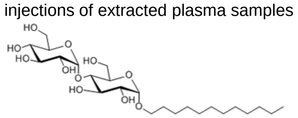
Improved Performance with Column Back-Flushing Between Injections: Two Case Studies

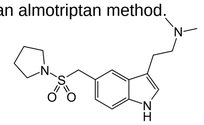
Jason Bilodeau, Nicolas Jean, Marie-Claude Theberge, Sylvain Lachance, Nadine Boudreau and Ann Lévesque (inVentiv Health Clinique, Québec, Canada)

Introduction

During the course of a bioanalytical project, the performance of the column might degrade drastically even after few batches. This is generally due to the cleanliness of the extracted samples. Sometimes, the extraction process may be improved for cleaner extract. For other cases, chromatography conditions might be optimized. Herein, we present two case studies in which a back-flush of the column was deemed necessary to improve the robustness of the column. First, an accuracy issue was observed with the analysis of dodecyl maltoside in human plasma. Degradation of the chromatography was also seen after consecutive injections of extracted plasma samples of an almotriptan method.



Dodecyl Maltoside 510.60 amu



Almotriptan 335.46 amu

Method

Dodecyl maltoside is extracted by an automated liquid-liquid extraction using ethyl acetate. The dry residue is reconstituted with the mobile phase, a mixture of methanol, water, ammonium formate and formic acid. The samples are injected onto a C18 column and analyzed by LCMSMS API 5000. Originally, there was a column flush with methanol between each injection. Almotriptan is extracted from human plasma by an automated evaporation-free protein precipitation extraction with methanol. The extracted samples are injected onto a CN column and analyzed using an API 4000 LCMSMS.

Extraction Procedure

	Dodecyl Maltoside	Almotriptan
Matrix	EDTA K_3	EDTA K_2
Analytical Range	0.5-2500 ng/mL	0.2-100 ng/mL
Internal Standard	Decyl Maltoside	Almotriptan-d_6
Sample Volume	0.120 mL	0.050 mL
Extraction Type	Automated Liquid-Liquid Extraction	Automated Protein Precipitation
Dilution Factor	2.08	20

LC-MS/MS Analysis

	Dodecyl Maltoside	Almotriptan
Chromatographic Mode	Reverse Phase	Reverse Phase
Analytical Column	C18 50x4.6mm 3µm	CN 50x4.6mm 3µm
Elution Mode	Isocratic	Isocratic
Mobile Phase A	MeOH/Water/Amm. Formate/Formic Acid	Methanol/Water/Amm. Formate
Flow Rate	1.00 mL/min	1.00 mL/min
Injection Volume	10 µL	20 µL
Retention Time	1.59 min	1.68 min
Acquisition Time:	3.00 min	3.00 min
Detector:	API 5000	API 4000
Source:	TurboIonSpray	TurboIonSpray
Ion Monitored:	Summation of 528→349 and 528→349	336→58

Results

During the routine analysis of dodecyl maltoside by LCMSMS, an accuracy issue was observed for the calibration standards and quality controls. Bias up to 48% was observed in some samples **(Table 1)**. The dodecyl maltoside peak area seemed more affected than the internal standard area (decyl maltoside). Re-optimization of the LCMSMS acquisition parameters was performed without seeing any improvement in accuracy. However, a back-flush of the column with methanol improved drastically the accuracy of the method without affecting the chromatography. The %biases ranged from -10 to 4% and precision was below 7.9% **(Table 2)**. ISR was assessed and near 100% of reassay confirmation rate was demonstrated.

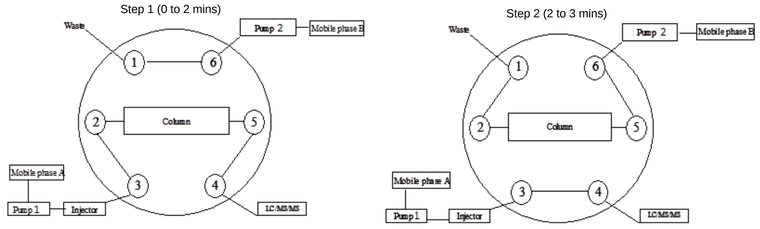


Figure 1. Switch-Valve Installation for Column Back-Flushing

Table 1. Dodecyl Maltoside Calibration Stanadards and Quality Controls Accuracy Without Column Back-Flush

Sample	Analyte Area	Analyte Ret. Time (min)	IS Area	IS Ret. Time (min)	Ana/IS Ratio	Conc. Found (ng/mL)	Accuracy (% Bias)
AHLX CONTROL_BLANK	363	1.56	80	1.05	4.5375		
AHLX CONTROL_BLANK	0	0	70	1.13	0.0000		
AHLX ZS	0	0	217245	1.06	0.0000		
AHLX ZS	1148	1.61	263243	1.06	0.0044		
AHLX CS1 1	5194	1.63	219824	1.06	0.0236	0.44	-12.0
AHLX CS1 2	7179	1.63	251630	1.06	0.0285	0.54	8.0
AHLX CS2 1	10113	1.62	218621	1.05	0.0463	0.93	-7.0
AHLX CS2 2	14056	1.62	251876	1.05	0.0558	1.14	14.0
AHLX CS3 1	454318	1.62	221604	1.05	2.0501	44.38	-11.2
AHLX CS3 2	623571	1.62	240509	1.05	2.5927	56.15	12.3
AHLX CS4 1	1218578	1.61	222074	1.05	5.4873	118.92	-4.9
AHLX CS4 2	1527782	1.62	239885	1.05	6.3688	138.04	10.4
AHLX CS5 1	2569189	1.61	218804	1.05	11.7420	254.56	1.8
AHLX CS5 2	2979273	1.61	236473	1.05	12.5988	273.14	9.3
AHLX CS6 1	5925830	1.61	244908	1.05	24.1961	524.63	4.9
AHLX CS6 2	5950465	1.61	235022	1.05	25.3188	548.97	9.8
AHLX CS7 1	10961914	1.62	246329	1.05	44.5011	964.95	-3.5
AHLX CS7 2	11589941	1.61	243258	1.05	47.6446	1033.12	3.3
AHLX CS8 1	21571292	1.63	254370	1.06	84.8028	1838.91	-8.1
AHLX CS8 2	22003344	1.61	256132	1.05	85.9063	1862.84	-6.9
AHLX CS9 1	26326202	1.63	250835	1.05	104.9543	2275.90	-9.0
AHLX CS9 2	27039033	1.62	265233	1.05	101.9445	2210.63	-11.6
AHLX LLQC 1	7376	1.61	236106	1.05	0.0312	0.60	20.0
AHLX LLQC 2	9560	1.6	254221	1.05	0.0376	0.74	48.0
AHLX LLQC 3	6091	1.62	225036	1.05	0.0271	0.51	2.0
AHLX LLQC 4	6152	1.63	234932	1.06	0.0262	0.49	-2.0
AHLX LLQC 5	8483	1.61	268603	1.05	0.0316	0.61	22.0
AHLX LLQC 6	9919	1.61	267532	1.05	0.0371	0.73	46.0
AHLX QC1 1	19887	1.62	235683	1.05	0.0844	1.76	17.3
AHLX QC1 2	15192	1.62	221560	1.05	0.0686	1.41	-6.0
AHLX QC1 3	20761	1.63	266164	1.06	0.0780	1.62	8.0
AHLX QC1 4	17195	1.62	239484	1.05	0.0718	1.48	-1.3
AHLX QC1 5	19287	1.62	240242	1.05	0.0803	1.67	11.3
AHLX QC1 6	15787	1.62	232902	1.05	0.0678	1.40	-6.7
AHLX QC2 1	11330236	1.62	220225	1.05	51.4485	1115.61	-10.8
AHLX QC2 2	13616110	1.61	230539	1.05	59.0621	1280.71	2.5
AHLX QC2 3	14007803	1.63	255187	1.06	54.8923	1190.29	-4.8
AHLX QC2 4	12517921	1.63	227364	1.06	55.0567	1193.85	-4.5
AHLX QC2 5	14481620	1.61	262666	1.05	55.1332	1195.51	-4.4
AHLX QC2 6	13149496	1.61	238494	1.05	55.1355	1195.56	-4.4
AHLX QC3 1	19442966	1.62	232648	1.05	83.5725	1812.23	-3.4
AHLX QC3 2	20616550	1.61	262503	1.05	78.5383	1703.06	-9.2
AHLX QC3 3	19684615	1.62	231355	1.05	85.0840	1845.01	-1.6
AHLX QC3 4	17518773	1.63	235932	1.06	74.2535	1610.14	-14.1
AHLX QC3 5	20770228	1.61	257426	1.05	80.6843	1749.60	-6.7
AHLX QC3 6	20775806	1.61	256490	1.05	81.0005	1756.45	-6.3

Table 2. Dodecyl Maltoside Calibration Stanadards and Quality Controls Accuracy using Column Back-Flush

Sample	Analyte Area	Analyte Ret. Time (min)	IS Area	IS Ret. Time (min)	Ana/IS Ratio	Conc. Found (ng/mL)	Accuracy (% Bias)
AHLX CONTROL_BLANK	269	1.64	36	1.06	7.4722		
AHLX CONTROL_BLANK	344	1.64	117	1.05	2.9402		
AHLX ZS	842	1.62	187321	1.05	0.0045		
AHLX ZS	75	1.64	193862	1.05	0.0004		
AHLX CS1 1	4770	1.63	189218	1.05	0.0252	0.51	2.0
AHLX CS1 2	5005	1.64	199162	1.05	0.0251	0.51	2.0
AHLX CS2 1	8338	1.63	196914	1.05	0.0423	0.94	-6.0
AHLX CS2 2	8604	1.64	197945	1.05	0.0435	0.96	-4.0
AHLX CS3 1	401815	1.63	189398	1.05	2.1215	52.12	4.2
AHLX CS3 2	412386	1.63	200550	1.05	2.0563	50.52	1.0
AHLX CS4 1	980017	1.63	188921	1.05	5.1874	127.60	2.1
AHLX CS4 2	1012638	1.63	202229	1.05	5.0074	123.17	-1.5
AHLX CS5 1	1991044	1.64	189475	1.06	10.5082	258.59	3.4
AHLX CS5 2	2091133	1.65	203614	1.06	10.2701	252.73	1.1
AHLX CS6 1	4065757	1.65	196490	1.06	20.6919	509.30	1.9
AHLX CS6 2	4243081	1.66	204426	1.06	20.7561	510.87	2.2
AHLX CS7 1	7820310	1.64	192189	1.06	40.6907	1001.63	0.2
AHLX CS7 2	8320073	1.65	210458	1.06	39.5332	973.14	-2.7
AHLX CS8 1	15793107	1.64	195053	1.05	80.9683	1993.20	-0.3
AHLX CS8 2	16810140	1.64	211397	1.06	79.5193	1957.53	-2.1
AHLX CS9 1	20129709	1.63	201515	1.05	99.8919	2459.07	-1.6
AHLX CS9 2	20803526	1.64	210447	1.05	98.8540	2433.52	-2.7
AHLX LLQC 1	4481	1.65	192217	1.06	0.0233	0.47	-6.0
AHLX LLQC 2	4541	1.64	194533	1.05	0.0233	0.47	-6.0
AHLX LLQC 3	4386	1.65	195129	1.06	0.0225	0.45	-10.0
AHLX LLQC 4	4741	1.66	201110	1.06	0.0236	0.47	-6.0
AHLX LLQC 5	4999	1.63	202720	1.05	0.0247	0.50	0.0
AHLX LLQC 6	5188	1.65	202642	1.06	0.0256	0.52	4.0
AHLX QC1 1	12713	1.63	192922	1.05	0.0659	1.52	1.3
AHLX QC1 2	12789	1.64	197045	1.06	0.0649	1.49	-0.7
AHLX QC1 3	13018	1.63	199488	1.05	0.0653	1.50	0.0
AHLX QC1 4	12254	1.63	196559	1.05	0.0623	1.43	-4.7
AHLX QC1 5	12666	1.64	202768	1.05	0.0625	1.43	-4.7
AHLX QC1 6	13157	1.63	207838	1.05	0.0633	1.45	-3.3
AHLX QC2 1	10149395	1.63	198809	1.05	51.0510	1256.69	0.5
AHLX QC2 2	10216439	1.65	200641	1.06	50.9190	1253.44	0.3
AHLX QC2 3	10444024	1.63	209028	1.05	49.9647	1229.94	-1.6
AHLX QC2 4	10323896	1.63	202969	1.05	50.8644	1252.09	0.2
AHLX QC2 5	10525433	1.63	205781	1.05	51.1487	1259.09	0.7
AHLX QC2 6	10530469	1.63	211360	1.05	49.8224	1226.44	-1.9
AHLX QC3 1	15023491	1.64	197625	1.06	76.0202	1871.39	-0.2
AHLX QC3 2	15348930	1.64	204295	1.06	75.1312	1849.50	-1.4
AHLX QC3 3	15279904	1.64	202559	1.06	75.4343	1856.97	-1.0
AHLX QC3 4	15510695	1.64	206647	1.06	75.0589	1847.72	-1.5
AHLX QC3 5	15798652	1.63	210234	1.05	75.1479	1849.92	-1.3
AHLX QC3 6	15797579	1.64	212009	1.06	74.5137	1834.30	-2.2

Results

A typical batch of extracted samples for the determination of almotriptan accounts for 260 samples. Within one batch, the internal standard peak height decreased from approximately 6000 to 2500. The peak area was not as affected as tailing was observed throughout the batch. Although the accuracy was not affected, the chromatography was not acceptable. Washing the column after each batch was not effective. A back-flush of the column with a mixture of water, methanol (90/10) and acetic acid 1% was proposed to improve the overall robustness of the column. No tailing was observed after multiple consecutive batches using the same column. Moreover, the assay was found highly reproducible with near 100% of confirming reassays.

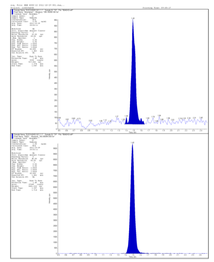


Figure 2. Representative Chromatogram of Low Quality Control at the Beginning of the Batch injected without Column Back-Flush

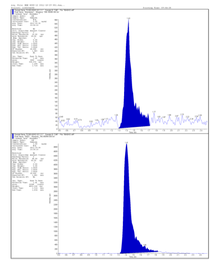


Figure 3. Representative Chromatogram of Low Quality Control at the End of the Batch without Column Back-Flush

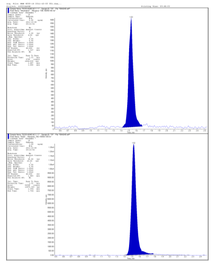


Figure 4. Representative Chromatogram of Low Quality Control at the Beginning of the Batch with Column Back-Flush

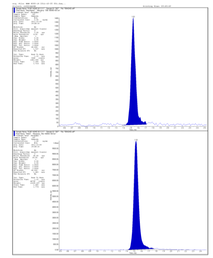


Figure 5. Representative Chromatogram of Low Quality Control at the End of the Batch with Column Back-Flush

Conclusion

Sometimes the chromatography is affected during the course of bioanalytical projects. In these two case studies, the column degradation caused the lack of accuracy at low concentrations for dodecyl maltoside and degradation of the peak shape for almotriptan. The addition of a column back-flush between each injection solved the issues and increased the column robustness.

TRANSFORMING PROMISING IDEAS INTO COMMERCIAL REALITY