Simultaneous GLC Determination of Methyl Salicylate and Menthol in a Topical Analgesic Formulation

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Abstract □ A simultaneous GLC determination for methyl salicylate and menthol in a topical analgesic formulation is reported. *n*-Nonadecane is used as the internal standard. The method is rapid, accurate, precise, and selective in the presence of salicylic acid and ethyl salicylate.

Keyphrases □ Methyl salicylate—GLC analysis, topical analgesic formulation □ Menthol—GLC analysis, topical analgesic formulation □ GLC—analysis, methyl salicylate and menthol, topical analgesic formulation □ Analgesics, topical—methyl salicylate and menthol, GLC analysis

Combinations of methyl salicylate and menthol are used in several commercially available topical analgesic products (1). Official analytical methods for the quantitation of each of these active ingredients have been published (2). Other GLC determinations of methyl salicylate and menthol also have been published (3–5).

Simultaneous quantitation of methyl salicylate and menthol involved separating camphor, menthol, and methyl salicylate within 16 min on a 2-m × 2-mm i.d. stainless steel column containing 10% (w/w) polyethylene glycol on silanized Chromosorb W (5). Prior to GLC analysis, each sample was steam distilled and each distillate was extracted with chloroform. The amounts of camphor, menthol, and methyl salicylate were quantitated by peak area normalization. The reported precision was not greater than ±4%.

The described method is more rapid, more accurate, and more precise than the method described by Bruno (5) for the simultaneous determination of methyl salicylate and menthol in a topical analgesic formulation.

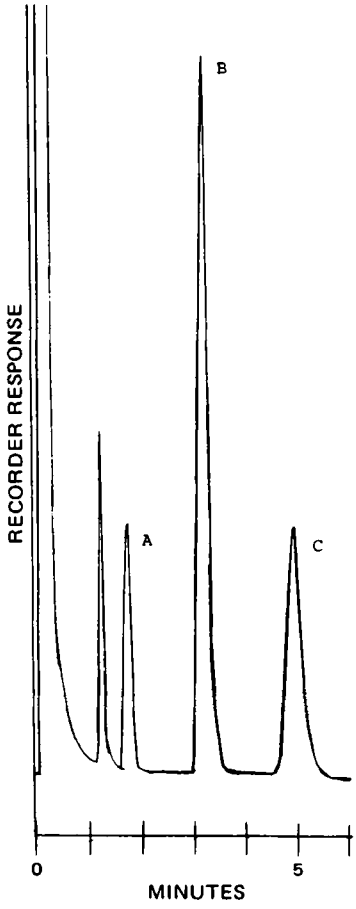

Figure 1—*Typical GLC separation of menthol (A), methyl salicylate (B), and* n*-nonadecane (C).*

EXPERIMENTAL

Chemicals and Reagents—Glass-distilled hexane[1], chloroform[1], and methanol[1] were used. Methyl salicylate[2], menthol[2], salicylic acid[2], and ethyl salicylate[3] were used as received. The internal standard was *n*-nonadecane[4].

GLC—A gas chromatograph[5] with a flame-ionization detector was used. A glass column, 180 cm × 3 mm i.d., was packed with 20% (w/w) polyethylene glycol[6] on 80–100-mesh Gas Chrom Q[6]. The carrier gas was helium[7] at a flow rate of 55 ml/min.

Analyses were carried out under the following conditions: injection port temperature, 200°; column oven temperature, 155°; and detector temperature, 220°. An electronic integrator[8] was used to determine peak areas.

Procedure—Approximately 1 g of the formulation was accurately weighed into a 100-ml volumetric flask. Then 10.0 ml of the internal standard solution [13 mg/ml in hexane–chloroform–methanol (50:50:3)] was pipetted into the flask, and the solution was diluted to volume with the same solvent. A standard solution was prepared similarly by accurately weighing proportional amounts of reference standard methyl salicylate and menthol. One microliter of the sample and standard preparations was injected into the gas chromatograph. Figure 1 shows a typical GLC separation.

Sensitivity—Calibration curves were determined for methyl salicylate and menthol in spiked placebo mixtures over the concentration ranges of interest.

Accuracy and Precision—The accuracy and precision of the GLC determination were evaluated from the recovery data for methyl salicylate (Table I) and menthol (Table II) from 10 spiked placebo mixtures.

The precision of injection for the GLC determination was evaluated from 20 injections of a placebo mixture spiked with methyl salicylate and menthol (Table III).

Selectivity—The selectivity of the GLC determination in the presence of salicylic acid and ethyl salicylate was evaluated by injecting standard mixtures containing these two compounds (Figs. 2 and 3).

RESULTS AND DISCUSSION

The calibration curves for methyl salicylate and menthol in this formulation were linear from 70 to 700 and 10 to 120 mg/g, respectively. No interference from a placebo mixture was observed.

The accuracy of this method, as measured from the recovery data, was 98.4 and 98.7% for methyl salicylate (Table I) and menthol (Table II), respectively. The precision, as measured from the standard deviations

[1] Burdick & Jackson Laboratories, Muskegon, Mich.
[2] USP reference standard.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Pfaltz & Bauer, Stamford, Conn.
[5] Model 3920 B, Perkin-Elmer Corp., Norwalk, Conn.
[6] Carbowax 20M, Alltech Associates, Arlington Heights, Ill.
[7] Liquid Carbonic Co., Chicago, Ill.
[8] Minigrator, Spectra-Physics, Piscataway, N.J.

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Table I—Recovery Data for the GLC Determination of Methyl Salicylate

Mixture	Theory, mg/g	Found, mg/g[a]	Recovery, %
1	300.06	297.92	99.3
2	299.25	292.48	97.7
3	306.00	300.26	98.1
4	305.09	302.56	99.2
5	300.06	295.55	98.5
6	303.23	295.12	97.3
7	304.40	296.06	97.3
8	303.08	297.28	98.1
9	300.07	296.11	98.7
10	300.50	298.79	99.4
Mean			98.4
SD, %			0.79

[a] Each value represents the average of duplicate sample injections.

Table II—Recovery Data for the GLC Determination of Menthol

Mixture	Theory, mg/g	Found, mg/g[a]	Recovery, %
1	51.15	50.98	99.7
2	31.78	31.34	98.6
3	49.97	49.24	98.5
4	50.06	49.92	99.7
5	49.24	48.63	98.8
6	50.83	49.66	97.7
7	50.53	49.18	97.3
8	51.78	51.16	98.8
9	48.94	48.56	99.2
10	51.09	50.60	99.0
Mean			98.7
SD, %			0.77

[a] Each value represents the average of duplicate sample injections.

of the recovery data, was ±0.79 and ±0.77% for methyl salicylate (Table I) and menthol (Table II), respectively.

The precision of injection (Table III), as measured from the standard deviations of the peak area ratios of the sample to the internal standard, was 1.428 ± 0.0201 and 0.356 ± 0.0048 for methyl salicylate and menthol, respectively. Such precision was obtained by filling the injection syringe in the following order: 0.5 μl of the solvent mixture, 0.5 μl of air, and 1 μl of the sample solution. This procedure assures that the sample will not be lost by volatilization through the rear seal of the syringe.

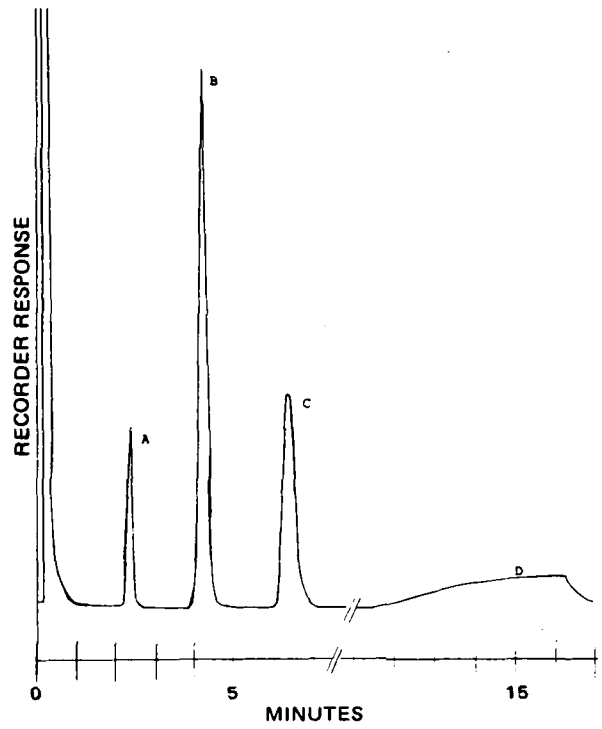

Figure 2—*GLC separation of menthol (A), methyl salicylate (B), n-nonadecane (C), and salicylic acid (D).*

Table III—Precision of Injection for the GLC Determination of Methyl Salicylate and Menthol

Injection	Area Methyl Salicylate / Area *n*-Nonadecane	Area Menthol / Area *n*-Nonadecane
1	1.443	0.359
2	1.431	0.356
3	1.431	0.357
4	1.399	0.351
5	1.447	0.361
6	1.431	0.357
7	1.409	0.351
8	1.415	0.353
9	1.451	0.363
10	1.405	0.352
11	1.419	0.354
12	1.404	0.350
13	1.421	0.355
14	1.454	0.363
15	1.421	0.355
16	1.475	0.367
17	1.426	0.355
18	1.408	0.351
19	1.452	0.362
20	1.423	0.354
Mean	1.428	0.356
SD, %	0.0201	0.0048

The selectivity of this method in the presence of salicylic acid (Fig. 2) and ethyl salicylate (Fig. 3) has been demonstrated. Such selectivity became important from the standpoint of product stability studies. The major hydrolysis product of methyl salicylate in this formulation would be salicylic acid. The major product from the transesterification between methyl salicylate and ethanol, a solvent used in the formulation, would be ethyl salicylate.

Prior sample preparations, such as steam distillation or solvent extraction, were not necessary for the analysis of this formulation. Elimination of these steps saved considerable analysis time and produced a chromatographic separation within 7 min. Column performance was maintained by periodically programming the column temperature to 220° to bleed off less volatile sample components.

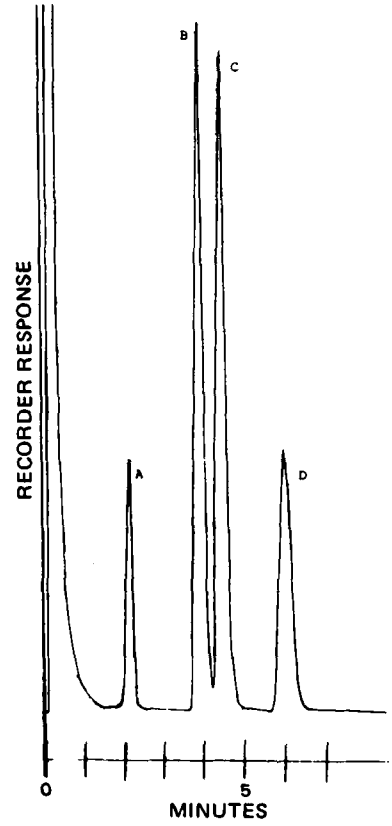

Figure 3—*GLC separation of menthol (A), methyl salicylate (B), ethyl salicylate (C), and n-nonadecane (D).*

REFERENCES

(1) "Handbook of Nonprescription Drugs," 5th ed., American Pharmaceutical Association, Washington, D.C., 1977, p. 293.

(2) "Official Methods of Analysis of the Association of Official Analytical Chemists," 12th ed., Association of Official Analytical Chemists, Washington, D.C., 1975, pp. 696, 753.

(3) S. Collings and R. Sinar, *J. Assoc. Public Anal.*, **4**, 59 (1966).

(4) W. Groebel, *Arch. Pharm.*, **300**, 226 (1967).

(5) E. Bruno, *Atti Congr. Qual.*, **6**, 121 (1967).

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Analysis of Dienestrol and Its Dosage Forms by High-Performance Liquid Chromatography

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Received September 22, 1977, from the *Department of Pharmaceutical Chemistry, College of Pharmacy, Rutgers—the State University of New Jersey, Piscataway, NJ 08854.* Accepted for publication October 3, 1978.

Abstract □ A high-performance liquid chromatographic (HPLC) analysis is described for dienestrol as a drug substance and in cream, foam, and tablet dosage forms. After incorporation of the drug or dosage form into a solvent mixture containing an internal standard, biphenyl, an aliquot was chromatographed using a reversed-phase medium, followed by UV spectrophotometric detection at 254 nm. The response of the chromatographic system was linear over a concentration range corresponding to 50–200% of the labeled amount of dienestrol. Satisfactory accuracy and precision were confirmed by analyzing cream by the standard addition method. The advantages of the HPLC method are its simplicity, speed, and sensitivity, which permit direct analysis of single-dose quantities of dienestrol.

Keyphrases □ Dienestrol—analysis by high-performance liquid chromatography, in various dosage forms □ High-performance liquid chromatography—analysis, dienestrol in various dosage forms □ Estrogens, synthetic—high-performance liquid chromatographic analysis in various dosage forms

Dienestrol [4,4′-(1,2-diethylidene-1,2-ethanediyl)bisphenol, I] is a synthetic estrogenic compound used for the treatment of osteoporosis and estrogen deficiency. Because of its high level of potency, the dose used is very small. Therefore, analysis of pharmaceutical preparations requires not only separation of the compound from the dosage form matrix but also detection of microgram quantities of drug.

BACKGROUND

Separation of dienestrol from associated interfering substances has been accomplished by TLC (1), solvent extraction (2), open column chromatography (2, 3), and reversed-phase high-performance liquid chromatography (HPLC) at elevated temperatures (4). Quantitation has been obtained by UV spectrophotomety of dienestrol itself or of the indene formed by acid-induced isomerization (2). A recent publication (5) discussed the optimization of a normal-phase HPLC separation of dienestrol but not its applicability to pharmaceutical dosage forms.

The method of analysis official in NF XIV (6) for dienestrol cream requires a preliminary solvent extraction followed by lengthy column chromatography. The isolated phenolic fraction is then reacted with nitrous acid, and the nitrosophenols are determined polarographically (7). Although the method is sensitive, it is time consuming and subject to wide variation due to the number of manipulations required.

The purpose of this study was to use the inherent speed and sensitivity of HPLC to develop a simple, direct, accurate, and sensitive enough method for dienestrol to determine single-dose quantities. Such an analysis would be particularly applicable in quality assurance and stability testing situations.

The proposed method involves only dissolution of the sample, addition of the internal standard, and introduction of an aliquot of the resulting mixture onto a liquid chromatograph with a reversed-phase column and a UV detector operated at 254 nm. The accuracy and precision are comparable to those reported for other methods, but the time required is significantly less.

EXPERIMENTAL

Reagents and Chemicals—Dienestrol NF reference standard was used as received. Biphenyl[1] was recrystallized twice from ethanol, dried *in vacuo*, and stored in a vacuum desiccator, mp 69–71°.

Solvents—Acetonitrile[2] (99 mole % pure) was used as received.

Apparatus—The HPLC system consisted of a pump[3] and a UV monitor[4] operated at 254 nm. Samples were introduced using a loop injector[5] with a fixed volume of 20 μl. The loop injector was equipped with a sample prefilter (8). The output of the detector was displayed on a recorder[6] having a full-scale range of 10 mv. The output signal was integrated, and results were calculated using an electronic integrator[7]. The 25-cm × 4.6-mm i.d. stainless steel column contained reversed-phase packing material[8]. The mobile phase was pumped at a pressure of about 1600 psig, which resulted in a flow rate of 3.0 ml/min.

A mechanical shaker[9] was used in the extraction of samples.

Mobile Phase—The mobile phase, a mixture of acetonitrile and water (40:60), was degassed *in vacuo* prior to use.

Standard Solutions—*Dienestrol Standard Solution*—An accurately weighed 0.0500-g sample of dienestrol was transferred to a 100-ml volumetric flask and diluted to volume with acetonitrile.

Internal Standard Solution—An accurately weighed 0.0500-g sample of biphenyl was transferred to a 100-ml volumetric flask and diluted to volume with acetonitrile.

Diluted Internal Standard Solution—Exactly 5.0 ml of internal standard solution was transferred to a 250-ml volumetric flask and diluted to volume with acetonitrile.

Dienestrol Drug Substance—*Standard Preparation*—Exactly 2.0 ml of dienestrol standard solution and 2.0 ml of internal standard solution were transferred to a 100-ml volumetric flask and diluted to volume with acetonitrile. A 20-μl sample was introduced into the liquid chromatograph. The areas under the two peaks were measured, and the ratio, R_s, was calculated by dividing the area of the dienestrol peak by the area of the internal standard peak.

Assay Preparation—About 50 mg of dienestrol was weighed and transferred to a 100-ml volumetric flask and diluted to volume with ac-

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Constametric IIG, Laboratory Data Control, Riviera Beach, Fla.
[4] Model 1203, Laboratory Data Control.
[5] Model HPSV-20, Spectra-Physics.
[6] Omniscribe model 521I-151, Houston Instruments, Austin, Tex.
[7] Model 3380A, Hewlett-Packard, Avondale, Pa.
[8] Partisil PXS-1025 ODS, Whatman, Clifton, N.J.
[9] Eberbach Corp., Ann Arbor, Mich.

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