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A SPME–GC procedure for monitoring peppermint flavor in tablets

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Abstract

A method was developed using solid-phase microextraction (SPME) and gas chromatography to monitor the peppermint flavor loss in a taste-masked tablet formulation. This was accomplished by headspace sampling of two major components of peppermint: menthone and menthol. It was found that the excipients from the tablet produced an important matrix effect and that standard addition analysis was necessary for improved accuracy of the determination. The method was shown to be specific and precise. Furthermore, the method produced acceptable results with adequate quantitation limits to determine peppermint flavors in taste-masked tablets. The optimized extraction procedure was successfully used to monitor the stability of peppermint flavor in an oral solid formulation. The accelerated stability studies of the tablet showed that the menthone and menthol was lost in an exponential manner and levels off after several days of heat exposure.

Keywords: Solid-phase microextraction (SPME); Peppermint; Polyacrylate fiber; Menthol; Menthone; Pharmaceutical tablet

1. Introduction

The active ingredients in pharmaceutical drugs often have an unpleasant and bitter taste. It is, therefore, common practice to mask the bitterness of the formulation with mint or fruit flavors, especially for chewable or tablets that dissolve on the tongue.

One of the concerns during the development of a peppermint taste masked tablet formulation is that over an extended period of time, the peppermint flavor will have reduced hence losing its functionality and consequently patient compliance. An analytical method is, therefore, needed to monitor the peppermint content during storage.

The commercial peppermint flavoring in the taste-masked tablet is supplied as peppermint oil that is spray-dried onto a food grade encapsulant [1]. Peppermint oil is made up of at least 29 components [2], menthone and menthol being the two predominant compounds. To evaluate the

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peppermint levels in the formulation, a method to determine the levels of menthone and menthol is developed. Menthone and menthol are both volatile making headspace sampling coupled to a GC-FID a suitable technique.

Conventional quantitative analysis methods for volatiles such as liquid–liquid extraction and purge and trap are often time consuming. An alternative to these methods is to collect the sample using solid phase microextraction (SPME). The SPME method relies on the mass transfer of analytes between the headspace (or a solution) and a polymer-coated fiber [3,4]. The fiber is first exposed to the sample. It is then allowed to adsorb the analytes for a given amount of time. Finally, the fiber is inserted into the GC inlet and the analytes bound to the fiber are thermally desorbed at high temperature into the GC column by the carrier gas [3,4]. SPME has already found a broad range of uses extending from environmental studies [5,6] to the food [7–13] and pharmaceutical industries [13–20]. In the pharmaceutical industry, SPME has been used in the analysis of blood [14,15] and urine [16–18] samples and on packaging materials [19]. There have also been some studies involving a tablet matrix [13,16,20], although these are much less common. Ligor and Buszewski [13] reported an SPME method where menthone and menthol in peppermint flavored food products as well as a pharmaceutical tablet were analyzed. This method reported a feasibility study using SPME to determine menthone and menthol at low levels.

In this paper, the objective is to develop and optimize a method using SPME–GC that is able to quantitate the levels of menthone and menthol in an oral solid dosage formulation. In addition this method will be used in the evaluation of flavor loss during an accelerated stability study of the formulation.

2. Experimental

2.1. Reagents/chemicals

Menthone-90%, menthol-99% standards were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The chemicals were used without any drying or further purification. The water used was filtered through a Milli-Q UV Plus Millipore water purification system. The methanol, acetonitrile, hexane and 2-propanol were of a high purity grade. The commercial peppermint flavoring agent was obtained from Bush Boake & Allen Inc. (Chicago, IL, USA). The taste-masked tablets were obtained from the Pharmaceutical Research and Development department of Merck Frosst Canada and Co. (Quebec, Canada).

2.2. Instruments

2.2.1. SPME extraction apparatus

Three different SPME fibers were used throughout the study, all of which were obtained from Supelco (Bellefonte, PA, USA). The coating materials were 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 85 μm polyacrylate.

The samples and standards were contained in 4 ml vials, which were fitted with siloxane septum screw caps. During the sampling process, the vials were held fixed by a vial holder and the fiber holder was stabilized above the vial using an SPME sampling stand.

The temperature of the system was controlled by immersing the vial and the vial holder in a jacketed vessel. This vessel was connected to a circulating bath to maintain the temperature at 45 ± 1 °C. The water level in the vessel was adjusted such that it came up to the neck of the vial.

Stirring was controlled using an 8×1.5 mm stir bar and a VWR-400 stirrer. The 4 ml vials, the SPME fiber, the vial holder and fiber holder were all obtained from Supelco (Bellefonte, PA, USA).

2.2.2. Gas chromatographic—FID system

All experiments were performed on a HP 5890 Series II Gas Chromatography unit (Agilent, Avondale, CA). A poly[5% diphenyl/95%dimethylsiloxane] (PTE-5), 30 m × 0.25 mm × 0.25 μm column from Supelco (Bellefonte, PA, USA) was used with the GC. An SPME inlet guide, also from Supelco, was placed above the inlet to stabilize the needle holder. A HP 7673 autosampler was used for direct injections.

2.2.3. GC analysis conditions

Two GC methods were used in the study. One was used for the analysis of the commercial peppermint flavoring agent by direct GC injection and the other was used for the analysis of tablet extracts by SPME–GC. The two methods differed in the oven program and the injection technique (i.e. manual injection for the tablet extract using SPME vs. automated injection for the peppermint flavoring agent by direct GC injection). All other parameters were identical.

For the analysis of peppermint flavoring agent, helium was used as a carrier gas with a flow rate of 1 ml/min. The inlet and detector temperatures were both set at 250 °C. Splitless injection was used and the purge valve was closed after 5 min. The injection volume was 1 μl. The initial oven temperature was held at 73 °C for 18 min. It was then ramped to 140 °C at a rate of 5 °C/min and to 230 °C at 30 °C/min (for 1 min).

For the analysis of the tablet extract, the same GC conditions were used as described for the peppermint flavoring agent with minor differences. In this analysis the initial oven temperature was held at 60 °C for 1 min. It was then ramped to 140 °C at a rate of 10 °C/min and to 230 °C at 30 °C/min (for 1min). This method was obtained from Supelco [21] with slight modifications to the oven program in order to shorten the run time.

2.3. Procedure

2.3.1. Analysis of menthol and menthone in the peppermint flavoring agent by direct injection

The peppermint flavoring agent sample was prepared by dissolving 1 g of the powder in 1 l of 70% methanol in water. It was then sonicated for 1 h and centrifuged at 14 000 rpm for 5 min. An aliquot was transferred into a 2 ml HPLC vial and loaded in the autosampler. Three aliquots of the powder were taken and two samples of each aliquot were prepared. Quantification was performed by the method of external standardization. A stock standard solution was prepared by dissolving 16 mg menthone and 20 mg menthol in 200 ml of 70% methanol in water. Since the reference standards used were not the same lot as the excipient used for the flavor the purity of the standards were considered for the quantitation i.e. for menthone and menthol a factor of 0.9 and 0.99, respectively, was used in the calculation. The stock standard solution was then diluted to prepare four standard solutions in the concentration range of 15–70 μg/ml menthone and 20–100 μg/ml menthol in 70% methanol in water. The standards and analytes in peppermint flavoring agent were injected into the GC and the area response of the analytes (the sum of the area for cis and trans menthone was used) were compared with that of the standard curve. The standards and the sample were injected in duplicate.

2.3.2. Analysis of the taste-masked tablet formulation using SPME

Quantification of menthone and menthol in tablets was performed by standard addition analysis. For the standard addition analysis, a stock standard solution was prepared by dissolving 60 mg menthone and 60 mg menthol in 10 ml of 100% methanol. The stock standard solution was then diluted to prepare standard solutions in the concentration range of 2–6 mg/ml for both menthone and menthol in 100% methanol. A 5 μl aliquot of the standard solution was then added to an accurately weighed 25 mg portions of crushed tablets and processed according to the procedure described below.

For the samples, two 200 mg taste-masked tablets were crushed using a mortar and pestle and a 25 mg aliquot was transferred into a 4 ml vial containing the stir bar. To this vial, 995 μl of water and, using a 10 μl syringe, 5 μl of a standard solution (or methanol) were added. The vial was then left in the 45 °C water bath for 10 min while being stirred at 1400 rpm. The 85 μm polyacrylate SPME fiber was inserted through the vial cap's septum with the needle length set at 2 cm, so that the fiber was in the headspace, and left to adsorb the analytes for 25 min. Finally the fiber was removed from the sampling stand, the needle length was increased to 4 cm and the fiber was immediately inserted into the GC. The fiber was left in the GC inlet for 5 min during which the analytes were thermally desorbed. It was then left to cool at room temperature for 5 min before the next sampling. A carry-over test showed less than

0.05% menthone or menthol remaining on the fiber. All sample-standard mixtures were prepared in duplicate.

2.3.3. Stability tests

The stability tests were carried out using taste-masked tablets. The tablets were stressed in a ventilated 40 °C oven for up to 8 days in ambient air, open dish (tablets placed horizontally with respect to the light source on a petri-dish in a single layer). The samples were then treated as outlined in Section 2.3.2. The determination at each time point was performed once.

2.4. Parameter optimization

Parameters for the SPME sampling of menthone and menthol were optimized for reproducibility and sensitivity. These parameters include solvent composition, stir speed, temperature, evaporation/adsorption time, sample weight and SPME fiber coating material. For the solvents, 0.5% concentrations of methanol, acetonitrile, 2-propanol and hexane in water were compared for their extraction efficiency. Also, solutions of 0.5–70.0% methanol in water were tested. The stir speed was tested between 700 and 2000 rpm. For optimization of the temperature, the system was maintained between room temperature and 90 °C. The evaporation time was varied between 1 and 10 min and the adsorption time was varied between 1 and 60 min to determine the length of time required for the system to reach equilibrium. The proportionality relationship of sample weight to peak area was evaluated between 5 to 200 mg. The three fibers used were PDMS, PDMS/DVB and polyacrylate. For the optimization of the stir speed and the sample weight, the samples were prepared in duplicate. For all other parameters a single measurement was taken per data point.

3. Results and discussion

3.1. Method development

3.1.1. GC analysis

Two methods were developed for this study. The first was used to quantify menthone and menthol in commercial peppermint flavoring agent by direct injection into the GC. The second was used for the menthone and menthol quantitation in tablets using SPME. Both methods developed for menthone and menthol detection provided adequate resolution with reasonable run times as shown in Figs. 1 and 2. The method used for the SPME injections, however, was not suitable for the direct injection of the aqueous methanol solution required for the analysis of peppermint flavoring agent. The initial oven temperature was too low to vaporize the solvent. Consequently, the flame of the FID detector extinguished. A separate method for direct injection was developed by increasing the initial oven temperature from 60 to 73 °C with a hold time of 18 min. All other parameters were unchanged. The peaks in Fig. 1 showed some fronting due to the aqueous nature

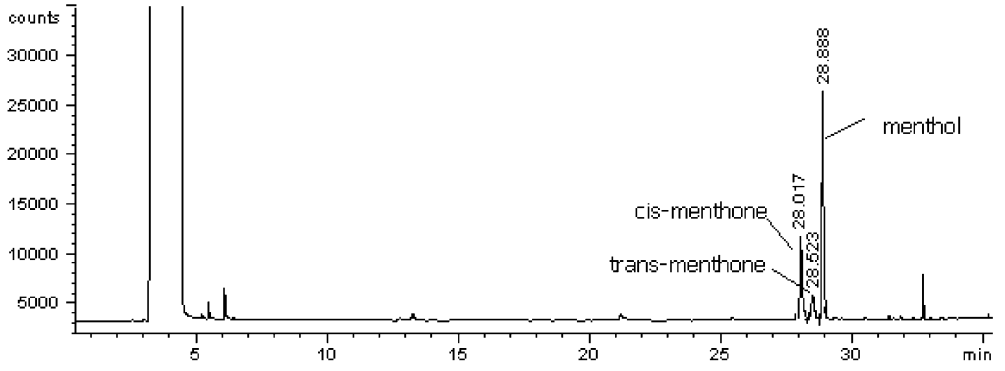

Fig. 1. Typical chromatogram of the peppermint flavoring agent.

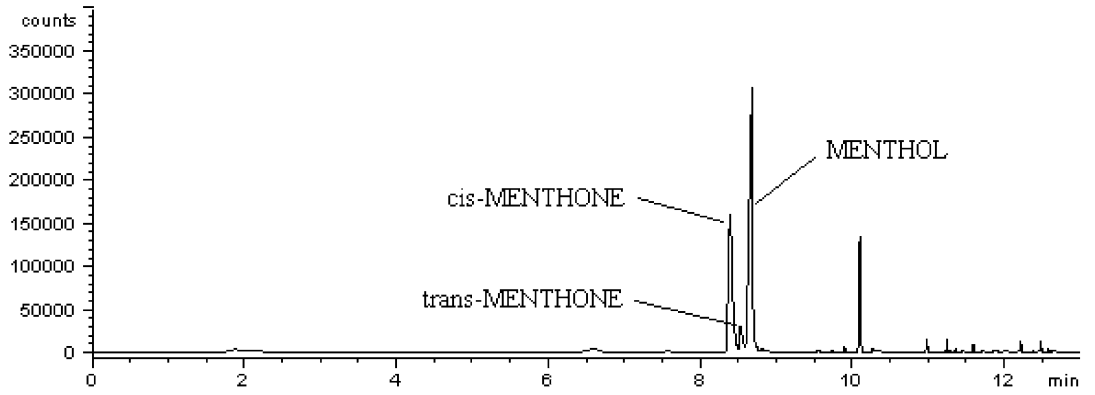

Fig. 2. Typical chromatogram of the taste-masked tablet.

of the solution, but the separation remains adequate for quantification.

3.1.2. Optimization of SPME extraction

There are many parameters of the SPME extraction that can affect the sensitivity, recovery, precision and accuracy of the analysis. These include solvent composition of the extract, temperature of the extraction, stir speed, sample weight, vaporization time, adsorption time and SPME fiber type.

3.1.2.1. Solvent composition of the extract. The solvent extraction of menthol and menthone proved to be most efficient in 100% water. The presence of any organic solvent resulted in significant decrease of the extraction efficiency as shown in Fig. 3. The organic solvents themselves

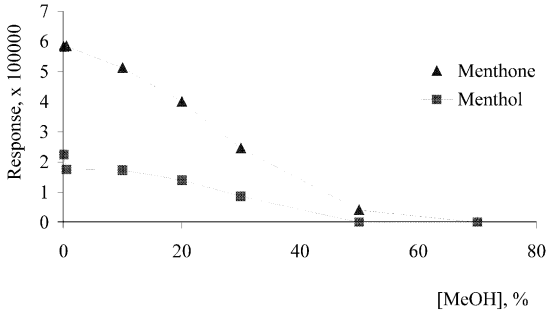

Fig. 3. The effect of methanol on the extraction sensitivity. Extraction conditions: sample; 25 mg taste-masked tablet; vaporization time, 5 min; adsorption time, 5 min, stir rate, 400 rpm; temperature, room temperature; fiber, PDMS.

are often volatile and have a high affinity for the fiber. In the case of increased organic solvent concentration, the solvent may saturate the fiber thus preventing adsorption of the analytes of interest. However, because the menthol and menthone standards used in the quantitation are not water soluble, they must be dissolved in a minimum quantity of organic solvent. At the concentration range of interest it was found that a minimum amount of 0.5% methanol was needed as the optimized methanol concentration.

Other solvents with higher boiling points were also tested to minimize the competitive binding between the analyte and the solvent. However, 0.5% concentrations of acetonitrile, hexane, 2-propanol and methanol in water showed no significant difference in their efficiency to increase response. Methanol was chosen because it has the lowest boiling point, making it ideal for the GC analysis.

3.1.2.2. Temperature of extraction. During the extraction, it was necessary for the solute to vaporize from the solvent to the gas phase by heating the sample. However, at temperatures higher than the optimum, the analytes were desorbed off the fiber. Thus an optimized temperature of 45 °C was observed as shown in Fig. 4. A further increase in the temperature resulted in loss of recovery.

3.1.2.3. Stir speed of extraction. During the vaporization and adsorption stages of the SPME

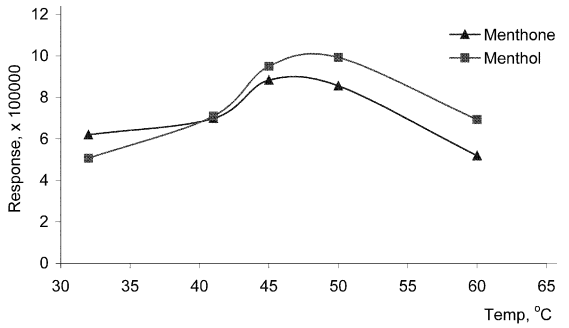

Fig. 4. Effect of extraction temperature on the extraction sensitivity. Extraction conditions: sample, 25 mg taste-masked tablet; vaporization time, 10 min; adsorption time, 25 min; stir rate, 1400 rpm; fiber, polyacrylate.

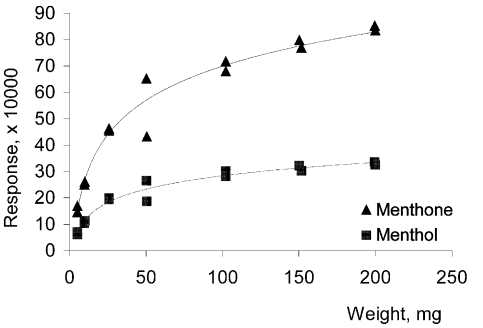

Fig. 5. Linearity between sample size to response. Extraction conditions: sample, taste-masked tablet; vaporization time, 5 min; adsorption time, 5 min, stir rate, 1400 rpm; temperature, 45 °C; solvent 0.5% MeOH; fiber, PDMS.

procedure the solution was stirred. This will keep the solution homogeneous and refresh the menthone/menthol depleted solution–gas phase interface. The stir rate was varied between 700 and 2000 rpm. In this study, the recovery of the extraction was not affected by the stirring rate. In order to control the extraction, the stir rate was set at 1400 rpm. This high stir rate was preferred to accelerate the mass transfer between the solvent and the matrix or between the solvent and the headspace. However, stir rates greater than 1400 rpm caused the stir bar to become erratic.

3.1.2.4. Sample weight used for extraction. To maintain the accuracy and precision of the extraction it is also necessary to determine the appropriate sample size to use for the extraction. The response and sample weight relationship is not linear as shown in Fig. 5. External standard calibration plots indicate that the linearity range of the FID detector and the recovery of analyte from the SPME fiber extends beyond area counts of 10 million for both menthone and menthol, and consequently the non-linearity is not due to limitations of the detector or of the fiber. This trend is likely due to matrix effect. In this study, the weight used is chosen at 25 mg. This weight is chosen because smaller sample sizes become difficult to weigh out accurately and precisely. In addition, higher sample sizes become difficult to transfer into the narrow opening of the 4 ml vial. Also, 25 mg is the upper limit of the linear range in Fig. 5. Although, it is later shown that the determination of menthol and menthone is independent of the sample size when using the standard addition method, the 25 mg weight was still chosen because of handling problems as explained above.

3.1.2.5. Vaporization time of extraction. Before the analytes are collected on the fiber, the sample is heated and the menthone and menthol are allowed to vaporize into the headspace. The vaporization is allowed to continue until an equilibrium between the condensed phase and the headspace is reached. This equilibrium is shown to occur rapidly. Vaporization times greater than 1 min (and followed by a 5 min adsorption time) does not increase the recovery of the analytes. The vaporization time is chosen to be 10 min because it takes 5 min for the sample to desorb from the fiber and 5 min for the fiber to cool before the next sampling.

3.1.2.6. Adsorption time of extraction. The adsorption time of the analyte onto the SPME fiber was also optimized. Unlike the vaporization time, the adsorption onto the fiber requires more time to reach equilibrium. The response showed that after 25 min the amount adsorbed is maximized, as shown in Fig. 6. This was chosen as the optimized adsorption time because it is the time point where the adsorption begins to level off and because quantitative analyses generally do not require a

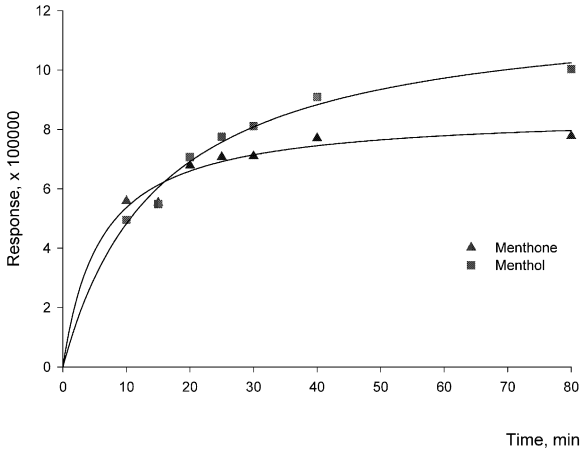

Fig. 6. Optimization of adsorption time for menthone and menthol. Extraction conditions: sample, 25 mg taste-masked tablet; vaporization time, 10 min; stir rate, 1400 rpm; temperature, 45 °C; solvent, 0.5% MeOH; fiber, polyacrylate.

full equilibrium between the sample and the fiber as long as this is reproducible [21].

3.1.2.7. SPME fibers used for the extraction. Throughout the study, three different fibers were used: PDMS, PDMS/DVB and polyacrylate. It is found that the optimal sampling conditions of menthone and menthol are similar for the different fibers. The polyacrylate fiber, however, was chosen because it yielded the most accurate recovery while maintaining sensitivity.

3.2. Quantitation of menthone and menthol

3.2.1. Menthol and menthone in peppermint flavoring agent

The menthone and menthol from peppermint flavoring agent are soluble in concentrations of up to 2 mg/ml in 70% methanol. The linearity range of the peppermint weight to detector response using the direct injection method is shown in Fig. 7. The R^2 values for menthol and menthone were both 1.000. It is, therefore, assumed that there is negligible matrix effect with the peppermint flavoring agent. Using the direct injection procedure, detection limits of 1 μg/ml for menthone and 0.1 μg/ml for menthol were found.

By using an external calibration curve it was shown that the peppermint flavoring agent contains 4.25% menthone (R.S.D. of 1.0%) and 6.86% menthol (R.S.D. of 1.1%). The peppermint flavoring agent content used in the taste-masked tablet formulation was 0.5% (w/w). Based on the results found, the expected label claim of menthone and menthol in the tablet was calculated as 0.0213% (w/w) and 0.0343% (w/w), respectively.

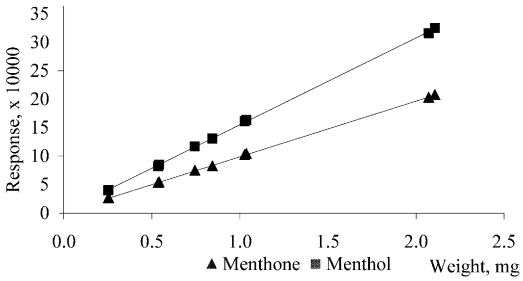

Fig. 7. Linearity range of menthone and menthol in the peppermint flavoring agent.

3.2.2. Menthol and menthone in peppermint taste-masked tablet

Initially, the external standard quantitation method was used on a crushed tablet sample. Using the external standard method and the SPME procedure, detection limits of 0.0003 μg/ml for menthone and 0.0002 μg/ml for menthol were found. When external standard quantitation was initially used for the tablets, the amount recovered was only 55.6% label claim for menthone and 45.4% label claim for menthol. However, it was also shown in Section 3.1.2.4 that the response to sample weight relationship is not linear and, therefore, the recovery is dependent on the sample size. These observations are indications of important interference by the matrix. To compensate for the matrix effect, the standard addition method was considered.

The standard addition technique accounts for the matrix effect by adding various standard concentrations directly to a fixed amount of sample. The concentration of the analyte is determined by plotting the amount of added standard (four data points) to the resulting response. The amount of analyte in the sample corresponds to the x-intercept of the plot. The R^2 values of the calibration plot were greater or equal to 0.996 for menthone and menthol. The linearity

of the plot indicates that the method is sufficiently precise.

Quantitation using a standard addition technique resulted in a recovery of 93.0% label claim for menthone (day-to-day R.S.D. of 2.8%) and 78.4% label claim for menthol (day-to-day R.S.D. of 5.3%) from the tablet matrix. To ensure that the effect of the matrix is overcome by using the standard addition, the determination of menthone and menthol was carried out using several sample sizes: 12.5, 25, 50 and 200 mg. All four determinations gave the same recovery with less than 7% R.S.D.. Thus, the choice of a 25 mg sample size as discussed in Section 3.1.2.4 is still valid.

3.3. Loss of menthone and menthol in tablets stored at high temperature

The stability profile of the menthol and menthone in the tablet proceeds via an exponential decay as shown in Fig. 8. Although there is a significant loss of the menthone and the menthol content, it is important to note that the peppermint has not necessarily lost its functionality. And because it levels off, no further loss is expected at a longer stress condition. The formulation contains an excess of the flavoring component, and so a 30% label claim of the flavoring components may still be sufficient to taste-mask the active ingredient.

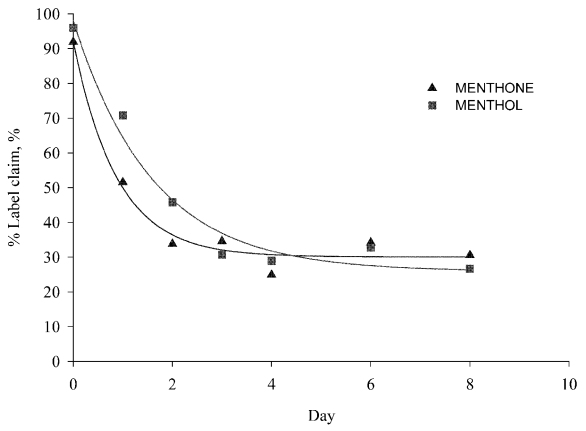

Fig. 8. Stability profile of menthone and menthol in the taste-masked tablet. The tablets were stressed in a ventilated 40 °C oven in ambient air, open dish.

4. Conclusion

A method which can follow the loss of peppermint in a taste-masked tablet containing a bitter drug is successfully developed and presented here. In addition the menthol and menthone content of a commercial peppermint flavoring agent was determined using direct injection into GC.

The SPME procedure using a polyacrylate fiber was optimized for maximum extraction and determination of menthone and menthol in taste-masked tablets. External calibration curves greatly underestimated the menthol and menthone content in the tablet. Standard addition method was, therefore, used and showed a 93.0% recovery for menthone and 78.4% recovery for menthol. The day-to-day reproducibility was acceptable given a R.S.D. of 2.8% for menthone and R.S.D. of 5.3% for menthol. The method was then used for an 8 day accelerated peppermint stability study where the tablets were stored at 40 °C, open dish. The results successfully showed an exponential decay of menthone and menthol in the tablet matrix.

Although it has been shown that SPME can be used to follow the potency of menthone and menthol in the stability study, the method is somewhat tedious. The preparation time can be shortened by using shorter adsorption times. Ai [22] had shown that quantitative analyses can be performed without reaching a full equilibrium. As well, autosamplers are commercially available that heat and adsorb the sample using the SPME fiber and which would significantly simplify the extraction.

Though SPME–GC is not yet a widely accepted technique, it offers many advantages over other methods. The SPME method avoids injections of solvent into the GC, therefore, substantially prolonging the life of the column. Also, the sample preparation requires very little solvent, thus reducing waste. The fiber can be reused approximately a hundred times before the precision deteriorates. There is no modification needed on the GC and the accessories needed for sampling that are not commonly found in the laboratory are inexpensive. Given these factors, SPME–GC is a simple and cost effective technique to monitor the levels of peppermint flavor in pharmaceutical products.

Acknowledgements

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