Original Article

TIPS Trends in Pharmaceutical Sciences 2019: 5(2): 103-110.

A Gas Chromatographic-Based Method for Determination of γ-terpinene in a herbal solid dosage form

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Abstract

Gastrointestinal reflux, as a physiologic process, commonly occurs following a daily meal with no sign. But in pathologic condition, it may be accompanied by some complications such as heartburn. In Traditional Persian Medicine (TPM), Heartburn has been defined as "*Harghat-e-me'adeh*" or "*Josha-e-hāmez*". There are many formulations for the management of this complication in TPM. Current work aimed to reformulate and standardized a multi-ingredient preparation from *Qarabadin* textbooks of Persian medicine, containing True cardamom, Ginger, Black cumin and Mastic. Following content authentication and microscopic characterization, various tablet formulations were prepared using related excipients. The final formulation was a tablet containing those ingredients, with avicel and Magnesium stearate. Subsequently, tablets were extracted via the hydrodistillation method to yield the essential oil. Chemical composition analysis of essential oil was carried out using Gas Chromatography/Mass spectrometry apparatus (GC/MS). α-terpinyl acetate (34.02%), 1,8- cineole (18.07), cumin-aldehyde (8.15), and γ-terpinene (11.74%) were identified as major constituents. The content determination of tablets was performed via Gas Chromatography/Flame ionization detector (GC/FID). Data on pharmaceutical assessment included friability (less than 1%), weight variation (less than 1%), hardness (6.5±0.5 kg/cm^2), disintegration time (less than 35 min) and thickness (3.02±.04 mm). The yield of essential oil extracted from the tablet was found 3%, and each tablet contained 1.33 mg γ-terpinene. This finished product can be introduced as medicine for the clinical trial and pharmaceutical industry.

Keywords: Gas chromatography, Formulation, Tablet, Quantification.

1. Introduction

As a recurrent and chronic complication, gastrointestinal reflux disease (GERD), is mentioned as regurgitation of the stomach contents into the esophagus (1). In addition to the high prevalence of this disorder around the world, in Iran, GERD is also increasing during recent decades (2).

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Among various conventional treatment lines, proton pump inhibitors, prokinetic agents and H_2 receptor antagonists are known common. In addition to these therapies, scientists and physicians have currently considered complementary and alternative aspects of treatment (3). Traditional Persian Medicine (TPM) encompasses complementary and integrative aspect to various diseases and complications. This school of holistic medicine, not only is a collection of prior experiments but also a summation of indigenous information

Recieved: 27/03/2019; Accepted: 28/04/2019

and findings of early Persian healers in diagnosis, prevention, and treatment (4).

Medical and pharmaceutical manuscripts of TPM have cited a large part for gastrointestinal disorders and allied treatments. GERD is a complication with plenty of symptoms, mostly indicated as heartburn or *herqat-al me'deh* in the Persian manuscript. Many medicinal plants have been mentioned effective for this complication in those concerned manuscripts (5). These medicaments have been applied solely or in addition to other natural medicines with gastrointestinal effective approaches. The combination of these natural effective medicines as multi-ingredient preparations have been commonly reported in specific traditional textbooks, known as *Qarābādins* or formulary books (6). There are many multi-ingredient or compound medications for GERD or related disorders in these textbooks. One of those highly cited preparations for such disorder in *Qarābādin* textbooks is the Mastic pill (*Habb-e-Mastaki*) (7). This formulation contains four main ingredients including fruit of *Bunium persicum* (Boiss.) B.Fedtsch. (Family; Apiaceae), rhizome of *Zingiber officinale* Roscoe (Family; Zingiberaceae), fruits of *Elettaria cardamomum* (L.) Maton (Family; Zingiberaceae), and gum of *Pistacia lentiscus* L. (Family; Anacardiaceae).

Among most compound preparations for various complications in TPM, very few of those have been reformulated, standardized and determined in regard to content and specific markers to be introduced as medicine for pharmaceutical markets. In this regard, the current study aimed to reformulate this compound preparation in terms of a conventional tablet in line with aspects of standardization of natural multi-ingredient medicines.

2. Material and methods

2.1. Selection of the product's ingredients

Fruit of Bunium persicum (BP), the rhizome of *Zingiber officinale* (ZO), fruits of *Elettaria cardamomum* (EC), gum of *Pistacia lentiscus* (PL) were prepared from medicinal plants markets and authenticated by Department of Phytopharmaceuticals (Traditional Pharmacy), Shiraz University of Medical Sciences.

2.2. Tablet preparation

All components were undergone the direct compression tablet preparation procedure using various expedients. For each prepared tablet, common pharmaceutical tests such as friability, hardness, and disintegration were considered.

2.3. Fourier-transform infrared spectroscopy (FT-IR)

The presence or absence of functional groups and chemical structure in herbal components or herbal medicines can be relatively indicated by FT-IR (8). For this purpose, this procedure was performed for all components and final products. Prior screening of herbal component, calibration of the spectrometer was carried out. To this, polystyrene or polyethylene films were employed. To prepare the samples, pure dry potassium bromide (400 mg) was mixed and pressed with 5-15 mg of powder of each tablet components as well as the tablet, individually to prepare a clear thin tablet.

2.4. Essential oil composition and Gas chromatographic analysis

The essential oil of the final product as a form of a compressed tablet was extracted using a Clevenger. The oil was isolated, and stored in dark amber glass bottles at 4 °C. Gas chromatography/Mass spectrometry (GC/MS) analysis was performed using Agilent technologies model 7890A gas chromatograph with a mass detector. The gas chromatograph was equipped with an HP-5MS capillary column (phenylmethyl siloxane, L×I.D. 30 m×0.25 mm, Agilent technologies (60 to 325/350 ºC). The oven temperature was programmed from 60 (at 0 min) to 250 °C at the rate of 5 °C/min and subsequently was held for 10 min at 250 °C. The carrier gas was helium and the flow rate was adjusted to 1 ml/min. The mass spectrometer (Agilent technologies 5975 C) operated in EI mode at 70 eV. The interface temperature was set at 280 °C and the mass range was from 30-600 m/z. components identification was carried out based on a comparison of calculated Kovats retention indices of each constituents using retention times of simultaneously injected normal alkanes (C_8-C_{18}) as well as their mass spectra with Willey

Table 1. Different tablet formulations from direct compression method.

No	BP (%)	EC (%)	PL (%)	ZO (%)	Mg stearate (%)	Avicel (%)	Outcome
1	31	31	31	7	-	-	Low hardness
2	30	30	30	9.8	0.2	-	Low hardness
3	28	28	28	6	-	10	Inappropriate
4	28.14	28.14	28.14	10.5	-	5	Inappropriate
5	23.5	23.5	23.5	9.3	0.2	20	Hardness ≥ 10
6	25	25	25	9.8	0.2	15	Hardness ≥ 10
7	25.5	25.5	25.5	9.5	0.2	14	Hardness ≥ 10
8	25	25	25	25	0.2	13	Hardness ≥ 10
9	25	25	25	25	0.2	12	Hardness ≥ 8
10	25	25	25	25	0.2	11	Hardness ≥ 8
11	28	28	28	6	0.2	9.8	Appropriate

(nl7) and Adams libraries spectra (9).

2.5. Determination of major-specific marker in the finished product

The essential oil extracted from the final formulation (Tablet) was injected to gas chromatograph/flame ionization detector (GC/FID) for content determination. GC/FID analysis was carried out via a gas chromatograph, Agilent technologies apparatus (7890A) with HP-5 column (25 m×0.32 mm, 0.52 µm film thickness) coupled with a flame ionization detector (FID). Nitrogen was as carrier gas with a split ratio of 1:30 flow and rate of 1 ml/min. The injector and detector temperatures were adjusted at 250 °C and 280 °C, respectively. The column temperature was linearly programmed from 60 to 250 °C (at the rate of 5 °/min) and then held for 10 min at 250 °C. The stock solution of reference compound (γ-terpinene) as a main constituent in the essential oil) was prepared separately in methanol at a concentration of 42.5 mg/ml. Dilutions containing 17, 8.5, 1.8 and 0.75 mg/ml of thymol in dichloromethane were then prepared from the stock solution and injected to GC-FID in order to set a calibration curve. In addition to the standard solutions, the essential oil yielded from the tablet was also prepared as the solution of 180 mg/ml to inject to GC. Approximately, 1µl of standard solutions was injected to GC/FID for three times. Calibration curves of γ- terpinene at mentioned concentrations were determined from the fresh stock solution (42.5 mg/ml).

3. Result and Discussion

3.1. Tablet Preparation

Results of proportion and types of ingredients and expedients were shown in Table 1. Out of 54 formulations, 11 of those have been presented in this table. Mainly, Avicel (microcrystalline cellulose) and Magnesium stearate were used in the preparation of tablets. The final formulation contained 9.8 % Avicel, 0.02 % Mg-stearate, mainly 28 % of each BP, EC, and PL, and 6% of ZO raw materials. Figure 1 represented tablet shapes and color. The hardness of 10 tablets for the selected formulation was found 6.5±0.5 Kg/Cm^2. The disintegration time for the finished product was about 35 min. The friability of prepared tablets was less than 1%. The mean weight of each finished tablet was found 650±33 mg out of 20 tablets.

3.2. Fourier-transform infrared spectroscopy (FT-IR)

Figure 2 represented a frame in which all spectra of components, as well as the spectrum of the finished product, are cited. Although in-detailed analysis of the IR profile of those components can-

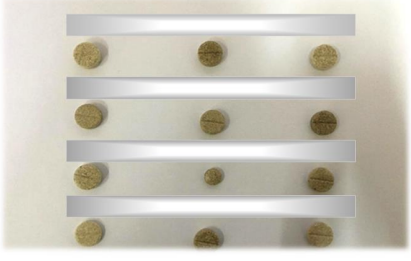

Figure 1. Shape and color of prepared tablets.

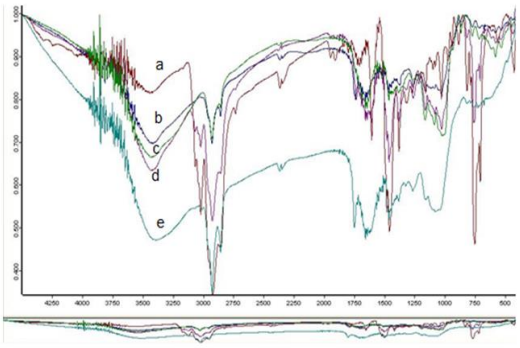

Figure 2. FT-IR fingerprint of Mastic tablet and four components. a: PL; b: EC; c:ZO; d:Tablet; e: BP.

non clearly be determined, this pattern can roughly show the complexity of functional groups of metabolites in those assessed components.

3.3. Essential oil composition

The yield of final tablets was determined by 3% W/W from the hydrodistillation procedure for 4 hours. According to GC/MS analysis, the main determined constituents were α- terpinyl acetate (34%), 1,8- cineole (18.07), cumin-aldehyde (8.15) and γ- terpinene (11.74%). Table 2 represents the chemical composition of yielded essential oil from the finished product. GC/MS profile of finished product essential oil with representing

Table 2. Chemical composition of the selected tablet essential oil.

No.	Component	Area (%)	KICal.	KIRef.	Ref.
1	α- thujene	0.35	930	930	(10)
2	α- pinene	1.54	939	939	(11)
3	Camphene	0.63	953	953	(12)
4	Sabinene	3.12	973	973	(11)
5	β- pinene	1.04	980	980	(13)
6	β- myrcene	2.2	991	991	(11)
7	ρ- cymene	0.3	1025	1026	(14)
8	1,8- cineole	18.07	1038	1037	(15)
9	Indene	0.32	1051	1051	(16)
10	γ- terpinene	11.74	1066	1067	(17)
11	α- terpinolene	0.42	1088	1089	(18)
12	Sabinene hydrate	0.49	1099	1095	(19)
13	Linalool	0.66	1103	1100	(20)
14	Borneol	0.36	1166	1165	(21)
15	4- terpineol	2.83	1179	1177	(9)
16	β- fenchyl alcohol	2.34	1180	1180	(9)
17	Cuminaldehyde	8.15	1246	1246	(9)
18	Geranyl alcohol	1.5	1259	1259	(9)
19	Trans- citral	0.45	1271	1271	(9)
20	Cumic alcohol	4.74	1308	1308	(22)
21	α- terpinyl acetate	34.02	1355	1355	(9)
22	Geraniol acetate	0.56	1386	1385	(9)
23	Zingiberene	0.79	1495	1492	(9)
24	Nerolidol	0.34	1565	1565	(9)
Identification		96.96			

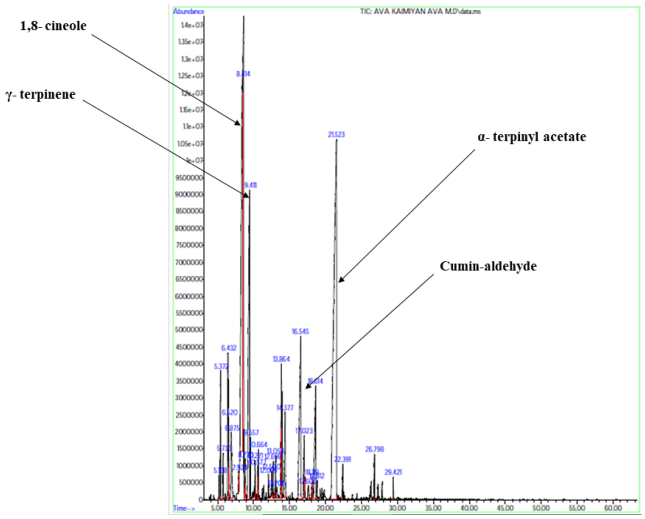

Figure 3. GC chromatogram of the final tablet.

the peak zone of major constituents was mentioned in Figure 3.

3.4. Quantification of γ- terpinene

Quantification of the specific or nonspecific markers in medicines is a need for standardization (23). Prior determination process, identification of γ- terpinene peak in the report graph for the essential oil extracted from the finished tablet was reconfirmed via comparison with an injection of 1 µl of this composition to GC/FID solely (Figure 4). Subsequently, a calibration curve was plotted and the respective equation was determined according to different concentrations of the standard (Figure 5). Based on the calibration curve, the R2 was found 0.99 and confirmed the linearity of the protocol. On the other word, the chromatogram was found linear over the concentrations ranging from 42.5 to 0.75 mg/ml. Afterward, 1 µl of prepared tablet essential oil (180 mg/ml) was injected to GC/FID in order to quantify the concentration of γ- terpinene in mentioned essential oil. Table 3 exhibited the determined γ- terpinene content in the essential oil of the finished product.

According to the finished product characteristics, the optimum formulation weight of the tablets was calculated as 650 mg, while the content of herbal ingredients in it was 585 mg. According to the essential oil extraction process, the volume of the yielded essential oil from each tablet is calculated by 0.003 ml and the amount of γ- terpinene in every 1 ml of the extracted essential oil was determined by 75.13 mg. In this regard, the amount of γ- terpinene in each tablet was 1.31 mg, when the purity of the standard γ- terpinene, employed in this assessment be 100%. However, the purity was mentioned as 96%. Accordingly, the actual amount will be 1.26 mg in each tablet.

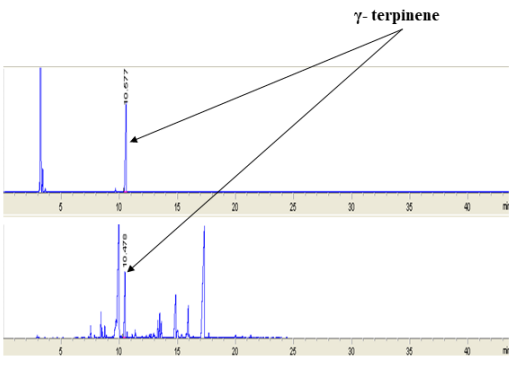

Figure 4. γ- terpinene peak, both in extracted essential oil and as a standard.

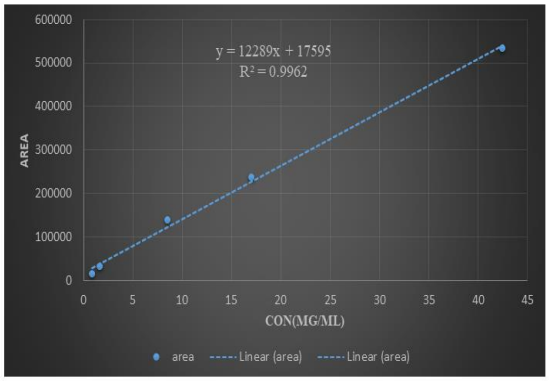

Figure 5. Standard calibration curve of γ- terpinene.

Table 3. Determined γ- terpinene content in the essential oil of the finished product.

Repeat	Area	γ- terpinene concentration (mg/mL)
1	940320	**75.13±2.1**
2	915290	
3	967320	
	940976±26021	

4. Conclusion

This study aimed to develop and perform some of the main parameters of the standardization of a traditional-based herbal medicine for the management of GERD. As described in related traditional manuscripts, a combination of Fruit of *Bunium persicum* (BP), the rhizome of *Zingiber officinale* (ZO), fruits of *Elettaria cardamomum* (EC), gum of *Pistacia lentiscus* (PL) in a solid form can be beneficial for the management of GERD. In order to facilitate the administration and patient's compliance, herein, a tablet form of this multi-ingredient medicine was developed and assessed in regard to a specific marker, via a gas-chromatographic based method.

Although traditional medicines have been prescribed for many centuries, however, standardization of those formulations is one of the main concerns in the field phytomedicine. In this survey, a Clevenger apparatus was utilized to extract the essential oil from developed Mastic tablets in order to be employed for quantifiable determination of a specific marker in the product. This current method was validated and thus can be suggested for quantitative assessment of markers in solid herbal formulations. According to the aforementioned report, this tablet with related determination can be introduced to industrial markets for complementary requirements and scale-up production.

Acknowledgments

The authors of this manuscript wish to express their thanks and appreciation to Shiraz University of Medical Sciences, Shiraz Iran (Project No: 1396-01-70-15703).

Conflict of Interest

None declared.

5. References

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