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Validation of Rapid Gas Chromatographic Method for Determination of Seven Volatile Compounds in a Urinary Tract Antiseptic Soft Gelatin Capsules

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ABSTRACT

A simple, rapid and precise gas chromatographic method has been developed for simultaneous determination of seven volatile compounds namely alpha pinene, camphene, beta pinene, cineole, fenchone, borneol and anethol in urinary tract antiseptic soft gelatine capsule. The seven compounds were analysed by Gas chromatography-flame ionization detector (GC-FID) on a (5% diphenyl and 95% dimethylpolysiloxane) (30m x0.25mm x 0.25µm) column and Helium as a carrier gas. The injector and detector port temperatures were maintained at 200°C and 250°C respectively. Results of assay and recovery studies were statistically evaluated for its accuracy and precision. The correlation coefficient (r) values ranged from 0.997 to 0.9998. The detection limits ranged from 0.0015 to 0.014 mg ml^{-1}. No interference from any components of pharmaceutical dosage forms was observed. According to the validation results, the proposed method was found to be specific, accurate and precise and could be applied to the simultaneous quantitative analysis of these seven volatile compounds in such pharmaceutical formulation.

Keywords: Pharmaceutical analysis; Volatile compounds; Gas chromatography.

1. INTRODUCTION

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. [1-4] Essential oils (also called volatile or ethereal oils) are aromatic oily liquids obtained from plant materials. The greatest use of essential oils is in food as flavourings, perfumes and pharmaceuticals (for their functional properties).[5] The presence of some of main constituents of Essential oils; alpha pinene, camphene, beta pinene, cineole, fenchone, borneol and anethol (Figure 1) together in a pharmaceutical formulation considered as a potential urinary tract antiseptic. The literature contains several methods; including gas chromatography mass spectrometry (GC-MS) [6] and headspace gas chromatography (HSGC) for determination of the previous components in essential oils but there is no specific and rapid method to determine these seven volatile organic components in a commercial

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pharmaceutical formula. In analytical chemistry, the trend is toward simplification and miniaturization of the sample preparation step, and decrease in the time and the quantities of organic solvents needed for the extraction. The aim of the present work is to develop a rapid, simple, accurate, specific and reproducible method of analysis that is capable of identification and simultaneous determination of the seven volatile compounds; alpha pinene, camphene, beta pinene, cineole, fenshone, borneol and anethol in a soft gelatin capsule formula as per International Conference on Harmonization (ICH) guideline [7] by GC-FID with only one injection.

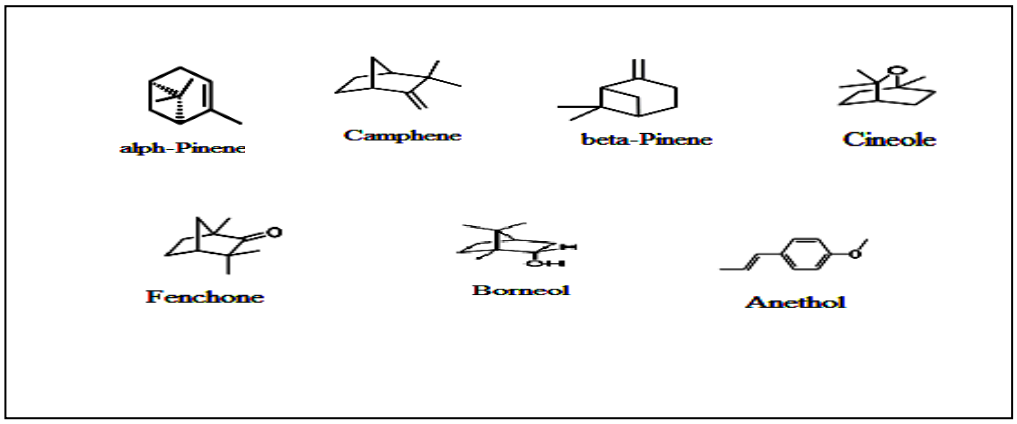

Figure 1. Chemical structure alpha pinene, camphene, beta pinene, cineole, fenchone, borneol and anethol.

2. MATERIAL AND METHODS

2.1. Apparatus

Hewlett Packard GC system 5890 II with flame ionization detector (FID) was used in this study.The compounds were separated on HP-5MS® (5% diphenyl and 95% dimethylpolysiloxane) (30m x 0.25mm x 0.25µm) column obtained from Arabian AGITECH Group for Integrated Technologies (Cairo, Egypt). The column initial temperature was 100°C then it was programmed at 10°C/minute to 190°C, which was maintained for 0.5 min. Helium was used as carrier gas with a flow rate of 1ml/min and split ratios 1:50. The injector and detector temperatures were 200 and 250°C respectively. The injection volume was 1 µL.

2.2. Chemicals and reagents

The solvent used for the analysis was n-Hexane 95% HPLC/Spectro (CAS 110-54-3) and was obtained from Tedia Company (USA). The standards of alpha pinene, camphene, beta pinene, cineole, fenchone, broneol, anethol and menthone were obtained from Destilaciones Bordas Chinchurreta, (Sevilla , Spain). Urinex® soft gelatin capsules was obtained from Pharco Pharmaceutical Coporation, Alexandria ,Egypt. Each soft gelatin capsule claims to 24.8 mg alpha pinene, 6.2 mg beta pinene , 15 mg camphene, , 3 mg cineole, 4 mg ®fenchone, 10 mg borneol and 4 mg anethol

2.3. Preparation of internal standard (IS)

Menthone (60mg) was accurately weight, transferred into a 25 ml volumetric flask, 20 ml n-hexane 95 % was added, shook well to dissolve, completed to volume with the same solvent and mixed well.

2.4. Preparation of standards

248 mg of alpha pinene, 150 mg of camphene, 62 mg of beta pinene, 30 mg of cineole, 40 mg of fenchone, 100 mg of broneol, 40 mg of anethol were accurately weighted and transferred into a 100 ml volumetric flask, dissolved in 50 ml n-Hexane 95%, The volume was made up to the mark with the same solvent and mixed well.

10 ml of the previous solution and 5 ml of internal standard solution were transferred into a 50 ml volumetric flask, diluted to volume with n-Hexane 95%, mixed well.

2.5. Preparation of pharmaceutical formulation

Twenty capsules were opened in a Petri dish. About 100 mg of medicine were accurately weighted, transferred into a 50 ml volumetric flask, 30 ml n-hexane 95% were added to dissolve, 5 ml of internal standard solution were added, diluted to volume with n-Hexane 95%, mixed well and filtered through membrane filter 0.45µm porosity.

3. VALIDATION OF THE METHOD

3.1. System Suitability

As per United States Pharmacopeia (USP) 29 [8] system suitability test was carried out on freshly prepared standard solution of the seven volatile compounds. The parameters coefficient of variation (% CV) for peak ratio, tailing factor, theoretical plates and resolution were evaluated for five replicate injections (Table 1).

Table 1. System Suitability and Chromatographic parameters

	%CV of peak Ratio n=5	Tailing Factor	Resolution	Theoretical Plates
Alpha pinene	0.446	0.95	-----	140069
Camphene	0.444	1.15	3.52	126266
Beta pinene	0.699	1.01	5.49	148479
Cineole	0.835	1.02	12.18	172625
Fenchone	1.324	0.97	15.14	193710
Borneol	0.602	1.04	2.24	237731
Anethol	1.746	0.94	31.71	326251

3.2. Linearity and Calibration Curve Characteristics

Linearity was demonstrated by preparing five standard solutions within the range of about 50% to 150% of the nominal sample concentration for the seven components. Each solution was prepared by serial dilution from a single stock and was injected in duplicate. Linear regression analysis was performed, excluding the origin as a point.

3.3. Accuracy

Accuracy and recovery of the method was demonstrated by analyzing data obtained from spiked placebo solutions at three concentration levels 80, 100, 120% of the nominal concentration value of each component then the percent recovery was calculated for each component at the three concentration levels also the pooled coefficient of variation (% CV) for the three concentration levels was calculated.

3.4. Precision

The repeatability of the method for assay was demonstrated by preparing six synthetic samples for at 100% of label claimed concentration of each component. The samples were analyzed according to the analytical method and the percent label claim for each compound was determined for each sample. Then the coefficient of variation (% CV) for the six samples for each component was calculated. The intermediate precision of the method was demonstrated by repeating the repeatability experiment with a second analyst in another day then Student t-test [9] (comparison between two experimental means) and F-test (comparison between Standard deviations) was calculated.

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

The LOD and LOQ of the method were determined by evaluating solutions containing the seven compounds at several different lower concentrations. Five injections were made at each concentration and the coefficient of variation (% CV) and signal to noise ratios for each compound were determined. LOD was reached when signal/noise (S/N) ratio is 3, while LOQ was defined as the point where S/N = 10.

4. RESULTS AND DISCUSSION

Figure 2 shows the peaks and retention times of the well resoluted seven compounds. The retention time of the last eluted compound is 6.127 minutes.

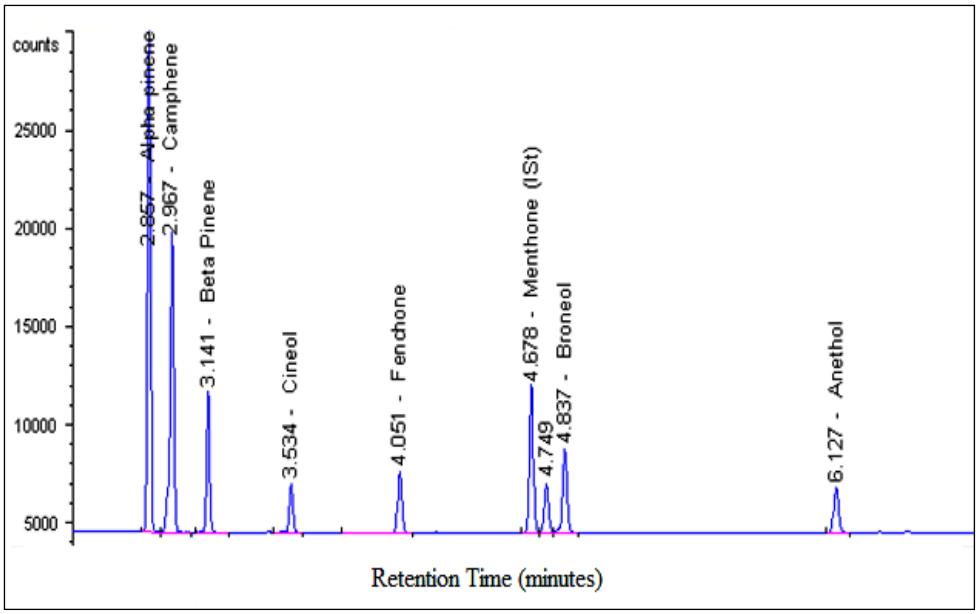

Figure 2. Typical chromatogram of separation of the seven volatile compounds; alpha pinene, camphene, beta pinene, cineole, fenchone, borneol and anethol.

Table 2 shows the linear range of the seven volatile compounds. The higher linear range value was for alpha pinene (0.24 ～0.73 mg ml $^{-1}$) while the lowest one was for cineole (0.027 ～0.081 mg ml $^{-1)}$ due to the high concentration of alpha pinene and low concentration of cineole in the tested soft gelatin capsule formula. There was a linear response at the mentioned concentration ranges for the seven compounds where (r) value ranged from 0.997 for anethol to 0.9998 for fenshone and beta pinene. Moreover, the line equations for the seven compounds as per (Table 2) illustrated that the *y* intercept did not show significant difference from zero. The method repeatability is presented in Table 3. The coefficient of variation (% CV) was ranged from 0.532 % for alpha pinene to 1.518 % for cineole and the mean recovery (%) ranged from 99.8% for Beta pinene to 100.9% for Alpha pinene which indicates adequate method repeatability. Moreover, Table 4 shows that there is no significant difference between the two means and the two standard deviations (SD) obtained by the two analysts. The t- test$_{calc}$ values were less than t- test $_{theo}$ values for all the seven compounds and F-test values were also less than F- test $_{theo}$ for all the seven compounds.

Table 2: Linearity of standard curves

	Mean Recovery (%) ± SD	**% CV**
Alpha pinene	100.90 ± 0.537	0.532
Camphene	99.79 ± 0.850	0.852
Beta pinene	99.80 ± 1.330	1.332
Cineole	100.23 ± 1.522	1.518
Fenchone	99.66 ± 0.849	0.852
Broneol	100.27 ± 1.172	1.169
Anethol	100.05 ± 1.284	1.283

n=6

Table 3. Precision of the method

	Linear range mg ml $^{-1}$	Regression Equation	r
Alpha pinene	0.24 ～0.73	Y = 0.021 + 5.412 X	0.9997
Camphene	0.14 ～0.44	Y = 0.014 + 5.752 X	0.9997
Beta pinene	0.064 ～0.19	Y = 0.005 + 5.424 X	0.9998
Cineole	0.027 ～0.081	Y = 0.0014 + 4.59 X	0.9997
Fenchone	0.038 ～0.11	Y= 0.003 + 4.434 X	0.9998
Broneol	0.10～0.30	Y = 0.032 + 2.863X	0.9997
Anethol	0.054 ～0.16	Y= 0.0480+3.884 X	0.9979
Y: Peak Ratio X: Concentration in mg ml $^{-1}$			

Table 4. Intermediate precision

	t- test $_{calc}$	F- test $_{calc}$
Alpha pinene	0.411	1.055
Camphene	0.384	1.153
Beta pinene	0.963	1.146
Cineole	0.172	1.982
Fenchone	0.255	1.014
Broneol	0.753	1.083
Anethol	0.175	1.96
t- test $_{theo}$= 2.228 F- test $_{theo}$= 5.055		

As shown in Table 5, the recoveries obtained were very good, indicating that the extraction procedure employed was satisfactory. The pooled coefficient of variation (%CV) for the three concentration levels 80,100, and 120% of the nominal concentration value of each component was less than 2%, the smallest (%CV) was for borneol and the highest one was for anethol.

Table 5. Recovery of the method

	Grand Mean (%)	Pooled Coefficient of Variation (% CV)
Alpha pinene	99.87	1.41
Camphene	99.96	1.37
Beta pinene	99.69	1.28
Cineole	99.94	1.22
Fenchone	100.03	1.21
Broneol	99.68	1.13
Anethol	99.43	1.44

Table 6. The limit of detection (LOD) and limit of quantification (LOQ)

	LOQ mg ml $^{-1}$	LOD mg ml $^{-1}$
Alpha pinene	0.0471	0.0141
Camphene	0.0312	0.0094
Beta pinene	0.0139	0.0042
Cineole	0.0049	0.0015
Fenchone	0.0051	0.0015
Broneol	0.0141	0.0042
Anethol	0.0374	0.0112

The limit of detection (LOD) and limit of quantification (LOQ) for the method are shown in Table 6. The detection limits (S/N = 3) ranged from 0.0015 to 0.0141 mg ml $^{-1}$ and the quantification limit (S/N = 10) ranged from 0.0049 to 0.0471 mg ml $^{-1}$ The lowest LOD was for cineole (0.0015 mg ml $^{-1}$) and the highest LOD was obtained for alpha pinene (0.0141 mg ml $^{-1}$).

5. CONCLUSIONS

A validated GC-FID method was developed and validated for identification and determination of the seven volatile components in pharmaceutical formulation. The proposed method is fast, cost effective and consumes a little quantity of solvents with a total run time of about 6.5 minutes. The proposed method was found convenient and reproducible for analysis of these compounds in such pharmaceutical dosage form.

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