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RESEARCH ARTICLE

Formulation, stability and analytical method validation of peppermint oil solution

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Abstract

Tension type headache is the most common type of headaches causing mild, moderate and intense pain in the forehead and neck. There are prescription medicines -usually nonsteroidal anti-inflammatory drugs- and herbal remedies to alleviate the pain. As peppermint (*Mentha piperita* L.) is one of those most used medicinal plants in the world, its essential oil has also a wide range of medical applications like in tension type headache. In this present study a peppermint oil solution preparation in ethanol was developed, and an analytical method was validated to evaluate the stability. GC-FID and GC/MS analyses were used for the standardization, where thymol served as an internal standard. To the best of our knowledge, the peppermint oil preparation was evaluated and validated in the present form for the first time.

Keywords: Peppermint, *Mentha piperita* L., Validation, Formulation, Stability

Introduction

Peppermint (*Mentha piperita* L.) -a hybrid of water mint (*Mentha aquatica* L.) and spearmint (*Mentha spicata* L.)- is one of the most used medicinal plants in the world. The word "*Mentha*" is originated from "*Mintha* or *Minthe*", a nymph beloved by Hades and metamorphosed into a garden mint by goddess Persephone in ancient Greek mythology (Graves, 1955; Grimal, 1996). Mint leaves have been used in medicine for several thousand years, according to records from the Greek, Roman, and ancient Egyptian eras (Evans, 1991). It is cultivated in many parts of the world and the whole drug, extracts or essential oils are widely used in food, cosmetic and pharmaceutical industries because of its fragrance and flavouring properties.

Chemical composition of steam distilled peppermint oil is widely studied in literature as İşcan et al. (2002) mentioned that it contains mainly menthol and menthone. European Pharmacopoeia (EP 8.0, 2013) also describes the content of peppermint oil as 30-55% of menthol and 14-32% of menthone. Peppermint essential oil has a wide range of medical applications against colonic spasm (Asao et al, 2001), cough (Morice et al, 1994), digestive disorders (Giachetti et al., 1986), esophageal spasm (Pimentel et al., 2001), gastric spasm (Hiki et al., 2003), irritable bowel syndrome (Grigoleit & Grigoleit, 2005) and post-herpetic neuralgia (Davies et al., 2002).

According to Gobel et al. (1996) 10% solution of peppermint oil in ethanol efficiently alleviated tension type headache in a randomized, placebo-controlled, double-blind crossover study against paracetamol. Community Herbal Monograph on *Mentha x piperita* L. aetheroleum of European Medicine Agency (2007) also describes the same indication as a well-established therapeutic use.

In this study, we report the preparation of the dosage form of peppermint oil solutions and validated analytical method to evaluate its stability by GC-FID and GC/MS, respectively. To meet the CTD requirement of EMA, which contains production and quality control data in module three, required for registering a product in European Union, to the best of our knowledge for the first time.

Materials and Methods

%96 ethanol was obtained from Merck (Darmstadt, Germany), menthol, thymol and peppermint oil were purchased from Frey & Lau (Henstedt-Ulzburg, Germany).

Formulating and preparation of the dosage forms

10% (v/v) peppermint oil was prepared by diluting in ethanol; 25 mL amber bottles with special sponge applicators was selected as primary packaging material. 5%, 7.5%, 12.5% and 15% peppermint oil solutions in ethanol were produced for linearity and accuracy studies for validations.

Stability testing

Accelerated and long term stability tests for the products in primary packaging material were performed in a Binder KBF 115 (Tuttlingen, Germany) stability chamber according to Q1A(R2); Stability testing of new drug substances and products guidelines (ICH, 2003). Accelerated stability conditions were as follows; 40 ± 2°C, % 75 ± % 5 R.H meanwhile long term stability conditions were 25 ± 2°C, % 60 ± % 5 R.H. Long term stability control test periods were 1, 3, 6, 9, 12, 18, 24th months, respectively.

Assay method

The method was developed using an Agilent 7890B (California, USA) gas chromatography system equipped with flame ionization detector (FID). Peak purity studies were performed with Agilent 5977E electron ionization mass spectrometer (MS). An Agilent DB-Wax capillary column (60 m, 0.25 mm, 0.25 µm) was used for separation and G4513A auto injector was employed for sample injections. All system parameters are given in Table 1.

5 mL of product is transferred to a 25 mL volumetric flask and filled up to the volume with internal standard solution. 1 µL of the test solution was injected to the system according to the method parameters. Only menthol and thymol peaks were integrated and the peak area percentage ratio of menthol to thymol should be between 0.6 to 1.13; corresponding to 30% to 55% menthol in peppermint oil respectively as described in European Pharmacopoeia 8.0.

Table 1. Method parameters

Internal Standard Solution	10 mg/mL thymol in ethanol
Column	Agilent DB-Wax (60 m, 0.25 mm, 0.25 µm)
Injection	Split (50:1), 1 µL
Injector Temperature	200 ^{0}C
Carrier Gas Flow	1.5 mL/min He
Hydrogen Gas Flow	30 mL/min
Dry Air Flow	400 mL/min
FID Temperature	220 ^{0}C
Oven Temperature Program	60 ^{0}C isothermal for 10 minutes 2 ^{0}C/min ramp to 140 ^{0}C 10 ^{0}C/min ramp to 210 ^{0}C 210 ^{0}C isothermal for 18 minutes

Results and Discussion

Method validation

Method validation was performed according to International Conference on Harmonization Q2(R1) Validation of Analytical Procedures: Text and Methodology guidelines (ICH, 2005)

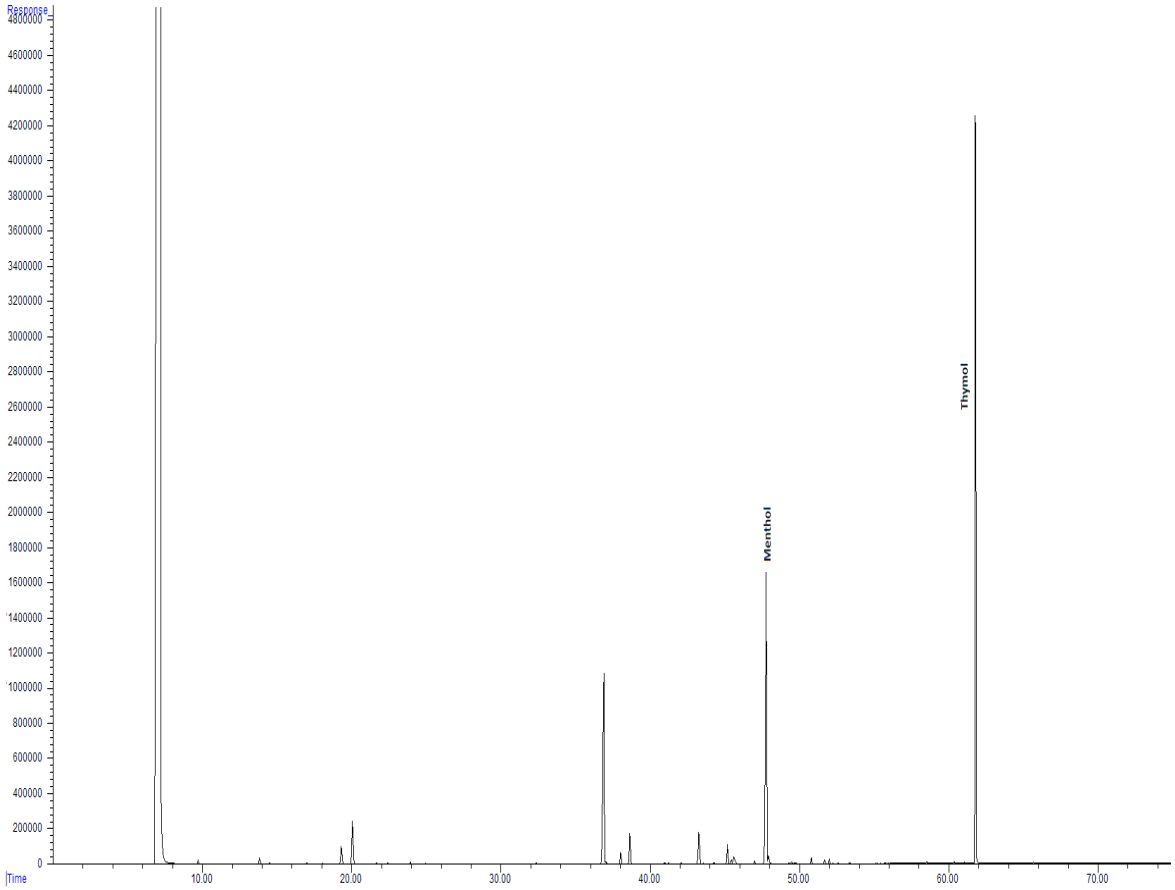
Specificity

The ICH documents define specificity as the ability to assess the analyte in the presence of matrix components. To achieve specificity; ethanol, thymol solution in ethanol, menthol solution in ethanol were injected to the system. Retention times for thymol and menthol were recorded. Products were analysed according to the method described above. Specificity results and the chromatograms of the product are represented in Table 2 and Figure 1 respectively. A 5977E MSD electron impact ionization mass detector was utilized to control peak purities of menthol and thymol to evaluate specificity.

Table 2. Specificity test results.

Sample	Menthol Retention Time (min.)	Thymol Retention Time (min.)
Ethanol	None	None
Menthol Solution	47,60	None
Thymol Solution	None	61,67
Peppermint oil solution	47,60	61,67

Figure 1. Chromatogram of the prepared product under optimum conditions.



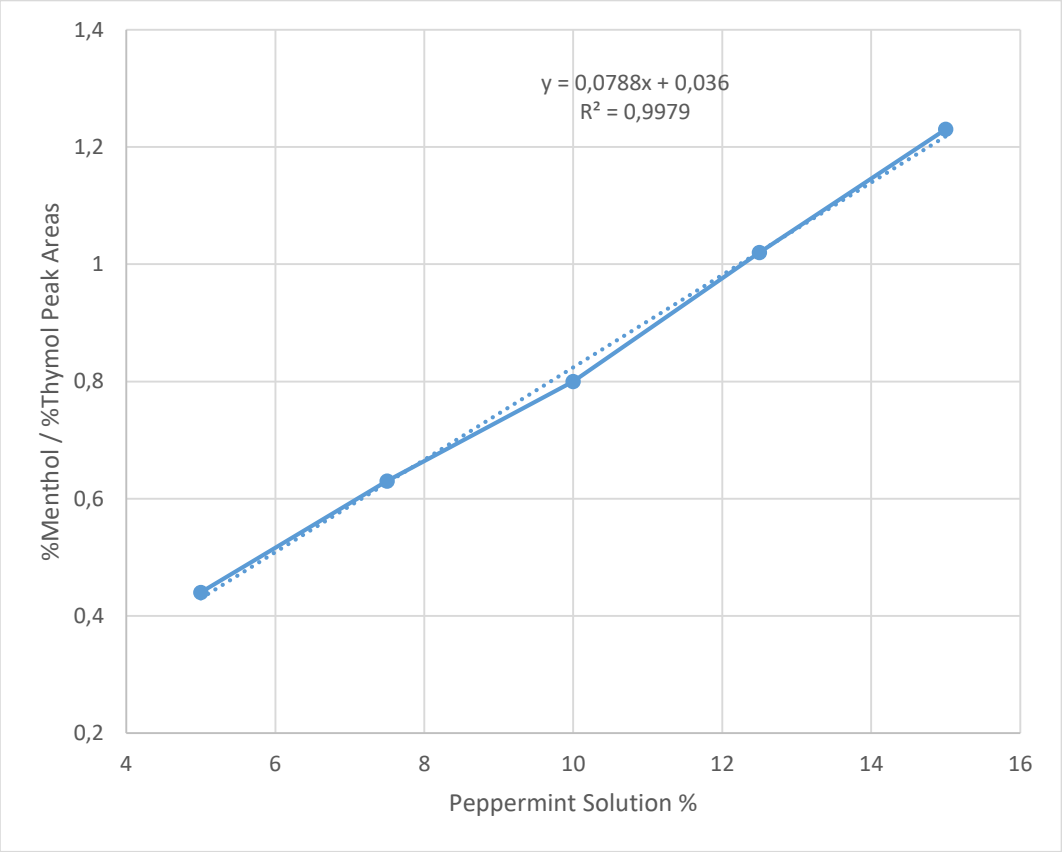
System repeatability, system suitability, range and linearity

To achieve system repeatability, system suitability and linearity; 5, 7.5, 10, 12.5 and 15% concentrations of peppermint oil solutions were prepared. All solutions were injected six times to achieve both linearity, system repeatability and system suitability. RSD% of the analyses resulted for each group were ≤2. The result of tests are listed in Table 3., and Figure 1. demonstrates the linearity.

Table 3. System repeatability, system suitability, linearity and range tests results.

Sample (n=6)	Menthol Peak Area (%)	Thymol Peak Area (%)	Menthol% / Thymol%
5%	30,45±0.17	69,55±0.11	0,44
7,5%	38,53±0.13	61,47±0.18	0,63
10%	44,43±.0.23	55,57±0.17	0,80
12,5%	50,49±0.16	49,51±0.14	1,02
15%	55,20±0.21	44,81±0.26	1,23

Figure 2. Calibration curve of linearity studies.



Accuracy

Because the final product is quantified as 10% peppermint oil in ethanol linearity studies complies with the accuracy tests too.

Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision of the method

assessed in terms of repeatability, intermediate precision and reproducibility. The results are acceptable if RSD%≤2. Table 4 shows the intermediate precision and repeatability data while Table 5 demonstrates the reproducibility of the method.

Table 4. Intermediate precision and repeatability tests results.

Sample	**Analyst 1** **Menthol% / Thymol% Peak Areas**	**Analyst 2** **Menthol% / Thymol% Peak Areas**
1	0,8055	0,7981
2	0,8025	0,8021
3	0,8031	0,8039
4	0,8043	0,7997
5	0,7981	0,8058
6	0,8040	0,8046
Mean	0,8029	0,8024
RSD%	0,3132	0,3398

Table 5. Reproducibility test results with a same type different column.

Sample	**Menthol% / Thymol% Peak Areas**
1	0,8055
2	0,8025
3	0,8031
4	0,8043
5	0,7981
6	0,8040
Mean	0,8029
RSD%	0,3132

Limit of detection and limit quantification (LOD and LOQ)

Analytical procedures for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products are classified as category 1. United States Pharmacopoeia states that LOD and LOQ test data elements are not required for this category (USP, 2016).

Robustness

The robustness of an analytical procedure is the measure of method capacity to remain stable with relatively small amount of changes in method parameters. Robustness test were evaluated by increasing the flow rate at 10% and increasing the temperature of the column for 5°C. The results are acceptable if RSD% and difference% ≤2. Table 6 shows the data of robustness tests including difference of the results from the normal method as percentage (%).

Table 6. Robustness test results with a same type different column.

Sample	Increased Flow Menthol% / Thymol% Peak Areas	Increased Temperature Menthol% / Thymol% Peak Areas
1	0,8232	0,8003
2	0,8205	0,8223
3	0,8182	0,8069
4	0,8140	0,8146
5	0,8092	0,8220
6	0,8222	0,8037
Mean	0,8179	0,8117
RSD%	0,5992	1,0601
Difference %	1,49	0,87

Solution stability

Test solutions used in linearity test were kept in 4^0C and 25^0C in the dark for 48 hours and analysed again to achieve solution stability. Solutions were stable according to analysis results.

Stability test results

Accelerated and long term stability tests were carried out to confirm the stability of the herbal product in primary packaging material according to ICH Q1A(R2); Stability testing of new drug substances and products guidelines (ICH, 2003). Accelerated stability conditions were as follows; 40 ± 2°C, % 75 ± % 5 relative humidity (R.H) meanwhile long term stability conditions were 25 ± 2°C, % 60 ± % 5 R.H. Table 7 illustrates the stability parameters and the test results. Long term stability control test periods were 1, 3, 6, 9, 12, 18, 24th months and the results were also in the limits.

Table 7. Accelerated stability test results.

Product Specifications		Time Periods		
Tests	Limits	1. month	3. month	6. month
Density	0,7980-0,8090	0,8015	0,8018	0,8015
Assay (Menthol% / Thymol%)	0,6 - 1,13	0,8043	0,8075	0,7992

As phytotherapy and aromatherapy is gaining popularity in modern world there is a high demand for herbal medicines. This demand increased the concerns about the safety, efficacy, standardization and quality. Herbal medicinal products are regulated by international or local authorities same way as the conventional medicines. Registration of herbal and conventional medicines requires application dossiers known as common technical document (CTD), a mandatory format for new drug applications. The CTD consists of five modules and the third one contains all the required manufacturing and production quality data about the product.

In this study, a successful dosage form of peppermint oil in ethanol was developed and analytical method was fully validated to control its stability parameters in the primary packaging material. Accelerated and long term stability data showed that the 10% peppermint in ethanol is stable at least for 24 months. The developed dosage form and analytical method could be used while registering the solution as herbal medicinal product as a part of CTD module 3.

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