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Application of GC-MS in Quantitative Analysis of Some Carminative Syrups

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Abstract: *Gas chromatographic analysis was applied for the quantitative estimation of the essential oil(s) present in some carminative syrups in the Egyptian market. Depending on the fact that each essential oil has its unique component(s);the unique component was used as a marker compound in the quantitative determination of such oil. A calibration curve for each standard material was constructed to be used in these analyses. Thymol(r^2=0.9981) for Thyme;R-(-)-Carvone(r^2= 0.9991)for Caraway or Dill separately;Cinnamaldehyde(r^2 =0.9998) for Cinnamon; Trans-Anethole (r^2= 0.9988) for Fennel;(±)Menthol (r^2=0.9988) for Peppermint;1,8-Cineol(r^2=0.9980) for Cardamom or Eucalyptus separately;(-)-α-Bisabolol(r^2=0.9984) for Chamomile and R-(+)-Limonene(r^2=0.9967) for Lemon oil. A validated GC-MS method was successfully applied for the quantitative determination of the components of different essential oils either as a separate raw material or within the pharmaceutical product(syrup).*

Key words:*Essential oils, syrups, GC-MS.*

I. Introduction

Volatile oils are known to have antispasmodic and carminative action as they stimulate the intestinal motility by increasing muscle tones. Over years, many medicinal herbs such as Cinnamon, Caraway, Fennel, Chamomile, Cardamom, Peppermint, Thyme, Eucalyptus, Lemon, and Dill have a focus of interest due to their volatile oil content. It was reported that the essential oils have various activities, such as carminative, anti-spasmodic, analgesic and other indications(1).The well-known properties of Dill in traditional medicine stated that it is used as carminative, stomachic and diuretic. Also, it was reported that Dill has antibacterial and antioxidant activity (2). Fennel is a well-known medicinal and aromatic plant the volatile oil of fennel has antispasmodic;antiflatulence properties, it is used for dyspeptic complaints and as hepatoprotective drug (3). The peppermint oil has smooth muscle relaxant activity; conforming its antispasmodic activity (4). Ethanolic extract of cardamom as well as its essential oil had shown antispasmodic activity (5). Also, antiulcer activity was observed upon use of the aqueous or methanol extracts of cardamom seeds to mice (6). Chamomile oil has been used for symptomatic treatment of digestive ailments such as dyspepsia, epigastric-bloating impaired digestion and flatulence (7). Thyme extract has been used to treat dyspepsia and other gastrointestinal disturbances; cough, bronchitis, laryngitis and tonsillitis (8). Cinnamon was used for treatment of dyspeptic conditions, fullness and flatulence, loss of appetite and abdominal pain with diarrhea, and amenorrhea and dysmenorrheapains (9).A double-blind study involving 45 patientswith non-ulcer dyspepsia assessed the change in pain intensity and ClinicalGlobal Impression after treatment with an enteric-coated capsule containinga combination of different essential oils (90 mg) and caraway oil (50 mg). After4 weeks of treatment, 63% of patients were free of pain; 89.5% had less pain; and94.5% showed improvements. Inanother study, oral administration of the essential oil (0.2 ml) delayed the gastricemptying time in healthy volunteers and in patients with dyspepsia (10).

II. Materials& Methods

1. **Three Pharmaceutical Products (syrups) containing volatile oils were purchased from the Egyptian Pharmaceutical Market, these are:**

- **Carminex syrup**, Three batches were used 050514, 060514 and 070514;each 100 g contains 0.1 g Cinnamon oil, 0.1 g Caraway oil and 0.1 g Fennel oil, manufactured by Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco-Medifood) (Cairo, Egypt).
- **Top Calm syrup**, Three batches were used83008, 83009 and 83011;each 100 g contains 0.01 g Chamomile oil, 0.005 g Dill oil, 0.005 g Cardamom oil, 0.005 g Cinnamon oil and 0.02 g Peppermint oil manufactured by EIMC United Pharmaceutical for Tetrapharm Company for Pharmaceutical & Chemical Industries (Cairo, Egypt).
- **Followcease syrup**, Three batches were used3556, 3680 and 3689;each 5 g contains 0.005 g Thymus Extract, 0.001 g Eucalyptus Extract, 0.0005 g Peppermint oil and 0.0015 g Citrus Lemon oil, manufactured

by Unipharma Pharmaceutical Industries for the National Arabian Company for Pharmaceutical Agencies (NAPHA) (Cairo, Egypt).

2. Equipments

- **Gas Chromatography-Mass Spectroscopy:** Shimadzu GC-MS, Model QP-2010 Ultra, equipped with head space AOC-5000 auto injector, Kyoto, Japan.
- **Column:**Rtx-5 MS (0.25mm x 30 m), 0.25 µmdf, Restek, USA.
- **Electronic Balance:** Model AUY220, Shimadzu Instrument, Kyoto, Japan
- **Ultrasonic bath:** NSXX Sonics Model NS-A-12-7H, Germany.

III. Chemical Reagents:

All chemical, reagents and standards used were GC grade. Pure standards of Volatile oil were obtained as follow:1,8-Cineol, Trans-Anethole 99%, (R)-(-)-Carvone 98% and (-)-α-Bisabolol 95% were purchased from **Sigma-Aldrich**. Thymol≥ 99%, (±)-Menthol ≥99% and (R)-(+)-Limonene 90% purchasedfrom**Fluka.**Cinnamaldehyde> 98% was obtained from **Merck.**Hexane≥97% was HPLC and GC grade from **Sigma Aldrich**.

IV. Experimental

4.1. Chromatographic conditions:

The GC/MS analysis was carried out using Shimadzu GC-MS, Model QP-2010 Ultra, equipped with head space AOC-5000 auto injector, under the following condition:

Column: Rtx-5 MS (0.25mm x 30 m), 0.25 umdf, Restek, USA.
Injection volume: 5 µl.
Flow rate: 0.99 ml/min.
Injection Temp.: 210°C.
Injection Mode: Splitless
Split ratio: 10
Carrier gas: Helium.
Oven Temperature Program:

Rate (°C/min)	Temperature (°C)	Hold Time (min)
----	40	2
5	210	5

[GCMS-QP2010 Ultra]
Ion SourceTemp: 230°C
Interface Temp.: 280°C
Solvent Cut Time: 2 min
Detector Gain Mode: Relative
Detector Gain: +0.00 kV
Threshold: 0

[MS Table]
Start Time: 2.5 min
End Time: 41 min
ACQ Mode: Scan
Event Time: 0.3 sec
Scan speed: 1666
Start m/z: 35
End m/z: 500

4.2. Method Validation:

4.2.1. Calibration curves:

The stock standard solution of each standard pure was prepared as follows: about 10-30 mg of each compound was accurately weighed and placed into a 25 ml volumetric flask. n- Hexane was added and the solution diluted to volume with the same solvent.

Calibration curves were established on five data points covering the concentration range of 60–1200µg/ml for Trans-Anethole, 60– 1200 µg/ml for Carvone, 60–1200µg/ml for Cinnamaldehyde, 40–800µg/ml for Menthol, 20–400µg/ml for 1,8- Cineol, 40–400 µg/ml for (-)-α-Bisabolol, 80–400 µg/ml for Thymol, 112–560 µg/ml for R-(+)-Limonene.

5 microliter aliquots of each standard solution were used for GLC analysis. Triplicate injections were made for each standard solution. Each calibration curve was obtained by plotting the peak area of the essential oils at each level prepared versus the concentration of the sample.

- (Dilution was done case related).

Table (1):Statistical analysis for the calibration curves of the standards in the essential oil

Compound	Linearity range (µg/ml)	Slope, a	Intercept, b	r^2
Cinnamaldehyde	60-1200	1.7645	1.2959	0.9998
Carvone	60-1200	1.6675	-0.2419	0.9991
T- Anethole	60-1200	1.3494	33.7650	0.9988
Bisabolol	40-400	9.8720	- 4.3253	0.9984
Menthol	40-800	5.0815	- 49.5837	0.9988
Cineol	20-400	5.3144	- 0.4788	0.9980
Limonene	112-560	3.7252	1.7430	0.9967
Thymol	80-400	7.8558	- 0.7241	0.9981

S.D. values are given in parenthesis.
a for each curve the equation is $y = ax + b$, where y is the peak area, x is the concentration of the analyte (µg/ml), a is the slope, b is the intercept and r^2 is the correlation coefficient.

4.2.2. Recovery

The accuracy of the method was evaluated with the recovery test. This involved the addition of known quantities of essential oils standards to known amounts of Carminative Syrup. The samples were then extracted and analyzed with the proposed GC-MS method. The percentage recovery was determined by subtracting the values obtained for the control matrix preparation from those samples that were prepared with the added standards, divided by the amount added and then multiplied by 100.

Table (2): Results of the recovery test for the used essential oil & extract

Compound	Spiked amount (mg)	Recovery (%)	Mean (n = 5)	R.S.D. (%)
Cinnamaldehyde	0.525	99.50-101.32	100.01	1.62
Carvone	0.552	98.82-102.42	101.62	1.82
T- Anethole	0.542	97.97-100.46	99.92	1.71
Bisabolol	0.742	98.68-101.88	100.88	1.46
Menthol	0.624	99.65-101.24	100.71	1.43
Cineol	0.764	98.26-100.58	99.17	1.29
Limonene	0.524	97.70-102.25	100.98	0.98
Thymol	0.662	98.22-101.87	100.85	1.69

R.S.D. (%) = (standard deviation/mean)×100.

4.2.3. Limits of detection and quantification

Limits of detection (LOD) were calculated according to the expression $3.3\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. Limits of quantification (LOQ) were established by using the expression $10\sigma/S$. LOD and LOQ were experimentally verified by injections of pure standard at the LOD and LOQ concentrations.

Table (3): Limit of detection (LOD) and limit of quantification (LOQ)

Compound	LOD (µg/ml)	LOQ (µg/ml)
Cinnamaldehyde	13.33	40
Carvone	13.33	40
T- Anethole	13.33	40
Bisabolol	10.00	30
Menthol	6.67	20
Cineol	5.00	15
Limonene	33.33	100
Thymol	20.00	60

4.3. Standard Preparation:

10-30 mg of each of the following standards was accurately weighed and transferred into 25 ml volumetric flask; then dissolved in hexaneand sonicated for 5 minutes, and then the volume was completed to 25 ml with hexane.

- (Dilution was done case related).

4.4. Raw Material:

Sample Preparation:

- **Essential oils:**

Accurately weighed separately 100mg Cinnamon oil, Caraway oil, Fennel oil, chamomile oil, Dill oil, peppermint oil, lemon oiland transfer each oil separately into 50 ml volumetric flask; then dissolved in hexane and sonicated for 5 minutes. Then completed to50 ml with hexane and mixed well.

- (Dilution was done case related).

- **Extracts:**

Accurately weigh separately50 gm of Thymus extract and Eucalyptus extract; transfer each extract separately to a separating funnel and extract three successive times each of 15 ml n-hexane. The combined hexane of each extract was then separately filtered over phase separation paper and transferred into 50 ml volumetric flask. Then the filtrate volume was completed to 50 ml volumetric flask with hexane.

- (Dilution was done case related).

Table (4): Results of the analysis of the active raw materials:

Raw material	Active principal	Limit%	Average %6 assays	Concentration mg/g
Cinnamon oil	Cinnamaldehyde	55-75	60	600
Caraway oil	Carvone	50-65	55	550
Fennel oil	T. Anethole	55-75	67	670
Chamomile oil	Bisabolol	10-65	45	450
Peppermint oil	Menthol	30-55	43	430
Dill oil	Carvone	43-63	51	510
Cardamom oil	Cineol	25-45	36	360
Lemon oil	Limonene	56-78	65	650
Thymus extract 1:1 1.2 % VO	Thymol	0.44-0.66	0.55	5.5
Eucalyptus extract 1:1 1.5% VO	Cineol	0.5-0.8	0.70	7.0

4.5. Syrups

Sample Preparation:

The content of six bottles (2 from each batch) of each syrupwere mixed well; then 100 gof each syrup was accurately transferred into 250 ml conical flask. Extraction of the volatile oil content of the syrup was done using three portion of hexane each of 15 ml. The combined hexane extract was then filtered over phase separation paper and transferred into 50 ml volumetric flask. Then the filtrate volume was completed to 50 ml volumetric flask with hexane and sonicated for 5 min. Both the samples and the standards were injected at least three times.

Table (5): Results ofcarminative syrups of analysis:

Carminex Syrup:					
Raw material RM	g/100g syrup	Active principal	mg/g RM	Expected mg/100g syrup	Determined Conc. mg/100g syrup
Cinnamon oil	0.1 g	Cinnamaldehyde	600	60.0	59.930
Caraway oil	0.1 g	Carvone	550	55.0	55.001
Fennel oil	0.1 g	T. Anethole	670	67.0	66.887
Top Calm Syrup:					
Raw material RM	g/100g syrup	Active Constituent	mg/g RM	Expected mg/100g syrup	Determined Conc. mg/100g syrup
Chamomile oil	0.01 g	Bisabolol	450	4.50	4.485

Peppermint oil	0.02 g	Menthol	430	8.60	8.540
Dill oil	0.005 g	Carvone	510	2.55	2.546
Cinnamon oil	0.005 g	Cinnamaldehyde	600	3.00	2.995
Cardamom oil	0.005 g	Cineol	360	1.80	1.788
Follow Cease Syrup:					
Raw material RM	g/100g syrup	Active Constituent	mg/g RM	Expected mg/100g syrup	Determined Conc. mg/100g syrup
Thymus Extract	0.1 g	Thymol	5.5	0.550	0.548
Eucalyptus Extract	0.02 g	Cineol	7.0	0.140	0.138
Peppermint oil	0.01g	Menthol	430	4.30	4.297
Citrus Lemon oil	0.03 g	R-(+)-Limonene	650	19.50	19.24

V. Results and Discussion

The used method GC-MS for the analysis of pharmaceutical product(syrup) proved that it is an accurate, precise, fast and easy method for the quality control of complex herbal pharmaceutical products containing volatile oils such as carminative syrups.

This fact is clear and can be deduced the obtained results of Calibration curves table (1) where we can found the upper and lower value of linearity (20-1200 µg/ml), slope (1.3494-9.8720), intercept (49.5837-33.7650) and the correlation coefficient (0.9967-0.9998).

The percentage recoveries table (2) range form (97.97-102.42%). Limit of detection (LOD) and limit of quantification (LOQ) table (3) where we can found the upper and lower value of LOD (5.00-33.33 µg/ml) and LOQ (15-100 µg/ml) as well as the reducibility of the obtained results for the different raw material as well as tested syrups table (5).

IV. Conclusion

The applied GC-MS method is of great value for the quality control of the herbal pharmaceutical products containing complex mixture of volatile oils.

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