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Determination of *l*-menthol in pharmaceutical products by high performance liquid chromatography with polarized photometric detection

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Abstract

A simple analytical method for *l*-menthol by high-performance liquid chromatography with a polarized photometric detector was established. The polarized photometric detector was constructed with two polarizers mounted on both sides of the flow cell in a conventional photometric detector and can be easily used for the detection of optically active compound, such as *l*-menthol. This study was conducted with a newly developed split-cell assembly in order to increase the sensitivity. The characteristic of this method is the ability to determine optically active compounds selectively among the other coexisting materials and the pretreatment of the sample can be very simple or not required at all. The detection of *l*-menthol by this method is 0.5 μg. A good agreement was shown between another commonly used GC method and the method described in this paper. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: *l*-Methanol; Chiral HPLC assay; Polarized photometric detector

1. Introduction

Menthol is an alcohol obtained from extracts of various mint oils or prepared synthetically. Currently, menthol is used as an ingredient of pharmaceutical products for its fragrance and flavor moreover, it is widely used in foods, beverages, cigarettes, tooth paste and food flavor for its particularly refreshing taste. Although both *l*-menthol and *dl*-menthol have been used in Japan, *l*-menthol is more commonly used. *d*-Menthol is scarcely used alone because of its lack of flavor. A great variety of chemically based techniques have been used for the analysis of *l*-menthol to compensate for the lack of chromophores in *l*-menthol. These include colorimetry [1–4], gas chromatography (GC) [4–9], normal-phase high performance liquid chromatography (HPLC) with refractive index detection [10], HPLC using fluorescence-labeling reagents [11] and indirect

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photometry [12]. However, these methods are low in sensitivity except for GC and HPLC using fluorescence-labeling reagents and lack applicability for real samples because of the lengthy and cumbersome sample preparation.

The objectives of our work were to establish a simple and rapid analysis of *l*-menthol (angle of rotation $\sim -50°$, 2.5 g, ethanol, 25 ml, 100 mm) in pharmaceutical products by HPLC using a polarized photometric detector (HPLC-PPD) [13–17] to facilitate the specific photometric detection of optically active compounds with no chromophores. The prototype split-cell assembly [15–17], consisting of twice the normal cell length, was installed in a polarized photometric detector in this system to increase the sensitivity.

2. Experimental

2.1. Materials and reagents

l-Menthol (99%) was pure grade from Wako (Osaka, Japan), (1*S*, 2*R*, 5*S*)-(+)-menthol (99%) and menthol (99%) were obtained from Aldrich (Tokyo, Japan). The mobile phase was filtered through a 0.45 μm membrane filter from Millipore (Milwaukee, WI). All the water used in this study was purified with a Millipore Milli-Q system (Millipore, Bedford, MA). Ten samples of over-the-counter drugs and clinical drugs identifying *l*-menthol as an active ingredient were purchased and prepared for analysis.

2.2. Apparatus

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-10 AD pump, a Rheodyne (Cotati, CA) Model 7125 injector (100 μl loop), a Shimadzu CTO-6A column thermostat and a Shimadzu SPD-10 AV UV-VIS detector. Also a Shimadzu SPD-M6A photodiode array detector was used for UV detection of *l*-menthol.

The prototype split cell assembly was installed in a SPD-10 AV detector mounting two polarizers (HN32, Polaroid, Norwood, MA) on both sides of the cell with a second polarizer on either side of the sample and reference cell at a tilted angle.

A Shimadzu GC-9A (Kyoto, Japan) with flame ionization detector (FID) and a Shimadzu C-R6A integrator were used for the GC analysis. The column used for the GC analysis was an open-tubular column G-205 (40 m × 1.2 mm i.d.) (df = 5.0 μm) from the Chemical Inspection and Testing Institute (Tokyo, Japan). For sample preparation a Branson model 5210 ultrasonic bath (Markham, Ontario, Canada) and Kubota KR-20 000 T centrifuge (Tokyo, Japan) were used. The solid phase extraction (SPE) columns used were Sep-pak C_{18} cartridge from Waters (MA, USA).

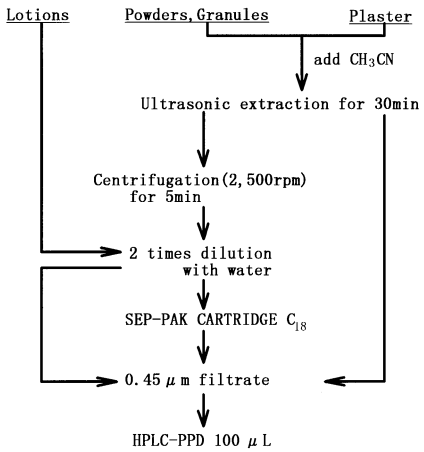
2.3. Sample preparation

2.3.1. Powders and granules

The powder or granule sample was dissolved in a small volume of acetonitrile, ultrasonicated for 30 min, then centrifuged for 5 min. The sample solution was quantitatively diluted 2-fold with water. The extract was passed through a Sep-pak C_{18} cartridge and filtered through a 0.45 μm membrane filter before analysis by HPLC (Scheme 1).

2.3.2. Lotions

An aliquot of the drug lotion was diluted 2-fold with water and filtered through a 0.45 μm membrane filter before analysis by HPLC (Scheme 1).



Scheme 1. The analytical procedure for determination of *l*-menthol from different samples.

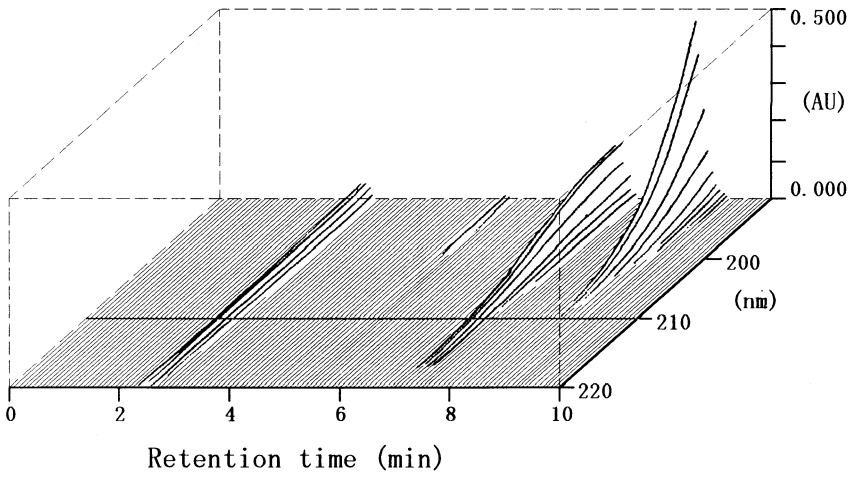


Fig. 1. HPLC chromatograms of *l*-menthol with a photodiode array detector.

2.3.3. Plasters

The plasters were extracted by ultrasonication with acetonitrile for 30 min and filtered through a 0.45 μm membrane filter before analysis by HPLC (Scheme 1).

An 100 μl aliquot of each sample solution was injected into the HPLC.

2.4. Chromatographic conditions

2.4.1. HPLC conditions

The analytical column used was a J'sphere ODS-H80 (YMC 4.6 mm i.d. × 150 mm). The mobile phase was water–acetonitrile (40:60 v/v) at a flow rate of 1.0 ml min^{-1} and column temperature of 40°C.

2.4.2. GC conditions

The GC column temperature was set at 108°C and both injector and detector temperatures were 250°C. The flow rate of the nitrogen carrier gas was 20 ml min^{-1}. The injection volume was 2 μl.

3. Results and discussion

3.1. UV absorption of l-menthol

l-Menthol absorbs sufficiently around 210 nm with a conventional spectrophotometer. However, the photodiode array detection of *l*-menthol by HPLC gave many peaks derived from structural isomers or other impurities (Fig. 1). *l*-Menthol has been contaminated with isomers and impurities during the process of production [18]. *l*-Menthol was confirmed to elute around 8 min by PPD. As shown in Fig. 1, this peak has no absorption above 210 nm, and it would be difficult to detect *l*-menthol at 210 nm with a UV detector.

3.2. Conditions of the detector

3.2.1. Detection wavelength and phase angle of the polarizers

Fig. 2 shows the energy distribution given by a tungsten lamp in the SPD-10 AV detector in

which these polarizers were set at fixed angles ($\pm$ 1.0 rad) and peak height of a 20 μl injection of 100 mM *l*-menthol under the same condition. The ordinate at the right represents light intensity expressed as the multiplication of the spectroscopic characteristics of the lamp, the grating and the photocell. That is, multiplying the current output value from the photocell of the detector by the transmittance of light at the polarizer, and the left is the peak intensity in absolute terms expressed in absorbance units. The increase in peak height as the detection wavelength moves toward the ultraviolet region suggests that the optical rotatory dispersion of *l*-menthol exhibits a plain curve. Whereas, in the short wavelength region there is an increase in base line noise, because the relative noise level is usually in inverse proportion to the light intensity. Therefore the wave length was set at 530 nm where excellent stability and the highest sensitivity can be obtained.

The authors have previously investigated the optimum phase angles of the polarizers for the determination of saccharides [17]. It was recognized that the SPD-10 AV detector used here showed a constant noise level at transmitted light energies above 700 mV as a result of these investigations. Consequently, the phase angle at the sample side was set at + 1.0 rad at which the light energy exceeded 700 mV at 530 nm. The circuit of reference side in the SPD-10 AV has a resistance which suppresses the output energy of the reference side by about one third of that of the sample side. When the absolute phase angle value of both the sample and reference sides are equal, the base

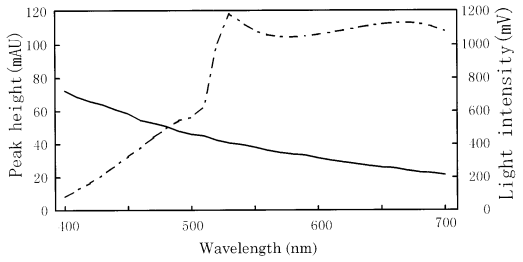


Fig. 2. Effect of wavelength with HPLC-PPD on the peak height of the *l*-menthol solid line:peak height of *l*-menthol, broken line:spectral energy distribution through the HN32 polarizers.

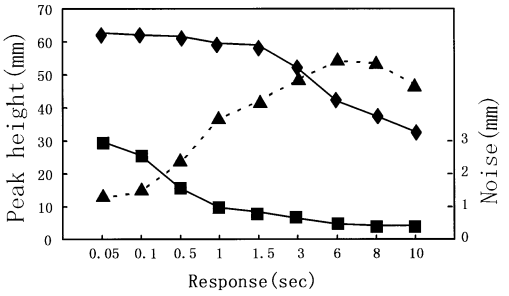


Fig. 3. Effect of response value on the peak height of *l*-menthol and on the level of baseline noise; -◆-, peak height of *l*-menthol; -■-, level of baseline noise; -▲-, S/N ratio.

line drift was observed due to the large difference in output energy between these two cells. Hence the base line drift was controlled by minimizing the difference in output energy between them, holding the phase angle of the reference side at + 0.9 rad. (see [15] for information on polarizer installation).

3.2.2. The response time and the calibration curve

By delaying the time constant of the detector, the effects on a decrease in noise level as well as a decrease in response of the sample peak were indicated. The effects of the time response of the detector on peak intensity of a 20 μl injection of 100 mM *l*-menthol and on the base line noise level are summarized in Fig. 3. Although a response time below 0.5 s appeared to provide good peak intensity as indicated in Fig. 3, the best sensitivity was observed at a response time of 6.0 s with respect to the signal to noise ratio (S/N). Therefore, the time constant of the detector was set at 6.0 s where a steady base line was observed.

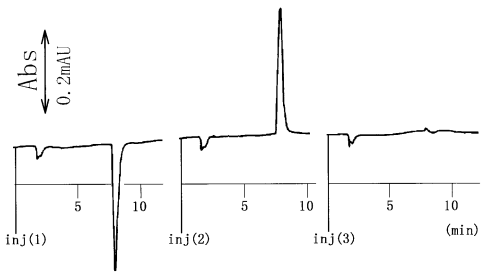


Fig. 4. HPLC chromatograms of standard compounds with PPD (1) *l*-menthol, (2) *d*-menthol, (3) *dl*-menthol.

Table 1
Determination of *l*-menthol in pharmaceutical products

Sample	Calculated (mg 100 ml^{-1})	Found (mg 100 ml^{-1})	R.S.D. (%)
Powder # a	10.00	10.50	4.4
Powder # b	15.00	15.50	1.3
Granule	35.00	34.12	1.3
Solution	30.00[a]	31.77[a]	1.6
Plaster # a	27.72	26.81	2.5
Plaster # b	28.67	28.76	3.3
Plaster # c	30.00	28.30	3.6
Plaster # d	32.76	31.17	1.8
Plaster # e	33.60	30.60	0.9
Plaster # f	52.21	49.85	2.2

$n = 6$.
[a] (mg ml^{-1}).

The chromatograms of standard materials detected under the preceding conditions are shown in Fig. 4. The peak of a 20 μl injection of 100 mM *d*-menthol (dextrorotatary) on the positive side and the peak of a 20 μl injection of 100 mM *l*-menthol (laevorotatary) on the negative side are shown in Fig. 4. The racemic form of menthol containing an equal ratio of the *d*- and *l*- form should not exhibit any peaks theoretically, by cancelling out their angle of rotation. Nonetheless, a slight positive peak was observed in the chromatogram of a 20 μl injection of 100 mM *dl*-menthol.

This seemed to be a residual refractive index artifact. The calibration curve obtained from the peak height and concentration of the analyte showed excellent linearity = 0.9998 going through the origin over a wide range (3–500 μg) of absolute injection volume and the reproducibility for each 30 and 200 μg gave R.S.D. of 2.4 and 0.3%, respectively. The detection limit of this method is 0.5 μg (S/N = 3).

3.3. Analysis of actual samples

The results from determinations of actual samples using the described extraction method (Scheme 1) are listed in Table 1. All samples gave good results against the indicated values in the pharmaceutical products. It should also be noted that the determination of menthol was carried out with extremely simple pretreatment of the samples without eliminating other ingredients in the pharmaceutical products. The chromatograms obtained under UV and PPD detection of powder sample (# b) and plaster sample (# f) are shown in Fig. 5. In spite of some interferences which appeared under UV detection, the specific determination of menthol was possible under PPD without any interferences from other co-extracts in the sample.

3.4. Correlation between the HPLC-PPC method and the GC method

l-Menthol in the plaster samples was determined by both the HPLC-PPD system and another commonly used GC method to compare the results. For sample preparation for GC analysis, the sample was extracted quantitatively with acetonitrile using ultrasonication, diluted 10-fold

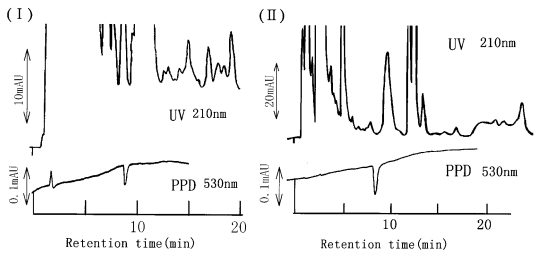


Fig. 5. HPLC chromatograms of *l*-menthol in pharmaceutical products with the PPD and UV detector (I) powder # b, (II) plaster # f.

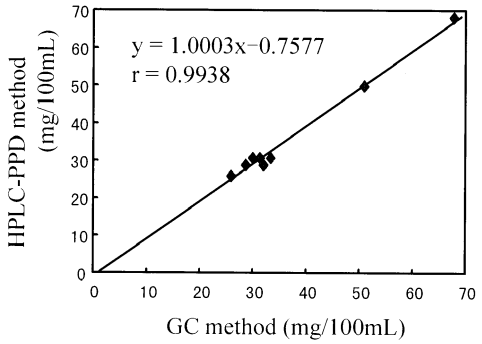


Fig. 6. Correlation between the GC method and the HPLC-PPD method.

with acetonitrile, followed by dehydration with anhydrous sodium sulfate. An aliquot of the sample solution was then filtered through a 0.45 μm membrane filter before analysis by GC.

The values $a = 1.0003$, $b = -0.7577$ and $r = 0.9938$ were obtained from the linear regression line ($y = ax + b$) (Fig. 6), and the values of the slope (a) and y-intercept (b) at 95% confidence limit using the t-value (2.45) with 6 degrees of freedom were,

$$a = 1.0003 \pm 0.0380, \quad b = -0.7577 \pm 4.4541$$

where slop = 1 and intercept = 0 are within the confidence limit. Hence, high correlation was recognized between these two techniques. As can be seen from the results above, the HPLC-PPD system described in this paper provides the effective determination of *l*-menthol with very simple sample pretreatment.

4. Conclusions

l-Menthol in pharmaceutical products and over-the-counter drugs was determined by HPLC with PPD. The specific analysis of *l*-menthol with no chromophores was possible by HPLC with only simple pretreatment of the sample. The PPD can be easily configured by pairing two polarizers with a conventional photometric detector for HPLC. The method described in this paper obtained good correlation with the commonly used GC method, and thus, HPLC-PPD can be a very useful technique for the analysis of other optically active compounds.

References

[1] N.G. Yurova, D.M. Popov, T.A. Denisova, Farmatsiya (Moscow) 30 (1981) 65.
[2] P.S. Kovtun, A.O. Kozireva, A.K. Bagrii, Farm. Zh. (Kiev) 6 (1983) 62–63.
[3] T.O. Safronova, D.M. Popov, T.G. Shevchenko, Khim. Farm. Zh. 16 (1982) 110–112.
[4] H.D. Spitz, Int. Flavours Food Addit. 8 (1977) 248–248.
[5] J.P. Sapio, K. Sethachutkul, J.E. Moody, J. Pharm. Sci. 68 (1979) 506–550.
[6] M. Sugrue, J.J. Brennan, Anal. Proc. 24 (1987) 191–192.
[7] U.S. Pharmacopeia National Formulary, USP XX III, US Pharmacopeial Convention, Rockville, MD, 1995.
[8] E.A. Tavss, S.G. Wiet, R.S. Robinson, J. Santalucia, D.L. Carroll, J. Chromatogr. 438 (1988) 273–280.
[9] F. Ortiz-Boyer, M.T. Tena, M.D. Luque de Castro, M. Valcarcel, J. Pharm. Biomed. Anal. 13 (1995) 1297–1303.
[10] S.A. Haut, M.T. Core, J. Liq. Chromatogr. 4 (1981) 1869–1874.
[11] Y. Tutaya, Y. Data, K. Kohashi, Anal. Sci. 7 (1991) 411–413.
[12] J.E. Parkin, J. Chromatogr. 303 (1984) 436–439.
[13] A. Yamamoto, A. Matsunga, K. Hayakawa, E. Mizukami, M. Miyazaki, Anal. Sci. 7 (1991) 719–721.
[14] K. Hayakawa, A. Yamamoto, A. Matsunaga, E. Mizukami, M. Nishimura, M. Miyazaki, Biomed. Chromatogr. 8 (1994) 130–133.
[15] A. Yamamoto, A. Matsunaga, E. Mizukami, et al., Analyst 120 (1995) 1137–1139.
[16] A. Yamamoto, A. Matsunaga, E. Mizukami, K. Hayakawa, M. Miyazaki, M. Nishimura, J. Chromatogr. A. 727 (1996) 55–59.
[17] A. Yamamoto, T. Wataya, K. Hayakawa, A. Matsunaga, M. Nishimura, M. Miyazaki, J. Pharm. Biomed. Anal. 15 (9–10) (1997) 1383–1387.
[18] The Japanese Pharmacopoeia, JP X III., 1996.