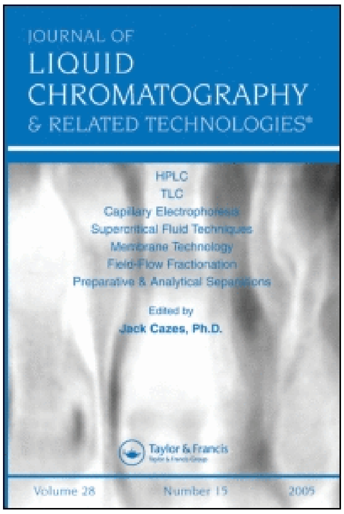

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Portable Slurry Packing Apparatus for High Performance Liquid Chromatography

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To cite this Article Kikta Jr., Edward J.(1979) 'A Portable Slurry Packing Apparatus for High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 2: 1, 129 — 144
To link to this Article: DOI: 10.1080/01483917908060053
URL: http://dx.doi.org/10.1080/01483917908060053

JOURNAL OF LIQUID CHROMATOGRAPHY, 2(1), 129-144 (1979)

A PORTABLE SLURRY PACKING APPARATUS FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A portable apparatus for slurry packing high performance microparticulate liquid chromatographic columns is described. The packer offers lab to lab portability and ease of operations. Columns up to 50 cm in length can be accommodated with the described configuration. Material costs per column are $40 - $70, offering a substantial saving over commercially prepared columns, in situations where labor costs are minimal. Efficiencies of columns packed with this apparatus have been equivalent to or better than those obtained with commercially available columns. Columns are packed using a staged pressurization/de-pressurization method. This method also leads to longer column life. Evaluation of relative column efficiency is also discussed.

INTRODUCTION

The popularization of high performance liquid chromatographic technology has led to the solution of many difficult separation problems in diverse scientific studies. However, high performance LC columns are relatively expensive. For example, a typical commercially available 10 um reversed phase packed column of 25 to 30 cm length costs in the neighborhood of $250. For many smaller companies or educational institutions such capital cost can be prohibitive when several columns are required. It has been the experience of this author that the reproducibility of results from column to column is often variable, requiring the reoptimization of analytical conditions when comparing columns of nominally the same type. This variation is most often found when comparing bonded phase columns from different manufacturers where variables such as the degree of derivatization can lead to quite different analytical properties under similar mobile phase conditions.

Many authors have utilized slurry packing methods to generate highly efficient columns. The basic slurry packing method as described by Kirkland (1-3) and further modified and used by others (4,5) has been used to prepare most high efficiency columns in use today. Recent developments, such as upward slurry packing (6), have shown great promise for further improving column efficiencies. Gonnet and Rocca (7) have discussed the design of the packing apparatus and the optimal conditions under which it should be used to make efficient columns.

If a laboratory decides to pack its own HPLC columns, it is often faced with the dilemma of what to do with the column packing apparatus. Since column packing is usually done infrequently, it is a waste of valuable bench space to dedicate a section to a packing instrument which sits idle 95% of the time. Often, more than one laboratory in an installation desires to pack its own columns. If this is the case, making the column packer portable is a great advantage. This paper discusses a compact portable column packer adaptable to the needs of many small and medium size laboratories' applications. The cost of this apparatus, ~$750, is offset by the average saving of $200 per column over the purchase of commercially available columns. In other words, four well-packed columns pay for the packer. Column to column reproducibility can also be greatly enhanced by packing several columns of a desired type, from a single batch of support material, at one time.

EXPERIMENTAL

Figure 1 schematically shows the components of the column packer. All fittings and tubing on the high pressure side, components 9-19, are stainless steel. Fittings and tubing on the low pressure side can be made from any convenient material capable of withstanding pressures up to 100 psi. The packer was statically tested to pressures in excess of 10,000 psi without encountering any leaks. The only component which may develop leaks, if closed when

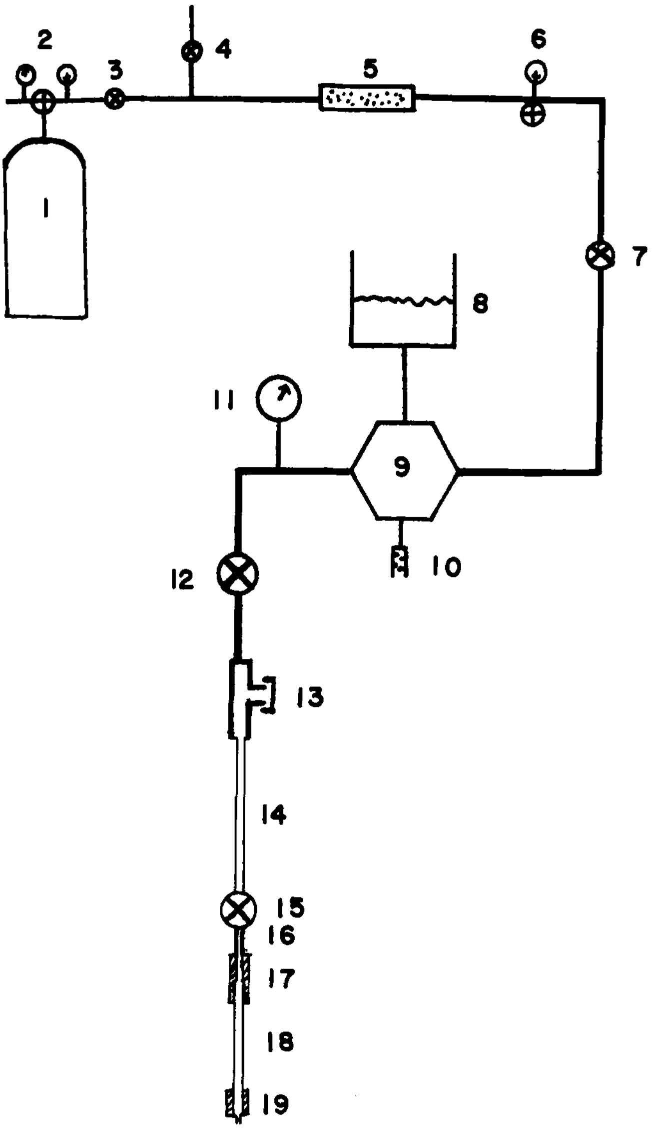
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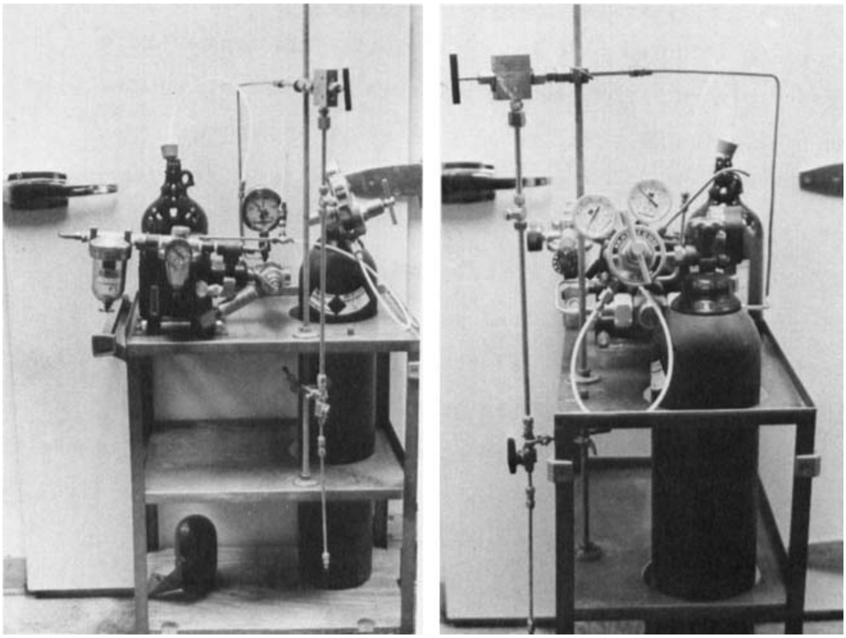
Figure 1 - Schematic representation of the hplc column packing apparatus.

1. Nitrogen cylinder - C size
2. Two-stage regulator - 0-250 psi
3. Nitrogen supply shut-off valve
4. Toggle valve and gas vent line
5. Gas filter (Arrow Scintered Products Co., Forest Park, Illinois - #3104 - 1/2)
6. Single stage regulator - 0-120 psi
7. Gas shut-off valve
8. Solvent reservoir
9. Pump, gas driven fluid model DST100C - Haskel Engineering and Supply Co., Burbank, California
10. Pump gas vent and muffler - C. W. Morris Co., Detroit, Michigan
11. Fluid pressure gauge - 0-15,000 psi, Enerpac, Butler, Wisconsin
12. Packing chamber shut-off valve - Sno-Trik® model SS-645-FPAR, Sno-Trik Co., Solen, Ohio
13. SS-Union T - 3/8" Swagelok®, Crawford Fitting Co., Cleveland, Ohio
14. Packing chamber - SS-3/8" O.D. x 9/32" I.D. x 35 cm L.
15. Column shut-off ball valve - Whitey SS-4354, Whitey Co., Oakland, California
16. Pre-column SS-1/4" O.D. x 4.2 mm I.D. x 4 cm L.
17. 1/4" SS-Union - drilled through - Swagelok®
18. Hplc column - 1/4" SS up to 50 cm L. I.D. range up to 4.2 mm
19. LDV or ZDV - 1/4" to 1/16" SS - reduction Union fitted with a 2 µ filter element - Swagelok®

head pressures exceed 5,000 psi, is the column shut off ball valve (item 15). Since this valve is never shut under high pressure, this problem is not encountered. The column packer is mounted on a stainless steel cart of the following dimensions: height - 80 cm, width - 60 cm, depth - 40 cm. Front and side views of the column packer and the cart are shown in Figures 2A and 2B. The cart has three evenly spaced shelves. In order to support a"C"size nitrogen cylinder the bottom shelf is reinforced by placing a 1.5 cm thick plywood board upon it. Two 20.5 cm holes, one above the other, are cut into the first and second shelves near the right rear of the cart. The nitrogen cylinder is placed through these holes onto the wooden base, positioning it securely. The Haskel DST-100C pump is securely bolted near the left front of the cart. The column packing chamber and immediately associated valving are supported by a 120 cm L. x 1.3 cm radius metal support rod located near the front of the cart (see figure 2). The rod passes through the upper two shelves and is attached to each shelf with a Fisher Brand Flexaframe® foot plate. The solvent reservoir consists of a one gallon glass jug. Under conditions of portability, it may be advisable to either build a restraining shield around the glass jug or to substitute a closed attached metal reservoir for the glass jug. Flexible 1/4" Teflon® tubing is used to make the liquid inlet connection to the pump and the initial connection from the two-stage regulator supply shut off valve (item 2) to the toggle valve and gas vent line. These Teflon lines, both in low pressure (<250 psi)



A. Front view B. Side view

Figure 2 - The column packing apparatus - a 1/4" O.D. x 30 cm L. column is shown in the packing position.

sections, allow for the easy manipulation of components which must be changed or refilled, such as the solvent reservoir or gas tank regulator.

A word of caution regarding the use of tube fittings when assembling this apparatus. In general, we have found that tube fittings utilizing a one piece ferrule assembly do not have adequate gripping power to insure pressure seal integrity for the system at the required pressures. This is especially true where 3/8" tubing is used. Static pressure tests at 10,000 psi led to repeated union failure with one piece ferrule assemblies. We recommend that all fittings be of a two piece, front and back, ferrule type such as the

Swagelok® or Sno-Trik® type. This type of two piece ferrule makes an indented bite into the tube. This type of bite leads to a much more secure pressure seal which resulted in no static testing failures. Most users of HPLC equipment do not usually worry about high pressure leaks because of the relatively incompressible nature of liquids and the low volumes involved. The Haskel DST-100C pump is capable of flow rates up to 1.2 litres per minute and pressures up to 16,500 psi. At such pressures (> 6,000) and fluid displacement rates, a joint failure can throw a section of the packer, such as the packing chamber, a considerable distance at a high velocity. The use of proper fittings and safety precautions around the column packer should eliminate any danger of a blow out. Our unit has been used for over a year without any leak problems or pressure failures. Approximately fifty columns have been packed with the system.

PROCEDURE

The following column packing procedure is used for both bonded phase and silica gel (nonuniform particle shape): (e.g., col. 30 cm L x 4.2 mm i.d.)

1) Prepare a slurry of the packing ~2-2.5 g in 50% methanol/50% ethylene glycol, total volume ~ 20 cc.
2) Through the side port of the 3/8" union T fill the solvent reservoir with the slurry. Note: The ball valve is open.
3) Fill the remaining volume (~ 10 cc) above the slurry with methanol.
4) Seal the union T with a 3/8" SS cap.

5) Pressurize the pumping system before the packing chamber shut off valve to 3,000 psi.
6) Open the chamber shut off valve.
7) Maintain this pressure until all the glycol/methanol mixture is eluted and only methanol is being eluted. This elution pattern generates a packing-solvent density gradient.
8) After glycol elution, stage the pressure up in 1,000 psi increments to at least 6,000 psi. Elute at least 10 cc of methanol between pressurized stages.
9) When the maximum desired pressure is reached allow the column to be subjected to this pressure at least 15 min.
10) Shut the packing chamber shut off valve. Leave the instrument in this state at least 1/2 hour.
11) Shut the column shut off ball valve. Leave the instrument in this state at least 15 min.
12) Remove the column and proceed to analytical evaluation. For any column to be used in a normal phase mode, condition the column with the series of solvents suggested by Scott and Kucera (8).

The packing of spherical particles is similar except it has been observed that when a polar packing slurry is used extreme foaming of the slurry is obtained. In this case we resort back to a more conventional halocarbon solvent balanced density slurry.

Analytical liquid chromatography is performed on an instrument assembled from components purchased from Waters Associates, Milford, Mass. The system and its associated temperature control devices have been previously described

(9). The only modification to the system was the incorporation of a Fisher Scientific Proportional Temperature Controller (Fisher Scientific, Fairlawn, NJ). With this controller temperature precision was ± 0.01°C with an accuracy better than ± 0.02°C. Columns are evaluated for efficiency using the following scheme:

Normal Phase Packings - Mobile phase n-heptane at 1 ml/min; T=40°C; solute - 10% v/v benzene in heptane; injection volume - 1 μl;

Reversed Phase Packings - Mobile phase methanol at 1 ml/min; T=40°C; solute -10% v/v acetophenone in methanol; injection volume - 1 μl. These conditions were chosen because they seem to give efficiency values (HETP) similar to efficiencies most often observed for operational analytical systems in our laboratory. The test sample concentrations and injection volumes are not especially critical as long as the operator avoids concentration extremes which could lead to column or detector overloading or too large injection volumes which could lead to excessively large elution zones for the rapidly eluting weekly retained solutes. The invocation of such extreme conditions could lead to the improper evaluation of a column.

RESULTS AND DISCUSSION

In order to evaluate the effectiveness of our column packing procedure we must establish efficiency criteria which will satisfy our minimum analytical requirements. Table 1 lists the minimum acceptable efficiencies we have decided upon in terms of plates per meter. These efficiencies have proven to be the lowest tolerable values for most routine separations performed in our laboratory. In order to adequately evaluate columns, one should extensively validate this or other criteria.

Table 1: Minimum acceptance specification in terms of plate count using the specified evaluation scheme. (Analytical columns 25-40 cm L)

Packing Type	Mean Particle Size-Microns	Minimum Plates/M
Silica	10	6,000
Silica	5	10,000
Bonded Phase	10	3,000
Bonded Phase	5	5,000

Perhaps a more valid comparison would be possible if reduced plate heights were compared at a set reduced velocity. But for a quick in-house check, we find the use of a set velocity and comparing plate per meter values adequate. Admittedly this makes the comparison of 5 and 10 μm columns difficult. In addition to these efficiency criteria we also specify that the base asymmetry be less than 1.5. To measure base asymmetry first drop a perpendicular to the base line from the peak maximum and then draw tangents to each side of the peak. Measure the distance from where the tangents bisect the base line to the perpendicular. The base asymmetry is the distance measured for the back half of the peak divided by the distance for the front half of the peak.

Table 2 lists some efficiencies found with columns prepared in our lab using our evaluation system. The importance of using the staged pressurization scheme is reflected in Table 2. The efficiencies of similar columns packed using the staged pressurization method are generally observed to be greater than those packed using a constant pressure method. In a conventional constant pressure packing procedure an initial high pressure is used throughout the entire packing process (1-7). Whether packed by either the constant pressure or staged pressure method, the same specified criteria shown in Table 1 are used for evaluative purposes. Efficiencies tend to drop as column length is increased. This well known effect (7) does not seem to be reduced by using the staged method. Comparing columns 2-5 listed in Table 2, a loss of 20,000 plates per meter is realized by doubling the column length. We have

Table 2: Plate count evaluations for columns prepared with the described packing system.

Col. No.	Packing Type/Col. Length	Stage Pressurization and/or Packing P	Plates/M
1	5 µ silica - 25 cm L	No (P=7,000 psi)	65,000
2	5 µ silica - 40 cm L	Yes	55,000
3	5 µ silica - 30 cm L	Yes	70,000
4	5 µ silica - 30 cm L	Yes	70,000
5	5 µ silica - 20 cm L	Yes	75,000
6	10 µ silica - 30 cm L	No (P=6,000 psi)	14,000
7	10 µ amine - 30 cm L	No (P=6,000 psi)	8,000
8	10 µ ODS - 25 cm L	No (P=6,000 psi)	3,000
9	10 µ ODS - 30 cm L	No (P=6,000 psi)	3,200
10	10 µ ODS - 30 cm L	No (P=4,000 psi)	2,400
11	10 µ ODS - 25 cm L	Yes	10,800
12	10 µ spherical ODS - 30 cm L	No (P=6,000 psi)	5,400
13	10 µ ODS - 25 cm L	Yes	8,400
14	10 µ octyl bonded - 30 cm L	No (P=5,000 psi)	5,100
15	10 µ octyl bonded - 30 cm L	No (P=5,000 psi)	5,000
16	10 µ octyl bonded - 25 cm L	Yes	8,000
17	5 µ ODS - 25 cm L	Yes	12,400
18	5 µ ODS - 25 cm L	Yes	16,500
19	10 µ benzylchloride (ref. 12) 30 cm L	No (P=5,000 psi)	4,800

found that columns packed using the staged system are more resistant to pressure shock disruptions of the bed and give a longer usable life. It should also be noted that columns packed with too low a packing pressure are observed to give poorer efficiencies (1-3).

Equivalent column evaluation between laboratories is often a difficult task. A user can be confronted with an evaluative chromatogram showing plate counts several times greater than those obtained for his analytical separation of interest. The column evaluation procedures chosen by us, gives plate counts similar to those obtained with the majority of our analytical systems and may or may not be typical of problems encountered by other users. Table 3 compares plate counts obtained for commercially available columns purchased by our laboratory (manufacturers are not named). Two trends can be noted. A large difference can be seen between in-house plate counts and those reported for several commercial columns. This does not mean that the manufacturer's evaluation is invalid. It is most likely a perfectly valid plate count for their system.

An extreme example of efficiency variability as a function of sample type can be abstracted from a paper by Grushka, Kikta and Naylor (10). A qualitative examination of figure 1 in this paper shows that peaks for uracil and uridine, unfortunately incompletely resolved in this example are significantly narrower than peaks for N-formylkynurenine and 3-hydroxykynurenine which elute respectively before and after the partially resolved pair. Examination of old data reveals a count of approximately 15,000 plates per meter for the uridine peak, 3,000 plates per meter for the earlier eluting N-formylkynurenine peak and

Table 3: Plate count comparisons - commercially available columns.

Manufacturer Designation	Column Type	Claimed N/M	Found N/M*
A	10 μ ODS 25 cm L	52,000	3,000
B	10 μ ODS 30 cm L	10,000	14,000
B	10 μ nitrile 30 cm L	10,000	7,000
B	10 μ nitrile 30 cm L	10,000	4,000
B	10 μ silica 30 cm L	10,000	12,000
B	10 μ ODS 30 cm L	10,000	2,900
C	5 μ silica 25 cm L	62,000	50,000
A	10 μ silica 25 cm L	60,000	14,000

*evaluation schemes outlined in the experimental section

3,200 plates per meter for the later eluting 3-hydroxykynurenine peak. This example illutrates that variations, most likely in retention mechanism, for compounds from two varied chemical families can lead to significantly different plate counts for the same column under the same condition. Secondly, variations between columns of the same type from the same manufacturer purchased at different times can be quite large, although quality control efforts are rapidly reducing such problems. To overcome some of these variabilities we derivitize our own silica gel to prepare large uniform batches of bonded phases much as large scale manufacturers do. The derivatization and utility of bonded phases has been reviewed elsewhere (11). We then pack several columns and store them for future use. We can see that a comparison of plate counts obtained for commercial columns and those packed using the staged pressurization method tend to favor the staged method by a small but significant amount for all but one case. Our best 10 µ ODS column had 10,800 plates per meter while the best commercial column gave 14,000 plates per meter. Both columns are among the best 10 µ ODS columns we have ever evaluated with typical columns being considerably poorer. One must qualify the above discussion by noting that changes in silica can generate as many problems as the packing method. The best packing methods yet devised cannot make a good column from a poor grade silica.

Problems other than those already described can be avoided by packing several columns at a time from a single batch of packing. At times a laboratory is fortunate or often unfortunate to obtain a so-called "super column". A column of this type is one which may provide two or three times the efficiency of a typical column of its manufacture. If a critical separation is developed around a column of this type, all is well until the column irreversibly fails. Then one is faced with the problem of finding a similar atypical column. In such a situation one may find himself out of business. By having evaluated several columns, one has just packed, one can have a reasonable chance of reproducing the separations obtained on the next column used from that lot.

CONCLUSION AND SUMMARY

The column packing apparatus described in this paper allows the user to prepare hplc columns of good efficiency at a most reasonable cost. Columns packed using the described system are of equivalent or better quality relative to commercially obtained columns. The portability features of the unit allow it to be easily stored and moved from one user's laboratory to another with a minimal set up procedure. During the past year and a half all hplc columns in our laboratory were prepared with this system. Commercially available column packing devices can be easily adopted to the portable configuration described in this paper (13). Further details regarding the construction or use of this apparatus will be furnished upon request.

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