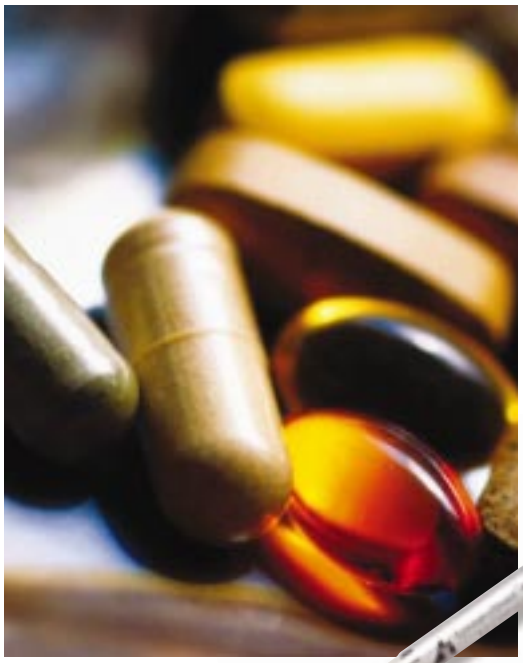
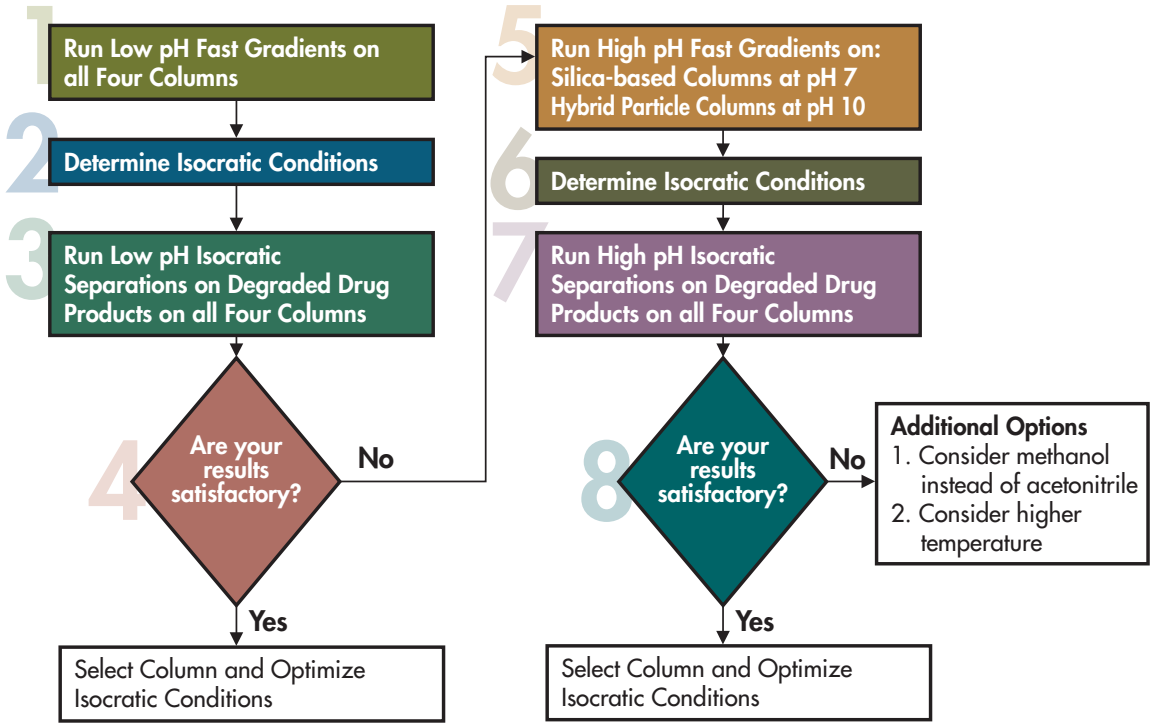
Stability-Indicating HPLC Method Development

A Systematic Approach using pH and Column Selectivity



Waters

Stability-Indicating HPLC method development



This method development system is based on Waters' years of HPLC application experience and industry-leading chromatography products. This protocol is designed to save time and money, while resulting in robust and reliable methods.

A Solution for analytical chemists

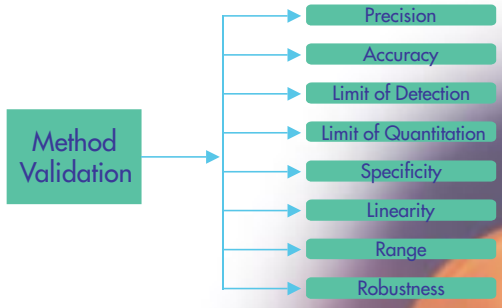
The need to save method development time and improve accuracy is forcing today's analytical chemists to look for better, faster ways to develop stability-indicating methods. Costly delays can be prevented by quickly establishing robust analytical methods that remain useful throughout the life of a drug product.

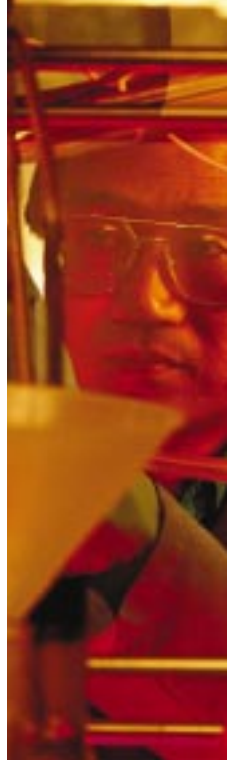
Waters has designed a method development approach and accompanying four-column kit to establish stability-indicating methods using the selectivity of HPLC columns. Starting with HPLC columns that offer excellent reproducibility, column lifetime, and sensitivity, this step-by-step protocol can save the method development chemist time and money required to establish new methods.

This approach is consistent with developing methods that meet ICH (International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines. The ICH established characteristics for validation of modern analytical methods that are part of US Pharmacopeia and other compendial volumes referenced by regulatory organizations throughout the world, as shown in Figure 1. As part of a methods validation package, the U.S. FDA defines stability-indicating assay as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. Column selection can have a major influence on each of these characteristics.

Figure 1

Method Validation
Analytical Performance Characteristics





Benefits of the Waters approach

Systematic steps

- Reduce development time
- Less than 1 week to complete work per compound
- Common retention factors (*k*)*

Unique Selectivity

- Maximize chances for best separation

Methods on "new generation" HPLC columns

- Best peak shape
- Excellent reproducibility

Run at low and high pH

- Maximize selectivity differences
- Work in pH zones that maximize robustness by running two pH units above or below the pk_a

Perform degradation studies

- Challenge the method to ensure resolution of all potential impurities

A Wide Range of Column Selectivity

The Waters process is designed to help you identify the proper column and optimum pH level for your separation to improve robustness.

The Waters Method Development Kit contains four columns that offer a wide range of selectivities. This kit includes straight-chain ligands and embedded polar groups to offer unique selectivities as shown in Figure 2, and provide excellent peak shape. All HPLC columns in the kit offer excellent reproducibility and column lifetime.

Hybrid particle based, straight chain alkyl

XTerra® MS C_{18}

Hybrid particle based, embedded polar ligand

XTerra® RP_{18}

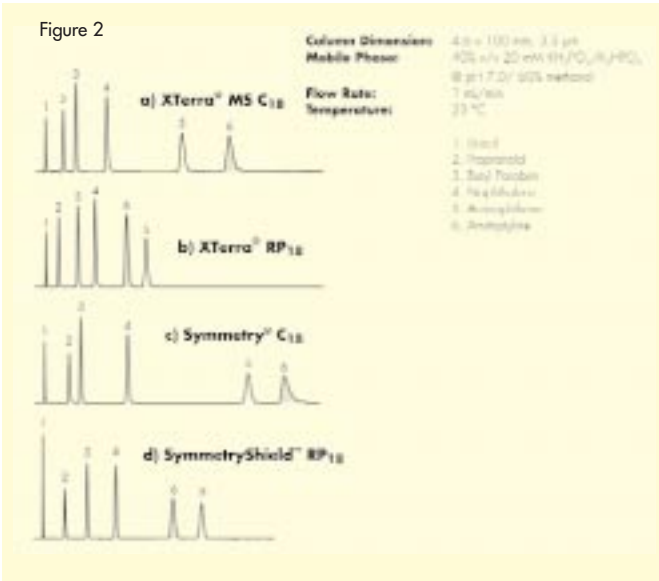
Silica based, straight chain alkyl

Symmetry® C_{18}

Silica based, embedded polar ligand

SymmetryShield™ RP_{18}

Figure 2



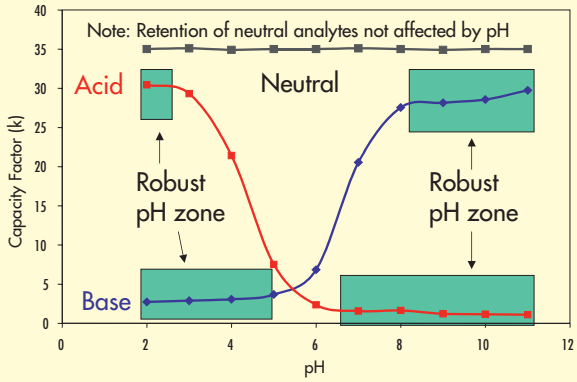
*$k = (t_R/t_0) - 1$, where t_R is the retention time and t_0 is the void time.

The columns contain 3.5 micron particles with dimensions of 4.6 x 100 mm. Shorter columns and smaller particle sizes provide shorter analysis time while maintaining efficiency.

The retention of an analyte can be influenced by pH. Ideally, it is best to work in a pH region where ionization, and therefore the retention, is weakly dependent on pH. Figure 3 illustrates the robust pH zones which shows the change in retention factor of an acid, base and neutral as the pH changes. Waters recommends avoiding retention changes by establishing pH conditions at least 2 pH units away from the pK_a.

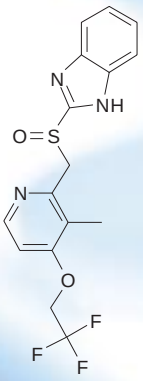
Figure 3

Behavior of Acidic, Basic and Neutral Compounds in Reversed-phase Chromatography



The following pages present the Stability-Indicating HPLC method development 8-step process. This is a general methodology which may be used with any compound; however, to help make the steps clear, we have used specific examples of the process as applied to the drug Lansoprazole indicated for treating ulcers.

The method development system is intended to be used with the Waters four-column **Method Development Kit, Part Number 186001100.**



Lansoprazole

STEP 1

Run low pH fast gradients on all four columns

Purpose: Perform fast gradient chromatographic runs to determine conditions required to generate retention factors of 4-6 on all four columns when converting to isocratic conditions in step 2.

Method:
Mobile phases (for low pH)
A: 10 mM ammonium formate, pH 3.0
B: acetonitrile
Initially 5% B
5 minute gradient to 95% B
Flow rate of 1.4 mL/minute

Rationale: This mobile phase is recommended because it:

- is mass spectrometry-compatible
- contains a good buffer
- is relatively simple

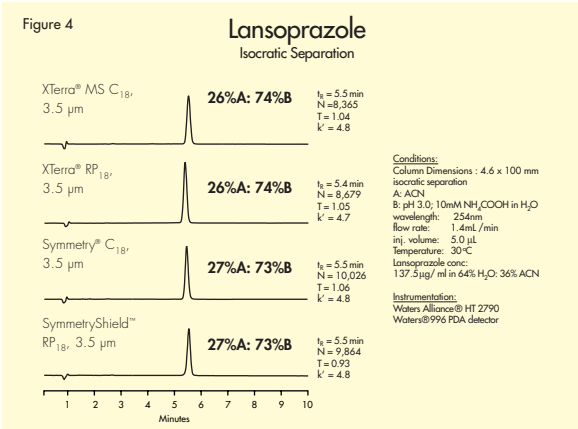
Evaluating results: The retention time for the analyte peak should be between 1 and 5 minutes.

STEP 2

Determine isocratic conditions

Purpose: Determine isocratic conditions to set a retention factor of 4-6 on all four columns, as in Figure 4.

Figure 4



Method: Theoretical equations for going to isocratic conditions from gradient runs have been reduced to a set of rules for these conditions

- To get a retention factor of 4-6, try the following range of percent acetonitrile:
  Low % organic = (t_e x 19) – 32, where t_e is the analyte elution time
  High % organic = (t_e x 19) – 22
- Generate isocratic conditions and adjust percent organic levels as necessary

Rationale: Speed up the process of converting to isocratic conditions with common retention factors.

Evaluating results:
The results can be evaluated by setting up and performing the isocratic runs for each column. If k' (retention factor) is either low or high, organic levels can be adjusted as necessary.

Example Calculation:
If t_e = 3.0 minutes and using the above equations
Range of acetonitrile to try: Low = 25%
High = 35%

Assumption: HPLC equipment should have a minimum gradient dwell volume of less than 100 µl or, for a Waters Alliance® 2690 System, the pre-column volume feature should be used.

Alternative approach for determining isocratic conditions
Establishing isocratic conditions for each HPLC column with common retention factors can be determined following a second approach. Using isocratic conditions on a single column at a time, consecutive runs can be performed where the organic level is lowered 10% each time. It is recommended that the initial run start with an organic level of 90%. Additional runs should be completed until a *k* of 5 is reached. Note: remember that *k* changes by a factor of three (3) for every change of 10% in the organic phase.

This same approach should be repeated for each column.

STEP 3

Run low pH isocratic separations on degraded drug products on all four columns

Purpose: Determine which HPLC column provides:

- a complete separation of the main analyte from the degradants and
- whether all degradants have been successfully separated.

Method: We recommend using the isocratic method developed for each HPLC column in step 2 to evaluate the degraded products.

- Active Pharmaceutical Ingredients (API): degrading the API by 10 to 30%. The examples shown include degradation studies using acid, base and peroxide.

Rationale: The multiple column approach provides selectivity differences which allow the chromatographer to rapidly determine if the main analytes and each degradant:

- are fully separated from each other (peak purity)
- have good peak shape and sensitivity.

Evaluating results: For each stress test (acid, base, oxidation, etc.), comparisons of the chromatography can be made of each HPLC column to determine if the column and conditions provide satisfactory results (Figures 5-7).

Figure 5

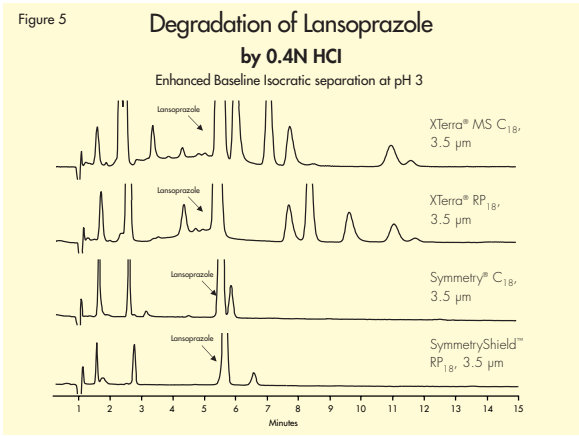


Figure 6

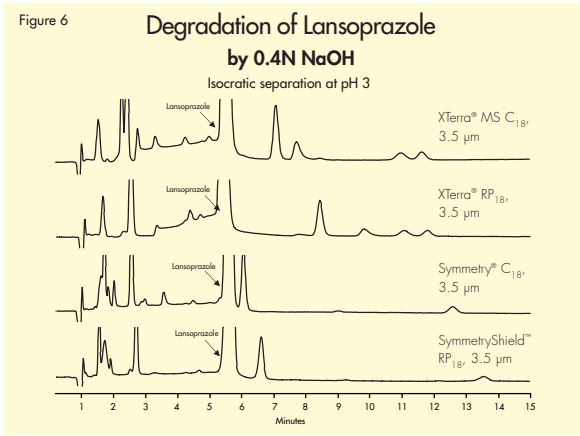
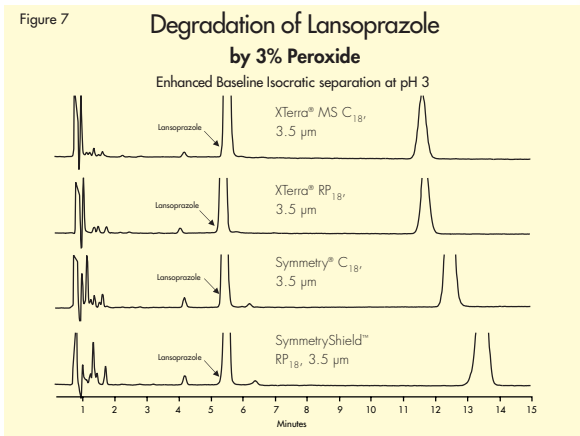


Figure 7



Examine results and select HPLC column

STEP 4

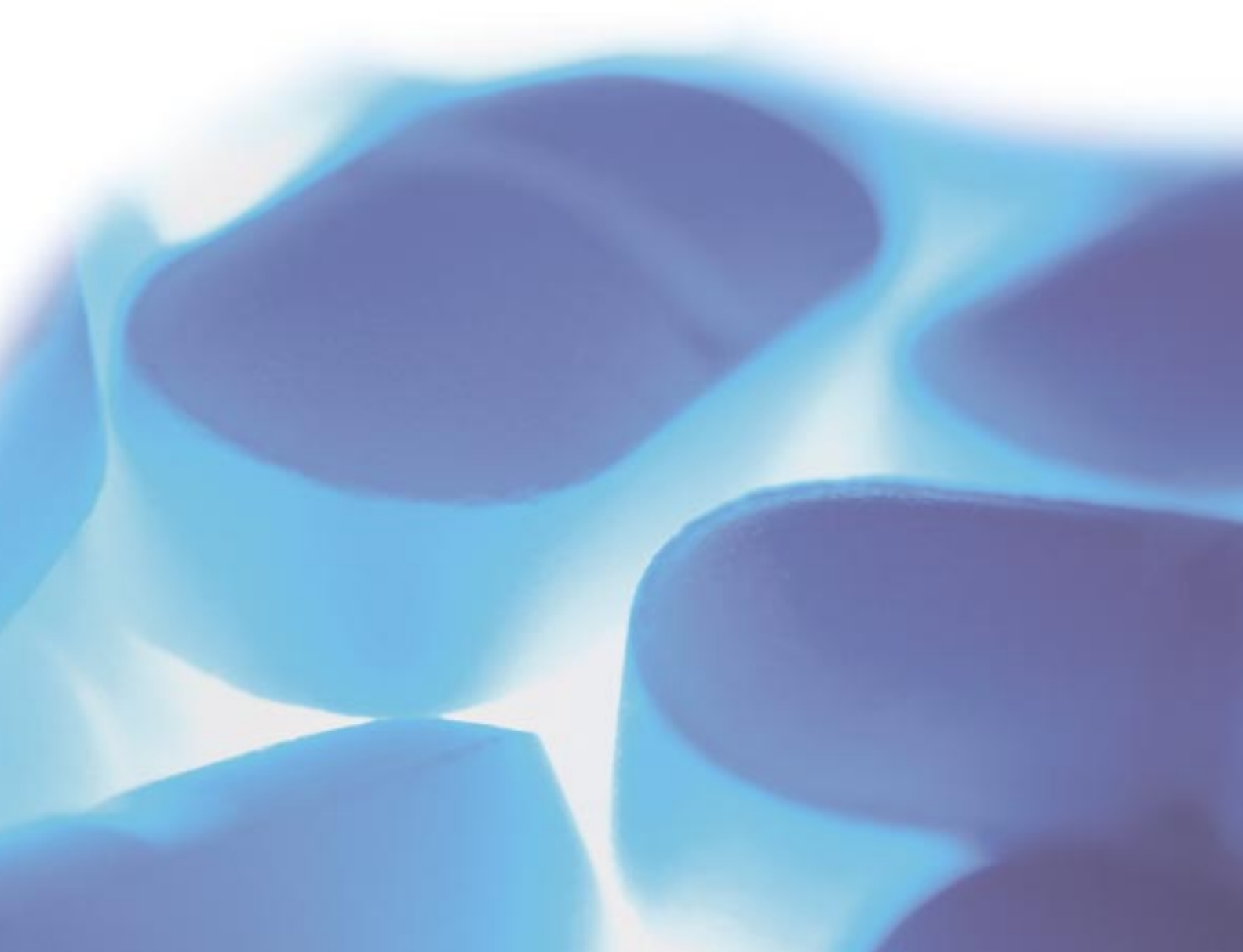
At this point, multiple stress tests on the four different columns will show the number of different degradants that are present from each type of chemical stress.

Do any/all of the columns offer:

- baseline resolution of all degradant peaks?
- good peak shape for all degradants?
- pure main peak using PDA or MS?
- sufficient signal-to-noise (S/N) ratio for the minor peaks?

If yes, select the individual column, further optimize percent organic.

If no, we recommend evaluating the drug substance or product at a higher pH to gain the added selectivity.



STEP 5

Run high pH fast gradient separations on silica-based columns at pH 7 and hybrid particle columns at pH 10

Purpose: As in step 1, the purpose/goal of this step is to perform fast gradient chromatographic runs to determine conditions required to generate retention factors of 4-6 on two silica-based columns at pH 7 and on two hybrid particle columns at pH 10.

Methods:

Mobile phase at pH 7

A: 10 mM ammonium bicarbonate, pH 7
B: acetonitrile

Mobile phase at pH 10

A: 10 mM ammonium bicarbonate, pH 10
B: acetonitrile
Initially 5% B
5 minute gradient to 95% B
Flow rate of 1.4 mL/minute
Note: In order to maintain a constant pH with ammonium bicarbonate, a helium sparge is recommended.

Rationale: These mobile phases are recommended because they:

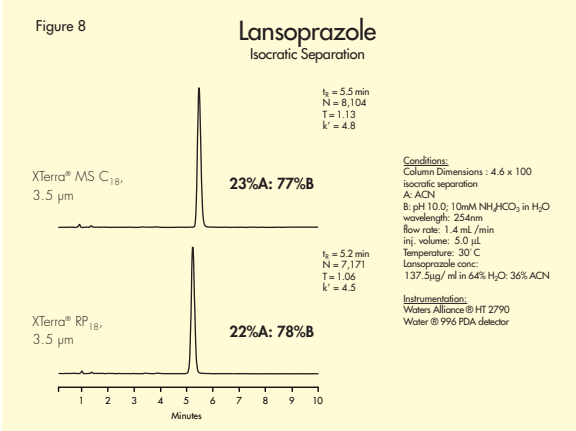
- are mass spectrometry-compatible
- contain a good buffer
- are relatively simple

Evaluating results: The retention time for main analyte peak should be between 1 and 5 minutes.

STEP 6

Determine isocratic conditions

Purpose: Determine isocratic conditions to set a retention factor of 4-6 on all four columns as in Figure 8.



Method: Theoretical equations for going to isocratic conditions from gradient runs have been reduced to a set of rules for these conditions

- To get a retention factor of 4-6, try the following range of percent acetonitrile:
  Low % organic = (t_e x 19) – 32, where t_e is the analyte elution time
  High % organic = (t_e x 19) – 22
- Generate isocratic conditions and adjust percent organic levels as necessary

Rationale: Speed up the process of converting to isocratic conditions with common retention factors.

Evaluating results:
The results can be evaluated by setting up and performing the isocratic runs for each column. If k (retention factor) is either low or high, organic levels can be adjusted as necessary.

Example:
If t_e = 3.0 minutes and using the above equations
Range of acetonitrile to try: Low = 25%
High = 35%

Assumption: HPLC equipment should have a minimum gradient dwell volume of less than 100 µl or, for the Waters Alliance® 2690 system, the pre-column volume should be used.

Alternative approach for determining isocratic conditions
As with the low pH conditions, establishing isocratic conditions for each HPLC column with common retention factors can be determined following a second approach. Using isocratic conditions on a single column at a time, consecutive runs can be performed where the organic level is lowered 10% each time. It is recommended that the initial run start with an organic level of 90%. Additional runs should be completed until a *k* of 5 is reached. Note: remember that *k* changes by a factor of three (3) for every change of 10% in the organic phase.

This same approach should be repeated for each column.

STEP 7

Run high pH isocratic separations on degraded drug products on all four columns

Purpose: Determine which HPLC column provides

- a complete separation of the main analyte from the degradants
- a successful separation of all degradents.

Method: We recommend using the method developed for each HPLC column to evaluate the degraded products.

- Active Pharmaceutical Ingredients (API): degrading the API by 10 to 30%. The examples shown include degradation studies using acid, base and peroxide.

Rationale: As with the low pH conditions, the multiple column approach provides selectivity differences which will allow the chromatographer to rapidly determine if the main analytes and each degradant:

- are fully separated from each other (peak purity)
- have good peak shape and sensitivity is established.

Evaluating results: For each stress test (acid, base, oxidation, etc.), comparisons of the chromatography can be made of each HPLC column to determine if the column and conditions provide satisfactory results, as shown at pH 10 at Figures 9-11.

Figure 9

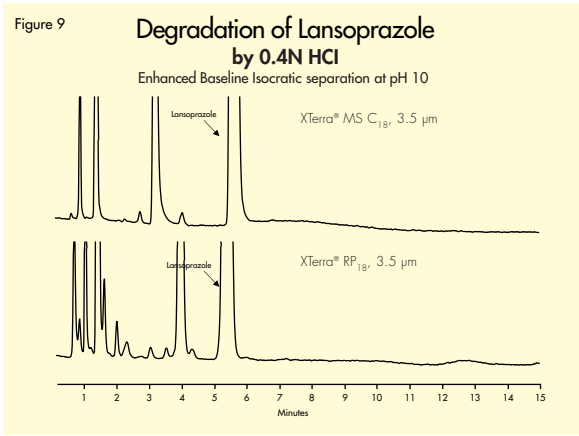


Figure 10

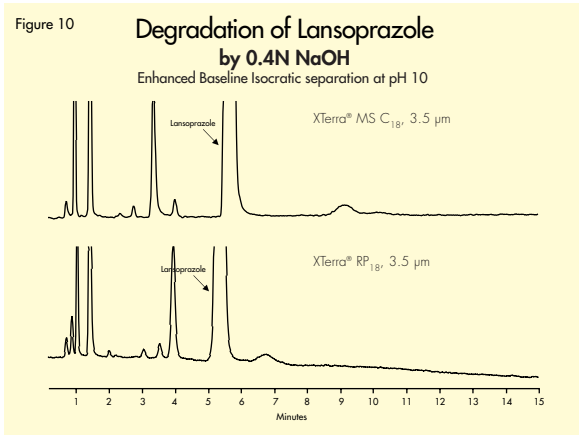
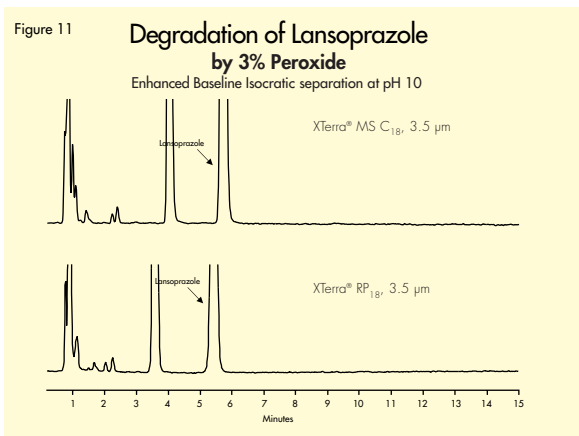


Figure 11



Examine results and select HPLC column

STEP 8

At this point, multiple stress tests on the four different columns at low and high pH will show the number of different degradants that are present from each type of chemical stress.

Do any/all of the columns offer:

- baseline resolution of all degradant peaks?
- good peak shape for all degradants?
- pure main peak using PDA or MS?
- sufficient S/N for the minor peaks?

If yes, select the individual column and chromatographic conditions, further optimize percent organic.

If no, we recommend evaluating the drug substance or product using

- methanol instead of ACN,
- higher temperature to gain the added selectivity.

Lansoprazole Example

In this specific set of examples for lansoprazole, the XTerra® RP_{18} 3.5 µm column 4.6 x 100 mm provided the best set of results when examining the degradant peaks formed in the acid, base and peroxide stress tests. Both studies at pH 3 and pH 10 provided excellent separations. We might suggest a method be developed using pH 10 where all the degradant retention times were less than the main peak, Lansoprazole. In degradation studies, this pH 10 method might prevent the main peak from coeluting with individual degradants, if the column became overloaded.

Equipment used in examples:

Waters Alliance® 2690 and 2790 HPLC system
Waters Millennium^{32} chromatography software, version 3.2
Waters® 996 PDA detector

The role of HPLC columns in method development

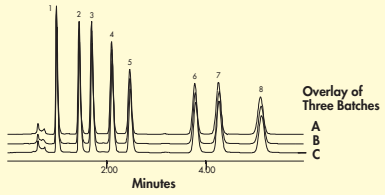
Today's analytical method requirements demand HPLC columns with excellent reproducibility and tight specifications, good column lifetime, pH and temperature stability, high efficiency, and good peak shape for acids, bases, and neutral compounds.

Reproducibility: Column-to-column reproducibility is controlled by the packing process and batch-to-batch reproducibility is controlled by the major steps in the production of the chromatographic packing material. Each column selected for the method development kit has similar batch-to-batch reproducibility as illustrated in Figure 12.

Figure 12

Batch-to-batch Reproducibility of 3.5 µm Symmetry® C_{18} Packing

1. Barbital
2. Allobarbital
3. Phenobarbital
4. Butabarbital
5. Butalbital
6. Amobarbital
7. Mephobarbital
8. Secobarbital



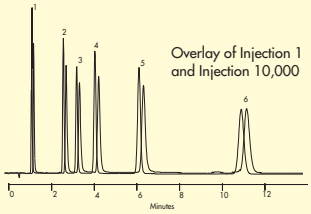
Column:	Symmetry® C_{18} 3.5 µm (4.6 x 100 mm)
Flow Rate:	1.8 mL/min
Mobile Phase:	0.1N potassium phosphate (pH 6.9)/acetonitrile/water 20:30:50
Detection:	UV at 214 nm
Sample:	10 µL of 25 µg/mL of each barbiturate

Column lifetime is important when selecting a column for validated methods. All four columns in the Waters method development kit exhibit excellent column lifetimes as illustrated in Figures 13 and 14.

Figure 13

Lifetime of a Symmetry® 3.5 µm Column: 10,000 Injections of Sulfa Drugs (2500 hrs)

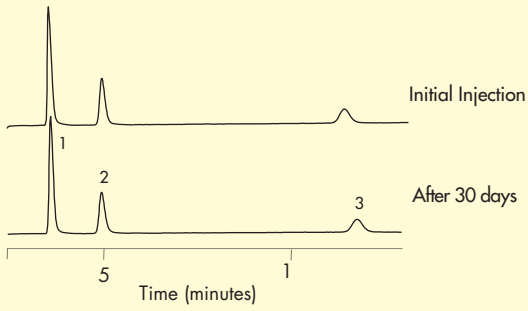
1. Sulfanilamide
2. Sulfadiazine
3. Sulfathiazole
4. Sulfamerazine
5. Sulfamethazine
6. Succinylsulfathiazole



Conditions	
Column:	Symmetry® C_8 3.5 µm 4.6 mm x 100 mm with Sentry™ Guard Column 3.9 mm X 20 mm
Mobile Phase:	water/methanol/glacial acetic acid 79:20:1
Flow Rate:	1.5 mL/min
Detection:	UV at 254 nm
Injection volume:	10 µL of mixture of six sulfa drugs at 10-39 µg/mL

Figure 14

Lifetime Study at pH 11.5



Conditions	
Column:	XTerra® RP_{18}, 5 µm, 4.6 x 150 mm with 3.9 x 20 mm XTerra® RP_{18}, 5 µm guard column
Mobile Phase:	0.05 M Pyrrolidine-Hydrochloric acid pH 11.5/acetonitrile, 50/50
Flow Rate:	1.0 mL/minute
Column Temp	30 °C
Detection:	UV–215 nm
Injection volume:	2 µL

Peak Number	Day 1 USP Tailing Factor	Day 30 USP Tailing Factor
1. Methamphetamine	1.2	1.2
2. Propranolol	1.3	1.3
3. Nortriptyline	1.1	1.1

Good peak shape is important in establishing stability-indicating methods. A column should be selected that exhibits good shape for all types of compounds, namely acids, bases and neutrals. Excellent peak shape is needed to detect and quantitate very small quantities as shown in Figures 15 and 16.

Figure 15

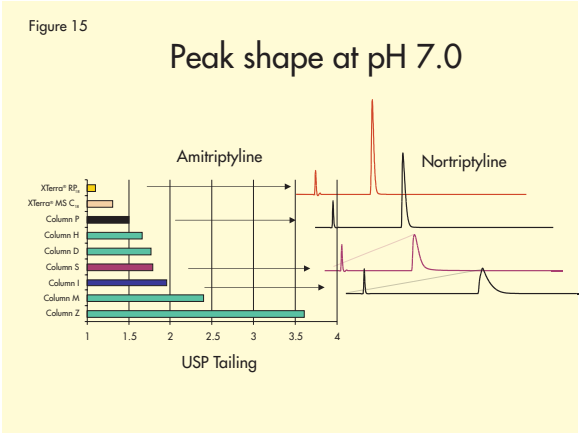
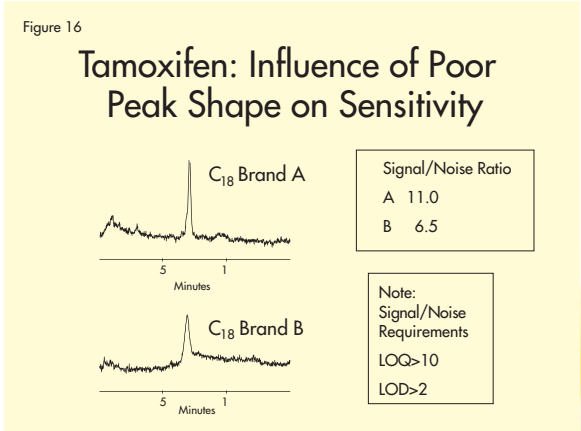


Figure 16



The Waters Method Development Kit meets all of these ideal column characteristics. Waters has two manufacturing operations in both the U.S. and Europe, which are registered with the FDA as a medical device manufacturer. Both facilities operate under cGMP and they are ISO-9002 registered.

Method Development Kit Part Number: 186001100

Now you can start developing robust analytical methods by ordering the Waters method development kit which includes four Waters HPLC columns and a comprehensive applications book with detailed applications on the following compounds:

- Diltiazem hydrochloride
- Fluconazole
- Lansoprazole
- Paclitaxel
- Pravastatin sodium
- Risperidone
- Simvastatin
- Terbinafine hydrochloride
- Zidovudine

The Method Development Kit contains:

Symmetry® C_{18} column 3.5 µm 4.6 x 100mm

SymmetryShield™ RP_{18} column 3.5 µm 4.6 x 100mm

XTerra® MS C_{18} column 3.5 µm 4.6 x 100mm

XTerra® RP_{18} column 3.5 µm 4.6 x 100mm

Applications Book

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