Determination of Ethanol in an Elastomeric Matrix by Gas Chromatography

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Abstract □ A gas chromatographic procedure is described for the complete separation and quantitative determination of trace amounts of ethanol in the presence of nine common pharmaceutical compounds. By using a column containing an ethylvinylbenzene polymer, the occluded ethanol content of a single dosage unit can be determined in 8 min.

Keyphrases □ Ethanol, traces—separation, determination, elastomeric matrix, GLC □ Elastomeric matrix—ethanol separation, determination □ GLC—separation, determination

Ethanol has been determined by gas chromatography in liquid cough syrups (1) on a column of styrene-divinylbenzene polymer[1] and in blood (2–5) on a column of ethylvinylbenzene polymer.[2] This polymer has also been used to determine ethanol by gas chromatography in a variety of pharmaceuticals such as paregoric, mercurochrome, elixer phenobarbital, and antihistamine syrups (6). To date, there have been no studies reported in the literature concerning the determination of ethanol in a pharmaceutical dosage form comprising a water-soluble elastomeric material.

This report describes a simple, rapid method for determining ethanol which was occluded in an elastomeric matrix containing lidocaine, propylene glycol, sorbitol, sodium saccharin, polyvinyl alcohol, polyvinylpyrrolidone, gelatin, methylparaben, propylparaben, and common coloring and flavoring agents. The method was developed as part of a quality control program to determine the presence and quantity of ethanol in the finished dosage form prepared from a liquid mixture of the aforementioned components, which was subjected to heat above the boiling point of ethanol during processing.

EXPERIMENTAL

Instruments—A Perkin-Elmer model 811 gas chromatograph with a flame-ionization detector was used. The sensitivity of the detector was 0.005 C./g. The 1.82-m. (6-ft.) glass column, i.d. 0.32 cm. (0.125 in.), o.d. 0.63 cm. (0.25 in.), was packed with ethylvinylbenzene polymer 50/80 mesh, using a vibrator and conditioned for 18 hr. at 200° with helium gas flowing through the column. A small pledget of glass wool was placed in each end of the column. The following gauge pressures were used: helium, 30; hydrogen, 15; and compressed air, 40. The flow rate through the column was 64 ml./min. The column temperature was 122° and the injector temperature was 185°. A Bristol Dynamaster Strip-Chart recorder with a chart speed of 2.54 cm. (1 in.)/min. was used.

Standard Solution—A stock solution containing 1 μl. of 95% ethanol[3]/ml. was prepared by pipeting 2.0 ml. of alcohol into a 2-l. volumetric flask and diluting to the mark with distilled water.[4] Standard solutions containing 0.3, 0.5, 1.0, and 5.0 μl. of ethanol/10 ml. were prepared by pipeting 0.3, 0.5, 1.0, and 5.0 ml. of stock solution, respectively, into 10-ml. volumetric flasks and diluting to the mark with water. Five microliters of each standard was injected.[5] Table I shows the relationship of peak height[6] to alcohol concentration.

A single dosage unit of 5 cm.^{2} of the solid elastomer mixture was cut into two pieces and introduced into a 10-ml. volumetric flask containing 5 ml. of water. The pieces were dissolved with vigorous shaking, and the flask was diluted to the mark with water. Five microliters of the prepared solution was injected. This procedure was carried out for 74 additional dosage units. Ethanol concentrations were calculated as follows: μl. of ethanol/dosage unit = peak height × av. μl. of ethanol/10 ml./unit of peak height.

A standard containing 1 μl. of ethanol/10 ml. of solution was injected throughout this investigation. An evaluation of 10 injections of the standard indicated that steady-state operating conditions were maintained ($p = 0.06$ for $2S$).

Identification of the Ethanol Peak—The ethanol peak was qualitatively identified by the following enrichment method: 8 ml. of a solution consisting of 1 dissolved dosage unit/10 ml. (containing 0.025 μl. of ethanol/ml.) was pipeted into a 10-ml. volumetric flask containing 1 μl. of ethanol. The flask was diluted to the mark with water. The solution thus prepared contained 1.2 μl. ethanol/10 ml. The expected peak height was 13.0, and the expected retention[7] distance was 16.98 ± 0.22 cm. Following are the results of two trials:

	Peak Height	Retention Distance, cm.
Trial 1	13.5	16.9
Trial 2	12.8	16.7

Table I—Relationship of Peak Height to Ethanol Concentration

Ethanol, μl./10 ml.	Peak Height	Ethanol, μl./10 ml./Unit of Peak Height
0.3	3.0	0.10
0.5	5.0	0.10
1.0	10.5	0.095
5.0	51.0	0.098
		Av. = 0.098

Table II—Ethanol Content of Three Batches of Product

	No. of Dose Units	Alcohol Content, μl./unit
Batch 1	4	0.05
	11	0.10
	2	0.15
	6	0.20
	2	0.25
Batch 2	13	0.00
	12	0.05
Batch 3	23	0.00
	1	0.05
	1	0.25

[1] Polypak-2, F. M. Scientific Division, Hewlett Packard.
[2] Porapak Q, Waters Associates, Inc., Framingham, Mass.

[3] Ethyl alcohol USP, 95%, U.S. Industrial Chemical Co. Referred to as ethanol in the following text.
[4] Referred to as water in the following text.
[5] All samples in this study were injected with a 10-μl. Hamilton syringe.
[6] All peak heights in this study were measured on chart paper which was divided into 100 equal units.
[7] The retention distance is the distance in centimeters between the leading slope of the water peak and the maxima of the peak under investigation. The average retention distance for 5 standard ethanol solutions was 16.98 ± 0.22 cm.

This experiment indicated that the observed peak was due to ethanol. A similar procedure was used to identify the first major peak. The peak resulted from the organic impurities in water.

RESULTS AND DISCUSSION

Table II shows a summary of the ethanol content of the three batches studied. The column material used in this investigation has outstanding separation properties for volatile, low-molecular weight compounds and was particularly valuable for this study since no signals were observed from any of the compounds in the dosage form except ethanol.

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Antimicrobial Activity of Some β-Nitrostyrenes

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Abstract □ Nine compounds were tested for antimicrobial activity, and eight of these exhibited varying spectra of inhibition.

Keyphrases □ β-Nitrostyrene derivatives—antimicrobial activity □ Antimicrobial activity, evaluation—β-nitrostyrene derivatives □ Paper disks—microbe-inhibition analysis

In the course of synthesizing some chemical compounds for testing as potential molluscicides, a number of β-nitrostyrene compounds were prepared. This report indicates the preliminary results obtained from testing nine of these compounds, seven of which are new (1), for antibacterial and antifungal activity.

The antimicrobial activity of β-nitrostyrenes has been previously noted by several workers, including Schales and Graefe (2), Bocobo *et al.* (3), and Huitric *et al.* (4).

An impregnated filter paper disk on an inoculated agar plate was selected as the testing method. This technique has the advantage over the hole or cup method because the solvent can be evaporated from the saturated paper disks prior to placing them on the inoculated agar plate. In this manner, any potential antimicrobial effects from the solvent used can be avoided.

EXPERIMENTAL

Materials and Methods—The test organisms used included the following: *Staphylococcus aureus*, strain 209, ATCC 6538; *Escherichia coli*, ATCC 4157; *Pseudomonas aeruginosa*, ATCC 10145; *Bacillus subtilis; Proteus vulgaris; Aspergillus niger*, ATCC 9642; *Trichophyton mentagrophytes*, ATCC 9129; and *Candida albicans*, ATCC 10231. The melted test agar media were inoculated with 0.1 ml. of a 24-hr.-old bacterial culture grown in nutrient broth or a 0.1 ml. saline suspension of a 48-hr.-old fungal culture grown on Sabouraud's agar. Nutrient agar was employed for the bacterial tests

Table I—Antibacterial and Antifungal Activity of Some β-Nitrostyrenes

R	Microbial Spectrum[a] 1	2	3	4	5	6	7	8
(structure: R on phenyl–O–phenyl with NO_2, bearing C(H)=C(NO_2)—H)								
H	++[b]	++	+	−	−	+	+	+++
p-CH_3O—	++	++	−	−	−	+	++	+++
p-CH_3CONH—	++	++	+	−	−	−	−	+
2,4-Cl_2—	++	+	−	−	−	−	+	+++
Cl_5—	+	−	−	−	−	−	−	−
Br_5—	−	−	−	−	−	−	−	−
(structure: R on phenyl bearing C(H)=C(NO_2)—H)								
m-CH_3CO_2—	++	++	++	++	++	+	++	++
p-$CH_3CH_2CO_2$—	++	++	+++	−	++	+	+++	+++
3-CH_3O-4-CH_3CO_2—	++	++	++	−	++	++	+++	+++

[a] Microbial spectrum: Gram-positive: 1, *S. aureus*, strain 209, ATCC 6538; 2, *B. subtilis*. Gram-negative: 3, *E. coli*, ATCC 4157; 4, *P. aeruginosa*, ATCC 10145; 5, *P. vulgaris*. Fungi: 6, *C. albicans*, ATCC 10231; 7, *A. niger*, ATCC 9642; *T. mentagrophytes*, ATCC 9129. [b] Zone of inhibition: − = less than 12.7 mm. (zone includes diameter of disk); + = less than 20 mm., ++ = 21–29 mm., and +++ = more than 30 mm.