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Investigation of critical factors for the resolution of SR695, a key impurity, from efavirenz in the reversed-phase assay of efavirenz dosage forms

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Abstract

An investigation of the critical factors effecting the resolution of SR695 from efavirenz in the assay of efavirenz by reversed-phase HPLC was performed. This study was implemented to address the inability of a subset of the Zorbax SB-CN columns used in this method to adequately perform this separation, which were otherwise indistinguishable from columns of this type that could. In this study, column temperature, detector time-constant, pre-gradient isocratic hold-time, pre-column mixing volume, column, and HPLC type were considered. Experimental Design methods were employed to find the relative importance of these factors and to find parameters that would optimize the resolution of SR695 and efavirenz on any HPLC, with any column of this type, for both efavirenz oral liquid and capsule samples. It was also desired that this method change be minimal, so that extensive revalidation would not be required. The most important factors were the column temperature, with lower temperatures giving better resolution, and pre-column mixing volume of sample with mobile phase, with higher mixing volumes giving better resolution up to an asymptote reached at around 150 μl. Added pre-gradient isocratic hold time was found to result in a small improvement in resolution, but was insignificant compared with the other factors mentioned above. A possible explanation is given for the mechanism by which temperature and pre-column mixing have this effect on the resolution obtained in this assay.

Keywords: Reversed-phase chromatography; Resolution; Efavirenz; Experimental design; Mixing volume; Sample solvent strength; Cyano column; Separation of double and triple bond homologs

1. Introduction

Efavirenz is a marketed non-nucleotide reverse-transcriptase inhibitor (NNRTI) used for treatment of HIV (Fig. 1) [1]. The major impurity is the related substance SR695, the *trans*-alkene re-

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duction product of efavirenz (Fig. 1). Methods were developed to determine SR695 in efavirenz drug substance and in both capsule and oral liquid dosage forms [2,3]. It was later observed that a minority of the Zorbax SB-CN columns were not capable of adequately separating these two species, and that some HPLC systems could provide better separation than others on a given column (the required minimum resolution is 1.2). It is, of course, well known that a sample solvent stronger than the mobile phase can cause chromatographic problems [4]. However, there was no way to solubilize the very hydrophobic efavirenz at the required concentration in a mobile phase of lower solvent strength [5], and the method did work well for the majority of columns. The oral liquid formulation required a higher sample solvent strength than the capsule formulation (73 vs. 50% acetonitrile, respectively, (Tables 1 and 2) to ensure complete recovery due to the excipient triglycerides in the oral liquid formulation [3]. To better understand the reasons for the column to column variation in resolution, the column manufacturer was consulted. According to their tests, there were no significant differences between the columns that would not adequately separate SR695 and efavirenz, and those that did (data not shown). Since efavirenz was an approved drug, it was desirable that any changes to the method implemented to overcome this column to column variation problem were not significant ones, such that extensive revalidation could be avoided.

Prior to the present study, it was discovered that using larger inner diameter and/or longer tubing between the autosampler and the column enhanced resolution in this method. It was also noted that a detector time-constant of 1 s gave somewhat better resolution than a time-constant of 4 s. These observations were implemented before the present studies were begun.

Existing data on factors affecting the resolution of SR695 from efavirenz were analyzed using statistical methods. The information obtained was used to determine the key factors controlling the resolution of this critical pair. Empirical observations on the effect of precolumn tubing length on the resolution of these peaks led to the hypothesis that the sample required at least partial dilution from the sample solvent by the mobile phase for efficient partitioning into the stationary phase. For this reason alternate ways of producing the required mixing were explored in the experimental designs which were employed. Whatever method change was finally implemented needed to be portable enough to be moved with the column

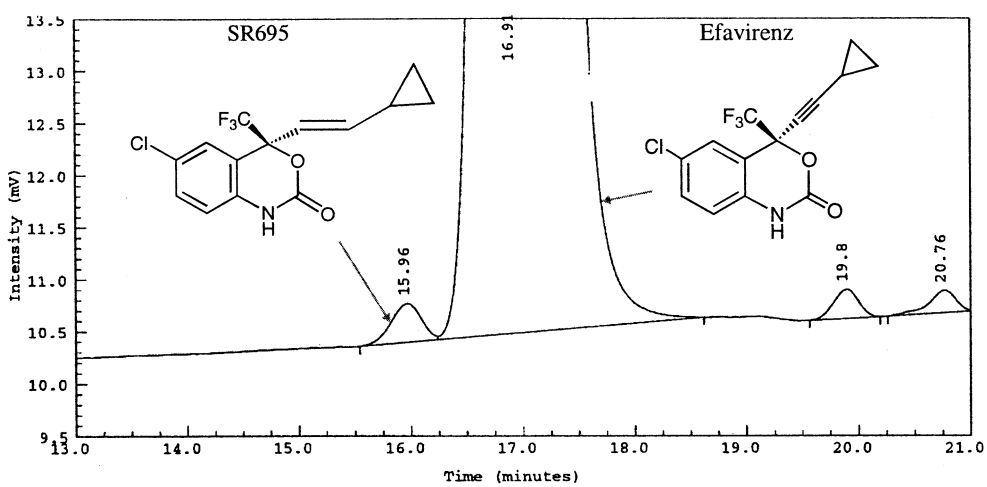

Fig. 1. Example chromatogram shows the relevant section of a typical run showing the structures of SR695 and efavirenz and their elution positions.

Table 1
Chromatographic conditions for efavirenz drug substance and capsule drug product assay and related substances or degradation products analysis

Column	Zorbax® SB-CN, 15 cm × 4.6-mm i.d.
Sample solvent	50/50 acetonitrile/water
Mobile-phases	(A) 90% water/10% methanol with 0.05% trifluoroacetic acid (B) 90% methanol/10% water with 0.05% trifluoroacetic acid
Gradient profile	A linear gradient from 60:40 (A:B) to 50:50 over 16 min, then to 35:65 over 7 min, then to 30:70 over 5 min, then to 20:80 over 1 min. Hold for 2 min and return over 1 min to 60:40 (A:B) and equilibrate for at least 8 min before the next injection
Flow rate	1.5 ml/min
Injection volume	35 μl
Column temperature	40 °C
Detector	UV at 250 nm
Run time	40 min

as different systems were used for this analysis so that systems would not require extensive reconfiguration to run the method.

2. Experimental

2.1. Materials and methods

The HPLC methods being studied are described in Tables 1 and 2, [2,3]. Table 1 shows the method used for drug substance and capsule drug product. The method for oral liquid drug product was adapted from this method by additional washing steps (5 min at 100% B) needed to remove excipient triglycerides which are present in this formulation (Table 2). Efavirenz reference standard, SR695 and capsule and oral liquid formulated efavirenz were produced by DuPont Pharmaceuticals Company, Inc., (Wilmington, DE). All reagents were HPLC grade. Capsule samples were prepared in 50/50 acetonitrile and water [2], whereas oral liquid samples were prepared in 73/27 acetonitrile and water.

2.2. Data analysis

Experiments were designed and statistically analyzed using linear models with the software JMP (SAS Institute, Inc., Cary, NC) and CARD (S-Matrix Corporation, Eureka, CA). The relative significance of the tested factors in the models was tested using a variety of tools, including leverage plots [6,7], which were also produced with JMP.

2.3. Historical data analysis

Historical data were analyzed as an experimental design (Table 3). The factors included in the linear model were HPLC type, column serial number, detector time constant, column temperature, and whether, or not, a 4 min isocratic hold segment was added to the beginning of the method.

2.4. Equipment

System 1: Hewlett Packard HP1050 system consisting of a VWD detector, column oven, pump and autosampler, using Zorbax SB-CN column # SJ5239 (Agilent Technologies, Wilmington, DE).

System 2: Spectra Physics SP8800 pump (ThermoQuest, San Jose, CA), Waters 717 autosampler (Waters Corporation, Milford, MA), an ABI 759A detector (ThermoQuest), Waters TCM column oven, and using Zorbax SB-CN column # SJ5239 (Agilent Technologies).

2.5. Investigation of pre-column tubing related effects

This work was done on System 1.

Two techniques for adding pre-column mixing

of sample with mobile phase were tested. The first was to attach a pair of PEEK static-mixing tees (Upchurch Scientific, Oak Harbor, WA) in series immediately before the column. The second approach was to add an empty 4.3 × 10-mm (145-μl) guard cartridge holder, with end frits, (Upchurch), immediately before the column.

2.6. Optimization of factors

A mixed-level experimental design was performed consisting of 21 experiments using three column temperatures and three levels of added pre-gradient isocratic hold-time (Table 4). For all experiments an empty guard column holder was present immediately before the column, as described in the previous paragraph. The location of the guard holder was tested both inside and outside of the column oven for each condition. This experiment was done on System 1. The column used was known to give poor resolution with efavirenz oral liquid samples, but to work well with efavirenz capsule samples. Efavirenz oral liquid sample was used as a worst case.

2.7. Verification of effects

The next experiment was designed to verify the results from the optimization experiment on a different HPLC using the same column as the previous experiment (Table 5). This was a two-level design, testing column temperatures of 35 and 40 °C, the presence and absence of the guard column holder, and two HPLC types (Systems 1 and 2). Both efavirenz oral liquid sample and efavirenz capsule standard were analyzed. System 2 was known to give better resolution than System 1.

2.8. Optimization of pre-column mixing volume

Efavirenz oral liquid sample was used on System 1 at a column temperature of 40 °C. Empty guard column holders (Upchurch) of: 2 × 10 mm (31 μl), 4.3 × 10 mm (145 μl), two of the 2 × 10 mm size holders in series (62 μl) and two 4.3 × 10 mm size holders in series (290 μl) were tested. In addition, packed single and dual series 4.6 × 12.5-mm (~ 87-μl void volume each) SB-CN guard columns (Agilent Technologies) were tested. The 4.6-mm guard columns were packed with 5-μM particles.

3. Results

3.1. Historical data analysis

From this analysis (Table 3) the most significant factor was found to be column temperature, wherein the lowest temperature (30 °C) gave about twice the resolution as the highest temperature (50 °C) (Fig. 2). The addition of isocratic hold-time to the beginning of the method also appeared to be significant, but resolution only improved about 20% with 4 min of added isocratic hold-time. Since the data set used was not a planned experimental

Table 2
Chromatographic conditions for efavirenz oral liquid drug product assay and related substances or degradation products analysis as used prior to the present investigation

Column	Zorbax® SB-CN, 15 cm × 4.6-mm i.d.
Sample solvent	73/27 acetonitrile/water
Mobile-phases	(A) 90% water/10% methanol with 0.05% trifluoroacetic acid (B) 90% methanol/10% water with 0.05% trifluoroacetic acid
Gradient profile	A linear gradient from 60:40 (A:B) to 50:50 over 16 min, then to 35:65 over 7 min, then to 30:70 over 5 min, then to 20:80 over 1 min. Hold for 2 min and then to 0:100 over 1 min, hold for 5 min, return over 1 min to 60:40 (A:B) and equilibrate for at least 8 min before the next injection.
Flow rate	1.5 ml/min
Injection volume	35 μl
Column temperature	40 °C
Detector	UV at 250 nm
Run time	46 min

Table 3
Existing resolution optimization data analyzed with experimental design tools to look for significant factors

HPLC	System HPLC (#)	Add 4 min IHT?	Column ID	Detector time constant (s)	Column temperature (°C)	Resolution
HP1100	A	No	A	4	40	0.7
HP1100	A	No	B	1	40	0.7
HP1100	C	No	C	1	50	0.7
Waters	B	No	C	1	50	0.7
HP1100	A	No	D	1	40	0.94
HP1100	C	No	E	1	40	1.2
HP1100	A	No	A	1	40	1.3
HP1100	C	No	E	1	40	1.3
HP1050	D	No	A	4	40	1.4
HP1100	A	No	F	4	40	1.4
HP1100	A	No	F	4	40	1.4
Waters	B	No	C	1	35	1.4
Waters	E	No	A	4	40	1.5
HP1100	A	No	G	1	40	1.5
HP1100	A	Yes	D	1	40	1.5
HP1100	C	No	E	1	35	1.5
HP1100	A	Yes	A	4	40	1.6
HP1100	A	Yes	A	4	40	1.6
HP1100	A	No	H	4	40	1.6
HP1050	D	No	H	4	40	1.7
HP1100	A	Yes	H	4	40	1.7
HP1100	A	Yes	G	4	40	1.7
HP1100	A	Yes	B	1	40	1.7
HP1100	C	No	E	1	30	1.7
Waters	E	No	H	4	40	1.8
HP1100	C	No	E	1	30	1.8
HP1100	C	No	C	1	30	1.8

Added isocratic hold-time (IHT) refers to whether or not a 4-min isocratic hold segment was included in the gradient after injection.

design, but an analysis of existing data, the power of the analysis was not high enough to place much weight on moderately significant results. This is due to the asymmetrical distribution of the experimental parameters over the experimental space (Table 3). The effect of added isocratic hold-time was further tested as described below.

3.2. Investigation of pre-column tubing related effects

To better understand the effect of added pre-gradient isocratic hold-time and sample solvent, the gradient parameters were examined. In the original efavirenz-assay method the elution gradient started at injection. By holding the mobile-phase composition at initial conditions during partitioning of the sample into the stationary phase, the stronger sample solvent could be washed from the analyte zone by the weaker mobile phase nearer the head of the column. This allowed the sample to partition into the stationary phase closer to the head of the column, and in a narrower band, than when the gradient was started at injection. This hypothesis was further explored as described below.

3.3. Investigation of pre-column mixing effects

Easily transportable methods of adding pre-column mixing were investigated to test the hypothesis that dilution of the sample prior to the column could allow the sample to partition into

the stationary phase with improved efficiency so that all columns could perform the separation adequately. The first method of adding pre-column mixing volume tested was to add two coupled static mixing tees in series before the analytical column. These devices are used to provide gradient mixing in some commercial HPLC systems. This method may have produced efficient mixing, but with only a small total mixing volume (~ 10 μl) relative to the sample volume (35 μl). This did not significantly enhance the resolution of SR695 from efavirenz. The second approach tried, using empty guard cartridge holders, significantly improved resolution. For example, a HP1050 (system 1) gave a resolution of approximately 0.7 without, and 1.5 with the guard cartridge holder at 40 °C for an oral liquid sample (Table 5).

3.4. Optimization of factors

The results of this experiment are shown in Table 4 and a graph of the linear model versus the actual data is shown in Fig. 3.

Column Temperature showed a strong effect on resolution in the leverage plot shown in Fig. 4, which plots the predicted resolutions for each experiment with the factor (in this case column temperature) included, versus excluded, from the model. In such a plot, the vertical distance from a data point to the fit-line is equal to the residual with the factor included in the model. The vertical distance from the point to the mean response line (horizontal line) is equal to the residual with the factor excluded from the model. If the factor has a significant effect in the model the slope of the fit-line will be significantly different than zero. In this case, since the fit-line and both 95% confidence intervals crossed the mean response line, the column temperature had a significant effect on the resolution. The F-ratio was relatively high (37.7). The statistic 'Prob > F' shows the probability that a higher F-Ratio could arise due to chance. It this case, that probability was very low ('Prob > F' = 0.0001), which showed that the high F-Ratio was a true indication that the factor had a significant effect ('Prob > F' was < 0.05). Post-

Table 4
Optimization experimental design, with data

Column temperature (°C)	Added IHT (min)	Guard in/out of column oven	Resolution
35	0	In	1.69
35	0	In	1.63
35	2	In	1.69
35	4	In	1.7
35	0	Out	1.81
35	2	Out	1.51
35	4	Out	1.63
37.5	0	In	1.47
37.5	2	In	1.53
37.5	4	In	1.58
37.5	0	Out	1.52
37.5	2	Out	1.51
37.5	4	Out	1.53
40	0	In	1.44
40	0	In	1.31
40	2	In	1.46
40	4	In	1.5
40	4	In	1.5
40	0	Out	1.39
40	2	Out	1.45
40	4	Out	1.51

System 1 used (see text). A 4.3×10-mm (145-μl) empty guard holder was used.

Table 5
Verification experimental design and data

Column temperature (°C)	Guard	System	Sample type	Resolution
35	Off	2	Capsule	1.42
35	Off	2	Capsule	1.52
35	Off	2	Liquid	1.24
35	Off	2	Liquid	1.51
40	Off	2	Capsule	1.41
40	Off	2	Capsule	1.3
40	Off	2	Liquid	0.8
40	Off	2	Liquid	0.8
40	On	2	Capsule	1.33
40	On	2	Capsule	1.4
40	On	2	Liquid	1.2
40	On	2	Liquid	1.2
35	On	2	Capsule	1.5
35	On	2	Capsule	1.5
35	On	2	Liquid	1.38
35	On	2	Liquid	1.68
40	On	1	Capsule	1.58
40	On	1	Capsule	1.63
40	On	1	Liquid	1.49
40	On	1	Liquid	1.58
35	On	1	Capsule	1.81
35	On	1	Capsule	1.76
35	On	1	Liquid	1.68
35	On	1	Liquid	1.73
35	Off	1	Capsule	1.76
35	Off	1	Capsule	1.82
35	Off	1	Liquid	1.27
35	Off	1	Liquid	1.3
40	Off	1	Capsule	1.74
40	Off	1	Capsule	1.66
40	Off	1	Liquid	0.7
40	Off	1	Liquid	0.7

System 2 used (see text). Guard on, or off, refers to whether or not an empty 4.3 × 10-mm (145-μl) guard column holder was used before the column.

injection isocratic hold-time showed little effect on resolution (not shown). Though the fit-line crossed the mean-line, the 95% confidence intervals included the mean-line within them. The *F*-Ratio was low (1.69), and there was a significant probability that a higher *F*-Ratio could have arisen by chance ('Prob > *F*' = 0.21). The guard column location, whether inside or outside the column oven, had no effect (not shown); the fit-line was almost coincident with the mean-line. The *F*-Ratio was also low (0.17) and there was a very high probability that a higher *F*-Ratio could have arisen by chance ('Prob > *F*' = 0.68).

3.5. Verification of effects

The results of this experiment are shown in Table 5 and graph of the linear model versus the actual data is shown in Fig. 5. Leverage plots were also used to evaluate this model (not shown). The model confirmed that the column temperature ('Prob > *F*' = 0.005) and the presence of the guard holder ('Prob > *F*' = 0.019) were most significant (fit-line and confidence intervals crossed the mean-line for both.) The HPLC was not a significant factor ('Prob > *F*' = 0.065 and fit-line was almost coincident with the mean-line), which

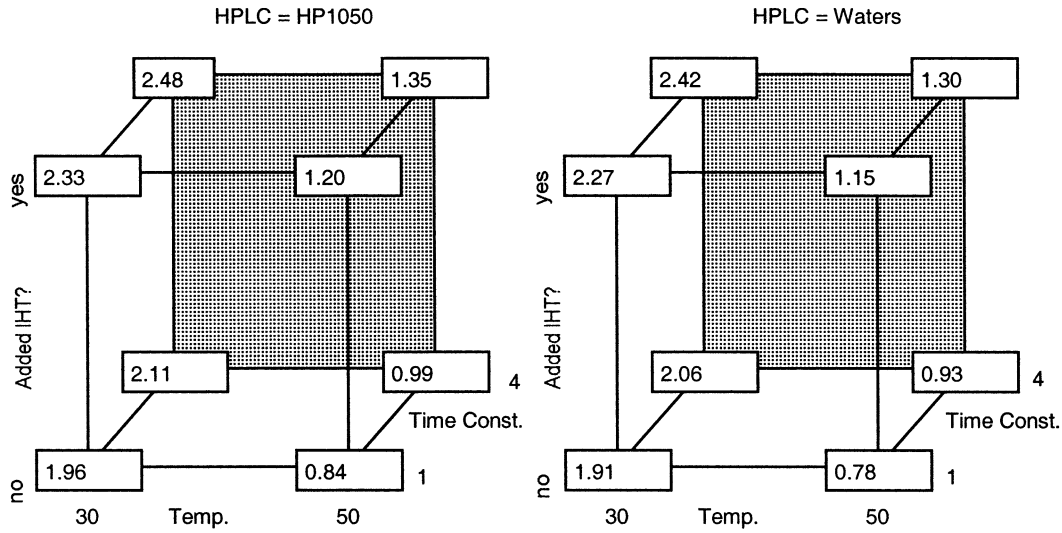

Fig. 2. Cube plot of the extremes of the screening model used to examine the existing historical data. The corners of the cube show the resolutions predicted by the model. It is clear that the largest effects are from column temperature and increased IHT (isocratic hold-time after injection). An increased IHT of '0' means no added IHT, '1' means 4 min of added IHT.

showed that the added guard holder eliminated type HPLC as an effect in the model.

3.6. Optimization of pre-column mixing volume

Since of the improvement seen with the addition of a pre-column mixing chamber in the optimization and verification experiments, the size of the mixing chamber was varied to determine its effect on resolution. The addition of pre-column mixing volume in the form of empty guard cartridge holders increased resolution in an asymptotic manner, with resolution of less than 1.0 with no added mixing and improving little after 145 μl of mixing volume were added (Fig. 6). A single 4.6×12.5-mm SB-CN packed guard column improved resolution to 1.3. This was less than the unpacked pair of 2 mm guard holders (resolution 1.6), though the mixing volume (void volume) in the packed guard column is larger. A pair of packed 4.6×12.5-mm SB-CN guard columns in series (174-μl void volume) gave a resolution of 1.6. The single and dual packed SB-CN guard configuration was also tested at a 35 °C column temperature, where the resolution for a single guard column was 1.5, and for dual guard columns was 1.6.

4. Discussion

Although lowering column temperature had the greatest effect on resolution for the subset of columns that could not adequately separate SR695 from efavirenz, the lowest practical temperature that can be reliably maintained with standard column ovens is 35 °C. A minimum amount of additional sample/mobile-phase mixing prior to sample binding in the column was neces-

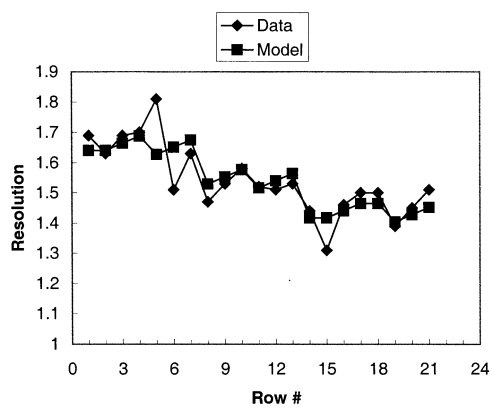

Fig. 3. Plot of model vs. actual data for the optimization experiment showing the resolution for each row in the experimental table (Table 4).

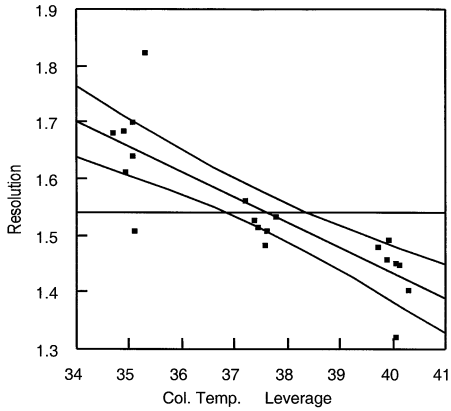

Fig. 4. Column temperature showed a strong effect on resolution, since the fit-line and the confidence intervals all cross the horizontal mean-line. See text for discussion on Leverage plots.

sary for adequate resolution of SR695 from efavirenz for this subset of columns with the original method. This was supported both by the observation that larger pre-column tubing volumes enhanced resolution, and by the data obtained using unpacked guard column holders.

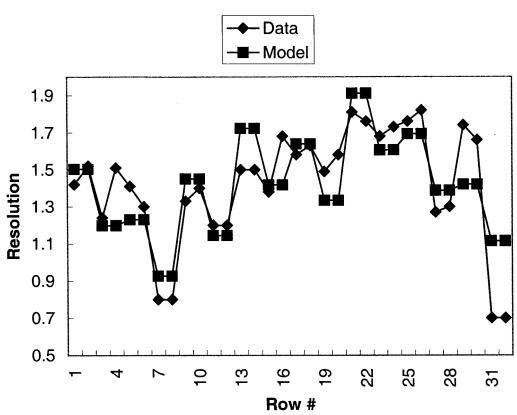

Fig. 5. Plot of model vs. actual data for the verification experiment showing the resolution for each row in the experimental table (Table 5). The four lowest values in the actual data are estimated (value = 0.8 and 0.7, respectively), since they were not sufficiently resolved to allow the SR695 width at half height to be accurately measured.

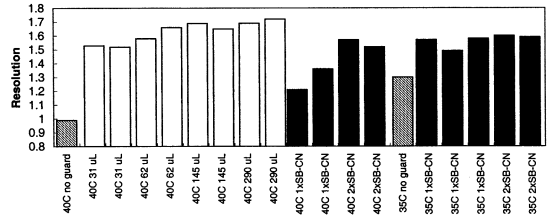

Fig. 6. Resolution as a function of method of pre-column sample/mobile phase mixing. The crosshatched bars are controls with no added mixing volume. The un-filled bars are runs with empty guard holders of the indicated volume, 1 × SB-CN represents a single 4.6 × 12.5-mm SB-CN guard column, 2 × SB-CN indicates two 4.6 × 12.5-mm SB-CN guards in series, all with 5-μM particles.

The presence of a relatively high concentration of acetonitrile in the sample interfered with the ability of the analyte to partition into the stationary phase on this column. This effectively shortened the column, since the sample had to be sufficiently diluted by the mobile phase before it could partition into the stationary phase. Since the oral liquid method used a higher percentage of acetonitrile, compared with the capsule method (Tables 1 and 2) [2,3], the sample partitioned into the stationary phase in a wider, less symmetrical band further into the column than was the case for capsule samples. This was consistent with the observation that lower resolution was obtained on the same column with oral liquid versus capsule samples.

Fig. 7 shows the peak width of the SR695 peak as a function of added pre-column mixing volume for empty guard holders and for filled guard columns. It is apparent that maximum improvement in peak width is obtained with a smaller mixing volume if added in the form of empty guard holders then filled guard columns. The selectivity (α) for SR695 and efavirenz remained constant when empty mixing volume was added (α varied non-systematically between 1.06 and 1.07). Therefore, the change in resolution was due primarily to the change in peak width (Fig. 7). The runs where no pre-column mixing volume was added showed fronting of the peaks, which improved with addition of mixing volume up to

145 μl of volume (Fig. 8). This observation is also consistent with the hypothesis that this column required extra dilution of sample with mobile phase before the analyte could properly partition into the stationary phase to give a more Gaussian peak shape and adequate resolution. Again, it should be remembered that the majority of these columns perform the separation well without any added mixing volume.

When column temperature is reduced, one normally expects a reduction in resolution in reversed-phase HPLC due to reduced efficiency from reduced mass transfer [8]. However, when lower column temperatures were used the retention time of the sample in the column was increased. This resulted in the analyte moving a shorter distance into the column before the sample solvent had been sufficiently diluted away from the analyte to allow partitioning into the stationary phase. In this experiment, the retention time was about 10% longer at 35 than at 40 °C. Apparently, this effect was more important for resolution in this method than the reduced efficiency due to reduced mass transfer.

By adding sufficient pre-column mixing volume, through either tubing or unpacked guard column holders, resolution was improved for this column. Presumably, this was because, the sample was already diluted with mobile phase by mixing with mobile phase in the added pre-column volume, thus allowing normal partitioning of the analyte into the stationary phase. Adding packed guard columns served to directly add length back to the column, with a small amount of mixing volume added due to the inlets, outlets, frits and inter-particular spaces of the guard cartridges. Fig. 7 shows that the peak widths versus mixing volumes obtained with SB-CN packed guard columns lie on a different curve from those obtained with empty mixing volumes. For empty column holders a smaller mixing volume was needed to produce a given reduction in peak-width-at-half-height than for filled columns. The reduced peak width for the empty guard column holders probably resulted from higher mixing efficiency. In the packed columns, the solution was only free to move laterally by diffusion, the packing preventing bulk mixing. In empty column holders the solution was free to flow turbulently in any direction, which allowed more efficient mixing.

Presumably, the reason that some columns were

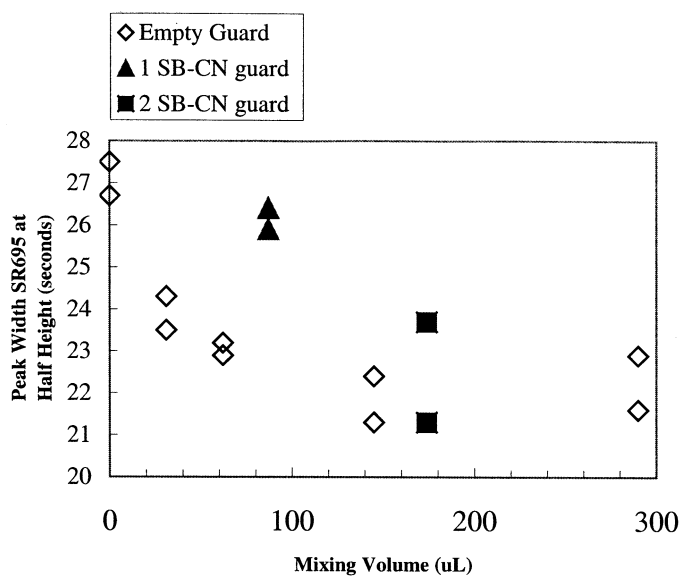

Fig. 7. Effect of mixing volume on the efavirenz peak width at half-height with different sized empty guard cartridge holders run at 40 °C (△), and with filled SB-CN guard cartridges, one (▲), or two in series (■). The mixing volume shown for the filled columns was the void volume, which was assumed to be 40% of the empty volume.

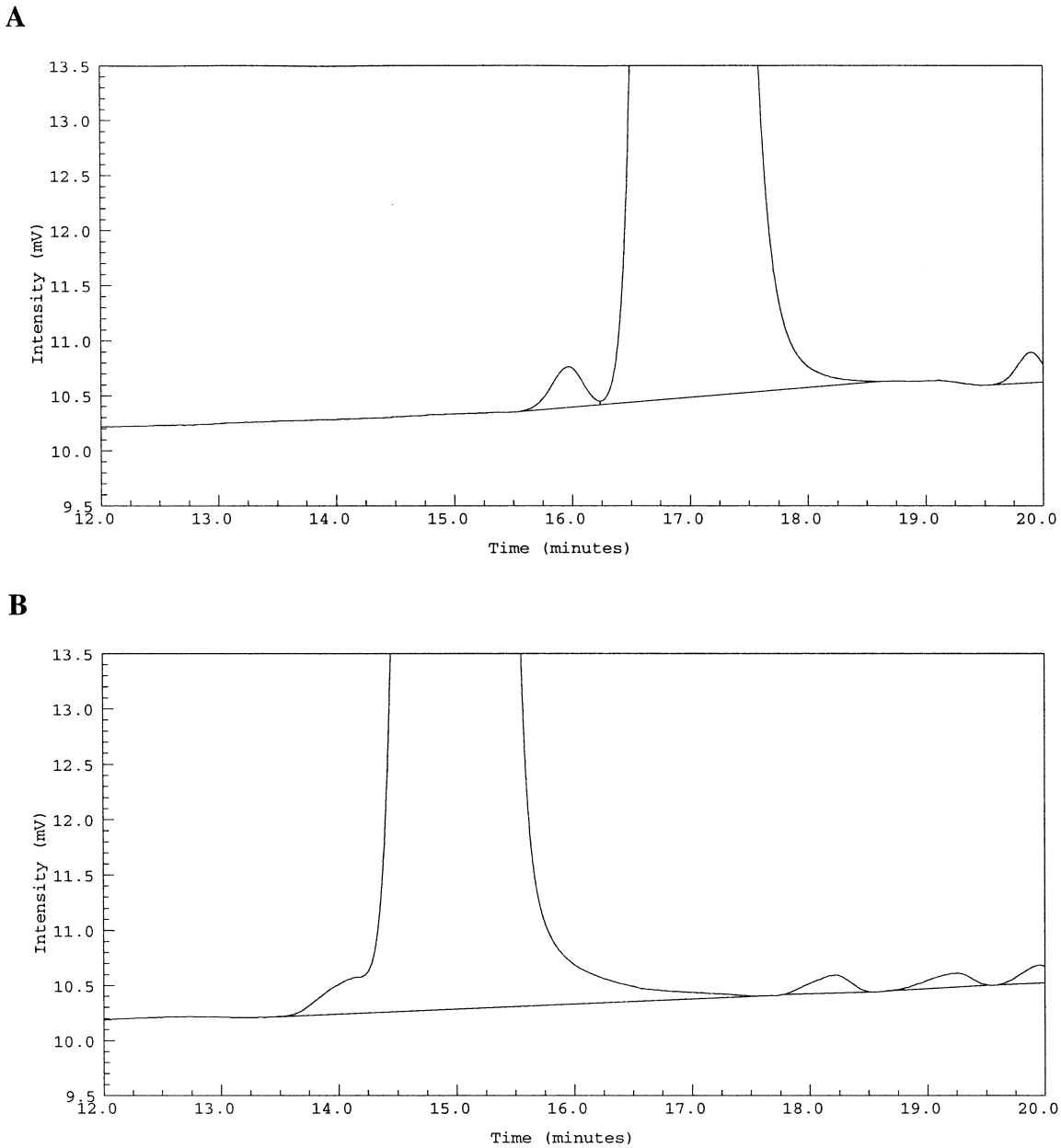

Fig. 8. Chromatograms showing runs with (A) and without (B) a 145-μl empty guard column holder attached before the analytical column. The column used is the same as used in the optimization and verification experiments (see text). The SR695 and efavirenz peak region of the chromatogram is shown (see Fig. 1). Both runs were done at a 40 °C column temperature with oral liquid efavirenz as sample. In addition to the resolution difference between SR695 and efavirenz, note the fronting of the small peaks in the run without added mixing volume (B).

not able to perform this SR695/efavirenz separation adequately with the original method was that some column property (i.e. lower carbon loading, different metal content, or packing density, etc.) reduced the binding efficiency, or capacity, of the column for oral-liquid efavirenz samples. The nature of this column property is not apparent in any of the manufacturer's quality control data. The result of this reduced binding efficiency was that the effective length of these columns was reduced compared with other SB-CN columns, the analyte moving further into the column before being sufficiently diluted with mobile phase for it to partition into the stationary phase. The combination of the band broadening and distortion caused by this partitioning process and the reduced effective working-length of the column produce a reduction in resolution.

5. Conclusion

The combination of reduced column temperature and added pre-column mixing volume allowed the SR695/efavirenz separation to be performed on any HPLC, with any column. This resulted from added mixing of sample with mobile phase prior to the analytical column and increased residence time in the column obtained by reducing the column temperature to 35 °C. Although maximum resolution was obtained with added 'empty' mixing volume, adding an unpacked guard holder to the system is undesirable in that the 'empty' space in the holder could accumulate gas bubbles. Therefore, a dual 4.6 × 12.5-mm SB-CN guard column configuration was chosen, since it provided adequate resolution on all HPLC systems and with all analytical SB-CN columns. This modified method was successfully validated for linearity, accuracy, precision, robustness and specificity. The validation steps were simplified by the relatively minor method changes imposed by this study, since the column and general method conditions were the same. Thus most of the accumulated specificity and robustness data were still relevant. The method has been transferred to three other analytical laboratories.

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