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Note

High-performance liquid chromatographic assay of menthol using indirect photometric detection

J. E. PARKIN
School of Pharmacy, Western Australian Institute of Technology, Kent Street, Bentley, Western Australia 6102 (Australia)
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Menthol is commonly found in pharmaceutical products and a number of analytical methods have been developed for its quantitation in both pharmaceutical products and volatile oils. These include colorimetric methods[1–4], gas–liquid chromatography[4–8] and normal-phase high-performance liquid chromatography (HPLC) with refractive index detection[9]. However, menthol lacks a chromophoric system and cannot normally be detected using UV detection systems.

Recently it has been demonstrated that non-UV absorbing compounds can be detected in reversed-phase HPLC by the addition of a suitable UV-absorbing compound to the running-solvent[10]. During a study of this phenomenon it was noted that menthol could be detected and quantitated by this method. This paper presents a simple HPLC assay for menthol in a simple pharmaceutical product [Menthol Inhalation A.P.F.[11], 2% (w/v) menthol in 90% ethanol] using indirect photometric detection.

EXPERIMENTAL

Reagents and materials

n-Heptyl-*p*-aminobenzoate and *n*-pentyl-*p*-aminobenzoate were prepared from *p*-nitrobenzoic acid and the corresponding alcohol by the method of Flynn and Yalkowsky[12] and recrystallised repeatedly from ethanol–water (60:40, v/v). The *p*-nitrobenzoic acid, *n*-heptanol, *n*-pentanol, natural (−)-menthol and synthetic (±)-menthol were obtained from Aldrich (U.S.A.). The methanol was HPLC grade (Ajax Chemicals, Australia).

Chromatographic equipment

The liquid chromatograph consisted of a pump and variable-wavelength detector (LC-3, Pye-Unicam, Cambridge, U.K.), 20-μl loop injector (Rheodyne 7125, Cotati, U.S.A.), integrating recorder (Hewlett-Packard 3380 A, Palo Alto, U.S.A.), and a μBondapak C_{18} column (30 cm × 6.4 mm I.D., 10 μm particle size) (Waters Assoc., Sydney, Australia).

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Chromatographic conditions

The mobile phase was methanol–water (75:25, v/v) containing $2 \cdot 10^{-5}$ *M* *n*-heptyl-*p*-aminobenzoate at a flow-rate of 1.5 ml min^{-1}. Monitoring wavelength was 290 nm.

Standard solutions

Menthol (2.5%, w/v) standards were prepared in 90% ethanol and serially diluted to prepare calibration curves.

Internal standard solutions used were either $5 \cdot 10^{-5}$ *M* *n*-pentyl-*p*-aminobenzoate in 90% ethanol or *n*-heptanol (5%, v/v) in 90% ethanol.

RESULTS AND DISCUSSION

It has been previously observed in this laboratory[10] that neutral compounds such as aliphatic alcohols, ethers and esters can be quantitated in the reversed-phase mode by indirect photometric detection following the addition of a neutral UV-absorbing detection compound (UV-ADC) to the chromatographic solvent. The presence of the alcohol on the column is sufficient to perturb the partitioning characteristics of the UV-ADC to enable detection and quantitation to be made. Compounds eluting before the UV-ADC give positive peaks and a negative "system-peak" at the retention time of UV-ADC[10]. The response was found to be greatest when a UV-ADC was used which eluted immediately after the compound being studied[10]. Further studies in this laboratory[13] have shown that this is a general phenomenon and can be applied to the detection of a wide range of compounds by the careful selection of a suitable UV-ADC. In this study *n*-heptyl-*p*-aminobenzoate was used as the UV-ADC. The *p*-aminobenzoate esters, which can be readily synthesized from the corresponding alcohols[12], afford a series of compounds with widely differing polarities which readily aid optimisation in the selection of UV-ADCs eluting immediately after the compound being analysed.

The injection of 20 μl of (±)-menthol in 90% ethanol over the range 0–2.5% (w/v) gave a linear calibration curve which passed through the origin:

$$\text{Area response} = 179.2 \cdot 10^3 \text{ (conc. \%, w/v)} - 0.89 \cdot 10^3$$
$$(n = 5, r = 0.9993)$$

with the menthol peak well separated from the system peak (Fig. 1a). Repeated injection of a sample of menthol inhalation A.P.F. (2%, w/v, menthol in 90% ethanol) afforded a single peak due to the menthol; the coefficient of variation based on six replicate determinations being 1.8%. It was found that the analysis could be modified to include an internal standard, either by dilution of the inhalation (5 ml) with (5%, v/v) *n*-heptanol (2 ml) (Fig. 1b) or $5 \cdot 10^{-5}$ *M* *n*-pentyl-*p*-aminobenzoate (2 ml) (Fig. 1c), with results of comparable precision. The *n*-pentyl-*p*-aminobenzoate is an internal standard quantitated by direct photometric detection whereas the *n*-heptanol is quantitated in the indirect photometric mode. It was also shown that natural (−)-menthol and synthetic (±)-menthol give identical responses. This is important as the British Pharmacopoeia 1980[14] allows the use of either natural or synthetic menthol in pharmaceutical products.

A disadvantage of the method is the lack of sensitivity, the method requiring

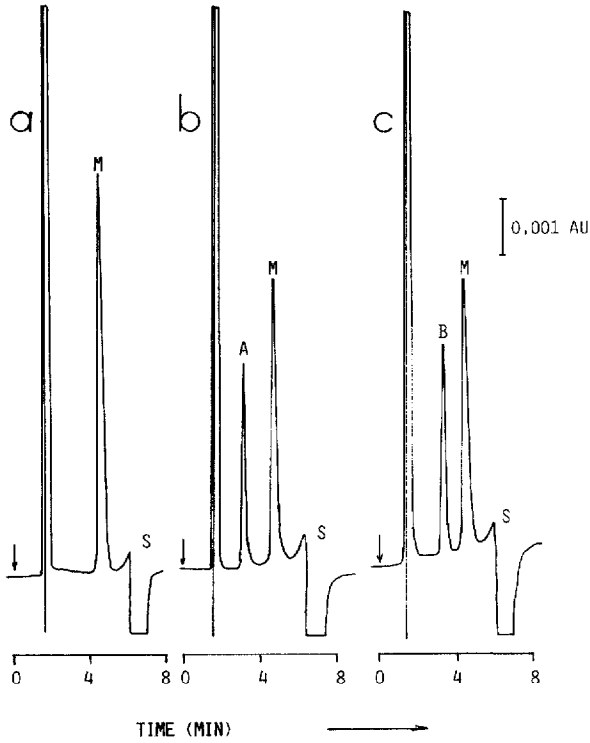

Fig. 1. Chromatograms of (a) menthol (2%, w/v); (b) menthol (2%, w/v) (5 ml) diluted with *n*-heptanol (5%, v/v) (2 ml); (c) menthol (2%, w/v) (5 ml) diluted with $5 \cdot 10^{-5}$ *M* *n*-pentyl-*p*-aminobenzoate (2 ml). All solutions in 90% ethanol. Volume of injection 20 μl. M = menthol; A = *n*-heptanol; B = *n*-pentyl-*p*-aminobenzoate; S = system peak. Flow-rate 1.5 ml/min; monitoring wavelength 290 nm.

the injection of relatively high concentrations of the compound to be analysed. This could result in interference from low concentrations of strongly UV-absorbing components in the case of more complex formulations of menthol. This problem may be circumvented by the use of detection compounds which absorb in the visible region of the spectrum with subsequent greater specificity. Studies on this and related problems are continuing in these laboratories.

REFERENCES

1 N. G. Yurova, D. M. Popov and T. A. Denisova, *Farmatsiya (Moscow)*, 30 (1981) 65; *Anal. Abstr.*, 42 (1982) 2E23.
2 P. S. Kovtun, A. A. Makarov and A. K. Bagrii, *Farm. Zh. (Kiev)*, 6 (1980) 68; *Anal. Abstr.*, 42 (1982) 3E49.
3 T. O. Safranova, D. M. Popov and T. G. Shevchenko, *Khim.-Farm. Zh.*, 16 (1982) 110; *Anal. Abstr.*, 43 (1982) 6E38.
4 H. D. Spitz, *Int. Flavours Food Addit.*, 8 (1977) 248; *Anal. Abstr.*, 34 (1978) 5F27.
5 J. P. Sapio, K. Sethachutkul and J. E. Moody, *J. Pharm. Sci.*, 68 (1979) 506.
6 F. de Fabrizio, *J. Pharm. Sci.*, 70 (1981) 1151.
7 K.-A. Kovar and A. Sakmann, *J. Chromatogr.*, 247 (1982) 356.
8 H. S. I. Tan, P. A. Kemper and P. E. Padron, *J. Chromatogr.*, 238 (1982) 241.

9 S. A. Hant and M. T. Core, *J. Liq. Chromatogr.*, 4 (1981) 1869.
10 J. E. Parkin, *J. Chromatogr.*, 287 (1984) 457.
11 *Australian Pharmaceutical Formulary and Handbook*, The Pharmaceutical Soc. of Aust., Canberra, Australia, 13th ed., 1983.
12 G. L. Flynn and S. H. Yalkowsky, *J. Pharm. Sci.*, 61 (1972) 838.
13 J. E. Parkin and L. H. Tai, unpublished results.
14 *British Pharmacopoeia 1980*, Vol. 1, HMSO, London, 1980.