnanensis* (Franch.) Rehder. Molecules. 2011;16(5):3875-84. doi: 10.3390/molecules16053875, PMID 21555977.
7. Zhang B, He XL, Ding Y, Du GH. Gaultherin, a natural salicylate derivative from *Gaultheria yunnanensis*: Towards a better non-steroidal anti-inflammatory drug. Eur J Pharmacol. 2006;530(1-2):166-71. doi: 10.1016/j.ejphar.2005.11.030, PMID 16375889.
8. David MR, Alexander P, Ilya R. The determination of salicylates in *Gaultheria procumbens* for use as a natural aspirin alternative. J Nutraceuticals Funct Med Foods. 2015;4(1):39-52.
9. Sims KL, Prabodh S, William S. The Volatile phytochemistry of seven Native American aromatic medicinal plants. Plants. 2021;10(1061):1-19.
10. Vijendra S, Gunjan MA. Isolation of volatile constituents and biological studies of aerial parts of *Gaultheria procumbens* L. Int J Green Chem. 2017;11(4):S784-8.
11. Mao P, Liu Z, Xie M, Jiang R, Liu W, Wang X, *et al*. Naturally occurring methyl salicylate glycosides. Mini Rev Med Chem. 2014;14(1):56-63. doi: 10.2174/1389557513666131211110004, PMID 24329991.
12. Rakhi MRS. Pharmacological aspects of essential oil–wintergreen oil. Int J Sci Res. 2017;6(7):1539-41.
13. Anna P, Ramesh G. A simple liquid chromatographic method for simultaneous determination of aceclofenac, methyl salicylate and benzyl alcohol in pharmaceuticals. J Pharm Res. 2018;12(3):283-7.
14. Nk D, M J, V K. Improved analytical method for estimation of essential oil in drug ointment through rapid static chromatography headspace for quality control analysis. Asian J Pharm Clin Res. 2018;11(5):290-4. doi: 10.22159/ajpcr.2018.v11i5.23351.

15. Subhash K, Bhavesh B, Hemang V. Analytical method development and validation of menthol and methyl salicylate content in topical cream and gel by gas chromatography. J Chromatogr Sep Tech. 2017;8(6):1-4.
16. Nikolić M, Marković T, Mojović M, Pejin B, Savić A, Perić T, *et al*. Chemical composition and biological activity of *Gaultheria procumbens* L. essential oil. Ind Crops Prod. 2013;49:561-67. doi: 10.1016/j.indcrop.2013.06.002.
17. ICH harmonized tripartite guideline, validation of analytical procedures: Text and methodology. 4th version. Vol. Q2(R1). 1994.
18. Makeen HA, Pancholi SS, Alhazmi HA, Ezzi AA, A Hazzazi AJA, Meraya AM. Stability testing of extemporaneous preparation of methyl salicylate ointment. J Health Spec. 2018;6(2):73-6. doi: 10.4103/jhs.JHS_14_18.
19. Dawn P, Christina M, Christina S, Jerry S, Lain M. The analysis of methyl salicylate and salicylic acid from Chinese herbal medicine ingestion. J Ana Toxicol. 2004;4:214-16.
20. Dhawal D. Development of UV-spectrophotometric method for the determination of methyl salicylate in bulk and semisolid formulation. Chemistry. 2012.

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