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Not Only Reverse Phase…

A Need to Use Alternative HPLC Techniques for Pharmaceutical Analysis

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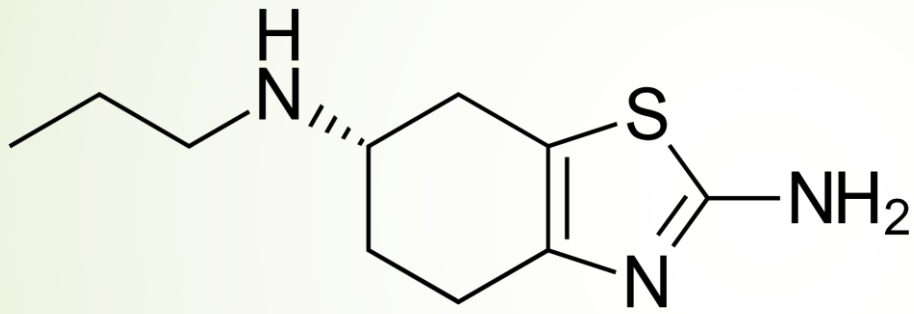
Drug Product under Development

- **P2B001** is a novel proprietary **F**ixed-**D**ose **C**ombination (**FDC**) of **E**xtended-**R**elease (**ER**) formulations of low doses of **pramipexole** (as dihydrochloride) and **rasagiline** (as mesylate) developed at **Pharma Two B, Ltd.**, Rehovot, Israel
- **Pharma Two B, Ltd**. announced that positive efficacy and safety data from its recently completed randomized, controlled **Phase 3 trial** of investigational **P2B001** in the management of early **Parkinson Disease** (PD)
- The data will be presented (in different formats) at the **MDS International Congress of Parkinson's Disease and Movement Disorders**, Sept. 15-18, in Madrid and published in the ***Movement Disorders*** journal supplement..

Drug Product under Development

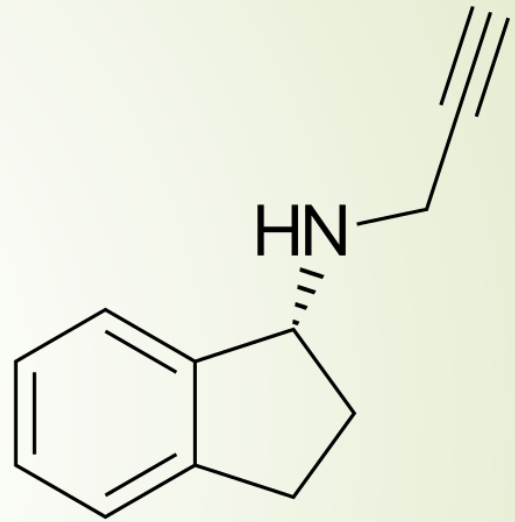
- The complexity of analytical test methods for **FDC** drug products containing two or more **A**ctive **P**harmaceutical **I**ngredients (**API**) follows from various reasons:
  - Difference in **physico-chemical characteristics** of API's requires sophisticated gradient HPLC methods to achieve **elution** and **detection** of all the components within a single chromatographic run
  - Assay and Impurities methods need to define, **which impurities / degradation products** are related to **which of the API's**
  - **Dissolution profile**, especially for **C**ontrolled **R**elease (**CR**) products, should be controlled **by formulation matrix**, rather than by the individual characteristics of the API's

Active Pharmaceutical Ingredients



Pramipexole

Physico-Chemical Properties	
LogP	1.42
pKa $_1$ (imine)	4.65
λmax	262 nm



Rasagiline

Physico-Chemical Properties	
LogP	2.30
pKa	8.40
λmax	210 nm

Analytical Methods Development

- The complexity of analytical test methods for **FDC** drug products containing two or more **A**ctive **P**harmaceutical **I**ngredients (**API**) follows from various reasons:
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  - Assay and **Impurities** methods need to define, **which impurities / degradation products** are related to **which of the API's**
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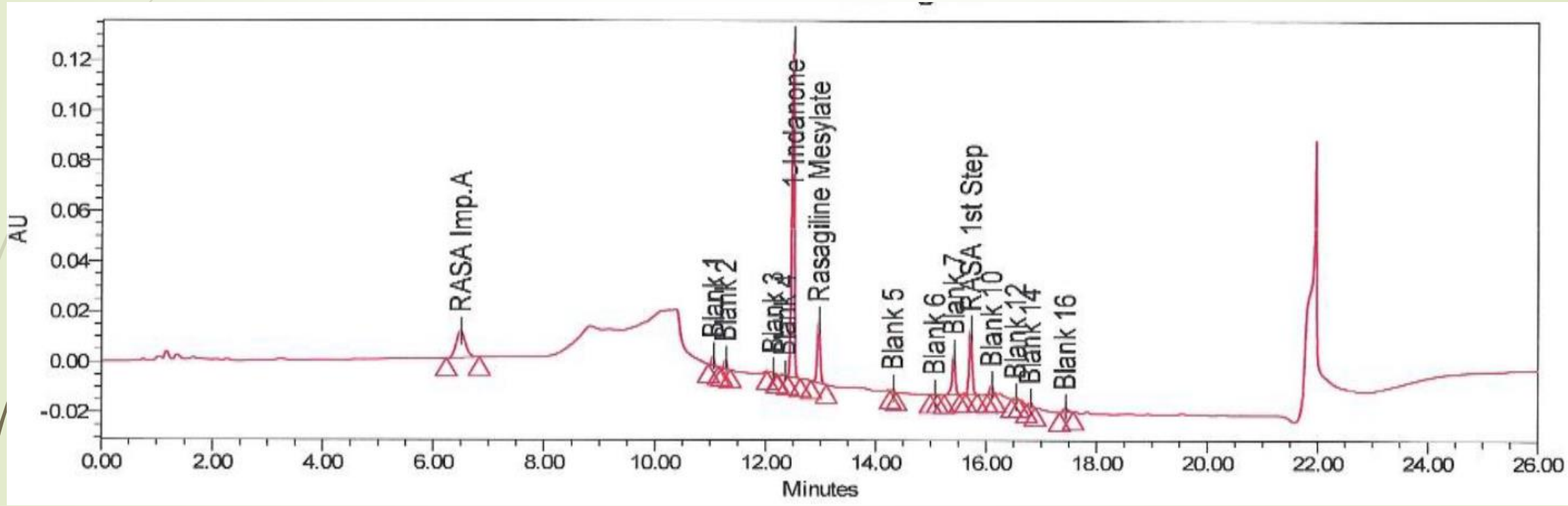
HPLC Parameters of the Method for Determination of Impurities

Mobile Phase	Mobile Phase A: (pH 6.0 Ammonium Phosphate Buffer/ACN/MeOH, 92/4/4 (v/v/v))			
	Mobile Phase B: Acetonitrile.			
Gradient Conditions	Time (min)	MP A	MP B	Curve
	0	100	0	
	6	100	0	6
	15	20	80	6
	20	20	80	6
	21	100	0	6
	26	100	0	6
Flow Rate	1.2 mL/min			
Column:	GL Science, Inertsil ODS-3, 5µm 150 x 4.0 mm			
Column Temperature	40 °C			
Autosampler Temperature	10 °C			
Injector Volume	100 µL			
Detector Wavelength	262 nm (PPX, PPHRCA) 210 nm (RASA, RASA Imp A, RASA Oxylate, I-Ind)			
Run Time	26 minutes			

Standard Chromatogram of the Method for Determination of Impurities at λ = 210 nm



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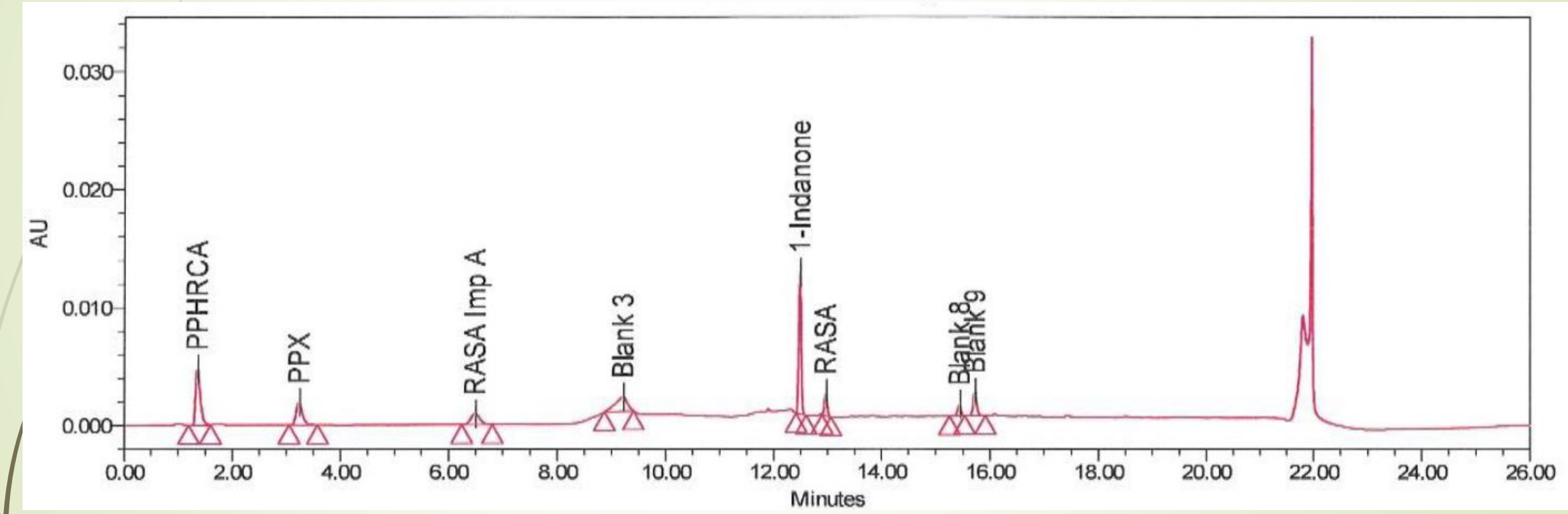


Dissolution Profile Test Parameters and its Monitoring HPLC Method

Apparatus	USP Apparatus I (Basket)
Dissolution Medium	pH 6.8 Potassium Phosphate buffer
Initial Medium Volume	500 mL
Temperature	37.0°C ± 0.5°C
Rotation Speed	100 rpm

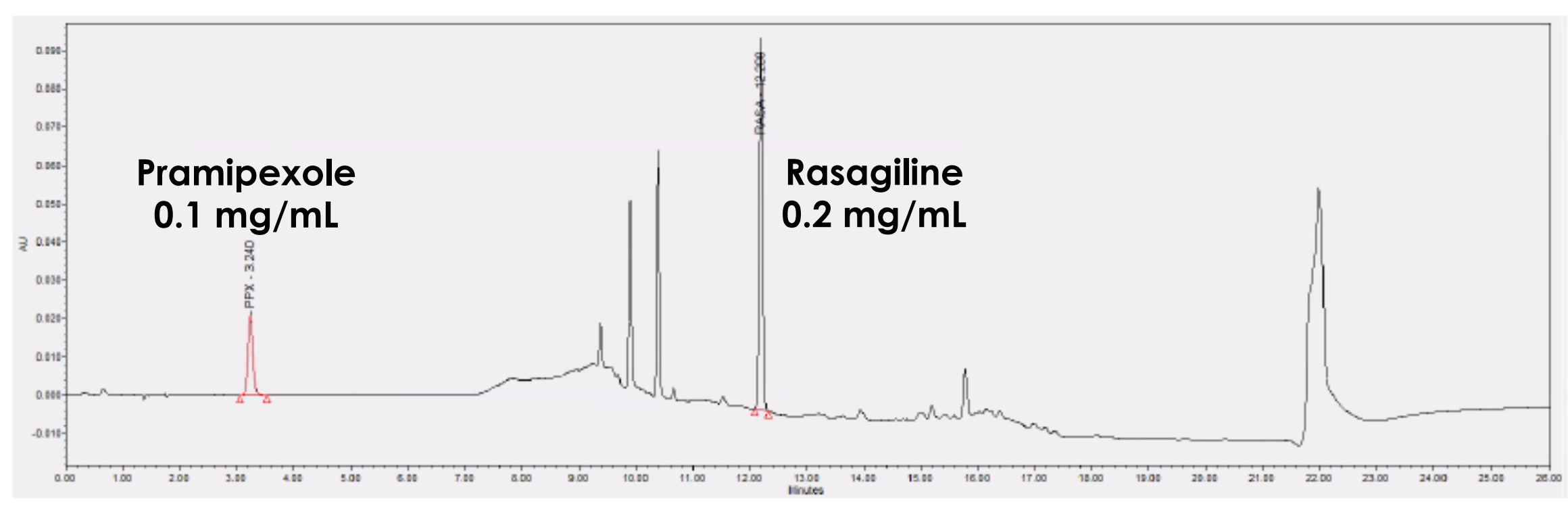
Sampling time points: 2 hr; 6 hr; 12 hr; 24 hr; 30 hr

- For monitoring the dissolution profile, **the same gradient RP HPLC** procedure, as for the **impurities method**, was adopted.
- Although having a long run duration, it worked perfectly for a standard dissolution medium at neutral pH used for the method

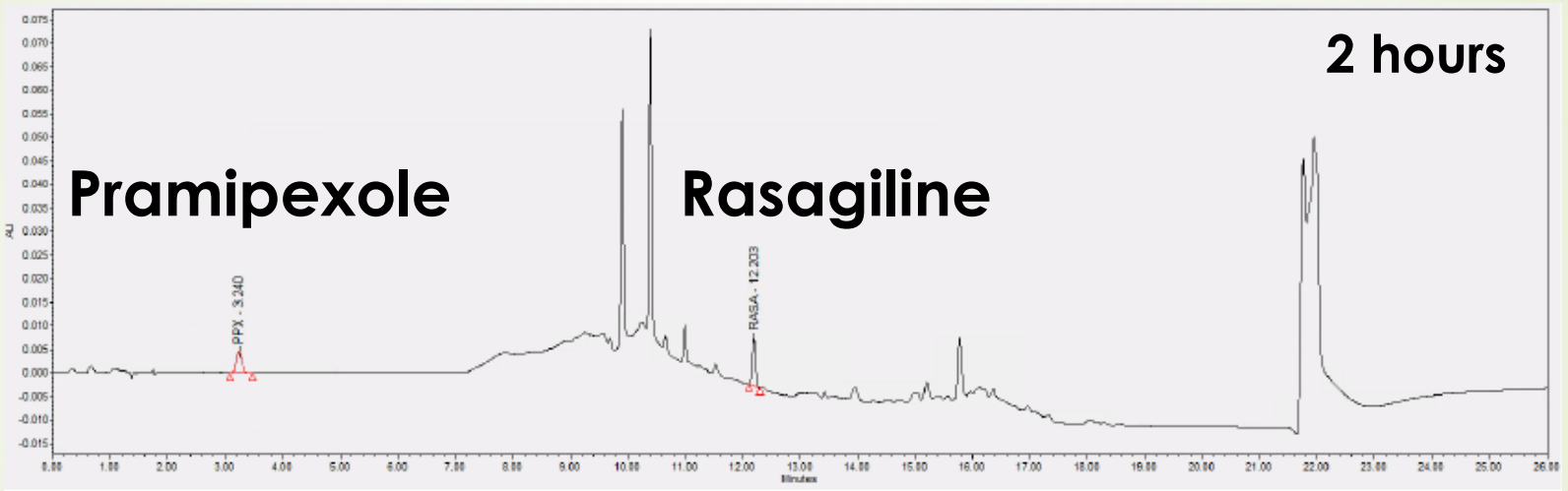
Existing HPLC Method Parameters

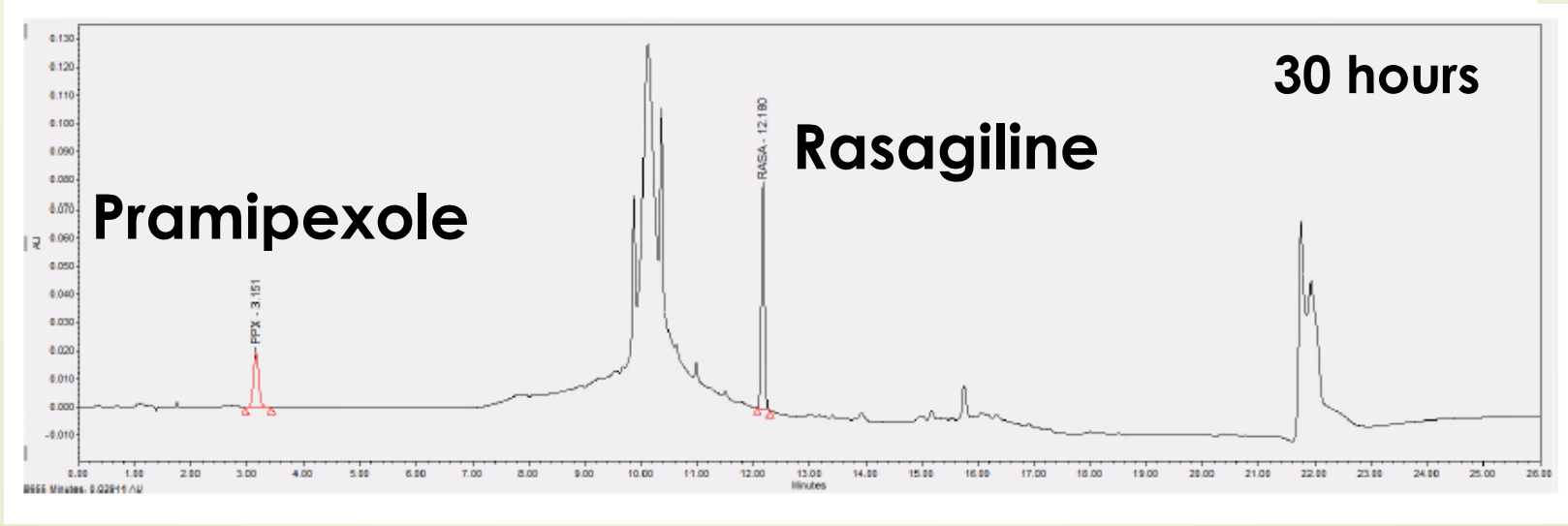
Column:	GL Sciences, Inertsil ODS-3, 5 µm, 4.0 × 150 mm
Mobile Phases	A: pH 6.0 Ammonium Phosphate buffer : ACN : MeOH (92 : 4: 4) B: 100% ACN
Flow Rate	1.2 mL/min
Gradient	Time (min) / %A / %B 0.0 / 100 / 0 5.0 / 100 / 0 15.0 / 20 / 80 20.0 / 20 / 80 21.0 / 100 / 0 26.0 / 100 / 0
Total Run Time	26 min
Column Temperature	40 °C
Autosampler Temperature	10 °C
Injection Volume	100 µL
Needle Wash	ACN: H_2O (50 : 50)
Detector Wavelength	210 nm for RASA 262 nm for PPX

Existing HPLC Method: Standard Solution Chromatogram

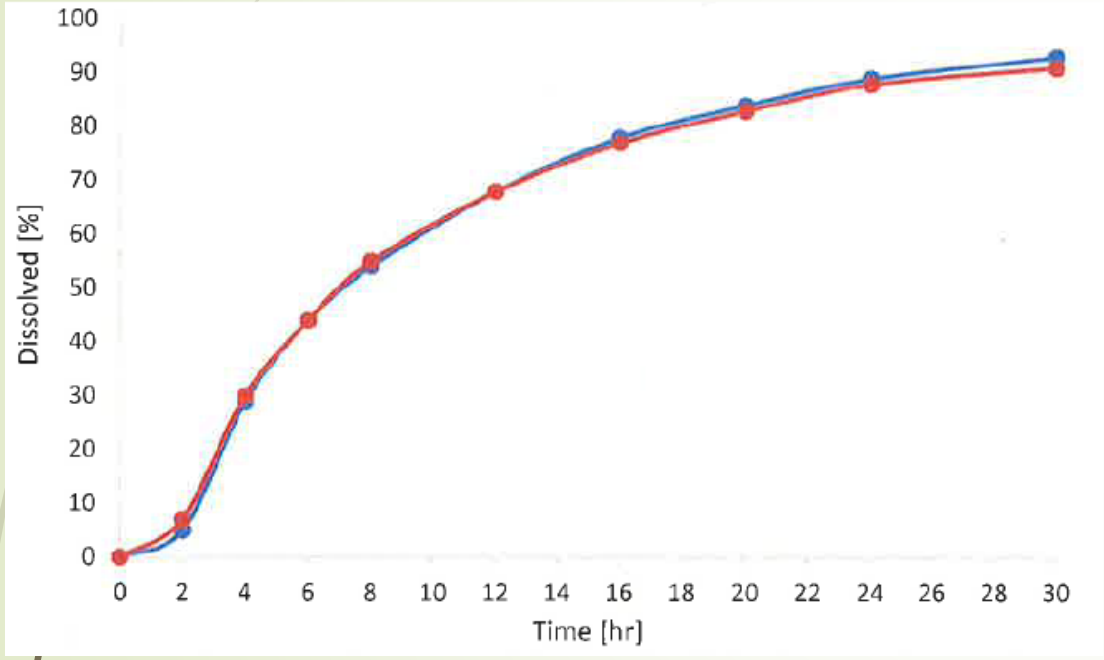


Existing HPLC Method: Dissolution Profile Chromatograms

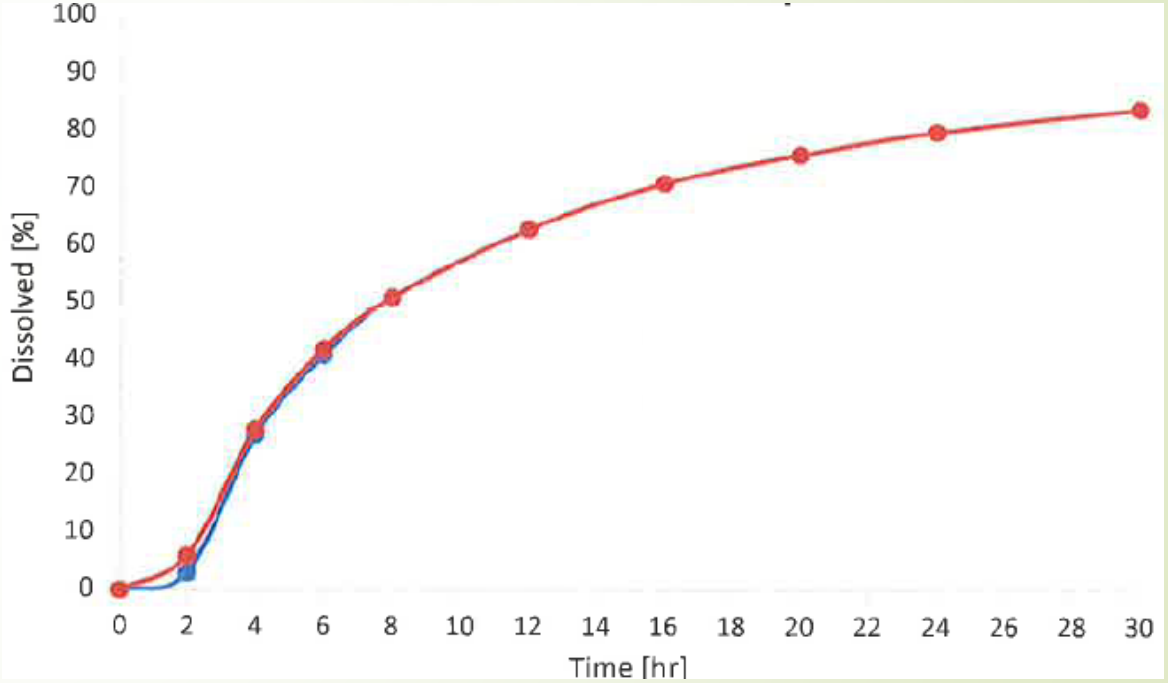




Dissolution Profiles of both API's of P2B001



Pramipexole

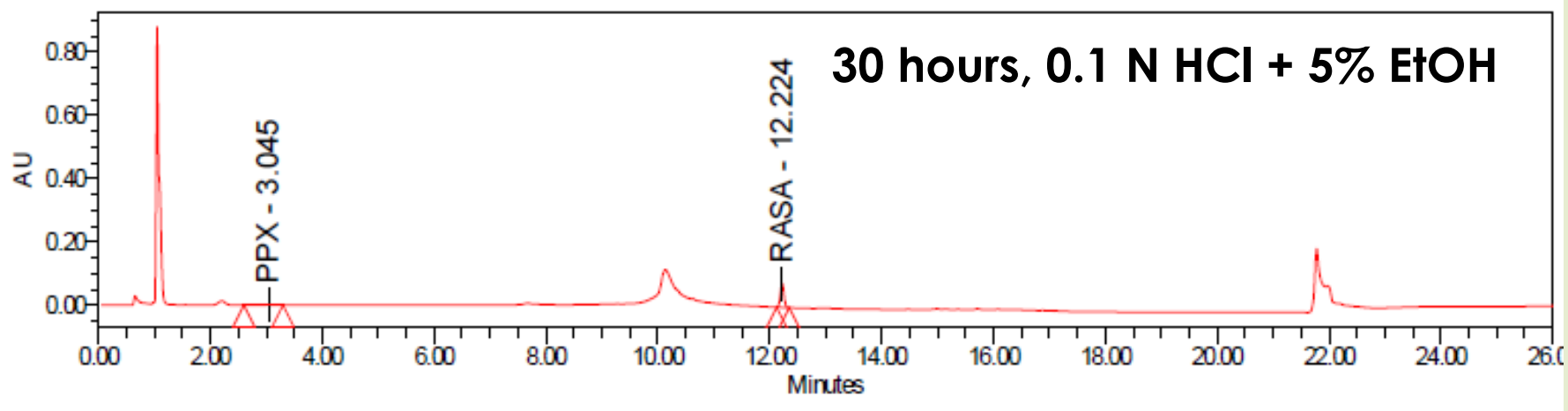


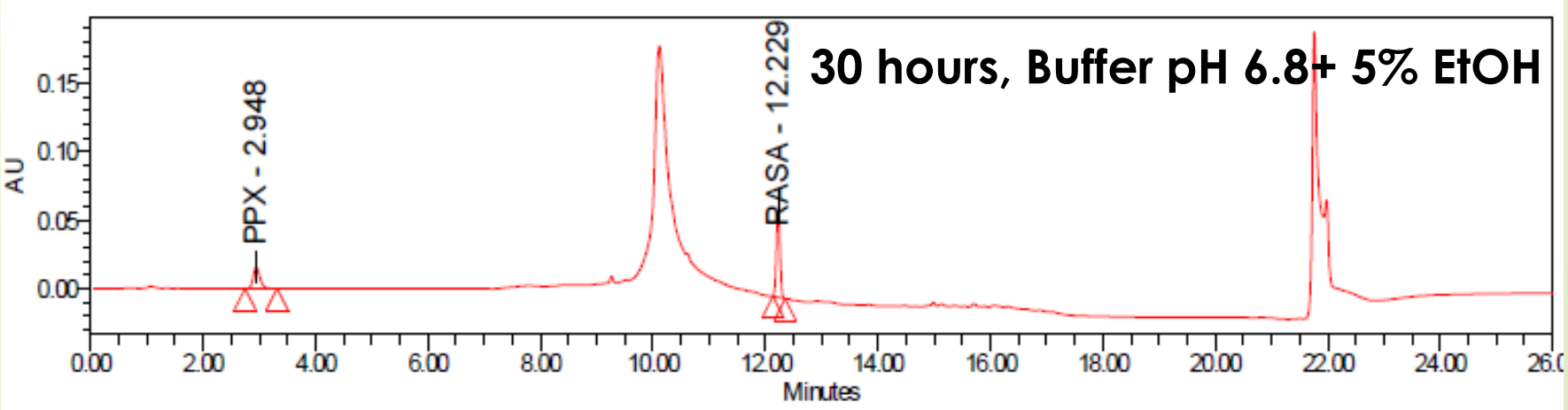
Rasagiline

Existing HPLC Method: Challenges for Alcohol Damping Study

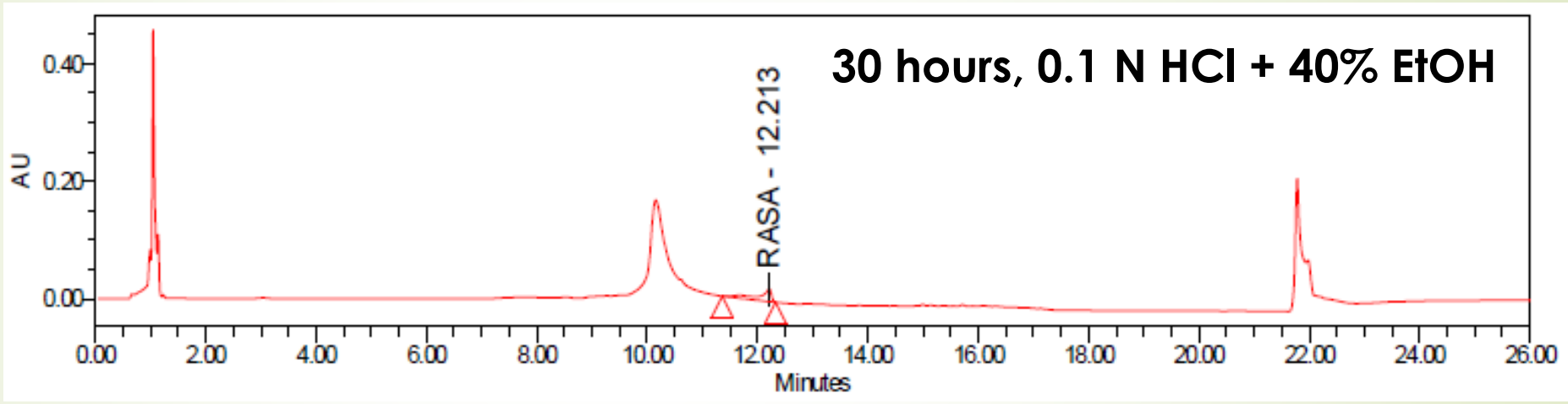
- However, when FDA required to conduct an **alcohol damping study**, the Company faced a serious problem:
- When alcohol concentration was **20% or above**, the **eluotropic strength** of the **sample diluent** was much **higher**, than that of the **mobile phase**, especially, at the beginning if the gradient (92% aqueous)
- This caused **deterioration of the peaks** of the analytes, especially of **Pramipexole**, which elutes very early.
- For **low pH** (0.1N HCl) dissolution medium with **high alcohol content**, even the much later eluting peak of **Rasagiline** **lost the shape and splitted**

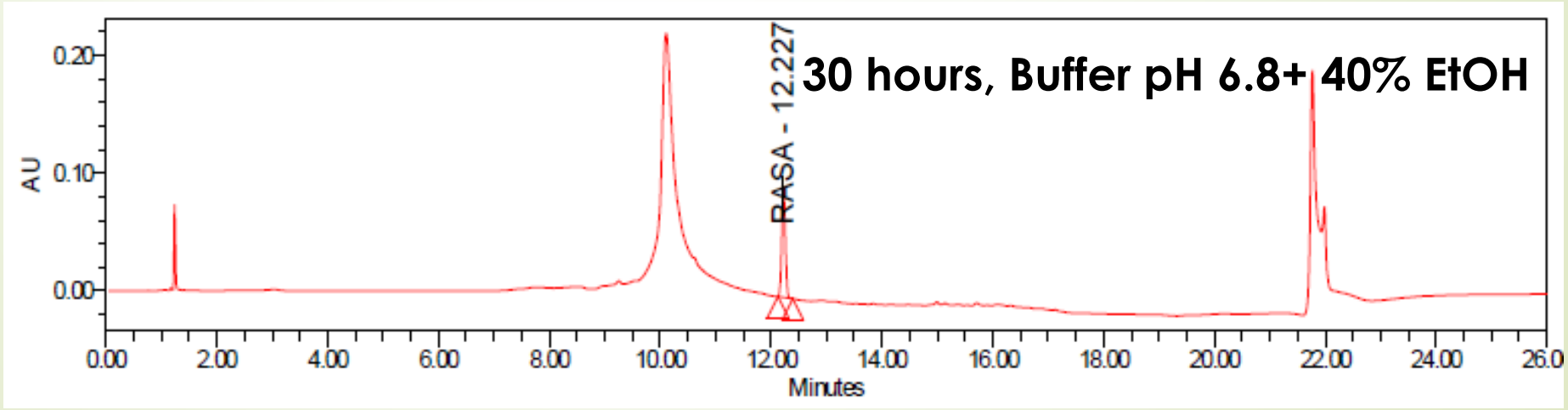
Existing HPLC Method: Chromatograms for Alcohol Damping Study





Existing HPLC Method: Chromatograms for Alcohol Damping Study





Challenges for Alcohol Damping Study: Do we need another method?

- This problem could not be solved within the same HPLC procedure
- Usually, to decrease the eluotropic strength of the diluent, a sample can be **diluted with water** / **aqueous solve**nt
- However, due to a **very low concentration** of both API's in the sample (due to their **low strengths** in the drug product), this cannot be performed without substantial **loss of the sensitivity**
- Therefore, the alternative way is to develop another HPLC procedure, which will **not depend** on the **high content of alcohol** (strong solvent) in the diluent (dissolution medium)

Mixed-Mode Chromatography as an Option

- Mixed-Mode chromatography combines **two** (**or more**) **retention mechanisms in one column**
- Mixed-Mode chromatography:
  - Ensures retention of polar compounds in reverse phase system
  - Improves shape of early eluted peaks and strong bases
  - Allows replacement of **complicated gradient** methods for compounds having different polarity with a **simple isocratic** method

Mixed-Mode Chromatography as an Option

- **Primesep™** columns (**SIELC**) for mixed-mode chromatography combine **two independent modes** of retention:
  - **Controlled ion-exchange** sites to interact with ionic species of the analyte
  - **Hydrophobic** chains of the stationary phase to interact with hydrophobic "portion" of the analyte

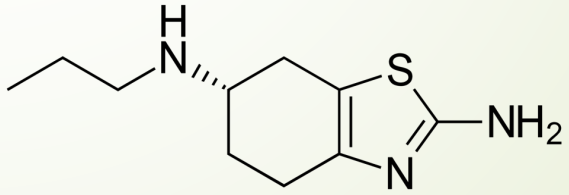
Development of Alternative Method Using Mixed-Mode Chromatography

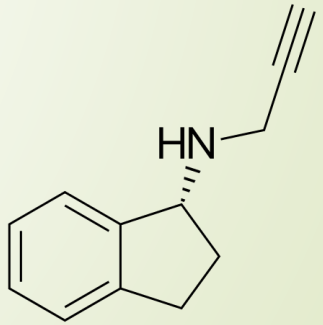
- Both drug substances are **weak bases**, having different **pKa**
- Therefore, a decision was taken to develop an alternative HPLC method using a **mixed-mode column**
- This development took **some time** since the laboratory had to:
  - Become familiarized with the mixed-mode chromatography when having no previous experience
  - Choose the right **column**
  - Optimize **Water / Acetonitrile ratio** in the mobile phase
  - Choose the **acid** (H_3PO_4; H_2SO_4; CF_3COOH; etc.) and optimize its **concentration** in the mobile phase
  - Learn how to **wash** and **store** the mixed-mode column

Alternative HPLC Method: Parameters

Mobile Phase	Water /Acetonitrile (60:40 v/v) with 0.2% Sulfuric acid
Column	Primesep 100 5µ, 100A, 4.6x150mm (SIELC)
Flow Rate	1.2 mL/min
Run Time	10 min
Column Temperature	40 °C
Autosampler Temperature	10 °C
Injection Volume	100 µL
Needle Wash	ACN: H2O (50:50 v/v)
Detector Wavelengths	210 nm for RAS; 262 nm for PPX

Diluent for Standard :
Dissolution Medium



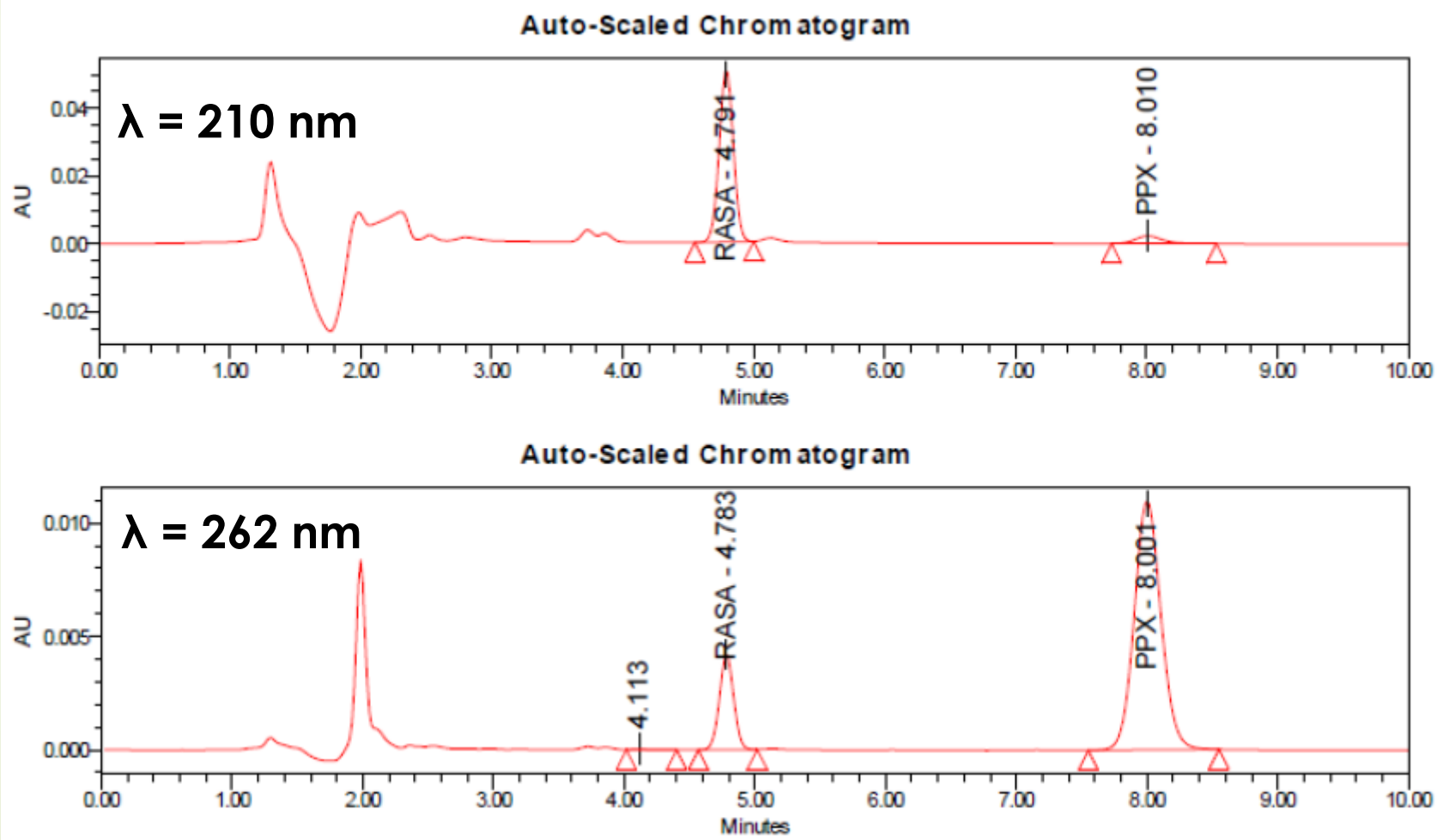


Working Standard Solution Concentrations:

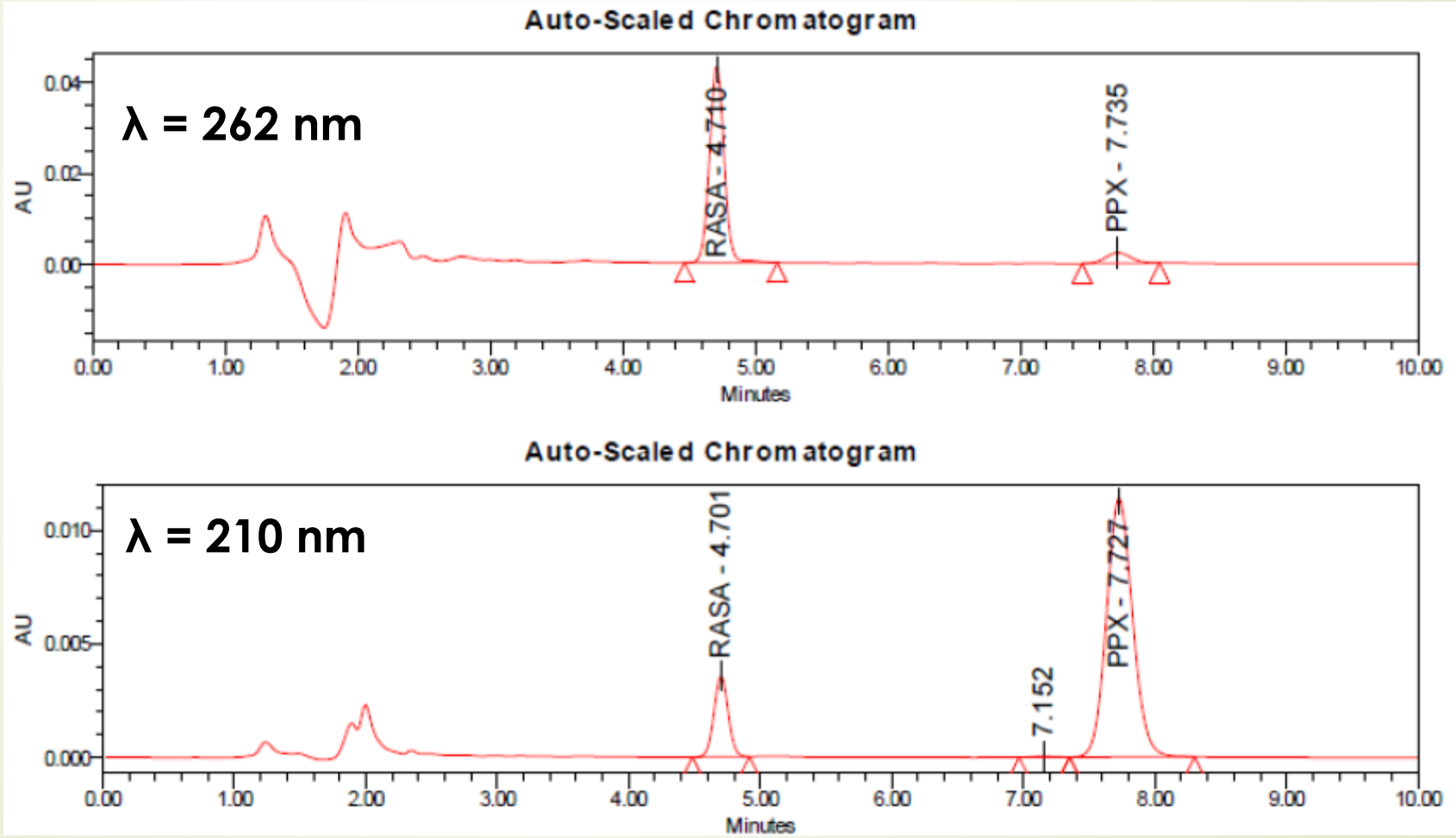
Pramipexole 0.1 mg/mL

Rasagiline 0.2 mg/mL

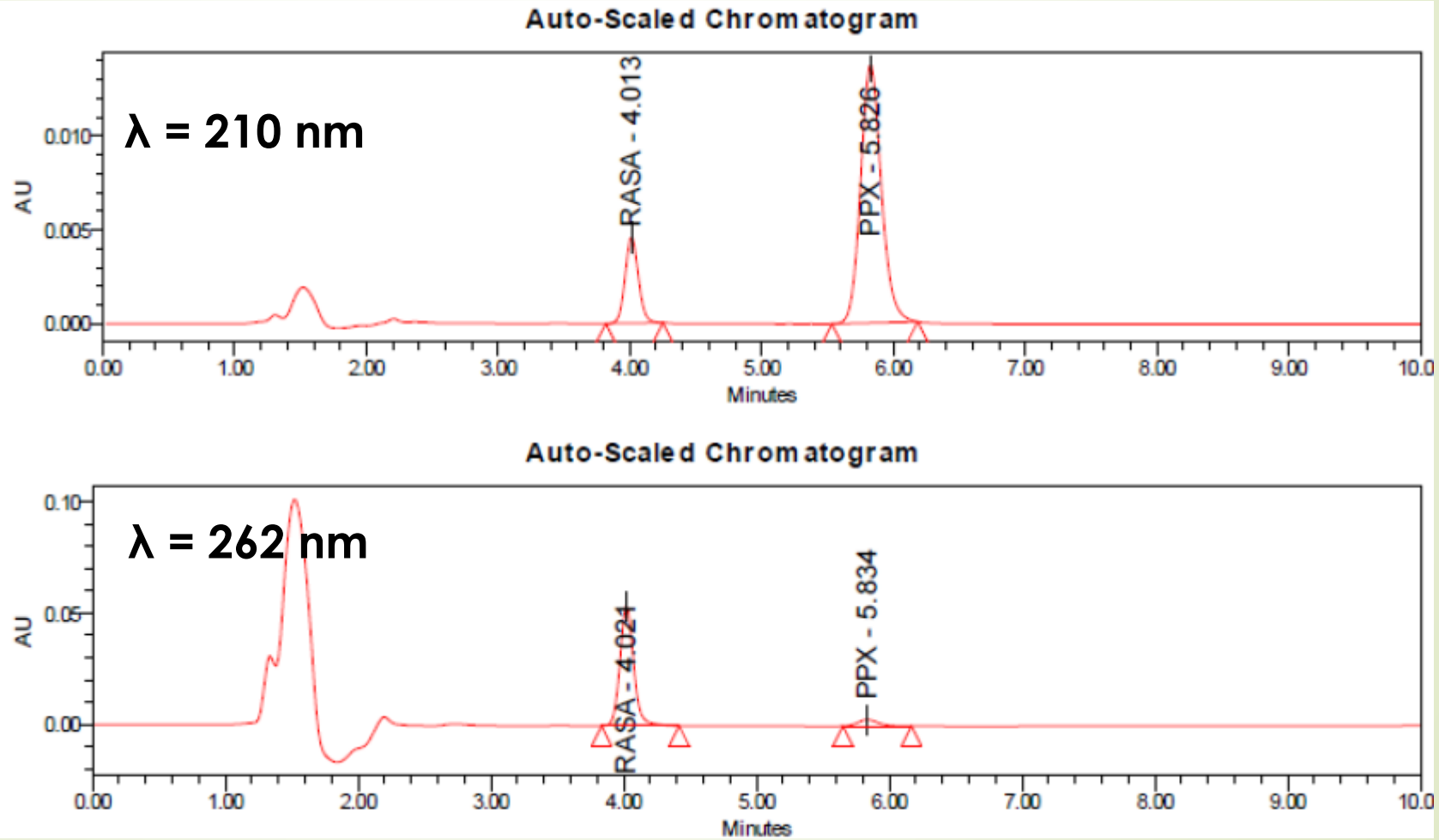
Diss. Medium: Phosphate Buffer, pH 6.8 Working Standard Chromatogram



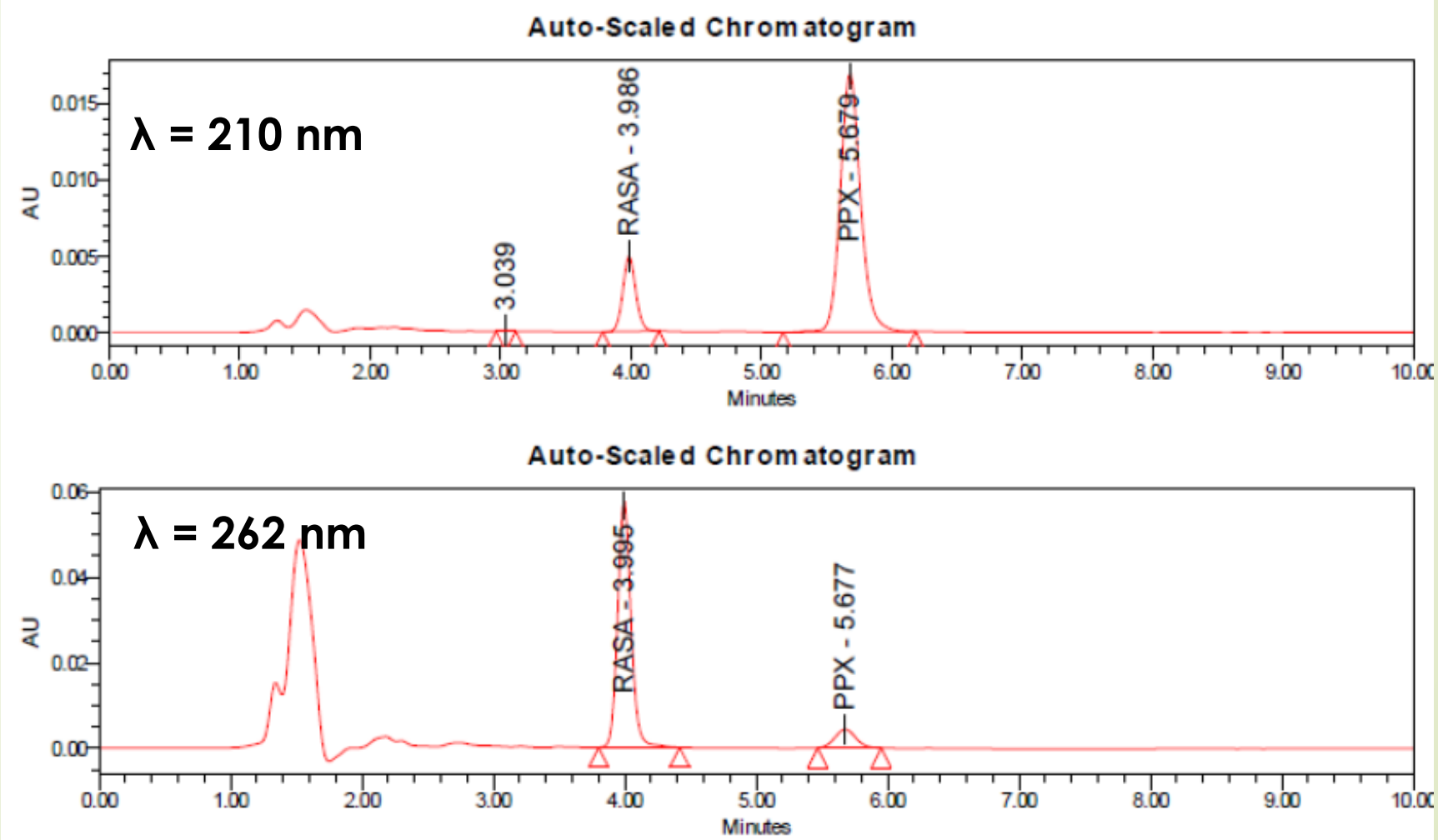
Diss. Medium: Phosphate Buffer, pH 6.8
Sample Chromatogram for 30 hours time point



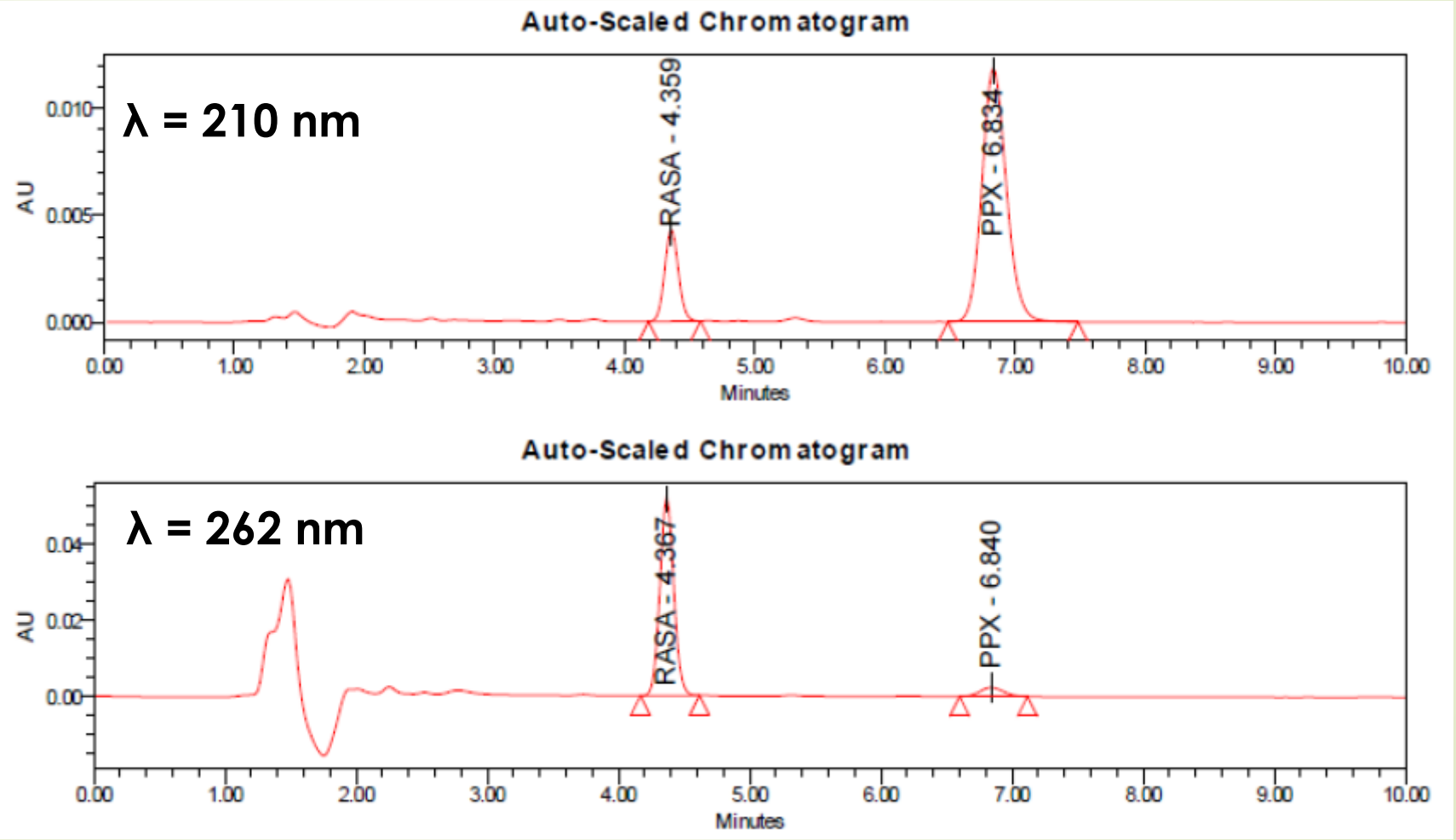
Diss. Medium: Phosphate Buffer, pH 6.8, 40% EtOH Working Standard Chromatogram



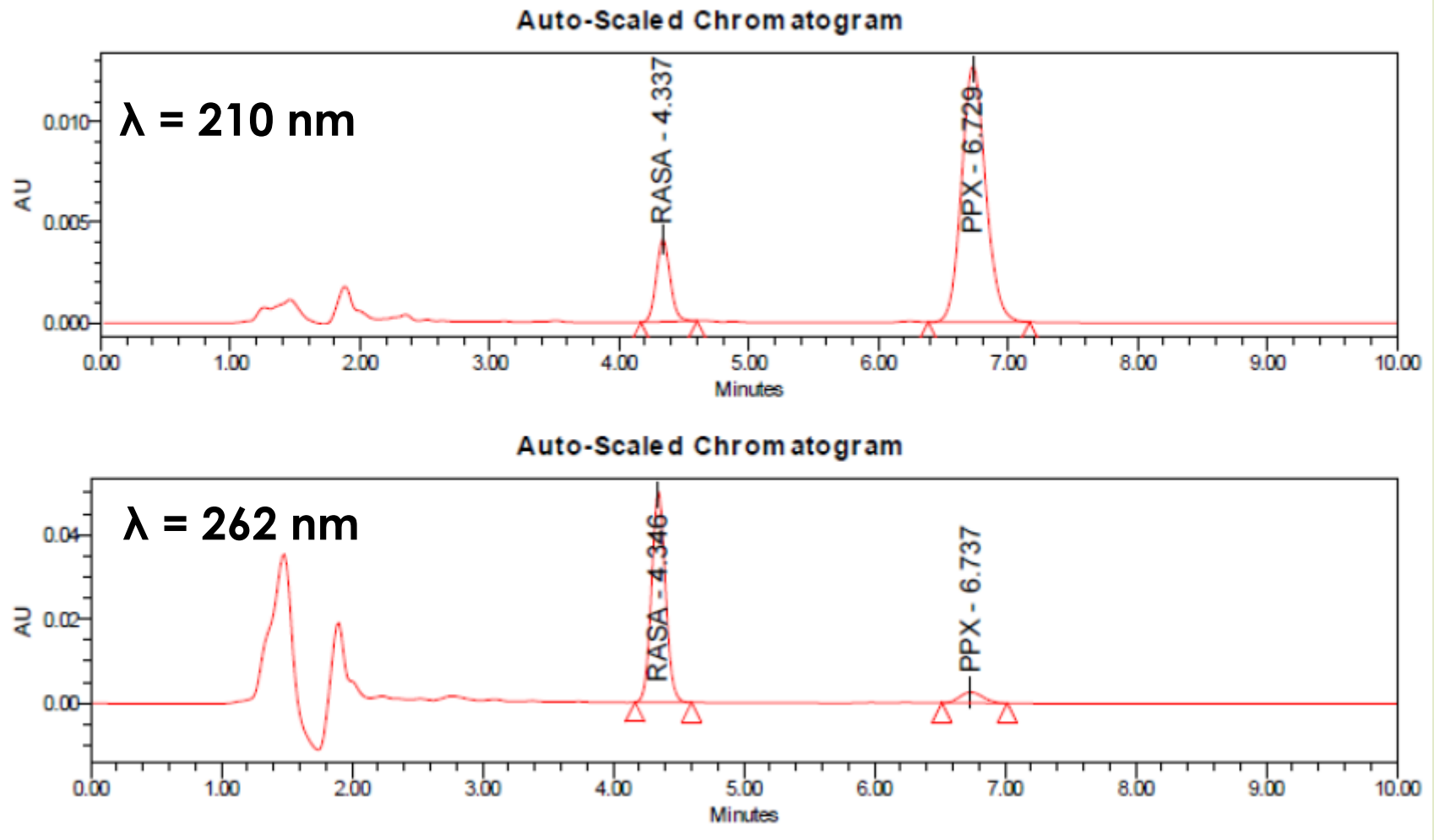
Diss. Medium: Phosphate Buffer, pH 6.8, 40% EtOH Sample Chromatogram for 30 hours time point



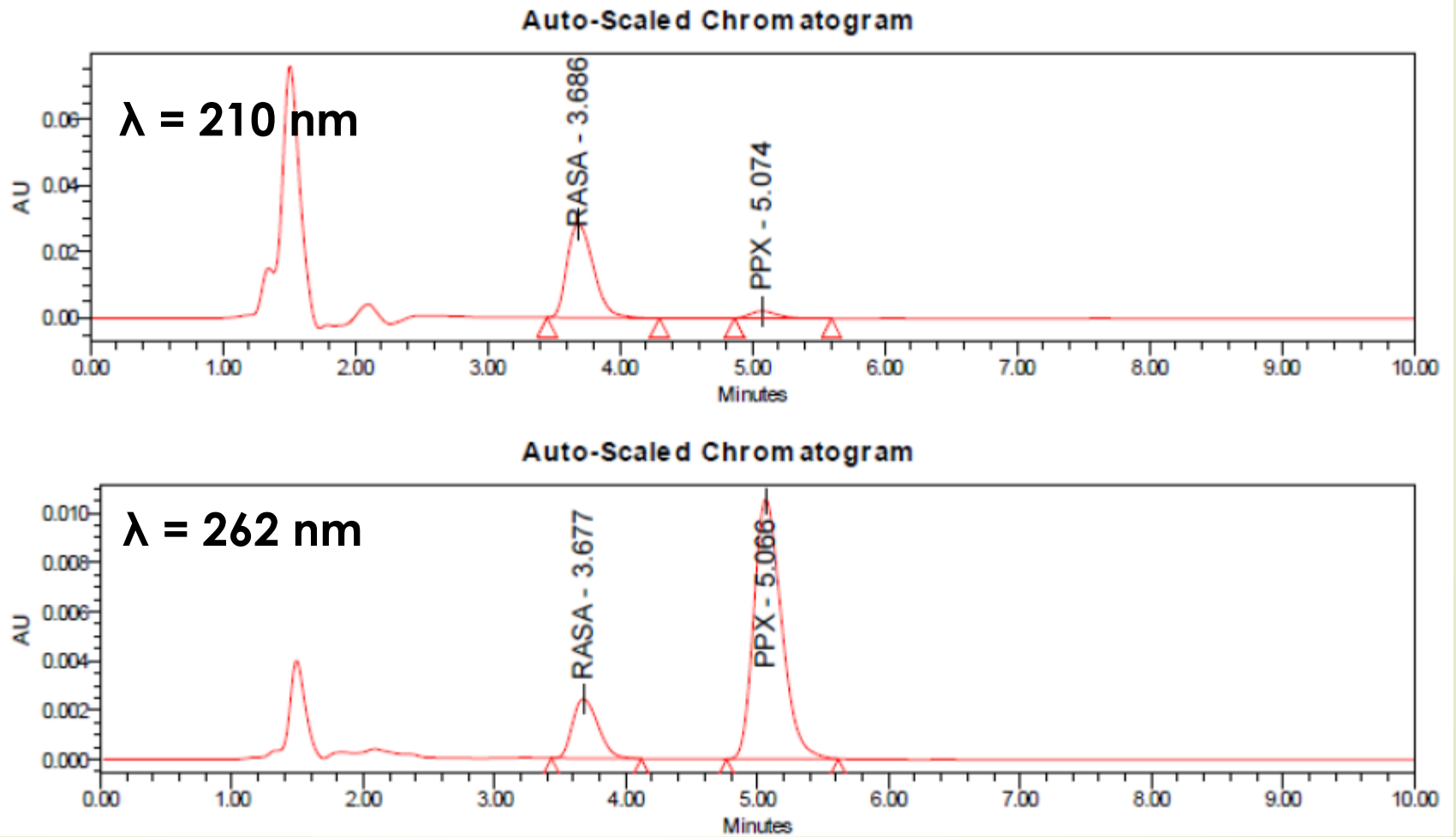
Diss. Medium: 0.1N HCl
Working Standard Chromatogram



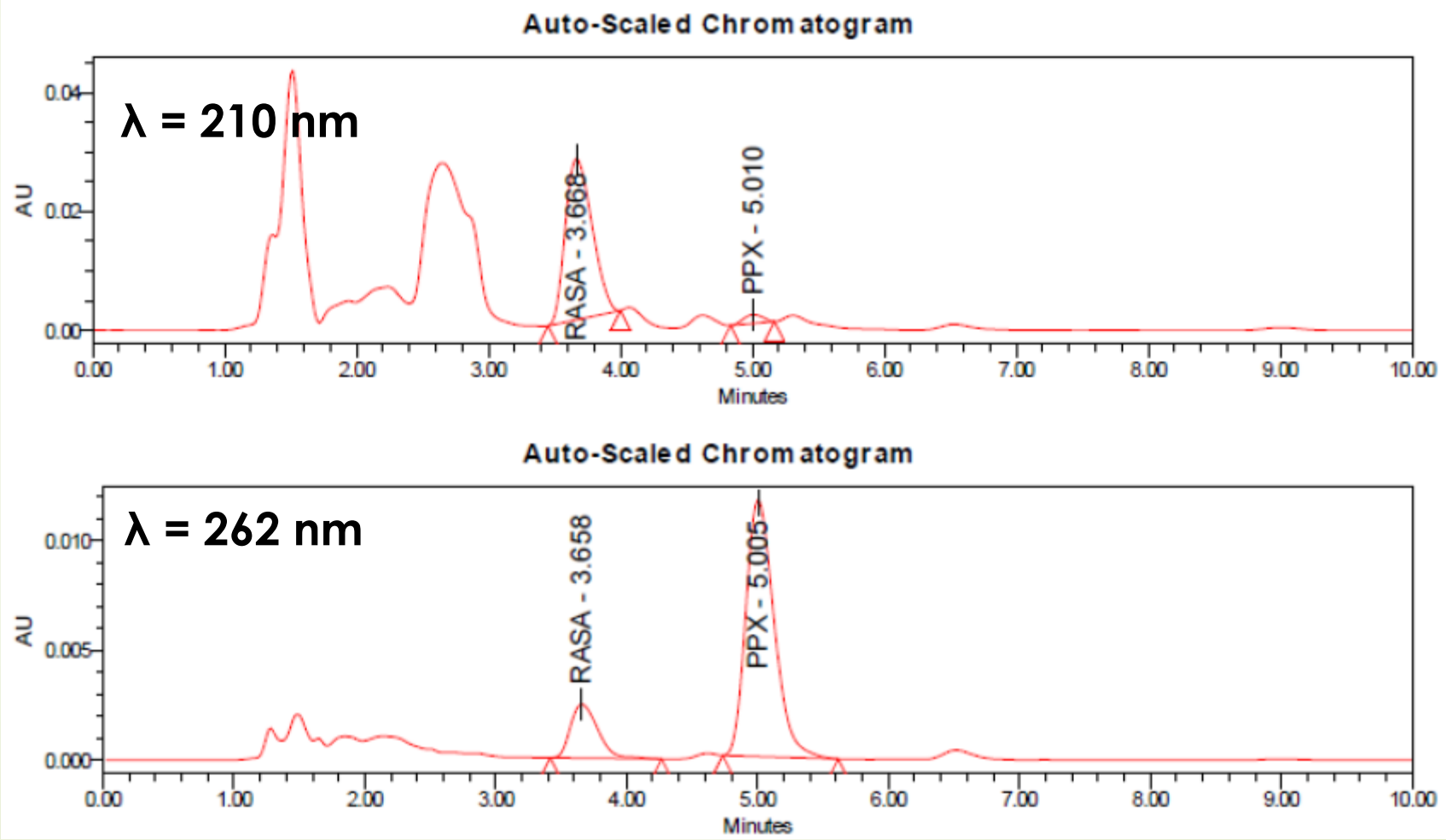
Diss. Medium: 0.1N HCl
Sample Chromatogram for 30 hours time point



Diss. Medium: 0.1N HCl, 40% EtOH Working Standard Chromatogram



Diss. Medium: 0.1N HCl, 40% EtOH
Sample Chromatogram for 30 hours time point



Summary

- Monitoring of dissolution profile was done using a gradient RP HPLC, having high aqueous content at the beginning of the gradient.
- The problem occurred when the drug product was subjected to the alcohol damping study.
- In the cases, where alcohol content was high, the strength of the diluent of the sample (dissolution medium containing ethanol) was higher than the strength of the eluent (mobile phase), the peaks were deteriorated.
- Both drug substances are weak bases, having different pKa. Therefore, a decision was taken to develop an alternative HPLC method using a mixed-mode column (a combination of cation exchange with reverse phase in one column).
- The method was successfully developed – being unaffected by the high alcohol content (up to 40%) in the sample solution.
- The method is isocratic and, as an added bonus, has a much shorter run time, than the previous one.