

## Difficult Separations: Resolution of Critical Pairs of Nifedipine Degradation Products

Co-eluting and/or closely eluting pairs are commonplace in complex (seven or more analytes) HPLC applications. For HPLC chromatographers, choosing a single column to separate all of the potential reaction products *a priori* is difficult at best. This document demonstrates a better approach in running a mixture on a series of HPLC columns that exhibit divergent selectivity and retention behavior. In this way, the risk of misidentifying two or more co-eluting peaks as a single entity is greatly reduced. This writing describes the analysis of accelerated degradation products of the coronary vasodilator, nifedipine, (see compound 9, Figure 3). The major points used in this successful method development strategy are presented.

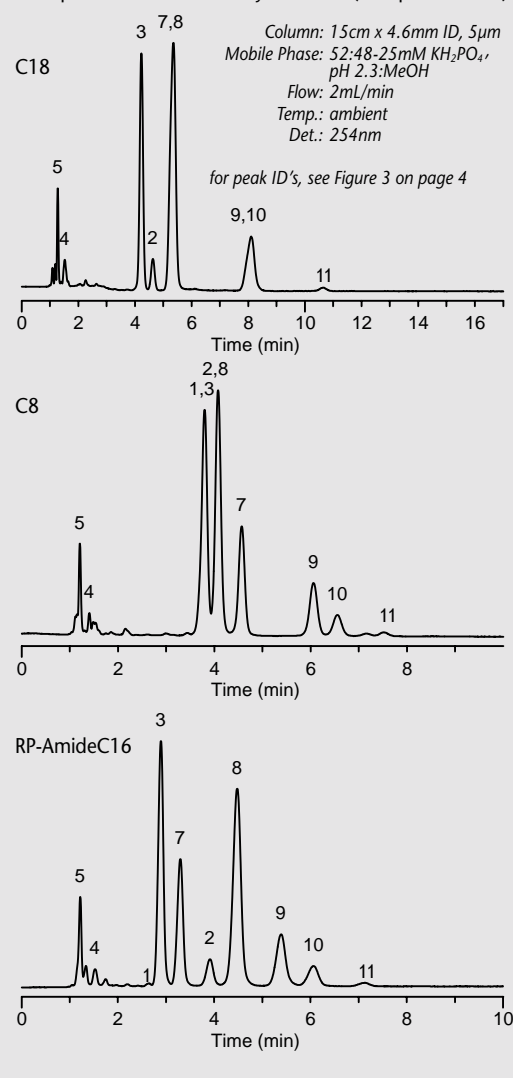
Pure nifedipine standards were submitted to thermal stress, and the resulting degradation was investigated. To ensure the successful separation and detection of all species formed, a series of three diverse columns were employed in the study: Discovery® C18, Discovery C8, and Discovery RP-AmideC16. The mobile phase was kept consistent for all columns, and a Rheodyne® column selector was used in the automated methodology. Methods can be more quickly developed in this way, rather than by switching mobile phases, which necessitates more time for column and system re-equilibration.

Nifedipine, subjected to 200°C thermolysis treatment, produced several degradation peaks as determined by three selected HPLC columns (Figure 1). If the C18 phase had been the only column used in this study, the co-elution of peak pairs 7,8 and 9,10 would not have been easily detected. Similarly, if the C8 column were the only column used, co-elution of peak pairs 1,3 and 2,8 may have gone unnoticed. The Discovery RP-AmideC16 appears to give the best overall separation of the peaks of interest under these conditions.

Following the same method development strategy, it was found that the Discovery C18 column provided the best separation for the 200°C thermolysis treatment sample, when a LC/MS-compatible buffer mobile phase was used (chromatograms not shown). The mobile phase conditions were then optimized on the C18 column to quickly obtain a high-quality separation for LC/MS analysis (Figure 2). With LC/MS data in hand, potential identities of the degradation products were then assigned (Figure 3).

In conclusion, separations of complex HPLC mixtures containing critical pairs are most easily accomplished using multiple complementary columns along with

Figure 1. Separation of Thermolysis (200°C) Products of Nifedipine on Three Discovery Columns (Phosphate Buffer)



an automated column switching system. This powerful approach was demonstrated in the rapid and high quality development of LC/UV and LC/MS methods for nifedipine degradants. It is recommended that all difficult separations be developed employing the automated, complementary column approach, to increase productivity. In future work, the authors plan to use this strat-

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## PRODUCTS

Discovery 15cm x 4.6mm ID  
HPLC Columns



### HPLC Columns

#### Discovery 15cm x 4.6mm ID

Discovery HPLC columns offer the highest level of base-deactivated type B silica packings available in the market today. The four bonded phase types provide a wide range of selectivity and retention for high quality separations and fast methods development.

RP-AmideC16 .....	505013
C18 .....	504955
C8 .....	59353-U
Cyano .....	59356-U

For more information, request T499127 (Pharmaceutical Applications), T499147 (Agricultural Applications), T199926 (Method Development Guidelines).

Rheodyne Column Selector



### HPLC Column Selectors

#### Rheodyne LabPro Column Selector

Select from up to 3 columns or up to 6 columns. Useful for column selectivity comparisons, other column selectivity applications. Includes mounting clips and cover. Rheodyne Nos: 3-column, stainless steel: PR-500-100; 3-column, PEEK: PR-550-100; 6-column, stainless steel: PR-500-104; 6-column, PEEK: PR-550-104.

#### 6-Column Selector

Stainless Steel .....	54379-U
PEEK .....	54380-U

Refer to the HPLC accessory section of the Supelco catalog for more information.

### HPLC Fittings

#### Upchurch PEEK Fingertight Fittings

One-piece fittings are convenient (there is no ferrule to drop onto the floor); two-piece fittings have lower replacement cost (the nut generally has a longer life than the ferrule). Except where noted, Upchurch PEEK Fingertight fittings can be used to 6000psi/420kg/cm<sup>2</sup>.

#### One-Piece Fittings

10-32, Pack of 10 .....	555067-U
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#### Two-Piece Fittings

Nuts & Ferrules, 10-32, Pack of 10 .....	57654
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#### Upchurch Precolumn Filter

**In-line installation.** Stainless steel body with inert polyetherether-ketone (PEEK) endfittings and a 0.5µm or 2µm PEEK frit in one endfitting.

#### Upchurch Precolumn Filter

0.5µm frit .....	55079
2µm frit .....	55078

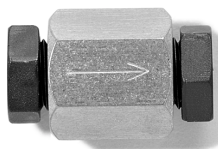
#### Frits

0.5µm, Pack of 10 .....	55080-U
2µm, Pack of 10 .....	55081

Upchurch PEEK One-Piece Fingertight Fittings



Upchurch Precolumn Filter



### Solid Phase Extraction Tubes

#### Discovery DSC-18, DSC-18Lt

Discovery DSC-18 and Discovery DSC-18Lt are polymeric C18 high carbon load (18%) and monomeric C18 moderate carbon load (11%) SPE phases, respectively. Each lot is carefully tested for optimum cleanliness and performance reproducibility.

#### Packing Characteristics:

Base Silica:	irregularly shaped, acid-washed
Mean Particle Size:	50µm
Mean Pore Diameter:	70Å
Total Pore Volume:	0.9cm <sup>3</sup> /g
Specific Surface Area:	480m <sup>2</sup> /g
Bonding:	polymeric, trifunctional octadecyl (DSC-18) or monomeric, octadecyl (DSC-18Lt)
Typical %Carbon Loading:	18% (DSC-18) 11% (DSC-18Lt)
Typical Surface Coverage:	2.50µmole/m <sup>2</sup> (DSC-18) 1.40µmole/m <sup>2</sup> (DSC-18Lt)
Endcapped:	yes

#### Discovery DSC-18 Tubes

50mg/1mL, Pack of 108 .....	52601-U
100mg/1mL, Pack of 108 .....	52602-U
500mg/3mL, Pack of 54 .....	52603-U
500mg/6mL, Pack of 30 .....	52604-U
1g/6mL, Pack of 30 .....	52606-U
2g/12mL, Pack of 20 .....	52607-U
5g/20mL, Pack of 20 .....	52608-U
10g/60mL, Pack of 16 .....	52609-U
bulk packing, 100 grams .....	52600-U

#### Discovery DSC-18Lt Tubes

50mg/1mL, Pack of 108 .....	52610-U
100mg/1mL, Pack of 108 .....	52611-U
500mg/3mL, Pack of 54 .....	52613-U
500mg/6mL, Pack of 30 .....	52615-U
1g/6mL, Pack of 30 .....	52616-U
2g/12mL, Pack of 20 .....	52618-U
5g/20mL, Pack of 20 .....	52621-U
10g/60mL, Pack of 16 .....	52622-U
bulk packing, 100 grams .....	52623-U

For more information, request T498367 (Discovery DSC-18) and T499129 (Discovery DSC-18Lt.).



All literature mentioned in this issue can be obtained from the website, [www.sigma-aldrich.com/TheReporter](http://www.sigma-aldrich.com/TheReporter), by completing the Literature Request section on the reply card, or by calling our Technical Service Department.

## UPDATED LITERATURE

### Custom Media Processing

A new brochure describes Supelco's capabilities for Custom Resin and Media Processing Services. This brochure explains the various types of processing available, the analytical testing services offered by Supelco and the process for receiving technical assistance and pricing from the company.

If you perform any processing of Low Pressure Liquid Chromatography resin before you use it, Supelco's custom media processing service may be a cost effective alternative.

For more information request T400049, Custom Resin and Media Processing Services.

## NEW APPLICATIONS

### Vitamin D<sub>2</sub> and D<sub>3</sub>: A Classic Case of a Critical Pair

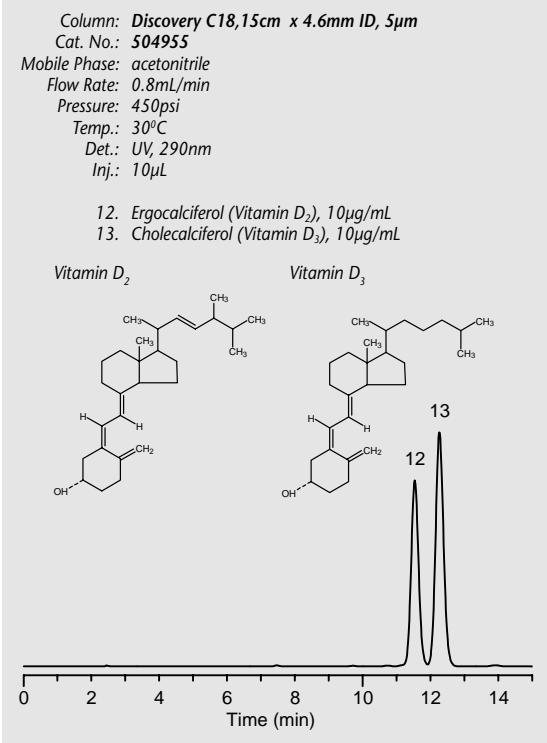
Vitamins are an extremely diverse range of compounds present in minute amounts in animals, humans and plants. They are vital in the enzyme reactions that are necessary for carbohydrate, fat, and protein metabolism. Vitamins are relatively unstable, affected by factors such as heat, light, air, other compounds, and food processing conditions. Because of their critical role in nutrition and their relative instability, qualitative and quantitative analyses are an important issue as well as a challenging task. HPLC is preferred for vitamin separations because of its powerful selectivity.

Vitamin D comprises two main forms. Cholecalciferol (Vitamin D<sub>3</sub>; 9,10-secocholesta-5,7,10(19)-trien-3-ol) is produced from the action of sunlight on a sterol in the skin. Ergocalciferol (Vitamin D<sub>2</sub>; 9,10-seco-ergosta-5,7,10(19),22-tetraen-3-ol) is produced in plants, fungi and yeasts by UV irradiation of another sterol, ergosterol. Vitamin D plays an important role in the body in the formation of bone by regulating calcium and phosphorus metabolism. The chemical structures of Vitamin D<sub>2</sub> and D<sub>3</sub> differ only in the C-17 side chain; in D<sub>2</sub> there is a double bond and an additional methyl group. Thus, these are closely related compounds that are difficult to separate. (See Figure 4 for the structures.)

We have developed a method to separate these structurally similar compounds on a Discovery C18 Column. Using a simple mobile phase of 100% acetonitrile, we were able to get baseline resolution of this critical pair of peaks in 13 minutes. Vitamin D<sub>2</sub> elutes first, then Vitamin D<sub>3</sub>.

For more information request T399149, Separating Fat-Soluble Vitamins by Reversed Phase HPLC, Using Discovery Columns.

Figure 4. Vitamins D<sub>2</sub> and D<sub>3</sub> on a Discovery C18 Column



## LC PERFORMANCE TIP

### Mobile Phase Buffer/% Organic Content Compatibility

Probably the single most often encountered column performance problem by both inexperienced and experienced HPLC chemists alike is high backpressure. Most analysts know that this can be caused by particulates from the sample, unfiltered mobile phase, HPLC system, or packing clogging the inlet and/or outlet frit of the column. However, the most common cause is often overlooked. When using and changing mobile phases in your system and column, always consider the compatibility of any buffer you are/were using with the amount of organic modifier. A column previously used with a phosphate or other buffer should be rinsed with 90:10-H<sub>2</sub>O:ACN be-

fore another mobile phase is used consisting of anything higher than about 50% organic content. A higher % organic mobile phase could precipitate buffer from the previous mobile phase, thereby, clogging the column frit(s) and causing high backpressure. Likewise, a buffer mobile phase should not be used on a HPLC system/column that previously had been run under high % organic content, without first being rinsed with 90:10-H<sub>2</sub>O:ACN. Also, be aware that some commercial HPLC columns, such as Discovery RP-AmideC16, ABZ, ABZ+, and Suplex® are shipped and/or stored in high % organic solvent. They should similarly be rinsed with 90:10-H<sub>2</sub>O:ACN before using a buffered mobile phase.

For more information contact Keith at [kduff@sial.com](mailto:kduff@sial.com)



Keith Duff,  
R&D LC Chemist

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 PEEK - Victrex plc  
 Rheodyne - Rheodyne, L.P.  
 Upchurch - Upchurch Scientific, Inc.

**S** For more information on the lead article, request T400152, Isolation of Nifedipine Pyrolysis and Photolysis Degradation Products.

## Difficult Separations:...

(continued from page 1)

egy to separate the most difficult of all mixtures to separate, enantiomers.

Figure 2. LC/MS Separation of Nifedipine 200°C Thermal Degradation Products (Ammonium Acetate Buffer)

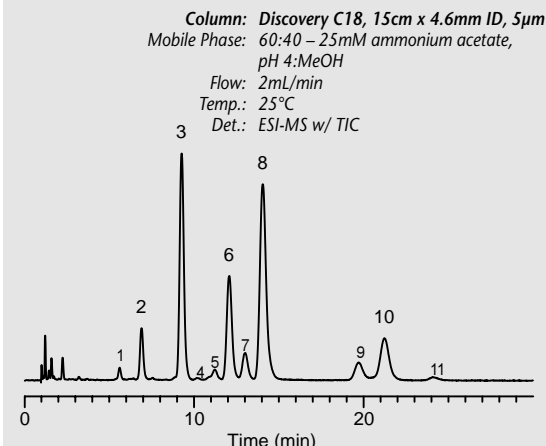
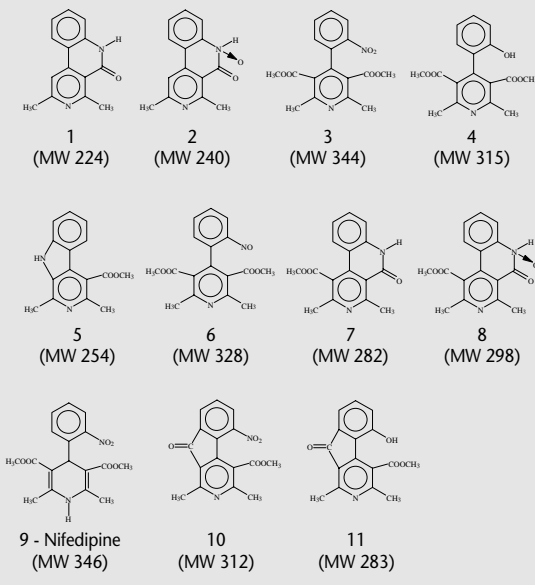


Figure 3. Potential Thermal Degradation Products of Nifedipine Based on LC/MS Data



## CASE STUDY 4

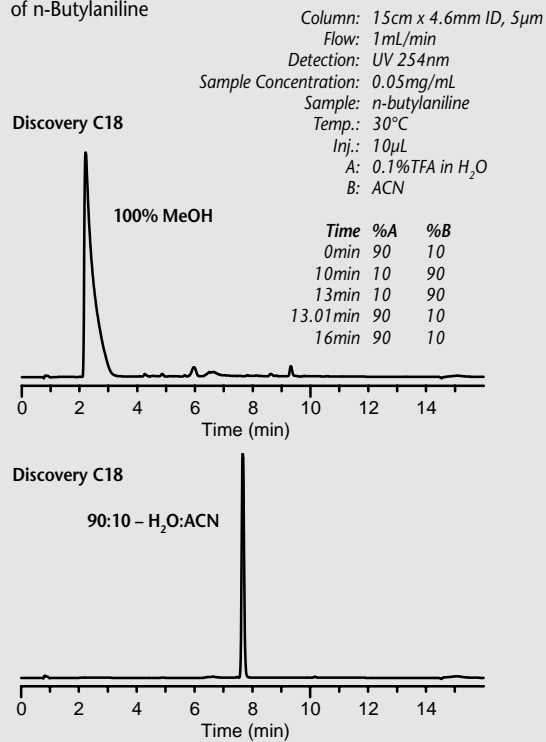
### Sample Solvent Effect on Chromatography

It is important not to overlook the sample solvent type and strength employed in relation to that of the mobile phase. In most cases, it is best to use a sample solvent of equal or less strength than the mobile phase. Also, the choice of sample solvent type (e.g., ACN, MeOH, water, etc.) generally should be the same as that used in the mobile phase. If a sample solvent of a higher strength and/or type than the mobile phase is used, peak splitting and/or tailing and faster elution times may occur. This results in a poorer separation and more difficult qualitative and quantitative measurements.

Figure 5 shows two chromatograms run under identical conditions except for sample solvent type and strength. The top chromatogram was run with the n-butylaniline analyte dissolved in 100% MeOH, which is too strong relative to the 10% organic mobile phase content at the beginning of the gradient run. A broad, tailing, fast eluting peak is noted. The same n-butylaniline analyte was then dissolved in a weaker 90:10 – water: ACN solvent at a concentration identical to the 100% MeOH sample. The modified sample solvent greatly improved the peak shape and retention as shown in the lower chromatogram.

**S** For more information request T100826, HPLC Troubleshooting Guide.

Figure 5. Effect of Sample Solvent on Peak Shape & Retention of n-Butylaniline



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