Impact of Food on Recovery of Carbidopa and its Internal Standard from Study Samples

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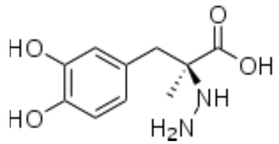
Introduction



MW: 226.2 g/mole

Carbidopa is a drug given to people with Parkinson's disease in order to inhibit peripheral metabolism of levodopa. Carbidopa reduces the amount of levodopa required to produce a given response. It was observed during study sample analysis that the recovery of carbidopa and its deuterated IS was impacted by the food intake during clinical study. An investigation was initiated to find the root cause of this phenomenon and the solution is described.

Method

The original method for the determination of carbidopa in human plasma is a protein precipitation with methanol/formic acid followed by derivatization with acetylacetone. The derivatized carbidopa is injected onto the ACE Excel2 column using a mobile phase mixture of methanol/water/IPA. The improved method is a protein precipitation with perchloric acid. The supernatant is then injected onto the ACE Excel2 column using a mobile phase mixture of methanol/water and acetic acid. Study samples were stabilized using sodium metabisulphite during collection.

Extraction Procedure

- Internal Standard: Carbidopa-d5
- Sample Volume: 50 µL
- Old Extraction Type: Protein precipitation with derivatization
- New Extraction Type: Protein precipitation using perchloric acid

LC-MS/MS Analysis

Old Method

Chromatographic mode:	Reversed Phase
Analytical Column:	ACE 3 C18-PFP
Column Temperature:	Room Temperature
Elution mode:	Isocratic with column flush
Mobile Phase A:	Milli-Q Type Water/Methanol (70/30), Ammonium formate 2mM, Formic Acid 0.2%
Flow Rate:	1.0 mL/minute
Retention Times:	2.75 minutes
Acquisition time:	5.5 minutes
Detector:	AB Sciex API 4000
Source:	TurboIonSpray, Positive mode
Masses:	291→139

Improved Method

Chromatographic mode:	Reversed Phase
Analytical Column:	ACE Excel2 C18-PFP
Column Temperature:	Room Temperature
Elution mode:	Isocratic with column flush
Mobile Phase A:	Milli-Q Type Water/Methanol (95/5), Acetic Acid 0.5%
Flow Rate:	0.5 mL/minute
Retention Times:	1.92 minutes
Acquisition time:	5.0 minutes
Detector:	AB Sciex API 5000
Source:	TurboIonSpray, Positive mode
Masses:	198→152

Results

The original method was validated over the dynamic range of 0.50-100 ng/mL. The IS responses in study samples were up to four times higher than the quality controls. However, for timepoints after a meal, the IS responses was lower for some subjects **(Table 1)**.

Table 1. Internal Standard Responses Summary

Samples	Mean Internal Standard Responses
Calibration Standards and QCs	455792
Volunteer A before Meal	1418074
Volunteer A after Meal	752604
Volunteer B before Meal	1430826
Volunteer B after Meal	806219
Volunteer C before Meal	1492607
Volunteer C after Meal	1420627

New chromatographic conditions were tested with study samples since QCs were not impacted. Although the effect of food was decreased to an acceptable level, there was a reproducibility issue with the assay: ISR gave less than 50% of confirmation rate. After investigation and complete modification of the method, the derivatization was found the main root cause of the irreproducibility and the food effect. The improved method without derivatization was validated and study samples were reanalyzed. ISR was evaluated with near 100% of confirmation rate. Food has no more effect on the recovery of carbidopa and its IS.

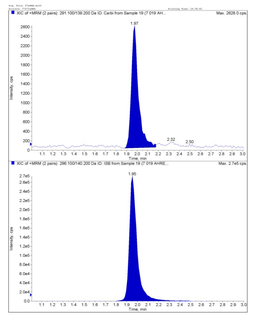


Figure 1. Representative Chromatogram of Low Quality Control with Old Conditions

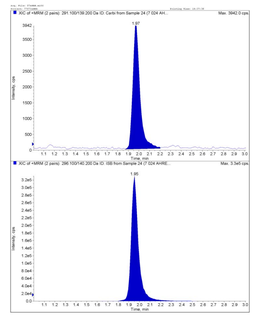


Figure 2. Representative Chromatogram of a Study Sample with Old Conditions

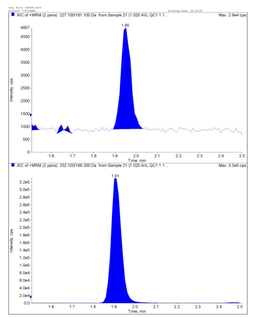


Figure 3. Representative Chromatogram of Low Quality Control with New Conditions

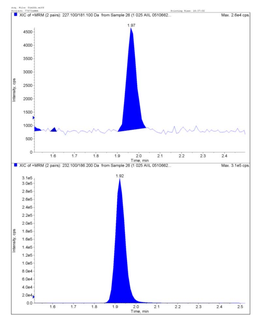


Figure 4. Representative Chromatogram of a Study Sample with New Conditions

Results

Table 2. Matrix Effect at Low Quality Control Level

Untreated Standard (MFQC1)		Reference Solution (RSQC1)		Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses			
21762	1629520	15750	1157394	1.339513	1.352792	0.990184
22048	1645637	15646	1196485	1.357117	1.366172	0.993372
22118	1672855	13304	988783	1.361426	1.388767	0.980313
21832	1646005	14512	1090599	1.343822	1.366477	0.983421
22058	1626823	19223	1405837	1.357733	1.350553	1.005316
21273	1606660	19042	1388268	1.309414	1.333814	0.981707
	Mean	16246.2	1204561.0			0.9890522
	SD(±)					0.00944975
	CV(%)					0.96

Table 3. Matrix Effect at High Quality Control Level

Untreated Standard (MFULOQ)		Reference Solution (RSULOQ)		Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses			
1587452	1595202	1577924	1591497	1.041141	1.045932	0.995419
1575779	1591625	1550277	1550784	1.033485	1.043587	0.990320
1651154	1633430	1522103	1515487	1.082920	1.070998	1.011132
1638591	1627108	1512331	1505103	1.074681	1.066852	1.007338
1646022	1645897	1507212	1503766	1.079554	1.079172	1.000354
1597197	1603083	1478494	1484252	1.047532	1.051100	0.996605
	Mean	1524723.5	1525148.2			1.0001947
	SD(±)					0.00779589
	CV(%)					0.78

Table 4. Between-Run Accuracy and Precision

	LLQC 0.5 ng/mL		QC1 1.5 ng/mL		QC2 50 ng/mL		QC3 75 ng/mL	
	Mesured Conc. (ng/mL)	% Bias	Mesured Conc. (ng/mL)	% Bias	Mesured Conc. (ng/mL)	% Bias	Mesured Conc. (ng/mL)	% Bias
N	30	30	30	30	30	30	30	30
Mean	0.568	13.6	1.514	0.96	50.986	1.97	73.594	-1.88
SD(±)	0.0278		0.041		1.6323		2.4179	
CV(%)	4.89		2.71		3.2		3.29	

Conclusion

Although that sometimes derivatization may bring more signal, in this case study it was demonstrated that removal of this tedious step was the solution for the food impact on the analyte and internal standard recovery. Investigation with study samples was mandatory in this case.

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