

# Efficient HPLC Column and Mobile Phase Screening Protocol for Developing Stability-Indicating Methods: Diphenhydramine Case Study

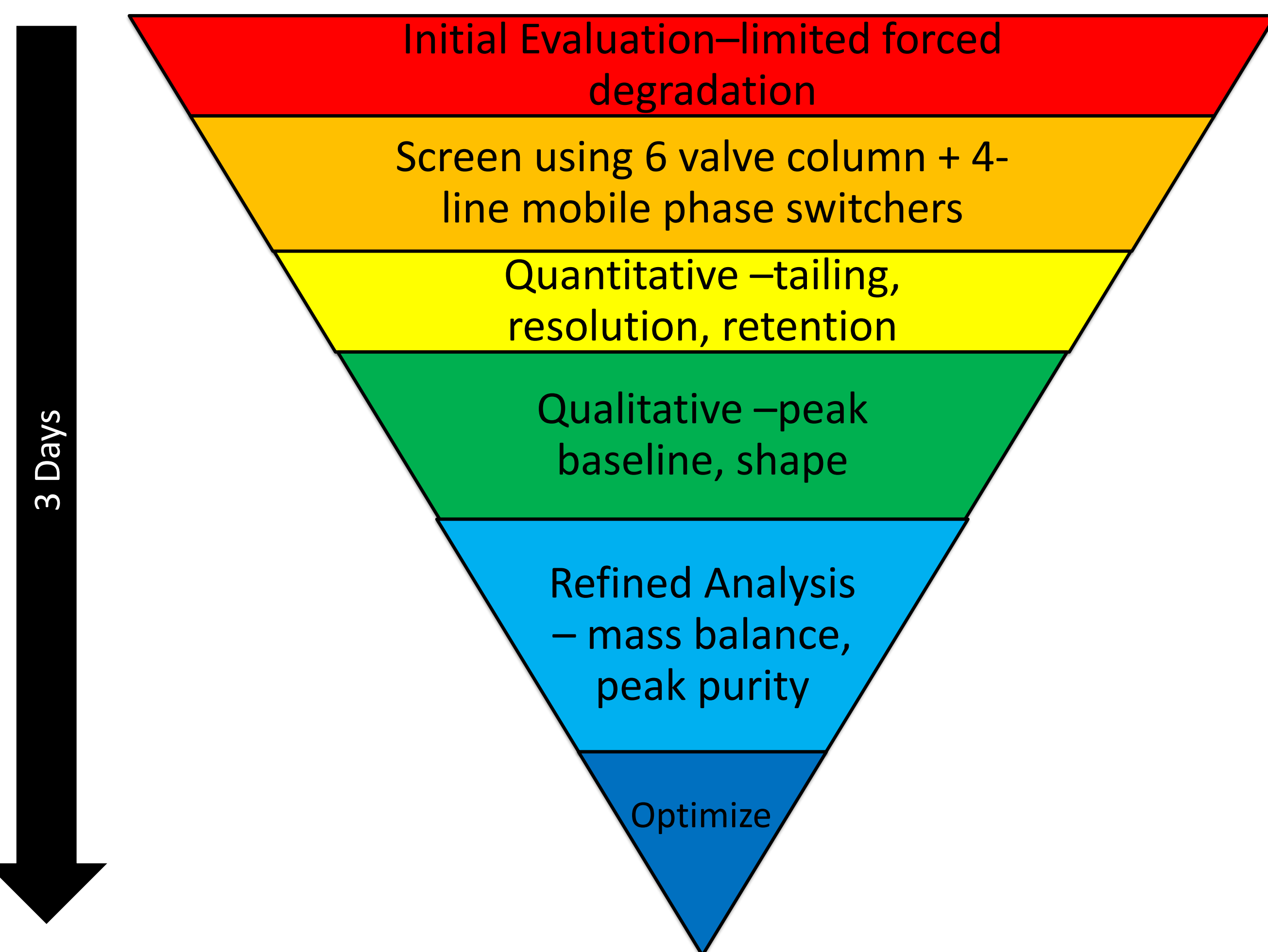
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## Background

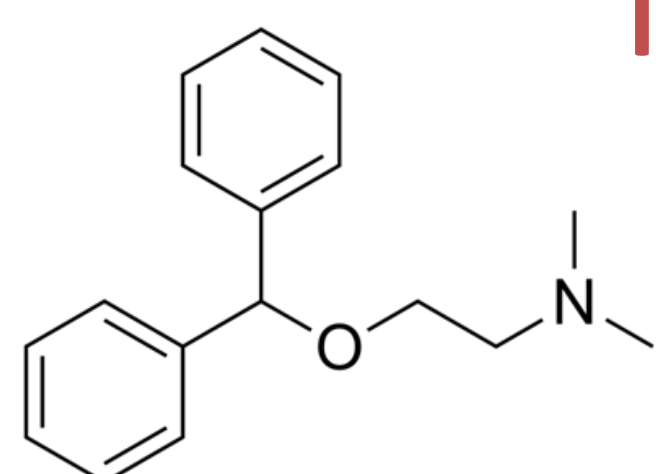
**STUDY GOAL:** Establish an efficient process for stability indicating HPLC method development

**PROCESS:** Use automated screening system with 6 HPLC columns + 4 mobile phases to generate data that enable efficient development of a stability indicating method



Case study: diphenhydramine (active ingredient in many OTC allergy medications)

## Initial Evaluation



Diphenhydramine

Functional Group	Susceptibility
Tertiary amine	Oxidation
Benzylic hydrogen	Oxidation
Ether	Acid

Acid exposure selected for initial forced degradation

## Columns and Mobile Phases

General protocol: columns with wide range of chemistries; mobile phases with a broad pH range and MS compatibility

### Columns + Mobile Phases

Columns	Mobile Phases
XSelect HSS T3	10 mM Ammonium Bicarbonate, pH 6.8
Shield RP18	0.05% Formic Acid, pH 2.5
Eclipse Plus Phenyl-Hexyl ZORBAX SB-AQ	0.1% Phosphoric Acid (50 mM Sodium Perchlorate + 0.1% Phosphoric Acid)
ZORBAX SB-C8 Symmetry C18	

## Quantitative Analysis

### Selection Criteria

Resolution	Retention	Peak Tailing	Plate Number
$R_s \geq 1.5$	$K' \geq 2.0$	$0.8 \leq T_f \leq 2.0$	$N \geq 2000$

Of the 24 combinations of columns + mobile phases, 12 selected to move forward

## Qualitative Analysis

### Selection Criteria

Retention	Elution	Baseline	Degradation
Main band + degradants <20 and >5 min	No coelution of peaks	No baseline drift, and low baseline noise	Parent + degradants separated

4 selected to move forward

## Refined Analysis

### Further Criteria

Mass Balance	Peak Purity
Mass balance $\geq 90\%$	Peak angle < Peak threshold

One selected to move forward

## Method Optimization

### Parameters Adjusted for Optimization

System flow rate	Gradient	Column temperature	Column particle size and length
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Following method optimization, a full forced degradation panel carried out to confirm selected method is stability indicating

## Conclusions

A screening process was demonstrated that enables rapid development of a stability indicating method

- 24 possible combinations of columns and mobile phases are used in an automated screen
- The options are narrowed based first on quantitative data then using qualitative factors to ideally select a single option for optimization
- The proposed methodology was successfully demonstrated using diphenhydramine where a method meeting all requirements was developed over 3 days with 3 hours of hands-on work

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