Application Note 72

Blood Alcohols Analysis by Packed Column GC

Despite many changes in gas chromatography over the last several decades, analysis of blood alcohols by packed column GC, using a packing Supelco specifically tests for this purpose, continues to be a simple, reliable procedure. Supelco packing 1-1766 enables you to simultaneously monitor ethanol, methanol, and isopropanol, and their metabolites, acetaldehyde and acetone, in either glass or stainless steel columns.

Key Words:
• blood alcohols • ethanol • methanol

Packed column GC is a specific, rapid, precise method for identifying and quantifying blood alcohols and related compounds. Supelco™ packing 1-1766 (Carbopack™ B/5% Carbowax® 20M) enables you to simultaneously monitor ethanol, methanol, and isopropanol, and their metabolites, acetaldehyde and acetone. This packing can be used in either glass or stainless steel columns.

GC analyses of blood alcohols are routinely done by one of two methods: direct aqueous injection or headspace analysis. There are advantages to each method, but packing 1-1766 improves the separation obtained by either.

In *direct aqueous injection*, the whole blood or serum sample is diluted with an aqueous solution of the internal standard, and an aliquot is injected directly onto the column. Many analysts prefer this method because the instrumentation required is simpler than that for headspace analysis.

Direct sample injection onto Carbopack C/0.2% Carbowax 1500, a general purpose industrial packing for C1-C5 alcohols, has been used to separate blood alcohols (1). This packing, however, does not resolve acetaldehyde, a product of ethanol metabolism, from methanol. Packing 1-1766 provides better resolution. Figure A shows the isothermal separation of the alcohols, their metabolites, and n-propanol, an internal standard, obtained by direct sample injection.

If you use direct injection, nonvolatile sample components will be deposited at the column inlet. Peaks may begin to tail after the column has been in use for some time. You can eliminate tailing from a glass column by replacing the silanized glass wool plug and the first few inches of packing in the column inlet. This process can be repeated several times before the column must be completely repacked or replaced. If you prefer, you can inject the samples into a glass lined inlet or a PureCol™ column inlet liner (see our catalog), rather than into the column inlet. It is easier to replace these disposable products, when necessary, than to repack a column inlet.

Figure A. Isothermal Separation of Blood Alcohols

Packing: **60/80 Carbopack B/5% Carbowax 20M**
Cat. No.: **11766** (15g)
Column: 6' x 2mm ID glass
Oven: 85°C
Carrier: helium, 20mL/min
Det.: FID
Inj.: 1µL, 0.05-0.10% each analyte

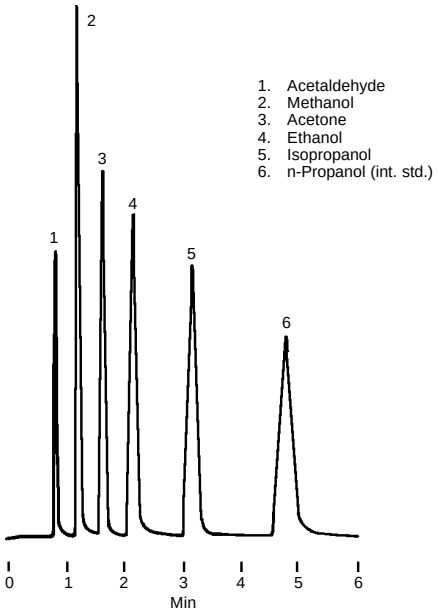

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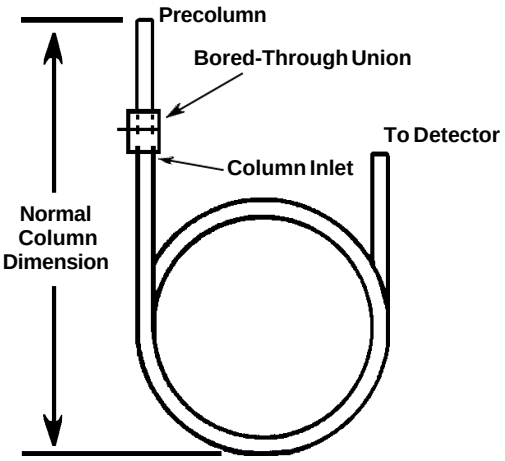
Figure B. Pre-Column Installation



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Alternatively, if you wish to use direct injections, we recommend a 6' x 1/8" stainless steel column connected to a 6" x 1/4" OD x 2mm ID glass precolumn through a 1/4" x 1/8" Swagelok® reducing union (Figure B). To maintain column performance, simply repack the precolumn with packing 1-1766 or replace it as necessary.

In the second approach, *headspace analysis*, the internal standard is added to the sample and the mixture is held at a fixed temperature to equilibrate the volatiles between the liquid and the headspace. Only the vapor above the sample is injected onto the column, so contamination from nonvolatiles is avoided. The procedure is well suited to automated analysis with specially designed injection equipment. Manual injection of the vapor via a syringe can yield erratic results because the components can condense in the syringe.

When headspace analysis is used, it is especially important that the column be capable of separating acetaldehyde and methanol. The vapor concentration of acetaldehyde in the headspace can approach that of a clinically significant amount of methanol. If the two compounds are not adequately separated, acetaldehyde might be incorrectly identified as methanol. Acetaldehyde levels in the aqueous phase usually are too low to cause this problem when direct aqueous injection is used.

Always order the blood alcohols packing by catalog number (Cat. No. 1-1766). We test each lot of packing 1-1766 with the mixture shown in Figure A and approve *only* this packing for blood alcohol analyses. Other packings prepared from Carbopack B coated with Carbowax 20M are for different applications and are sold under different numbers.

Reference

1. Manno, B.R., and J.E. Manno, *J. Anal. Toxicol.,* **2:** 257-261 (1978).

Trademarks

Carbopack, PureCol, Supelco – Supelco, Inc.
Carbowax – Union Carbide Corp.
Swagelok – Crawford Fitting Co.

Ordering Information:

Description	Cat. No.
60/80 Carbopack B/5% Carbowax 20M Packing 15g	**11766**❑
Glass precolumn packed with Cat. No. 1-1766 6" x 1/4" OD x 2mm ID, pk. of 6 (for use with SS columns only)	**12494**
Swagelok bored-through reducing union 1/4" x 1/8" SS	**22061**

For packed columns, please refer to our catalog or call our Order Processing personnel.

❑ **NOTE**: Supelco cannot guarantee results obtained with similar packings, including custom-made packings of identical composition.

Contact our Technical Service Department
(phone 800-359-3041 or 814-359-3041, FAX 814-359-5468)
for expert answers to your questions.