# Guide for Use: BAS UniJet Microbore Columns

(revised 10-01)

When compared to conventionally sized columns, UniJet columns provide much higher mass sensitivity, lower sample size requirements, and reduce mobile phase consumption.

## *Typical Kit Inventory (C8, C18, polymeric ODS, alumina bonded phases)*

Be sure that you identify each component in this kit before installation. See Figure 1. You will reuse the same fittings and tubings for all three columns. Some kits may have slightly different supplies.

| Qty. | Description |
|---|---|
| 3 | Microbore column |
| 1 | Long sleeve PEEK nut and ferrule (MR-4416) |
| 1 | PEEK nut and ferrule (MR-4409) |
| 1 | PEEK Union and 2 fittings (MR-4403) |
| 1 | Fused silica in PEEK tubing (ca. 20 cm) (MR-4411) |
| 3 | Microbore gasket for amperometric detector (13 µm) (MF-1044) |

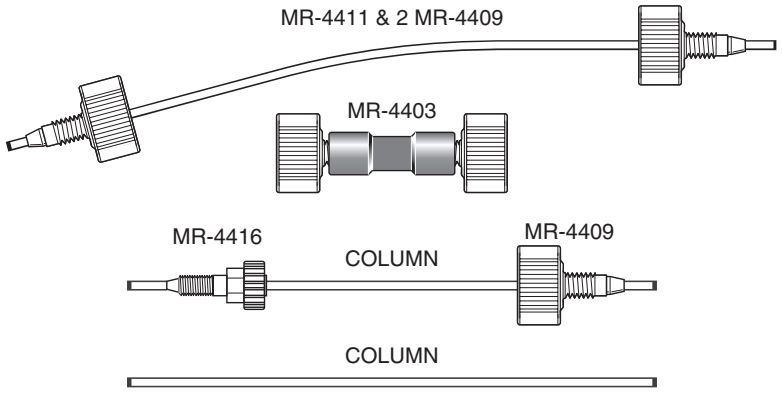



*Figure 1. Inventory of most reverse phase kits*

## *UniJet Microbore ACh/Ch Kit Inventory*

Please refer to Figure 2. You will reuse the same fittings for both enzyme reactors.

| Qty. | Description |
|---|---|
| 1 | Microbore ACh/Ch analytical column (MF-8904) |
| 2 | Microbore ACh/Ch IMER (MF-8903) |
| 1 | 1% ProClin® reagent (CF-2150) |
| 1 | Long sleeve PEEK nut and ferrule (MR-4416) |
| 1 | PEEK Union and 2 fittings (MR-4403) |
| 1 | Instruction Manual for ACh/Ch Assay Kit (MF-9053) |

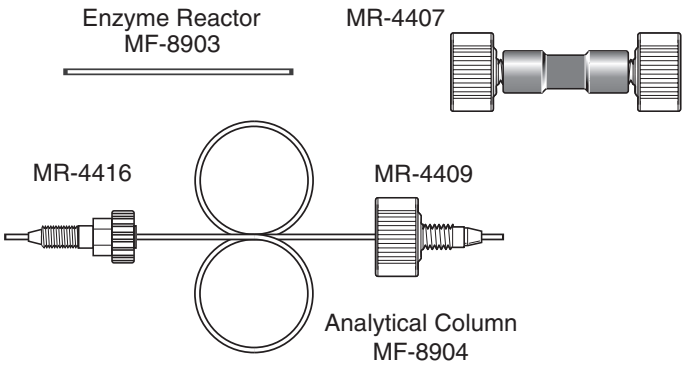



*Figure 2. UniJet microbore ACh/Ch analytical column and IMER.*

## *Converting a Conventional System to Microbore*

The essential points of conversion are: (1) creation of a quality flow rate in the 50-100 µL/min. range, (2) protection against particulates in the mobile phase or samples, (3) elimination of dead volume, and (4) electrical isolation of certain EC detectors from earth ground. Each of these topics is covered below.

Quality Flow Rates at 50-100 µL/min.

A flow splitter is an inexpensive way to convert a conventional pump to the microbore regime. The splitter consists of a tee and a restrictor in the form of either a capillary or a packed column. Flow will pass through the restrictor as well as the sample injector and analytical microbore column. The resistance to flow of the restrictor relative to the microbore column determines the split ratio. Normally, we advise a split ratio of about 9:1 (restrictor:microbore).

The BAS Flow Splitter Kit (MF-8947) provides both a packed column and capillary restrictors. The capillary restrictor is advised for methods involving alkaline mobile phases (e.g., acetylcholine).

In either style, the splitter tee is inserted between the LC pump and the sample injector. Remove the tee when converting back to "large" columns. Operating the pump at 1 mL/min., as in Figure 3, yields a microbore flowrate of approximately 100 µL/min.

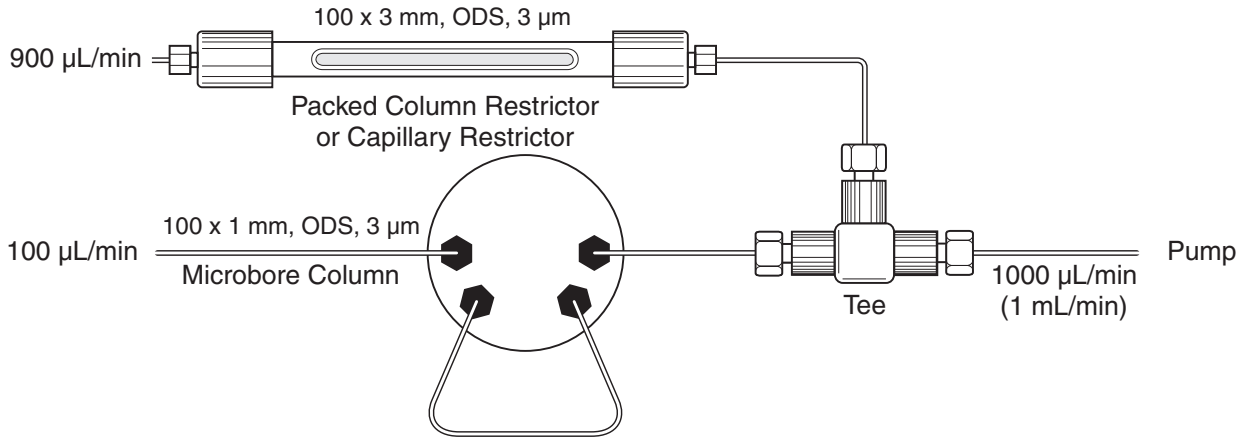



*Figure 3. Principle of split flow operation. The split ratio will remain constant as long as both branches maintain the same flow resistance. The split is determined by the ratio of cross-sectional areas in this case.*

Instead of a flow splitter, you may also use your pump to achieve the lower flow rates. There is thus a direct connection from your pump to the injector to the analytical microbore column. This option is the best choice for “dirty” samples, as constant flow is maintained even when increased pressure is experienced in the microbore column.

Protection Against Particulates

Particulate matter should be stringently avoided, particularly at the splitter and the column inlet. Sources of debris include mechanical devices such as pumps and autosamplers, as well as the samples themselves.

To trap debris from the pump, an in-line filter between the pump and splitter tee is mandatory. Use BAS MR-4135.

Particulates from the sample or injector will be deposited on/in the column inlet frit and will reduce column efficiency and change the splitter ratio. Use a microbore in-line filter kit (MF-8952) to trap these particulates. While an in-line filter will prevent debris from reaching the column, the fact remains that the split ratio will increasingly favor the bypass restrictor. Retention times will shift to longer values.

Accordingly, “dirty” samples should be thoroughly deproteinized and filtered through 0.2 µm membranes if this occurs. In general, microdialysates are clean and present no clogging.

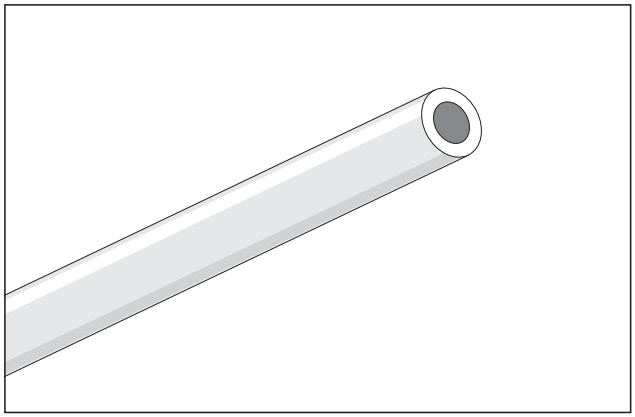


*Figure 4. The 1 mm frit is permanently mounted in the column. If your samples are not filtered, an optional guard column or in-line filter is recommended.*

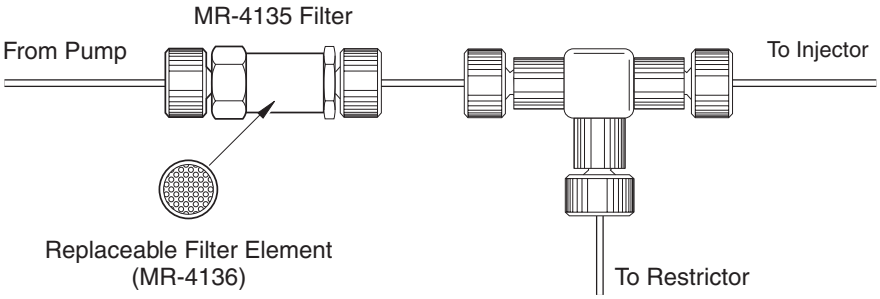



*Figure 5. Installation of MR-4135 in-line filter. This device has a larger surface area and acts as a mobile phase final filter.*

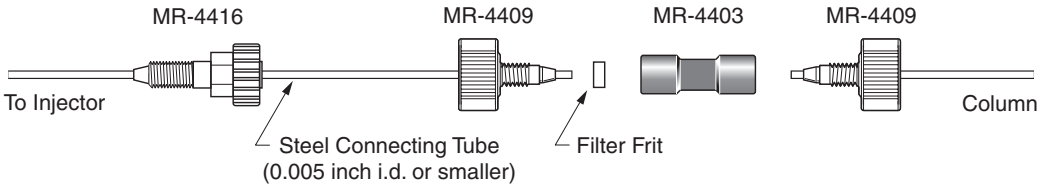



*Figure 6. Installation of MF-8952 microbore in-line filter kit. This filter has a very low dead volume and is the only filter recommended between the injector and column inlet.*

Elimination of Dead Volume

As you tighten a fitting, be sure that the tube or column is continually being pushed into its port to avoid dead volume. Use the reusable plastic ferrules provided so that you avoid damaging the microbore column. Use direct connections between injector, column, and detector. If this is not possible, use the fused silica/PEEK tubing supplied in the kit between the column outlet and the detector. Its internal diameter is 50 µm, which is 60% smaller than the best 0.005" i.d. stainless steel tubing. Always use this tubing on the outlet side of the column.

A major source of dispersion is the sample injector. The best manual injector for microbore chromatography is BAS' adaptation of the Rheodyne 8125. This exclusive hybrid (BAS MF-4161) features plastic ports for connections and the lowest dispersion internal passages.

Note: On most BAS products (except BAS 200 series), you will need to provide electrical isolation between the potential of the steel detector block (auxiliary electrode) and earth ground. The plastic ports of the BAS MF-4161 Injection Valve do provide this isolation, and so direct hookups are recommended. If you use a metal valve, then provide the necessary electrical isolation between it and the detector by using a union and the fused silica tubing between the column outlet and the detector. For reference, see Figure 11 and 12 in the following section.

## Installation Diagrams

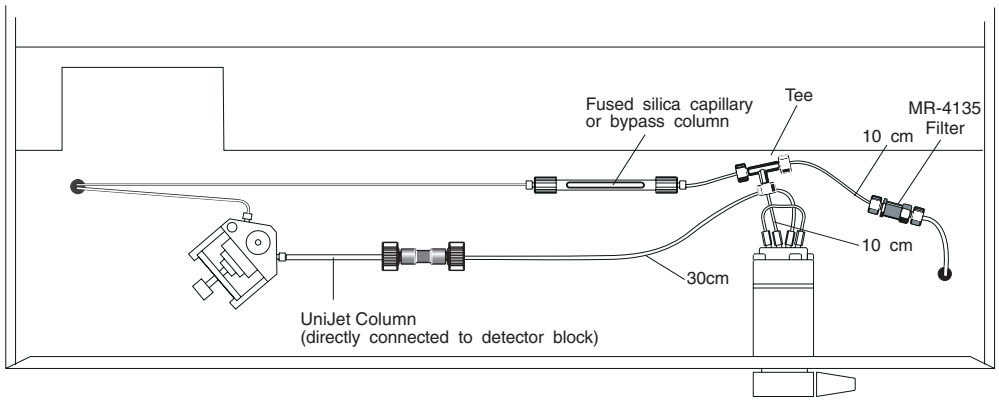



*Figure 7. BAS 200A*

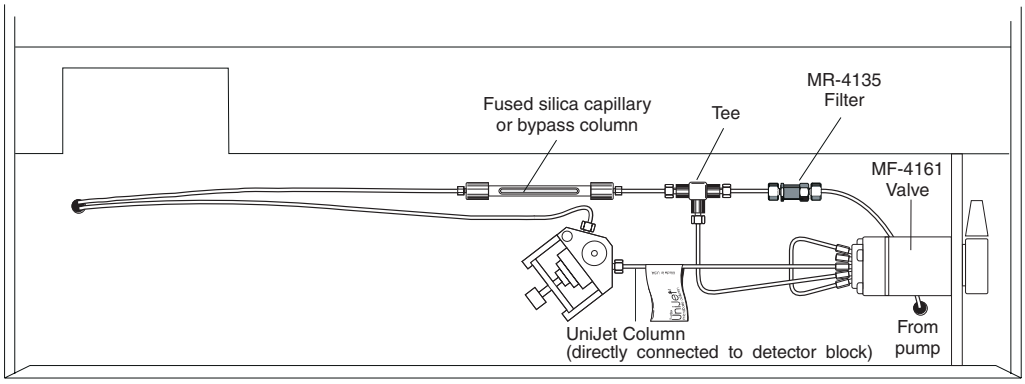



*Figure 8. BAS 200B using a flow splitter*

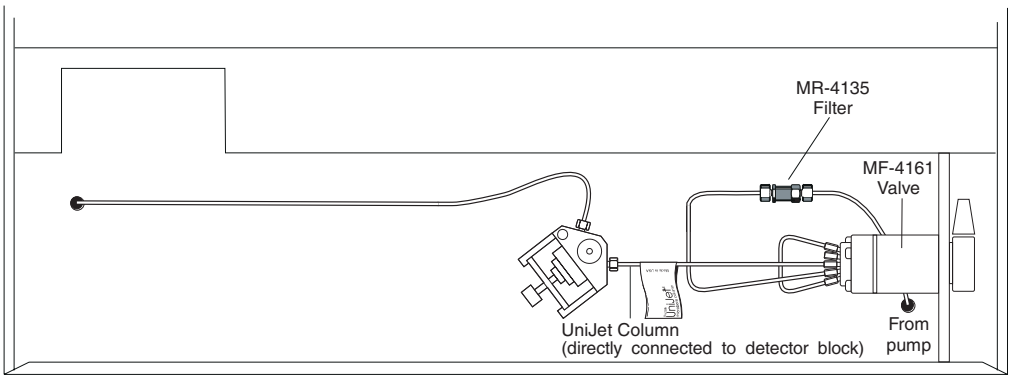



*Figure 9. BAS 200B – direct connection to pump*

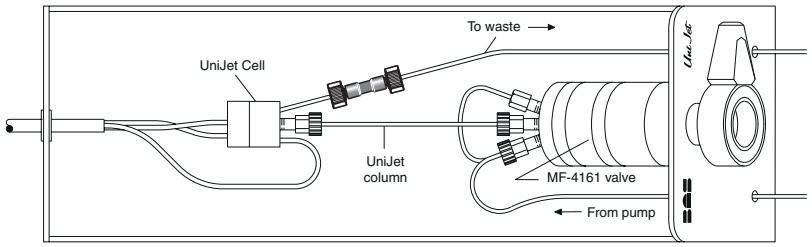



*Figure 10. BAS UniJet Cell Assembly*

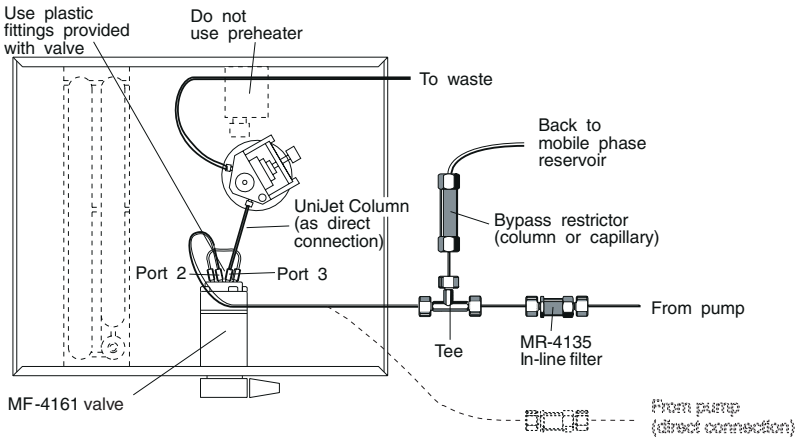



*Figure 11. BAS 481*

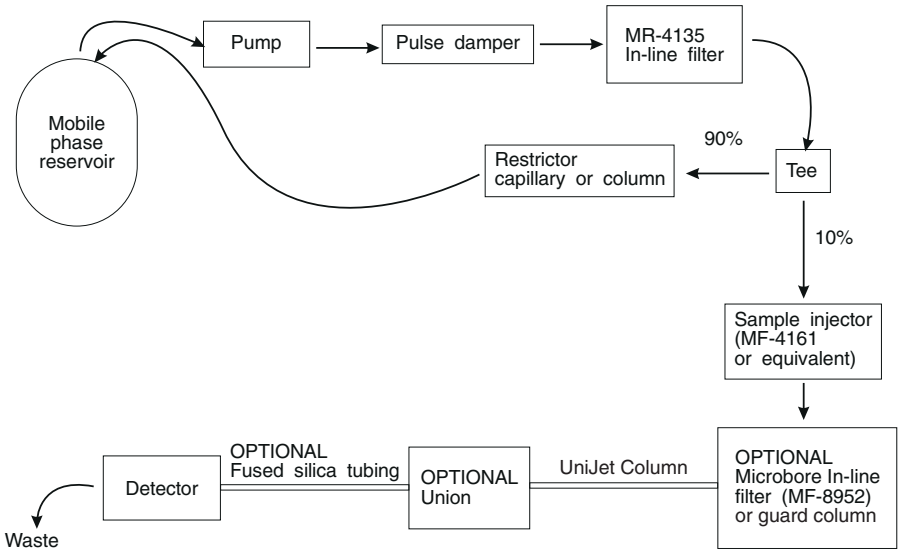



*Figure 12. Installation with flow splitter*

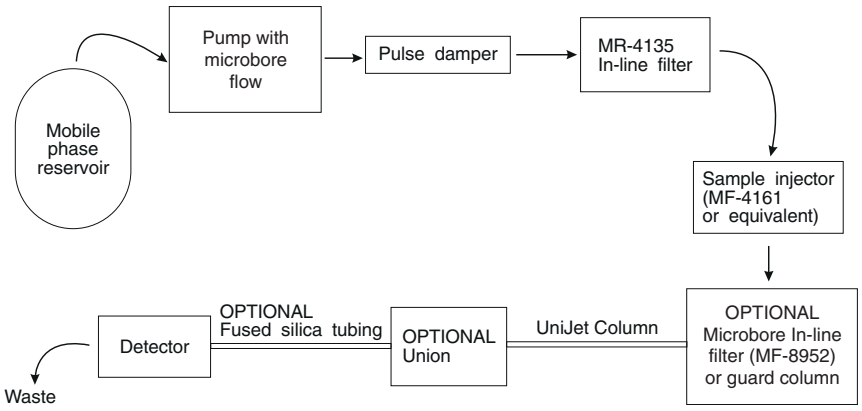



*Figure 13. Installation – direct flow*

## *Installing a UniJet Column Into Your Chromatograph*

Note: Do not bend the UniJet column when making connections. This may damage the column and affect performance.

1. Remove the plastic end caps from the column. If you are using the fused silica tubing, assemble as shown in Figure 14 before installing any portion of it in your chromatograph. As you hand-tighten the fittings into the union, be sure that the tubing and column are pushed into the ports tightly in order to avoid dead volume. It's a good idea to push the nuts and ferrules back at least 1 cm away from the tube end before making a connection.

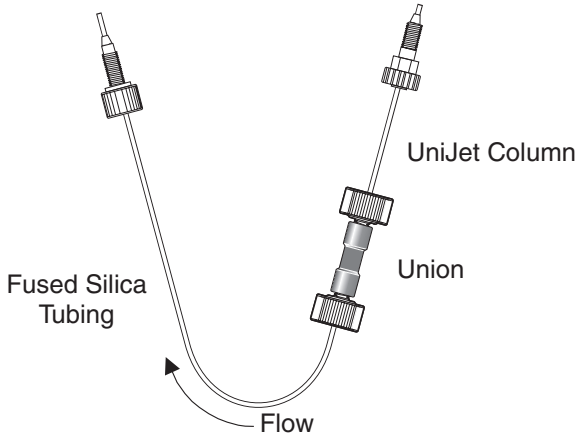



*Figure 14. Generic assembly of microbore column with the reusable fittings. The long nut at upper right fits into the injection valve.*

2. The long PEEK nut supplied with the microbore column kit is used to mount the column into most injectors.

Push back the nut and the plastic ferrule. Insert the column straight into the injector port as far as it will go. Hold the column firmly in place with one hand and tighten the nut with the other. Afterwards, tighten the nut another 1/8 turn with a wrench. Overtightening is unnecessary and can degrade column performance.

3. If you are using a metal injector, follow the same procedure to attach the plastic/fused silica tubing to the detector (Figure 13). No wrench should be used. The fused silica connecting tube may be unnecessary if you use the special MF-4161 valve; see BAS 481 installation diagram, Figure 11.

Note: In the next step, wetting the hydrophobic packing material with an organic solvent permits all of the surface area to be involved in the separation. If you omit this step, retention times will be too short. If your application kit involves a **specialty packing** or **immobilized enzymes**, **omit** the flushing instructions below and refer to the specialized instructions that came with the kit.

4. Always calibrate your pump gravimetrically or with volumetric glassware, since the accuracy of many pumps at these low flow rates is unreliable. An easy method of measuring microbore flow rates is shown in Figure 15.

*Please also consider the system volume from the mobile phase reservoir to the injector. This can be a few milliliters. Allow enough time for mobile phase changeovers to reach the column!*

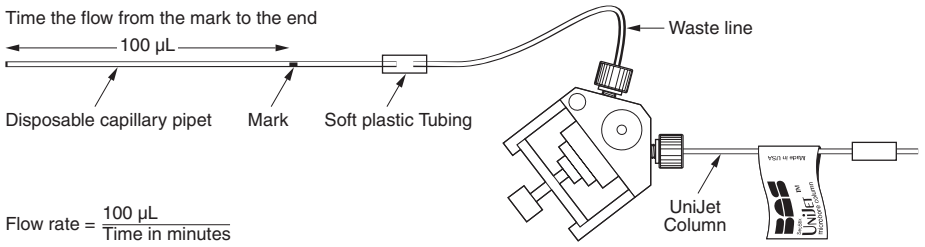



*Figure 15. Measuring microbore flow rate.*

5. If you are using this column with a BAS amperometric detector, install the special thin-layer gasket supplied with this kit in order to reduce the detector volume.

6. Follow the column wetting procedures (see Note under 3., above).

7. Equilibrate the system with your mobile phase.

Column Wetting Procedure

UniJet reverse phase columns are shipped in 40% acetonitrile. During storage and shipping, columns may become dry or partially dry. Follow the wetting procedure before using the columns with mobile phase.

1. Run 100% acetonitrile or methanol through the microbore column at 100 µL/min. for 15 minutes.

2. Run 40% acetonitrile:water through the column at 100 µL/min. for another 10 minutes.

Now the column is ready to be equilibrated with your mobile phase.

**NOTE:** Microbore columns for acetylcholine and choline determination do not need to be wetted. Do not run organic solvents through these columns. Read the ACh/Ch manual prior to use.

## *Important Notes on the Care of Your Columns*

1. Avoid sudden pressure shocks. Allow system pressure to gradually fall to zero rather than suddenly, by opening a high pressure fitting. Turn the sample injector rapidly for the same reason.

2. Use clean, filtered mobile phases. Components must be compatible with each other. Avoid combinations where buffer salt precipitation is likely.

3. The PEEK/fused silica outlet tubing is quite flexible but must not be sharply bent. Do not overtighten the fused silica tubing. Overtightening may squeeze the tubing shut and cause high backpressure.

4. Keep a log of system backpressure. When a significant increase occurs, determine the source by removing components one at a time, starting with the column and moving upstream. After each step, restart the pump and allow the pressure to stabilize. Calculate the pressure drop across each component and determine whether the samples/injector or pump/mobile phase is the problem.

5. If the pressure increase is attributed to the column try reversing the direction of flow. During this time do not use the fused silica outlet tubing; go directly to waste from the bottom of the column. Watch the pressure. If it falls back to initial values within an hour, the integrity of the packed bed is probably okay. Install a guard column or an in-line filter as per Figure 6 as a precaution. Inspect the rotor seal in your injection valve, since particles may be wearing off.

If the back pressure remains high, the problem might be due to on-column protein precipitation, which is generally irreversible. You must either de-proteinize your samples prior to injection or replace the columns more frequently. Using a guard column is strongly recommended.

6. Keep in mind that the injection volume should only be a small fraction of the column void volume, which is about 80 µL! This is particularly true if you need very low detection limits and cannot tolerate large baseline artifacts. Please realize that a 20 µL injection of 0.1 M $HClO_4$ tissue homogenate will momentarily fill 1/4 of the column with pH 1 sample solution. Weakly buffered mobile phases will thus drift from the desired control pH. Peak skewing, splitting, and tailing may occur.

7. When microbore columns become contaminated, wash them at 100 µL/min with 40% acetonitrile (in water) for 15 minutes, followed by 100% acetonitrile (15 minutes) and 40% acetonitrile (15 minutes). Remember to account for system delay/dead volume (mobile phase reservoir to top of column).

8. Microbore columns should be washed with 40% acetonitrile (in water) for 15 minutes prior to capping and storage.

## *UniJet Guard Columns and UniNut Fitting*

UniJet guard columns are 14 mm long, 1 mm i.d. and are packed with 3 or 5 µm particle size stationary phase. These less expensive columns will protect microbore analytical columns with negligible peak dispersion (Figure 16). The BAS patented UniNut fitting adapter directly connects a guard column and an analytical column to an injector valve without extra tubing or a union. The connection dead volume is zero.

UniJet guard columns are very efficient. Even a single 14 mm long column can rapidly separate compounds (Figure 17). However, guard columns are not routinely tested as analytical columns. The exception is MF-8948; these columns are tested for analytical purposes and the separation quality is guaranteed.

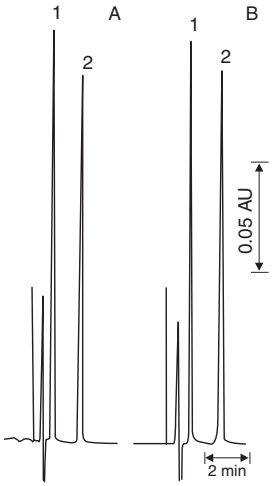



*Figure 16. Comparison of performance with and without using a UniJet guard column. The same standard solution of phenyl (1) and methyl benzoate (2) was injected. A: UniJet analytical column only (C18, 3µm, 100 x 1.0 mm); B: Plus a UniJet guard column (C18, 5µm, 14 x 1.0 mm)*

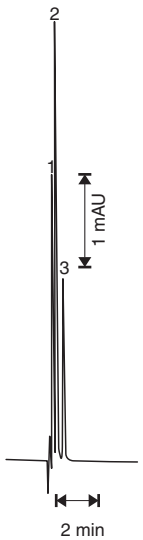



*Figure 17. Separation of void peak (1), phenol (2), and methyl benzoate (3) on a C8, 5µm, 14 x 1.0 mm UniJet column only.*

## *How to Connect the Guard Column to an LC System*

1. Insert a guard column into a PEEK ferrule and insert them both into a UniNut. Leave 10 mm of column outside the front of the ferrule. (See Figure 18 for connection procedures.)

2. Screw the UniNut into the port of an injector valve of an LC system. Make sure the guard column bottoms out in the injector port (push it in with the aid of a piece of tubing). Hand tighten slightly. Since the UniNut is made of stainless steel and the microbore injector valve port is made of PEEK, take care when screwing the UniNut into the injector. Do not press heavily on the UniNut when screwing. After hand tightening, make 1/4 turn using a wrench. Do not overtighten.

3. Connect an analytical column to the female end of the UniNut. Push a PEEK nut (or another UniNut) and ferrule 1 cm away from the column end before making the connection. Be sure the end of the analytical column touches the end of the guard column.

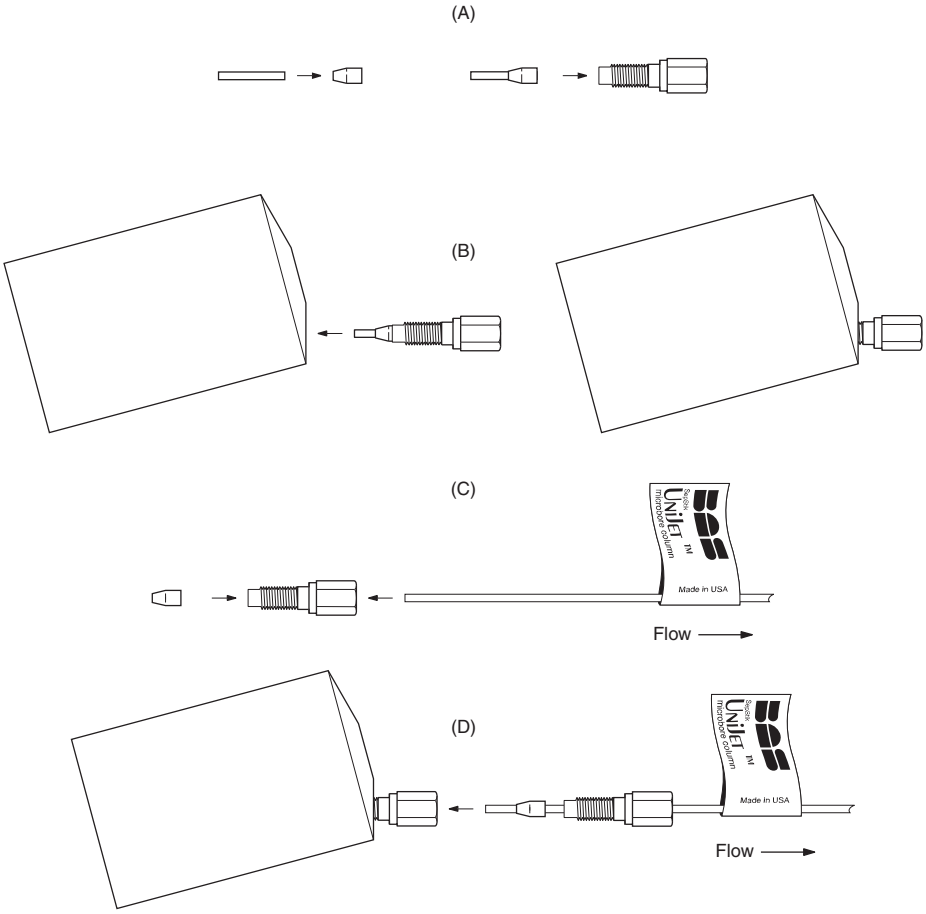



*Figure 18. UniJet guard column installation procedures: A) Insert the guard column into a PEEK ferrule and connect to the special UniNut; B) Mount the nut and guard column into an injector valve; C) Insert a UniJet analytical column into another UniNut and ferrule and; D) Insert into the first UniNut which is attached to the injector valve.*

## *Column Storage*

Reverse phase microbore columns should be stored in 40% acetonitrile when not in use. Run 40% acetonitrile through the columns for 15 minutes to wash out any mobile phase. Cap the columns and store in a sealable test tube. Add a few drops of 40% acetonitrile in the tube to prevent column drying. Columns stored longer than 10 days should be wetted before use. Follow the wetting procedures on p. 10.

### *1 mm i.d. UniJet Columns*

| **part number** | **material** | **particle size (µm)** | **pore Å** | **column dimension** |
|---|---|---|---|---|
| MF-8948 | C18 | 3 | 80 | 14 x 1 mm |
| MF-8901 | C8 | 5 | 100 | 100 x 1 mm |
| MF-8949 | C18 | 3 | 80 | 100 x 1 mm |
| MF-8902 | C18 | 5 | 100 | 100 x 1 mm |
| MF-8912 | C18 | 5 | 100 | 150 x 1 mm |

### *UniJet SepStik 2 mm i.d. PEEK Column*

| **part number** | material | **particle size (µm)** | **pore Å** | **column dimension** |
|---|---|---|---|---|
| MF-8957 | C18 | 3 | 80 | 100 x 2 mm |
| MF-8956 | C18 | 5 | 100 | 50 x 2 mm |

## *UniJet Guard Columns*

| **part number** | material | **particle size (µm)** | **pore Å** | **column dimension** |
|---|---|---|---|---|
| MF-8943 | C8 | 5 | 100 | 14 x 1 mm |
| MF-8946 | C18 | 3 | 80 | 14 x 1 mm |
| MF-8945 | C18 | 5 | 100 | 14 x 1 mm |

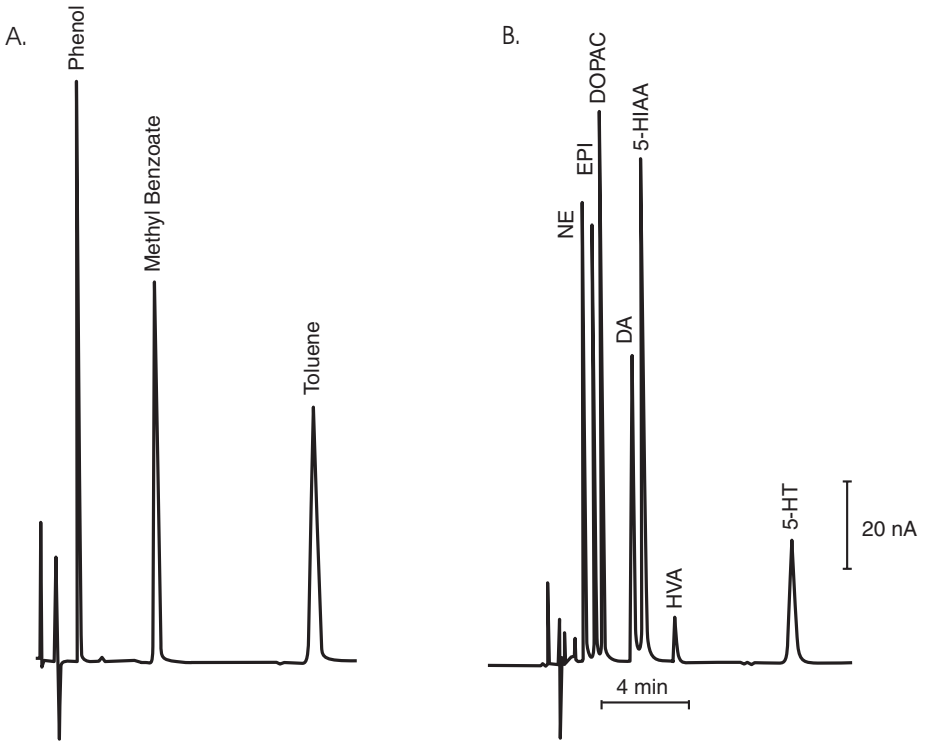
## *UniJet Columns for Special Applications*

| **part number** | description |
|---|---|
| MF-8908 | Acetylcholine/choline microbore kit |
| MF-8904 | Acetylcholine/choline analytical column, 530 x 1 mm, 10 µm |
| MF-8903 | ACh/Ch IMER, 50 x 1 mm, 10 µm |
| MF-8907 | Ch/catalase IMER, 55 x 1 mm, 10 µm |
| MF-8935 | ACh/Ch microbore guard column, 14 x 1 mm, 10 µm |
| MF-8958 | Amino acids microbore kit |

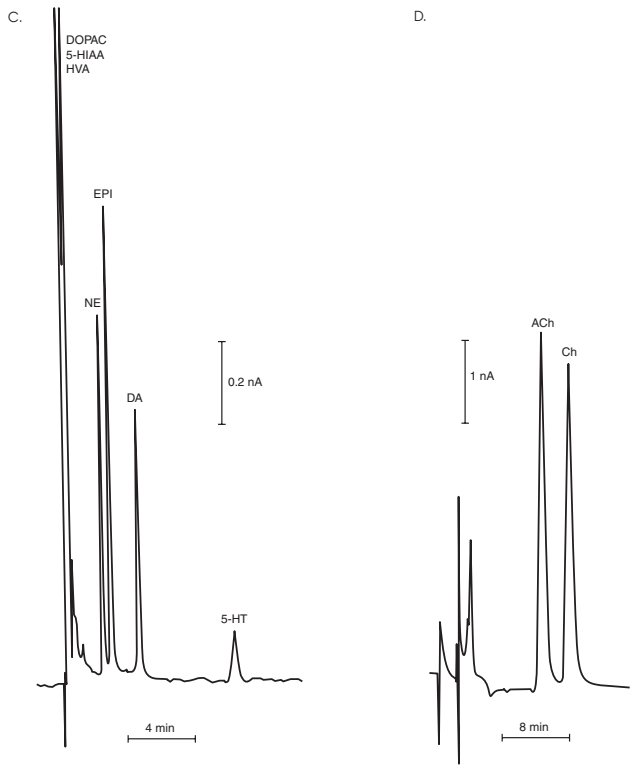
**Table 1**. *Useful spare or supplemental parts*

| Part No. | Description | Quantity |
|---|---|---|
| MF-8952 | Microbore in-line filter kit (includes package of 10 0.5 µm x 1.5 mm frits, union, stainless steel connecting tube, nuts, and ferrules) | 1 set |
| MF-8955 | Replacement 0.5 µm frits for in-line filter above | 10/pkg |
| MR-4135 | Normal in-line filter kit (includes 0.5 µm x 3 mm frit, frit holder, stainless steel connecting tube, nuts and ferrules). This kit is used prior to the sample injector. | |
| MR-4136 | Replacement frit for MR-4135 | 1 each |
| MR-4410 | Replacement plastic ferrule for MR-4409 | 1 each |
| MF-1044 | Special thin-layer gasket for microbore LCEC | 3/pkg. |
| MF-8947 | Flow splitter kit (includes capillary and column-style splitters, fittings, and instruction sheets for installation) | 1 each |
| MF-4161 | Microbore injection valve (includes valve, tools, fittings, 5 µL loop) | 1 each |
| MF-4147 | 20 µL loop for MF-4161 | 1 each |
| MF-4165 | PEEK nuts and ferrules for MF-4161 | 5 each |
| MF-4167 | PEEK ferrules for MF-4161 | 5 each |
| MR-4068 | UniNut™ adapter for UniJet guard columns | 1 each |

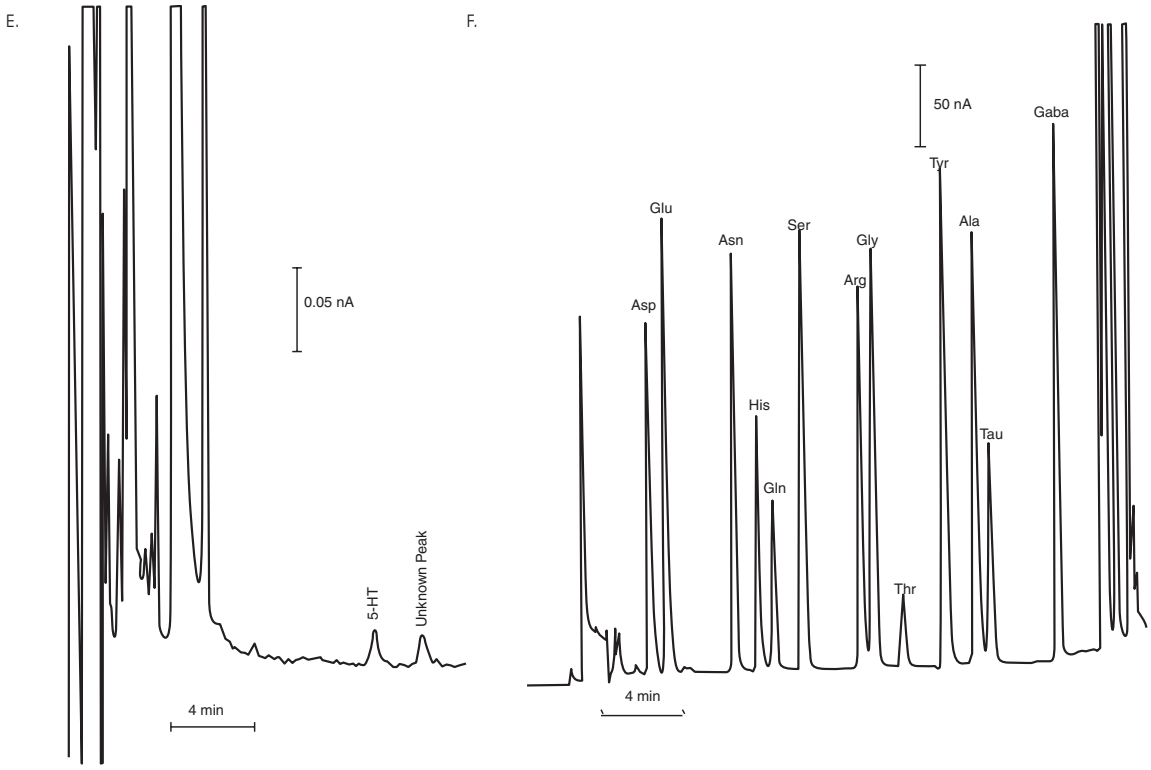
## *Representative Separations (please call BAS for updated applications)*




| | | |
|---|---|---|
| A. | Column: | MF-8949, 3 µm, C18, silica |
| | Detector: | BAS UV-8, 254 nm, special fused silica flowcell, 1.0 sec. rise time, 0.01 AUFS |
| | Pump: | BAS PM-60 microhead |
| | Flowrate: | 70 µL/min. measured flow |
| | Mobile phase: | acetonitrile/$H_2O$/acetic acid = 40/60/0.08 (v:v:v) |
| | Injector: | Rheodyne 7413, 1 µL loop |
| | Backpressure: | typically 2300-2800 psi |
| B. | Column: | MF-8949, 3 µm, C18, silica |
| | Detector: | BAS LC-4C prototype, 650 mV vs. Ag/AgCl, glassy carbon electrode |
| | Pump: | BAS PM-48 microhead |
| | Flowrate: | 80 µL/min. measured flow |
| | Mobile phase: | mix 475 mL $H_2O$ with 4.7 gm monochloroacetic acid, 75 mg sodium octylsulfate, and 93 mg disodium ethylenediamine-tetraacetate dihydrate. Adjust pH to 3.2 with 6 M NaOH. Add 25 mL acetonitrile and 4.0 mL tetrahydrofuran. |
| | Injector: | Rheodyne 7125 5 µL loop |
| | Backpressure: | typically 3000-3500 psi |




| | | |
|---|---|---|
| C. | Column: | MF-8901, 5 µm, C8, silica |
| | Detector: | BAS LC-4C, 650 mV vs. Ag/AgCl, glassy carbon electrode |
| | Pump: | BAS PM-60 conventional pump with a splitter, a 100 x 3.2 mm BAS Phase II cartridge column as a restrictor (ODS, 3 µ, MF-6213) |
| | Flowrate: | 0.7 mL/min. set at the pump, giving 80 µL/min. through the UniJet column |
| | Mobile phase: | Add 80 mg disodium ethylenediaminetetraacetate dihydrate to 1 liter $H_2O$. When dissolved, add 0.5 g 1-decanesulfonic acid, sodium salt and 2.8 g sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$). Adjust pH to 5.8. Mix the above with acetonitrile in the volume ratio of 200 to 30. |
| | Injector: | BAS MF-4161 injector with 5 µL loop |
| | Backpressure: | 1500-2000 psi |
| | Analytes: | NE, EPI, DA: 250 femtomoles; 5-HT: 100 femtomoles; HVA, DOPAC, 5-HIAA: 2 picomoles |
| D. | Columns: | MF-8908 Acetylcholine kit |
| | Detector: | BAS LC-4C, 500 mV vs. Ag/AgCl, platinum electrode, 10 nA f.s. |
| | Pump: | BAS PM-60 with microhead |
| | Flowrate: | 134 µL/min. (set pump at 0.4 mL/min.) |
| | Mobile phase: | mix 500 mL $H_2O$ with 3.5 g sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) and 2.5 mL 1% ProClin, adjust pH to 8.5 with 6 M NaOH |
| | Injector: | BAS MF-4161 injector, 5 µL loop |
| | Backpressure: | 3200-3700 psi |
| | Analytes: | 5 pmoles each of acetylcholine and choline |




| | | |
|---|---|---|
| E. | Column: | MF-8949, 3 µm, C18, silica |
| | Other conditions: | Refer to chromatogram B. The retention time of 5-HT is slightly different due to evaporation losses of THF solvent. |
| | Analyte: | Serotonin taken from dialysate of rat striatum 5 hours after implanting the probe. Samples at later times demonstrated same magnitudes and presumably represent basal levels. 4.3 femtomoles of serotonin was observed in 5 µL of dialysate collected over 2.5 minutes. |
| F. | Column: | MF-8949, 3 µm, C18, silica |
| | Detector: | BAS 200A system, +0.7 V vs. Ag/AgCl, at glassy carbon electrode |
| | Sample: | 2 µM mixture of amino acids, 10 µL taken for derivatization, 10 µL of the 14 µL derivatization mixture injected (14 pmoles injected). |
| | Flowrate: | set flowrate was 0.8 mL/min. and the packed column splitter was used. |
| | Pump: | BAS 200A system delivering binary gradient involving acetate buffer, dimethylacetamide, and methanol |
| | Oven temperature: | 40 °C |
| | Injector: | CMA 200 Microsampler, 10 µL injected. |

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