DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF EUCALYPTUS OIL IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Micro or nanoencapsulation of eucalyptus oil may be a mechanism for protecting and promoting the controlled release of its bioactive compounds. To optimize the encapsulation process it is necessary to accurately quantify the oil present in the micro or nanosponges. The main objective of the study was to develop and validate a simple UV spectrophotometric method to determine the encapsulation efficiency of eucalyptus oil-loaded micro and nanosponges. The method was developed using acetonitrile as a suitable solvent and the maximum absorption was observed at 233nm. The method showed linearity within a concentration range of 100-500mcg/mL with an R^2 value of 0.998. LOD and LOQ values were calculated as 0.206mcg/mL and 0.625mcg/mL respectively. The RSD values for repeatability, intermediate, and method precision were found to be within the limits of 2%. The recovery ranged between 99.48 to 103.4% and robustness was indicated by negligible variations in absorbance with deliberate variations in λmax. The entrapment efficiency of micro and nanosponges was found to be 32.26 to 99.32%. The validation procedure confirmed that the method is reliable for the estimation of eucalyptus oil as the method was proved to be specific, simple, fast, sensitive, precise, accurate, and robust.

KEYWORDS: Eucalyptus oil, UV spectrophotometry, Validation, Micro and nanosponges

INTRODUCTION

Microsponges are patented polymeric delivery systems consisting of porous microspheres that can entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti-infective, antifungal and anti-inflammatory agents. Eucalyptus oil (EO) is one of the essential oil obtained from fresh leaves of Eucalyptus globulus belongs to the family myrtaceae. It has diverse pharmacological activities like antibacterial, antiviral, anthelmentic, analgesic, anti-inflammatory and antioxidant. These activities are contributed by the major volatile components being 1, 8-cineol (eucalyptol) (61.46%), limonene (13.68%), ρ-cymene (8.55%), ϒ-terpinene (5.87%) and α-pinene (4.95%) [1]. The possible established mechanism for analgesic and anti-inflammatory effect of principal component eucalyptol (1,8-cineole) (fig.1) is the inhibition of production/synthesis of tumour necrosis factor-α, interleukin-1β, leukotriene B4, and thromboxane B2 in inflammatory cells, thus shows analgesic and anti-inflammatory property [2-3] . Inspite of availability of variety of analytical methods to determine entrapment efficiency of micro or nanosponges through quantification of essential oils, there is lack of simple methods for routine estimation of essential oils due to their complexity in their composition. Some of the analytical methods reported in the literature include, gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), high performance thin-layer chromatography (HPTLC), gas chromatography (GC) and reverse phase high performance liquid chromatography (RP-HPLC) [4-11] . Moreover, to the best of information obtained in the literature, there is no UV spectrophotometric method available for the quantification of EO. Hence, objective of the present study was to

develop a simple UV spectrophotometric method and validate the same for quantifying EO in micro/nanosponges and also *in-vitro* release samples.

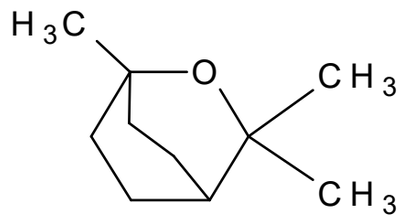

Figure 1.Structure of Eucalyptol

MATERIALS AND METHODS

Materials

The EO was purchased from Falcon, exporters of 100 % Pure & Essential oils, Bangalore, and acetonitrile were obtained from Hi-Media Laboratories Pvt Ltd., Mumbai.

Instrumentation

A Shimadzu UV-Visible Spectrophotometer (UV-1800) with a matched pair of 10 mm quartz cuvettes and Shimadzu electronic weighing balance were used for the study. Ultrasonicator (Servewell Instrument Pvt. Ltd.) was used for sample preparation.

Method development

Selection and Optimization of Solvent

The solvent does exert an intense influence on the quality and shape of the peak [12]. Initial trials were carried out to check the solubility of EO in various solvents such as ethanol, dichloromethane, a mixture of ethanol-dichloromethane, and acetonitrile. Since the oil is completely soluble in acetonitrile and produced stable solutions and also the said solvent was found to be satisfied relative to peak quality and peak consistency at the specified wavelength. Hence a UV spectrophotometric method was developed for EO using acetonitrile as a solvent.

Preparation of standard stock solution

The standard stock solution was prepared by dissolving 500mg of EO in 100 mL of acetonitrile to obtain a concentration of 5mg/mL. Aliquots of 0.25mL, 0.5 mL, 0.75mL, 1.0mL, 1.25mL, 1.75mL, 2.0mL, 2.25mL, 2.5mL and 2.75mL were taken from stock solution and diluted with acetonitrile to 25ml separately to prepare series of concentration from 50-500 mcg/mL.

Determination of wavelength of maximum absorbance (λmax) of EO

The maximum wavelength of absorption of EO was determined by scanning the concentration of 350mcg/mL solution using a UV-visible double beam spectrophotometer within a wavelength range of 400-200nm against acetonitrile as blank. The λmax was obtained at 233nm. The method was validated according to ICH guidelines for parameters viz, specificity, linearity, precision, ruggedness, LOD (Limit of detection), LOQ (Limit of quantification), accuracy, and robustness.

Specificity

Specificity was confirmed by UV spectrophotometric scanning of each concentration of EO ranging from 100 to 550 mcg /mL within a wavelength gamut of 200-400nm against acetonitrile as blank.

Linearity

Linearity was determined by a multipoint calibration method consisting of a series of concentrations from 100 to 550mcg/mL and the absorbance of all the dilutions was measured respectively at 233nm. The calibration curve was constructed by plotting concentration on the x-axis and absorbance on the y-axis. A regression equation and coefficient were determined by subjecting the data to regression analysis.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest concentration of an analyte that can be detected by an analytical method. LOQ is the least concentration of analyte that can be quantified reliably with acceptable accuracy, precision, and variability. LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve using the following equation.

$LOD=3.3\sigma/S$

$LOQ=10\sigma/S$

Where σ= standard deviation of response

S=slope of the calibration curve

Precision

The precision of the analytical method was determined based on the repeatability, intra- day and inter-day precision. Three concentration points were selected for the study, which include low 100mcg/mL, middle-350mcg/mL and high-550mcg/mL. Precision was indicated in terms of percentage relative standard deviation (%RSD) values.

Repeatability

Repeatability is indicative of method precision. It can also be termed as intra-assay precision as it expresses the precision obtained over a short period under the same operating conditions.

Intermediate precision

Intermediate precision was demonstrated by intra-day and inter-day variation studies to determine the effect of random events during the study. Intraday precision was determined by analyzing dilution of low concentration 100 mcg/mL, mid concentration 350mcg/mL, and highest concentration 550mcg/mL at three-time points within the same day, and for inter-day determination was done with the same concentrations on three consecutive days. All the measurements were done in triplicate and %RSD was calculated.

Ruggedness

Ruggedness should be considered during the development phase of an analytical method. It shows the reliability of an analytical method concerning variation in external factors, such as instruments and analysts.

Robustness

The influence of change in wavelength of measurements is one of the variations made to establish the robustness of the analytical method.

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 such as 80%, 100%, and 120 %. Accuracy was determined by performing recovery studies by spiking concentrations of 350mcg/mL at 3 different levels such as 80%, 100%, and 120 %. The concentration of oil was calculated at each level and % recovery was determined.

RESULTS AND DISCUSSION

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

The maximum absorption was found at 233nm (fig.2 and 3), thus same wavelengths were used for the analysis throughout the validation procedure.

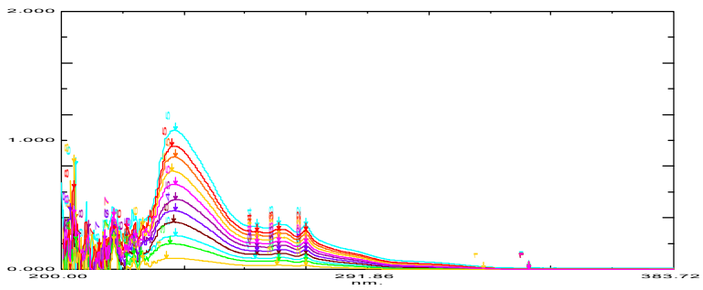

Figure 2.Linearity of EO

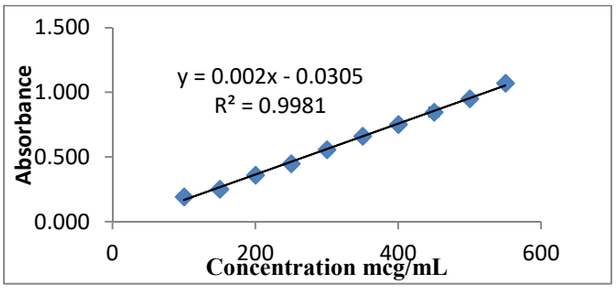

Figure 3.Calibration curve EO

Specificity

The maximum absorption was found to be 233nm. Retention of the same λmax at all concentration levels for the oil is an indication of the specificity of the method.

Linearity

Linearity of an analytical method is the ability, within the given range of obtained test results which are directly proportional to the concentration of the analyte. The linearity was established within the selected concentration ranges of 50-550mcg/mL. The results of regression analysis gave regression coefficient (R^2) values of 0.998 (table 1 and 2) which demonstrated an excellent correlation between absorbance and concentration of oil (fig. 1 and 2).

Table 1- Validation parameters

Parameters	EO
Absorption maxima(nm)	233nm
Linearity range (mcg/ml)	50-550 mcg /mL
Regression coefficient (R^2)	R^2=0.998
Standard regression equation	Y=0.002x - 0.030
LOD	0.206 mcg/mL
LOQ	0.625mcg/mL

Table 2- Linearity data EO

Concentration mcg/mL	Absorbance	
	Average	%RSD
100	0.193	1.66
150	0.252	0.92
200	0.361	0.16
250	0.449	1.11
300	0.556	0.21
350	0.661	0.31
400	0.751	0.27

450	0.867	0.31
500	0.952	0.28
550	1.072	0.46

Precision

The precision of an analytical method expresses the amount between a series of measurements obtained from multiple sampling under prescribed conditions. Thus the precision of the method was determined based on repeatability and intermediate precision (inter-day, intra-day), and ruggedness (method precision). The precision measurements were expressed in % RSD. The % RSD of repeatability, inter-day, intra-day, and ruggedness was found to be 0.16-1.66, 0.30-1.66, 0.45-1.85, 0.11-1.98, which indicated the precision of the method as it complies with acceptable limits of <2% (table 3,4 and 5).

Table 3-Inter-day precision EO

Concentration mcg/mL	**Absorbance**		
	Day 1	**Day 2**	**Day 3**
100 (Lowest concentration)	0.197	0.199	0.184
	0.195	0.193	0.178
	0.191	0.196	0.181
Average	**0.194**	**0.196**	**0.181**
%RSD	**1.57**	**1.53**	**1.66**
350 (Mid concentration)	0.663	0.687	0.687
	0.662	0.683	0.676
	0.65	0.686	0.671
Average	**0.658**	**0.685**	**0.678**
%RSD	**1.1**	**0.3**	**1.21**
550 (Highest concentration)	1.075	1.184	1.188
	1.074	1.194	1.201
	1.066	1.187	1.177
Average	**1.072**	**1.188**	**1.189**
%RSD	**0.46**	**0.43**	**1.01**

Table 4-Intra-day precision EO

At 9.00am					
Concentration mcg/mL	**Absorbance 233 nm**				
	Trial 1	**Trial 2**	**Trial 3**	**Average**	**%RSD**
100	0.197	0.192	0.191	0.193	1.66
350	0.694	0.681	0.687	0.687	0.95
550	1.089	1.096	1.094	1.093	0.33
At 2.00pm					
Concentration mcg/mL	**Absorbance 233nm**				
	Trial 1	**Trial 2**	**Trial 3**	**Average**	**%RSD**
100	0.184	0.178	0.181	0.181	1.66
350	0.687	0.676	0.671	0.678	1.21
550	1.088	1.082	1.096	1.089	0.65
At 6.00 pm					
Concentration mcg/mL	**Absorbance 233 nm**				
	Trial 1	**Trial 2**	**Trial 3**	**Average**	**%RSD**
100	0.189	0.193	0.186	0.19	1.85
350	0.687	0.683	0.186	0.518	0.45
550	1.075	1.074	1.066	1.071	0.46

Table 5-Ruggedness data of EO

Concentration mcg/mL	Analyst 1					Analyst 2				
	Trial 1	Trial 2	Trial 3	Average	% RSD	Trial 1	Trial 2	Trial 3	Average	% RSD
100	0.184	0.178	0.181	0.678	1.207	0.197	0.192	0.191	0.19	1.66
350	0.687	0.676	0.671	1.189	1.011	0.622	0.627	0.62	0.62	0.58
550	1.188	1.201	1.177	0.678	1.207	1.042	1.071	1.064	1.06	1.43

Robustness

The variation in the λmax of ± 2.0nm showed a % recovery between 99.48-103.4%. The RSD of 0.11-1.98 with deliberate changes in λmax of 233±2 describes the robustness of the method (table 6).

Table 6: Robustness data for EO

Concentration mcg/mL	Absorbance at 231 nm				
	Trial 1	Trial 2	Trial 3	Average	%RSD
100	0.171	0.172	0.172	0.172	0.34
350	0.648	0.664	0.674	0.662	1.98
550	1.089	1.119	1.089	1.099	1.58
Concentration mcg/mL	Absorbance at 233 nm				
	Trial 1	Trial 2	Trial 3	Average	%RSD
100	0.184	0.181	0.178	0.181	1.66
350	0.681	0.685	0.687	0.684	0.45
550	1.087	1.089	1.089	1.088	0.11
Concentration mcg/mL	Absorbance at 235 nm				
	Trial 1	Trial 2	Trial 3	Average	% RSD
100	0.188	0.189	0.188	0.188	0.31
350	0.674	0.677	0.39	0.675	0.26
550	1.158	1.163	1.164	1.161	0.28

Accuracy

The accuracy of the analytical method is the closeness of the test results to actual values. Thus the accuracy indicates the recovery efficiency of the method, which was determined by the standard addition method and ranged between 97.14% -107.82%, confirming that the method is accurate (table 7).

Table 7 Accuracy study for EO

Accuracy level	Amount of EO taken (mcg/mL)	Amount of EO added (mcg/mL)	Total amount of EO recovered (mcg/mL)	Quantity of EO recovered (mcg/mL) (n=3)	Mean % recovery (n=3)
80%	350	280	630	651.5	103.4
100%	350	350	700	707	101
120%	350	420	770	760	99.48

CONCLUSION

UV spectrophotometric method was successfully developed and validated in terms of validation parameters as per ICH guidelines for EO. The developed method was found to be simple as it involves a single solvent, specific, precise, robust, and accurate. Hence, this method can be used for the quantification of EO in bulk and micro/nanosponges *in-vitro* release

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