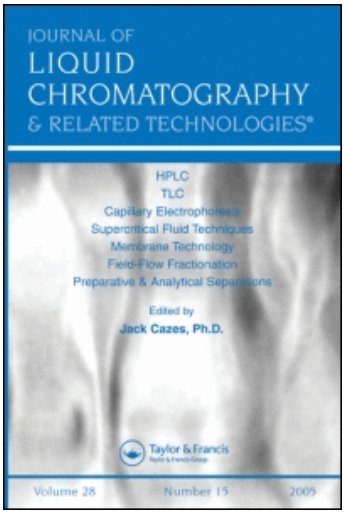

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Method Transfer to Narrow Bore Columns: An Evaluation

T. W. Ryan[a]
[a] Ganes Chemicals, Inc., Pennsville, New Jersey

To cite this Article Ryan, T. W.(1995) 'HPLC Method Transfer to Narrow Bore Columns: An Evaluation', Journal of Liquid Chromatography & Related Technologies, 18: 1, 51 — 62
To link to this Article: DOI: 10.1080/10826079508009220
URL: http://dx.doi.org/10.1080/10826079508009220

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(1), 51-62 (1995)

HPLC METHOD TRANSFER TO NARROW BORE COLUMNS: AN EVALUATION

TIMOTHY W. RYAN
Ganes Chemicals, Inc.
33 Industrial Park Road
Pennsville, New Jersey 08070

ABSTRACT

The use of organic solvents is common in high performance liquid chromatography (HPLC). Some of these solvents, such as acetonitrile, have a very high purchase price. In addition, because of the toxic nature of these solvents, waste elimination costs have soared. The use of narrow bore HPLC columns (2mm ID or less) represents an attractive solution to the problem of excessive solvent costs by reducing the amount of HPLC mobile phase consumed. An evaluation of analysis integrity following method transfer from a standard bore column (3.9 mm ID) to a narrow bore column (2 mm ID and 1 mm ID) is necessary to determine feasibility in a QC/analytical environment.

INTRODUCTION

High performance liquid chromatography (HPLC) is the preferred method of analysis for a number of important industries. Most HPLC mobile phases incorporate the use of an organic solvent. Generally, these solvents are toxic materials. They can also be quite expensive, both in terms of initial purchase cost

and in terms of the ever increasing cost of disposal. As such, solvents for HPLC can represent a significant portion of an analytical laboratory's annual budget.

A number of approaches have been developed to address both the costs of the solvents and the elimination of the waste. Commercially available (or laboratory fabricated) distillation apparatus allows the analytical lab to purchase less expensive technical or reagent grade solvents, which are then refined to an acceptable purity level. While this technique reduces the initial solvent purchase cost, it does not address the problem of disposal. It does, however, represent an increase in the routine lab housekeeping functions, something most analytical and quality control laboratories would rather avoid. Potentially, the same distillation technique could be applied to the waste mobile phase. But the possible difficulties are prohibitive. These include separating and purifying complex mixtures (even in the case of a single solvent with an aqueous phase, formation of an azeotrope would interfere with purity), and avoiding contamination from spent sample in the waste. This also represents an increase in housekeeping functions. Mobile phase recycling systems have also been commercially available for some time. These devices work on the principle that most of the mobile phase elutes from the HPLC column essentially uncontaminated, and that only those volumes that contain the injection void or the actual sample peaks are true waste. These systems can be very useful in an analysis where only a single peak in a product assay is encountered. Even in a QC lab, such an ideal situation is rare, almost all samples have some low level of impurities. As the number of peaks in the sample increases, the efficiency decreases. As the solvent is recycled, contamination eventually takes its toll. Increasing levels of chromaphoric "non-sample" material affects sensitivity and detection linearity. The presence of non-chromaphoric background material will affect the separation. Also there is some question as to whether or not the regulatory agencies would require additional testing of the

recycled mobile phase to demonstrate that it does not interfere with the integrity of the analysis. More importantly, the technique is useless for HPLC methods that requires any degree of gradient elution. Because of these inadequacies, this technique cannot be seriously regarded as a solution to the problem.

The easiest way to address both the costs of the solvents, and the cost and environmental impact of disposal, is to decrease the amount of solvent initially purchased and hence the amount of waste mobile phase generated. Liquid chromatography using narrow bore columns (2 mm internal diameter or less) represents an attractive solution to this problem. Column flow is proportional to the column internal diameter (ID). Thus the same linear velocities (and therefore retention times) are preserved when using a lower flow rate and a column with a reduced ID (if column ID is the only parameter changed). The following equation is commonly used to calculate flow rate changes based on a change in the column ID:

$$\frac{(\text{ID col 2})^2}{(\text{ID col 1})^2} \times \text{flow col 1} = \text{flow col 2}$$

The use of a 2 mm ID column in place of a traditional 3.9 mm ID column can reduce mobile phase consumption by a factor of 3-4 (assuming a typical flow rate of 1-2 ml/min). There are some difficulties with the technique, most of which are associated with incompatibilities between the narrow bore column and the chromatographic system.

The purpose of these experiments is to document the transfer of an HPLC method from a standard analytical column to a narrow bore technique. The object is to determine how much adjustment is required in order to execute an accurate method transfer. In order for the transfer to be practical, the only considerations addressed will be minor adjustments in flow rate and in injection volume. The only

hardware modification was the use of a semi-micro flow cell (volume 8 µl) in place of the standard flow cell (volume 14 µl) to maintain UV detector sensitivity with the narrow sample bandwidths that the smaller ID columns produced. The cell could also be used with the standard bore column

MATERIALS AND METHODS

The equipment used included a Model 1050 HPLC system (Hewlett Packard Co., Palo Alto CA). This system included an autosampler, pump and UV detector. The only other important change was slowing the syringe draw speed from 200 µl/min to 10 µl/min. This change was incorporated to accommodate the small injection volumes. Connecting tubing lengths were kept at an absolute minimum and the connecting tubing used had a 0.005" ID. Although even smaller ID's would be achieved using fused silica capillary tubing, the fragile nature of exposed capillary tubing on an LC precluded its use. Three different HPLC columns were evaluated. The first was the "standard" column, a 300 x 3.9 mm µBondapak C18 (Waters Division of Millipore, Milford MA). This column is routinely used for a number of our purity profile analyses and sample assays. The first narrow bore column evaluated was a 300 x 2 mm µBondapak C18 (Waters). The final narrow bore column evaluated was a 300 x 1 mm Hyperbond C18 (Keystone Scientific, Bellefonte PA). The Hyperbond column was used since the µBondapak is not available with a 1 mm bore (the Hyperbond packing is marketed as a comparable packing to the µBondapak). The sample was a mixture of 10 barbiturates made from in-house material (Ganes Chemicals, Inc., Pennsville NJ) and standards purchased from Aldrich (Milwaukee WI). The sample was prepared by dissolving 6-8 mg of each barbiturate in a single 25 ml volumetric flask and

diluting to volume with mobile phase. The mobile phase was 70/30 water/acetonitrile pH 3.0 (H_3PO_4). The mobile phase was filtered and degassed using sonication before use.

RESULTS & DISCUSSION

Ten injections were performed for each sample series. After calculation of the mean, the two injections with the highest deviation were eliminated. The sample series mean, standard deviation and % realtive standard deviation were then calculated using the remaining eight injections. The elimination of the two outliers is statistically acceptable given the number of injections. Figure 1 is an example analysis of the barbiturate sample analyzed using the 3.9 mm ID column. The flow rate was set at 1 ml/min, injection volume was 3 µl. All of the

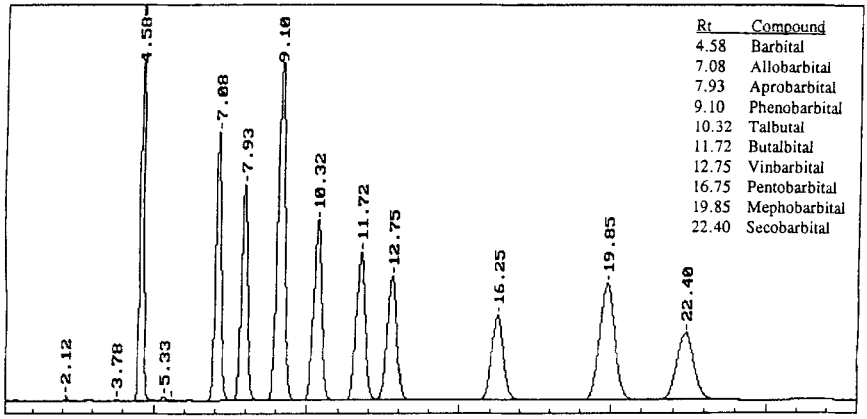

Figure 1. Analysis of barbiturate sample. Flow: 1 mL/min; Mobile Phase: 70/30 Water/Acetonitrile, pH 3.0 (H_3PO_4); Column: 300 x 3.9 mm µBondapak C18.

barbiturates are baseline resolved from one another. From Table 1, the reproducibility of the analysis is evident. This represents important information concerning the injection system. Injection volumes commonly used are 10 µl, so these analyses establish that at a 3 µl injection volume, the autosampler does provide reproducible injections. Because of the accuracy and reproducibility achieved using this column, no further experimentation was performed.

Figure 2 is an example of the barbiturate analyses using the 2 mm ID column. The flow rate was set at 263 µl/min, a comparable flow rate reduction based on the decrease in column cross sectional area. Table 2 lists the results of the initial analyses that used an injection volume of 0.8 µl. These results were not as good as those achieved using the 3.9 mm column. Although peak separation is comparable, area reproducibility is between 1 - 2 %. Most analytical methods specify RSD's of 2% or less, our methods typically provide RSD's of less than 1%.

TABLE 1
HPLC ANALYSIS OF BARBITURATES
3.9 MM ID COLUMN, 3 µl INJECTION

INJ#	Barb.	Allo.	Apro.	Pheno.	Tal.	Butal.	Vin.	Pento.	Mepho.	Seco.
1	354439	329744	295414	546253	322946	297960	270036	230622	386894	250018
2	355392	330048	295751	547646	322980	297605	270152	231460	387110	250280
3	353272	328179	**293816**	543556	**321211**	**295899**	269054	**228678**	386399	**248424**
4	357277	331686	296747	549010	324795	298964	271180	231990	387705	250947
5	356205	330881	296491	549307	324111	298636	270585	231131	387184	250124
6	**358591**	**332562**	**298061**	**551400**	324976	299811	**272299**	231769	389172	251889
7	353798	329046	294852	546220	322399	298158	269654	**229801**	385847	**249503**
8	354246	329822	294187	547557	324324	298900	270726	231316	386295	252395
9	357736	332030	297572	550862	**325553**	299492	271364	232464	388447	252035
10	**352436**	**327326**	294130	**546031**	321992	**296941**	**268841**	231123	386449	250180
mean	355296	330180	295643	547551	323565	298691	270344	231484	387067	250984
s	1643	1299	1239	2255	1130	757	776	576	729	980
% RSD	0.46	0.39	042	0.41	035	025	0.29	0.25	0.19	0.39

Bold entries were not used in calculations.

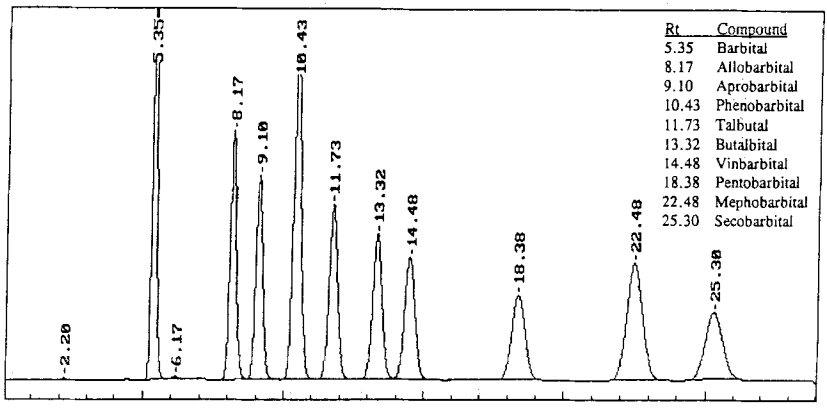

Figure 2. Barbiturate analysis. Flow: 263 µL/min; Mobile Phase: 70/30 Water/Acetonitrile, pH 3.0 (H_3PO_4); Column: 300 x 2 mm µBondapak C18.

TABLE 2
HPLC ANALYSIS OF BARBITURATES
2.0 MM ID COLUMN, 0.8 µl INJECTION

INJ#	Barb.	Allo.	Apro.	Pheno.	Tal.	Butal.	Vin.	Pento.	Mepho.	Seco.
1	**312776**	**290289**	**264096**	**478181**	**283012**	**262301**	**237853**	**201640**	**335389**	**217668**
2	323506	299375	272242	495702	293032	270145	244938	210161	346932	226372
3	318195	295330	266253	486908	287740	266648	242700	207522	342187	219542
4	325213	306145	274920	499073	295964	273365	248489	210809	349008	224665
5	325346	305271	271043	496477	291330	272896	249248	211785	348827	226549
6	324917	303052	271236	495983	291543	270825	246081	210408	346006	225606
7	327762	308805	275666	501941	297179	273329	249126	212101	346933	226096
8	**313069**	**290447**	**262277**	**478151**	**283071**	**260632**	**237232**	**200893**	**333866**	**215658**
9	320947	297574	268652	489582	290219	267857	243918	206032	341662	220065
10	328906	305145	276445	502219	298395	274501	250668	212485	350476	227587
mean	324349	302587	272058	495986	293175	271196	246896	210163	346504	224560
s	3427	4681	3534	5446	3692	2832	2885	2277	3165	3053
% RSD	1.07	1.55	1.30	1.01	1.26	1.04	1.17	1.08	0.91	1.34

Bold entries were not used in calculations.

From these results, it is apparent that the deviation is due to the small injection volume. This conclusion was reached by observing that in specific injections, all of the peaks have an area that is either higher or lower than the mean area. If the high RSD was due to chromatographic variations, a much more random distribution of peak areas would be expected (see Table 1). Since the smaller column has a lower sample capacity, and since the experimentation using the 3.9 mm ID column indicates that 3 µl is an accurately reproducible injection volume, the sample was diluted by a factor of 5 (2ml in a 10ml volumetric flask) and re-analyzed using a 3 µl injection volume. Results are listed in Table 3. Using the larger injection volume, the reproducibility of the injections is improved dramatically, and are now clearly comparable to those results achieved using the 3.9 mm ID column. This concluded the experimentation using the 2 mm ID column.

TABLE 3
HPLC ANALYSIS OF BARBITURATES
2.0 MM ID COLUMN, 3.0 µl INJECTION

INJ#	Barb.	Allo.	Apro.	Pheno.	Tal.	Butal.	Vin.	Pento.	Mepho.	Seco.
1	**270295**	**252049**	**228685**	**416014**	**246305**	**226476**	**204804**	**175611**	**295651**	**190130**
2	**269058**	**249865**	**224709**	**416720**	**245837**	**226386**	**204765**	**174795**	**296289**	**189795**
3	264284	245862	221237	409598	241707	222910	201261	171248	290096	184315
4	265923	247611	222732	412789	243220	224035	202811	174139	293082	187031
5	267414	247975	223143	413350	243675	225794	204522	174136	294271	187940
6	263759	245017	220857	409170	241544	223000	201344	171965	290063	185977
7	265397	246110	221874	410386	242093	223463	202956	173308	293004	185023
8	264960	246008	221422	410725	242413	223355	202398	173217	291959	185880
9	265293	246208	221518	410586	242182	223637	202073	173014	291986	185340
10	264899	245849	221453	409706	241895	223692	202821	173091	291995	186037
mean	265241	246330	221780	410789	242341	223736	202523	173015	292057	185943
s	1103	977	777	1511	744	909	1039	991	1448	1137
% RSD	0.42	0.40	0.35	0.37	0.30	0.41	0.51	0.57	0.50	0.61

Bold entries were not used in calculations.

Figure 3 is an example of the barbiturate sample analyzed using the 1 mm ID column. The flow rate was reduced to 65 µl/min, and the initial sample volume was 0.3 µl. It is immediately apparent that the separation is not as good as that achieved using the other two columns. Unfortunately, the difference in separation could be due to the Hyperbond packing not performing as well as the µBondapak, or if the system void volumes are responsible. In either case, using a weaker mobile phase would probably restore baseline resolution, although this option was not explored. From Table 4, the effect of the 0.3 µl injection on area reproducibility is evident, RSD's ranged from 3 - 6%. This was not suprising since an injection volume of 0.8 µl was already observed to cause problems. In order to note changes in injection reproducibility, the sample was diluted by a factor of 25 (1ml in a 25ml volumetric flask) and re-analyzed using the 3 µl injection volume. These results are listed in Table 5. When using the larger injection volume, the area reproducibility improved significantly. Although the numbers are not quite as good as those achieved using the other two columns, the low RSD's indicate that the information from such an analysis should be quantitatively accurate.

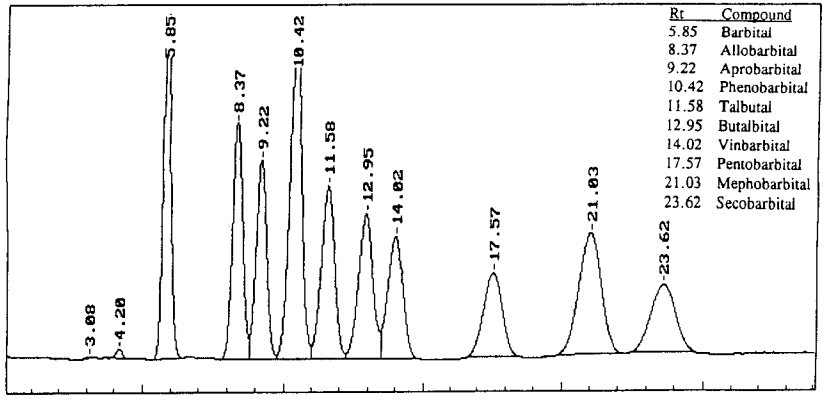

Figure 3. Barbiturate analysis. Flow Rate: 65µL/min; Mobile Phase: 70/30 Water/Acetonitrile, pH 3.0 (H_3PO_4); Column: 300 x 1 mm Hyperbond C18.

TABLE 4
HPLC ANALYSIS OF BARBITURATES
1.0 MM ID COLUMN, 0.3 µl INJECTION

INJ#	Barb.	Allo.	Apro.	Pheno.	Tal.	Butal.	Vin.	Pento.	Mepho.	Seco.
1	1180899	1168686	1001255	1782814	1067950	988876	899738	752711	1243252	848519
2	1188151	1209883	1022421	1815420	1085823	986471	891049	751654	1238571	838530
3	1088485	1056942	949435	1687601	1018341	927431	856532	714585	1170595	774813
4	1086278	1059138	961452	1680237	966416	882976	835252	713120	1186391	779738
5	1114745	1052680	961708	1725738	1081658	996259	895273	707251	1227165	858523
6	**872298**	**826099**	**752096**	**1338772**	**774842**	**709189**	**651416**	**579065**	**901454**	**596107**
7	1082325	1025078	927694	1658494	977675	874262	804929	716522	1133828	725930
8	1092671	1032814	948666	1682105	1008704	909205	806974	720034	1184685	764879
9	1168352	1123035	1022947	1824025	1090636	980115	874148	770788	1271588	812134
10	**865476**	**829858**	**739636**	**1315729**	**773823**	**688403**	**636016**	**568594**	**892777**	**580259**
mean	1125238	1090907	974447	1732054	1037150	943199	857987	730833	1207009	800383
s	45909	68290	36213	66108	50516	50597	38590	23796	45499	46559
% RSD	4.08	6.26	3.71	3.82	4.87	5.36	4.50	3.25	3.77	5.82

Bold entries were not used in calculations.

TABLE 5
HPLC ANALYSIS OF BARBITURATES
1.0 MM ID COLUMN, 3.0 µl INJECTION

INJ#	Barb.	Allo.	Apro.	Pheno.	Tal.	Butal.	Vin.	Pento.	Mepho.	Seco.
1	**214109**	194014	176690	328618	**186741**	177005	157636	**136472**	230514	**144910**
2	**207737**	**190827**	**174217**	**318102**	194756	181631	**161603**	131158	**231459**	143923
3	209962	191997	177449	323914	191052	175491	156977	134451	223297	142899
4	212537	196203	180028	334001	198095	182593	**160881**	132978	227464	140119
5	209456	194394	176827	325807	192397	178029	156970	132884	226795	143604
6	210955	193832	176202	326900	192778	178567	156970	134984	226623	**137042**
7	209141	195468	176661	324439	191505	**174372**	156893	132816	228121	142953
8	211669	192307	177304	324971	193372	179575	157672	132506	227996	142452
9	212909	**197011**	**181182**	**331647**	196925	181253	158131	**130784**	223450	141671
10	209650	194776	176222	322053	**186653**	**174552**	156369	134753	**230603**	139876
mean	210785	194124	177173	326338	193860	179268	157702	133316	226782	142178
s	1455	1442	1237	3669	2539	2452	563	1309	2418	1515
% RSD	0.69	0.74	0.70	1.12	1.31	1.37	0.36	0.98	1.07	1.07

Bold entries were not used in calculations.

CONCLUSION

Transfer of the HPLC method from the 3.9 mm ID column to the 2 mm ID column was very successful. The only consideration necessary was appropriate reduction in sample concentration in order to accommodate the injection volume without exceeding the column capacity, and the use of the semi-micro flow cell. This method is immediately applicable to any of the analytical methods performed using the 3.9 mm ID μBondapak columns, assuming that instrument performance is comparable. Based on availability of other C_{18} packings in a 2 mm ID column, other methods should transfer as successfully (this probably represents at least 90% of the HPLC analyses performed in most HPLC laboratories). To provide some idea of the potential savings, this analytical and associate QC lab spends approximately $1000 per month on acetonitrile which is used almost exclusively for LC. This figure could easily be reduced to less than $200 per month, yielding an annual savings of over $9000. Corresponding savings in methanol and other HPLC solvents would also be realized. This does not consider the savings realized from reduced waste disposal.

The transfer to the 1 mm ID column was not as successful. Although the separation could have been restored with some method development, the primary objective was method transfer without additional development. The evaluation of the performance must be tempered with the very rigorous separation that this sample requires. Most methods do not demand this degree of resolution for this many peaks. It is also possible that the sample bandwidths required a flow cell smaller than the 8 ul volume used, particularly to improve resolution between peaks 2-7. When comparing peak shape of pentobarbital, mephobarbital, and

secobarbital (which are clearly resolved), the broader bandwidth is obvious and is definately not due to the UV cell volume.

It is probable that the difficulties encountered with the low injection volume (< 1 µl) were beyond the instruments capabilities. More accurate injections with low volumes would be achieved fixed volume internal sample loop. The injection devices are not common on typical analytical HPLC's.

If a narrow bore column is substituted for a standard bore column, at least some aspects of the method validation package will need to be addressed. It is conceivable that in a rigorously regulated environment, such as pharmaceutical manufacturing, complete re-validation of the method may be necessary.

Narrow bore column analyses are extremely sensitive to extra-column volumes (commonly referred to as "dead" volumes), particularly those in the injection system. These volumes contribute to sample dispersion that, coupled with the low flow rates, can ruin peak shape and resolution. Narrow bore columns require that the liquid chromatograph can deliver low flow rates, have small internal volumes and handle small injection volumes. To facilitate widespread method transfer, it is important that the associated hardware be capable of performing the analysis in an unmodified state since the expertise to execute in-house modifications may be lacking in some laboratories. Some LC's, particularly older ones, do not provide the necessary minimum performance characteristics needed to accommodate narrow bore columns. Although future savings incurred by reduced solvent costs would more than pay for instrument modification, in those cases where modification is not possible it is difficult to consider outright instrument replacement based on projected budgetary surpluses.

Received: June 23, 1994
Accepted: September 7, 1994