COLORIMETRIC METHODS OF DETERMINATION OF MENTHOL IN AIR

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The two described colorimetric methods are based on the development of colour on dilution of the reaction product of menthol with dimethylaminobenzaldehyde (DMAB) or vanillin in concentrated sulphuric acid medium. The analytical techniques for determination of menthol by the described methods have been optimised. Both the methods are sensitive to 2 μ g of menthol in 2.5 ml aliquote of sample in concentrated sulphuric acid. The coefficient of variation of the DMAB and vanillin method has been found to be 4.4 and 4.0% respectively. Concentrated sulphuric acid has been found to be a suitable air sampling medium for menthol vapours.

Menthol vapours are irritant to eyes, nose and throat, and ingestion of this chemical has been reported by Von Oetingen (1958)¹⁾ to cause abdominal pain, vomiting, staggering gait and sopor. The workers exposed to high concentrations of menthol vapours in an industry manufacturing menthol crystalls from the plant extracted mentha oil complained of drowsiness, headache, nausea and vomiting. Till 1979 no TLV²⁾ has been assigned to menthol. In order to conduct industrial hygiene survey in such a plant with a view towards establishing a TLV, there is need of simple, sensitive and reliable methods for determination of menthol in air.

There is qualitative method of detection of menthol in British Pharmacopia (1973)³⁾ based on the development of a purple colour on dilution of the reaction product of menthol and vanillin in concentrated sulphuric acid medium with water in the ratio of 2 : 1 (v : v).

The other method described by Masamune (1933)⁴⁾ is based on the visual colorimetric estimation of the red colour developed by heating menthol with DMAB in (1.6 : 1) sulphuric acid : water (v : v) medium.

Guerin (1905)⁵⁾ and Ekkert (1928)⁶⁾ stated that the higher alcohols and phenols dehydrated by concentrated sulphuric acid combine with aromatic aldehydes such as DMAB to give colour. According to Snell and Snell (1957)⁷⁾ the factors increasing colour intensity in addition to amount of higher alcohol are; increase in aldehyde, increase in sulphuric acid, heating and time. In the present paper it was intended to study the various factors influencing the sensitivity of DMAB and Vanillin methods so that the analytical techniques of determination of menthol are optimised. To find out a suitable

air sampling medium concentrated sulphuric acid was evaluated for its collection efficiency for menthol vapours. Ethanol was found to be unsatisfactory sampling medium because of its positive interference with the described analytical methods.

MATERIALS AND METHODS

To analyse each of the menthol samples in concentrated sulphuric acid the analytical procedure followed was;

1) *DMAB method* :

To 2.5 ml of a sample taken in a test tube calibrated to 5 ml mark, added 1 ml of 5% w/v DMAB reagent prepared in concentrated sulphuric acid, mixed and diluted to the 5 ml mark by dropwise addition of water. Heated the test tube in boiling water bath for 8 minutes, cooled to room temperature and read the absorbance at 520 nm against the reagent blank treated according to the procedure described. A reference curve was drawn by analysing 2-50 μ g quantities of menthol separately.

2) *Vanillin method* :

To a sample volume of 2.5 ml added 0.5 ml of 1% (w/v) vanillin reagent and proceeded exactly as described for the DMAB method but no external heating in the boiling water bath was carried out. Read the absorbance at 525 nm against the reagent blank treated according to the described procedure. A reference curve was drawn by analyzing 2-50 μ g quantities of menthol separately.

To find out the reagent concentrations required to develop maximum colour intensity, each of the sample containing 50 μ g of menthol was analysed by both the described methods wherein 0.5, 2.0 and 3.0 ml of 2% (w/v) DMAB and 0.5, 1.0 and 1.5 ml of 1% (w/v) vanillin reagents were separately used.

To study the optimum range of dilution of the reaction product of menthol with DMAB or vanillin in concentrated sulphuric acid required to develop the maximum colour intensity, each of the sample containing 50 μ g at menthol was analysed by both the described method at the different sulphuric acid concentrations of 100, 90, 80, 70, 60, 50 and 40% (v/v) in the volume made up finally.

In order to find out the effect of using prediluted sulphuric acid from a standard solution of menthol prepared in 2 : 1 sulphuric acid : water (v : v) 25, 50, 100 and 150 μ g quantities were analysed by the DMAB method and 5, 10, 20, 50 and 60 μ g quantities by the Vanillin method. In both the determinations for reagents preparation as well as for making up the final volume (prediluted) 2 : 1 sulphuric acid : water (v : v) was used.

To find out the effect of storing of menthol samples in concentrated sulphuric acid the different 5, 10, 20, 30, 50 and 60 μ g quantities of menthol from freshly prepared and overnight stored solutions were analyzed by both the described method. In the other series of experiments the freshly prepared menthol solution in concentrated sulphuric

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acid was heated for fifteen minutes in a boiling water bath, cooled to room temperature and the 5~60 μg quantities of menthol taken from this solution were also analyzed by both the described methods.

The heating period in a boiling water bath required to develop the maximum colour intensity was studied by analyzing each of the samples containing 50 μg quantity of menthol according to both the described methods for the different heating time in the range of 0~12 minutes. The experiment was repeated with the menthol samples in 2 : 1 sulphuric acid : water (v : v) reaction medium.

Air sampling :

To find out the collection efficiency of concentrated sulphuric acid for menthol vapours two small crystals of pure menthol were taken into a 250 ml capacity all glass bubbler. The outlet tube of the bubbler was connected to a train of two inter connected midget impinger tubes each containing 10 ml of concentrated sulphuric acid, a rotameter and an electrically driven pump. Air sampling of the volatalised menthol vapours in both the midget impinger tube was done at the different rates of 1, 2 and 3 lpm. The volume of air samples drawn ranged from 15 to 20 l. The collection efficiency of the concentrated sulphuric acid in the first midget impinger was calculated by analyzing the concentration of menthol in both the sampling tubes by the described Vanillin method. For each sampling rate the experiments were replicated five times.

RESULTS AND DISCUSSIONS

The results of influence of DMAB and Vanillin concentrations on the intensity of the colour are shown in Table I. The absorbance value for 50 μg menthol by DMAB method increased from 0.58 to the maximum value of 0.95 with the increase of the reagent concentration from 2.0 to 10.0 mg/ml or more in the final volume. Hence the DMAB concentration of 10 mg/ml in the final volume is required to develop the maximum colour

Table 1. Effect of reagent concentration.

S. No.	Reagent	Concentration mg/ml	Absorbance for 50 μg menthol	
			520 m/m μ	525 m/m μ
1	DMAB	2.0	0.58	—
2	"	4.0	0.68	—
3	"	8.0	0.82	—
4	"	10.0	0.95	—
5	"	12.0	0.95	—
6	Vanillin	1.0	—	1.2
7	"	2.0	—	1.2
8	"	2.5	—	1.2

Fig. 1. Effect of sulphuric acid dilution.

A(●) : Recommended DMAB method.

B(×) : Recommended Vanillin method.

Fig. 2. DMAB method.

1. Reaction medium (2 : 1) H₂SO₄ : H₂O (vol/vol).
2. Freshly prepared menthol sol. in conc. H₂SO₄.
3. Recommended procedure.

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intensity. For the Vanillin reagent concentration ranging from 1~3 mg/ml the absorbance value of 1.2 for 50 μg of menthol analysed was found to be same. Therefore 1.0 ml of 5% DMAB and 0.5 ml of 1% Vanillin reagent concentration (w/v) in concentrated sulphuric acid medium are suffice to develop maximum intensity of colour by the respective described methods.

The results of the effect of dilution of the respective reaction products of menthol with DMAB and Vanillin in concentrated sulphuric acid on the intensity of the colour developed are shown in the Fig. 1. For the 50 μg of menthol analysed by DMAB method the absorbance of 0.24 obtained at 100%(v/v) sulphuric acid concentration (1.84 sp.gr.) increases sharply to its maximum of 0.95 at the sulphuric acid concentration of 70%. On further dilutions at the respective sulphuric acid concentrations of 60 and 50% the absorbance decreases to 0.85 and 0.62. Below 50% sulphuric acid concentration the colour-intensity is unstable. Hence the maximum colour intensity with the DMAB method is obtained at 70% sulphuric acid concentration.

The absorption value of 0.5 for 50 μg of menthol analyzed by Vanillin method at the 100%(v/v) sulphuric acid concentration sharply increases to 1.2 at the sulphuric acid

Fig. 3. Vanillin method.

1. Reaction medium (2 : 1) H_2SO_4 : H_2O (vol/vol).
2. Freshly prepared menthol solution in conc. H_2SO_4 .
3. Recommended procedure.

concentration of 70%, and this remains same at the sulphuric acid concentration levels ranging from 50 to 70%. At 40% sulphuric acid concentration the absorbance is reduced to 0.9 and at this dilution the intensity of the colour is unstable.

The results shown in Figs. 2 and 3 indicate that the recommended DMAB and Vanillin methods achieve the maximum colour intensity when the menthol is dehydrated by concentrated sulphuric acid by storing overnight or by heating in a boiling water bath. The intensity of the colour is reduced by 24 and 25% respectively for DMAB and Vanillin methods when freshly prepared menthol solution in concentrated sulphuric acid is analyzed. The sensitivity of DMAB and Vanillin methods is further reduced by 65 and 35% respectively when the menthol samples are analyzed in prediluted sulphuric acid (2 : 1) medium. Hence to achieve the maximum colour intensity the menthol in concentrated sulphuric acid should be completely dehydrated by storing overnight or by heating in a boiling water bath for 15 minutes.

The results of the experiments carried out to study the influence of heating time in boiling water bath on the sensitivity of the DMAB method are shown in the Fig. 4. The heat produced by the dilution of the concentrated sulphuric acid to 70% (v : v) level initiates the quick development of colour, so as a result of this the initial absorbance of 0.66 is obtained for 50 μ g of menthol. This initial absorbance gradually increases with the time of heating upto 8 minutes and thereafter this attains a maximum of 0.95. When the same experiments were carried out in prediluted (2 : 1) v : v sulphuric acid medium the maximum absorbance was also found after heating period of 8 minutes.

Fig. 4. Effect of heating time (DMAB method).

A(●) : Recommended procedure.

B(×) : (2 : 1) H₂SO₄ : H₂O (vol/vol) Reaction medium.

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The heat evolved during the dilution of the concentrated sulphuric acid is sufficient to develop the maximum colour intensity by Vanillin reagent so further heating is not required. However in (2 : 1) sulphuric acid : water medium 2 minutes of heating time in a boiling water bath was found to be sufficient to develop the maximum achievable intensity of the colour.

Both the described methods are sensitive to $2\mu\text{g}$ of menthol. The coefficient of variation of the DMAB and the Vanillin method has been found to be 4.4 and 4.0%. The vanillin method has been found to be better than the DMAB method because; (I) this is 1.27 times more sensitive; (II) no external heat is required to develop the maximum colour intensity, hence the estimation is rapid; and (III) under the described experimental conditions the different vanillin concentrations has no effect on the sensitivity of the analytical method.

The collection efficiencies of 10 ml of the concentrated sulphuric acid in the midjet impinger tube for the generated menthol vapour concentrations ranging from 12 to 20 mg/m³ were found to be 97% (SD-0.6), 94% (SD-0.6) and 90% (SD-1.2) for respective sampling rate of 1, 2 and 3 lpm. Hence concentrated sulphuric acid is an efficient and as well as suitable sampling medium for evaluation of menthol vapour concentration in air by the recommended methods.

Interference

Higher alcohols of paraffin series, esters of these alcohols, hydroaromatic alcohols, phenols and other terpenes would interfere positively with the described analytical methods.

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