

Minimizing Purification Bottlenecks Using a Multi Detector Approach to Flash Chromatography While Achieving Maximum Sample Recovery in Post Synthetic Workups

**Scott Anderson, Kathy Lawrence
Dennis McCreary, Melissa Wilcox**

Grace Davison Discovery Sciences
2051 Waukegan Rd.
Deerfield IL 60015 U.S.A.
Phone: 1-800-255-8324
Website: www.discoverysciences.com

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Objective

Purification bottlenecks encountered when using flash chromatography may include (1) the necessity of collecting everything eluted during a run, (2) checking the composition of every fraction collected, and (3) replacing an interfering solvent with a more compatible one.

By using the RevealX™ detection technology of the Reveleris® system, which independently triggers fraction collection from multiple detectors including UV and ELSD, this work will demonstrate that these bottlenecks can be reduced or eliminated without reducing the recovery of compounds of interest.

To quantify the reduction in bottlenecks, a process map for flash purification was designed and tasks required assigned to each segment of the map. Time required for each of these tasks was then measured. Using these time measurements a comparison of total time required can be made between traditional flash technology using only UV detection and the RevealX™ technology of the Reveleris® system. The traditional flash technology is simulated by turning off the ELSD detector.

Using the methodology required by each technology to combine fractions, a comparison of amounts recovered of each component separated can be made.

Time Measurements for Tasks

Map Segment	Task	Time (minutes)
Pre Run Workup	Replace reaction solvent ¹ (100mL) by evaporating, find and reconstituting in more compatible solvent	37
	TLC Tank preparation ²	4
	Develop TLC plate ³ and UV check (254nm)	30
	Visualize by iodine ⁴	15
Instrument Setup	Prime pumps, mount cartridge, prepare injection, and set run parameters	14
Perform Separation	Depends on equilibration and run time of the separation	Run Dependent
Analyze Fractions	Develop TLC plate and UV check	30
	Visualize by iodine	15
Combine and Evaporate	Evaporate 20mL of solvent from fraction ⁵	5

¹For this work the reaction solvent is toluene

²Involves preparing mixture on hexane and ethyl acetate

³For this work 20cm x 20cm Reveleris® silica plates (hard layer, organic binder, fluorescent indicator, 254nm) were used

⁴After evaporation of solvent, plate is inserted into a tank containing iodine crystals. Compounds are visible after absorbing the iodine vapor

⁵For this work the solvent is a mixture of hexane and ethyl acetate (may contain toluene for fast eluting components)

Flash Separation Conditions

Column	12g Reveleris® silica
Sample Size	10mL
Injection Mode	Liquid
Flow Rate	36mL/min
Equilibration	Varies
Run Length⁶	Varies
Air Purge	Varies
Slope Detection	Varies
ELSD Threshold Detection⁷	3mV
UV Threshold Detection	0.02au
Collection Mode⁸	Varies
Per Vial Volume Maximum	29mL
Per Vial Volume Peaks	20mL
Per Vial Volume Non-Peaks	20mL
UV1 wavelength	254nm
UV2 wavelength⁹	280nm
ELSD Carrier Solvent	Isopropanol
Solvent A	Hexane
Solvent B	Ethyl acetate
Color of ELSD Signal Trace	Green
Color of UV1 Signal Trace	Blue
Color of UV2 Signal Trace	Red
Color of Gradient Trace	Gray

⁶Determined by the gradient profile

⁷Not used when simulating traditional flash chromatography

⁸Either "Collect All" or "Collect Peaks"

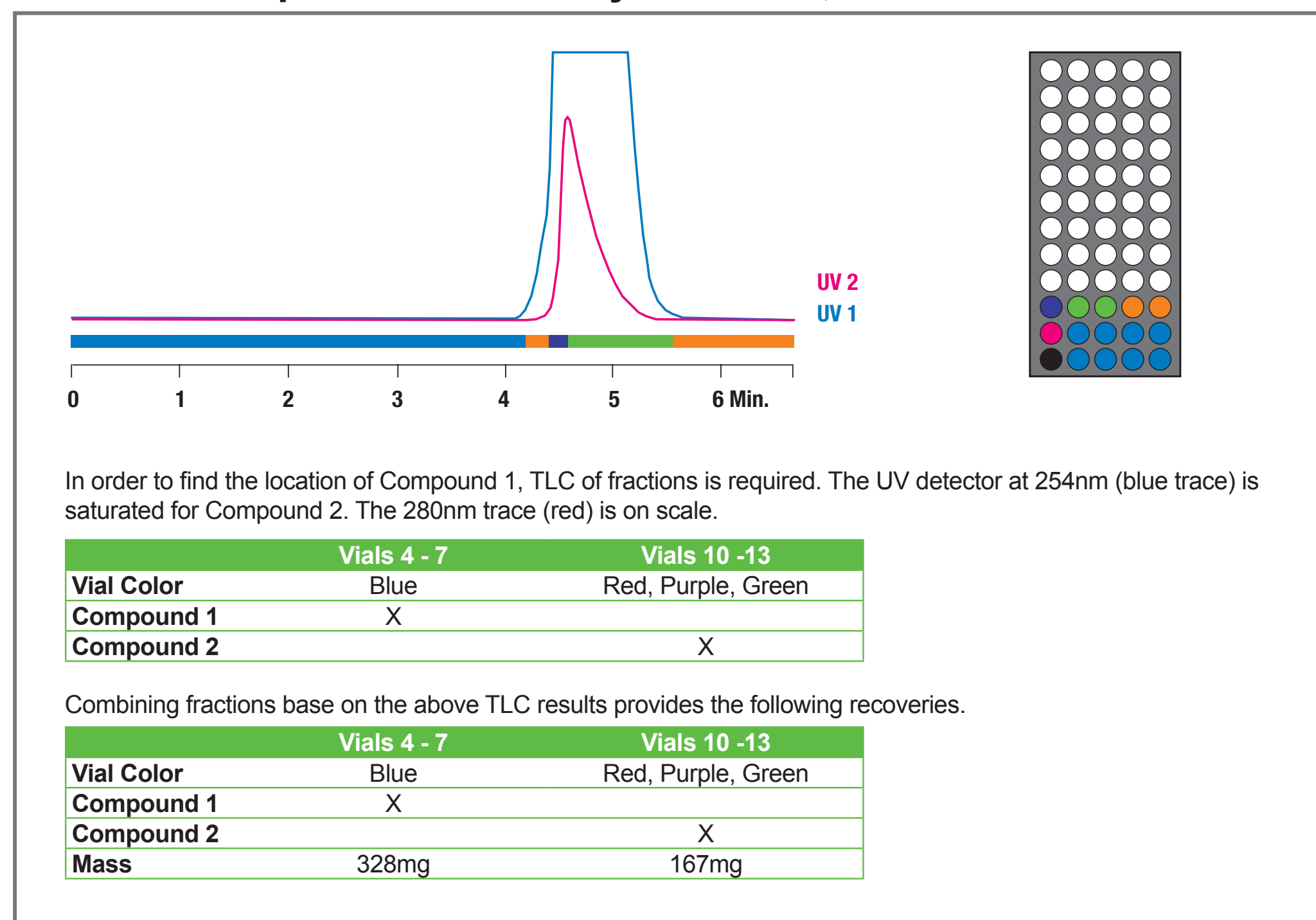
⁹When used

Separation Challenge 1

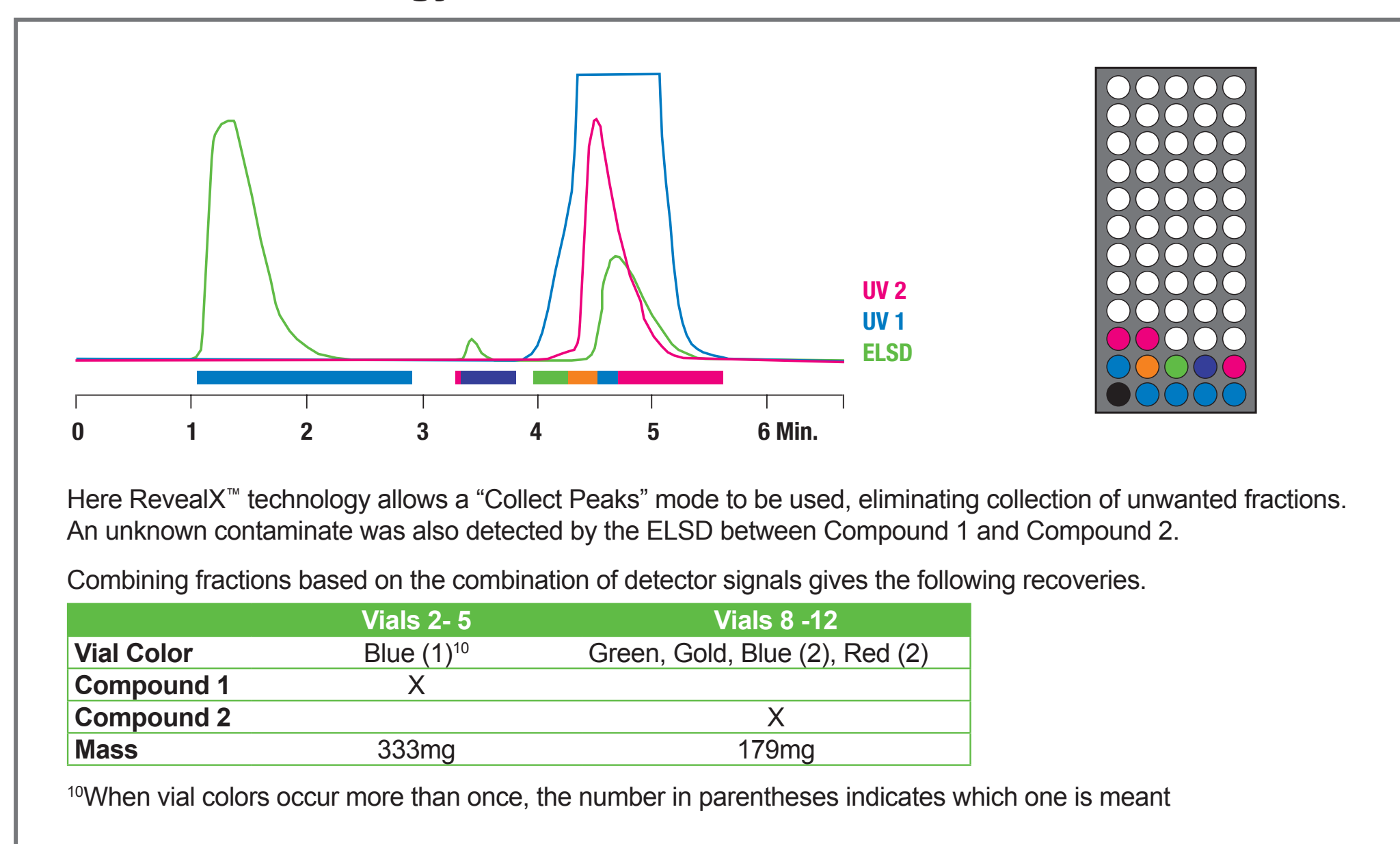
This challenge is the separation of a non-UV absorbing Compound 1 (cholesterol acetate) from a UV absorbing Compound 2 (butyl paraben) in a non-UV absorbing solvent (hexane/ethyl acetate, 90/10). Both these compounds have good peak shape, which allows "Collect Peaks" mode for the RevealX™ technology. Also the strong UV absorbance of Compound 2 allows the retention time when the UV threshold is met to compare well with the retention time when the ELSD threshold is met for this compound.

TLC Rf's for the Mixture			Other Conditions	
Spot	20% B	30% B	Equilibration	6 min
Compound 1	0.73	0.75	Run Time	6.7 min
Compound 2	0.35	0.50	Air Purge	1.5 min
Gradient for Flash Separation			Slope Detection	High Sensitivity
Time (min)	%B			
0	0%			
1	0%			
2.4	20%			
3.3	20%			

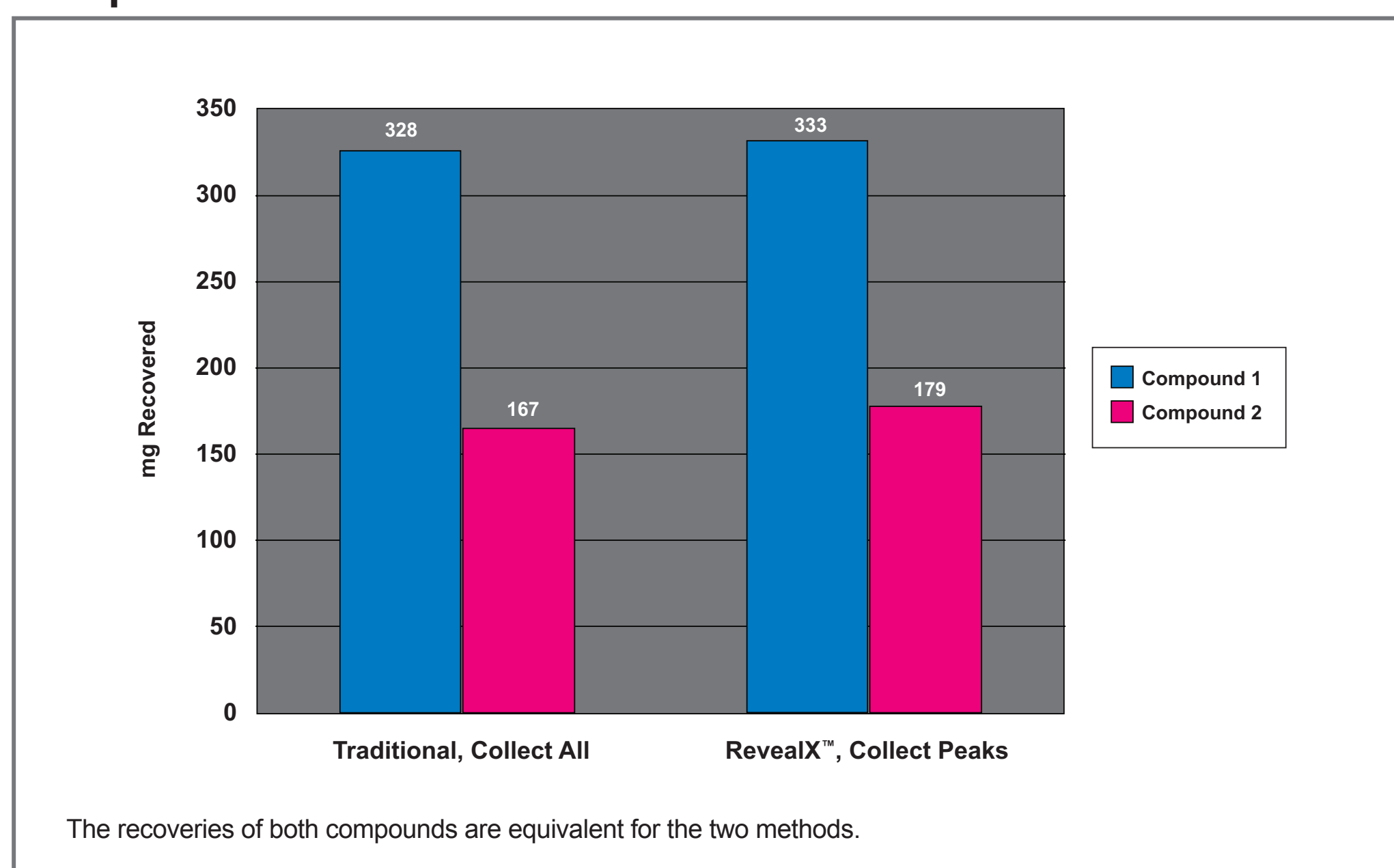
Traditional Separation – UV only detection, Collect All Mode



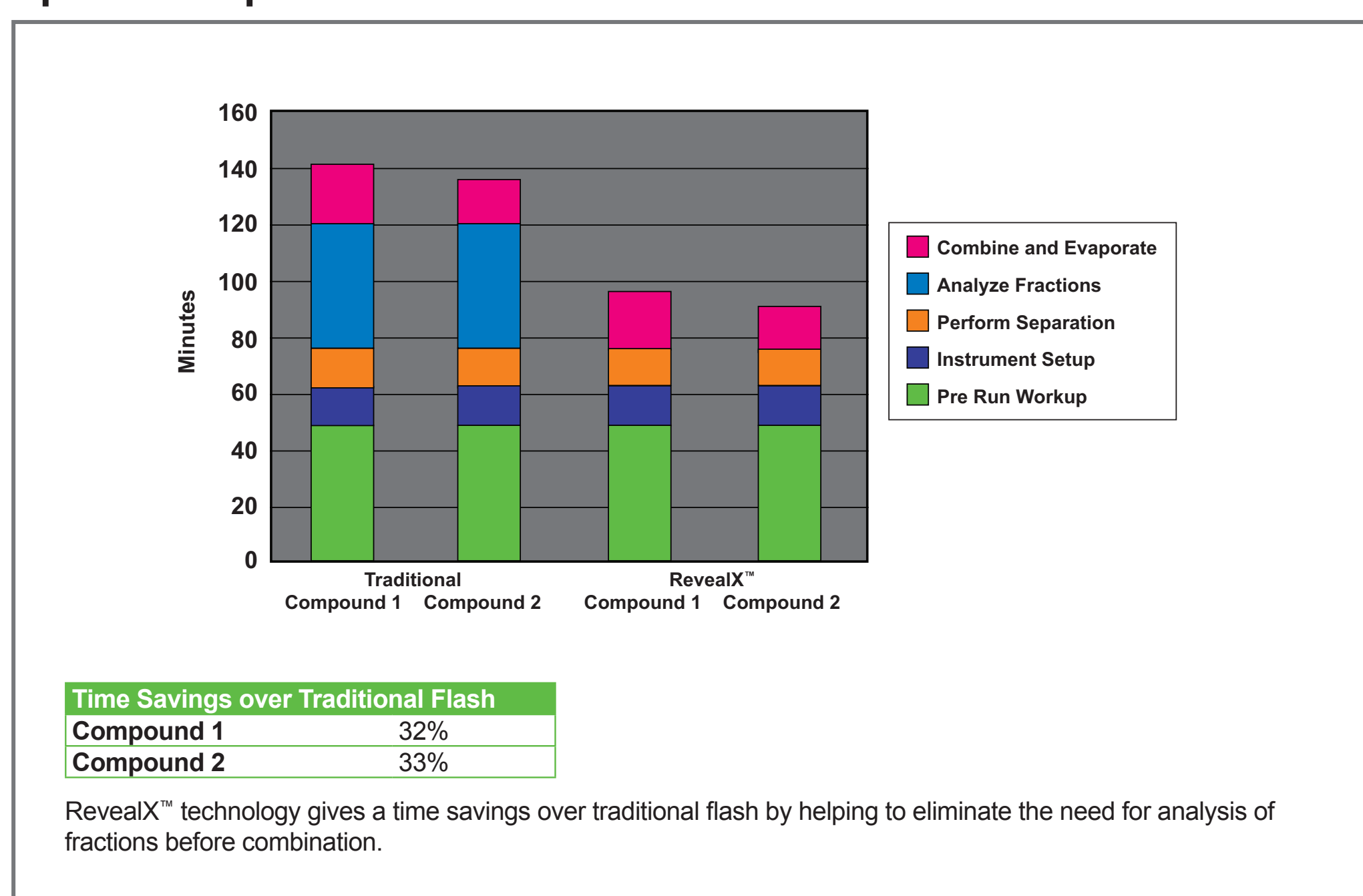
RevealX™ Technology – UV and ELSD detection, Collect Peaks



Comparison of Recoveries



Speed Comparison



Separation Challenge 2

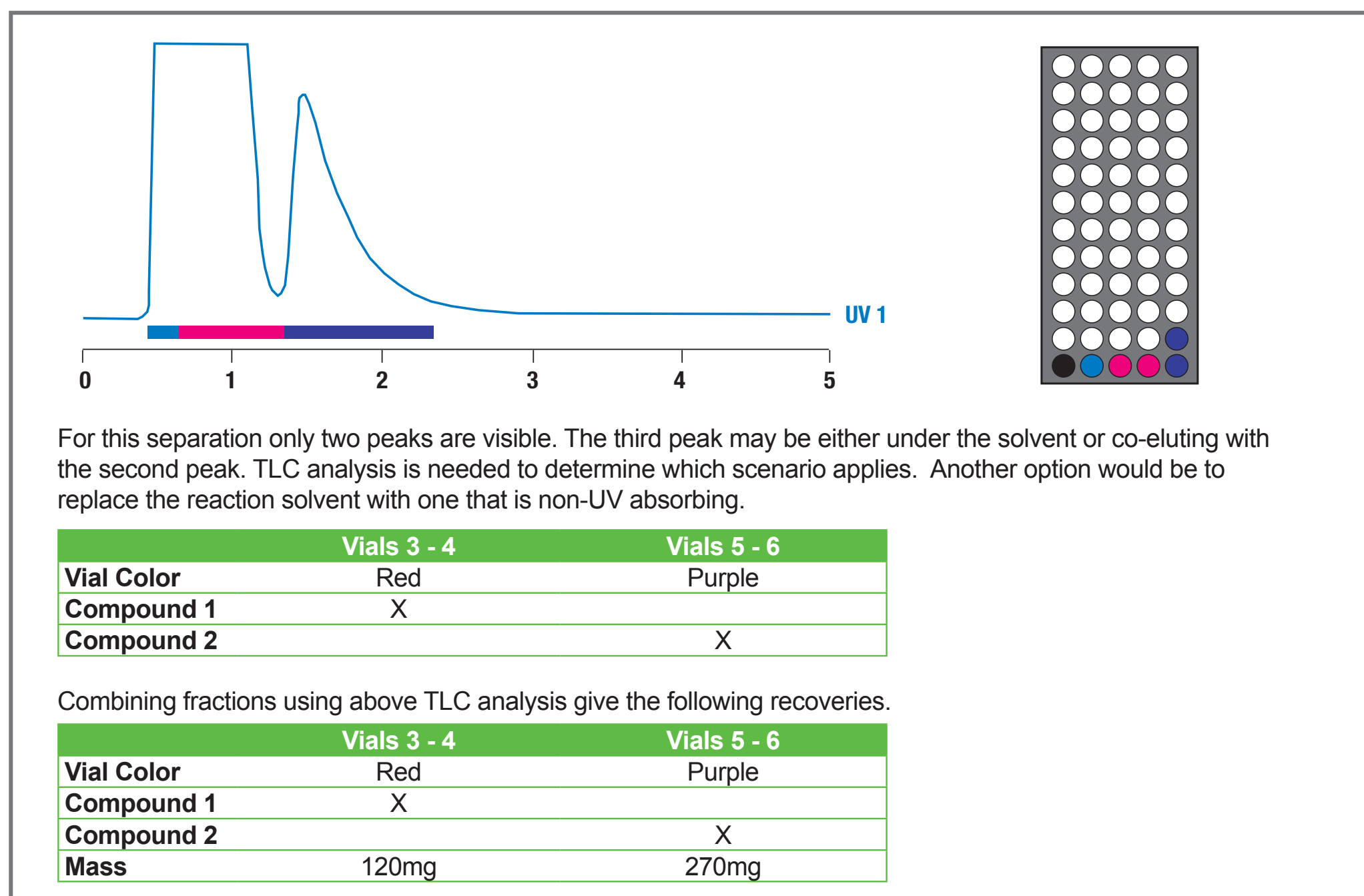
This challenge is the separation of two UV absorbing compounds, Compound 1 (2-chloro-2', 6'-dimethylacetanilide) and Compound 2 (lidocaine) in a UV absorbing solvent (toluene). Because of the tailing of Compound 2, a strong starting concentration was selected.

TLC Rf's for the Mixture		
Spot	30% B	50% B
Compound 1	0.27	0.53
Compound 2	0.16	0.31

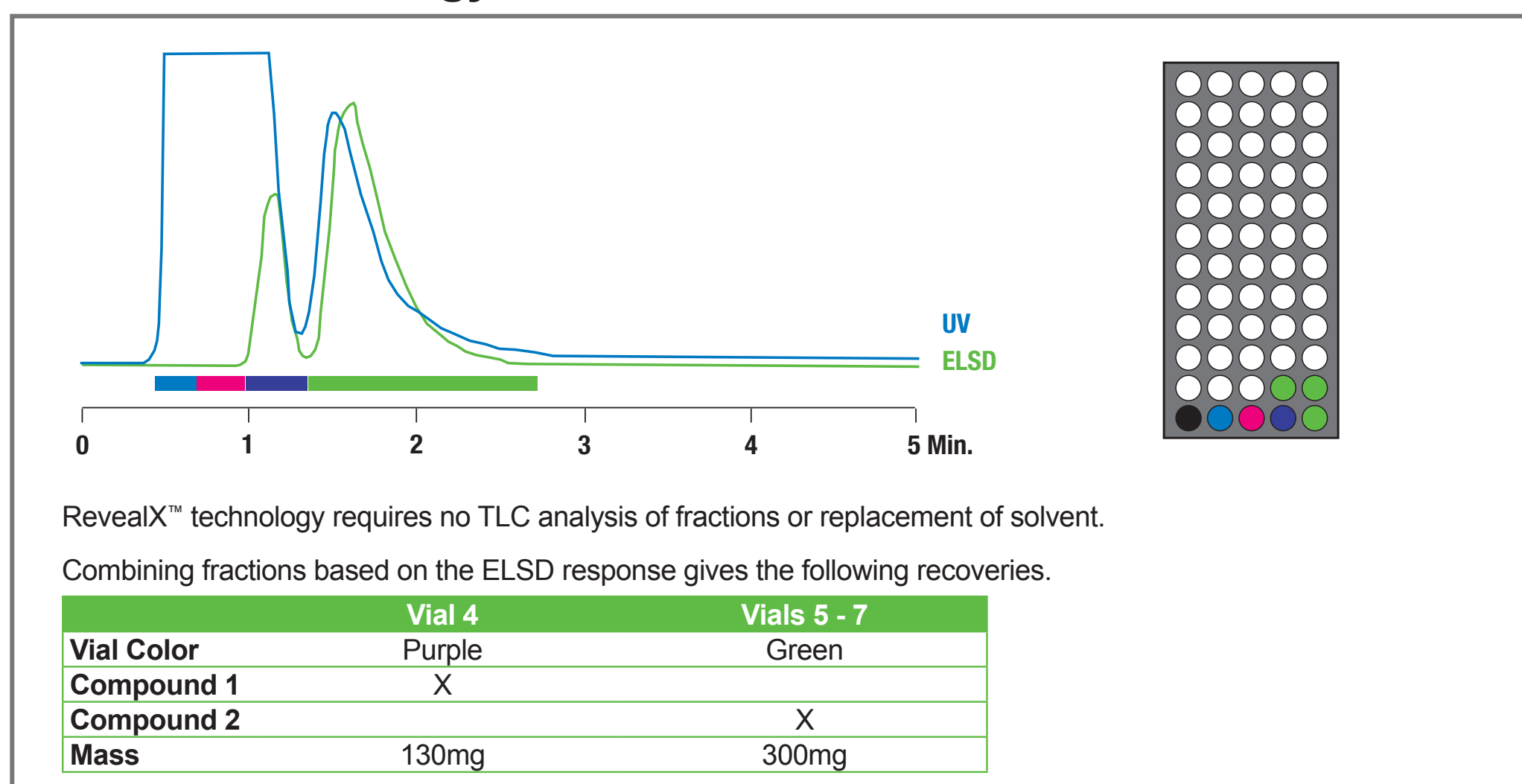
Gradient for Flash Separation	
Time (min)	%B
0	60%
1	60%
3	100%
1	100%

Other Conditions	
Equilibration	2.8 min
Run Time	5 min
Air Purge	0.5 min
Slope Detection	High Sensitivity
Collection Mode	Collect Peaks

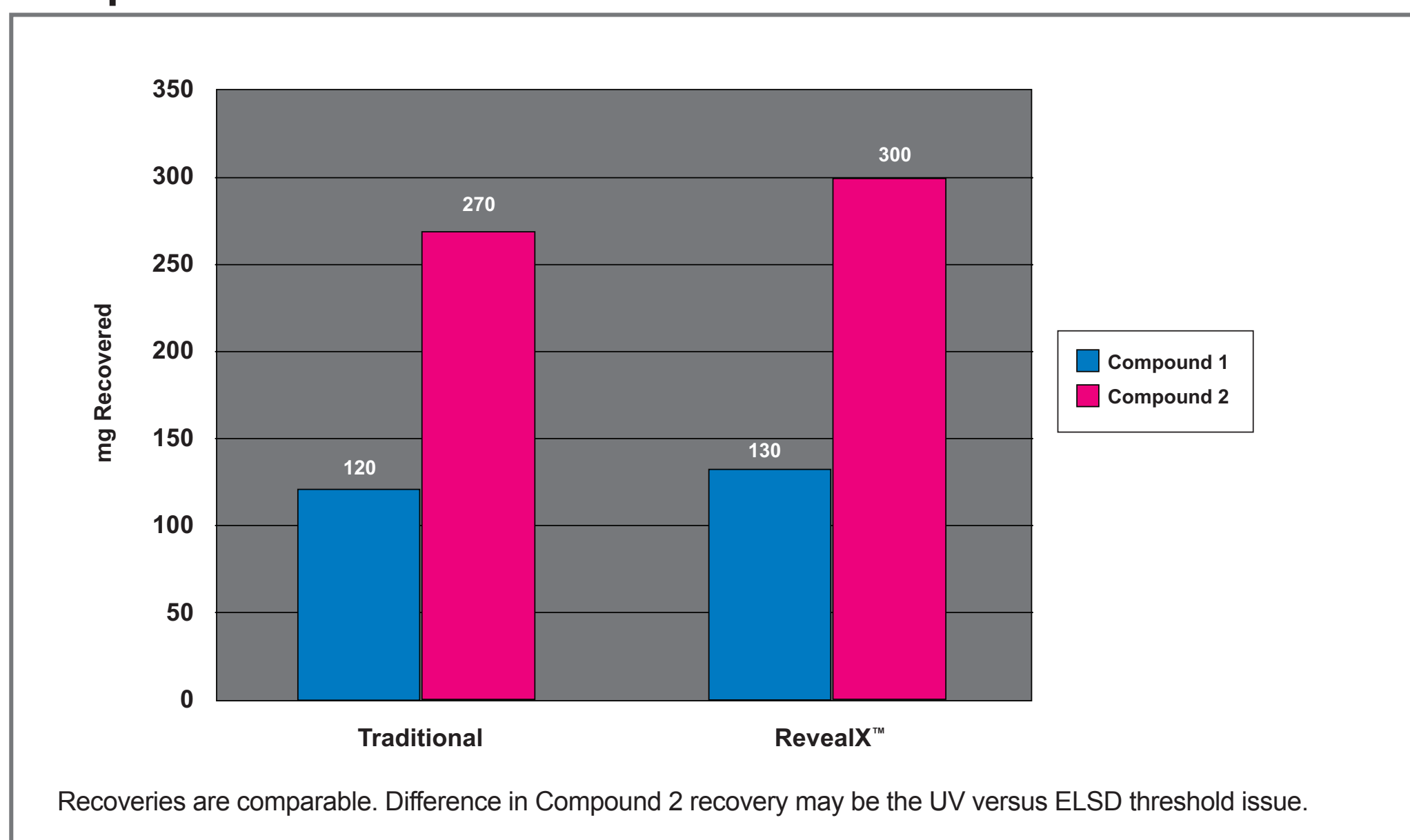
Traditional Separation – UV only detection



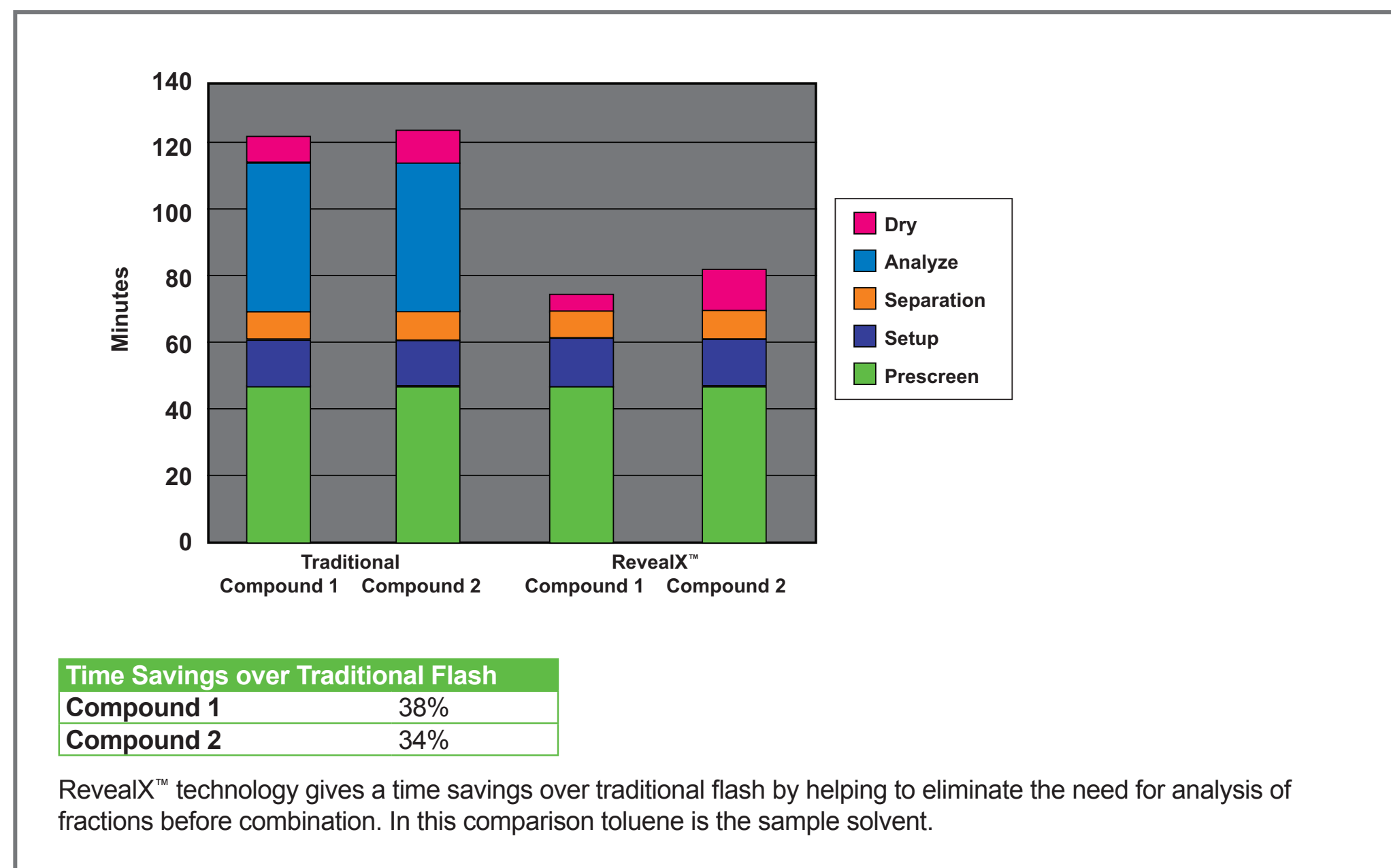
RevealX™ Technology – UV and ELSD detection



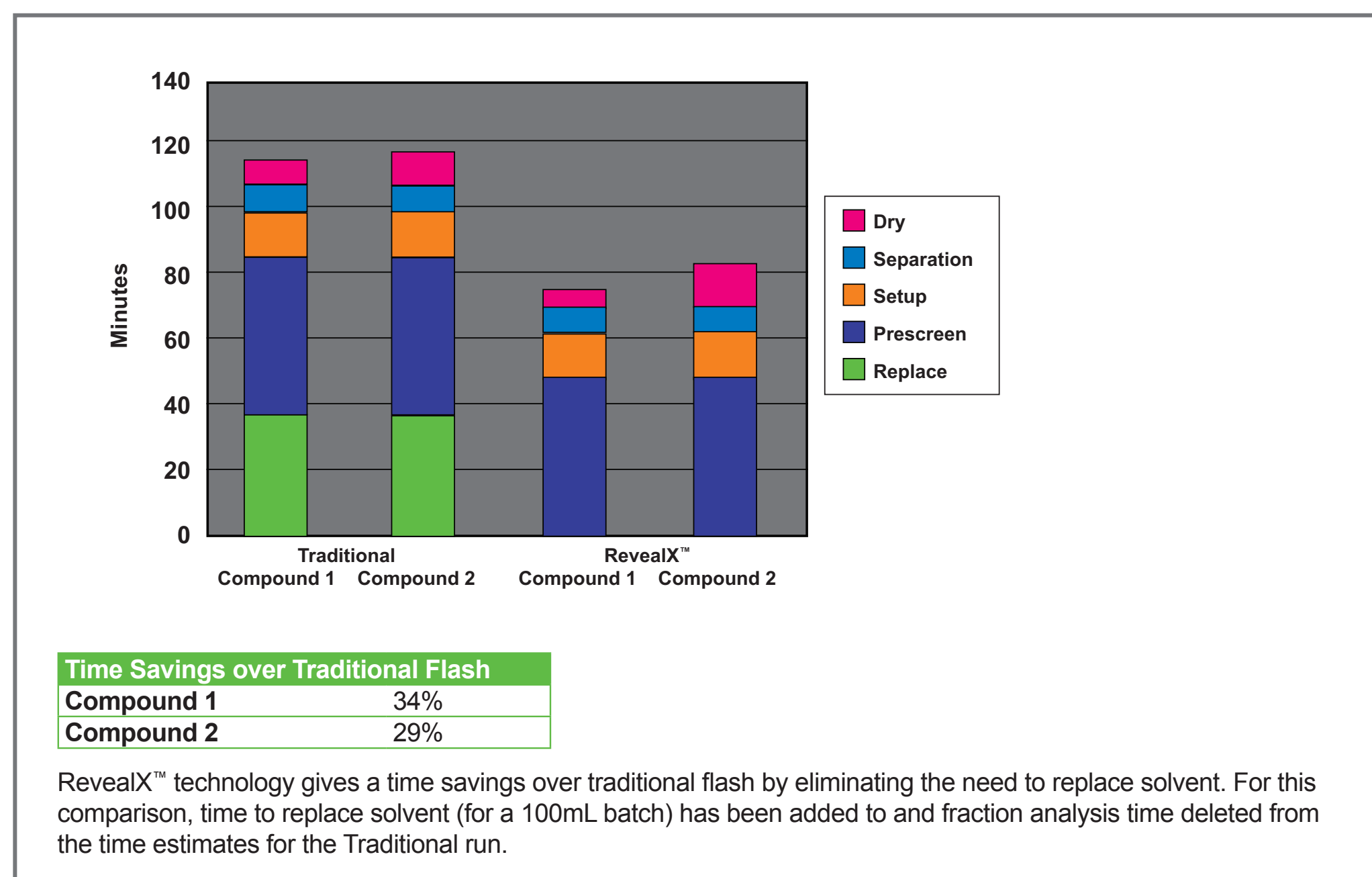
Comparison of Recoveries



Speed Comparison (TLC analysis of Fractions)



Speed Comparison (Solvent Replacement)



Conclusion

Independent triggering of fraction collection from multiple detectors including UV and ELSD incorporated in the RevealX™ technology of the Reveleris® system allows the elimination of fraction analysis before combination, saving 32% to 38% of the time needed to isolate components of the mixtures studied.

For separations of UV absorbing compounds in an interfering UV absorbing solvent, this system can eliminate the need to replace the solvent resulting in a 29% to 34% reduction in isolation time.

By allowing regular use of the "Collect Peak" mode, the system helps to eliminate the need to collect fractions containing no components.

These enhancements in productivity are achieved without the sacrifice of recovery of components of the mixtures studied.

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