Gas Chromatographic Determination of Ethanol in Pharmaceuticals

Comparison of Polyethylene Glycol 400 and Divinylbenzene Polymer Columns

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Gas chromatography with a flame ionization detector was used with an official NF XII and a divinylbenzene polymer (DVBP) column to determine ethanol in pharmaceuticals. The DVBP column separated ethanol from methanol and gave results comparable to the polyethylene glycol 400 (PEG 400) column for various types of pharmaceuticals.

GAS CHROMATOGRAPHY has been used for the quantitative determination of alcohol in pharmaceuticals and other products for several years (1–5). Because of its numerous advantages, this method was investigated by the Pharmaceutical Manufacturers Association (6) and was adopted by the National Formulary (7). Elefant *et al.* (8) showed the advantages of the flame ionization detector over the thermal conductivity cell for the determination of ethanol.

Most earlier GLC methods for separating alcohols have utilized a polyethylene glycol column. Although this column was the most satisfactory of those available, ethanol could not be separated from methanol under the conditions suggested in NF XII (9). Another disadvantage is that the solvent peak elutes after the sample and internal standard peaks, unnecessarily prolonging the analysis time (8). The presence of water will affect the over-all sensitivity of the flame detector, although it does not give a response itself (10).

Recently a divinylbenzene polymer (DVBP) column[1] has been used for the separation of ethanol and chloroform in pharmaceuticals (11).

Hollis (12) introduced the use of porous polymer beads in gas chromatography. DVBP beads[2] more easily solved the problem of separating low molecular weight alcohols, acids, and glycols. Porous polymer beads have many advantages; they need no liquid phase, are hard and do not break during packing, require no pretreatment, have a large number of theoretical plates per unit volume, and condition in a relatively short time (approximately 1 hr.).

DVBP, unlike polyethylene glycol 400 (PEG 400),[3] does not retain water. When an aqueous solution is used, water will be eluted in the solvent peak or will be the first compound eluted from the DVBP column.

EXPERIMENTAL

Instrument—A Barber-Colman 5000 gas chromatograph equipped with a model 5062 oven, a flame ionization detector, and a 1-mv. recorder was used throughout this study.

PEG 400 Column—A 183 cm., 3-mm., U-shaped glass column was packed with 20% PEG 400 on 80/-100 mesh Anakrom ABS prepared by the slurry procedure (13), and conditioned at 125° for approximately 48 hr.

DVBP Column—A 120 cm., 3-mm., U-shaped glass column was packed with 80/100 mesh DVBP and conditioned at 235° for approximately 1 hr.

Operating Conditions—The column temperatures were 100° for PEG and 150° for DVBP. The temperatures of the detector and injector were 210° and 225°, respectively. The flow rates were nitrogen, 50 ml./min.; oxygen, 400 ml./min.; hydrogen, 50 ml./min. The voltage was 300 v., the sensitivity 100, the attenuation 5, and the AFS 1.0×10^{-9}.

Solutions—A standard alcohol solution was prepared according to the NF XII procedure (9), then diluted to approximately 2% (v/v). When necessary, samples were diluted with distilled water to approximately 2% alcohol (v/v). A 2% (v/v) solution of acetone in distilled water was used as an internal standard.

The final solution for injection was prepared by transferring 10.0 ml. of the diluted sample or stand-

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[1] Polypak-2, available from F and M Scientific Division, Hewlett-Packard Corp., Avondale, Pa.

[2] Available commercially as Porapak Q from Waters Associates, Inc., Framingham, Mass.

[3] Carbowax 400.

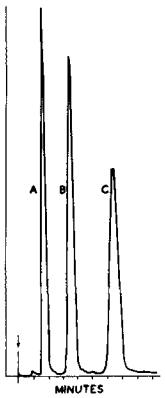

Fig. 1—Separation of alcohol standards by gas chromatography on DVBP column. Key: A, methanol; B, ethanol; C, isopropyl alcohol. DVBP column, 122 cm., 3 mm. i.d.; column temperature, 150°; injector temperature, 225°; flow rate, 50 ml. nitrogen 1 min.; flame ionization detector; detector temperature, 210°; sensitivity 1 × 10^{-9} AFS.

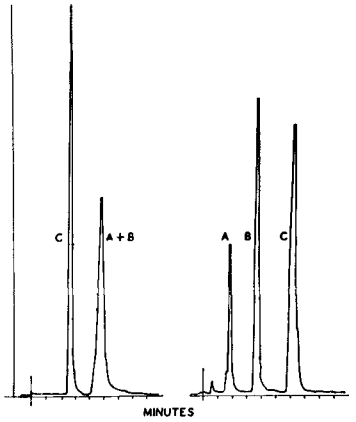

Fig. 2—Comparison of columns for gas chromatographic separation of alcohols. Key: A, methanol; B, ethanol; C, acetone. Left: PEG 400 column, 183 cm. ,3 mm. i.d.; column temperature, 100°. Right: DVBP column, 4 ft., 3 mm. i.d.; column temperature 150°. All other conditions identical: injector temperature, 225°; flow rate, 50 ml. nitrogen/min.; flame ionization detector; detector temperature, 210°; sensitivity 1 × 10^{-9} AFS.

ard alcohol solution and 10.0 ml. of the diluted acetone standard to a 100-ml. volumetric flask, diluting to volume with distilled water, and mixing.

If the sample contains acetone, two identical dilutions can be made, eliminating the internal standard from one solution and making the appropriate corrections, or another internal standard can be used.

Calculations—All calculations were made by using net integrator counts and the following formula:

$$\% \text{ alcohol} = \frac{\text{net sample counts}}{\text{net std. counts}} \times \frac{\text{net std. acetone counts}}{\text{net sample acetone counts}} \times \text{dilution factor} \times \text{concn. of std. (\%)}$$

RESULTS AND DISCUSSION

DVBP was used for about 6 months in this laboratory; no major difficulties were incurred and results are reproducible. However, solutions containing borates should not be injected into either column (14). Figure 1 shows that the three commonly used alcohols are completely separated on DVBP columns.

Retention times for ethanol and acetone were 4.0 and 6.0 min., respectively, on DVBP under the conditions stated in the experimental section (see Fig. 1).

If peak height is used as the method of quantitation, raising the column temperature to 200° will further reduce the retention time, with little loss in resolution. A complete chromatogram can then be obtained in approximately 4 min.

A comparison of the PEG 400 and DVBP is shown in Fig. 2. Methanol and ethanol are not separated on the PEG column with the conditions described in NF XII (9).

Varying amounts of the standard alcohol solution were injected onto the columns to determine the effect of injection size on response. Figure 3 shows a plot of the ratio of net alcohol counts to net acetone counts *versus* sample size. The DVBP column gave a higher net number of integrator counts/μl. of injected acetone than the PEG column.

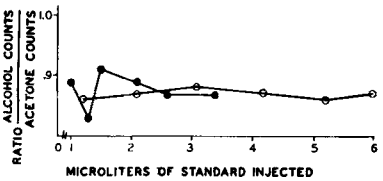

Fig. 3—Response ratio as a function of μl. injected: comparison of the two columns. Key: ○, DVBP; ●, PEG.

TABLE I—COMPARISON OF ETHANOL DETERMINATIONS ON PEG 400 AND DVBP COLUMNS

Product Type	—% Ethanol— PEG	DVBP	% Declared
Brown mixture NF	11.5	11.5	9–11
Mercurochrome	56.7	56.7	52
Antihistamine expectorant	3.0	3.0	3
Barbiturate expectorant	15.2	15.2	15
Paregoric USP	47.5	50.0	45
	47.0	46.4	...
	47.4	...	...
Terpin hydrate with codeine elixir	40.0	42.5	40
	41.2	39.6	...
	39.6	...	...
Elixir phenobarbital (synthetic)	12.7	13.8	12–15
Mouthwash	19.2	18.9	18.5
	18.1	17.4	17.0

Table I compares the analyses of ethanol content of various products on both the PEG and DVBP columns. Results from the two columns were similar. Table II lists the analyses of ethanol from

TABLE II—RESULTS OF ETHANOL DETERMINATION ON VARIOUS COMMERCIAL PRODUCTS USING THE DVBP COLUMN

Product Type	% Ethanol Declared	% Ethanol Found	% of Amt. Decl.
Antihistamine elixir	2.3	2.4	103
Antihistamine expectorant	3.5	3.8	109
Antihistamine syrup	15	14.6	97
Cough syrup	10	11.0	110
Cough syrup	3.0	3.0	100
Cough syrup	14	14.7	105
Three bromides elixir NF	3–5	4.3	107
Three bromides elixir NF	3–5	4.0	100
Phenobarbital with bromides	20	21.5	107
Corn remedy	42.5	42.0	99
Tincture merthiolate	50	51.4	103
Alcohol soln. of coal tar	83	93.8	113

TABLE III—RESULTS OF ANALYSIS OF SYNTHETIC PHENOBARBITAL ELIXIR BY VARIOUS OFFICIAL METHODS

Method	% Ethanol	Found	Av.
Pycnometer (25°)	13.9	14.2	14.0
Immersion refractometer	13.2	13.5	13.4
GLC direct on DVBP	13.8	13.7	13.7
Distillate on GLC	13.1	13.4	13.3

different types of pharmaceuticals on the DVBP column.

A synthetic mixture of elixir of phenobarbital USP was prepared and analyzed in duplicate on the DVBP column, by the distillation procedure (7), and by the immersion refractometer method (15). The results are shown in Table III. The amount of alcohol determined by refractometry on the distillate was in good agreement with the amount determined when the distillate was injected into the gas chromatograph.

CONCLUSIONS

Columns of DVBP (porous polymer beads) offer advantages for alcohol determinations, and give results as good as or better than the commonly used PEG 400 columns.

With a 150° column temperature, a sample can be injected every 7 min., resulting in 8 analyses/hr. By raising the column temperature to 200°, the time can be reduced to approximately 4 min. with little loss in resolution.

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Keyphrases

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GLC analysis
Divinylbenzene polymer column
Acetone–ethanol separation