Effective Carryover Reduction by Derivatization of Residual Analyte in HPLC System During LC-MS/MS Quantification

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OVERVIEW

Purpose

To investigate derivatization of the residual analyte on the HPLC injection needle to reduce carryover issues during LC-MS/MS analysis of *S*-adenosyl-L-methionine (SAMe).

Method

- Propionic anhydride was chosen as a derivatization reagent.
- Different washing solutions were tested.
- Carryover measurement was done by injection of a ULOQ solution followed by two subsequent non-extracted internal standard solution injections using different washing solutions and comparing to an LLOQ.

Results

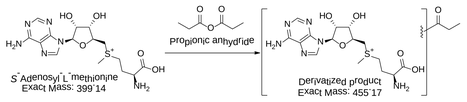
- The optimized wash cycle allowed a 44% decrease of carryover at LLOQ following the injection of ULOQ samples as compared to using a standard wash procedure.
- The needle was immersed for 5 seconds into a vial containing the derivatization solution to sufficiently remove carryover.
- This procedure can be applied to an analytical method with minimal impact on its efficiency and throughput.

INTRODUCTION

During the LC-MS/MS quantification of *S*-adenosyl-L-methionine (SAMe), carryover issues were observed. Since standard carryover reduction methods proved to be inefficient in this case, alternatives were considered. Derivatization of the analyte itself was excluded due to instability of one diastereomer of the analyte. Derivatization of the residual analyte on the HPLC injection needle, a main source of carryover, was investigated to prevent carryover to the next injection while minimizing the impact on the quantification method.

In the present work, we describe the investigation of a propionic anhydride derivatization process (**Figure 1**) and demonstrate its impact on carryover reduction in order to obtain a robust and high throughput assay.

Figure 1: Structures of *S*-Adenosyl-L-methionine and its Derivatization Product



METHODS

SAMPLE EXTRACTION

25µL plasma was extracted by protein precipitation using trichloroacetic acid. Specific precautions were taken to prevent contamination.

DETECTION

- AB SCIEX API3000
- (ESI)(+)
- Mass transition 399/250 was monitored.

CHROMATOGRAPHY

- Agilent Technologies Series 1100 pumps and autosampler
- Zorbax reversed-phase column, 3.5µm, 2.1X50mm
- Mobile phase consisted of ammonium formate and methanol in isocratic mode.

RESULTS

To determine if the derivatization was efficient, the following tests were done:

- A 200 µL aliquot of *S*-adenosyl-L-methionine solution (10000.00 ng/mL) and 100 µL of 1.0 N aqueous HCl were combined.
- 10 µL of propionic anhydride was added.
- Vortex for 5 seconds.
- An aqueous solution of 2M ammonium bicarbonate (100 µL) was added and mixed.

This procedure was highly efficient. Approximately 99.9% of the analyte was derivatized during a very short timeframe (data not shown). The derivatization of the residual *S*-adenosyl-L-methionine on the injection needle was then tested using three different needle wash procedures.

Wash Procedure A (see **Figure 2**):

a) Immersion of needle in ULOQ for 15 seconds
b) Immersion in reconstitution solution (H_2O:0.01N HCl:TCA 7.7:15.4:76.9% v/v) for 15 seconds
c) Injection

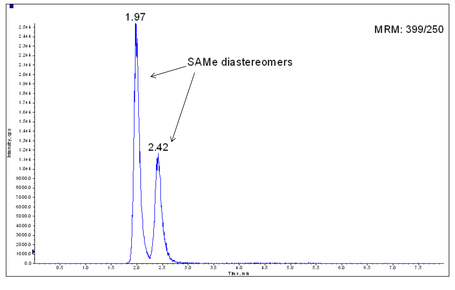
Wash Procedure B (see **Figure 3**):

a) Immersion of needle in ULOQ for 15 seconds
b) Immersion in propionic anhydride for 5 seconds
c) Immersion in reconstitution solution (H_2O:0.01N HCl:TCA 7.7:15.4:76.9% v/v) for 15 seconds
d) Injection

Wash Procedure C (see **Figure 4**):

a) Immersion of needle in ULOQ for 15 seconds
b) Immersion in propionic anhydride for 5 seconds
c) Immersion in 2M ammonium bicarbonate solution for 5 seconds
d) Immersion in reconstitution solution (H_2O:0.01N HCl:TCA 7.7:15.4:76.9% v/v) for 15 seconds
e) Injection

Figure 2: Chromatogram Following Injection After Wash Procedure A



The chromatograms in Figures 2 to 4 show that the best procedure for residual SAMe derivatization on the HPLC immersion needle is procedure C (**Figure 4**). The high pH neutralization solution ensures high conversion to the derivatized compound (**Figure 3** vs **Figure 4**).

No improvement was observed by increasing the needle immersion time to 30 seconds. Therefore the procedure with 5 seconds of needle immersion in each vial was determined as optimal for effective carryover reduction.

Figure 3: Chromatogram Following Injection After Wash Procedure B

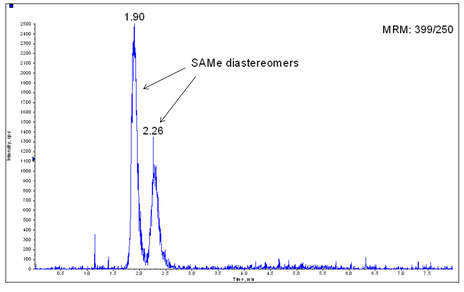
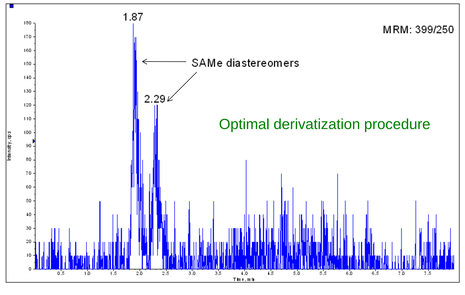


Figure 4: Chromatogram Following Injection After Wash Procedure C



Different washing solutions were also tested (**Table 1**). The propionic anhydride and ammonium bicarbonate wash cycle proved to be highly efficient, allowing a 38% decrease in carryover at LLOQ, to a level which meets our internal acceptance criteria for method development (comparison between entry 1 vs entry 2). The results in **Table 1** also demonstrate that a 2M ammonium bicarbonate (entry 9) or other high pH wash (entries 4 and 8) alone is not sufficient to decrease carryover by itself and propionic anhydride is necessary in order to obtain maximum impact .

Table 1: Carryover Tests Results with Different Washing Solutions

Entry	Wash Vial Solution	Sample Name	Analyte Peak Area (counts)	% of LOQ
1	None	ULOQ	1938971.5112	N/AP
		ULOQ	1788488.7405	
		N.-E. IS solution	700.1018	73.6
		N.-E. IS solution	339.4150	35.7
2	Vial 1: propionic anhydride Vial 2: 2M ammonium bicarbonate	ULOQ	3987641.6441	N/AP
		ULOQ	4134956.0470	
		N.-E. IS solution	1259.6097	60.8
		N.-E. IS solution	353.3046	17.1
3	mobile phase	ULOQ	4037356.6529	N/AP
		ULOQ	3929900.2595	
		N.-E. IS solution	1455.4549	92.0
		N.-E. IS solution	449.0895	28.4
4	10% NH_4OH in MeOH	ULOQ	3895139.5723	N/AP
		ULOQ	3860727.0267	
		N.-E. IS solution	1730.1639	109.4
		N.-E. IS solution	565.5677	35.8
5	CH_3COOH:ACN 50:50% v/v	ULOQ	3890630.9415	N/AP
		ULOQ	3942191.2264	
		N.-E. IS solution	1684.5153	106.5
		N.-E. IS solution	681.3907	43.1
6	CH_3COOH:MeOH 50:50% v/v	ULOQ	3800602.5120	N/AP
		ULOQ	3781374.7320	
		N.-E. IS solution	1608.9283	101.7
		N.-E. IS solution	633.1293	40.0
7	10% HCOOH in water	ULOQ	3717981.0345	N/AP
		ULOQ	3751062.3131	
		N.-E. IS solution	1692.0158	107.0
		N.-E. IS solution	669.1324	42.3
8	50 mM sodium carbonate	ULOQ	3770907.5795	N/AP
		ULOQ	3719075.4856	
		N.-E. IS solution	1652.1833	104.5
		N.-E. IS solution	674.5713	42.7
9	2M ammonium bicarbonate	ULOQ	3677796.8956	N/AP
		ULOQ	3721975.9867	
		N.-E. IS solution	1648.4470	104.2
		N.-E. IS solution	543.4440	34.4
10	350 mM oxalic acid	ULOQ	3727664.0101	N/AP
		ULOQ	3736110.4621	
		N.-E. IS solution	1433.0214	90.6
		N.-E. IS solution	735.4660	46.5
11	CH_3COOH	ULOQ	3694390.7509	N/AP
		ULOQ	3765842.2178	
		N.-E. IS solution	1887.2447	119.3
		N.-E. IS solution	646.0921	40.8

CONCLUSION

In conclusion, the derivatization of residual analyte in the HPLC injection needle is an effective means to resolve carryover issues during LC-MS/MS analysis with minimal impact on the quantification method throughput and robustness.

* Currently at Angelini Pharma.