Journal of Chromatography, 238 (1982) 241–246
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,652

Note

Gas–liquid chromatographic assay of mixtures of camphor, menthol, and methyl salicylate in ointments*

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(Received December 15th, 1981)

Many over-the-counter (OTC) preparations to relieve pain due to strains, sprains, sore muscles and joints, rheumatism, neuralgia and similar disorders by topical application contain mixtures of camphor, menthol, and methyl salicylate as active ingredients. These compounds serve as counterirritants and by rubbing them into the skin stimulate circulation and relax muscle tension. Since preparations containing these three active ingredients are marketed by numerous manufacturers there is need for a simple, rapid, and reliable assay method to aid in the quality control of these OTC products. This is particularly important for methyl salicylate because U.S. federal law requires that preparations containing more than 5% of methyl salicylate must be dispensed in child-resistant containers with warning to use only as directed for external use.

Assay methods for the quantitative determination of combinations of any two of these drugs have been reported[1–3]. These methods were based on gas–liquid chromatography (GLC) since these drugs are volatile substances. However, only a few methods were published which allowed the assay of mixtures containing all three drugs[4–6]. Bruno[4] isolated the three compounds from the sample by steam distillation, followed by extraction of the distillate into chloroform. After washing with sodium bicarbonate and drying, the chloroform solution was injected onto the gas chromatograph. The isolation step was time-consuming and great care must be taken to prevent the loss of the compound(s) during distillation. Although Stevens and Warren[5] analyzed methyl salicylate in the presence of menthol and camphor by GLC after extraction or steam distillation, the latter two compounds were not quantitated. Douglas[6] assayed mixtures of all three compounds by GLC using phenol as internal standard. However, this method dealt with oily solutions and hydrophobic ointments only and was not applicable to many hydrophilic ointments containing the three compounds.

This paper describes a simple, rapid, and reliable GLC internal standard method for the quantitation of camphor, menthol, and methyl salicylate in both hydrophobic and hydrophilic OTC preparations.

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* To be presented at the meeting of the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Las Vegas, NV, U.S.A., April 1982.

0021-9673/82/0000–0000/$02.75

EXPERIMENTAL

Apparatus

We used a Varian Aerograph Model 2740 flame ionization gas chromatograph equipped with Disc chart integrator (Model 200, Disc Instruments, Santa Ana, CA, U.S.A.), a Sargent-Welch Model SRG strip-chart recorder, and 10-μl Hamilton Series 700 syringes.

Reagents and materials

The following reagents and materials were used: *d*-camphor, *l*-menthol, methyl salicylate, and benzyl alcohol (Eastman Kodak, Rochester, NY, U.S.A.); cyclohexane and methanol (99 mole % pure, Fisher, Fair Lawn, NJ, U.S.A.); Carbowax 20M and Chromosorb W-HP 80–100 mesh (Supelco, Bellefonte, PA, U.S.A.).

GLC conditions

A 6 ft. × 1/8 in. I.D. glass column packed with 5% Carbowax 20M on 80–100 mesh Chromosorb W-HP was used. Nitrogen was the carrier gas delivered at a flow-rate of about 30 ml/min. The flow-rates for hydrogen gas and air were approximately 30 and 300 ml/min, respectively. The injector port was heated at 200°C and the detector was maintained at 200°C. The temperature of the oven was programmed between 130° and 170°C at 6°/min. A flame ionization detector current of 1×10^{-9} A was used. Attenuation was adjusted to keep all peaks on the chart paper.

Internal standard solution

One ml of benzyl alcohol was diluted to 25.0 ml with methanol.

Standard solution preparation

About 0.5 g each of camphor, menthol, and methyl salicylate were weighed accurately and transferred into a 50-ml volumetric flask. After 1.0 ml of the internal standard solution was added, the solution was diluted to volume with methanol. For most applications, this solution was diluted again ten-fold using a volumetric flask with methanol. A different dilution might be necessary to accomodate a particular sample.

Sample solution preparation

Hydrophilic products (creams and ointments with water-soluble bases). A sample of about 3 g was weighed accurately in a 100-ml volumetric flask and dissolved or suspended in methanol. After 1.0 ml of internal standard solution was added, the mixture was diluted to volume with methanol and then filtered through dry filter paper, if necessary, discarding the first 5 ml of filtrate. Five milliliters of the filtrate were pipetted into a 50-ml volumetric flask and diluted to volume with methanol.

Hydrophobic products (ointments with water-insoluble bases). A sample of about 3 g was weighed accurately in a 25-ml beaker and dissolved in 7 ml of cyclohexane. The solution was transferred quantitatively into a 50-ml volumetric flask. The beaker was rinsed twice with 3-ml portions of cyclohexane, and the washings were combined with the cyclohexane solution in the volumetric flask. After 1.0 ml of

internal standard solution was added, methanol was slowly added to the mark while the mixture was shaken vigorously. The precipitated ointment base was allowed to settle and the supernatant was filtered through dry filter paper, if necessary, discarding the first 5 ml of filtrate. Five milliliters of the filtrate were pipetted into a 50-ml volumetric flask and diluted to volume with methanol.

Chromatographic procedure

Using a 10-μl syringe, 1.5 μl of the sample solution and 1.5 μl of the standard solution were injected into the gas chromatograph under the operating conditions described above. Quantitation was based on relating the compound–internal standard peak area ratio of the sample to that of the standard.

RESULTS AND DISCUSSION

The almost quantitative removal of water-insoluble bases from hydrophobic products prolonged column life. With the method of Douglas[6], for example, a solution of the ointment was directly injected onto the column. Since a relatively high carrier gas flow-rate was used, in time the deposited ointment base diffused through the column, thereby changing the column characteristics.

Under the proposed experimental conditions camphor, menthol, methyl salicylate, and benzyl alcohol eluted as symmetrical sharp peaks and were well-separated from one another (Fig. 1). The approximate retention times were for camphor 1.4, menthol 1.8, methyl salicylate 2.8, and benzyl alcohol 3.6 min. The entire elution can be completed within 4 min which is much faster than previously reported methods[1,5,6].

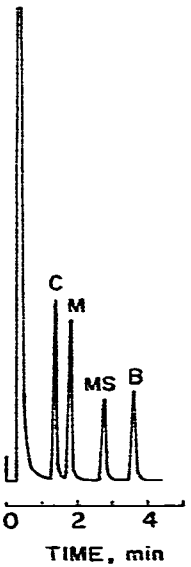


Fig. 1. Gas chromatogram of a standard solution run under conditions described in text. C = camphor; M = menthol; MS = methyl salicylate; and B = benzyl alcohol.

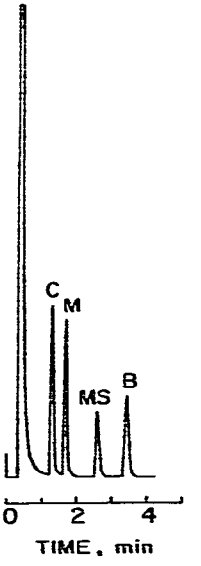


Fig. 2. Gas chromatogram of the extract from a hydrophilic synthetic mixture run under conditions described in text. C = camphor; M = menthol; MS = methyl salicylate; and B = benzyl alcohol.

TABLE I

RECOVERY DATA FROM SYNTHETIC OINTMENTS

Sample	*Camphor*			*Menthol*			*Methyl salicylate*		
	Amount (mg/g)		*Recovery (%)*	*Amount (mg/g)*		*Recovery (%)*	*Amount (mg/g)*		*Recovery (%)*
	Weighed	*Found*		*Weighed*	*Found*		*Weighed*	*Found*	
Hydrophilic									
1	165.0	167.8	101.7	161.4	166.0	102.9	179.4	183.8	102.5
2	222.3	215.6	97.0	160.3	152.4	95.1	223.6	220.2	98.5
3	214.7	215.3	100.3	156.7	154.7	98.7	223.4	218.5	97.8
4	175.3	173.9	99.2	159.0	159.0	100.0	164.5	159.1	96.7
5	162.8	156.7	96.2	166.4	166.1	99.8	183.0	177.9	97.2
Overall recovery (%)			98.9			99.3			98.5
S.D.			2.3			2.8			2.3
Hydrophobic									
1	165.9	167.0	100.7	166.2	163.2	98.2	172.9	168.2	97.3
2	174.9	176.1	100.7	152.6	151.2	99.1	222.5	215.8	97.0
3	165.1	158.5	96.0	168.6	169.1	100.3	165.6	166.1	100.3
4	158.2	148.8	97.8	152.6	156.4	102.5	154.8	157.6	101.8
5	166.3	164.3	98.8	167.2	166.1	99.3	187.8	180.3	96.0
Overall recovery (%)			98.8			99.9			98.5
S.D.			2.0			1.6			2.5

The relationship between compound-internal standard peak area ratio and amount of compound injected was established. Linearity was obtained between 10.4–83.0 μg of camphor, 10.2–81.5 μg of menthol, and 9.4–93.6 μg of methyl salicylate injected. Typical regression equations were for camphor: $A = 0.026\ C + 0.160$; for

TABLE II

RECOVERY DATA FROM COMMERCIAL OTC PRODUCTS

Product	*Component*	*Label claim (mg/g)*	*Amount found* (mg/g)*	*Label claim (%)*
Hydrophilic A**	Camphor	50	49.5 ; 53.8	103.6
	Menthol	70	72.7 ; 75.8	106.1
	Methyl salicylate	70	73.6 ; 73.4	105.1
Hydrophilic B**	Camphor	50	47.9 ; 50.4	98.2
	Menthol	50	54.7 ; 56.3	110.9
	Methyl salicylate	50	17.6 ; 20.7	42.8
Hydrophobic A	Camphor	–	–	–
	Menthol	70	73.4 ; 74.3	105.5
	Methyl salicylate	150	150.1 ; 146.3	99.0
Hydrophobic B	Camphor	90	70.8 ; 69.4	77.9
	Menthol	13.5	20.1 ; 20.7	153.2
	Methyl Salicylate	–	–	–

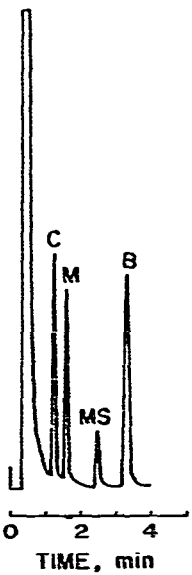
* Values of duplicate assays.

** Contains eucalyptol 10 mg/g.

menthol: $A = 0.029\ C + 0.099$; and for methyl salicylate: $A = 0.020\ C. + 0.047$, where A = compound-internal standard area ratio, and C = amount of compound injected in micrograms. The correlation coefficients r were for camphor 0.9986; for menthol 0.9997; and for methyl salicylate 0.9997.

Recovery studies were performed on synthetically prepared mixtures containing all three compounds. The hydrophilic base consisted of a prepared mixture of cetyl alcohol, white wax, sodium lauryl sulfate, polyethylene glycol 400, and water. The hydrophobic base consisted of white petrolatum. Fig. 2 shows a typical gas chromatogram from a synthetic hydrophilic mixture. Overall percent recoveries ($\pm$ S.D.) ($n = 5$) from hydrophilic synthetic mixtures for camphor, menthol, and methyl salicylate were 98.9 $\pm$ 2.3, 99.3 $\pm$ 2.8, and 98.5 $\pm$ 2.3%, respectively (Table I). The overall percent recoveries ($\pm$ S.D.) from hydrophobic synthetic mixtures were ($n = 5$) 98.8 $\pm$ 2.0, 99.7 $\pm$ 1.6, and 98.5 $\pm$ 2.5% for camphor, menthol, and methyl salicylate, respectively. Addition of eucalyptol in concentrations usually found in commercial products did not interfere with the assay. The eucalyptol peak was hardly noticeable

The method was applied to commercial hydrophilic and hydrophobic products. Table II shows the recovery data. The overall percent label-claim from these commercial preparations varied considerably from one brand to another. This would confirm the need for better quality control of these OTC products. In one brand the methyl salicylate content was only 42.8% of label claim (Hydrophilic B) whereas in another brand (Hydrophobic B) the menthol was 153.2% of label claim. Fig. 3 shows the gas chromatogram of Hydrophilic B. The gas chromatogram of Hydrophobic A is shown in Fig. 4.



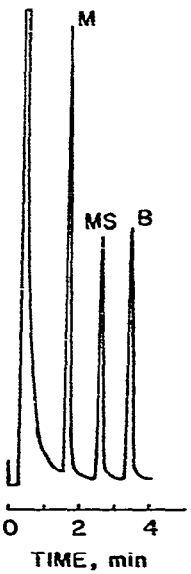


Fig. 3. Gas chromatogram of the extract from a commercial hydrophilic preparation run under conditions described in text. C = camphor; M = menthol; MS = methyl salicylate; and B = benzyl alcohol.

Fig. 4. Gas chromatogram of the extract from a commercial hydrophobic preparation run under conditions described in text. M = menthol; MS = methyl salicylate; and B = benzyl alcohol.

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