Solving Interference Issues in Blank Samples for Dalfampridine by Choosing the Appropriate Solid Phase Extraction Cartridges Characteristics

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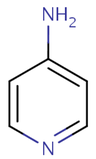
Introduction



MW: 94.1 g/mol

An interfering peak was detected in dalfampridine blank samples during method development and prevented proper quantitation of the analyte. The source of this interference was investigated and found to be the solid phase extraction cartridges. Choice of manufacturer, amount of packing and optimization of activation and washing steps were crucial to obtain interference free dalfampridine extracted blank samples.

Method

Dalfampridine is extracted from EDTA K_2 human plasma using 10 mg reverse phase solid phase extraction. Chromatography was performed on a reversed phase UPLC using an Atlantis HILIC Silica 50x4.6 (3µm) analytical column operated at 25°C. A mobile phase consisting of water and acetonitrile (18/82), 10 mM ammonium formate and 0.1% formic acid exhibited a retention time of 2.00 minutes and an analytical run time of 2.50 minutes. The triple quadrupole mass spectrometer (AB Sciex), model API 4000, equipped with an electrospray (ESI) ionization source in positive mode, was set up in multiple reaction monitoring mode, monitoring the transitions 95.0 → 78.0 and 99.0 → 81.0 for dalfampridine and labeled internal standard respectively. The method was validated over the range of 0.3-150 ng/mL.

Extraction Procedure

- Internal Standard: Dalfampridine-d_4
- Sample Volume: 50 µL
- Extraction Type: Solid-Phase Extraction Silia prepx HLB
- Concentration factor: 0.125

LC-MS/MS Analysis

Chromatographic mode:	Reversed Phase
Analytical Column:	Atlantis HILIC Silica 50 x 4.6mm
Column Temperature:	Room Temperature
Elution mode:	Isocratic
Mobile Phase A:	Milli-Q Type Water/Acetonitrile (18/82), Ammonium formate 10mM, Formic Acid 0.1%
Flow Rate:	1.0 mL/minute
Retention Times:	1.91 minutes
Acquisition time:	2.5 minutes
Detector:	AB Sciex API 4000
Source:	TurboIonSpray, Positive mode
Masses:	94.8→78.0 amu

Results

A careful investigation of solid phase extraction cartridges was done in order to identify the source in the interfering peak. Different cartridges manufacturer and packing sizes were tested as well as different washing steps. 30 mg cartridges offered the cleanest extracts. 60 mg cartridges, although they gave better %CV, showed more interference. Also, for the same size, interference level varied between cartridges manufacturers. Interferences could still be detected in extracted blank samples even using the most optimized washing conditions. Extensively activating cartridges gave cleaner blank samples than regularly activated ones. The cartridge activation step was then increased from 400 µL to 2 mL of methanol, followed by 400 µL of water. This increase in methanol activation volume was essential in providing cleaner extracts.

Table 1. Comparison of Interference in Blank for Different Cartridge Types

Cartridges	% Interference vs. LLOQ	% Interference vs. IS
Oasis HLB 1cc 30mg	7-10%	< 1%
Plexa 3cc 30mg	< 5%	> 5%
Silia PrepX HLB 1cc 30mg	8%	< 1%

Table 2. Comparison of Packing Sizes

Cartridges	% Interference vs. LLOQ	% Interference vs. IS
Oasis HLB 1cc 30mg	10-25%	< 1%
Oasis HLB 1cc 60mg	18-30%	< 1%

Table 3. Evaluation of Washing Solutions with the Plexa 3cc 30mg

Type of Washing Solution	% Interference vs. LLOQ	% Interference vs. IS
Water	68%	< 1%
Methanol 2%	69%	< 1%
Methanol 2% + NH4OH 2%	81%	< 1%
Methanol 5% + NH4OH 2%	36%	< 1%

Table 4. Impact of Activation Volume and Volume Transfer on Interference using the Silia

Steps	% Interference vs. LLOQ	% Interference vs. IS
Activation 2mL Methanol	0-10%	< 1%
Activation 0.4mL Methanol	6-15%	< 1%
Transfer 1mL of Loading Mixture	0-10%	< 1%
Transfer 0.5mL of Loading Mixture	6-15%	< 1%

The final extraction procedure was validated. Briefly, the Silia PrepX HLB 1cc 30mg cartridges are activated with 2mL of methanol followed by 0.400 mL of water. The loading mixture (plasma, IS and buffer) is added to the cartridges. The cartridges are washed with 0.400mL of methanol 2% + NH4OH 2% in water. The analyte is eluated with 0.400 mL of methanol. The dry residue is finally reconstituted and analyzed by LC-MS/MS.

Table 5. Recovery of Analyte and Internal Standard

Quality Control	Recovery %
Low 900pg/mL	87.3
Middle 75000pg/mL	82.6
High 112500pg/mL	85.4
Internal Standard	77.0

Table 6. Selectivity of the Method

Matrix Identification	Gender	Peak Responses		% Interference	
		Analyte	Internal Standard	Analyte	Internal Standard
100037244	F	464	2112	6.4	0.4
100037246	F	312	2587	4.3	0.5
100037301	M	102	1720	1.4	0.3
100037304	M	403	2167	5.6	0.4
100034376	M	357	3076	5.0	0.5
100037306	M	182	2040	2.5	0.4

Table 7. Between-Run Accuracy and Precision

Run Number	LLQC 300 pg/mL		QC1 900 pg/mL		QC2 75000 pg/mL		QC3 112500 pg/mL	
	Mesured Conc. (pg/mL)	% Bias	Mesured Conc. (pg/mL)	% Bias	Mesured Conc. (pg/mL)	% Bias	Mesured Conc. (pg/mL)	% Bias
1AIPR	331.7	10.6	888.6	-1.3	73618.2	-1.8	111239.3	-1.1
	282.3	-5.9	873.6	-2.9	72284.3	-3.6	109631.1	-2.6
	296.6	-1.1	883.0	-1.9	72954.2	-2.7	107942.1	-4.1
	301.4	0.5	875.6	-2.7	72206.5	-3.7	107272.7	-4.7
	287.1	-4.3	879.3	-2.3	72748.8	-3.0	107365.3	-4.6
	305.6	1.9	852.4	-5.3	72511.3	-3.3	107792.0	-4.2
2AIPR	313.1	4.4	931.7	3.5	69382.3	-7.5	112744.5	0.2
	310.3	3.4	910.6	1.2	68436.4	-8.8	111251.4	-1.1
	281.9	-6.1	1006.3	11.8	68109.5	-9.2	110926.2	-1.4
	288.6	-3.8	923.4	2.6	66637.1	-11.2	110865.4	-1.5
	299.3	-0.3	917.3	1.9	67426.9	-10.1	110066.3	-2.2
	287.1	-4.3	880.1	-2.2	67373.0	-10.2	108705.5	-3.4
3AIPR	322.1	7.4	867.8	-3.6	75325.7	0.4	118739.9	5.6
	292.1	-2.6	845.0	-6.1	76867.4	2.5	118660.7	5.5
	293.7	-2.1	859.3	-4.5	76058.5	1.4	120525.8	7.1
	286.6	-4.5	864.4	-4.0	76304.5	1.7	117139.9	4.1
	284.3	-5.3	857.2	-4.8	75806.3	1.1	117957.3	4.9
	287.2	-4.3	851.7	-5.4	76955.8	2.6	118058.9	4.9
5AIPR	300.6	0.2	895.7	-0.5	75480.5	0.6	112864.8	0.3
	277.3	-7.6	860.2	-4.4	75431.9	0.6	111612.2	-0.8
	290.4	-3.2	876.2	-2.6	74777.3	-0.3	112116.9	-0.3
	318.5	6.2	899.8	0.0	73449.8	-2.1	110740.4	-1.6
	282.9	-5.7	888.2	-1.3	73998.1	-1.3	110855.3	-1.5
	269.0	-10.3	887.4	-1.4	73513.4	-2.0	111517.6	-0.9
N	24	24	24	24	24	24	24	24
Mean	295.4	-1.5	886.4	-1.5	72819.1	-2.9	112358.0	-0.1
SD(±)	15.19		34.43		3249.98		3980.41	
CV(%)	5.1		3.9		4.5		3.5	

Chromatography

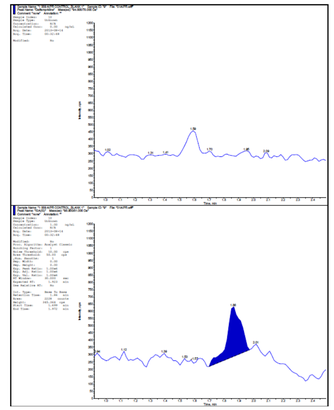


Figure 1. Representative Chromatogram of a Blank Sample

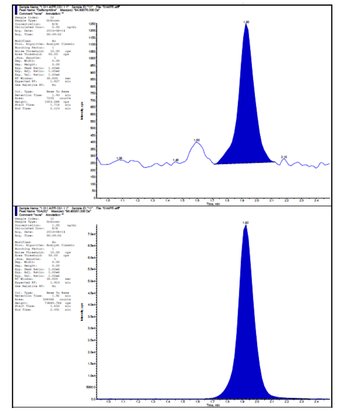


Figure 2. Representative Chromatogram of a Low Calibration Standard (300 pg/mL)

Conclusion

Overall, a careful choice of extraction cartridges (brand, size, packing amount) and optimal conditioning and washing steps procedures were successful in obtaining interference free dalfampridine extracted blank samples. This assay was found to be selective during the course of method validation.

TRANSFORMING PROMISING IDEAS INTO COMMERCIAL REALITY