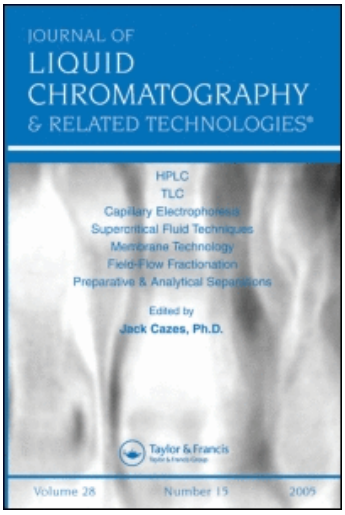

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G. R. Padmanabhan[a]; N. Mellish[a]; I. Becue[a]; J. B. Smith[a]
[a] Drug Development Department, Pharmaceuticals Division, New York

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APPLICATIONS OF FAST HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN PHARMACEUTICAL ANALYSIS

G. R. Padmanabhan, N. Mellish, I. Becue, and J. B. Smith

Drug Development Department
Pharmaceuticals Division
CIBA-GEIGY Corporation
Suffern, New York 10901

ABSTRACT

Fast HPLC offers a definite time advantage for analyses such as content uniformity which requires, in general, a routine analysis of as many as 30 samples and for the analysis of dosage forms of developmental drugs. The columns for Fast HPLC can be used with a minimal amount of modification to conventional HPLC hardware. The resolution obtainable with a Fast HPLC column is poorer than that of a conventional column. (All references to conventional columns in the article imply, unless otherwise indicated, those columns 15-30 cm in length and 3.9-4.6 mm i.d.) Attempts to modify a mobile phase to improve the resolution of a closely eluting peak resulted in retention times not too different from typical conventional columns. Although microbore columns offered improved resolution and a savings in both solvent and sample consumption, the utility of these columns is limited by the need for specialized hardware. Since the sample volume is not a limiting factor in a typical pharmaceutical analysis and overall cost savings from lesser solvent consumption are not significant in a majority of

cases as solvent can be recycled, it is concluded that the Fast HPLC columns can play a more important role than the currently available microbore columns in laboratories engaged in pharmaceutical analysis, particularly at the product development stage.

Fast high pressure liquid chromatography (1, 2) (Fast HPLC) is a technique in high pressure liquid chromatographic (HPLC) analysis in which the components of analytical interest are eluted in the order of 30 to 100 seconds, which is about 5 to 10 times faster than conventional HPLC. [This technique should not be confused with Technicon "Fast-LC"®, which is a sample pretreatment system (3).] Fast HPLC and the columns employed with it have been referred to in the literature by terminologies such as "Fast Analysis by HPLC" (4), "Fast LC Separations" (5), "High Speed Chromatography" (6), "High Speed, Low Dispersion LC" (7), "Very High Speed Liquid Chromatography" (8, 9, 10), "High Speed LC Using Short Columns" (7), "Short High Efficiency HPLC Column" (11), "Very Short LC Column" (12), and "Little Champ®" (13). These columns are typically short and packed with smaller particles with the following column parameters: length - 3 to 5 cm; diameter - 4.6 mm and particle size - 3 or 5 microns. In the past, Fast HPLC was not preferred mainly for the following reasons (14): the gain in chromatographic run time was not significant in terms of total analysis time, which includes sample clean-up and post HPLC data processing times; columns with 3 micron packings were not available commercially and the 5 micron or larger column

packings available at that time had, in general, poor resolution characteristics; and the hardware available were not suitable for Fast LC.

With improvements in data processing technology and with the availability of devices such as switching valves, the real average elapsed times in sample clean-up and data reduction have been reduced significantly, particularly for the analysis of a large number of samples. The availability of improved hardware, minor modifications of conventional HPLC hardware and use of conventional hardware with a small sacrifice in the chromatographic performance are options available at present to carry out Fast HPLC. In addition to providing high sample throughput, Fast HPLC operations consume less solvent, and cost less. Fast HPLC columns exhibit optimal plate height per unit length and per unit time, although due to the high pressure drop they have a low plate height per unit pressure (15). We have come across several instances in which the total elapsed chromatographic run time for the analysis of a larger number of samples for several developmental drugs has been as long as 24 - 36 hours using conventional HPLC columns. With the use of Fast HPLC, we can reduce this time by a factor of 2 to 5, depending upon the sample, mobile phase composition and desired degree of resolution. In this research article we will show examples of the utility of Fast HPLC, using slightly modified conventional hardware, in: content uniformity analysis of a

new drug capsule formulation; analysis of active drugs for estimation of impurities; analysis of a new drug in corn-starch formulation employed in toxicological studies; and identity of a large number of clinical packaged dosage forms.

Since there are some similarities in the advantages of Fast HPLC and microbore HPLC (1 or 2 mm diameter, 10 - 25 cm length, 3, 5, or 10 micron particle size and flow rate 10 - 100 μL/min.), data obtained with Fast HPLC were compared with those obtained with microbore columns, again using conventional hardware with minimal modifications and these results are also included in the manuscript.

EXPERIMENTAL SECTION

Chemicals and Reagents - All chemicals and reagents were ACS reagent grade, GC grade or HPLC grade and used without further purification. The purity of the drugs employed were established by several techniques such as HPLC, TLC, GC, etc.

Apparatus - The chromatograph was a modular unit equipped with a variable or fixed wavelength detector (Model 450 and Model 440, Waters Associates, Milford, MA), electronic integrator (Model 730, Waters Associates), system controller (Model 720, Waters Associates), automatic sample injector (Model 710B, Waters Associates), and pump (Model 6000A, Waters Associates). The columns were all octadecylsilane bonded (μBondapak®, 10 μm particles, 30 cm x 3.9 mm, Waters Associates; Little Champ®, 3 μm particles, 5 cm x 4.6 mm, Regis Chemical Company, Morton

Grove, IL; 3 x 3®, 3 μm particles, 3.2 cm x 4.6 mm, Perkin-Elmer, Norwalk, CT; Partisil®10 ODS-3, 10 μm particles, 25 cm x 1 mm, Whatman, Inc., Clifton, NJ).

RESULTS AND DISCUSSION

Applications in Pharmaceutical Analysis

Content Uniformity Analysis - Ten capsules of an experimental drug (A) were individually shaken with methanol, filtered and 10 μL of the solution was injected using a 3 cm Fast HPLC short column and a 30 cm conventional column, the results of which are tabulated in Table 1. (Typical chromatograms for both are shown in Figure 1.)

TABLE 1

Content Uniformity Data for Drug A, 10 mg Capsules Using Conventional and Fast HPLC Columns

	Assay, Percent Label	
	Conventional Column	Fast Column
Caspule Number	C_{18} 30 cm x 3.9 mm	C_{18} 3.2 cm x 4.6 mm
1	101.0	103.4
2	103.5	102.0
3	102.0	102.0
4	103.0	102.8
5	102.5	103.1
6	101.5	101.4
7	100.4	101.3
8	102.2	102.4
9	101.4	101.8
10	102.2	101.8
Average:	102.0%	102.2%
High:	103.5%	103.4%
Low:	100.4%	101.3%
Relative Standard Deviation:	± 0.9%	± 0.7%
Typical R.T. (minutes)	6	0.6
Mobile Phase Consumption, mL/run:	10	4

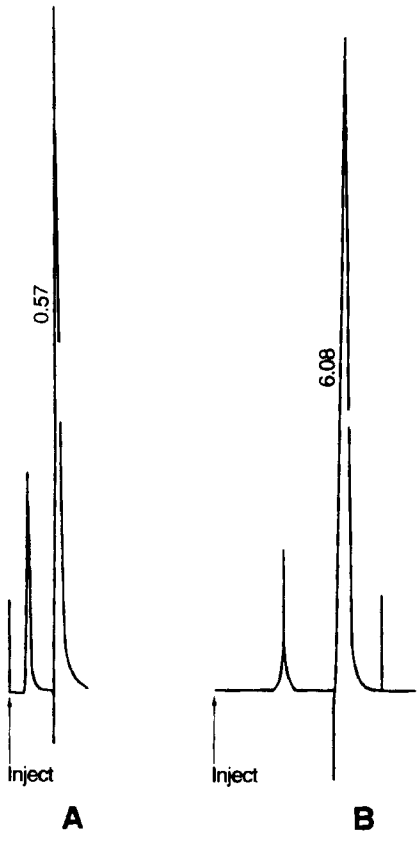

Figure 1 -- Typical chromatograms from the analysis of Drug A in a capsule formulation on: A. Fast HPLC Column: 150:147:3:1 methanol/0.01M pentanesulfonic acid sodium salt (PSASS)/acetic acid/triethylamine as mobile phase; 2.0 mL/min. flow rate; 16 µL flow cell with 280 nm UV detection; 0.5 AUFS sensitivity; 3.2 cm x 4.6 mm 3 µm ODS column; 10 µg of drug in 10 µL of injected sample and B; Conventional Column: 49:50:1 methanol/0.01M PSASS/acetic acid as mobile phase; 1.0 mL/min. flow rate; 16 µL flow cell with 280 nm UV detection; 0.2 AUFS sensitivity; 30 cm x 3.9 mm 10 µm ODS column; 10 µg of drug in 10 µL of injected sample.

The average assay, percent relative standard deviation, high and low values were 102.2% label, ±0.7%, 103.4% and 101.3%, respectively, for the Fast LC column. The corresponding values for the conventional column were 102.0% label, ±0.9%, 103.5% and 100.4%. The retention times of the basic form of the drug were 0.57 minutes and 6.08 minutes, respectively, for the Fast and conventional columns thereby showing a 10-fold decrease in elution time for the Fast HPLC column. The mobile phase in the Fast HPLC method was slightly modified by the addition of triethylamine in order to compensate for the differences between the conventional column and the Fast HPLC short column.

Ten capsules each of another experimental drug (B), 10 mg and 20 mg, were analyzed for content uniformity by conventional HPLC. Data from 20 mg strength were compared with a Fast LC column. The 10 mg capsules were compared with data from a microbore HPLC column. The results are shown in Table 2 (Figure 2). Again, the results from 20 mg capsules indicate that Fast HPLC columns gave results comparable to conventional columns. However, the results from the microbore column were poorer in terms of average assay and standard deviation values. Although one would expect the microbore columns to give results poorer than the conventional column, the microbore column results could have been improved had we used better hardware, such as pulse dampened pumps, a better injection system, low volume detection cell and an internal standard. Hence, the

TABLE 2

Content Uniformity Data for Drug B in 2 Capsule Formulations
% Assays of Drug: Microbore vs. Conventional and Fast vs. Conventional Columns

	10 mg Capsules		20 mg Capsules	
Sample Number	Microbore Column 25 cm x 1 mm	Conventional Column 30 cm x 3.9 mm	Fast Column 3.2 cm x 4.6 mm	Conventional Column 30 cm x 3.9 mm
1	92.6	99.2	90.5	96.1
2	99.0	102.7	104.3	103.8
3	101.7	102.9	96.7	96.3
4	99.2	100.0	98.6	98.6
5	100.7	103.1	86.7	87.0
6	100.3	99.2	101.6	101.2
7	101.9	98.4	99.6	99.4
8	93.3	98.8	95.6	95.5
9	88.2	99.2	98.6	99.5
10	99.4	99.8	102.2	103.5
Average	97.6	100.3	98.0	98.1
Relative Standard Deviation:	4.7%	1.8%	5.0%	5.0%
Typical R.T., Minutes:	7.3	7.2	1.4	7.2
Mobile Phase Consumption mL/run:	0.6	18	4.5	18

reported values reflect only the kind of values one can expect for a microbore column using conventional hardware with minimal modification. These results are by no means a reflection of the microbore technique itself.

Another interesting aspect of Fast HPLC is revealed in Table 2. The consumption of mobile phase per run by the Fast HPLC method is reduced by a factor of 4 when compared to conventional HPLC. The corresponding factor for microbore is about 30.

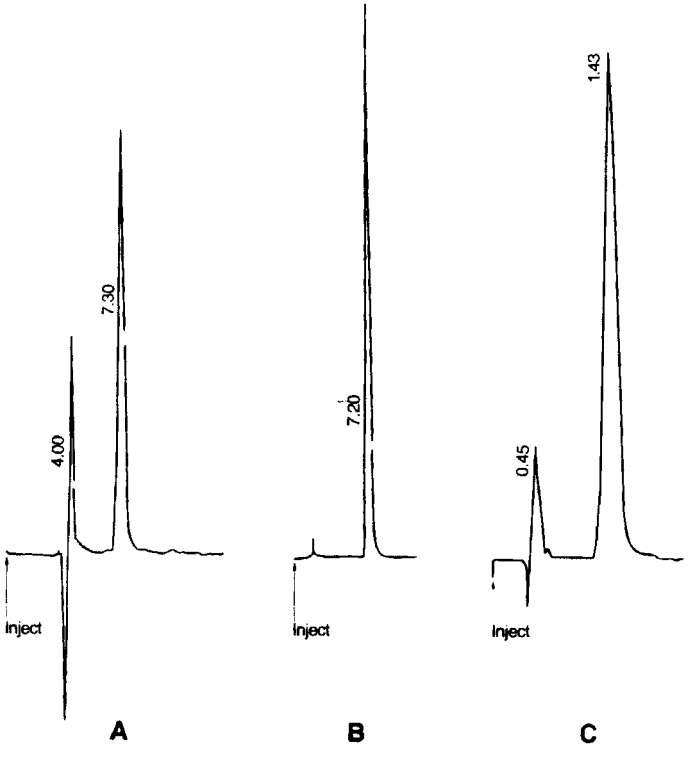

Figure 2 -- Typical chromotograms from the analysis of Drug B in a capsule formulation on: A. Microbore column: 75:20:5 methanol/water/tetrahydrofuran as mobile phase; 60 µL/min. flow rate; 1 µg of drug in 1 µL of injected sample; 1.95 µL flow cell with 280 nm UV detection; 1.0 AUFS sensitivity; 25 cm x 1 mm 10 µm ODS column. B. Conventional column: 60:35:5 methanol/water/tetrahydrofuran as mobile phase; 1.8 mL/min. flow rate; column, flow cell and detection same as in Figure 1B; 0.5 AUFS sensitivity; 2 µg of drug in 10 µL of injected sample and C. Fast HPLC column; 60:35:5 methanol/water/ tetrahydrofuran as mobile phase; 1.5 mL/min. flow rate; column, flow cell and detection same as in Figure 1A; 1.0 AUFS sensitivity 2 µg of drug in 10 µL of injected sample.

Uniformity of a Suspension Formulation of a Drug Employed in Toxilogical Studies - Results obtained from the analysis of experimental drug (C) in a 3% cornstarch formulation are shown in Table 3 (typical chromatograms for an individual sample are shown in Figure 3).

Again, the results indicate that the data from a Fast HPLC column are comparable to that of a conventional column. With this drug and using the indicated mobile phase, an eight-fold reduction in retention time was observed. In order to illustrate typical total analysis time for a large number of samples, ten individual samples of suspension of drug C were extracted with methanol, filtered and injected into a HPLC system with a Fast HPLC column. The results in Figure 4 show

TABLE 3

Percent Recovery of Drug C in a Suspension Formulation: Fast Column vs. Conventional Column

Sample Number	% Recovery Fast Column 3.2 cm x 4.6 mm	% Recovery Conventional Column 30 cm x 3.9 mm
1	98.4	98.6
2	100.1	100.2
3	98.6	99.8
4	99.3	99.0
5	100.4	101.7
Average Recovery:	99.4	99.9
Relative Standard Deviation:	0.9%	1.2%
Retention Time, Minutes:	1.2	10
Mobile Phase Consumption, mL/run	5	20

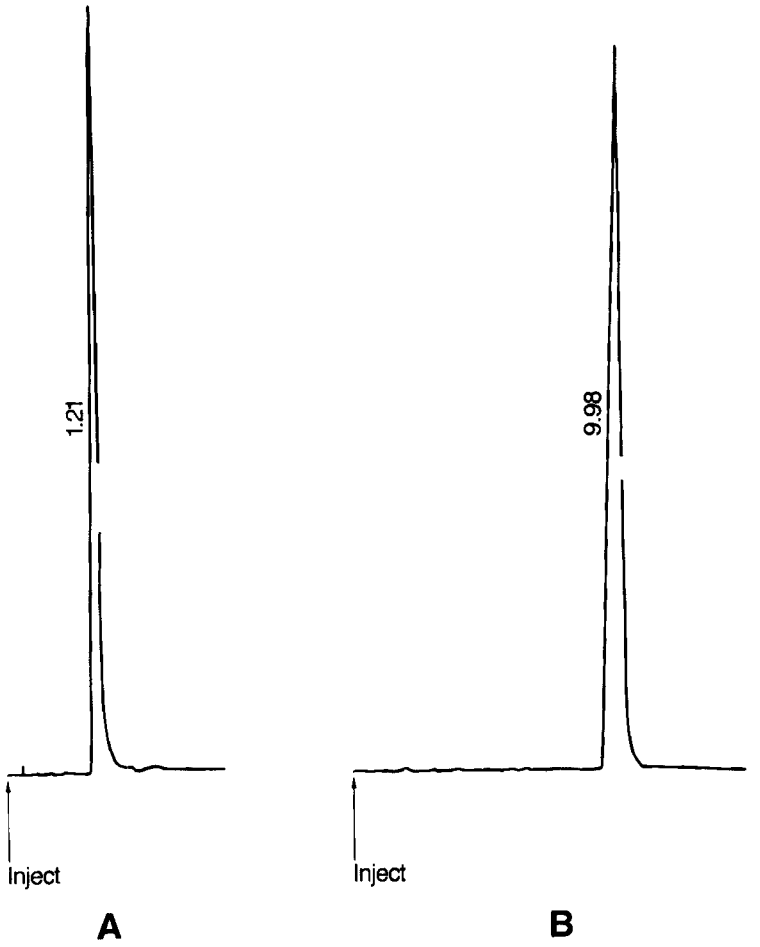

Figure 3 -- Typical chromatograms from the analysis of Drug C in a suspension formulation on: A. Fast HPLC column: 140:150:2:1 methanol/0.01M PSASS/acetic acid/triethylamine as mobile phase; 2.0 mL/min. flow rate; flow cell and detection same as in Figure 1A; 2.0 AUFS sensitivity; 10 µg of drug in 10 µL of injected sample and B. Conventional column: 140:150:2 methanol/0.01M PSASS/acetic acid mobile phase; 1.5 mL/min. flow rate; column, flow cell and detection same as in Figure 1B; 1.0 AUFS sensitivity; 10 µg of drug in 10 µL of injected sample.

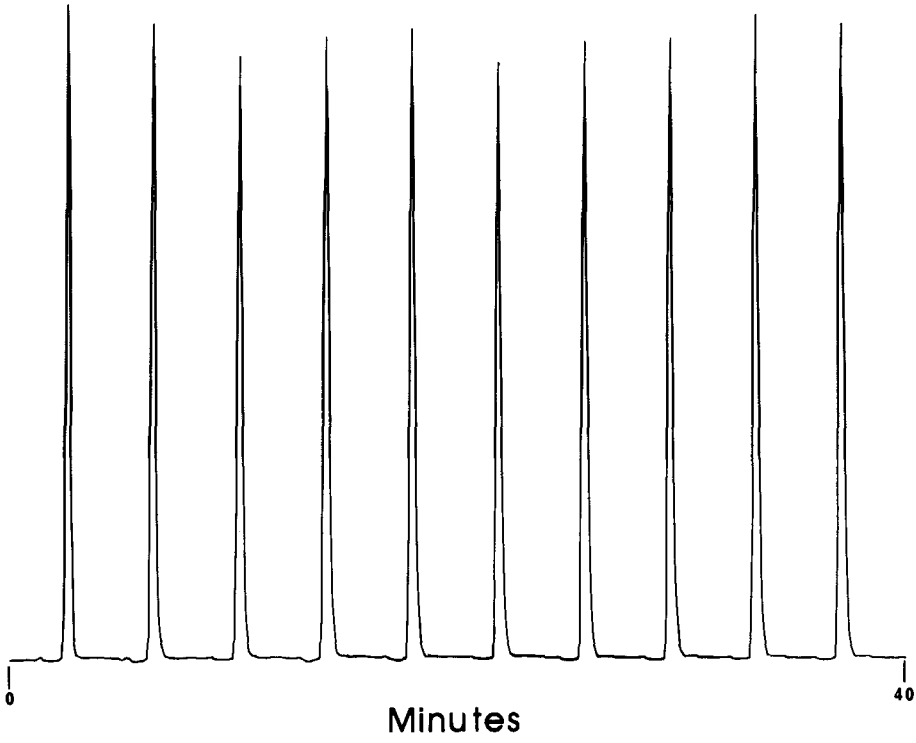

Figure 4 -- Typical chromatogram from the content uniformity of 10 replicate samples of Drug C in a suspension formulation on Fast HPLC column; all conditions same as in Figure 3A.

that the total analysis time is less than 40 minutes for the ten samples.

Analysis of Synthetic Mixtures of Three Drugs - In order to evaluate the application of Fast HPLC columns for the estimation of impurities in drug substances, synthetic mixtures of three structurally related developmental drug active ingredi-

TABLE 4

Percent Recoveries of "Impurities" Added to Three Drugs: Conventional Column vs. Fast Column

Sample Identification	Approximate Ratio in Mixture			Percent Recovery Conventional Column 30 cm x 3.9 mm			Percent Recovery Fast Column 3.2 cm x 4.6 mm		
	Drug X	Drug Y	Drug Z	Drug X	Drug Y	Drug Z	Drug X	Drug Y	Drug Z
A	100	1	1	100.0	99.2	107.5	101.5	103.0	113.6
B	100	5	5	98.7	99.0	99.6	101.1	99.9	102.5
C	1	100	1	95.9	100.5	104.3	106.0	100.3	111.6
D	5	100	5	99.5	100.2	99.0	106.1	101.3	105.5
E	1	1	100	103.5	101.6	101.0	104.5	98.1	102.3
F	5	5	100	99.9	99.0	100.2	107.0	103.3	103.6
G	100	100	100	101.9	101.4	100.8	100.5	99.7	98.9

ents were prepared (no significant impurities in each active ingredient) as indicated in Table 4. The recovery data obtained using both a conventional HPLC column and a Perkin-Elmer Fast HPLC column are also shown in the table. The results indicate that the recovery values for low level impurities in the Fast HPLC analysis are higher than theory when a conventional detector and data system are employed. However, we expect the recovery results to be improved with a fast response detector and a correspondingly improved data system.

Identification of Individual Active Ingredients in Large Numbers of Clinical Samples - Provided the clean-up procedure is simple and one is able to perform it quickly, Fast HPLC has a potential application in the identification of the individual active ingredients in clinical samples. Often, clinical samples require the identification of several different drugs from placebo. The contents of a large number of such clinical capsule samples were individually shaken with methanol and the extract filtered and injected into a chromatograph. The identity of three different active ingredients and placebo samples (Figure 5) could be established at a rate of no more than 2.5 minutes of chromatographic run time per sample using Fast HPLC.

Factors Affecting Fast HPLC Separations

Resolution - Resolution data for an impurity that can be present in a drug substance using a conventional column and a

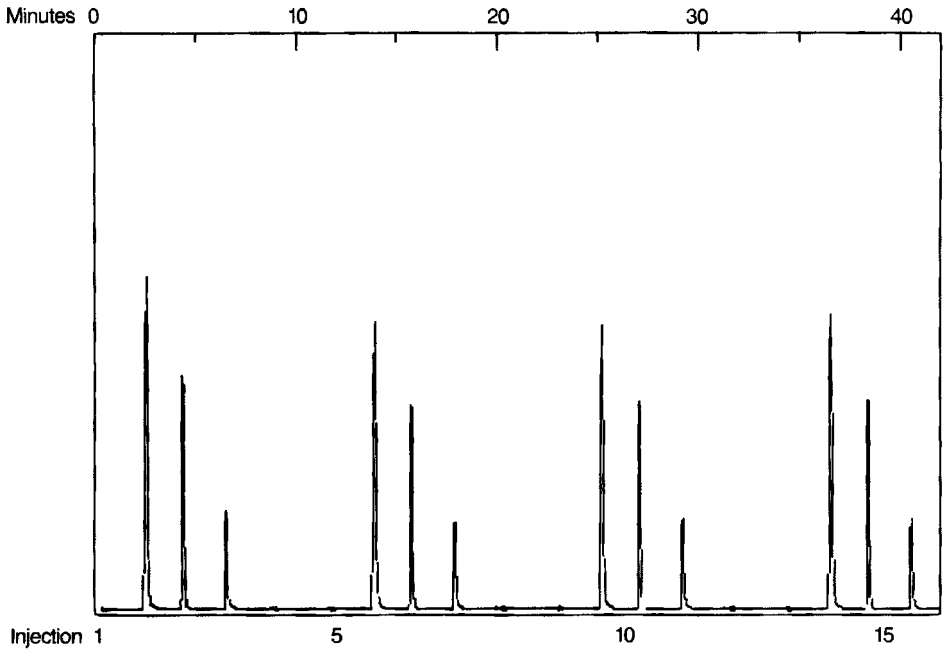

Figure 5 -- Typical chromatogram from the analysis of 12 clinical capsule samples for the identification of placebo and three active ingredients using Fast HPLC; 65:35 methanol/water as mobile phase; 1.5 mL/min. flow rate; column and flow cell same in in Figure 1A; 254 nm UV detection; 0.75 AUFS sensitivity; Injections 1, 2, and 3 are respectively standards for Drugs M, N and O; 4, 8 and 12 are placebo; 5, 9 and 13 are Drug M; 6, 10 and 14 are Drug N and 7, 11 and 15 are Drug O.

Fast HPLC column are shown in Table 5. The results indicate that for a pair of closely eluting peaks, improvement in the resolution by modifications in mobile phase is attained at the expense of total chromatographic run time for both conventional and Fast HPLC columns.

Amine Modifiers - The peaks obtained from Fast HPLC ion-pair methods that did not employ amine modifiers in the mobile phase (Table 5) exhibited increased tailing as confirmed by Lurie et al (16).

Column Life - Since the packings of a Fast HPLC column are generally made up of 3 micron particles and the void volume of a short Fast HPLC column is much smaller than that of a conventional column and the amount of packing is much less than a conventional column, any change in column conditions such as recrystallization of large column particles, reduced packing volume and dissolution of column particles in a mobile phase will affect in theory the performance of a Fast HPLC column much more than a conventional column. Hence, a limited study was carried out to determine the column performance over a short period of time. The chromatographic characteristics of a pair of compounds in a particular system was not changed when a cartridge based Fast HPLC column was subjected to about 600 injections of a solution of the two compounds. The results indicate that the Fast HPLC column could be expected to perform as well as their conventional counterparts. However, more extensive testing has to be done to confirm this result.

TABLE 5

Chromatographic Peak Parameters as a Function of Column and Mobile Phase Conditions for a Drug and an Impurity

HPLC Conditions	Retention Time, Min.		Peak Width, Sec.		Assymety Factor		Resolution Factor
	Impurity	Drug	Impurity	Drug	Impurity	Drug	
1	8.0	10.3	27	25.5	1.14	1.03	2.9
2	3.5	4.5	4.5	13.5	1.06	1.00	2.4
3	2.7	5.6	10.5	16.5	1.00	1.00	6.8
4	N.R.*	2.91	--	15.0	--	1.64	--
5	1.5	3.7	19.5	9.0	1.39	1.00	4.7

*N.R. -- Not Resolved

1. 30 cm x 3.9 mm - 10 µm ODS Column with 140:150:2 methanol/0.01M pentanesulfonic acid sodium salt/acetic acid; 1.5 mL/min. flow.
2. Column as in (1) with 200:150:2 methanol/0.01M pentanesulfonic acid sodium salt/acetic acid; 2.0 mL/min. flow.
3. Column as in (1) with 200:150:2:1 methanol/0.01M pentanesulfonic acid sodium salt/acetic acid/triethylamine; 1.5 mL/min. flow.
4. 5 cm x 4.6 mm 3 µm column with mobile phase as in 1; 1.5 mL min. flow.
5. Column as in (4) with 140:150:2:1 methanol/0.01M pentanesulfonic acid sodium salt/acetic acid/triethylamine; 1.5 mL/min. flow.

Fast HPLC with Conventional HPLC Columns - Since a minor modification of mobile phase may be necessary when one switches from a conventional column to a Fast HPLC column, a question can be raised whether Fast HPLC can be achieved with a conventional column itself by modifying the mobile phase and/or flow rate appropriately. The results obtained for such an investigation for a conventional column are also included in Table V. Although the retention time of the drug of interest was reduced from 10.3 minutes to 4.5 minutes when the mobile phase was changed from 140:150:2 to 200:150:2 methanol/0.01 M pentanesulfonic acid sodium salt/acetic acid, and the flow rate was increased from 1.5 ml/min to 2.0 ml/min, the column back pressure was increased beyond the system's recommended limits. Hence, the use of a conventional HPLC column for a Fast HPLC run can be limited by the hardware.

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