Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Suppl 2, 2014

Research Article

DEVELOPMENT AND VALIDATION OF A GC/FID METHOD FOR IDENTIFICATION AND QUANTIFICATION OF MAIN COMPONENTS OF SATUREJA MONTANA L. ESSENTIAL OIL

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Received: 08 Nov 2013, Revised and Accepted: 28 Jan 2014

ABSTRACT

Objective: *Satureja montana* L. is well-known for its essential oil contents and dermatological benefits. Main components of its essential oil are borneol, carvacrol, thymol, γ-terpinen, p-cymen. Our objective is to develop a Gas/Fid analytical method for quantification and identification of main components of *Satureja montana* L. essential oil.

Methods: Essential oils were obtained by hydrodistillation of *Satureja montana* L. using Clevenger apparatus. Analyses of essential oil and validation method were developed by GC/FID apparatus tip Varian 3800.

Results: The validation process; linearity, optimization of GC/FID parameters of method proposed, precision and accuracy results were statistically significant.

Conclusion: The method proposed were found appropriate for GC/FID analyses of *Satureja montana* L.

Keywords: *Satureja montana* L, essential oil, Gas/Fid,validation.

INTRODUCTION

Essential oils are lipid soluble well-known ingredient often applied to the skin for their important properties that ranges from antimicrobial to antinflammatory and skin whitening. Current applications of these volatile compounds turn out to be complicated because of chemical and physical properties.[6,7].

This is one of the major problems for their uses; therefore, microencapsulation could be the solution to problems of stability, evaporation and controlled release.[11] *Satureja montana* L. provides of interesting antimicrobial properties an on the other hand analytical method development and validation play important role in discovery, development and manufacture of herbal medicinal products. [8] The results from the validated test methods are used to ensure identity, purity, potency and performance of drug product. During encapsulation process is necessary the identification and quantification of main components of *Satureja montana* L. through a validated analytical method.

Identification and quantification of main components of *Satureja montana* L. essential oils by GC/FID method is our aim in this study. As far as we know there are no validated methods of performing such analyses of this essential oil. *Satureja montana* L. essential oil main components are [12,13,14,15] carvacrol (2.21 - 55.95%), thymol (0.38 - 40.51%), p-cymene (1.13 - 17.40%), borneol (1.35-9.64%), γ-terpinene (0.31 - 8.86%). As result we studied the GC/FID analytical method of identification and quantification of these components and developed the method validation.

MATERIALS AND METHODS

Plant Material

Herbal plants of *Satureja montana L*. were collected from different zones of Albania and were identified from our botanist Skerdilaid Xhulaj in Botanic Department, Faculty of Natural University of Tirana, Albania. Sample of drug is recorded in Herbarium Deposit in Natural University of Tirana.

Isolation of the essential oil

The hydrodistillation was carried out with a Clevenger-type apparatus according to the Hungarian Pharmacopiea VII. (1986). Drug quantity of 20 g was used; it was distilled with 500 ml of water for 3 hours. The resulting essential oil was dried over anhydrous sodium sulphate and stored at 4°C. [2,16,10]

Reagents

All reagents and solvents used were obtained from Sigma Aldrich Company. GC/FID Tip Varian CP3800, Stationary, Phase Capillary VF: 1ms, Film thickness 0.25 μm(L) 25 mx (ID) 0.25mmx(OD) 0.39mm. Mobile Phase is helium. Standards of carvacrol, p-cymen, y-terpinen, borneol, thymol were obtained from Sigma Aldrich company.

Gas/Fid method

GC/FID conditions GC analysis of the essential oil was performed using a Varian CP-3800 instrument equipped with a capillary column. Helium was used as the carrier gas at the constant flow of 1.2 ml/min and split ratio 1:30. The oven temperature was held at 50 °C for 1 min, and then programmed to 280°C at a rate of 5°C /min. Helium flux is 30ml/min and air flux is 300ml/min. The injector temperature is 280 and detector (FID) temperature is 300°C. Injection volume is 1μl. [3]

METHOD VALIDATION

Standard and sample Stock Solutions

Satureja montana L. essential oil stock solution was prepared dissolving 5 mg essential oil in 5 ml hexane and was stored in refrigerator (-4°C) for stability. Six samples were prepared and each one was injected three times. The standards stock solution were prepared in following concentration p-cimen 2mg/ml carvacrol 2mg/ml, γ-terpinen 2mg/ml, thymol 8mg/ml, borneol 0,5mg/ml.

Linearity – Calibration Curbes

We prepared serial dilutions of each standard. The calibration lines were constructed by plotting the areas of p-cymen, borneol, carvacrol, γ-terpinen and thymol against their corresponding concentration [5]. The concentration studies ranges between 0.5-5mg/ml for borneol, 1.0-8.0 mg/ml for γ-terpinen, 0.1-2.0mg/ml for carvacrol, 0.4-2mg/ml for p-cymen and 2.0-10 mg/ml for thymol. The statistical parameters slope, intercept, residual standard on deviation response correlation co-efficient and p- values were calculated by GraphPad 6.02 version. (Table 1). Their correspondative graph is shown below. (Fig.1)

Table 1: It shows slope, residual standard and intercept

Component	Slope	r	r^2	p
Borneol	0.08463 ± 0.015	0.9559	0.9138	0.0110
y-terpineni	0.1137 ± 0.0103	0,9929	0,9759	0.0016
Carvacrol	0.4205 ± 0.0975	0.9880	0.9885	0.0200
p-cymen	0.1307 ± 0.0143	0.9825	0.9653	0.0028
Thymol	0.0925 ± 0.0073	0.9963	0.9813	0.0011

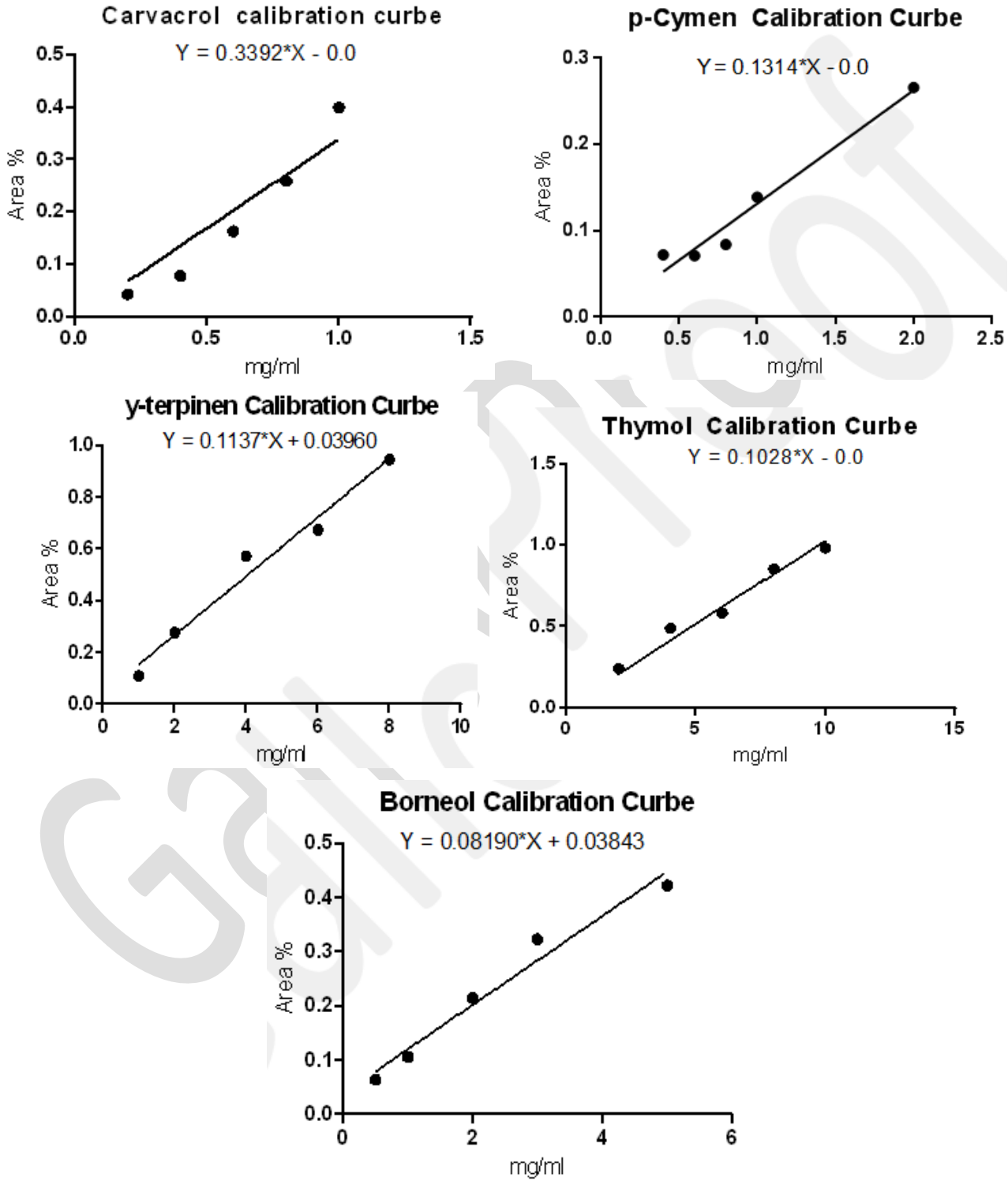
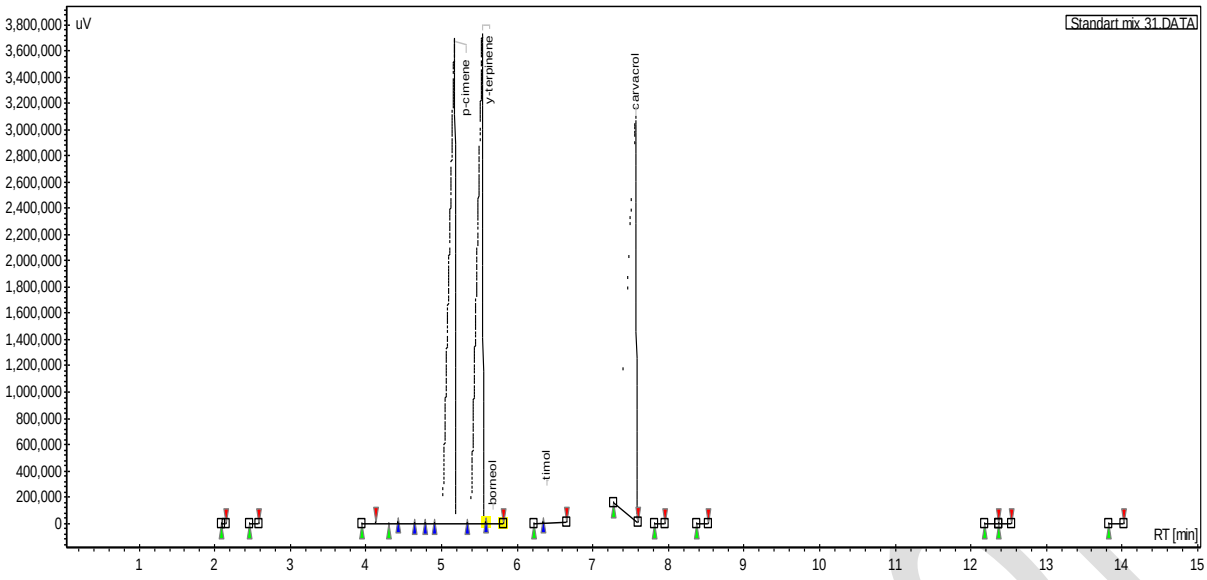


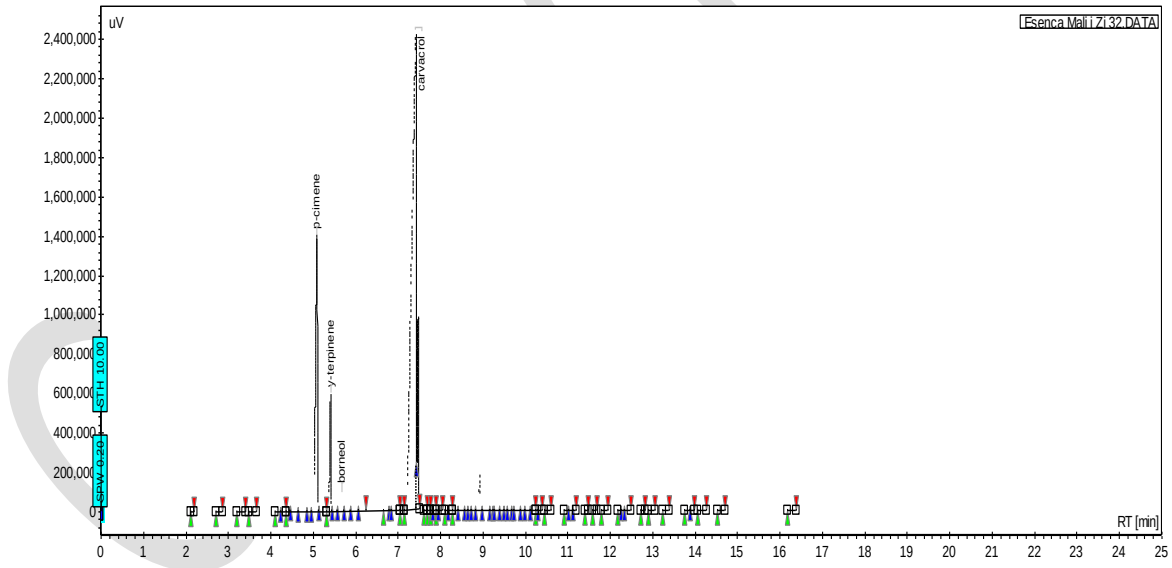
Fig. 1: It shows Calibration cubes



Peak results :

	Name	Time [Min]	Quantity [% Area]	Height [uV]	Area [uV.Min]	Area % [%]
	p-cimene	5.17	30.44	3696089.7	335666.9	30.436
	y-terpinene	5.54	29.22	3746505.7	322289.4	29.223
	borneol	5.68	0.24	94135.5	2621.2	0.238
	timol	6.40	1.08	284537.3	11897.8	1.079
	carvacrol	7.56	38.77	3080362.0	427577.3	38.770
Total			100.00	10950572.4	1102853.3	100.000

Fig. 2: It shows Typical chromatogram of separation of our standarts ; p-cymen, γ-terpinen, borneol, carvacrol, thymol



Peak results :

	Name	Time [Min]	Quantity [% Area]	Height [uV]	Area [uV.Min]	Area % [%]
	p-cimene	5.09	14.69	1403968.9	68072.2	14.687
	y-terpinene	5.41	3.93	600081.9	18200.2	3.927
	borneol	5.67	0.76	92925.5	3513.4	0.758
	carvacrol	7.42	62.97	2434205.2	291830.0	62.966
Total			100.00	6955483.4	463470.9	100.000

Fig. 3: It shows typical chromatogram of separation of our sample ; p-cymen, γ-terpinen, borneol, carvacrol, thymol

Optimization of GC Condition

For giving the most chemical information and better separation in the chromatograms, the column, mobile phase, detection wavelength and conditions for gradient elution were investigated in this study. Two kinds of kinds of temperature regimes were investigated, gradient and non gradient ones. Temperature gradient regime was found to be more suitable and gave good peak separation and sharp peaks (Fig. 2, 3).

Precision and Accuracy study

The results of precision and accuracy determination were obtained from the recoveries of the ratios of found quantities to the injected quantities. The precision of the proposed method was verified by calculation of their repeatability's RSD preparations done successively during one day and the following 3 consecutive days. The accuracy was determined by calculation of the mean recoveries ± SD of five levels of concentrations (Table 2)

Table 2: It shows recovery (%) and RSD values within day and between days

Component		In Day(N=5)			Between day 3 days,(N=5)		
	Sample mg/ml	Found	Recovery	R.S.D %	Found	Recovery	R.S.D %
Carvacrol	0.41	0.41	100 %	2.4	0.40	97.5%	2.3
Thymol	1.60	1.59	99.3 %	0.62	1.58	96.6%	0.62
Borneol	0.10	0.09	92.0%	0.85	0.1	100%	0.86
Y-terpinen	0.45	0.43	89.0 %	2.2	0.42	93.3%	2.1
P-cymen	0.45	0.44	97.7%	2.1	0.43	97.7%	2.2

Limit of Dedection –Lowering injection volume

Table 3: It shows limit of quantitation

Compound	LOD
Carvacrol	0.6 mg/ml
Thymol	1.2 mg/ml
Borneol	0.5 mg/ml
γ-Terpinen	0.6 mg/ml
p-Cymen	0.3 mg/ml
Carvacrol	0.2 mg/ml

The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated by serial dilutions of five standards stock solutions in order to obtain signal to noise ratios of 3:1 for LOD and 10:1 for LOQ. The LOD values for analyte were found to be as in (Tab.3).

Robustness

1- Change oven temperature. We changed the oven temperature from 280 °C to 290 °C

2- Change the flow rate from 30 ml/min to 25 ml/min

In both two cases we didn't have statistically differences in results obtained conducting recovery at different level of thymol and the average percentage and recovery was found to be in the range

RESULTS AND DISCCUSION

In this paper the validation of the GC/FID method for the determination of borneol, p-cymen, thymol, γ-terpienen and carvacrol in *Satureja montana* L. essential oil was carried out. No papers concerning this determination have been found. From the data it is suggested that GC/FID method is suitable and acceptable. The method demonstrated a wide linearity range from 0.95 – 0.99 (Table 1). A wide linearity range guarantees that good results are obtained. The recovery and precision of the method were satisfactory for quantitative analyses and good repeatability of the results has been achieved. The RSD values were 89% to 100% which indicated good precision and intermediate precision.

The obtained high recovery value was 100 %. Good precision and accuracy of the method were confirmed. Peak areas versus concentrations were linear, the resulting regression had a good slope and correlation coefficient was r = 0.99833.

The limit of detection and limit of determination are shown in Table 2. The validation of the GC/FID method along with statistical analysis indicates high sensitivity under established conditions.

The developed GC/FID method enables easy qualitative and quantitative analyses of main components of *Satureja montana* L. essential oil.

The validation data show that the results are accurate and precise so the method can be widely applied. It seems that the developed gas chromatographic method is especially suitable.

CONCLUSION

The method was found to be precise specific, sensitive and accurate and can be used for routine quality control of of borneol, p-cymen, thymol, γ-terpienen and carvacrol in *Satureja montna* L. essential oil was found to be in the range 90%-110%. The Linearity was established over a range of seven different concentrations of the analyte.

ACKNOWLEDGEMENTS

Authors are thankful to the board of Tirana University, Aldent University, National Drug Control Laboratory

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