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OVERVIEW

We have developed an automated approach to column and solvent evaluations with an emphasis on carryover assessment that has potential for reducing the time required to develop a robust and effective bioanalytical LC/MS/MS method.

INTRODUCTION

Excessive drug carryover is a common problem that can severely limit the dynamic range of calibration and degrade the precision and accuracy of results obtained during method validation and sample analysis. To address this problem, we have expanded our typical chromatography optimization procedures to also include a simultaneous carryover assessment in addition to column and mobile phase evaluations using an automated system with the ability to evaluate up to 11 different analytical columns and 2 different mobile phase systems per automated run.

METHODS

The techniques and strategies described here are generally applicable to compounds that are amenable to LC/MS/MS using reversed phase chromatography. For this particular work, we evaluated the optimal conditions for chromatography and carryover for a low molecular weight (MW ~ 450) polar amine drug extracted from rat plasma by protein precipitation. Quality control samples and blanks were prepared from rat control matrix that previously had been shown to be free of interference. The sampling scheme to evaluate carry over was BLANK, LLOQ, ULOQ, BLANK, BLANK.

Automated evaluations were performed using a TLX-2 system (Figure 1) with a Multiple Column Module (MCM; Figure 2), coupled to Applied Biosystems API 4000™ triple quadrupole mass spectrometer with Analyst 1.4.2™ software. The system control software was Aria OS 1.5.1™ (Figure 3).

For each of two runs, the TLX-2 with MCM was set up with the same 6 columns and 2 different mobile phase systems to evaluate a total of 6 different columns and 4 different mobile phase systems. The LC program was isocratic, where % B was increased by 10% each subsequent injection with a % B range of 40% B to 90% B. Mobile phase systems evaluated were:

- 1) 0.1% NH₄OH and MeOH with 0.1% NH₄OH
- 2) 0.1% formic acid (FA) and ACN with 0.1% FA;
- 3) 0.1% FA and MeOH with 0.1% FA;
- 4) (95:5 H₂O:MeOH) with 2 mM NH₄OAc and (85:10:5 ACN:H₂O:MeOH) with 2 mM NH₄OAc.

Figure 1: TLX-2 System



Figure 2: Multiple Column Module (MCM)

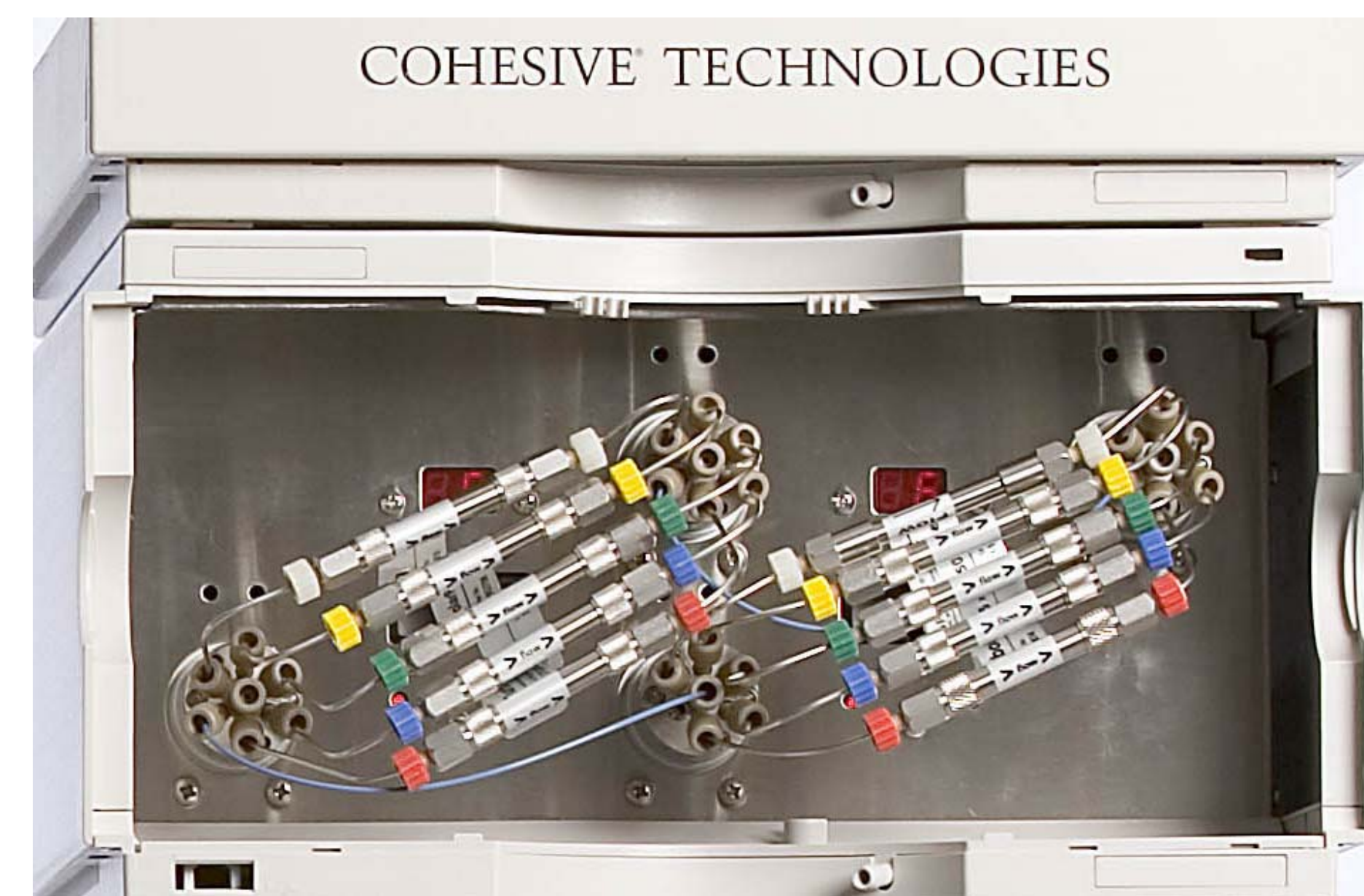
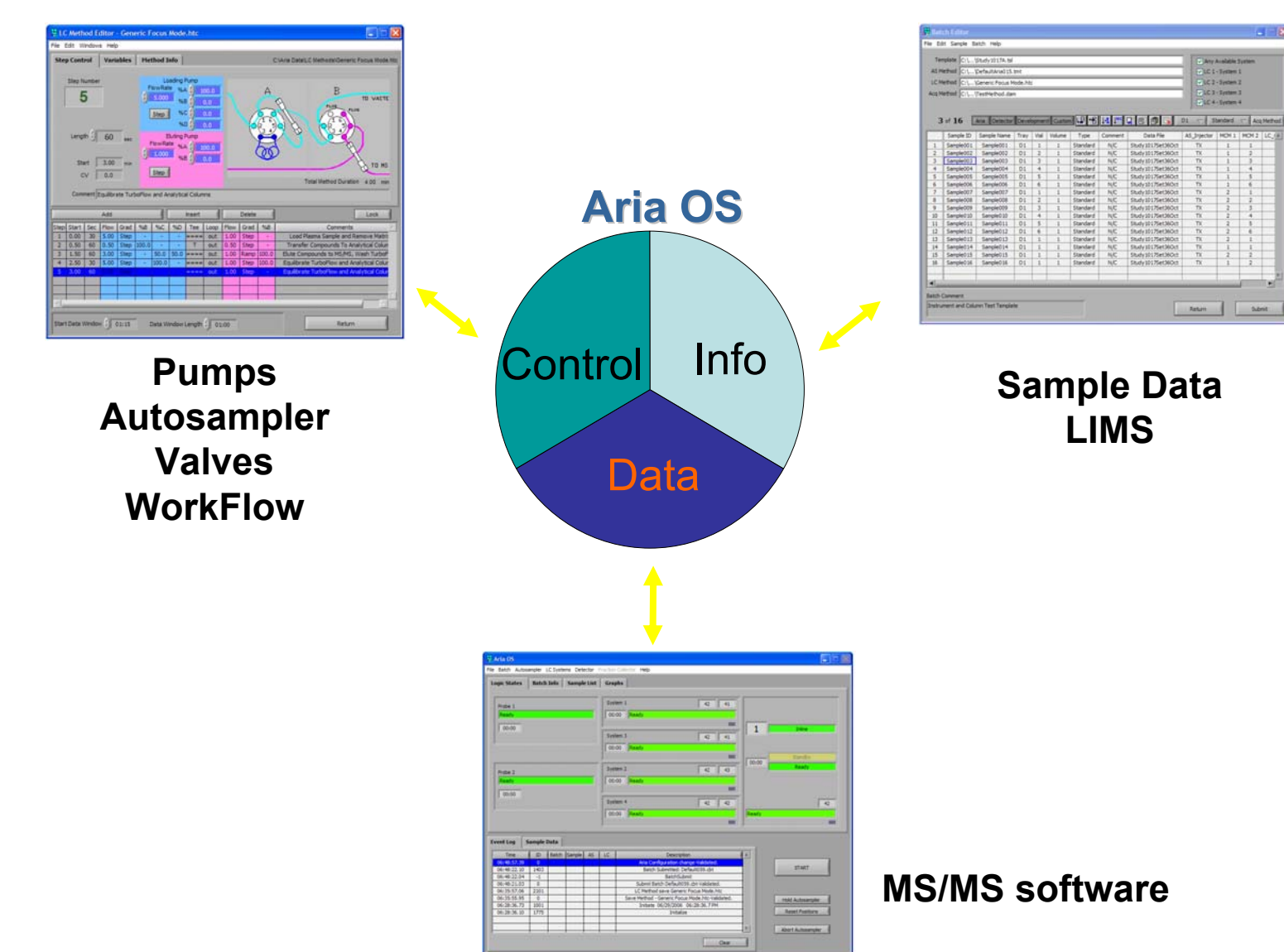


Figure 3: Aria OS System Control Software



RESULTS AND DISCUSSION

Using our automated system (TLX-2 system with MCM), the total time required to set up and execute two separate runs was approximately 2.5 hours.

In addition to the automated optimizations described here, manual evaluations were performed using 6 columns and 4 different mobile phase systems. The time required for manual evaluations of carryover, columns and mobile phases was approximately 4-fold greater (10 hours) than evaluations using our automated system.

Under acidic mobile phase conditions, drug carryover for the compound studied was observed to be as high as 136% of the LLOQ (Figures 4 & 5; chromatogram of LLOQ is not shown).

Using the final optimized conditions (Waters XBridge C18 column, 0.1% NH₄OH and MeOH with 0.1% NH₄OH mobile phases, 50-65% B gradient), the drug carryover was eliminated. (Figs. 6-8).

The bioanalytical method (represented here by Figures 6-8) developed using our automated system for chromatography screening has been validated and meets or exceeds all acceptance criteria (Table 1).

Figure 4: Chromatograms of Drug at ULOQ (top) and IS in Rat Plasma Extract Using Acidic Mobile Phases.

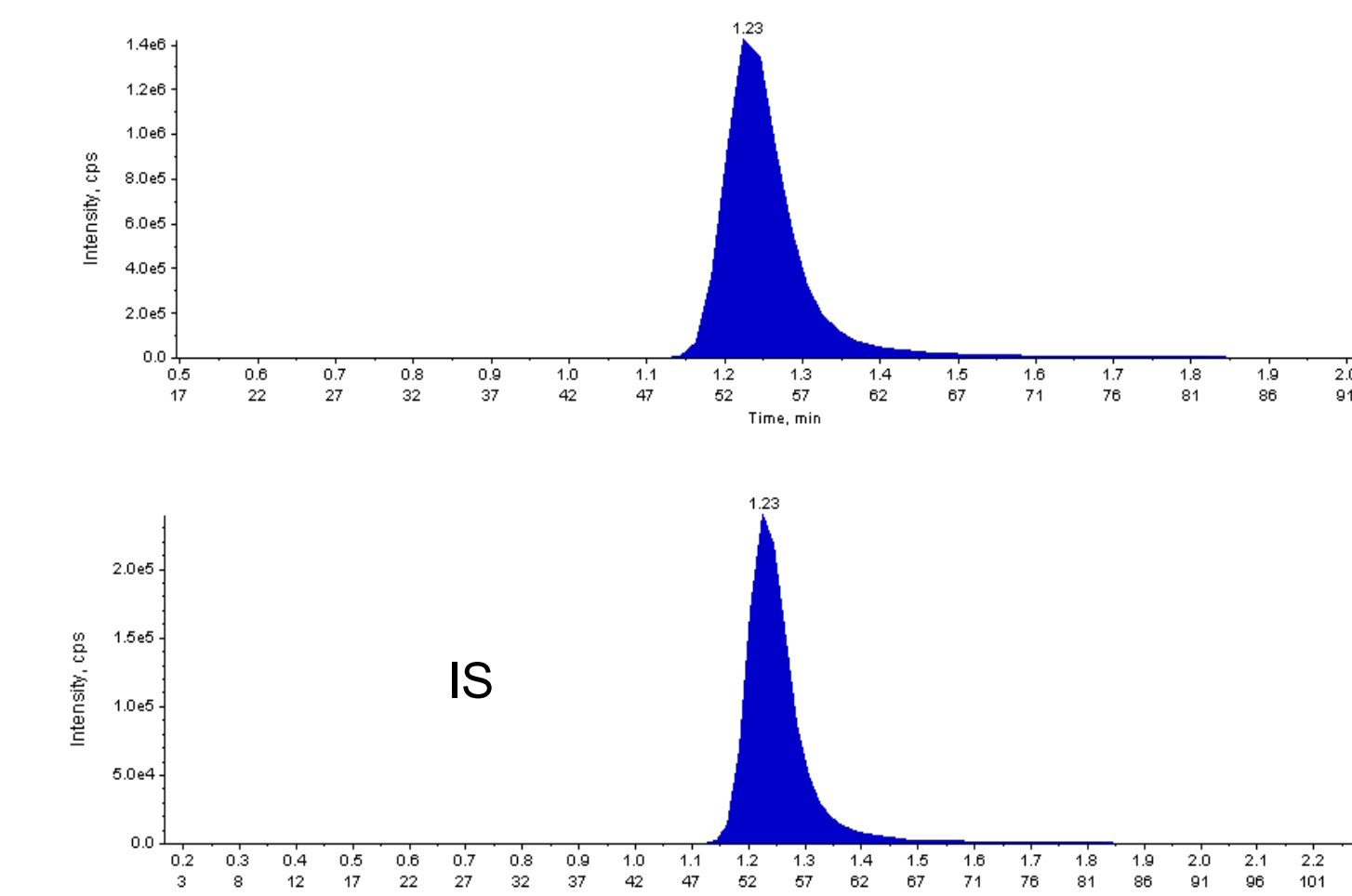


Figure 5: Chromatograms Showing Carryover of Drug (top) and IS in Blank Following ULOQ Injection Using Acidic Mobile Phases.

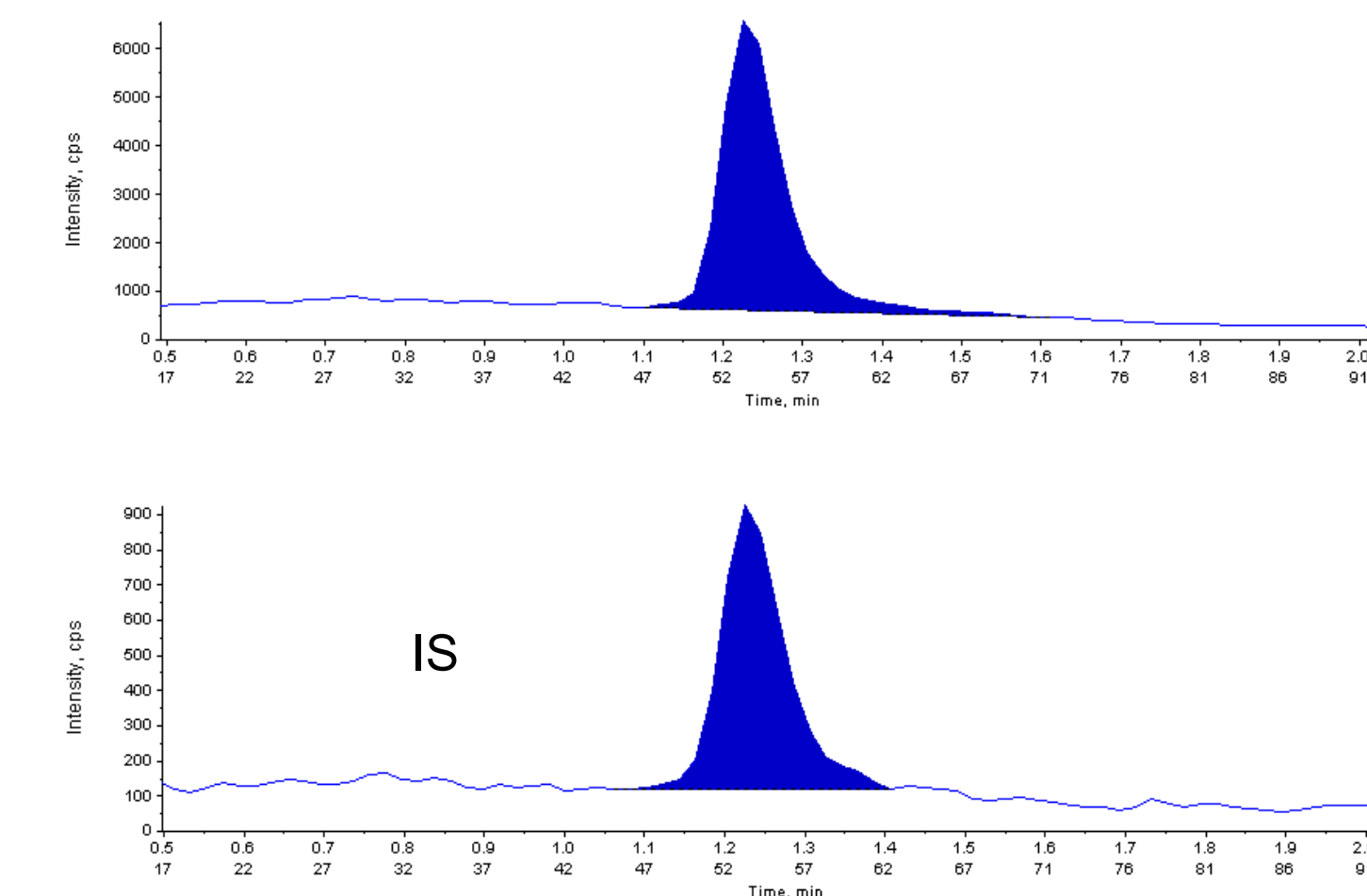


Figure 6: Chromatograms of Drug at LLOQ (top) and IS in Rat Plasma Extract Using Optimized Conditions.

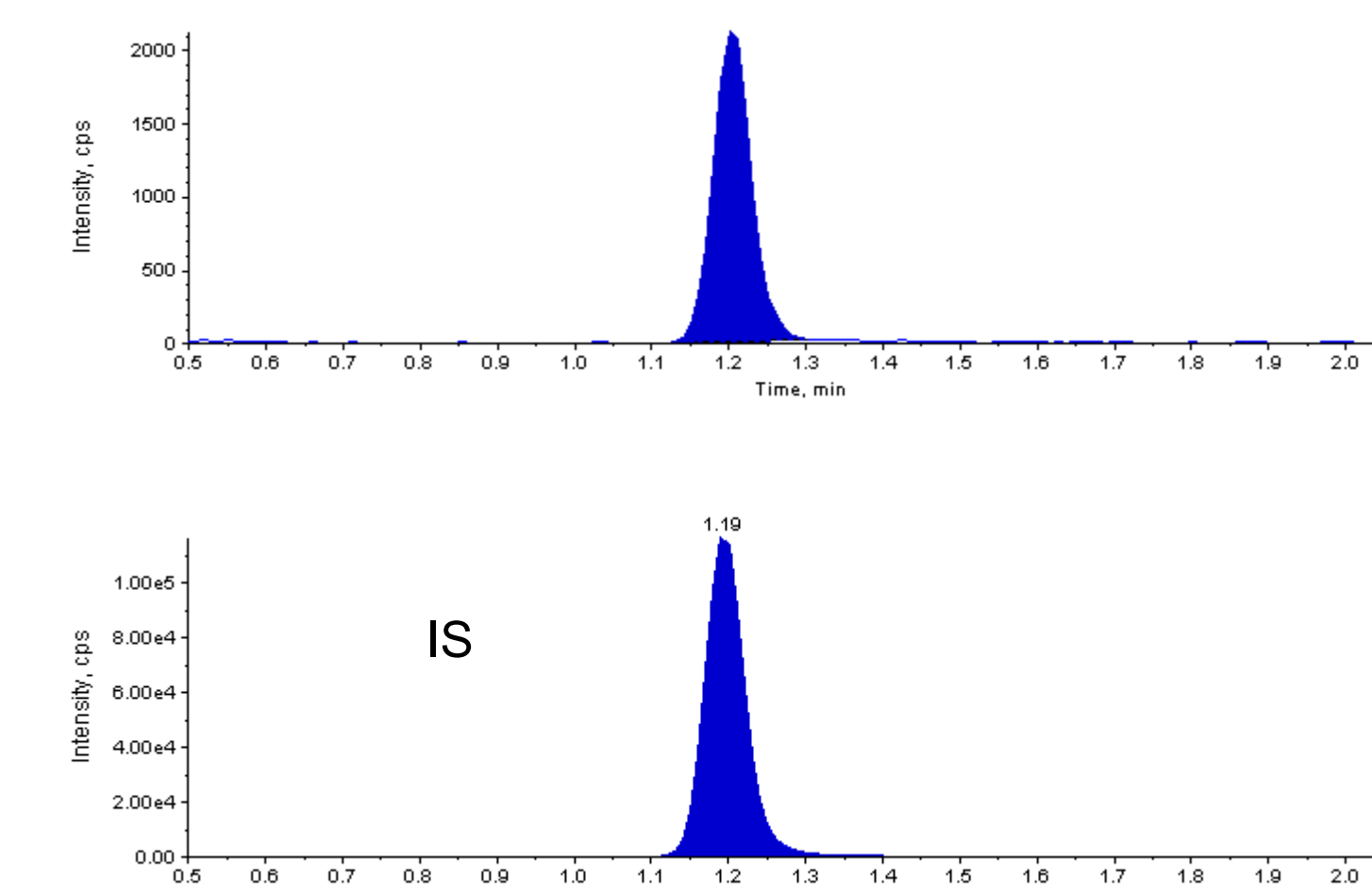


Figure 7: Chromatograms of Drug at ULOQ (top) and IS in Rat Plasma Extract Using Optimized Conditions.

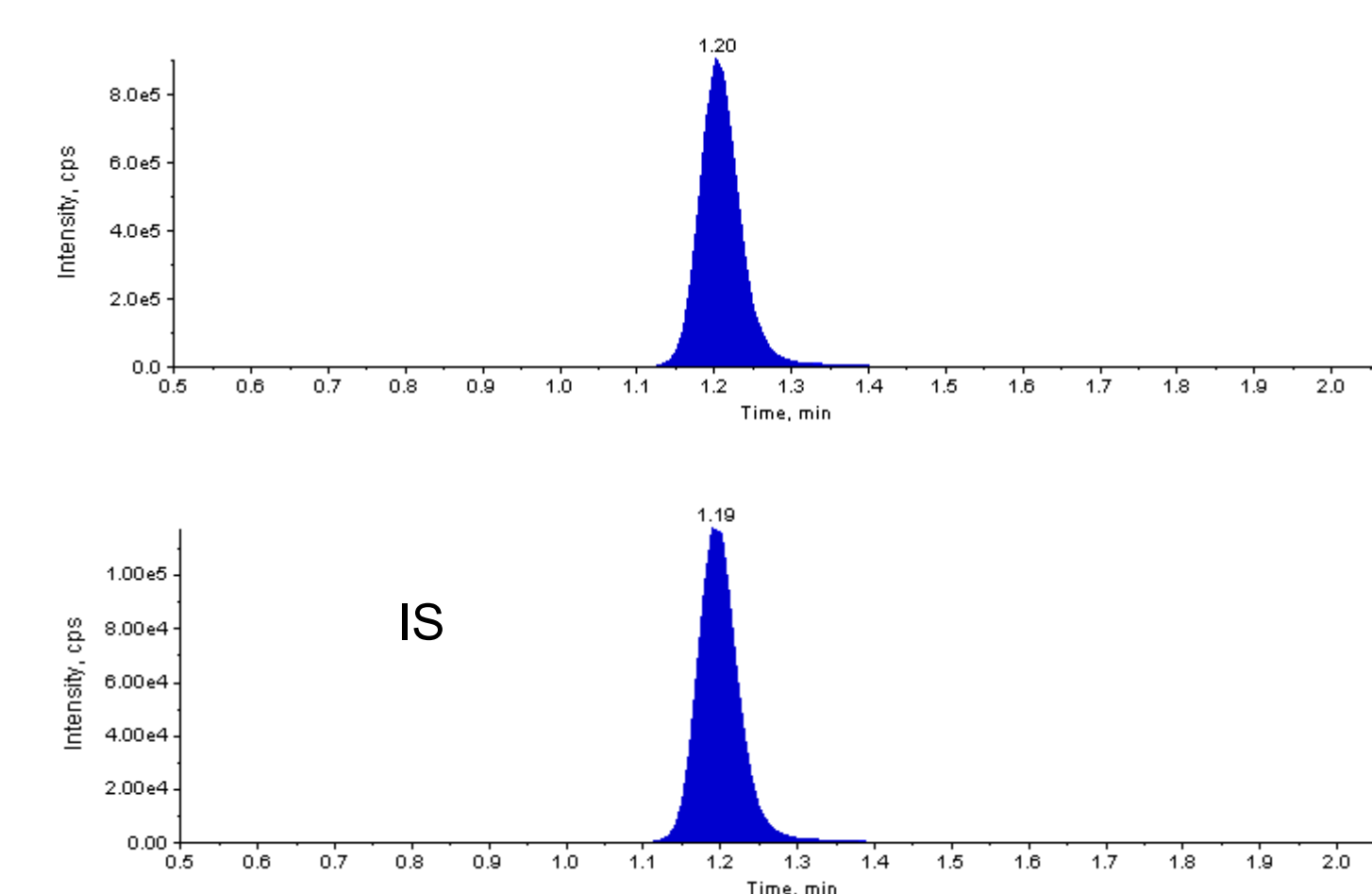


Figure 8: Chromatograms Showing No Carryover of Drug (Top) and IS in Blank Following ULOQ Injection Using Optimized Conditions.

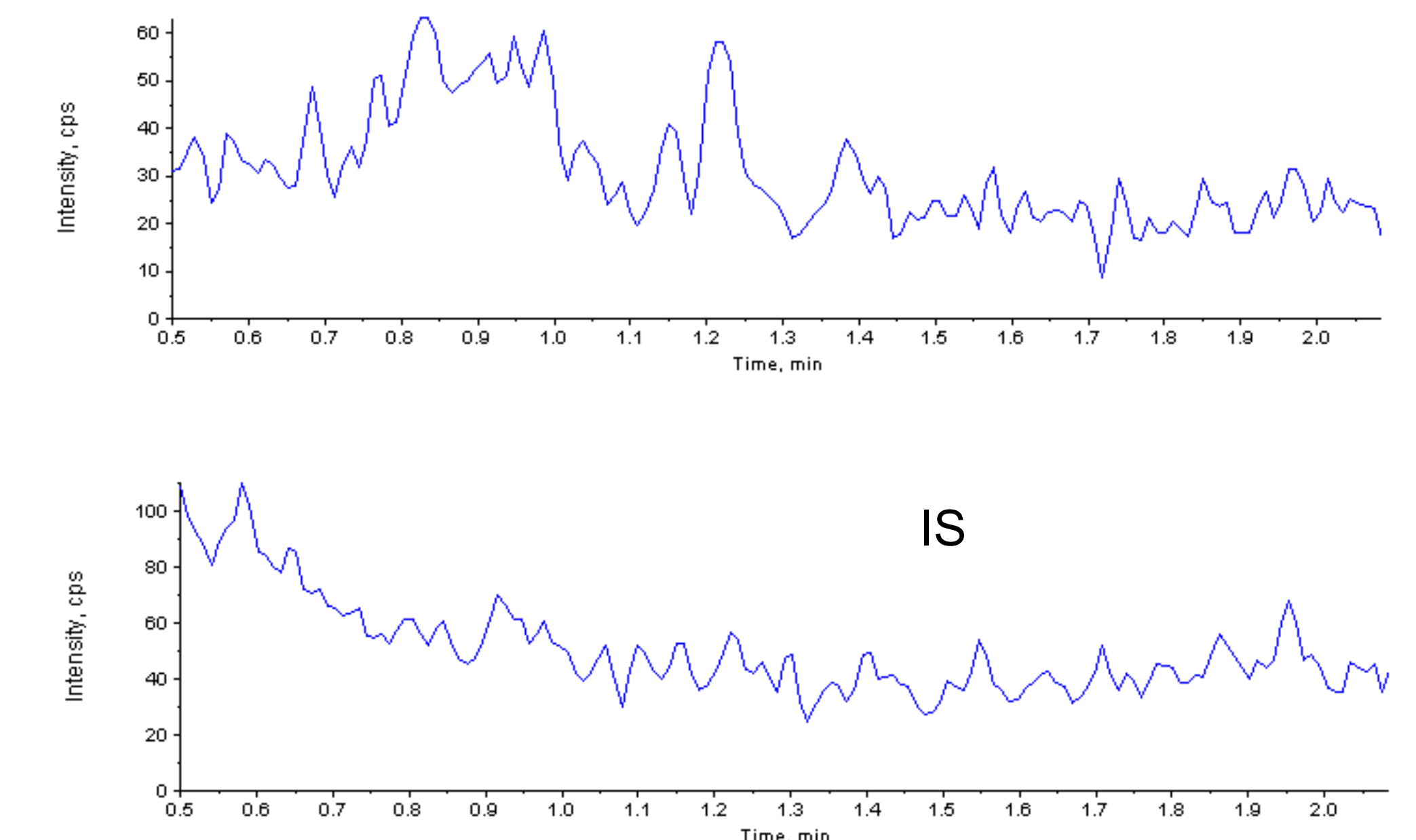


Table 1: Validation Summary

Sample Preparation	Protein Precipitation (3:1 with ACN/MeOH)	
Mobile Phase A	~0.1% NH ₄ OH	
Mobile Phase B	MeOH with ~0.1% NH ₄ OH	
LC Program	Time (min.)	Solvent B (%)
	0.00	50
	1.70	65
	1.71	100
	2.70	100
LC Program	2.71	50
	3.70	Stop
	Column/ Temp/ Flow Rate	Waters XBridge C18, 2.1 x 50 mm, 5 μm/ 50°C/ 0.6 mL/min.
Detection	Applied Biosystems API 4000 (TurboSpray™)	
Validation Range	1-500 ng/mL	
Matrix Effect	0.30% to 10.76%	
Inter Assay Precision (%RSD)	2.34% to 4.93%	
Inter Assay Accuracy (%RE)	-2.83% to 5.80%	

CONCLUSIONS

Although both manual and automated methods independently provided similar information for column and solvent selections, the data from the automated method allowed for conclusive selections of column and solvent systems that were optimal for the best chromatography with the least amount of carryover in 1/4 the time of the manual approach.

ACKNOWLEDGEMENT

Instrument pictures (Figures 1-3) were provided by Thermo Fisher.