BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI
Publicat de
Universitatea Tehnică „Gheorghe Asachi" din Iaşi
Tomul LIX (LXIII), Fasc. 2, 2013
Secţia
CHIMIE şi INGINERIE CHIMICĂ

DIRECT DETERMINATION OF MENTHOL USING A SIMPLE SPECTROPHOTOMETRIC METHOD

BY

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Received: September 1, 2013
Accepted for publication: September 15, 2013

Abstract. A simple, selective and accurate spectrophotometric method is proposed for rapid and direct determination of menthol and the optimal experimental conditions along with other analytical parameters have been evaluated. This method is based on the reaction between menthol and salicylaldehyde, in concentrated sulphuric acid media (96-98%), when a red-orange coloured product is obtained. The visible spectra of this reaction product, recorded against a blank solution, show a maximum at 510 nm, and the absorbance remains stable for at least 12 h. The method allows the menthol determination over the range 1.33 – 13.33 $\mu g \cdot mL^{-1}$, with a molar absortivity of $1.1533 \cdot 10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$ and a detection limit of 0.17 ppm. The interferences caused by several organic compounds (acetic acid, citric acid and ascorbic acid) which can be present along menthol in various samples were also determined. The validation of the spectrophotometric method was done by recovery test of menthol from green tea. The results show that the proposed method can be successfully used for direct determination of menthol and the main advantage is that the coloured reaction product appears immediately after mixing the reagents.

Key words: menthol determination, salicylaldehyde, spectrophotometric method.

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1. Introduction

Menthol is a flavouring agent (Lasekan & Lasekan, 2012), derived from natural and synthetic sources, that is added to various cosmetic or food samples, such as: body lotions, face creams, anti-perspirants, shampoo, shower gels, liqueurs, candy, etc. (Bhatia *et al*., 2008). Due to the sensory properties of menthol, like the enhancement of taste and soothing effect (Ahijevych & Garrett, 2010), its use worldwide is in the region of 100 to 1000 metric tones per annum (Lin *et al.,* 2005).

Although not itself harmful (only a high intake of menthol can cause abdominal pains and diarrhea (Burt, 2004)), the content of menthol from commercially available products is established by standards and should be respected by all the producers. Under these conditions, the rapid and direct quantitative determination of menthol concentration in various commercially products is still a subject of interest, since this is an important indication in the quality control of some products from market (Rickert *et al.,* 2009), and a high concentration of this can be a potential risk to human health.

The determination of menthol, regardless of analyzed sample, ca be done chromatographically or using NMR spectrometry (Ortiz-Boyer *et al.,* 1995; Hartner & Reinscheid, 2008). Various chromatographic methods can be used for the analysis of menthol content, including chiral analysis, such as gas chromatography – flame ionization (Ligor & Buszewski, 1999; Nozal *et al.,* 2002), gas chromatography – mass spectrometry (Valdez *et al.,* 1999), liquid chromatography with refractometric detection (Valdez *et al*., 2002), liquid chromatography with UV detection (Hamasaki *et al*., 1998; Rohloff, 2002), liquid chromatography with fluorimetric detection (Caraballo *et al.,* 1994). Most of these methods are either time consuming, or require sample pre-treatment, expensive instruments, which limits their application in laboratory practice.

Spectrophotometry is adequate for the development of a rapid, simple and inexpensive analytical method. The simplicity of the system design, low cost, easy automation and feasibility of wide-range determination are the main favourable characteristics of the spectrophotometric methods. But, the main inconvenience is that, in case of menthol, lack of chromophore groups makes necessary to use a colour reagent. In addition, due to its low reactivity, the most of colour reactions must occur in strong acid media and from this reason are needed supplementary precautions.

In this study, the reaction between menthol and salicylaldehyde, in concentrated acid media, was spectrophotometrically studied. The red-orange reaction product is stable and has a good behaviour from the point of view of the spectrophotometric study. The proposed method is simple, sensitive and selective, required no control of temperature and can be a good alternative for rapid and direct determination of menthol in various cosmetic and food samples.

In addition, the salicylaldehyde is a common chemical reagent, the use of which does not require additional precautions, which means that the proposed method does not present a significant risk to human beings. In order to emphasize its applicability, the proposed method was evaluated for menthol determination in two types of green tea, commercially available on our marked.

2. Experimental

2.1. Materials

Stock solution of 100 μg·mL^{-1} menthol was prepared by dissolving solid menthol (purchased from Aldrich) in ethanol (96% v/v). Working solutions were prepared from stock solution by dilution with ethanol. The salicylaldehyde solution (1%) was obtained by diluting an appropriate volume of 5% solution with ethanol. The required reaction media was obtained using concentrated sulphuric acid (96-98%), purchased from Aldrich.

All the reagents were of analytical reagents grade and were used without further purifications. All the dilutions were performed with ethanol.

2.2. Methods

Spectrophotometric measurements were performed with a Digital Spectrophotometer S 104 D, with a 1.0 cm glass cell. The ChemSketch® software provided by ACD Labs was used to measure and record visible absorption spectra.

2.3. General Procedure

Volumes of 0.2 – 2.0 mL of menthol solution were transferred in dry glass flasks and a final volume of 4.0 mL was adjusted with ethanol. 1.0 mL of salicylaldehyde solution (1%) and 10 mL of concentrated sulphuric acid solution (96-98%) were added carefully, drop by drop. The flasks were gently stirred to mix the solution and let it stand for 30 min, to reach the room temperature. The absorbance was measured at 510 nm, in a 1.0 cm glass cell against a blank solution prepared similarly, but without menthol. The menthol concentration in an unknown sample was determined using a prepared calibration graph, obtained as describes above.

The selectivity coefficients ($a_{menthol,j}$) were calculated as the ratio between menthol concentration and the interfering compounds concentration which gives a 5% absorbance change in a reference solution (6.65 μg menthol·mL^{-1}) (Dean, 1995).

The recovery test was done using two types of green tea (commercially available on our marked). In this case, an exact amount of green tea (1.0 g) was

weighed, and the menthol was extracted in 10 mL of ethanol, after 2 h of shaking. A known volume of obtained solution was measured and the menthol analysis was performed according with the procedure described above.

3. Results and Discussions

Under conditions of preliminary investigation of the proposed procedure, the red-orange coloured product is formed only in strong acid media. The best results were obtained in concentrated sulphuric acid (96-98%) media. Thus, in a total volume of 15 mL, the volume required for the maximum colour development was in the range of 9.0 – 12.0 mL. Hence, 10 mL of concentrated sulphuric acid (96-98%) was considered as reaction media, and this value was used in all further experiments.

In order to establish the optimum volume of salicylaldehyde solution (1%) used as colour reagent, two samples were prepared by adding 1.0 and 1.5 mL of reagent solution to 3.5-2.5 mL of ethanol solution contained 6.65 μg·mL menthol and 10 mL of concentrated sulphuric acid (96-98%). The absorption spectra in the visible region, recorded against a blank solution (prepared similarly, but without menthol) are illustrated in Fig. 1.

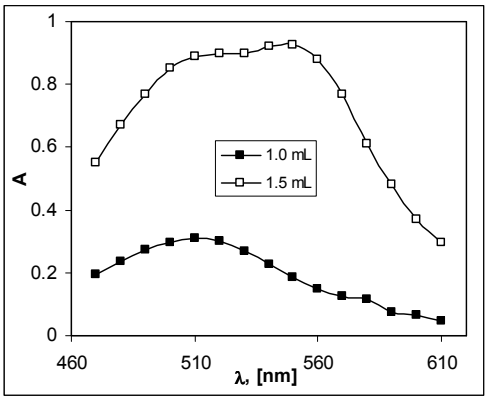

Fig. 1 − Effect of salicylaldehyde concentration used in the reaction on the absorption signal (menthol concentration = 6.65 $\mu g \cdot mL^{-1}$).

As can be seen from Fig. 1, for menthol concentration of 6.65 $\mu g \cdot mL^{-1}$ the measured absorbance is almost equal with unit, when a volume of 1.5 mL of salicylaldehyde is added. Under these conditions, there is a risk that for higher concentration of menthol, the measured absorbance to exceed the unit, which means that the accuracy of experimental measurements decrease significantly. From this point of view, more adequate is the utilization of 1.0 mL of salicylaldehyde solution (1%), when for a concentration of 6.65 $\mu g \cdot mL^{-1}$ the

measured absorbance is around 0.32. Thus, 1.0 mL of salicylaldehyde solution (1%) was considered sufficient for the total transformation of menthol in red-orange coloured product, and this value was chosen as optimal volume of reagent solution.

The absorption spectra of red-orange coloured product, recorded against a blank solution, for different menthol concentrations are presented in Fig. 2. The absorbance measurements against a blank solution instead of distilled water, was done in order to eliminate the errors in the experimental measurements caused by differences in density of solutions, which appear due to their preparation way. The blank solution was prepared similarly as described in the general procedure, but without adding menthol.

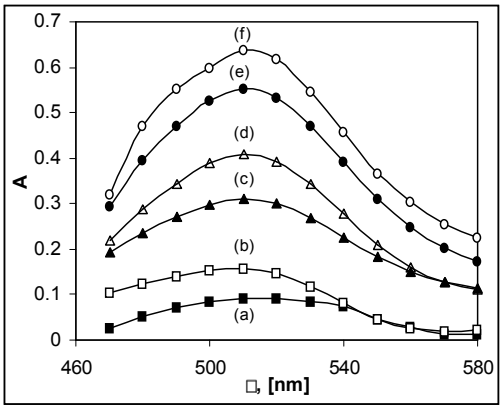

Fig. 2 − Absorption spectra of red-orange product recorded against blank solution at different menthol concentrations ((a): 1.33 μg·mL^{-1}; (b): 4.00 μg·mL^{-1}; (c): 6.65 μg·mL^{-1}; (d): 9.33 μg·mL^{-1}; (e): 12.00 μg·mL^{-1}; (f): 13.33 μg·mL^{-1}).

Under these conditions, the maximum of absorption is obtained at 510 nm, and can be successfully used for the quantitative determination of menthol in alcoholic solutions. The salicylaldehyde react immediately with menthol, forming a red-orange coloured reaction product, in concentrated sulphuric acid media, and the absorbance, measured after 30 min of stand-by, remains stable for at least 12 h.

The calibration curve (Fig. 3) was obtained as described in the general procedure using six standard solutions whose concentration of menthol is included in the linear dynamic range (Table 1). The linear regression equation (inside of Fig. 3) was obtained by use the mean values of six replicate measurements, where x represents the menthol concentration (μg·mL^{-1}) and R^2 is the correlation coefficient.

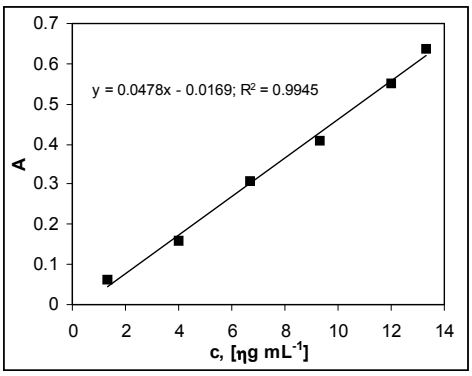

Fig. 3 − Calibration curve obtained for spectrophotometric determination of menthol with salicylaldehyde, in concentrated sulphuric acid media (the absorbance measurements was done against a blank solution, at 510 nm).

The detection limit calculated as three times of standard deviation (σ) of six replicate measurements, the calibration sensitivity obtained from the slope of calibration curve, the precision (RDS, %) as well as other analytical characteristics (Christian, 1994) of the proposed spectrophotometric method are summarized in Table 1.

Table 1
Analytical Characteristics of Proposed Spectrophotometric Method

Analytical parameter	At 510 nm, against a blank solution
Molar absortivity	$1.15 \cdot 10^4$ [L·mol^{-1}·cm^{-1}]
Spectral band width	89.5 [nm]
Correlation coefficient (R^2)	0.9945
Calibration sensitivity	0.05
Limit of detection (3 σ)	0.17
Limit of quantification (10 σ)	0.56
Linear dynamic range	1.33 – 13.33 [μg·mL^{-1}]

The Lambert-Beer law is obeyed from 1.33 to 13.33 μg·mL^{-1} of menthol, with a molar absorption coefficient of $1.1533 \cdot 10^4$ L·mol^{-1}·cm^{-1}, at 510 nm against a blank solution.

On the basis of analytical characteristics presented in Table 1 it can say that the proposed spectrophotometric method is rapid, sensitive and accurate, nor requires expensive reagents, has a reasonable linear dynamic range, and can be a good alternative for conventional analysis methods of menthol in various samples.

Another important step in characterization of the applicability of proposed spectrophotometric method is the selectivity towards menthol, in comparison with other interfering compounds. For the quantification of this characteristics the selectivity coefficients ($a_{menthol, j}$) were used, that are defined as the ratio of menthol concentration ($c_{menthol}$) to the interfering compound concentration (c_j) which gives a 5% absorbance change from the reference solution. In this study, the interfering compounds were added to an identical reference solution, with a constant menthol concentration (6.65 $\mu g \cdot mL^{-1}$), until a 5% change in absorbance was obtained. The calculated values of the selectivity coefficients are summarized in Table 2.

Table 2
The Values of Selectivity Coefficients

Interfering compound, J	C_J, [$mg \cdot mL^{-1}$]	LG $A_{MENTHOL, J}$
Acetone	0.03	0.28
Acetic acid	1.37	− 2.31
Citric acid	12.06	− 3.26
Ascorbic acid	0.21	− 1.48

The obtained values of selectivity coefficients indicate that the proposed spectrophotometric method is selective for menthol in the presence of several organic compounds that can appear in the food or cosmetic products. Thus, it can be observed from Table 2 that acetic acid, citric acid or ascorbic acid practically does not interfere in the spectrophotometric determination of menthol. This means that the proposed spectrophotometric method can be used for the quantitative determination of menthol in various commercial food and cosmetic products, where these compounds are also present.

Only the acetone gives a significant positive interference in the menthol determination, when its concentration is five times higher that menthol concentration. This observation is important because even if in the composition of most food and cosmetic products the acetone is not present, in the quantitative determination of menthol, the analyzed samples do not need to be dissolved in acetone.

The proposed method was validated by using recovery test and measuring the menthol concentration from two types of green tea (commercially available in our marked). The menthol from the green tea samples (around 1.0 g) was extracted in 10 mL of ethanol, after 2 h of mechanically shaking. To investigate the recovery of menthol, a known volume of obtained solution (2.0 mL) was transferred to each of the three dry glass flasks, and 0.5 mL, 1.0 mL and 1.5 mL of menthol standard solution (100 $\mu g \cdot mL^{-1}$) was added to each flask. In all the cases, the samples were analyzed according to the general procedure.

The menthol content was determined using a calibration graph at 510 nm, and the obtained results are summarized in Table 3.

Table 3
The Recovery Test

$C^{MENTHOL}_{ADDED}$ [μg·mL^{-1}]	Green tea 1		Green tea 2	
	$c^{menthol}_{added}$ [μg·mL^{-1}]	Recovery [%]	$c^{menthol}_{added}$ [μg·mL^{-1}]	Recovery [%]
0.00	10.21	–	11.15	–
3.33	13.46	97.59	14.39	97.29
6.65	17.02	102.41	17.86	100.91
10.00	20.81	104.76	21.43	102.81

Average of six determinations, calculated based on the calibration graph.

The good recovery of menthol was obtained for both types of green tea, indicating that the constituents of tea samples do not interfere significantly with the determination of menthol. Therefore, this method can be a good alternative device for the direct determination of menthol, in food and cosmetic samples. In addition, this method is advantageous in terms of accuracy and simplicity, is cheap and ready available in most laboratories of quality control.

4. Conclusions

The proposed method using salicylaldehyde as spectrophotometric reagent for direct determination of menthol is simple, selective, reproducible and accurate. The red-orange reaction product is formed in presence of concentrated sulphuric acid (96-98%), and its absorbance (measured at 510 nm, against a blank solution) remains stable for at least 12 h. The method allows the determination of menthol over the range 1.33-13.33 μg·mL with a molar absorption coefficient of $1.1533 \cdot 10^4$ L·mol^{-1}·cm^{-1} and a detection limit of 0.17 ppm.

The good selectivity of the proposed method to menthol in the presence of several organic compounds, which can be presents in the food and cosmetic samples, point out the applicability of proposed method. This observation is sustained also be the good results obtained in case of recovery test. The proposed method is rapid, requires low-cost equipments and therefore can be a good alternative for the direct determination of menthol in food and cosmetics samples.

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DETERMINAREA DIRECTĂ A MENTOLULUI FOLOSIND O METODĂ SPECTROFOTOMETRICĂ SIMPLĂ

(Rezumat)

În acest studiu, este propusă o metodă spectrofotometrică simplă, selectivă şi precisă pentru determinarea directă şi rapidă a mentolului, şi au fost stabilite condiţiile experimentale optime, împreună cu alţi parametri analitici. Această metodă se bazează pe reacţia dintre mentol şi aldehida salicilică, în mediu de acid sulfuric concentrat (96-98%), când se obţine un produs de reacţie de culoare roşu-portocaliu. Spectrul în domeniul vizibil al produsului de reacţie obţinut, înregistrat faţă de o probă martor, prezintă un maxim la 510 nm, iar absorbanţa rămâne stabilă pentru cel puţin 12 ore. Metoda permite determinarea mentolului în domeniul de concentraţie cuprins între 1,33-13,33 $\mu g \cdot mL^{-1}$, cu un coeficient molar de absorbţie de 1,1533 10^4 $L \cdot mol^{-1} \cdot cm^{-1}$ şi o limită de detecţie de 0,17 ppm. Interferenţele cauzate de unii compuşi organici (acidul acetic, acidul citric sau acidul ascorbic), care pot fi prezente împreună cu mentolul în diferite probe reale, au fost de asemenea determinate. Validarea metodei spectrofotometrice propuse a fost făcută prin teste de recuperare a mentolului din ceai verde. Rezultatele obţinute arată că metoda propusă poate fi folosită cu succes pentru determinarea directă a mentolului, iar principalul avantaj este faptul că produsul de reacţie colorat apare imediat după amestecarea reactivilor.