Automated Kinetic Forced Degradation Development and HPLC Method Validation

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Why Automated Forced Degradation?

- **Why perform forced degradation studies?**
  - Evaluate API bulk and solution stability at acid, base, oxidation, thermal conditions
  - Identify degradation route
  - Provide guidance for bulk and formulated sample storage conditions
- **Current Issues**: all forced degradation done manually, conditions and time are different, generate different degradates
- **When to perform forced degradation studies?**
  - Lead optimization
  - HPLC API development/validation
  - IND/NDA filing
- **Why automated forced degradation studies?**
  - Standardized workflow
  - Consistent/automatic data sharing
  - Increase productivity and efficiency
- **Why kinetic forced degradation studies**
  - Differentiate primary and second degradates
  - Estimate degradation rate constants
  - Define if the degradation pathway follows 1^{st} order/ 2^{nd} order reactions

Bench-top Automation Platform Robotics Development Strategy

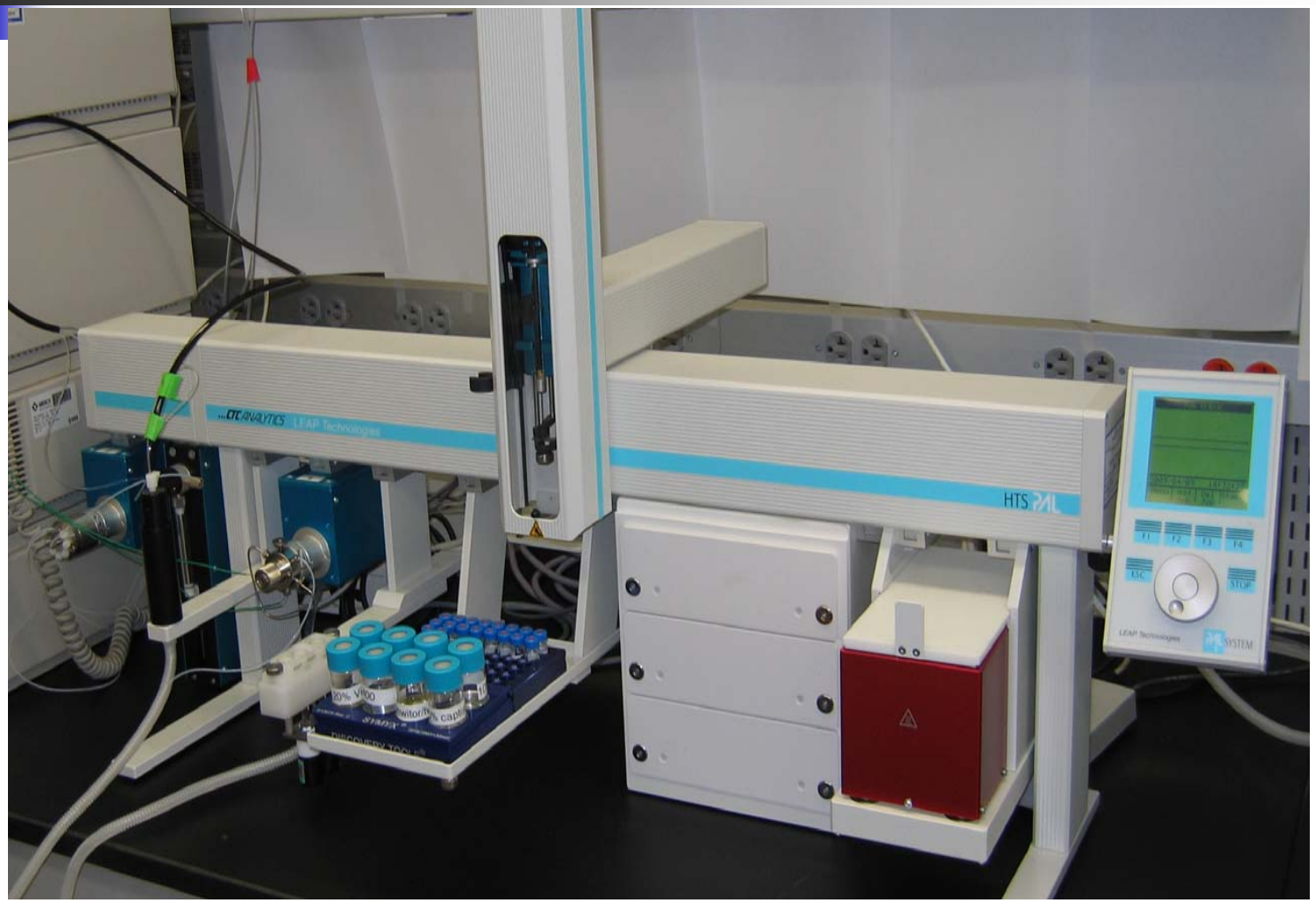
- User friendly and walk-up open access system
- Robust, standardized, easy to operate, straightforward to maintain
- Serves multiple purposes
- Design to work with existing data systems when required
- Cost effective solution that minimizes impact of multiple deployments
- Cycle time reduction needs outweigh high throughput centralized HTS service
  - Lower volume and sporadic activities that require rapid response
  - Increase productivity of time-sensitive work

Rationale for Selection Automated System

System Internal Capabilities	**Agilent HPLC**	**Symyx**	**Tecan**	**Leap**
Precision with < 1.0%; RSD% with ± 1.5% and linearity R^2>0.999 range from 2 – 2000 dilution factor	✓	✗	✓	✓
Temperatures, heat up to 200 ° C	✗	✓	✗	✓
Online filter	✗	✓	✓	✓
Online injection HPLC analysis	✓	✗	✗	✓
Cost (critical criteria for Bench top system)	✓	✗	✗	✓

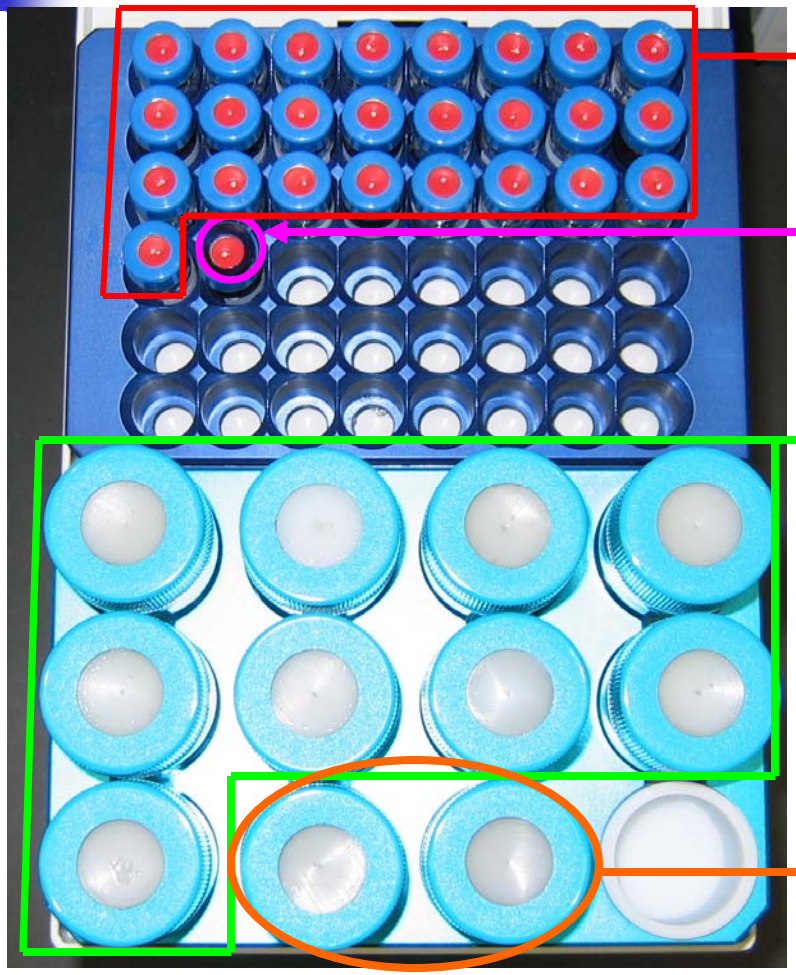
LEAP PAL WorkStation

System Overview



LEAP PAL WorkStation

Sample Setup



HPLC Empty Vials

0.1 mg API Powder

Forced Deg Reagents

API Stock Solutions

1 N HCl	1N NaOH	pH 5 100 mM Buffer	pH 7.4 100 mM Buffer
pH 9 100 mM Buffer	50 mM AIBN Solution	3% peroxide pH7.4@50 mM	50% PEG 400 MeOH
20% Tween 80 MeOH	120 % API in acetonitrile	120 % API in MeOH	open

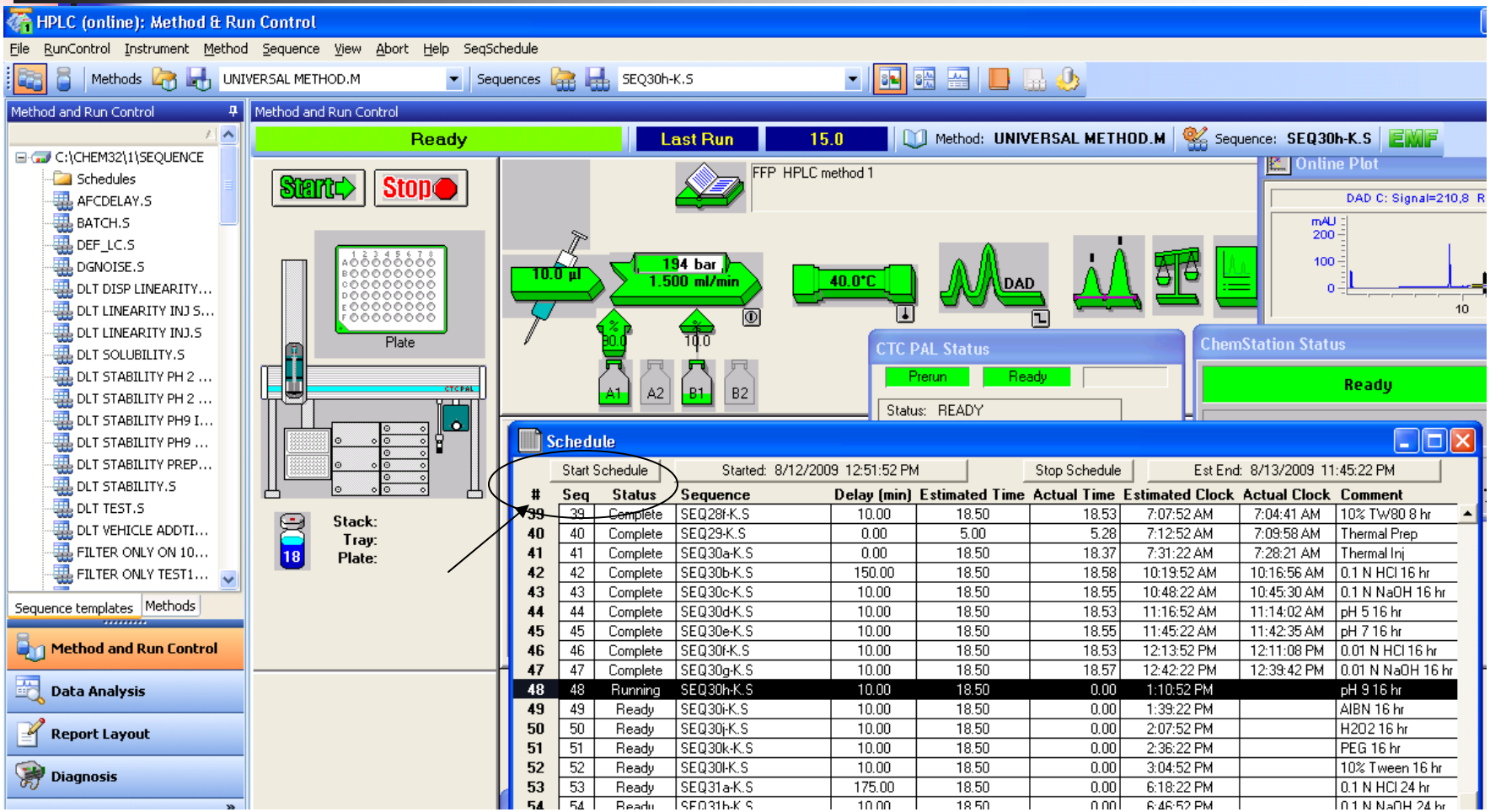
Kinetic Forced Degradation Conditions

Major Degradation Pathway	Reagents	Co-Solvent	Temp. (°C)	Time Points (hr)
Hydrolysis	0.1 N HCl	MeCN/water	40 °C	0, 8, 16, 24
	0.01 N HCl			0, 3, 8, 16, 24
	pH 5 buffer			0, 8, 16, 24
	pH 7.4 buffer			0, 8, 16, 24
	pH 9 buffer			0, 8, 16, 24
	0.01 N NaOH			0, 3, 8, 16, 24
	0.1 N NaOH			0, 8, 16, 24
Oxidation	H_2O_2	MeOH/water	25 °C	0, 8, 16, 24
	AIBN*		40 °C	
	Tween 80		40 °C	
	PEG 400		40 °C	
Thermal	N/A	MeCN/water	100 °C	4

* AIBN - 2,2''-azobisisobutyronitrile

Kinetic Forced Degradation Setup

- Sample Preparation Setup in ChemStation - **SeqSchedule**
- Online sample preparation and HPLC Analysis
- Data collected in local PC and Atlas*, automated report



#	Seq	Status	Sequence	Delay (min)	Estimated Time	Actual Time	Estimated Clock	Actual Clock	Comment
39	39	Complete	SEQ28f-K.S	10.00	18.50	18.53	7:07:52 AM	7:04:41 AM	10% TW80 8 hr
40	40	Complete	SEQ29-K.S	0.00	5.00	5.28	7:12:52 AM	7:09:58 AM	Thermal Prep
41	41	Complete	SEQ30a-K.S	0.00	18.50	18.37	7:31:22 AM	7:28:21 AM	Thermal Inj
42	42	Complete	SEQ30b-K.S	150.00	18.50	18.58	10:19:52 AM	10:16:56 AM	0.1 N HCl 16 hr
43	43	Complete	SEQ30c-K.S	10.00	18.50	18.55	10:48:22 AM	10:45:30 AM	0.1 N NaOH 16 hr
44	44	Complete	SEQ30d-K.S	10.00	18.50	18.53	11:16:52 AM	11:14:02 AM	pH 5 16 hr
45	45	Complete	SEQ30e-K.S	10.00	18.50	18.55	11:45:22 AM	11:42:35 AM	pH 7 16 hr
46	46	Complete	SEQ30f-K.S	10.00	18.50	18.53	12:13:52 PM	12:11:08 PM	0.01 N HCl 16 hr
47	47	Complete	SEQ30g-K.S	10.00	18.50	18.57	12:42:22 PM	12:39:42 PM	0.01 N NaOH 16 hr
48	48	Running	SEQ30h-K.S	10.00	18.50	0.00	1:10:52 PM		pH 9 16 hr
49	49	Ready	SEQ30i-K.S	10.00	18.50	0.00	1:39:22 PM		AIBN 16 hr
50	50	Ready	SEQ30j-K.S	10.00	18.50	0.00	2:07:52 PM		H2O2 16 hr
51	51	Ready	SEQ30k-K.S	10.00	18.50	0.00	2:36:22 PM		PEG 16 hr
52	52	Ready	SEQ30l-K.S	10.00	18.50	0.00	3:04:52 PM		10% Tween 16 hr
53	53	Ready	SEQ31a-K.S	175.00	18.50	0.00	6:18:22 PM		0.1 N HCl 24 hr
54	54	Ready	SEQ31b-K.S	10.00	18.50	0.00	6:46:52 PM		0.1 N NaOH 24 hr

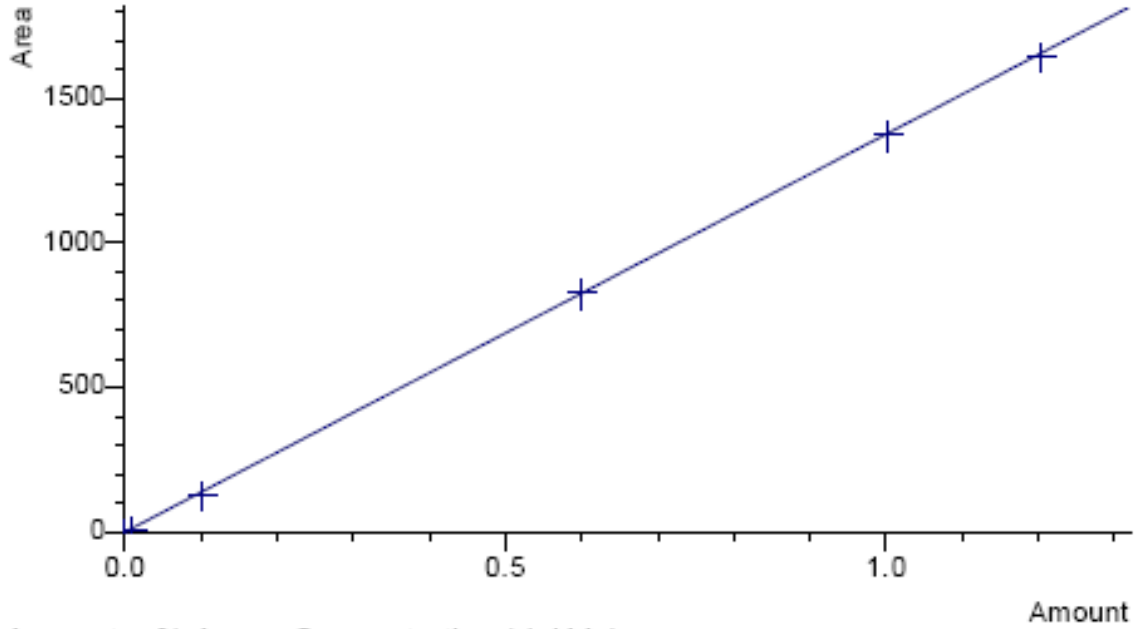
* Atlas is Thermo Chromatography data system

Case Study - Linearity Compd. A

Method Linearity

0.05% Std 2
RF Mode: Standards

Compd. A



Amount = % Assay Concentration * inj Vol

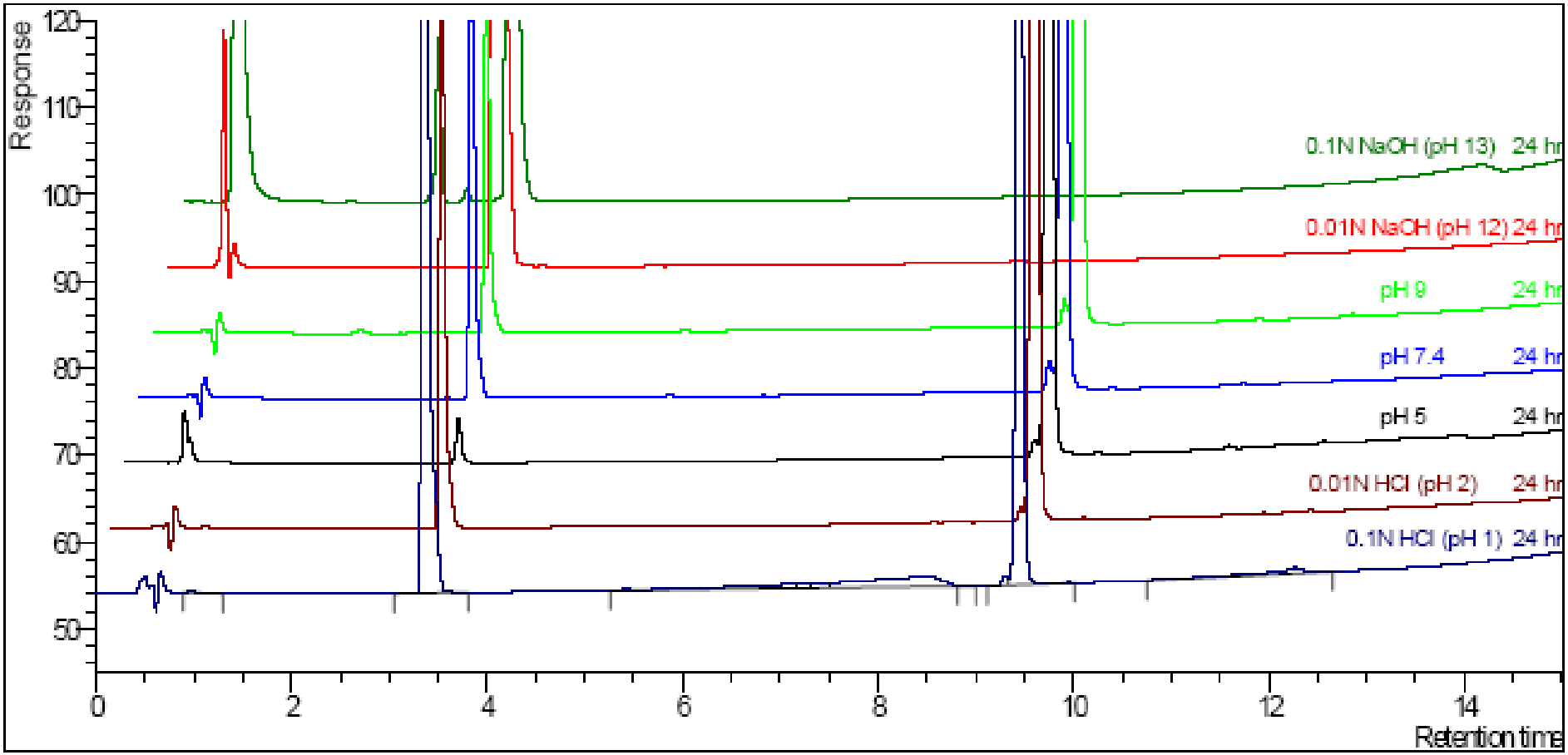
Calibration Information:

	Equation	Coeff of Det
Compd. A	y = 1376.2137x	1.0000

Acid/Base Forced Degradation

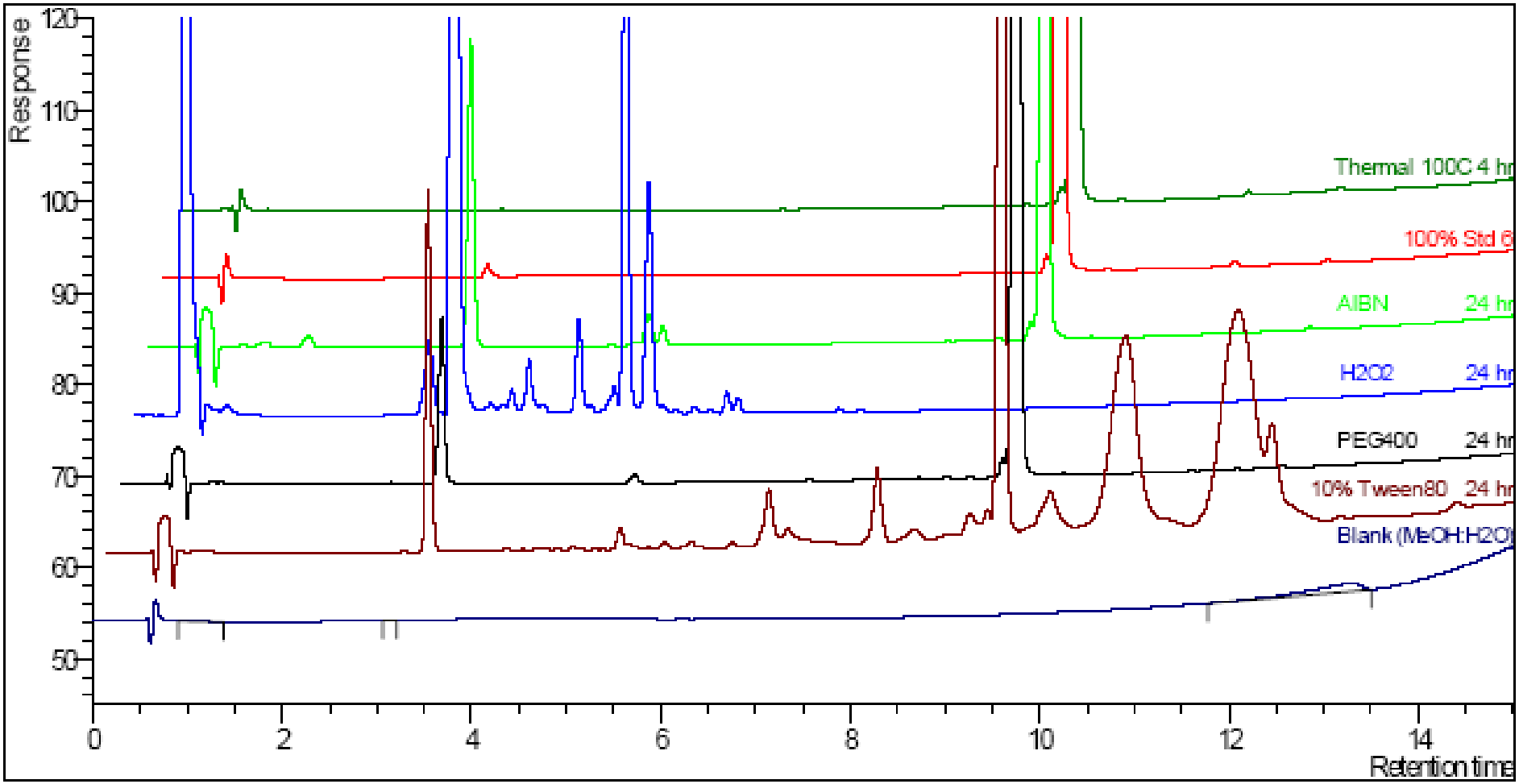
Chromatograms Overlay at 24 hr

Forced Degradation Overlay 24 hr. Data Summary



Oxidative Forced Degradation

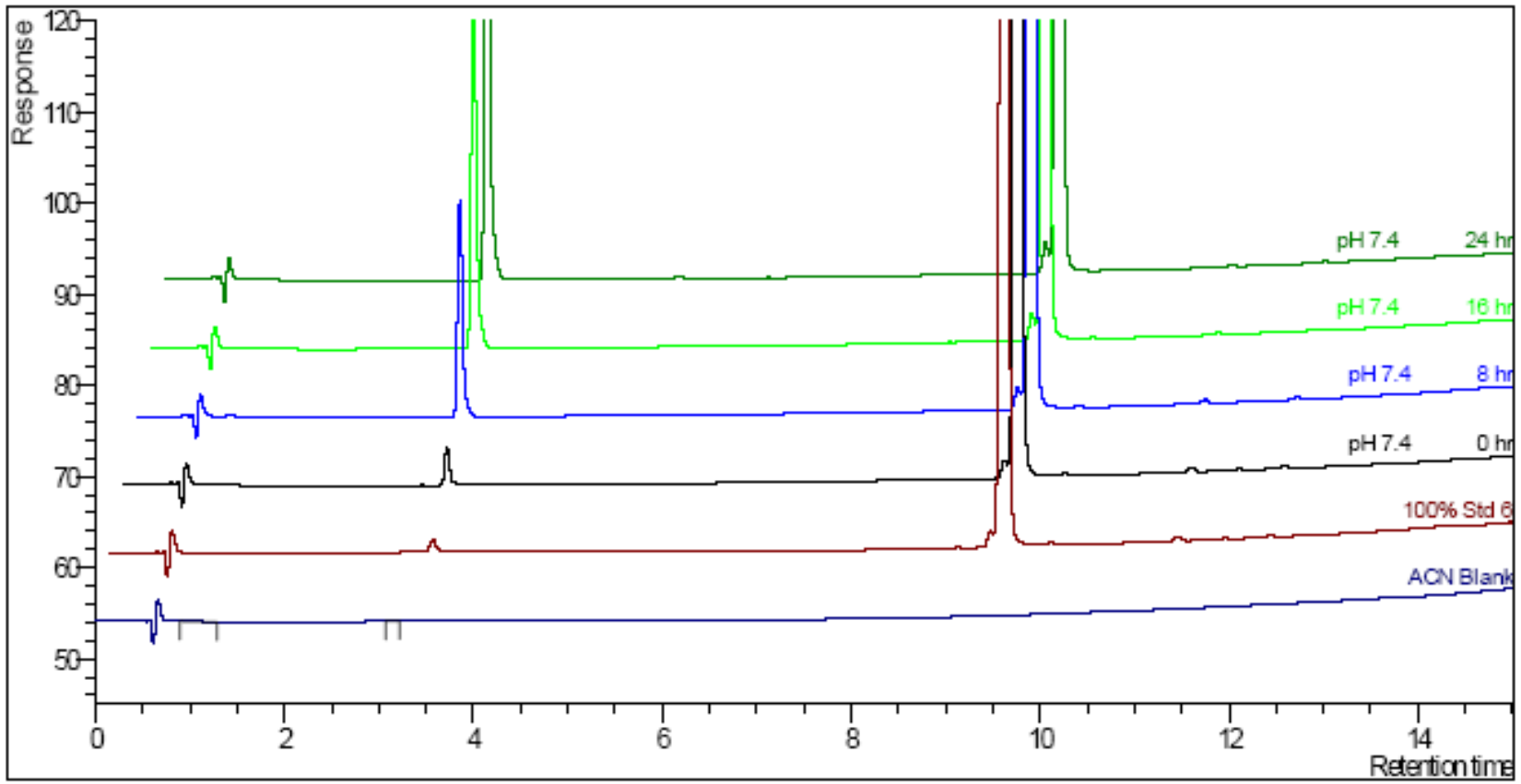
Chromatograms Overlay at 24 hr



Kinetic Forced Degradation

pH 7, t = 0 ,8, 16 and 24 hrs

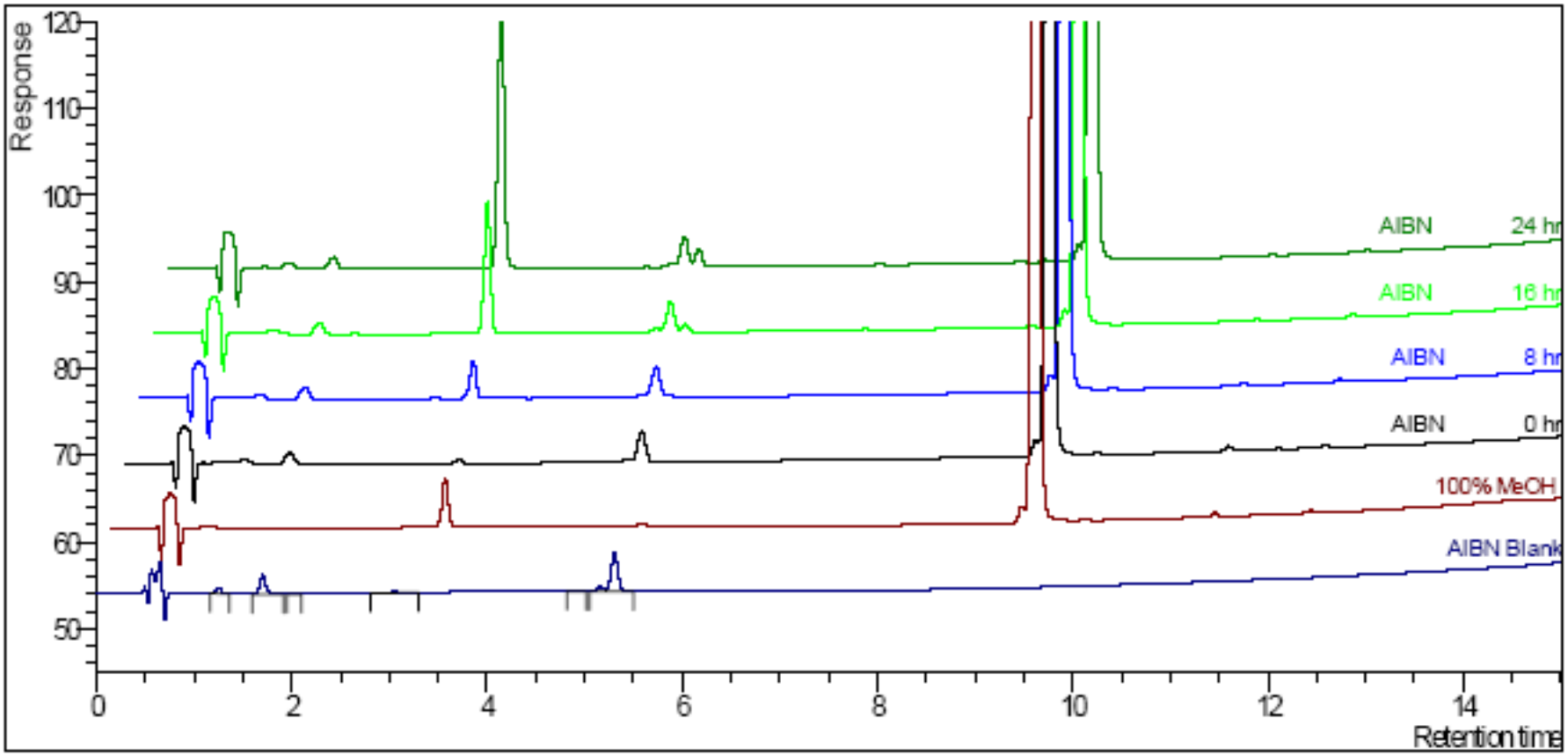
Forced Degradation Overlay pH 7 Data Summary



Kinetic Forced Degradation

AIBN* Oxidation

Forced Degradation 5mM AIBN Overlay Data Summary

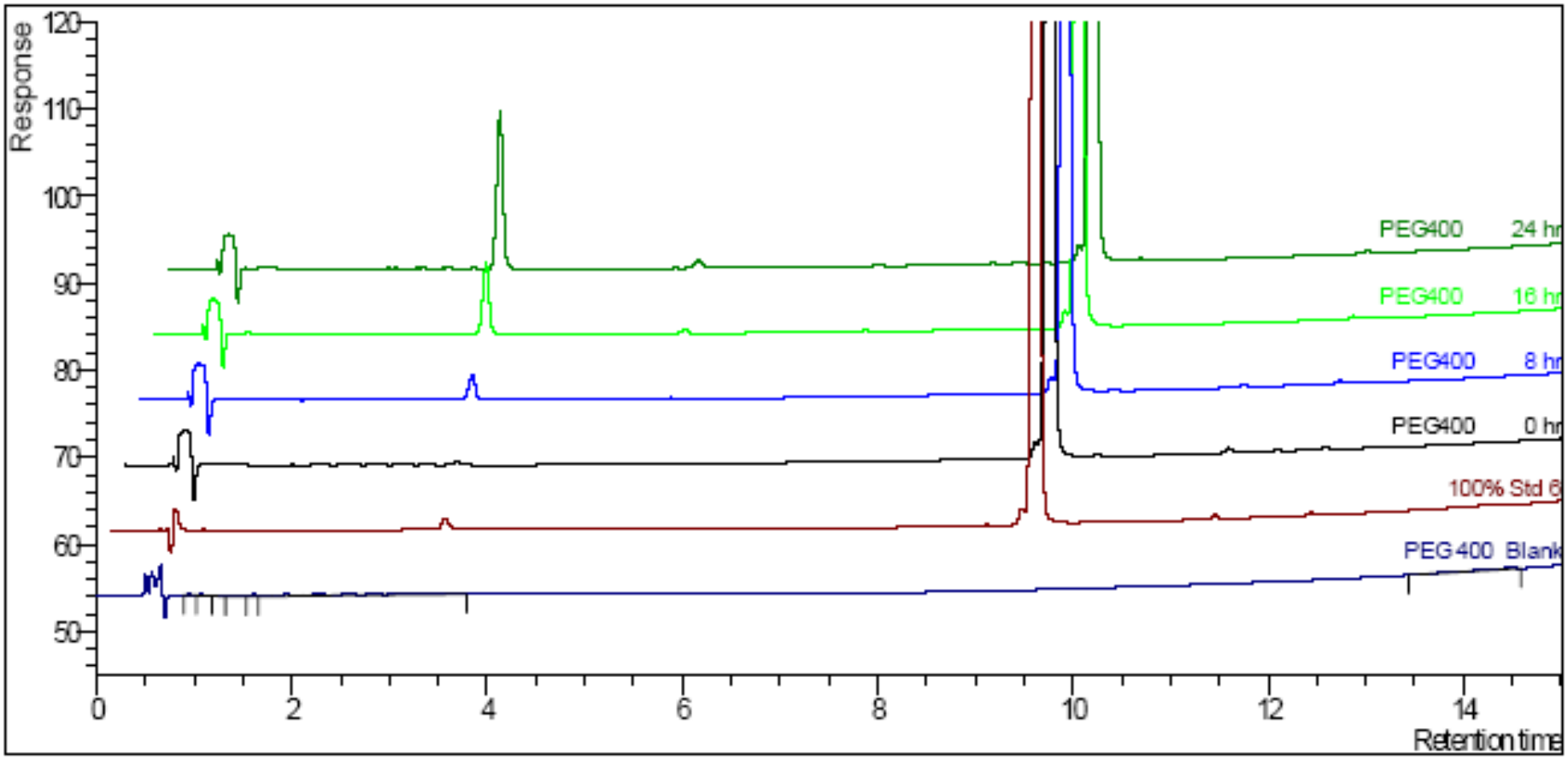


* AIBN - 2,2''-azobisisobutyronitrile

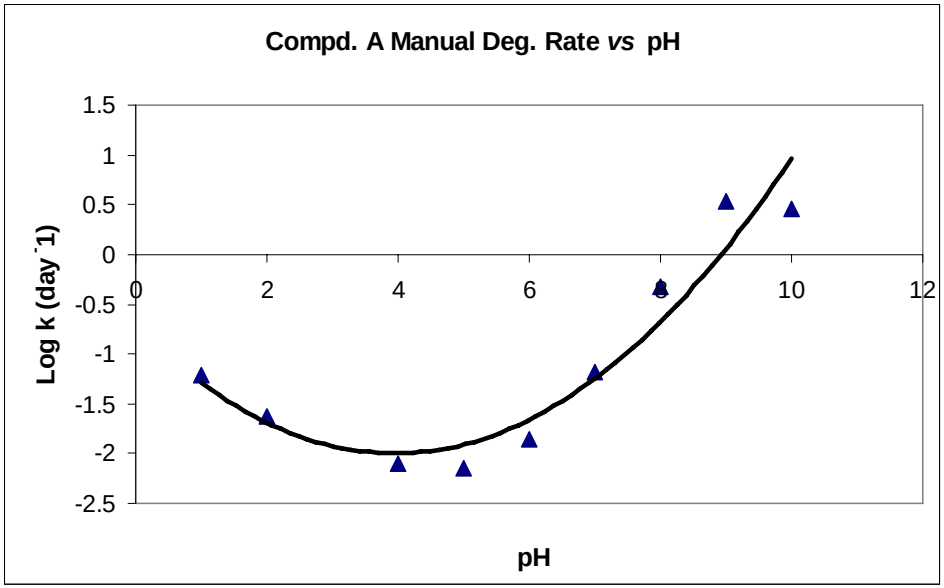
Kinetic Forced Degradation

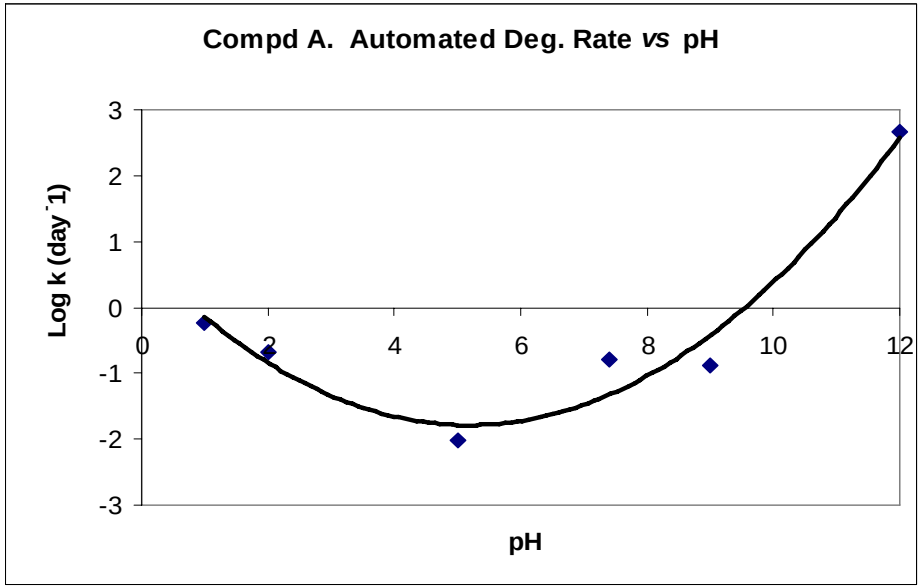
Oxidation in PEG 400 Solution

Forced Degradation 5% PEG 400 Overlay Data Summary



Kinetic Automated pH Rate Profile Forced Degradation





- Manual experiment ~ 1 FTE 2 days at 1 week run time
- Automated experiment ~ 15 minutes and 36 hr run time

Summary

- LEAP PAL system is user friendly and is intended to be used as “Bench top walk-up system”
- Automated linearity preparation and calibration
- Automated stress samples of pH profile hydrolysis, nucleophilic oxidation, auto-oxidation, vehicle related oxidation, and thermal
- Kinetic forced degradation and real time online analysis
- Automated report for easy data review and decision making
- It is cost efficiently, robust and requires minimal maintenance
- Most importantly, harmonize the forced degradation procedures from early drug development to IND/NDA filing
- Save chemist’s time and increase productivity

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