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Excellence in Science

Method Development and Approval Process for New and Alternative Test Procedures

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EPA may consider two types of methods for approval at 40 CFR Part 136

- **Alternative Test Procedures (ATP)**
  - Manufacturer's
  - Laboratories
  - Industries

- **New Methods**
  - Standards organizations
    - ANSI
    - ASTM
    - Standard Methods
    - USGS
    - EPA

In this presentation we are referring to "Standard" Methods

- **Technique**
  - Scientific Principle, such as GCMS
- **Method**
  - Adaptation of a technique to a measurement problem
- **"Standard" Method**
  - A method of known precision issued by a standardization organization

STANDARDMETHODS

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

ASTM INTERNATIONAL
Standards Worldwide

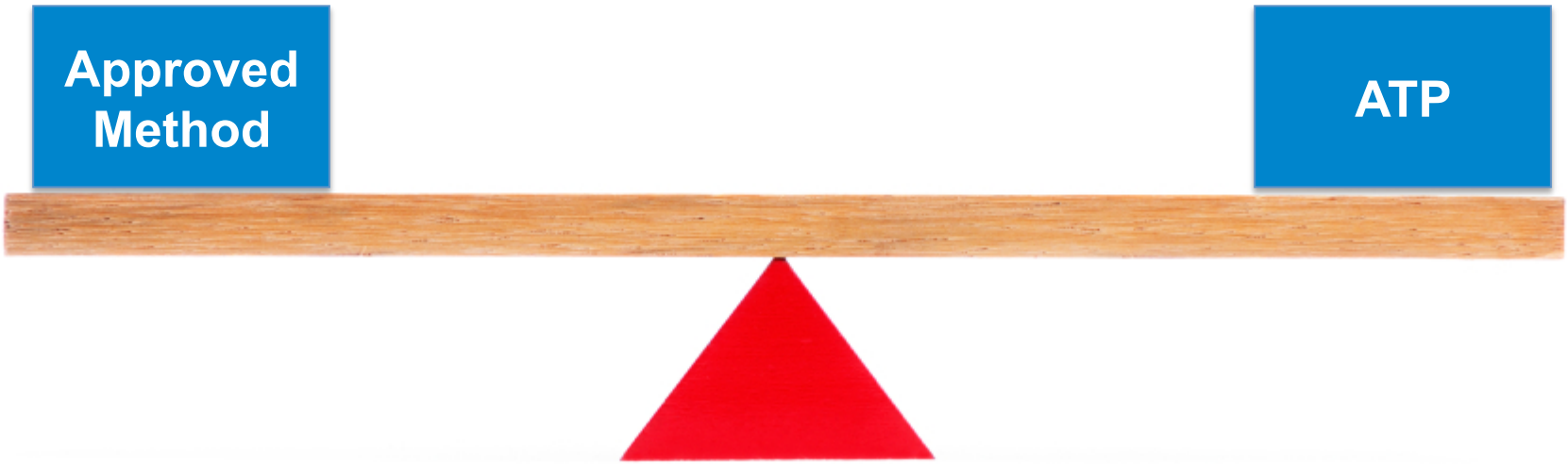
There are also “rational” methods and “empirical” methods

- **Rational**
  - **Same result by other techniques**

- **Empirical**
  - **“Method Defined”**
  - **Different techniques get different results**

Validation “Alternative Test Procedures”

- **Equivalency**
  - Same result as approved method (interference free)
  - Same QC
  - Same detection (wet chemistry)
  - Same extraction (GC)



Validation of the ATP

- Calibration
- MDL
- DOC
- Single Lab Study (matrices)
  - Precision
  - Spike Recoveries
- Multi-Lab study
  - Precision
  - Spike Recovery

Checklist

Validation of a New Method

- Preliminary Literature Search
- Design Phase
- Development Phase
- Validation Phase
- Evaluation Phase

The validation phase is the most overlooked part of method validation

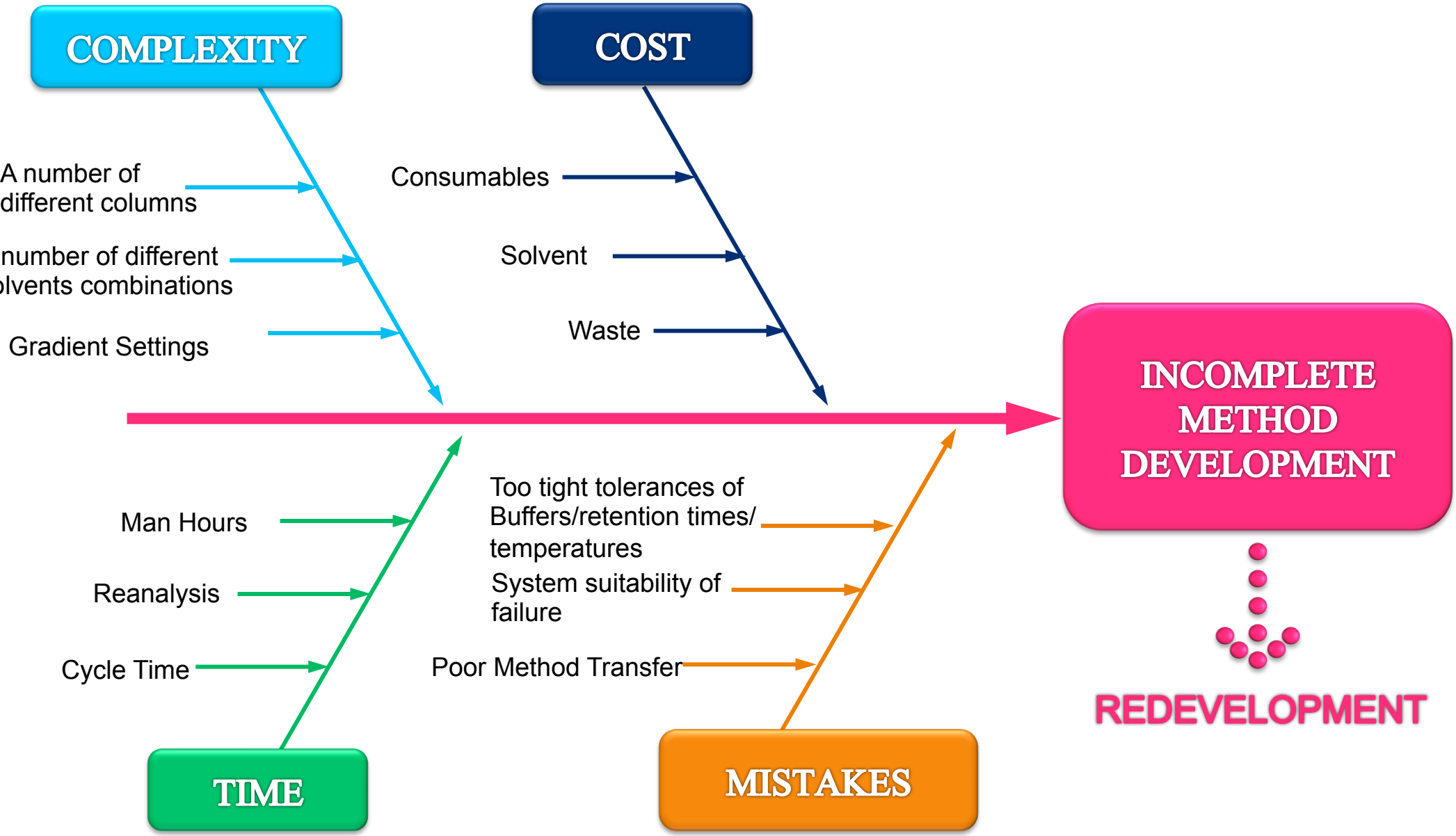
- Start with literature and write a “method”
- Design
  - Scope
  - Summary
  - Technique
  - Matrices
  - Concentration Range (estimate)
- Development Phase
  - Proof of concept
  - “preliminary data”
- Skip to Evaluation (inter-lab study)
  - If done at all

The Validation phase includes collection of the following information:

- **Selectivity**
  - Correctly ID analyte in matrices
- **Calibration**
  - Technique and model
- **Repeatability**
  - At a range of concentrations
  - In numerous matrices
- **Bias**
  - Compare to known matrices
  - Spike samples
  - Compare to other techniques
- **Ruggedness**
  - What can change results

A look at HPLC method development/validation

HPLC Method development/validation limiting factors



There are thousands of potential columns

Over 10,000 columns available

Manufacturers

Column Options

Manufacturers

- Restek
- Agilent
- Phenomenex
- Supelco
- Thermo
- MacMod
- Waters
- Agela
- YMC
- Sepax
- ES Industries
- Zirchrom
- Shinwa
- Tosoh
- Grace
- Shodex

Particles

- 5u FPP
- 3u FPP
- 2.Xu FPP
- 1.Xu FPP
- 5u SPP*
- 3u SPP*
- 2.Xu SPP*
- 1.Xu SPP*
- Monolithic
- Large Pore
- Polymer
- Polymer Hybrid

Stationary Phase

- C18
- Aq C18
- C8
- Phenyl
- Polar
- Cyano
- Amino
- PFP
- Si
- Ion Exchange
- HILIC
- Chiral

*SPP = Superficially Porous Particle = Fused Core = Core/Shell

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- C8
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- nge
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Is this going to be the best one???
How do we know?
Did we check every option?

*SPP = Superficially Porous Particle = Fused Core = Core/Shell

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*SPP = Superficially Porous Particle = Fused Core = Core/Shell

There are hundreds of different Mobile Phase combinations

Reverse Phase Solvents

Normal Phase Solvents

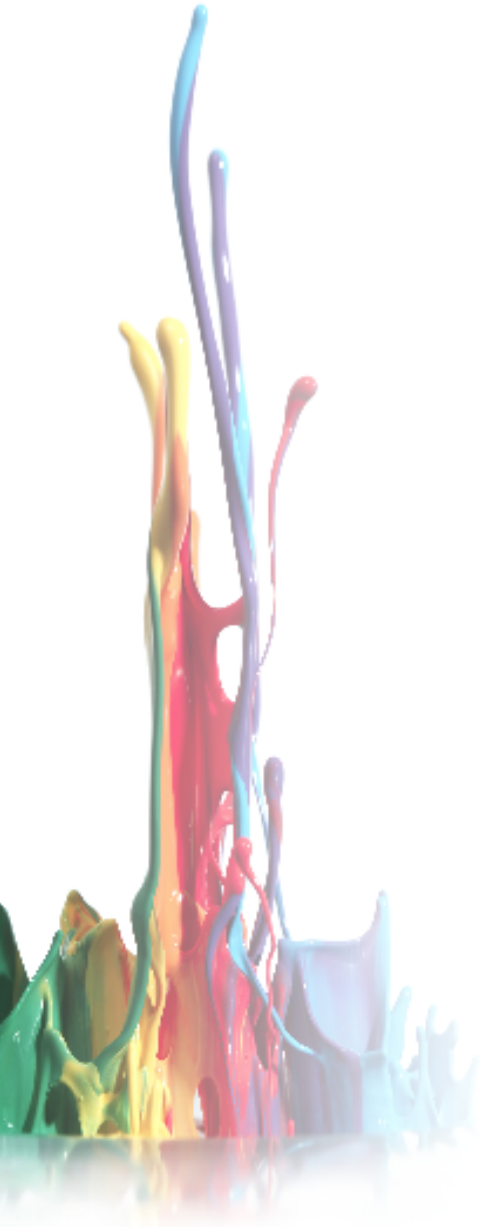
Over 30 solvents available

The Short List – 6 Pairs

Water
Water + Formic
Water + Ammonium Acetate
Acetonitrile
Methanol

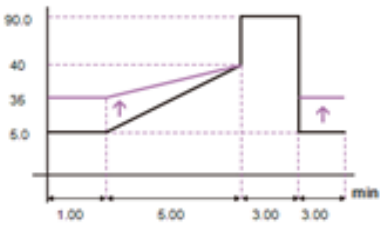
A: Water B: Acetonitrile
A: Water B: Methanol
A: Water/Formic B: Acetonitrile
A: Water/Formic B: Methanol
A: Water/Ammonium Acetate B: Acetonitrile
A: Water/Ammonium Acetate B: Methanol

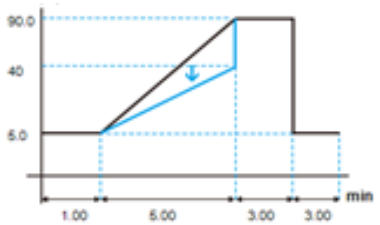
There are hundreds of possible gradients

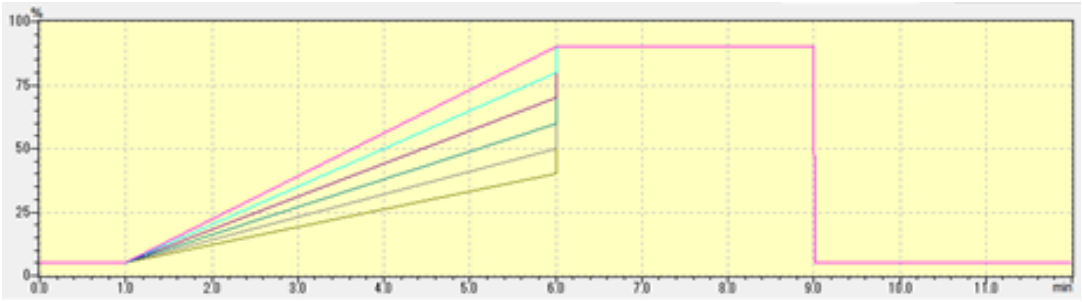


Gradients

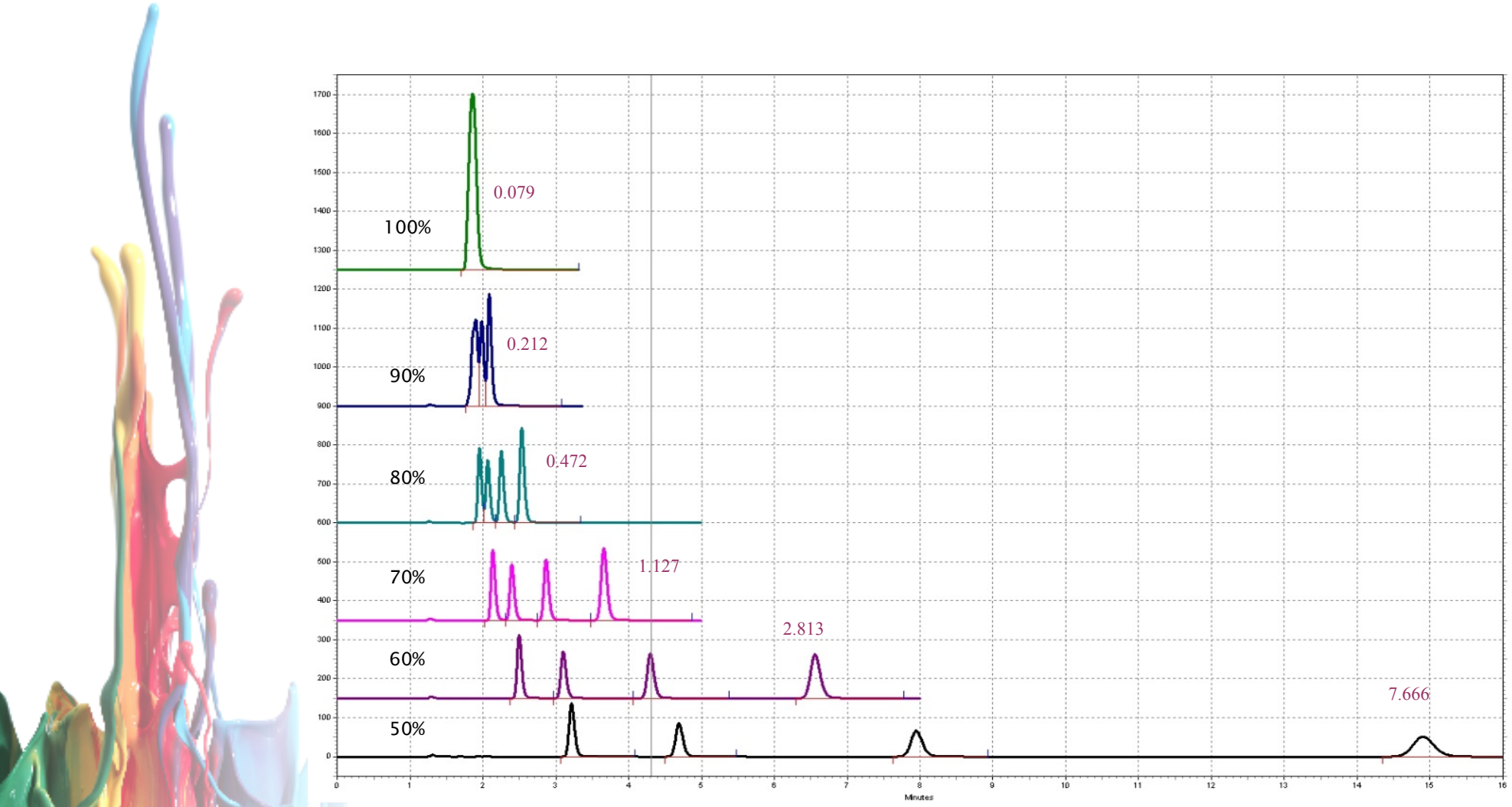
Scouting Gradients 5 – 95% etc.
Gradient based on 1st and last peak
Isocratic test/check
Steps added for critical pairs
Initial conditioned honed in, capacity factor calculated
Column clean up added and equilibration step
Robustness testing







The effect of different percentages of solvent



The following slides demonstrate what is necessary to determine correct method conditions

1 Column

+

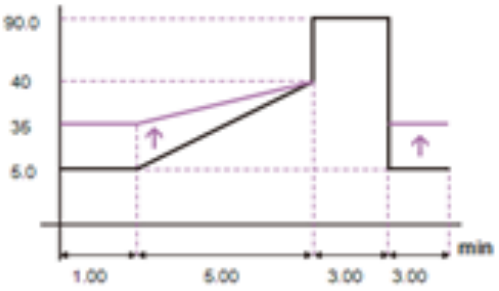
1 Mobile Phase Pair

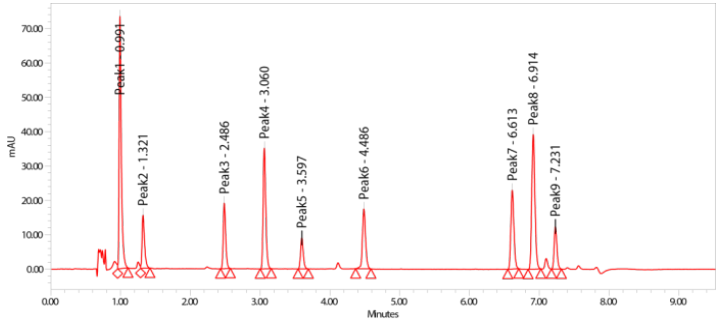
+

Pair

1
Gradient

1 Result





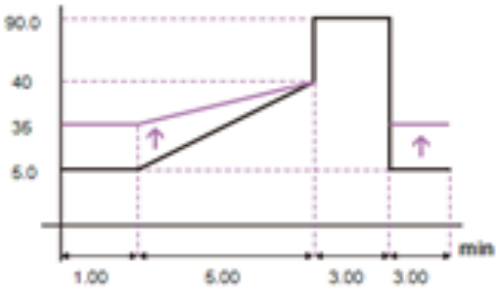
1
Column

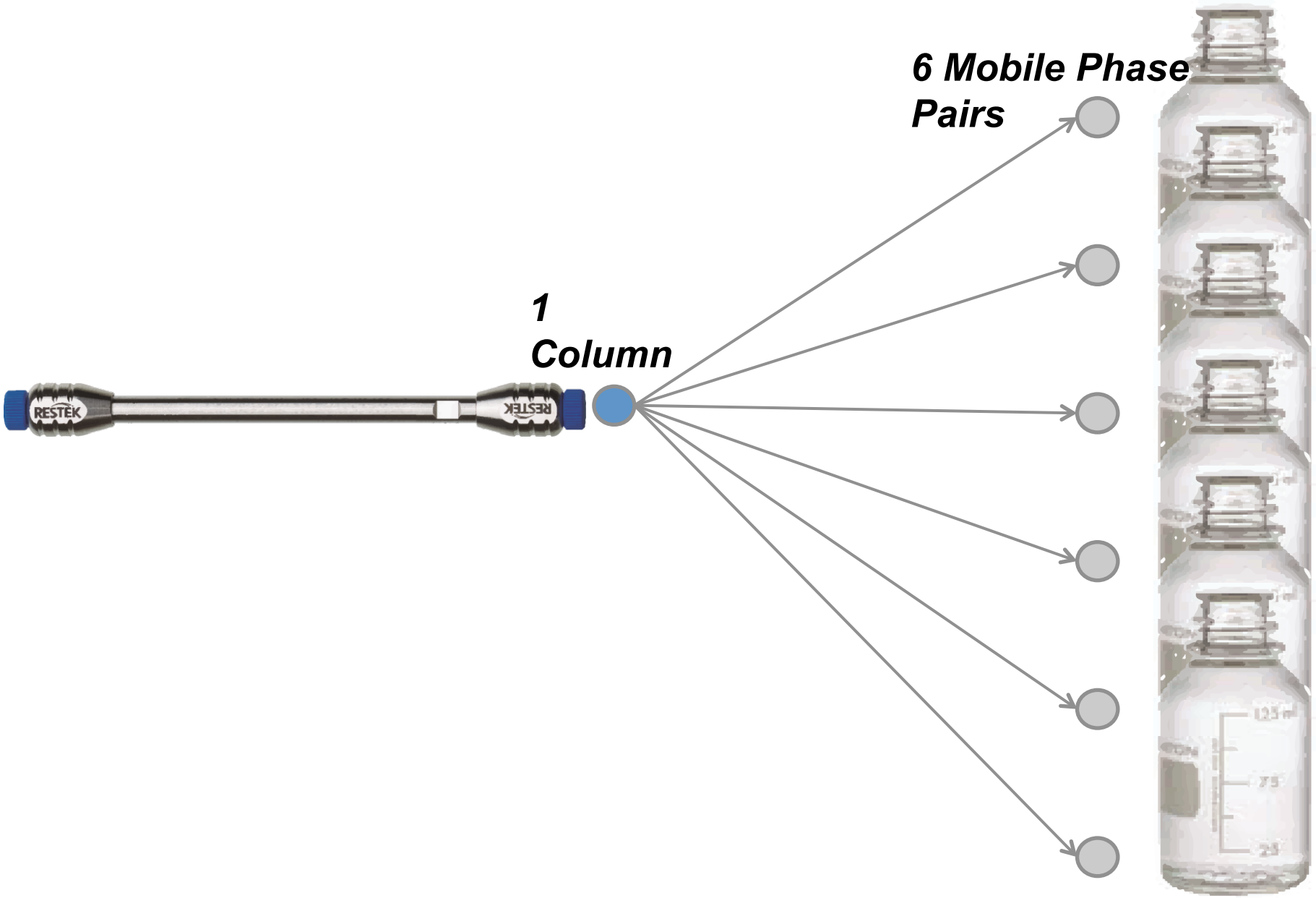
+

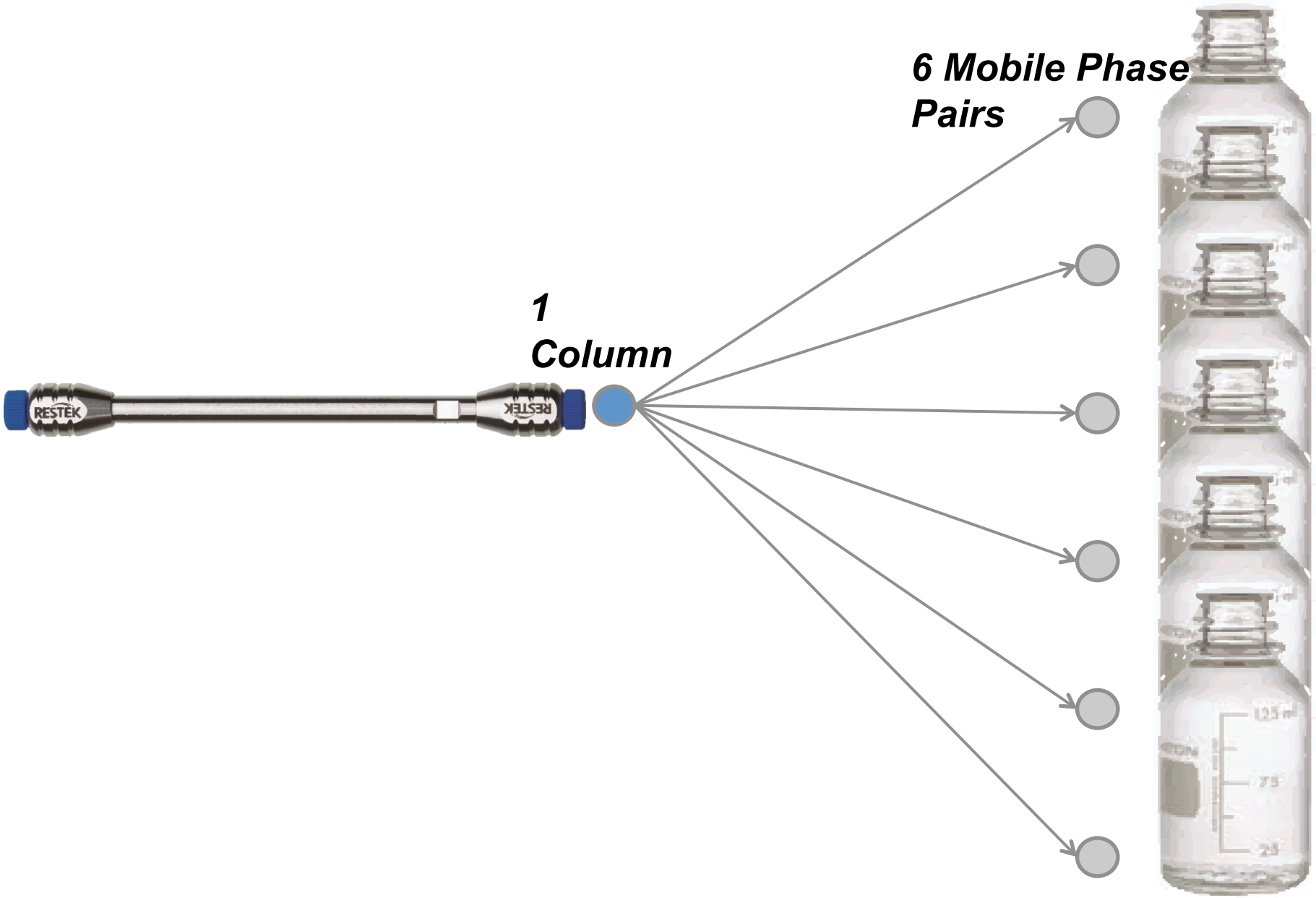
1 Mobile Phase Pair

+

1
Gradient




6 Mobile Phase Pairs
1 Column
RESTEK
RESTEK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1 Column
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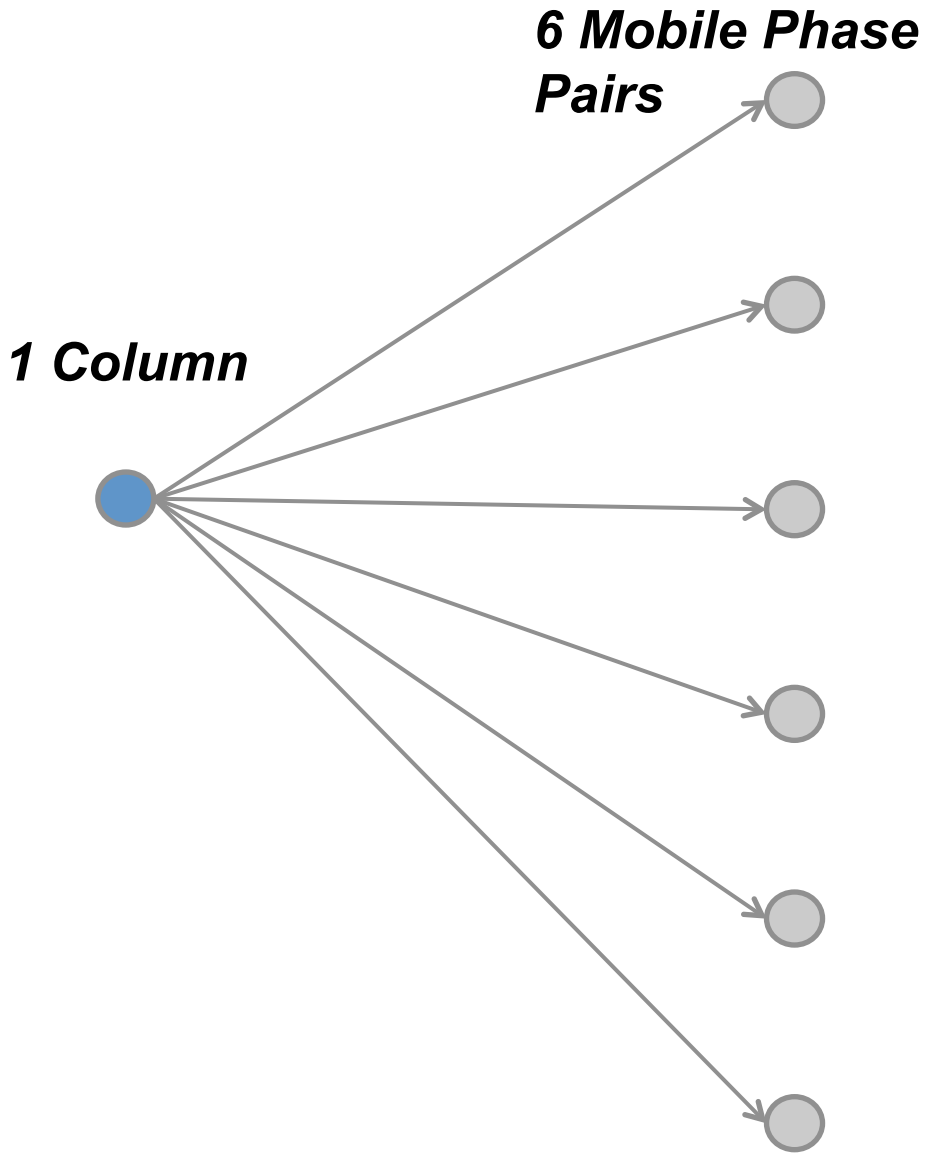
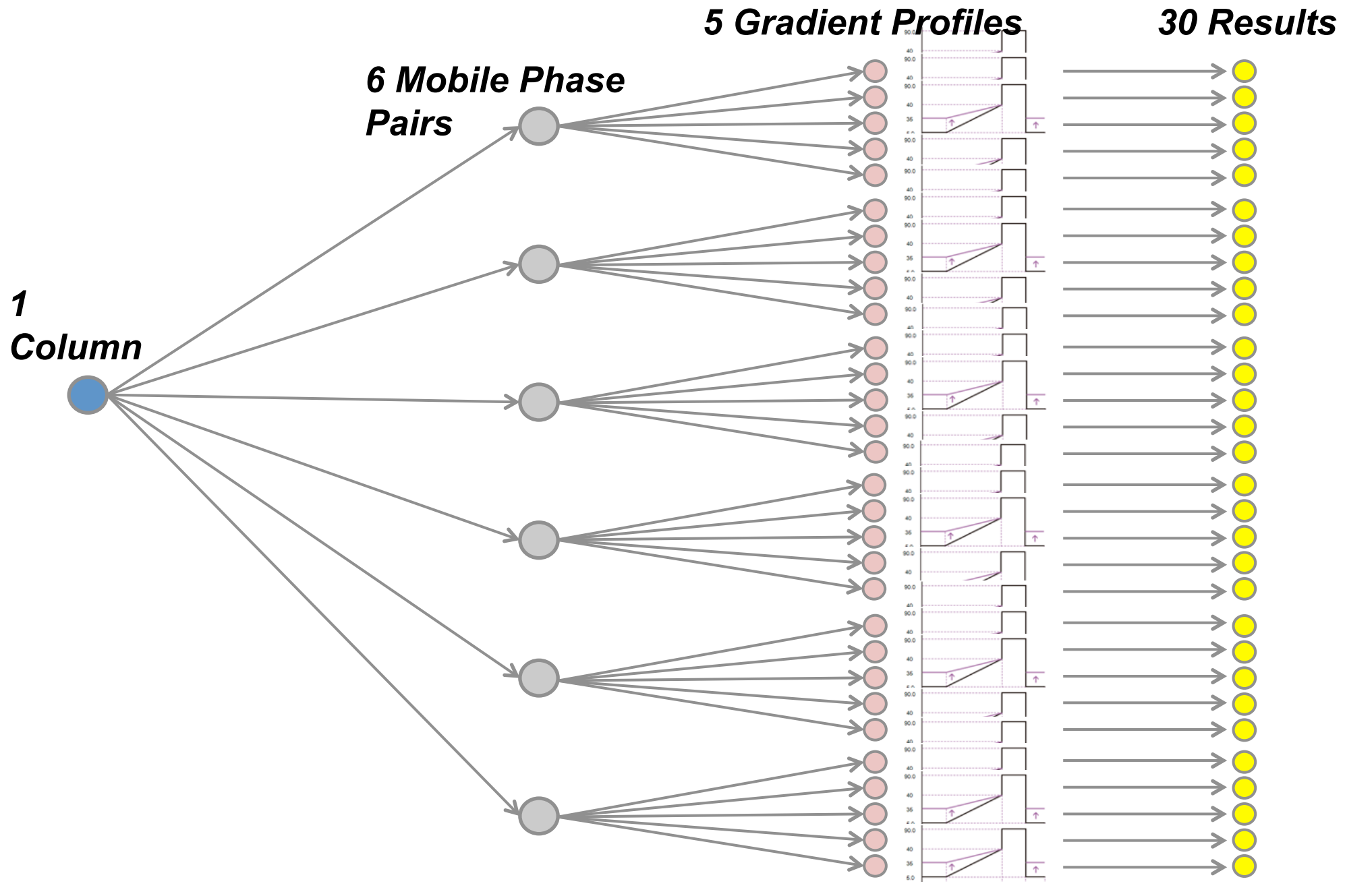
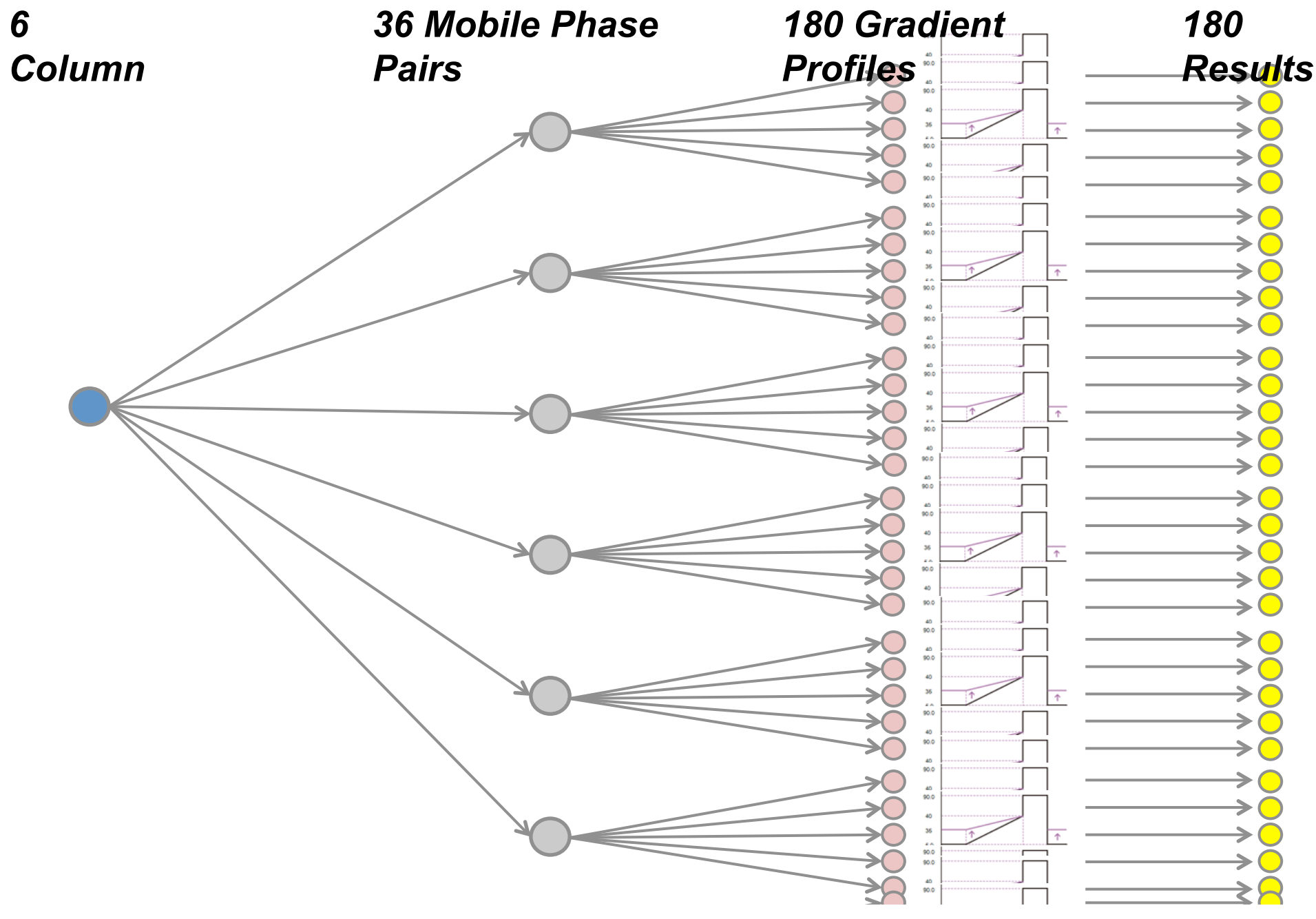

6 Mobile Phase Pairs
1 Column


5 Gradient Profiles
30 Results
6 Mobile Phase Pairs
1 Column


6
Column
36 Mobile Phase
Pairs
180 Gradient
Profiles
180
Results

This Degree of Method Development is important in validating nationwide/international standards

However, this level of testing is neglected. Generic Defaults are used which result in other issues down the road…

Validation of EPA 527 Pesticides and Flame Retardants

- Solid Phase Extraction GCMS Method

- Literature Search
  - A total of 45 candidate parameters chosen
    - Solubility
    - Purity
    - Applicable to scope of method (complexity)

The Validation studied 6 different SPE media

- Recovery in reagent water
  - Target analytes
  - Surrogates
- Recovery in 3 matrices (after optimization)
  - Preserved
  - De-chlorinated

The Validation studied the effect of 4 different pH buffers

- pH 3.8
- pH 5.0
- pH 7.0
- pH 9.0
- No buffer

Fortified, extracted, analyzed

More stable at pH 3.8

The Validation studied the effect of 4 different de-chlorination agents

- Ascorbic Acid
- Sodium Sulfite
- Sodium Thiosulfate
- Glycine
- None added

Spiked with Cl_2, Fortified, extracted, analyzed

Ascorbic Acid chosen

The Validation determined the holding time

- Tap water
  - spiked
  - De-chlorinated
  - Max temperature allowed

14 days chosen

The Validation determined the effect of trace metals

- 4 sets of samples buffered at pH 3.8
  - with Cu and no EDTA
  - With no Cu and no EDTA
  - With Cu and EDTA
  - With no Cu and with EDTA

Needs EDTA

Hidden Long Term Costs of a Sub-Optimal Method Validation

- ***Method fails on real samples***
- **Questions come rolling in**
- **Standards organization gets “black eye”**

If you evaluate methods thoroughly then, you:

- Reduce Human Error
- Develop robust method
- Minimize effects of an inferior method

Thank You, for more information contact me

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