Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 280–292

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A systematic approach to development of liquid chromatographic impurity methods for pharmaceutical analysis

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ARTICLE INFO

Article history:
Received 11 March 2011
Received in revised form 13 May 2011
Accepted 13 May 2011
Available online 20 May 2011

Keywords:
HPLC
Method screening
Strategy
Software control
Pharmaceutical

ABSTRACT

A strategy for developing chromatographic methods designed to determine impurities and degradation products in active pharmaceutical ingredients and drug products is presented. Selectivity is achieved by evaluating a chromatographic space comprised of 12 stationary/mobile phase combinations. Stationary phases predicted to be orthogonal based on their hydrophobic subtraction model parameters used. The particle sizes, column dimensions, and gradient times chosen provide high peak capacities and allow operation at backpressures that can be achieved with standard instrumentation. The mobile phases utilized are compatible with MS detection and cover a wide range of pH, solvent strength, and solvent selectivity. Analyte detection is accomplished using a combination of diode array and mass spectroscopic detectors which allow mixtures of project compounds to be injected and selectively detected. Automation of data acquisition and processing is accomplished using AutoChrom software from ACD\Labs. The strategy is illustrated with detailed data from two case studies and summary data from nineteen pharmaceutical projects.

1. Introduction

There is continued pressure within the pharmaceutical industry to reduce cycle times and increase the number of products that make it to market. Analytical chemists responsible for developing chromatographic methods have responded to this challenge by implementing strategic approaches that consist of standardized programs to identify the key components of a small molecule, reversed-phase HPLC method (e.g. stationary phase, mobile phase, and gradient profile) [1–5]. It is through these standardized experimental plans that the chromatographer is able to rapidly identify key method parameters and then is able to move on to method optimization which generates the finalized method conditions. More recently these programs have been refined to incorporate higher efficiency columns (those packed with smaller particles) and/or instrumentation capable of operating at higher pressure. There are several published reports where these technologies have been applied to the development of methods for pharmaceutical analysis [6–8]. The incorporation of higher pressure instrumentation into the entire development through commercial network (e.g. development lab, contract research lab, and multiple quality control release labs), may be difficult and is something that in most cases does not occur quickly. This was a design feature as this strategy was developed and implemented. In addition, software tools have been developed and incorporated to automate parts of the HPLC method screening and development process [9–13].

We previously published an HPLC method development strategy that utilizes four stationary phases, two organic modifiers, two pH modifiers, and two gradient profiles [14]. This paper builds on the previous effort through the incorporation of more efficient columns packed with smaller particles and software tools to enable data collection, signal processing, and decision making. The stationary phases employed in this screen were selected to cover a broad range of selectivity as demonstrated by differences in hydrophobicity, steric resistance, hydrogen-bond acidity, hydrogen-bond basicity, and cation-exchange capacity [15–20]. Our experiences with our original strategy indicated that separations performed at high pH were among the most successful column/mobile phase combinations in the experiment screen space, though the ruggedness of the Xterra MS C18 was poorer than desired. An evaluation of the XBridge C18 indicated that it would provide similar selectivity and enhanced ruggedness compared to the Xterra MS C18. In addition, the XBridge Phenyl phase performance indicated that it would provide selectivity similar to that of the Ace Phenyl phase at low pH and additional selectivity when used with the high pH mobile phases. This replacement was made and the screen space was expanded from eight to twelve column/mobile phase combinations by adding two experiments at high pH with the XBridge Phenyl phase and two experiments at low pH with the XBridge C18 phase. Other modifications included enhancements to peak capacity while maintaining the ability to perform the method with conventional HPLC instrumentation (400 bar pressure limit). This

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doi:10.1016/j.jpba.2011.05.028

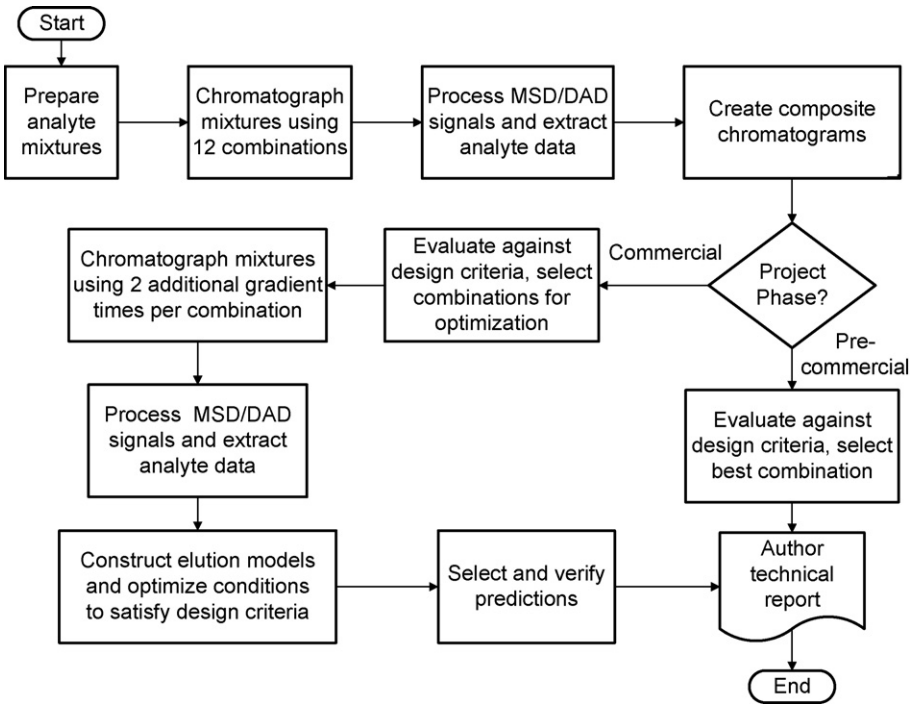


Fig. 1. Impurity method development workflow.

was accomplished by replacing the 3.5 μm Zorbax SB-C8 and Bonus RP phases with columns containing 1.8 μm particles, which results in a doubling of peak capacity for these screen conditions. The XBridge C18 and Phenyl phases were not available in a sub-2 μm particle and 4.6 mm ID column format. Therefore, the revised strategy is performed using these phases in a 2.5 μm particle size that yields peak capacity increases of approximately 40%. The selected column format (4.6 mm id × 75 mm) and particle sizes (1.8 μm and 2.5 μm) are amenable to both HPLC and UHPLC platforms. Maximum backpressures on a conventional HPLC under column screen conditions do not exceed 350 bar. Results obtained with these column configurations demonstrated that separation performance is not compromised when columns are utilized on a UHPLC instrument platform at higher flow rates to shorten analysis times. Originally trifluoroacetic acid had been used as a low pH modifier, but due to mass spectrometry ionization suppression issues, a more MS friendly modifier (formic acid) has been incorporated. In the previous version of the HPLC screening strategy, data were collected at two different gradient times (9.5 and 38.1 min) for every column/mobile phase combination. These data enabled the construction of elution models for each separation system. Given that approximately 75% of column/mobile phase combinations did not provide the needed selectivity, considerable resources were consumed collecting data on non-viable separation systems. In this revision, the screen is accomplished for all columns and mobile phases using a single 15 min gradient for each column/mobile phase combination. Those conditions that exhibit the best performance as judged by the design criteria are promoted to a second wave of experiments that are focused on optimizing separation conditions. Performing the screening and optimization activities separately shortens overall cycle time, minimizes mobile phase consumption and data processing, while effectively identifying those column/mobile phase combinations that are most suitable for further development.

The revised strategy is enabled by a suite of software tools and a clearly defined workflow is illustrated in Fig. 1. The software provides full instrument control with automated sequence execution, automated signal processing with reconciliation between detectors and test preparations, retention modeling and predictive simulation of chromatographic separations, and a single user interface that allows the user ready access to the project data.

In the remainder of this paper, the details of the HPLC screen are presented as well as results from two case studies that further illustrate the approach.

2. Experimental

2.1. Materials and reagents

HPLC grade water, acetonitrile, and methanol were purchased from Burdick and Jackson (Muskegon, MI). Formic acid (98% purity) was supplied by Sigma-Aldrich (St. Louis, MO). ACS reagent-grade ammonium hydroxide solution (28.0–30.0% w/w in water) was obtained from Mallinckrodt (Phillipsburg, NJ). Formic acid solutions were prepared at 27 mM in water, acetonitrile, and methanol by adding 1 ml of acid to 1 l of solvent and mixing well. Ammonium hydroxide solutions were prepared at 22 mM in water, acetonitrile, or methanol by adding 3 ml of base to 1 l of solvent and mixing well.

Test mixtures were prepared using the active pharmaceutical ingredients and their potential synthetic impurities by dissolving and diluting the analytes in acetonitrile and water (1:1, v:v). Degradation products were prepared by stressing the active pharmaceutical ingredients as indicated in the discussion of example projects that follows.

The Zorbax SB C-8 (4.6 mm id × 75 mm, 1.8 μm) and Zorbax Bonus RP (4.6 mm id × 75 mm, 1.8 μm) columns were obtained from Agilent Technologies (Palo Alto, CA). The XBridge C18 (4.6 mm id × 75 mm, 2.5 μm) and XBridge Phenyl (4.6 mm id × 75 mm, 2.5 μm) stationary phases were obtained from Waters (Milford, MA).

2.2. Instrumentation and software

Experiments were performed using an Agilent 1200SL series liquid chromatography system equipped with a binary pump (dwell volume = 1 ml), vacuum degasser, auto-sampler, column thermo-

Table 1
Design screen experimental conditions.

Exp #	Stationary phase	Mobile phases	Initial %organic	Final %organic	Gradient time (min)
1	XBridge C18	A1, B1	5	100	15
2	XBridge Phenyl	A1, B1	5	100	15
3	Zorbax SB-C8	A1, B1	5	100	15
4	Zorbax Bonus RP	A1, B1	5	100	15
5	XBridge C18	A2, B3	5	100	15
6	XBridge Phenyl	A2, B3	5	100	15
7	XBridge C18	A1, B2	4	81	15
8	XBridge Phenyl	A1, B2	4	81	15
9	Zorbax SB-C8	A1, B2	4	81	15
10	Zorbax Bonus RP	A1, B2	4	81	15
11	XBridge C18	A2, B4	4	81	15
12	XBridge C18	A2, B4	4	81	15

Columns were equilibrated with 10 column volumes of mobile phase at the initial gradient composition prior to sample injection

Mobile phase	Composition
A1	27 mM formic acid in water
A2	22 mM ammonium hydroxide in water
B1	27 mM formic acid in methyl alcohol
B2	27 mM formic acid in acetonitrile
B3	22 mM ammonium hydroxide in methyl alcohol
B4	22 mM ammonium hydroxide in acetonitrile

Component	Setting
Binary pump	Mobile phase flow rate 1.0 ml/min
Autosampler	Sample injection volumes ranged from 1 to 10 μl
Column compartment	Temperature maintained at 40 °C
Diode array	Wavelength range = 210–400 nm, peak width > 0.05 min, slit = 4 nm
Mass spectrometer	Ion source operated in ESI or ESI + APCI modes with conditions that favored molecular ion formation

stat, column and solvent switching valves, diode array detector (DAD), quadrupole mass spectrometric detector (MSD) with multimode source, and ChemStation software (Agilent Technologies, Palo Alto, CA). Instrument control, detector signal processing, peak tracking, data analysis/visualization, and component retention modeling were accomplished using ACD/AutoChrom for ChemStation software (ACD/Labs, Toronto, Canada).

2.3. Procedure

The screening experiments were performed by chromatographing pharmaceutical test mixtures and stress degradation solutions using the conditions described in Table 1. Elution conditions were selected to cover a wide range of solvent strength and be isoelutropic. The range of methanol composition was set at 5–100%. The corresponding range in solvent strength for acetonitrile is approximately 4–81%. Experimental execution and data collection were automated using the ACD/AutoChrom console. MSD signals were processed using the IntelliXtract tool in ACD/AutoChrom. This processing routine extracts component information from individual mass chromatograms using a sensitive peak detection algorithm. Ion peaks are clustered by retention time and individual components are defined by considering expected 12C/13C ratios, adducts, multimers, isotopes, and neutral losses. Components detected in the MSD signals are matched across the different screen experiments based on spectral similarity. The result is a table for each MSD signal that contains retention, peak width, asymmetry, and response data associated with each tracked component and their mass spectra with fragment ion assignments.

The DAD signals were processed using the LC-UV Peak Picking tool in ACD/AutoChrom. Peaks in the UV data matrix are detected, co-eluting analytes are deconvoluted, and pure component spectra and peak data are extracted from the DAD signal. Components detected in the DAD signals are matched across screen experiments based on spectral similarity and peak size. The retention, peak width, asymmetry, and response data associated with each tracked component are summarized in a table and individual component spectra are available for review.

Component data from the MSD and DAD signals in each experiment were combined into a single table of results. The redundancies that arise when individual analytes are present in multiple test solutions or respond in both detectors were removed using a reconciliation tool in the software. The retention, peak width, asymmetry, and response data associated with components tracked in the MSD and DAD signals were used to construct a composite chromatogram for each experiment that illustrates the separation of all analytes of interest to the separation.

3. Results and discussion

An essential part of the design screen strategy is the set of criteria used to evaluate quality of the chromatographic separations. Choices between the different stationary and mobile phase combinations are made by assessing selectivity, peak asymmetry, peak retention, peak distribution, and analysis time. Phase-appropriate design screen criteria are provided in Table 2. The target values designated as “Pre-commercial Phase” are suited for early phase method development where the objective is to define an impurity profile method that can be used to quantitatively characterize active pharmaceutical ingredient or drug product batches and verify that they are suitable for toxicology and Phase I–II human studies. The primary goal is to separate potential synthetic impurities and degradation products from the main component. The additional quality attributes are secondary in this phase of development. The target values labeled as “Commercial Phase” are more demanding and reflect the requirements of impurity methods designed to quantify specified impurities in the active pharmaceutical ingredient or drug product. Elution conditions satisfying these criteria are essential in the definition of impurity methods to support primary stability studies or release batches intended for use in pivotal clinical studies. Use of the design criteria is illustrated in the two examples that follow.

Table 2
Design criteria.

Quality attribute	Target value[a]	
	Pre-commercial phase	Commercial phase
Selectivity	Impurities and degradation products separated from main peak with Rs ≥ 1.5	Impurities and degradation products separated from main peak with Rs ≥ 2.0 Impurities and degradation products separated from each other with Rs ≥ 1.5
Peak symmetry	0.8 ≤ Tailing factor ≤ 1.5	0.8 ≤ Tailing factor ≤ 1.5
Peak retention	Analyte peaks separated from solvent front	k' > 1 for first eluting analyte peak
Peak distribution	Analyte peaks distributed throughout chromatogram	Analyte peaks distributed throughout chromatogram
Analysis time	≤20 min	≤20 min

Secondary criterion are indicated by shaded cells.
[a] Desired performance of optimized final method.

Table 3
Pre-commercial phase analytes.

Name	Description	Test preparation
Main peak	Active pharmaceutical ingredient	Compound mix
Imp A	Synthetic intermediate	Compound mix
Imp B	Synthetic intermediate	Compound mix
Imp C	Synthetic intermediate	Compound mix
Imp D	Synthetic intermediate	Compound mix
Deg A	Product of solution hydrolysis	pH 10 stress
Deg B	Product of solution hydrolysis	pH 10 stress and 0.1 N NaOH stress
Deg C	Product of solution hydrolysis	pH 10 stress and 0.1 N NaOH stress
Deg D	Product of solution hydrolysis	pH 10 stress and 0.1 N NaOH stress
Deg E	Product of solution hydrolysis	0.1 N NaOH stress
Deg F	Product of peroxide oxidation	Peroxide stress
Deg G	Product of peroxide oxidation	Peroxide stress
Deg H	Product of peroxide and free radical oxidation	Peroxide stress and radical initiator stress
Deg I	Product of free radical oxidation	Radical initiator stress

3.1. Pre-commercial phase example

The candidate active pharmaceutical ingredient in this example is an unsaturated carboxylic acid produced using a multi-step synthetic route. Amounts of the main component and its synthetic intermediates were combined in a single test mixture. Stress studies were conducted to identify degradation products of potential interest in the active pharmaceutical ingredient. Hydrolysis products were produced in solutions containing phosphate buffer pH 10 and 0.1 N sodium hydroxide. Active pharmaceutical ingredient oxidation by-products were generated in solutions containing 0.3% hydrogen peroxide and the radical initiator 2,2′-azobis(2,4-dimethylpentanenitrile). Table 3 describes the analytes and test preparations included in the design screen studies for this pre-commercial phase candidate. These five test preparations were evaluated using the HPLC screen experimental conditions of Table 1.

Labeled composite chromatograms containing relevant components detected in the five test preparations were created by processing the mass spectrometric and diode array detector signals from each screen experiment using the tools in the AutoChrom software. The resulting chromatograms are provided in Fig. 2. Performance characteristics for each screen experiment were summarized and compared with the design criteria to identify stationary and mobile phase combinations best suited for analysis of the active pharmaceutical ingredient and its potential impurities and degradation products (see Table 4). The Zorbax Bonus RP column used with 0.1% formic acid and methanol or acetonitrile and the XBridge C18 column used with 0.1% formic acid and acetonitrile provided acceptable separations of all components of interest from the main peak. Band spacing of individual impurities was better using the Zorbax Bonus RP column with 0.1% formic acid and methanol and this combination was judged to provide the best overall separation of these analytes.

Table 5
Commercial phase analytes.

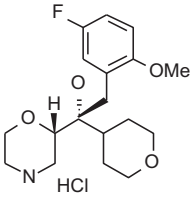
Name	Description	Test preparation
Main peak	Active pharmaceutical ingredient 	Compound mix
Imp A	Synthetic intermediate	Compound mix
Imp B	Synthetic impurity	Compound mix
Imp C	Drug product impurity	Compound mix
Imp D	Drug product impurity	Compound mix
Imp E	Drug product impurity	Compound mix
Imp F	Drug product impurity	Compound mix
Imp G	Drug product impurity	Compound mix
Imp H	Drug product impurity	Compound mix
Imp I	Drug product impurity	Stressed tablet

Table 4
Pre-commercial screening experiment results.

Separation system	Main peak resolution (pre/post)	Main peak asymmetry	Impurity resolution (critical pair)	k' (first peak)	Peak distribution[a]	Analysis time (min)
XBridge C18, low pH and acetonitrile	2.4/8.9	1.1	0.1	5.7	63	17.4
XBridge Phenyl, low pH and acetonitrile	1.4/5.7	1.2	0.3	5.6	42	15.3
Zorbax SB-C8, low pH and acetonitrile	0.9/8.4	1.0	1.1	6.1	48	16.5
Zorbax Bonus RP, low pH and acetonitrile	2.0/12.6	1.1	0.2	6.4	76	16.2
XBridge C18, high pH and acetonitrile	1.3/4.4	1.6	1.9	2.2	115	17.6
XBridge Phenyl, high pH and acetonitrile	1.4/3.3	0.9	1.7	1.0	110	15.3
XBridge C18, low pH and methanol	0.5/5.4	1.0	0.4	8.0	45	15.2
XBridge Phenyl, low pH and methanol	1.6/0.4	1.2	0.4	8.1	28	15.0
Zorbax SB-C8, low pH and methanol	1.3/0.8	1.0	1.0	8.4	31	15.0
Zorbax Bonus RP, low pH and methanol	1.7/2.5	1.1	1.2	8.4	52	15.2
XBridge C18, high pH and methanol	0.4/0.4	1.5	1.0	3.4	84	15.3
XBridge Phenyl, high pH and methanol	1.9/1.1	1.0	0.1	2.0	79	15.0

[a] Peak distribution is a measure of the deviation from equal peak resolution calculated as the sum of differences between the resolution values for every possible pair of neighboring peaks and the mean resolution of all peak pairs. Equal band spacing is indicated by low values.

3.2. Commercial phase example

HPLC screening and optimization work was undertaken to define impurity method conditions for a new drug product. The active pharmaceutical ingredient, a norepinephrine reuptake inhibitor, is formulated as a tablet with excipients commonly used in solid oral dosage forms. Potential drug product impurities identified through stress stability and excipient compatibility studies were available as authentic samples. Amounts of the active pharmaceutical ingredient and its potential drug product impurities were prepared as a single test mixture in 0.1 N hydrochloric acid/methanol (1:1 v:v). A second test solution was prepared in this sample solvent by dissolving drug product tablets that had been stressed at 40 °C/70% relative humidity for 6 months. Table 5 describes the analytes and test preparations associated with this project. These test preparations were chro-

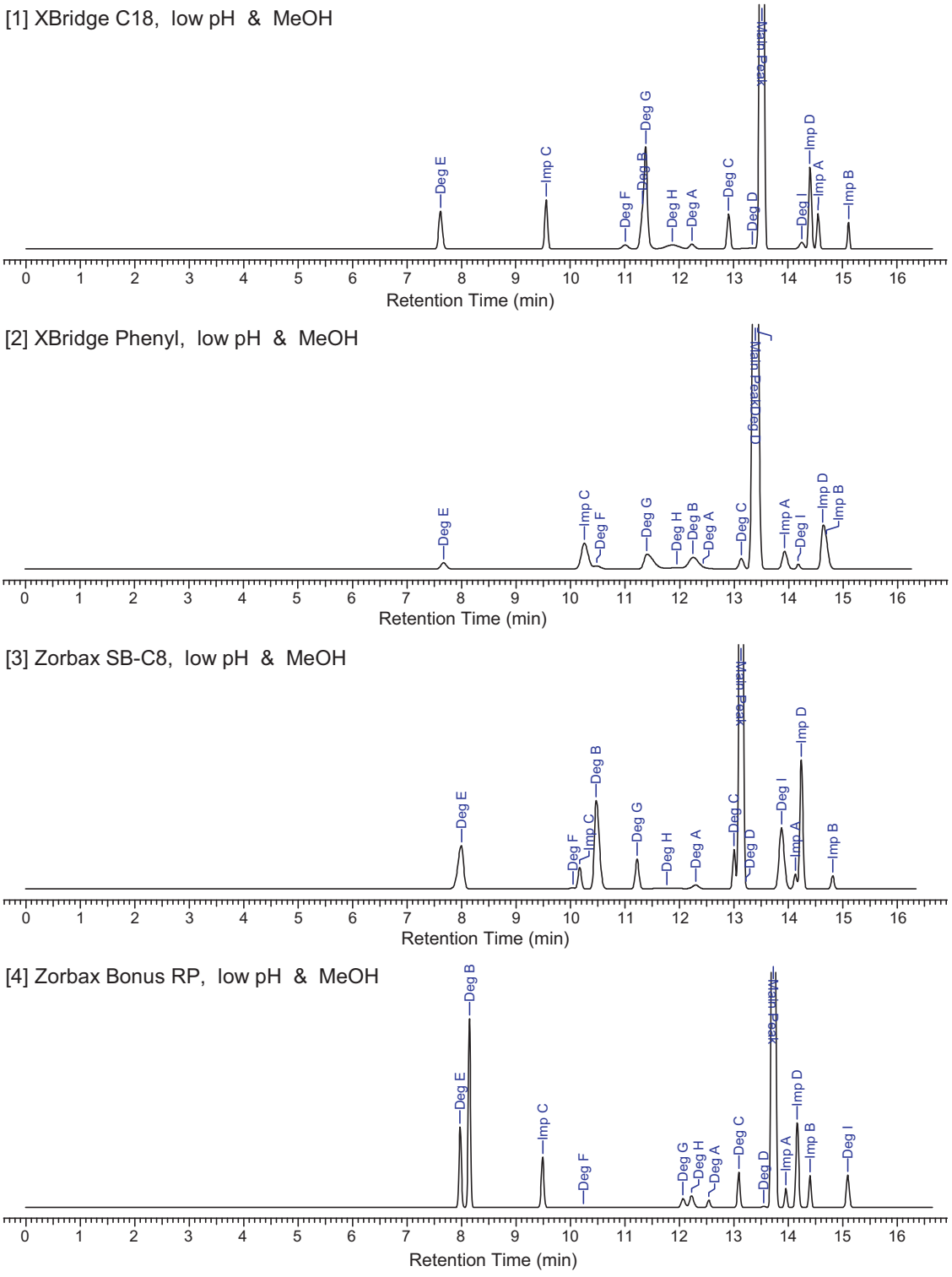


Fig. 2. Screen composite chromatograms for pre-commercial phase example (experimental conditions of Table 1).

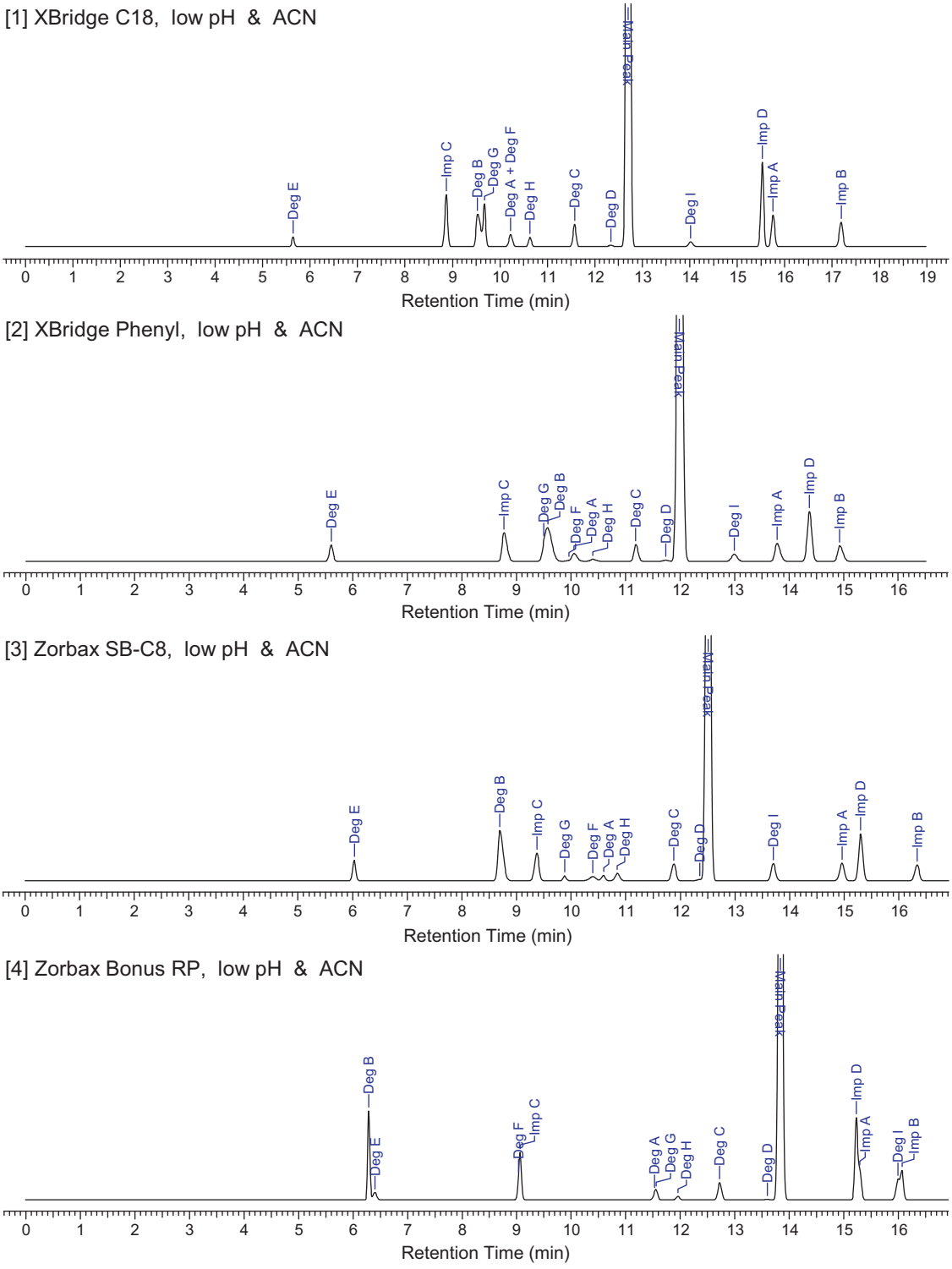


Fig. 2. (*Continued*)

matographed using the design screen experimental conditions of Table 1.

The labeled composite chromatograms of Fig. 3 containing relevant components detected in the two test preparations were created from the mass spectrometric and diode array detector signals of each screen experiment using the AutoChrom processing tools. Results for each of the design criteria used to identify stationary and mobile phase combinations best suited for analysis of these analytes are summarized in Table 6. The XBridge C18 and Phenyl columns used with 0.1% ammonium hydroxide and acetonitrile were judged to be candidates for optimization.

Additional data were generated using different gradient times as indicated in Table 7. The optimization runs on the Phenyl phase were performed at a lower column temperature to improve retention of the earliest eluting component. The resulting peak data for each component were fit with solvent strength to an elution model of the form $\ln k' = a + bB$ where B is the fraction of organic

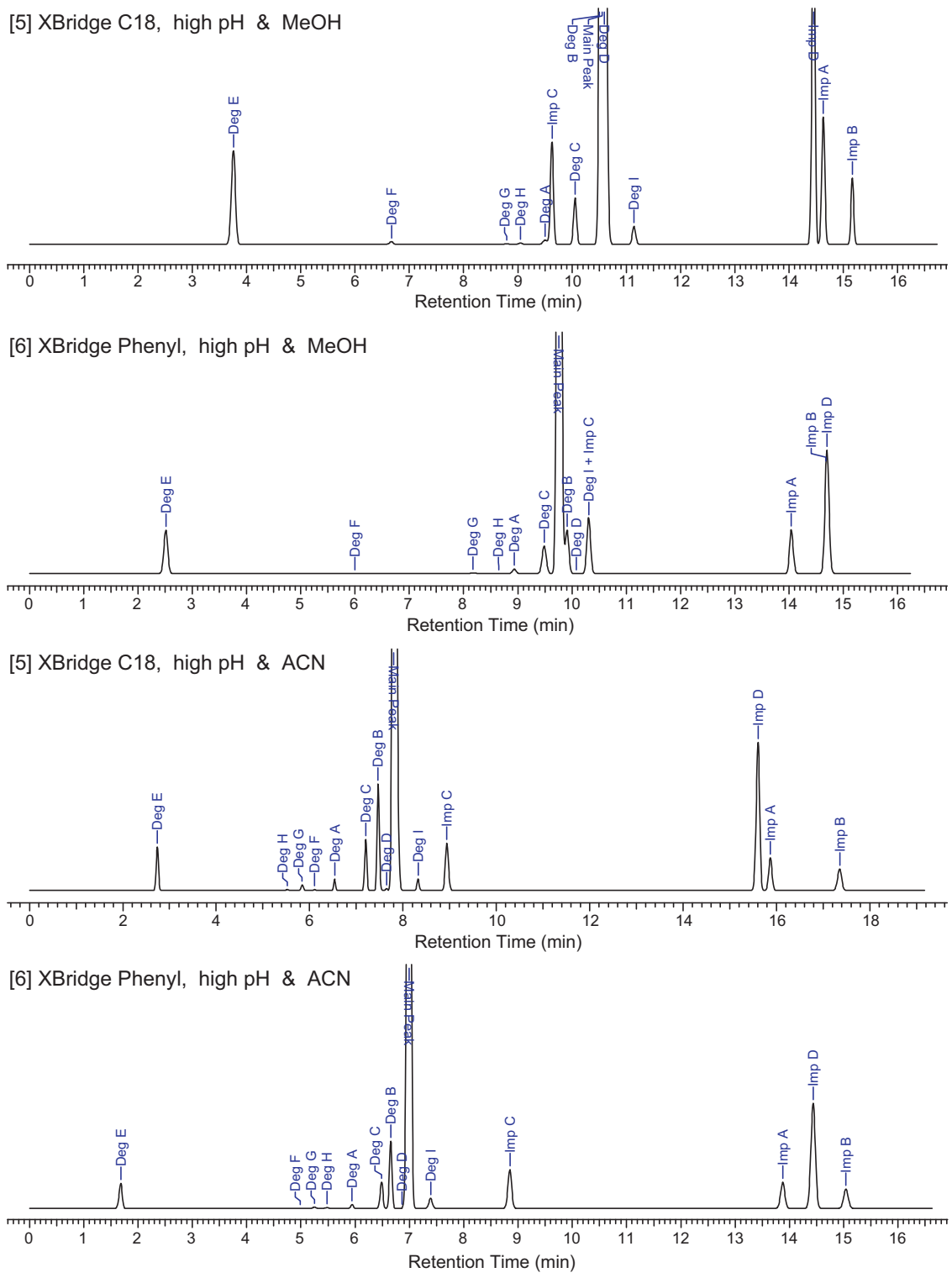


Fig. 2. (*Continued*)

modifier. The elution models for each component were used to simulate separations as a function of different gradient elution profiles. Operating conditions predicted to satisfy the design criteria were selected from simulation results and confirmed by analysis of the test preparations. Fig. 4 illustrates the optimized separations that were achieved. Table 8 summarizes performance characteristics for each of the design attributes.

3.3. Summary of project results

This design screen strategy has been utilized for nineteen development projects that encompass pre-commercial and commercial phases of development, cover a diverse range of analyte chemistry, and are of varying complexity. Descriptive project attributes are summarized in Table 9. The success achieved with each of the

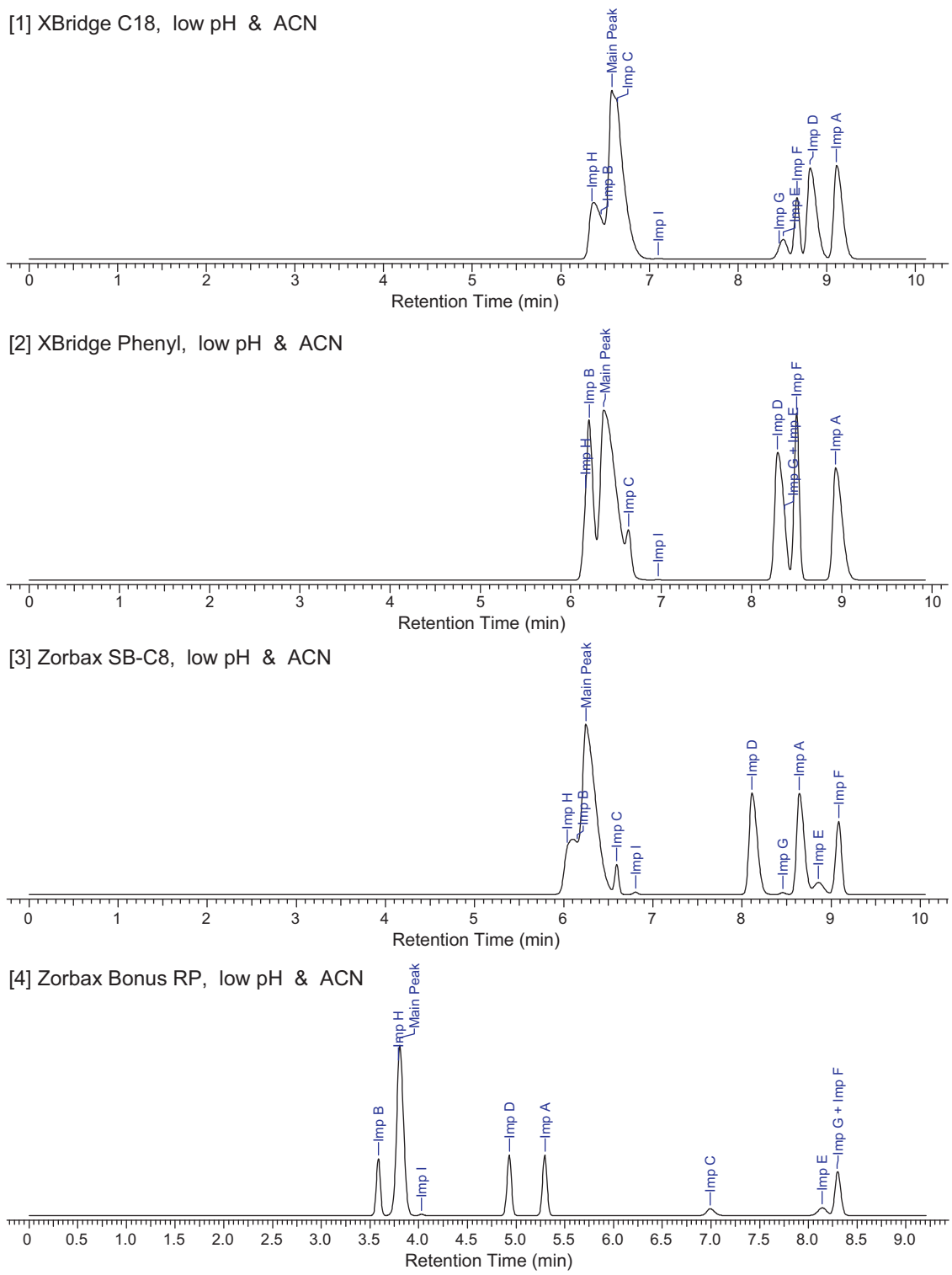


Fig. 3. Screen composite chromatograms for commercial phase example (experimental conditions of Table 1).

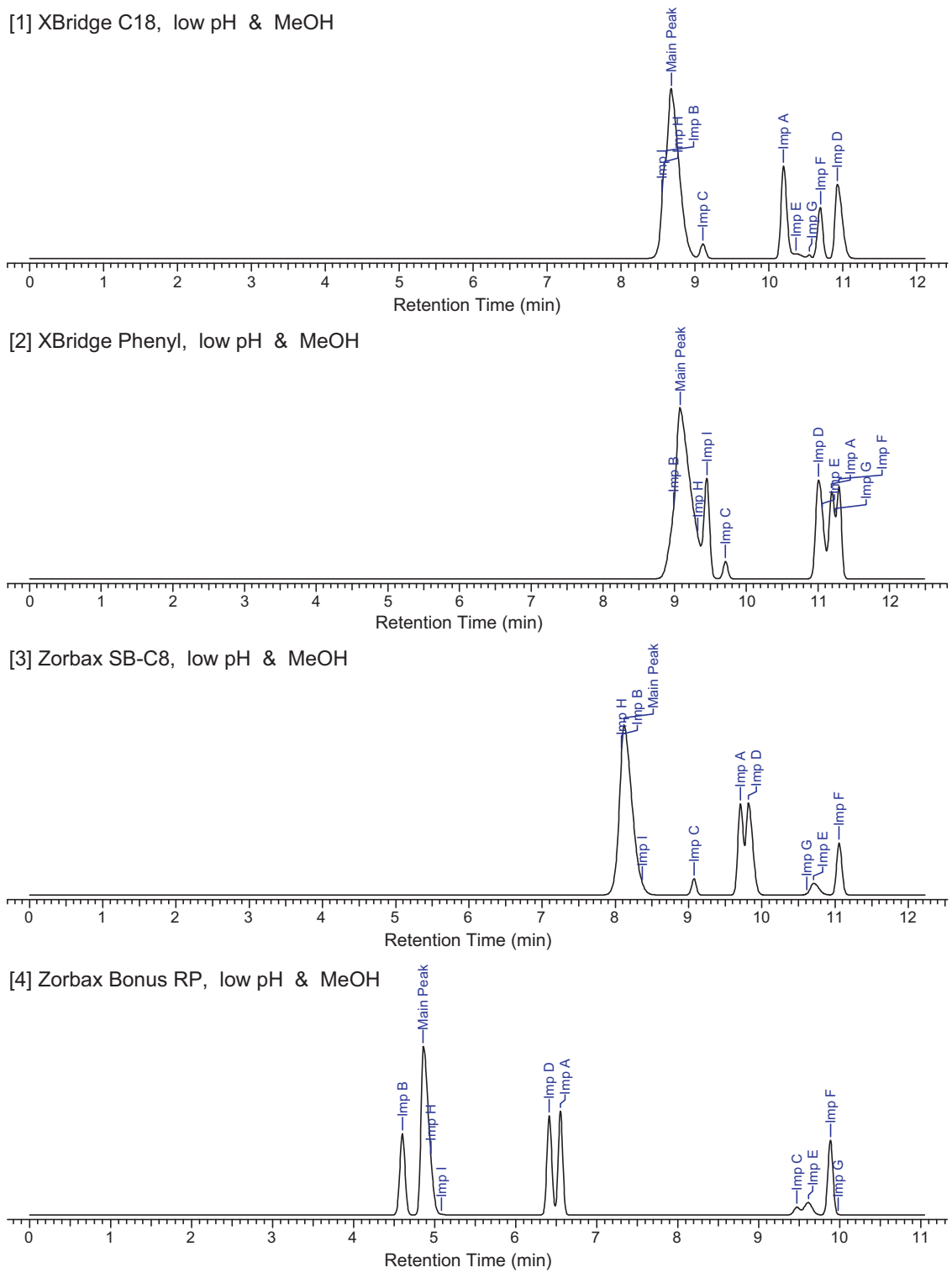


Fig. 3. (*Continued*)

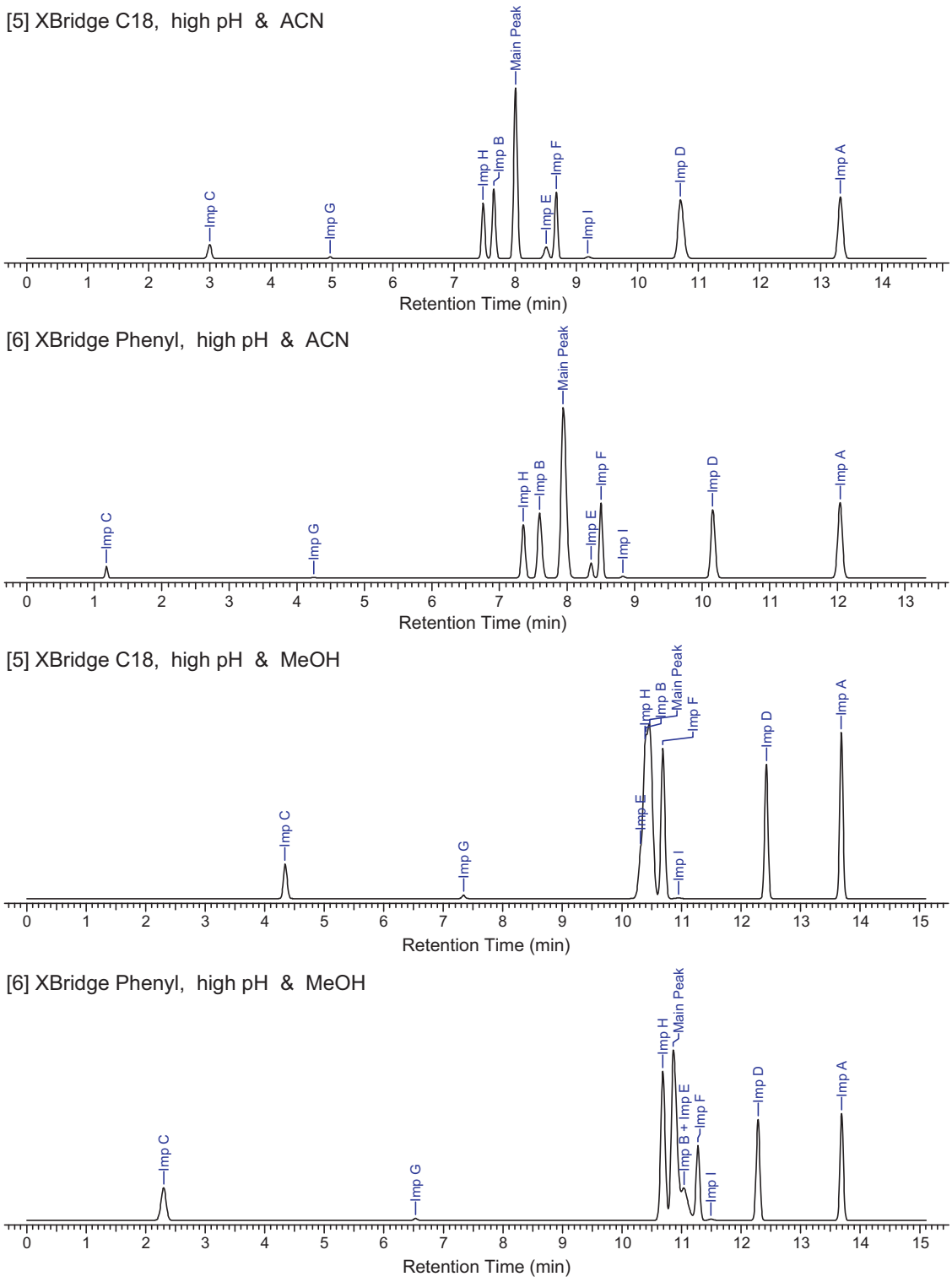


Fig. 3. (*Continued*)

Table 6
Commercial phase screening experiment results.

Separation system	Main peak resolution (pre/post)	Main peak asymmetry	Impurity resolution (critical pair)	k' (first peak)	Peak distribution	Analysis time (min)
XBridge C18, low pH and acetonitrile	0.3/0.4	4.0	0.3	6.5	20	9.5
XBridge Phenyl, low pH and acetonitrile	0.7/1.3	3.5	0.3	6.3	24	9.3
Zorbax SB-C8, low pH and acetonitrile	0.3/1.9	3.0	0.4	6.1	20	9.3
Zorbax Bonus RP, low pH and acetonitrile	0.1/2.2	1.4	0.9	3.2	39	8.6
XBridge C18, high pH and acetonitrile	3.3/4.1	1.1	1.5	2.5	72	13.5
XBridge Phenyl, high pH and acetonitrile	2.7/3.6	1.3	1.6	0.4	93	12.2
XBridge C18, low pH and methanol	0.2/2.0	2.7	0.1	9.1	13	11.3
XBridge Phenyl, low pH and methanol	0.3/0.7	2.9	0.3	9.6	17	11.5
Zorbax SB-C8, low pH and methanol	0.1/1.0	2.3	0.0	8.5	17	11.3
Zorbax Bonus RP, low pH and methanol	1.8/0.6	2.0	0.7	4.4	53	10.2
XBridge C18, high pH and methanol	0.3/1.5	1.1	0.1	4.1	84	13.8
XBridge Phenyl, high pH and methanol	1.1/0.8	1.7	0.0	1.7	100	13.8

Table 7
Optimization experiment elution conditions.

Exp #	Stationary phase	Mobile phases	Initial %organic	Final %organic	Gradient time (min)	Column temp (°C)
1	XBridge C18	A2, B4	4	81	15	40
2	XBridge C18	A2, B4	4	81	40	40
3	XBridge C18	A2, B4	4	81	10	40
4	XBridge Phenyl	A2, B4	4	81	15	30
5	XBridge Phenyl	A2, B4	4	81	40	30
6	XBridge Phenyl	A2, B4	4	81	10	30
Verify	XBridge Phenyl	A2, B4	10	60	16	30

Columns were equilibrated with 10 column volumes of mobile phase at the initial gradient composition prior to sample injection. Mobile phase flow rate was 1.0 ml/min in all experiments.

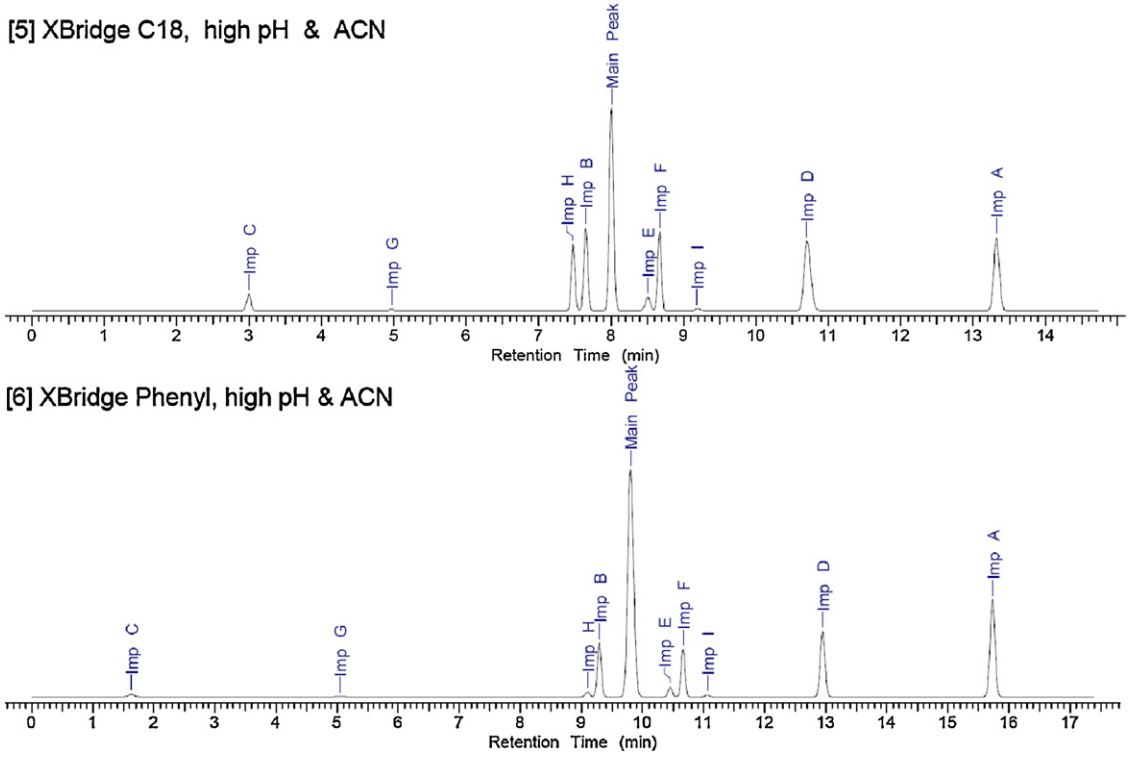


Fig. 4. Optimization composite chromatograms for commercial phase example.

Table 8
Optimization experiment results.

Separation system	Main peak resolution (pre/post)	Main peak asymmetry	Impurity resolution (critical pair)	k' (first peak)	Peak distribution	Analysis time (min)
XBridge C18, high pH and acetonitrile	3.3/4.1	1.1	1.5	2.5	72	13.5
XBridge Phenyl, high pH and acetonitrile	3.3/4.3	1.2	1.5	0.9	73	16

Table 9
Project descriptions.

Main component characteristics						Number of analyte peaks
Chemical family	Molecular mass	clog P[1]	Rotatable bonds	H Bond donor/ acceptor	pK_a[a] (most acidic/ most basic)	
Carboxylic acid	255	0.5	3	1/6	3.9/−5.5	16
Carboxylic acid	556	8.3	10	2/5	3.9/−0.4	15
Amide	566	7.6	9	2/7	4.9/−0.4	15
Amide	505	5.0	5	1/5	13.7/6.3	19
Amide	436	2.0	5	3/8	10.5/8.2	21
Nitrile	365	0.9	8	3/9	10.7/9.9	21
Amine	507	4.3	7	1/8	10.3/8.4	15
Lactam	518	4.0	6	1/8	10.7/9.0	14
Sulfonamide	439	2.5	10	2/6	11.2/0.4	20
Carboxylic acid	303	−0.1	4	1/6	3.3/8.5	8
Carboxylic acid	435	5.5	9	3/6	4.0/−3.4	14
Lactam	494	2.7	6	1/9	11.8/9.0	14
Alcohol	339	1.4	5	2/5	13.4/8.1	10
Amine	280	5.1	5	0/1	−/9.8	11
Amide	416	1.7	7	1/7	11.4/0.1	10
Amide	369	2.6	3	1/6	14.6/3.4	13
Carboxylic acid	380	0.8	4	1/6	3.1/8.4	14
Sulfonamide	512	3.4	10	3/9	8.4/9.2	21
Amine	255	3.8	6	1/2	−/9.8	11

[a] Values calculated from chemical structure (ChemAxon).

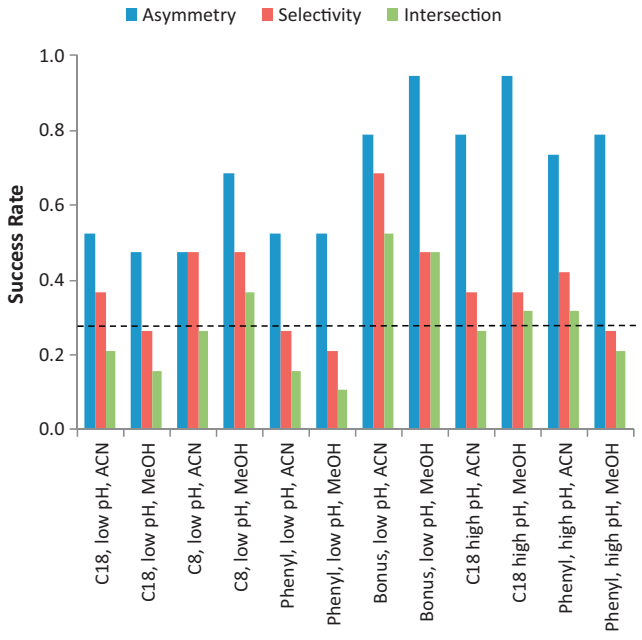


Fig. 5. Screen success rates (dashed line indicates mean for intersection of asymmetry and selectivity).

screen conditions in terms of providing acceptable peak asymmetry and selectivity is summarized in Fig. 5. The column/mobile phase combinations that most frequently satisfied the asymmetry criterion include the C18 and Phenyl phases when used with high pH mobile phases and the Bonus RP column used at low pH. Those systems most likely to provide the desired selectivity included the C8 or Bonus RP columns used with low pH/acetonitrile or methanol containing mobile phases. The mean overall success rate was 0.28 for this set of projects which translates into 3.4 successful combinations identified per project.

4. Conclusions

The strategy presented provides a rational approach to HPLC impurity method development. The procedure offers a common starting point for both active pharmaceutical ingredient and drug product methods. The workflow is tailored to match the specific needs of a project based on phase of development. The use of sample mixtures and the specificity of MS and UV detection dramatically reduces experiment cycle time by more than 80%.

Column and mobile phase combinations exhibiting the required performance characteristics for pre-commercial or commercial phase projects are identified in a screening step. Gradient profile optimization of separation systems identified in the screen provides final method conditions suitable for commercial phase use in the analysis of active pharmaceutical ingredients and drug products.

The strategy is enabled by the suite of software tools. ACD AutoChrom provides full instrument control with automated sequence execution, automated signal processing with reconciliation between detectors and test preparations, retention modeling and predictive simulation of chromatographic separations, and a single user interface that allows the user ready access to the project data.

Acknowledgements

The authors would like to thank Dr. Bernie Olsen, Dr. Eric Jensen, and Mr. Thomas Gunter for providing insight, technical guidance, or execution assistance. In addition, the authors appreciate the assistance of the following vendors: ACD/Labs, Agilent Technologies, and Waters Corporation.

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