



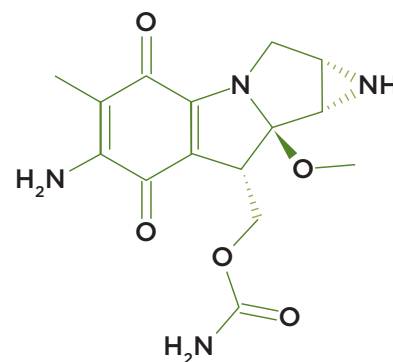
The Hidden Costs of Using a Surrogate Internal Standard

Background

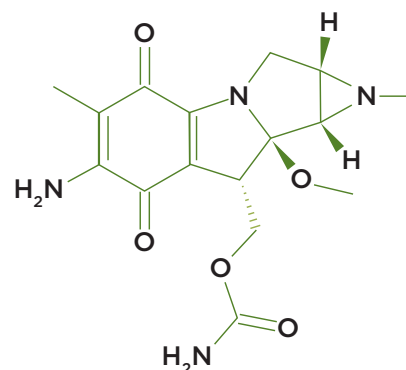
- Mitomycin C being dosed via local administration
- Goal was to prove there was no systemic (circulating) bioavailability/exposure, thus reducing overall tox development program
- Required a highly sensitive robust assay to prove this claim
- No stable isotope labeled internal authentic standard (SIL-ISTD) commercially available. Custom synthesis quoted ~ \$20,000

Problem

- Due to high cost of SIL-ISTD, a closely related surrogate standard, Porfiromycin, was chosen. Only difference being a methyl group in place of H on 5 membered ring.
- Assumption was the two compounds would mirror chemical, chromatography, and mass spectrometer properties.
- Assay variability, seen during Incurred Sample Reproducibility (ISR), testing was too high to meet FDA acceptance criteria, but in using a surrogate internal standard it became challenging to pinpoint the issue to the drug, study design, co-administered drug interference, plasma matrix, or internal standard. *See Table A6 below*, specifically % Bias data, which should be within +/- 15%.
- Weeks of assay development investigations, testing various matrices, were inconclusive. The program encountered delays and costs for added development investigations reached > \$20,000.



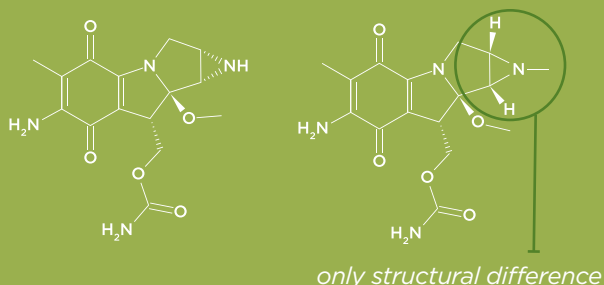
Mitomycin C Structure



Porfiromycin Structure

Client Challenge:

Making the assumption that a close but not identical molecule will act the same as your molecule can be costly.



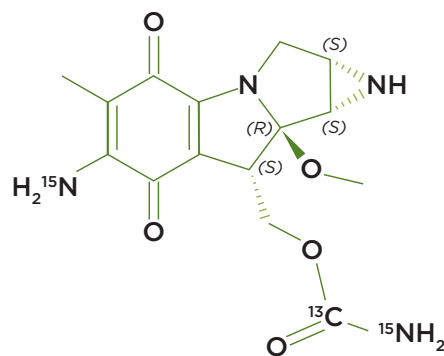
Our Solution:

Invest upfront in an authentic standard to save time and money because if not using an authentic standard, you won't know if the problem is with the internal standard or the assay.



Solution

- Several months into the program, a commercially available Mitomycin C SIL-ISTD was found.
- The key benefit of using an authentic SIL-ISTD is that it is chemically equivalent to the drug, so it extracts, chromatographs, and ionizes identically to the drug, but due to mass difference (addition of neutrons in the stable isotope label) it is differentiated in the mass spectrometer.
- Thus, any assay variability seen with the SIL-ISTD response is identical to the drug's variability and the peak ratio of SIL-STD/Drug does not change.
- The result of re-testing was a consistent ratio and a robust validated assay. *See Table A5, % Bias data.*
- Had the client incurred the initial cost to get an SIL-ISTD custom labeled initially, no delays in the program would have occurred and more than \$20,000 of method development and additional re-testing of 540 samples could have been saved.



Mitomycin C Isotope Structure

Table A6. Run 14 Results Using Porfiromycin as the Internal Standard

Replicate No.	LOW QC	MID QC	HIGH QC	AITB MID QC	Grp 1 0hr MID QC	Grp 1 0.5hr MID QC	Grp 1 2hr MID QC	Grp 5 0hr MID QC	Grp 5 0.5hr MID QC	Grp 5 2hr MID QC
#1	0.311	7.35	76.6	6.42	5.4	5	5.65	5.37	5.1	5.69
#2	0.291	7.08	72.8	6.39	5.41	4.98	5.62	5.09	5.25	5.69
#3	--	--	--	6.51	5.96	5.24	5.44	5.43	5.11	5.57
#4	--	--	--	6.54	5.74	5.12	5.47	5.07	5.3	5.51
#5	--	--	--	6.47	5.72	5.62	5.48	5.37	5.44	5.28
#6	--	--	--	6.77	5.88	5.12	5.76	5.4	5.33	5.85
Mean	0.301	7.22	74.7	6.52	5.69	5.18	5.57	5.29	5.26	5.6
%CV	--	--	--	2.1	4.1	4.5	2.3	3.1	2.5	3.5
%Bias	0.3	-3.7	-0.4	-13.1	-24.1	-30.9	-25.7	-29.5	-29.9	-25.3
n	2	2	2	6	6	6	6	6	6	6

Red text indicates a deviating value.

Table A5. Run 14 Results Using Isotope Internal Standard

Replicate No.	LOW QC	MID QC	HIGH QC	AITB MID QC	Grp 1 0hr MID QC	Grp 1 0.5hr MID QC	Grp 1 2hr MID QC	Grp 5 0hr MID QC	Grp 5 0.5hr MID QC	Grp 5 2hr MID QC
#1	0.316	7.67	74.5	7.5	7	7.1	7.68	7.29	7.67	7.49
#2	0.298	7.76	74.6	7.19	6.92	7.1	7.29	7.32	7.44	7.49
#3	--	--	--	7.26	7.04	7.11	7.29	7.59	7.34	7.43
#4	--	--	--	7.35	7.27	7.38	7.39	7.41	7.59	7.37
#5	--	--	--	7.46	7.73	7.44	7.45	7.75	7.64	7.11
#6	--	--	--	7.16	7.11	7.35	7.69	7.59	7.57	7.62
Mean	0.307	7.72	74.6	7.32	7.18	7.25	7.47	7.49	7.54	7.42
%CV	--	--	--	1.9	4.1	2.2	2.4	2.4	1.7	2.3
%Bias	2.3	2.9	-0.5	-2.4	-4.3	-3.3	-0.4	-0.1	0.5	-1.1
n	2	2	2	6	6	6	6	6	6	6

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