

Amines Analysis by Packed Column GC

Amines must be analyzed on a deactivated gas chromatography column, or these highly active analytes will adsorb to the support, adsorbent, or tubing, causing the peaks to tail. Three types of packings can be used to separate amines: graphitized carbons coated with a stationary phase and deactivated, coated and uncoated porous polymers, and conventional partition columns consisting of a diatomaceous earth support coated with a stationary phase and deactivated. The column usually is deactivated by adding a base to the packing. Column deactivation, analyses of various types of amines, at trace and higher levels, and derivatization of amines to form less active analytes are described in this bulletin.

Key Words:

amines • aliphatic amines • amino acids • aromatic amines
ethanolamines • fatty amines

Amines are difficult to analyze by gas chromatography. These active compounds often adsorb to the chromatographic support or adsorbent, causing the peaks to tail badly. Tailing becomes increasingly severe as the basicity of the amine increases – the highly basic primary aliphatic amines (RNH_2) and polyfunctional amines are the most difficult to analyze. Secondary aliphatic amines (R_2NH) are less basic and, consequently, are a lesser problem. Aromatic amines, which are weakly basic, tail less than primary aliphatic amines but, like primary amines, must be analyzed on a deactivated column. Tertiary amines (R_3N) are the least difficult to analyze. Column deactivation, which reduces amine peak tailing, usually is accomplished by adding a base to the packing. Deactivation is discussed in this bulletin.

Three types of GC packings can be used to separate amines:

- graphitized carbons (e.g., Carbowax™ packings) coated with a stationary phase and deactivated
- porous polymers, coated and uncoated
- conventional diatomaceous earth supports coated with a stationary phase and deactivated (partition columns)

Carbowax graphitized carbon packings and porous polymer packings are well suited for separating C1-C10 compounds, but retention times for larger molecules are excessive. Conventional packings based on diatomaceous earth supports, when properly deactivated and coated, are better suited to analyses of higher molecular weight amines.

Coated Carbowax Packings

Carbowax B coated with 4% Carbowax® 20M and 0.8% KOH was developed (10) specifically for monitoring small aliphatic amines at ppm levels in water (Figure A). More highly concentrated amines, or neat compounds, also can be separated readily by using this

packing. Heterocyclic amines can be separated on Carbowax B/4% Carbowax 20M/0.8% KOH packing (Figure B), but aromatic amines exhibit excessively long retention times. Acidic compounds in the sample will be irreversibly adsorbed by the KOH. Figure C, a separation of eleven neat C2-C9 amines, shows the same packing can be used to separate complex mixtures of higher boiling amines. Table 1 summarizes retention times for a variety of amines at several column temperatures on Carbowax B/4% Carbowax 20M/0.8% KOH.

Figure A. Trace Aliphatic Amines in Water

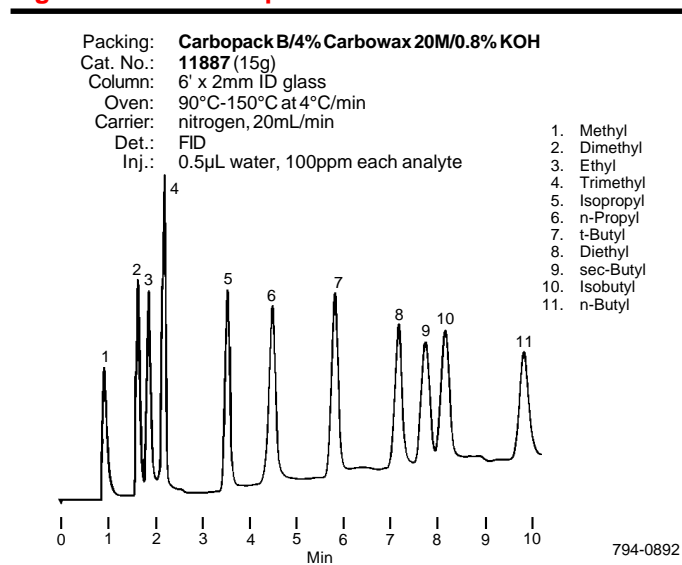


Figure B. Heterocyclic Amines in Water

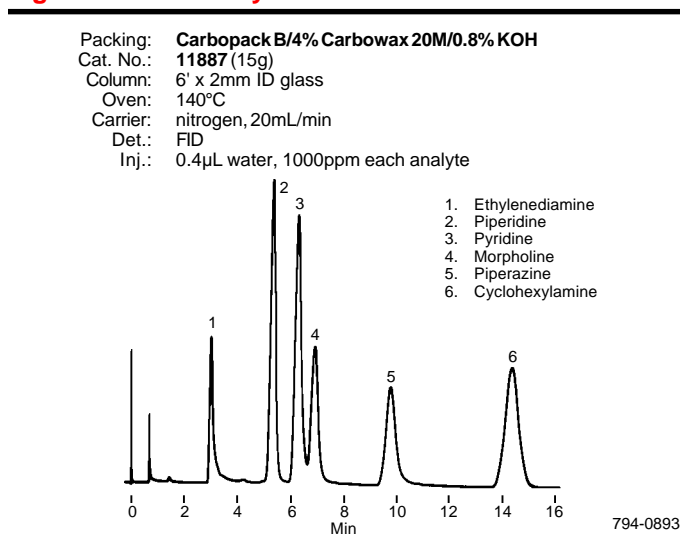


Figure C. C2-C9 Aliphatic Amines

Packing: **Carbopack B/4% Carbowax 20M/0.8% KOH**
 Cat. No.: **11887** (15g)
 Column: 6' x 2mm ID glass
 Oven: 200°C
 Carrier: nitrogen, 20mL/min
 Det.: FID
 Inj.: 0.01µL

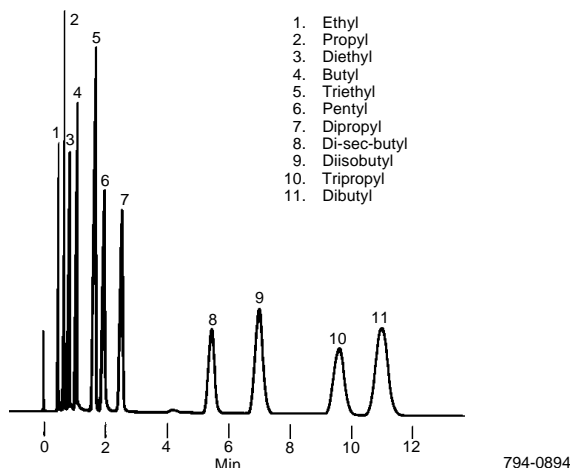


Table 1. Retention Times for Amines at Several Temperatures (Uncorrected)

Amine	Column Temperature/Retention Time (Min)				
	75°C	100°C	125°C	150°C	175°C
Methyl	1.1	0.8	0.6	—	—
Dimethyl	2.2	1.3	1.0	—	—
Ethyl	2.6	1.5	1.0	—	—
Trimethyl	3.3	1.7	1.2	0.8	—
Isopropyl	6.0	2.9	1.6	1.0	0.8
n-Propyl	8.4	3.9	2.0	1.2	0.8
t-Butyl	12.6	5.6	2.8	1.6	1.0
Diethyl	19.6	7.6	3.6	1.8	1.2
sec-Butyl	21.3	8.5	3.9	2.0	1.3
Isobutyl	23.5	9.3	4.2	2.2	1.4
n-Butyl	—	13.2	5.4	2.7	1.6
Isoamyl	—	—	12.3	5.4	3.0
n-Amyl	—	—	—	6.6	3.4
Diisopropyl	—	—	11.0	4.9	2.7
Triethyl	—	—	—	5.0	2.8
Dipropyl	—	—	—	9.8	4.7
Ethylenediamine	—	—	4.5	2.3	1.5
Piperidine	—	—	8.3	3.8	2.2
Pyridine	—	—	10.2	4.4	2.6
Morpholine	—	—	11.3	4.9	2.8
1,3-Propanediamine	—	—	—	5.4	3.0
Piperazine	—	—	16.4	6.8	3.7
Cyclohexyl	—	—	25	9.6	5.1
1,4-Butanediamine	—	—	—	—	—

Column: Carbopack B/4% Carbowax 20M/0.8% KOH, 6' x 2mm ID glass,
 Carrier: nitrogen, 20mL/min.

Column Characteristics

Separations obtained with Carbopack B/4% Carbowax 20M/0.8% KOH packing are primarily the result of the surface properties of the Carbopack graphitized carbon. Carbowax 20M modifies the surface of the carbon, while KOH makes it basic, to eliminate tailing of the amine peaks. The polarity of the packing is very low, relative to other packings, as shown by the McReynolds constants (determined at 120°C):

x' (benzene) 58 z' (2-pentanone) 11
 y' (butanol) 40 z (Pyridine) 52

Note that McReynolds Constants for Carbopack B/Carbowax 20M packings normally are determined with 20% Carbowax 20M, rather than 4%, and with no KOH.

A characteristic of Carbopack-based columns is that sample components are separated by carbon number and are eluted in the order C1 → C2 → C3, and so forth. This is seen in the separation of methylamine, dimethylamine, trimethylamine, and ethylamine shown in Figure D. Both C2 amines (dimethylamine and ethylamine) are eluted before the C3 amine (trimethylamine). Other classes of compounds, such as alcohols, acids, and phenols, also exhibit this elution pattern on other Carbopack-based columns.

Sample Size

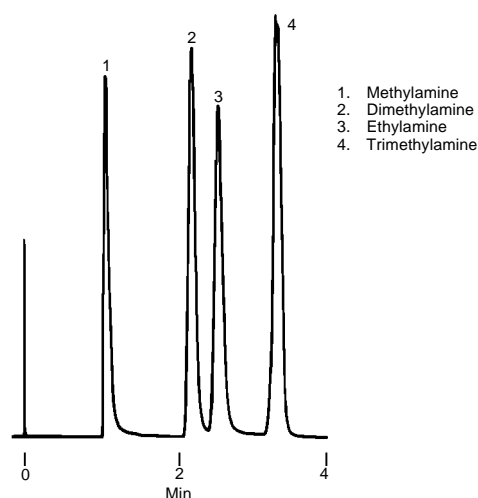
When analyzing neat amines, it is necessary to use small samples, e.g., 0.05-0.1µL. Larger samples will overload the column, and peak shapes will be poor. Overload on Carbopack-based packings is seen as a tailing peak, rather than as a leading peak. (Leading peaks are typical for an overloaded conventional partition column.) With dilute solutions, larger samples (e.g., 1µL) can be used.

Aqueous Solutions – Methylamines at ppm Levels in Water

Carbopack B/4% Carbowax 20M/0.8% KOH packing can be used to separate the three methylamines at 10ppm in water (Figure E). When analyzing amines in aqueous solution, the column must be conditioned by injecting a number of relatively large aliquots of water. This treatment converts any K₂CO₃ in the column to KOH, making the column more basic and improving its inertness for amines. Also, when water passes through the column a certain amount of stationary phase is hydrolytically decomposed and appears as the "water peak" in the chromatogram – even when a flame detector is used. Conditioning cleans the column and minimizes the water peak when standards and samples subsequently are injected. Details of this conditioning are discussed in *Column Preparation and Conditioning*.

Figure D. Methylamines and Ethylamine in Water

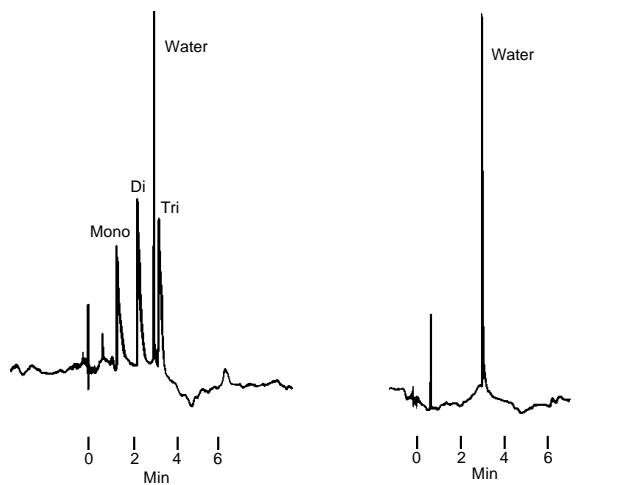
Packing: **Carbopack B/4% Carbowax 20M/0.8% KOH**
 Cat. No.: **11887** (15g)
 Column: 6' x 2mm ID glass
 Oven: 75°C
 Carrier: nitrogen, 20mL/min
 Det.: FID
 Inj.: 0.2µL water, 1000ppm each analyte



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Figure E. Trace Methylamines in Water

Packing: Carbowax 20M/0.8% KOH
 Cat. No.: 11887 (15g)
 Column: 6' x 2mm ID glass
 Oven: 100°C
 Carrier: nitrogen, 20mL/min
 Det.: FID
 Inj.: 1.0µL water;
 water containing 10ppm each analyte



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Column Preparation and Conditioning

When packing a column with Carbowax 20M/0.8% KOH packing, add the packing to the column slowly, while tapping the tubing with a spatula or vibrating it very gently. Leave several centimeters at the inlet empty, so that when the sample is injected the needle does not penetrate the packing bed. If it does, the crushed packing particles will plug the column, stopping the flow of carrier gas. Carbowax 20M/0.8% KOH packing and packed columns should not be exposed to air for excessive periods of time – the packing will adsorb carbon dioxide and lose its deactivation.

Install the column in the chromatograph. Check the system for leaks and, if necessary, replace the septum. Condition the column overnight at 220°C with a flow of 20mL/minute of carrier gas. If the column is to be used for aqueous samples, maintain the oven temperature at 220°C and make 20 10µL injections of 1% ammonium hydroxide in water in a period of 30 minutes. **The water used should be distilled or deionized, and freshly boiled to remove CO₂.** Adjust the oven temperature to a level appropriate for your analysis. If your calibration standards tail, or if you have not used the column for some time, repeat the 1% ammonium hydroxide treatment.

When using this packing for trace analyses, inject 1 µL of water and monitor at 1.6×10^{-12} AFS to see what a water peak looks like (Figure E). Do not use more water or a lower sensitivity. A 1µL sample minimizes the water peak and more accurately indicates what you will see with your samples.

Porous Polymer Packings

Porous polymers – large surface area resins – often are used as column packings in GC (9, 17). Because the surface of the resin causes the separation, a porous polymer need not be coated with a stationary phase. One porous polymer, Chromosorb® 103, was developed specifically for separating the amines listed in Table 2 (19). Chromosorb 103 has been used to separate methylamines in marine fish (15) and for analyses of short chain aliphatic amines (1, 24).

Amines tail on other porous polymers, but performance can be improved by coating such polymers with a basic material, such as 10% triethylenephosphoramidate (TEPA) or 10% polyethylenimine (PEI) (16). In reducing tailing of amines on Porapak® Q and Par-1, PEI and TEPA were more effective than KOH (21). A coating of 5% PEI provided good deactivation. At lower loading levels there was little change in retention time. At higher levels (e.g., 10-20%) retention time decreased and separation characteristics gradually changed from that of the resin to that of the PEI. Retention times were shorter for Par-1 polymer than for Porapak Q polymer, because Par-1 has a much lower surface area. Par-1 is no longer commercially available, but Chromosorb 101 and Porapak P are similar polymers.

Partition Columns

Aliphatic Amines

A conventional partition column consists of a support, generally a diatomite material, coated with a stationary phase. Diatomite supports, however, will interact with active analytes, such as amines, causing the peaks to tail. To prevent this, the support is made strongly basic in one of three ways:

- by adding 1-2% KOH or NaOH to the packing
- by adding 0.1-1% of an amine to the packing
- by using an amine as the stationary phase.

Table 2. Retention Times for Amines (Uncorrected)

Amine	Retention (Min)	Amine	Retention (Min)
Methyl	0.43	Diethyl	2.84
Dimethyl	0.71	sec-Butyl	3.64
Trimethyl	0.79	Isobutyl	3.94
Ethyl	0.79	n-Butyl	5.20
Isopropyl	1.34	Triethyl	7.64
n-Propyl	2.05	Isoamyl	10.72
t-Butyl	2.05	n-Amyl	12.61

Column: 50/60 Chromosorb 103, 2m x 4mm, Oven: 130°C, Flow Rate: 120mL/min. Data from Mosier *et al.* (24).

Smith and Radford were the first to demonstrate that KOH could be used to deactivate the support (30). Sie, *et al.* used high boiling amines to deactivate the support and evaluated their ability to separate smaller amines (29).

In the first two approaches to deactivating the packing, the stationary phase must be compatible with the basic material (silicones and polyesters, for example, are destroyed by bases). Polyglycols, such as the Carbowax and UCON® series, and certain hydrocarbons, have been used successfully with basic materials. Packings and column materials that have been deactivated with a base should not be exposed to the atmosphere – CO₂ will convert the base to the carbonate, which is ineffective for deactivation.

Table 3 lists stationary phases which have been used successfully with KOH. Apiezon L is a low polarity hydrocarbon; the other phases are moderately polar polyglycols. Table 4 lists several nitrogen-containing stationary phases which have been effective in deactivating a support. These compounds can be used, at a loading of 0.1%, with the stationary phases in Table 3. The McReynolds constants indicate most of these materials are very polar. Tables 3 and 4 also list minimum and maximum column temperatures. When using one of the amines with one of the stationary phases, observe the lower of the two temperature maxima (generally that of the amine).

Table 3. Stationary Phases Compatible with KOH

Stationary Phase	Min./Max Temp. (°C)	McReynolds Constants				
		x'	y'	z'	u'	s'
Apiezon L	50/250	32	22	15	32	42
Triton X-100*	0/200	203	399	268	402	360
UCON 50-HB-5100	0/200	214	418	278	421	375
Triton X-305	0/200	262	467	314	488	430
Carbowax 20M	60/225	322	536	368	572	510

*Comparable to Dowfax 9N9

Table 4. Amine Stationary Phases

Stationary Phase	Cat. No.	Min./Max Temp. (°C)	McReynolds Constants				
			x'	y'	z'	u'	s'
Quadrol®	2-1092	0/150	214	571	357	472	489
THEED	2-1118	0/150	463	942	626	801	893
TEPA	2-1117	0/125	—	—	—	—	—
PEI	2-1195	0/175	322	800	—	573	524
PPI	2-1198	0/200	122	425	168	263	224

Amine or caustic deactivation of diatomite supports appears to be more effective than silanization for analyses of amines. Generally, the white, lightweight, Chromosorb W type diatomite supports are used with adsorptive materials, but the pink, Chromosorb P type materials appear to be equally well deactivated by caustic agents. Teflon® supports are widely regarded as very inert, but they do not appear to be especially inert to amines.

For more details on deactivation, see reference 26.

Amine Adsorption on Metal

For analysis of free primary and secondary amines, particularly at low levels, we strongly recommend use of glass columns, rather than metal columns. Metal can be highly adsorptive of free amines, causing severe peak tailing. At low concentrations, the sample can be totally lost. Stainless steel and copper appear to be adequate for higher concentrations of less active amines, but aluminum tubing should be avoided. A metal column inlet also can adsorb sample. Samples should be injected directly into the (glass) column, or into a glass inlet liner.

Applications

10% Carbowax 20M/2% KOH on 80/100 Chromosorb W AW packing has been used for separating diamines (30), and for nicotine (Figure F). A 10% UCON 50-HB-5100/2% KOH packing was used to separate polyfunctional amines, guanidines, amidinoguanidines, and maleamines (7). Apiezon L/KOH has been widely used for separating amphetamines in low concentrations. 20% Dowfax 9N9/2.5% NaOH on Chromosorb P was used to separate a series of aliphatic amines (25). The Dowfax material is no longer available, but a similar packing separated a mixture of amines at 0.1% each in water (Figure G).

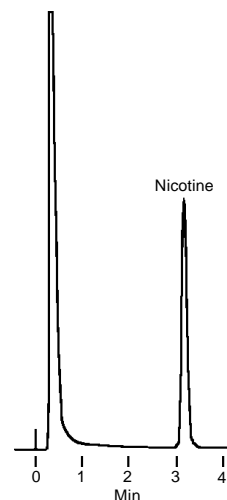
Poulson studied the performance of Carbowax 20M, Triton X-305, and Apiezon L for separating aliphatic amines, pyridines, quinolones, indoles, etc., comparing retention time, bleed rate, and capacity (28). Umbreit, *et al.* used Amine 220 and KOH to separate and quantify smaller aliphatic amines, releasing the amine salts from a caustic precolumn (31). Dunn, *et al.* found an Amine 220/KOH packing most effective for monitoring trace amounts of the methylamines (12). These investigators improved column deactivation by saturating the carrier gas with ammonia.

Polyamines

A 10% Apiezon L/10% KOH on 60/80 Chromosorb W packing can separate free forms of compounds such as tetra-ethylene pentamine (TEPA), triethylene tetramine (TETA), etc. (3). Polyamines can be

Figure F. Nicotine

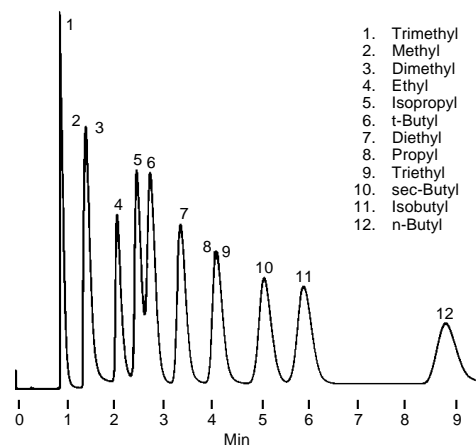
Packing: 10% Carbowax 20M/2% KOH on 80/100 Chromosorb W AW
 Cat. No.: 11805 (20g)
 Column: 6' x 2mm ID glass
 Oven: 200°C
 Carrier: nitrogen, 20mL/min
 Det.: FID
 Inj.: 1.0µL (0.1µg/µL)



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Figure G. Aliphatic Amines in Water

Packing: 20% Triton X-100/2% NaOH on 80/100 Chromosorb P AW
 Column: 6' x 2mm ID glass
 Oven: 75°C
 Carrier: nitrogen, 20mL/min
 Det.: FID
 Inj.: 0.2µL water, 0.1% each analyte



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readily separated on several columns if the analytes are methylated using formaldehyde and sodium borohydride (14).

Aromatic Amines

Aromatic amines are generally less basic than aliphatic amines and, consequently, present less of an adsorption problem. They can be separated on the highly basic columns described here, but the analysis generally is carried out with a silicone stationary phase on an acid washed, dimethyldichlorosilane treated (AW-DMCS) support. The SP-2100-DB, SP-2250-DB, and SP-2401-DB packings listed in this bulletin are deactivated for basic compounds and were developed specifically for separating alkaloids at low concentrations. Figures H and I show typical separations obtained with these packings. When analyte concentrations are very low, even AW-

DMCS treatment is inadequate, and it is necessary to use a specially deactivated column or derivatize the analytes.

Acylation of Amines

The other approach to avoiding peak tailing is to derivatize the primary and secondary amines. The choice of derivative is often a compromise between the relative volatility of the derivative (which affects retention time and analysis temperature) and the relative response of the detector. If an electron capture detector is available, trifluoroacetyl, pentafluoropropionyl, and heptafluorobutyryl reagents merit consideration. These reagents can be used as either anhydrides or imidazoles. The imidazoles must be used when mild

conditions are required (i.e., to prevent dehydration of certain compounds, or when using a DB (deactivated for base analysis) column packing, where the free acid generated by using the anhydride would ruin the packing. The *Handbook for Derivatives for Chromatography*, by Blau and King (4), contains excellent descriptions of the use of the trifluoroacetyl, pentafluoropropionyl and heptafluorobutyryl reagents.

Clarke, *et al.* compared ECD responses for several derivatives of benzylamine (8). The heptafluorobutyrate gave the best response (Table 5), but the pentafluoropropionate frequently can be analyzed at a lower temperature (27).

When a flame ionization detector is used, the reaction mixture can be injected directly onto the column, but with an ECD the derivative should be extracted into a nonpolar solvent. Reaction conditions

Figure H. Aromatic Amine Drugs

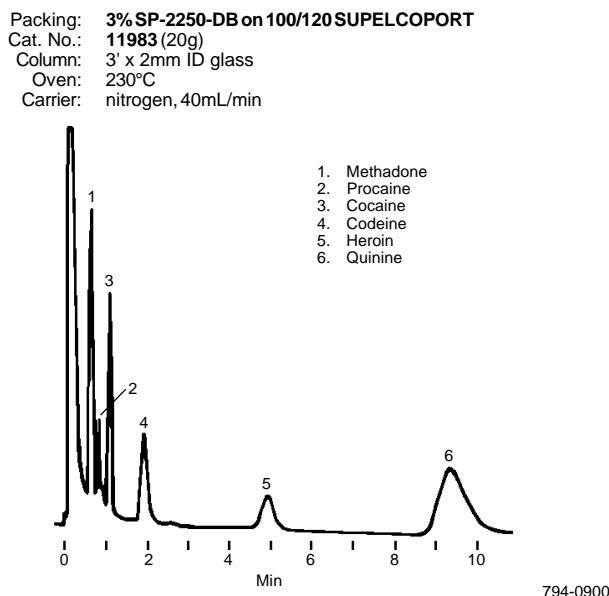


Figure I. Aromatic Amine Drugs

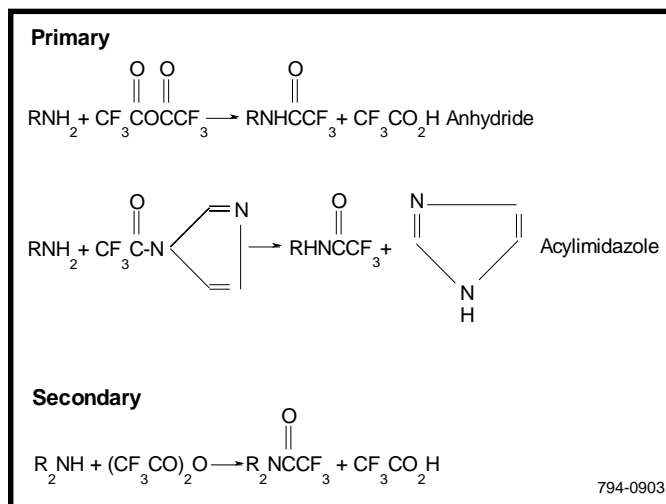
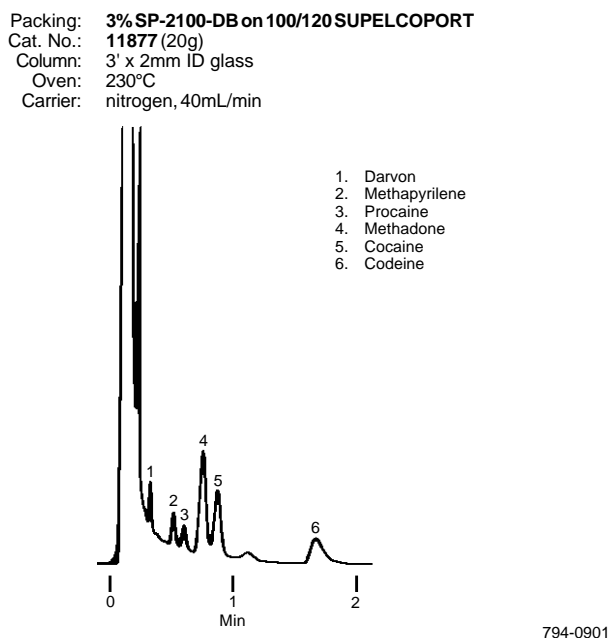


Table 5. Electron Capture Detector Responses for Amine Derivatives

Derivative	Relative Response
Acetate	0.05
Trifluoroacetate	1.0
Pentafluoropropionate	286
Heptafluorobutyrate	894

vary with the sample type, but these are all powerful, rapid acylating reagents.

Water or an alcohol in the sample will react with trifluoroacetic anhydride. In some instances a small amount of alcohol or water is added to the sample after the reaction, to react with excess TFA. N-trifluoroacetyl derivatives are stable in the presence of water, whereas O-trifluoroacetyl derivatives are somewhat labile (8).

Use of Perfluoroacylimidazoles

Indoleamines and indole alcohols have been analyzed as heptafluorobutyrylates on 5% SE[®]-30 at 200°C. The indole (1-2mg) is dissolved in 100-200µL of heptafluorobutyrylimidazole, heated in a micro reaction vessel for 2-3 hours at 80°C, and extracted into hexane for analysis (2). Similarly, phenolic acids have been derivatized with pentafluoropropionylimidazole by heating at 70°C for 10 minutes (20).

Amino Acids

To be separated on a GC column, amino acids must be converted to volatile derivatives. Gehrke, *et al.* have studied the TFA derivatization in detail to optimize the conversion, as well as the separation of the resulting mixture of derivatives (13).

Fatty Amines

Morrisette and Llnk derivatized both fatty amines and amides with TFA (23). Derivatized fatty amines can be separated by degree of unsaturation, using a polyester stationary phase. TFA derivatives of fatty amines can be detected at low concentrations with a flame ionization detector (22).

Ethanolamines

Brydia and Persenger developed a quantitative procedure for determining ethanolamines as TFA derivatives (5). Both the amine and hydroxyl groups react. A 5' x 1/4" column with 5% neopentyl glycol succinate on 60/80 Chromosorb G was used for the separation, and a thermal conductivity detector was necessary for good quantitative results.

Volatile Amines

Irvine and Saxby studied the separation of a large number of volatile amines from Latika tobacco leaves (18). The amines were steam distilled, collected in an HCl solution, dried, treated with KOH, and extracted into ether. The amine hydrochlorides were added to an ion exchange column and the TFA derivatives were prepared by treating the column with TFA. Relative retention times for the TFA amine derivatives on five GC columns are listed in the reference.

Aromatic Amines

Dove separated more than twenty aromatic amines, including aniline, toluidine, ethylaniline, and n-methyltoluidine isomers as TFA derivatives, using an 18' x 1/8" stainless steel column packed with 9.5% Apiezon L/3.6% Carbowax 20M (11). The column was operated at 152°C with a carrier gas flow of 100mL/minute.

Brydia and Willeboordse developed two procedures for separating the various diaminotoluene isomers as TFA derivatives (6). The 2,4 and 2,5 isomers were separated on a 12' x 1/4" column packed with 5% OS-138 on 100/120 Chromosorb G AW-DMCS, at 225°C. The other analysis, on a 3' x 1/4" column packed with 2% neopentyl glycol succinate on 60/80 mesh Chromosorb G, operated at 200°C, does not provide this separation. In these procedures, the TFA derivatives were prepared by adding TFA directly to the sample.

Ordering Information:

Column Packings

60/80 Carbopack B/4% Carbowax 20M/0.8% KOH, 15g	11887
Chromosorb 103 60/80, 50g	20216
Chromosorb 103 80/100, 50g	20217
Chromosorb 103 100/120, 50g	20218
10% Carbowax 20M/2% KOH on 80/100 Chromosorb W AW, 20g	11805
10% UCON 50-HB-5100 2% KOH on 80/100 Chromosorb W AW, 20g	11806
10% Apiezon L/2% KOH on 80/100 Chromosorb W AW, 20g	11893
GP* 3% SP-2401-DB** on 100/120 SUPELCOPORT, 20g	custom
GP 5% SP-2401-DB on 100/120 SUPELCOPORT, 20g	custom
GP 3% SP-2100-DB on 100/120 SUPELCOPORT, 20g	11877
GP 3% SP-2250-DB on 100/120 SUPELCOPORT, 20g	11983

Derivatization Reagents

Acetic anhydride, 10 x 2mL ampuls	33085
Trifluoroacetic anhydride, 25mL ampul	33164
Trifluoroacetic anhydride, 10 x 1mL ampuls	33165
Pentafluoropropionic anhydride, 10 x 1mL ampuls	33167
Pentafluoropropionic anhydride, 25mL ampul	33168
Heptafluorobutyric anhydride, 10 x 1mL ampuls	33170-U

*GP – Packing tested for specific analysis shown in this bulletin.

**DB – Packing specially deactivated for basic compounds.

Trademarks

Apiezon – Biddle Instruments
Carbopack – Supelco, Inc.
CARBOWAX – Union Carbide Corporation
Chromosorb – Manville Corporation
Porapak – Waters Associates, Inc.
Quadrol – Wyandotte Corporation
SE-30 – General Electric Company
SUPELCOPORT – Supelco, Inc.
Teflon – E.I. du Pont de Nemours & Co., Inc.
UCON – Union Carbide Corporation

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