Rapid, high-resolution high-performance liquid chromatographic analysis of antibiotics

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ABSTRACT

A HPLC column devised for high separation speed combined with highly practical operating features has been found useful for separating antibiotics. Important characteristics involve compromises in packing particle size, column configuration and support–stationary phase combinations. We determined that these columns are useful for rapid, high-resolution separations with unmodified state-of-the-art HPLC equipment without the extra-column band-broadening effects typical of so-called "fast" HPLC columns. The proposed columns feature efficient sterically-protected monofunctional silane stationary phases that provide good separation reproducibility and high column stability. The combination of these unique bonded silanes and a highly purified, less-acidic silica support give superior peak shapes for antibiotic compounds. The proposed column configuration can halve separation times and double peak heights without loss in resolution, compared to widely used analytical columns. Increased mobile phase flow-rates permit even faster separations of antibiotics with only modest loss in resolution and peak heights for trace analyses in biological systems.

INTRODUCTION

HPLC is effective and convenient for analyzing antibiotic compounds in many types of samples. This method also is often preferred for determining trace quantities of antibiotics and their metabolites in a variety of animal tissues and fluids [1,2]. Typically, silica-based reversed-phase columns with bonded alkyl ligands are used for these applications. These columns generally are preferred because of their high plate numbers for superior separating efficiency, and their convenient availability from many reliable sources.

Current needs in HPLC method development for antibiotics and other drugs stress easily reproduced, rugged methods that are developed

rapidly. Often required are fast separations that permit the analysis of large numbers of samples at minimum cost. But these fast separations also must provide needed separation resolution, especially in trace analyses where potentially interfering sample components are a special problem. Further, the methods need to be conducted on existing instrumentation to minimize capital investment cost. Finally, decreased solvent consumption is becoming increasingly important, to minimize cost and waste disposal problems. This study evaluates a column configuration that has the potential to meet these goals.

It has been known for some time that the separation goals stated above could be attained by using columns with particles smaller than the widely used 5-μm materials [3–5]. Smaller particles permit higher column bed efficiency for better separations. Also, higher mobile phase

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flow-rates are allowed without sacrificing improved column efficiency. Although commercial columns of high-efficiency 3-μm silica particles have been available for many years, this particle size accounts for only about 10% of columns in use [6]. Users typically complain that columns of 3-μm particles have a tendency to "plug", greatly shortening their lifetime compared to columns of larger particles. Also, 3-μm columns often exhibit plate numbers that are lower than predicted. The stability of columns with 3-μm particles also is often faulted, as well as different selectivity, relative to columns of larger particles. Another complaint against columns of 3-μm particles is the unusually high back pressure. Finally, extra-column effects from some HPLC apparatus broadens the sharp, low-volume peaks from columns of 3-μm particles, decreasing their potential advantage [7].

We evaluated a small-particle column designed to minimize the disadvantages of conventional columns with 3-μm particles. Small-particle columns certainly are not new in HPLC. However, the unique feature of the proposed column is a combination of desirable and practical characteristics that have not previously been available. A general goal of this study was to increase the speed and detectability of analyzing antibiotics without sacrificing separation resolution. Of special interest was the effect of mobile phase flow-rate and temperature on column efficiency and separation speed. Also, a study of the effect of sample injection, guard columns/filters on the observed performance of these small-particle columns was required. Finally, comparison of the new column type with conventional columns was desired for analyzing trace antibiotics in biological matrices.

The experimental difficulties often associated with using small-particle ($\leqslant 3$ μm) columns apparently can be minimized by appropriate design compromises, as described in ref. 8. Briefly, a very narrow particle-size distribution of 3.5-μm porous silica microspheres without "fines" permits the use of 2-μm porosity column inlet frits. These replace the easily plugged 0.5-μm frits commonly used in columns of 3-μm particles. This combination provides a column that exhibits high efficiency without the usual

tendency to "plug". The use of 3.5-μm particles allows a short (7.5 or 8 cm) column to develop the plate number required for difficult separations, often halving separation time with the same resolution as conventional 15 cm columns of 5-μm particles. The proposed column also was prepared with a high-purity, low-acidity silica support and efficient monomeric sterically protecting stationary phases whose stability and reproducibility have been documented [9–12].

Of particular interest was the potential increase in peak height and solute detectability that might be available from the small-particle column, because of the small peak volumes and predicted sharper peaks that should occur. However, because of the low-volume peaks expected from these columns, a practical question arose: Can existing HPLC equipment be used without the undue extra-column band broadening and resultant loss of column efficiency that often is associated with conventional short, small-particle columns? To answer these questions about the proposed column configuration, studies were designed to determine the effect of important separation parameters on separation performance: flow-rate, temperature, sample injection procedures, and detector response time [13].

EXPERIMENTAL

Columns

Prototype 8.0 × 0.46 cm columns were prepared by slurry packing within Rockland Technologies (Newport, DE, USA). The high-purity (>99.995%) [14], low-acidity type B porous silica microspheres (Zorbax Rx-SIL) used for these columns had a pore diameter of 8 nm and a nitrogen surface area of 152 m^2/g. These particles were prepared by the coacervation of silica sols using methods previously described [15–17]. The number-average particle size of these particles was 3.65 μm as measured with a Coulter Multisizer (Coulter, Hialeah, FL, USA). These silica microspheres were densely bonded with sterically-protecting diisopropyl-n-octylsilane ligands [9,10]. Carbon value for this packing was 4.94%, resulting in a ligand surface coverage of 2.10 μmol/m^2 . Similar 7.5 × 0.46 cm and 15 ×

0.46 cm columns of both 3.5- and 5-μm particles are available from Mac-Mod Analytical (Chadds Ford, PA, USA) as Zorbax SB-C$_8$. Additional characteristics of columns with this and other highly stable sterically protecting silane stationary phases have been described recently [11,12]. The C-270 guard column cartridge holder, C-280 1.0×0.20 cm ODS cartridge, and A-318 precolumn filter were from Upchurch Scientific (Oak Harbor, WA, USA).

Apparatus

HPLC separations were performed either with a Hewlett-Packard Model 1050 instrument (Avondale, PA, USA), or with a modular instrument consisting of a Hewlett-Packard Model 1050 pump, an ABI Model 783 variable-wavelength detector (ABI, Foster City, CA, USA), a Model 7125 valve injector with 20-μl sample loop (Rheodyne, Cotati, CA, USA), and a DuPont column heater compartment (DuPont Instruments, Wilmington, DE, USA). The detector cell on the Hewlett-Packard Model 1050 was 1 μl, and 12 μl for the ABI detector. Mobile phase flow-rates were 1.0 ml/min, except as noted. Detector wavelength usually was set at maximum absorption for each antibiotic compound. Detector rise (response) time was 0.2 s, except as given. Sample sizes typically were 50–100 ng, except as noted. Chromatographic data were monitored and processed with a Multichrom Version 2.0 data system (VG Laboratory Systems, Manchester, UK) or a ChromPerfect Version 5.0 (Justice Innovations, Palo Alto, CA, USA) data station. Data collection with these units was 2 and 10 points/s, respectively.

Reagents and chemicals

HPLC-grade solvents were from J.T. Baker (Phillipsburg, NJ, USA). Trifluoroacetic acid and triethylamine were obtained from Pierce (Rockford, IL, USA). Antibiotic compounds were procured from Sigma (St. Louis, MO, USA). Ciprofloxacin and imipenem were from Glaxo Pharmaceuticals (Research Triangle Park, NC, USA) and Merck, Sharp and Dohme (West Point, PA, USA), respectively.

Extraction procedure and HPLC analysis of chloramphenicol from dog plasma and rat urine

Aliquots (0.2 ml) of control dog plasma and rat urine were spiked with 10 μl of a 10 μg/ml aqueous solution of chloramphenicol. Non-spiked samples were processed in parallel. The rat urine samples were made basic with 50 μl of a reagent-grade ammonium hydroxide–water (1:1) mixture. All samples were extracted with 1.0 ml of methyl tert.-butyl ether and centrifuged to separate the phases. The organic layer was taken to dryness under nitrogen and reconstituted in 100 μl of mobile phase for injection into the column using a 20-μl sample loop. Mobile phase for both separations was 0.1% trifluoroacetic acid adjusted to pH 3 with ammonium hydroxide and acetonitrile in the ratio of 75:25 (v/v). Mobile phase flow-rate was 1.0 ml/min and UV detection was at 278 nm.

RESULTS AND DISCUSSION

The prototype column type was tested with different classes of antibiotics and a range of mobile phase types, as summarized in Table I. Different concentrations of acetonitrile modifier were required to maintain the solute capacity factor at $k' \approx 3$. Plate numbers for these antibiotics varied from a low of about 3000 (imipenem) to more than 8000 (chloramphenicol) under the conditions used with this 8.0-cm column. Asymmetry factors, A_s, ranged from 1.02 to 1.27, indicating good peak shapes for these compounds.

A simple acetonitrile/0.1% trifluoroacetic acid (pH $\approx$ 1.8) mobile phase produced good results with the first five compounds listed in Table I. These compounds and the silanol groups remaining on the silica support are fully protonated under these conditions. Low-pH operation often produces the best results for basic compounds in terms of column plate number, peak shape, and recovery [19]. However, under these low-pH conditions, the highly water-soluble imipenem, which is zwitterionic at pH 4–9.5, is unstable. A pH 7 acetic acid–triethylamine mobile phase produced satisfactory retention for imipenen, although a very low concentration of acetonitrile was required. Under these conditions, imipenem

TABLE I

SUMMARY OF ANTIBIOTICS TESTED

Column, 8×0.46 cm Zorbax SB-C$_8$ with 3.5-μm particles. Samples, 50–100 ng in 20 μl; flow-rate, 1.0 ml/min; ambient temperature. k' = Capacity factor; N = column plate number; A_s = peak asymmetry factor; ACN = acetonitrile; TFA = trifluoroacetic acid.

Class	Antibiotic	Mobile phase	UV (nm)	k'	N	A_s
Penicillin	Nafcillin	ACN–0.1% aqueous TFA (44:56)	235	2.7	4425	1.02
Cephalosporin	Cefazolin	ACN–0.1% aqueous TFA (20:80)	272	2.9	6767	1.12
	Cefotaxime	ACN–0.1% aqueous TFA (18:82)	254	2.6	6320	1.22
	Ceftazidime	ACN–0.1% aqueous TFA (12:88)	254	2.4	5172	1.26
Quinolone	Ciprofloxacin	ACN–0.1% aqueous TFA (20:80)	277	3.1	7063	1.27
Carbapenem	Imipenem	ACN–0.1% aqueous acetic acid pH 7 with triethylamine (0.5:99.5)	296	3.5	3001	1.20
Sulfonamide	Sulfamethoxazole	ACN–0.1% aqueous TFA pH 3 with triethylamine (27:73)	267	2.8	7883	1.13
Chloramphenicol	Chloramphenicol	ACN–0.1% aqueous TFA pH 3 with triethylamine (25:75)	278	3.8	8431	1.06
Trimethorprim	Trimethorprim	ACN–0.1% aqueous TFA pH 3 with triethylamine (16:84)	220	3.0	7120	1.12

apparently is unionized or only partially protonated. (Note that this particular acetic acid mobile phase at pH 7 has poor buffering capacity, so sample size must be kept very small —a phosphate buffer probably would have been a better choice).

The last three compounds in Table I produced excellent results with a mobile phase prepared by titrating 0.1% trifluoroacetic acid to pH 3.0 with triethylamine. Plate numbers were especially favorable for these compounds with this mobile phase.

The plate height *vs.* mobile phase velocity plots for ciprofloxacin in Fig. 1 illustrate the effect of particle size on column performance. Theory predicts that smaller particles should produce higher column efficiency for well packed columns [4,5]. Although plate heights (column efficiencies) are significantly enhanced with the column of 3.5-μm particles, the improvement was somewhat less than predicted. The reduced plate height (in particle diameters) for the column of 5-μm particles was 2.1, compared to 2.5 for the column of 3.5-μm particles. The lower performance of the smaller particles could be due to a non-optimized procedure of packing the prototype columns. An equally likely reason

could be secondary band broadening that is known to occur especially in packed beds. This secondary band broadening caused by temperature gradations across the column bed with increased flow is especially a factor with columns of very small particles [20,21].

The data in Fig. 1 show another known characteristic of operating with columns of smaller particles: the plate height minimum is shifted to higher mobile phase velocities, as predicted by
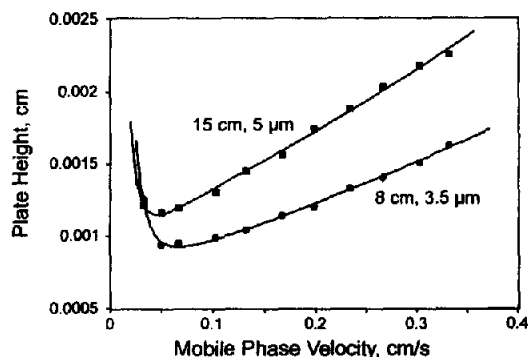

Fig. 1. Effect of column configuration on plate height. Columns: Zorbax SB-C$_8$, 0.46 cm I.D.; solute: ciprofloxacin; mobile phase: acetonitrile–water with 0.1% trifluoroacetic acid (80:20); 22°C; each data point represents the average of duplicate measurements.

theory [3–5]. A result of this effect is that columns of these smaller particles can be operated at higher mobile phase velocities (or flow-rates) without sacrificing column efficiency. Therefore, faster separations can be achieved at higher flow-rates without degrading separation resolution because of the superior kinetics associated with smaller particles. Fig. 2 gives an illustration of a rapid separation of an antibiotic mixture with the column of 3.5-μm particles by operating at 60°C and a flow-rate of 3.0 ml/min. Even at this high mobile phase velocity (≈ 0.3 cm/s), the plate number for this 8.0-cm column was still about 6000. The homogeneity of the packed bed and the rapid kinetics of the system is verified by the near-Gaussian peaks that were formed at this high flow-rate. Peak asymmetry factors approximated unity for all antibiotic peaks with $k' > 1$.

The chromatogram in Fig. 2 was obtained with standard state-of-the-art HPLC equipment (Hewlett-Packard Model 1050). No special modifications were required. The configuration of this column is such that extra-column effects apparently are minimal when this column is used with well designed, modern equipment. Significant extra-column effects would have caused tailing peaks rather than the near-Gaussian peaks actually observed. Note also that the pressure required for this high-flow-rate separa-
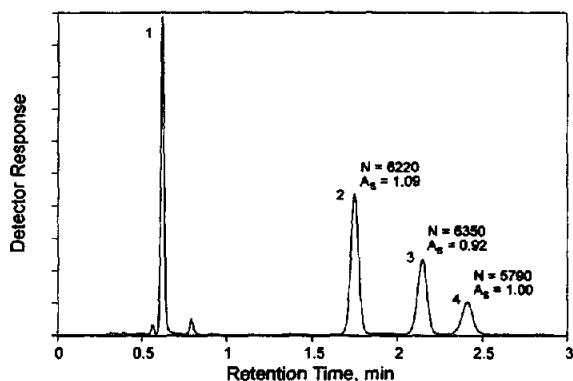
tion was well within the practical operating range for routine analyses (111 bar).

The effect of temperature on the plate height of the column of 3.5-μm particles is shown in Fig. 3. One might predict that operation at higher temperatures should improve solute diffusion, decrease mobile phase viscosity, and, therefore, decrease plate height with increase in column efficiency. The data for ciprofloxacin in Fig. 3 actually shows a slight increase in plate height (decreased column efficiency) as the column temperature was increased from 20 to 60°C. This effect suggests that the secondary band broadening effects discussed above [20,21] negate the expected improvement in solute diffusion. These and other results not given here indicate that higher temperature operation with very-small-particle columns may not be desirable for separating small molecules, unless special operating procedures are adopted [21]. On the other hand, high-temperature separations of macromolecules with such columns often are both advantageous and practical [11]. Apparently, the significant improvement in diffusion with macromolecules more than overcomes any secondary band broadening effects that occur.

A study was made on the effect of sample injection volume on the efficiency of the new column type. Because of the low-volume, sharp peaks generated, it was important to determine the sample volume limit. This information is particularly useful for trace analysis applications where large sample volumes often are required
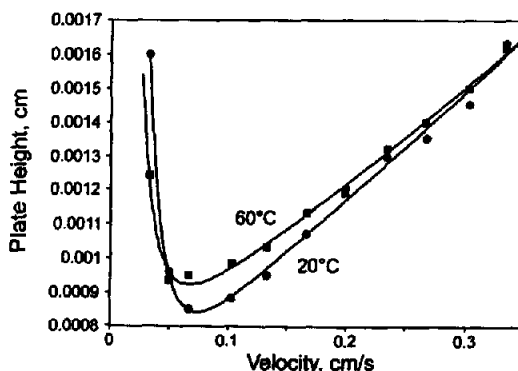

Fig. 2. High-speed separation of antibiotics mixture. Column: 8.0 × 0.46 cm Zorbax SB-C$_8$ (3.5 μm); mobile phase: acetonitrile–0.1% aqueous trifluoroacetic acid (8:92); flow-rate: 3.0 ml/min; sample: 1 μl containing 0.40, 0.36, 0.10 and 0.35 μg each of ceftazidime (1), cefotaxime (2), ciprofloxacin (3) and cefazolin (4); 60°C; UV detector: 260 nm.

Fig. 3. Effect of column temperature on plate height. Conditions as in Fig. 2, except: 0.10 μg ciprofloxacin; mobile phase velocity and temperatures as shown.

to obtain adequate measurement sensitivity. Table II shows the results of various sample volumes of ciprofloxacin solution injected into the 3.5-μm column with different loop sizes (volumes). Plate numbers and plate heights were essentially constant, within experimental error, with volumes up to 20 μl with the solute at $k' = 2.1$. Partially filling the 10- and 20-μl loops maintained observed column efficiency. Increases in peak heights were commensurate with sample volumes injected with completely or partially filled loops up to 20 μl. However, increasing the sample volume to 50 and 100 μl

increasingly degraded observed column efficiency. Also, expected increases in peak heights were not obtained with the larger sample sizes, because of band broadening.

The Table II data indicate that, for components with $k' \approx 2$, highest column efficiency and peak heights are obtained with sample volumes of no more 20 μl. Note, however, that sample volumes can be increased for solutes with larger k' values, since peak volumes increase with k' increase. As peak volume increases, the potential for band broadening is reduced. Other studies not reported here used 100-μl injections

TABLE II

EFFECT OF SAMPLE VOLUME ON COLUMN EFFICIENCY

Column, 8 × 0.46 cm Zorbax SB-C$_8$ (3.5 μm); sample, 100 ng ciprofloxacin $k' = 2.1$; mobile phase, acetonitrile–0.1% aqueous trifluoroacetic acid (20:80); 22°C. HETP = Height equivalent to a theoretical plate. Peak height in Multichrom counts.

Sample loop volume (μl)	Peak height	Injection volume (μl)	Plate number	HETP × 10^3 (cm)
5	4803	5	8574	0.933
	4929		8555	0.935
10	9313	10	8719	0.918
	9303		8613	0.929
	5161	5	8810	0.908
	5135		8455	0.946
20	17 739	20	8228	0.972
	18 156		8431	0.949
	10 188	10	8441	0.948
	10 137		8450	0.947
	5103	5	8560	0.935
	5122		8603	0.930
50	46 434	50	7589	1.05
	47 037		7593	1.05
	21 063	20	8237	0.971
	21 029		8265	0.968
	10 652	10	8361	0.957
	10 677		8384	0.954
100	83 756	100	6501	1.23
	86 931		6578	1.22
	50 818	50	7661	1.04
	50 705		7580	1.06
	21 238	20	8333	0.960
	21 179		8219	0.973
	10 763	10	8589	0.931
	10 801		8370	0.956

with this column for a solute of $k' \approx 4$ with no noticeable change in column efficiency.

Decreasing particle size and column length has an important effect on increasing the peak height of solutes [22,23]. This increased peak height increases the detectability of components of interest, particularly in trace analyses. Fig. 4 shows relative peak heights obtained for ciprofloxacin at varying flow-rates on a 15×0.46 column of 5-μm particles. These values are compared to those for a 8×0.46 cm column of 3.5-μm particles, both with the same stationary phase. Peak heights for the column of 3.5-μm particles were about twice that for the 5-μm particle column, resulting in a significant potential increase in solute detectability. Also, the peak height optimum for the column of 3.5-μm particles is about 0.6 ml/min, compared to about 0.4 ml/min for the 5-μm particle column. With the column of 3.5-μm particles, flow-rates apparently can be increased for faster analyses, without a large sacrifice in peak heights or detectability.

In the routine application of HPLC columns for "real" samples, guard columns and column filters are often recommended to protect the analytical column from strongly retained endogenous contaminants and particulates [24]. A question arose as to the contribution of the mixing volumes associated with these devices to the efficiency of the 3.5-μm column. Therefore,

a study was undertaken to determine possible extra-column band-broadening effects of appropriate guard columns and in-line column filters. Table III shows the results of this study using chloramphenicol as the test solute at a $k' \approx 4$. These results indicate that there is no detrimental influence of this column filter on peak height, plate number or column pressure. The guard column also had no significant effect on observed column performance, with only a slight increase in pressure. Combining the column filter and the guard column still produced no problems, with essentially the same column performance. The data suggest that appropriate column filters and guard columns often can be used without sacrificing column performance. Of course, observed column efficiency could increasingly degrade for components eluting with sharper peaks at lower k' values.

The narrow, sharp peaks generated by the short column of 3.5-μm particles requires that the response time of the detector is sufficiently fast to follow the true shape of the peak. Accordingly, we studied the effect of the Model 783 variable-wavelength detector response (rise) time on peak height and plate number measurements for chloramphenicol. This detector was equipped with a "standard" 12-μl flow cell. Measurements were made with three different concentrations of acetonitrile mobile phase modifier, to generate peaks with varying k' values and resulting differ-
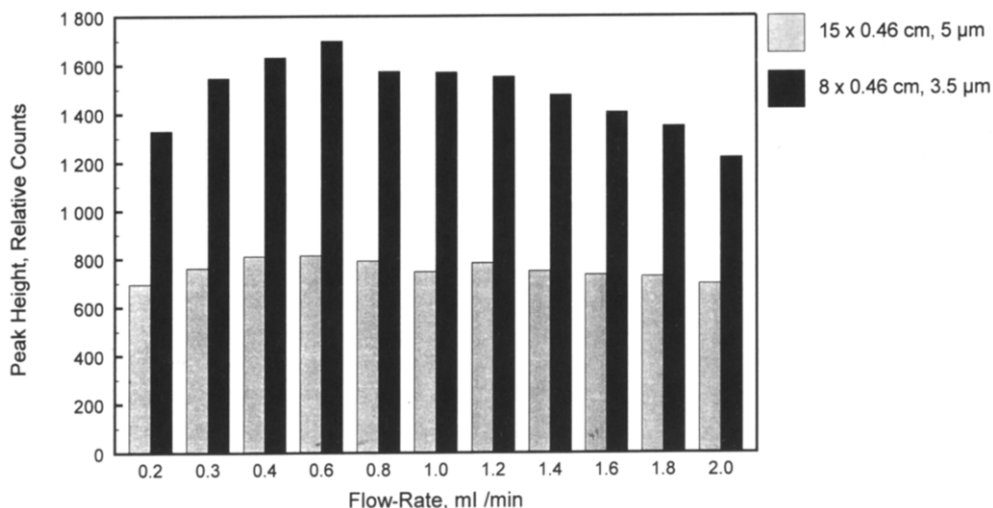

Fig. 4. Effect of column configuration on peak height. Conditions as in Fig. 1 except: 0.10 μg ciprofloxacin; $k' = 2.1$.

TABLE III

EFFECT OF COLUMN FILTER AND GUARD COLUMN ON EFFICIENCY AND BACK PRESSURE

Column, 8×0.46 cm Zorbax SB-C_8 (3.5 μm); 20 μl sample, 100 ng chloramphenicol; mobile phase, acetonitrile–0.1% aqueous trifluoroacetic acid pH 3 with triethylamine (75:25); flow-rate 1.0 ml/min; 22°C. Peak height in Multichrom counts.

Status	Peak height	k'	Pressure (bar)	Plate number
No guard	18 865	3.99	81	8251
With filter	18 892	4.01	82	8185
With guard	18 412	4.09	92	8083
With filter and guard	17 947	4.18	96	8013

TABLE IV

EFFECT OF DETECTOR RESPONSE RISE TIME ON PEAK-HEIGHT MEASUREMENTS

Column, 8.0×0.46 cm Zorbax SB-C_8 (3.5 μm); sample, 100 ng chloramphenicol in 20 μl. Mobile phase: acetonitrile–0.1% aqueous trifluoroacetic acid, pH 3 with triethylamine: I = 45:35, II = 35:65, III = 25:75; flow-rate, 1.0 ml/min; ambient temperature. Peak height in Multichrom counts.

Detector rise time (s)	I		II		III	
	Peak height, $k' = 0.6$	Plate number	Peak height, $k' = 1.4$	Plate number	Peak height, $k' = 3.9$	Plate number
0.02	38 036		31 595		18 390	
	37 451	3964	31 392	5733	18 559	8280
0.05	37 968		31 974		18 451	
	37 768	3958	32 063	5845	18 299	8336
0.10	38 383		31 563		18 639	
	37 711	3694	31 803	5935	18 077	8289
0.20	37 632		31 537		18 390	
	38 035	3707	31 112	5666	18 279	8379
0.50	37 453		32 133		18 181	
	37 269	3593	30 816	5767	17 870	8351
1.00	35 940		29 561		18 234	
	36 125	3433	30 096	5581	18 272	8240
2.00	31 551		27 785		17 881	
	31 545	2614	27 846	4643	17 635	7906
5.00	20 833					
	20 837	1136				

ent peak widths. Table IV summarizes the data obtained in this study. For the narrowest peak at $k' = 0.6$, rise times of > 0.2 s increasingly degraded plate numbers and peak heights. The peak at $k' = 1.4$ showed no degradation as long as the rise time did not exceed 0.5 s. For the widest and most retained peak at $k' = 3.9$, rise times up to 1.0 s could be used without degrading column performance. These results confirmed that the prototype column can be used without band-broadening difficulties with state-of-the-art detection systems having modest response characteristics.

The studies described so far suggested that the new column configuration should be useful for measuring trace antibiotics in matrices of interest in drug metabolism investigations. Accordingly, trace separations were conducted with chloramphenicol in two matrices using a 8.0×0.46 cm column of 3.5-μm particles and a 15×0.46 cm of 5-μm particles with the same type silica support and the same stationary phase. Recovery of chloramphenicol under the described experimental conditions was 85% for dog plasma and 70% for rat urine, relative to a calibration curve determined with standard solutions. Fig. 5 gives results obtained with extracts of dog plasma. Fig. 5A shows chromatograms with the longer column of 5-μm particles obtained with control extract and an extract containing chloramphenicol. Fig. 5B shows results on the same samples obtained of the shorter column of 3.5-μm particles. As anticipated, using the same flow-rate, separation time was nearly halved. Component k' values were essentially identical for the two columns with the same silica–stationary phase combination. But the height of the cleanly separated chloramphenicol peak for the column of 3.5-μm particles was about twice that of the longer column of 5-μm particles. This enhanced peak height effectively doubles the detectability of the trace component.

Results with a more complex sample are shown in Fig. 6 with extracts of rat urine. Fig. 6A gives chromatograms with the 5-μm column obtained with a control extract and an extract containing chloramphenicol. Fig. 5B shows results obtained on the same extracts with the 3.5-μm column. Again, k' values are compar-
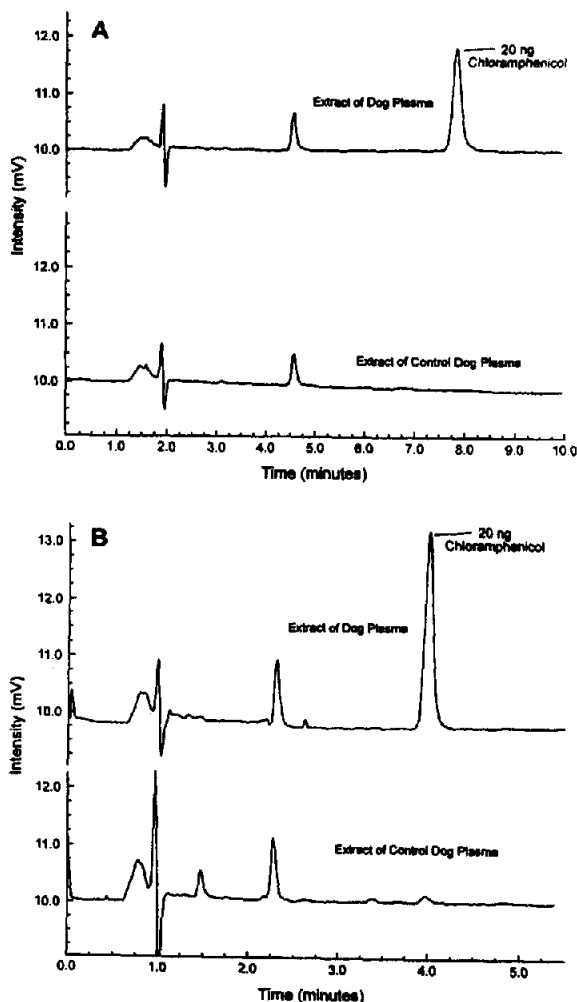

Fig. 5. Effect of column configuration on separation of chloramphenicol in extract of dog plasma. Sample: 20 μl of extract; mobile phase: acetonitrile–0.1% aqueous trifluoroacetic acid (25:75), pH 3 with NH_4OH; 22°C; UV detector: 278 nm; flow-rate: 1.0 ml/min. (A) Column: 15×0.46 cm Zorbax SB-C_8 (5 μm); upper, extract with 100 ng of chloramphenicol; lower, control extract. (B) Column: 8×0.46 cm Zorbax SB-C_8 (3.5 μm); upper, extract with 100 ng of chloramphenicol; lower, control extract.

able while separation time was about halved with the shorter column; peak height for chloramphenicol was about doubled. As before, a faster separation was performed with the potential for higher sensitivity. This new column type has been used in our laboratories for hundreds of sample injections without significant change in separation and efficiency characteristics. Based
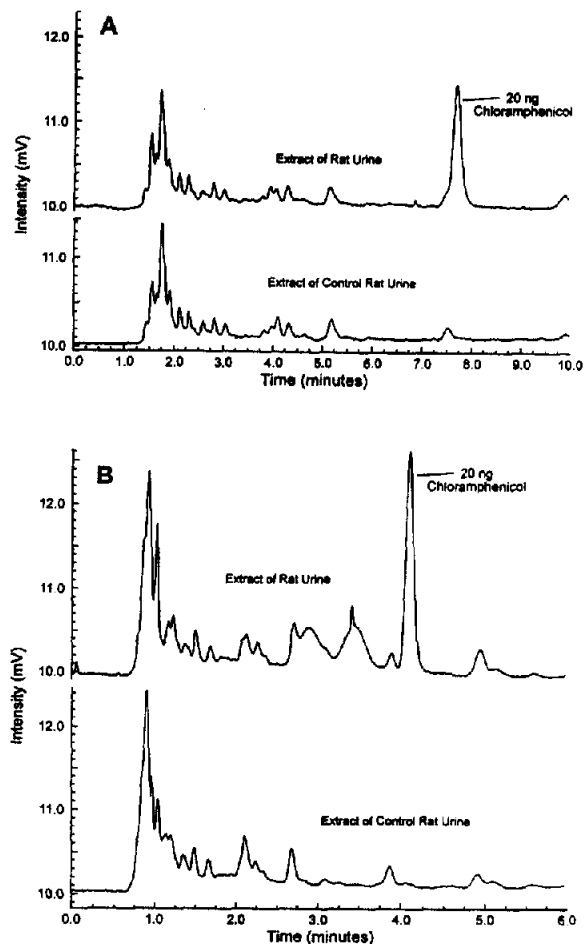
Fig. 6. Effect of column configuration on separation of chloramphenicol in extract of rat urine. Conditions as in Fig. 5.

on experience to date, the long-term stability of this column type appears comparable to equivalent columns of 5-μm particles in routine applications.

CONCLUSIONS

A new 8.0 × 0.46 cm column type with 3.5-μm particles and monomeric sterically protected silane stationary phase has been found effective for the rapid analysis of antibiotic drugs. Compared to conventional 15 × 0.46 cm columns of 5-μm particles, separations were performed in one-half the time, with no significant loss in separation resolution. The faster separations

means that solvent consumption per analysis is significantly reduced. Peaks heights also were increased about two-fold for higher component detectability. Higher operating temperatures lowers mobile phase viscosity so that higher flow-rates can be used at modest back pressures for still faster separations. The new column configuration was utilized with state-of the-art HPLC equipment without special modifications. No significant extra-column band broadening was observed for component peaks with $k' \geq 1$. Appropriate guard columns and inlet filters also were used with the new column configuration without extra-column band-broadening problems. Sample volumes of at least 20 μl were permitted.

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