



»» TRANSFORMING PROMISING IDEAS INTO COMMERCIAL REALITY

The re-emergence of GC-MS/MS as a tool for the analysis of small molecule biomarkers

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Name a biomarker that is best measured with a hammer.





Introduction

How has LC-MS/MS come to so completely dominate bioanalysis?

Why are some labs starting to reconsider GC-MS/MS as an important bioanalytical tool?



Definitions:

- **Selectivity** is a tool or set of tools that we use in the hope of achieving specificity.
- **Specificity** occurs if a detector response is caused by one and only one analyte.



Our Selectivity Tool Box

- Protein precipitation, liquid-liquid or solid phase extraction
- Column chromatography
- Mass selective detection
- MS/MS



Demonstrating Specificity—PK Studies

- For PK studies we can demonstrate specificity.



Typical Matrix Blank

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Quan: 530yk009
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Area: 5, 8.00, 10 Baseline : 30, 3

30-MAY-13 Start : 19:02:30 465
Study: RFA7844
Inlet: GC Vial 9
Label: 5, 200.0

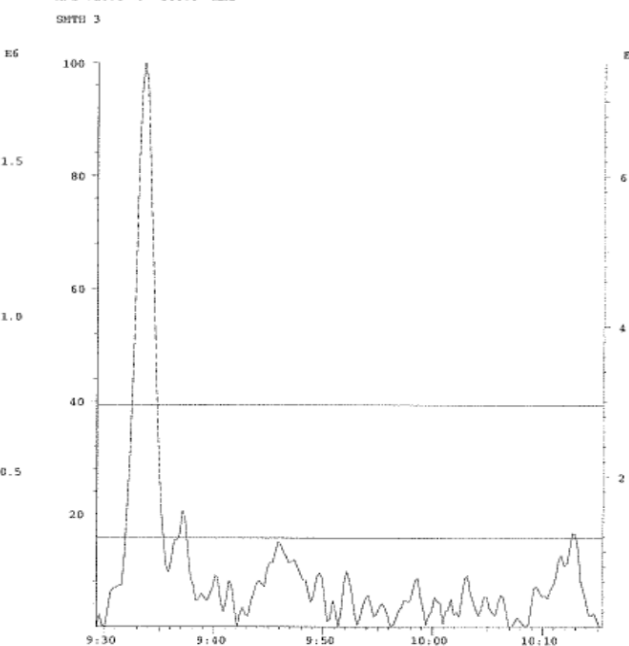
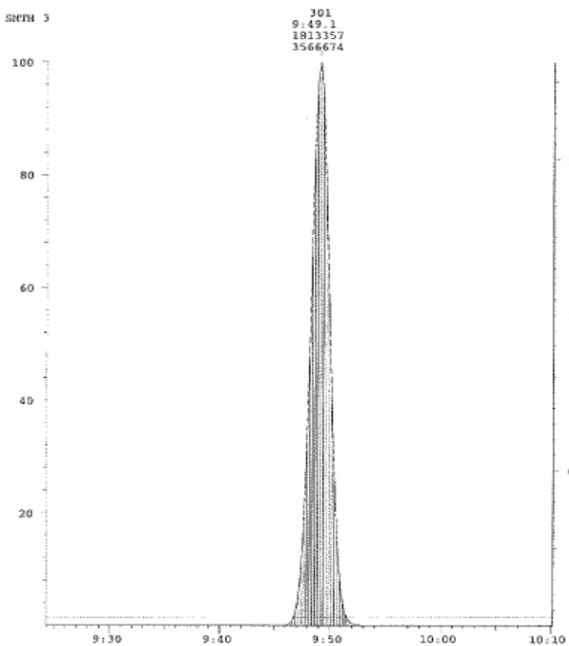
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30-MAY-13 Start : 19:02:30 465
Study: RFA7844
Inlet: GC Vial 9
Label: 3, 20.0

11d74BHOC
M/Z 721.4 +/- 500.0 m/z

301
9:49.1
1813357
3566674

1U4BHOC
M/Z 714.4 +/- 500.0 m/z



Date: Fri May 31 08:54:23 2013 ICIS: 8.3.0 SP2 for OSF1 (V4.0) build 98-238 from 26-Aug-98



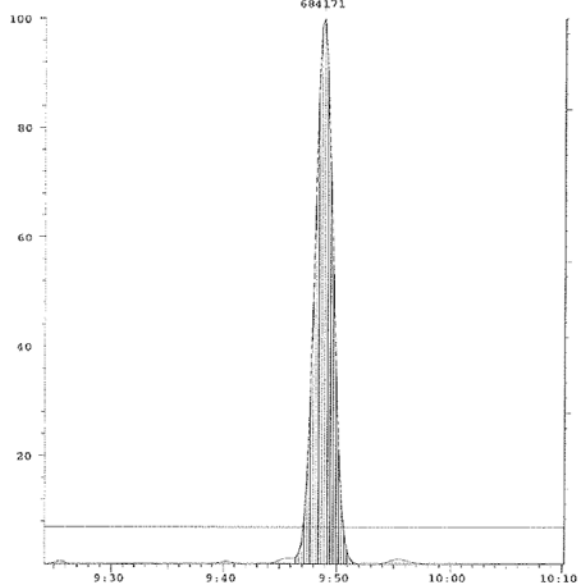
Matrix with Drug Present

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                                                Start : 21:03:45 466
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                                                Area: 5.800, 12 Baseline : 30.3
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                                                Label: 3, 20.0
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11d74BHC
M/Z 721.4 +/- 500.0 mmu

SMTH 3

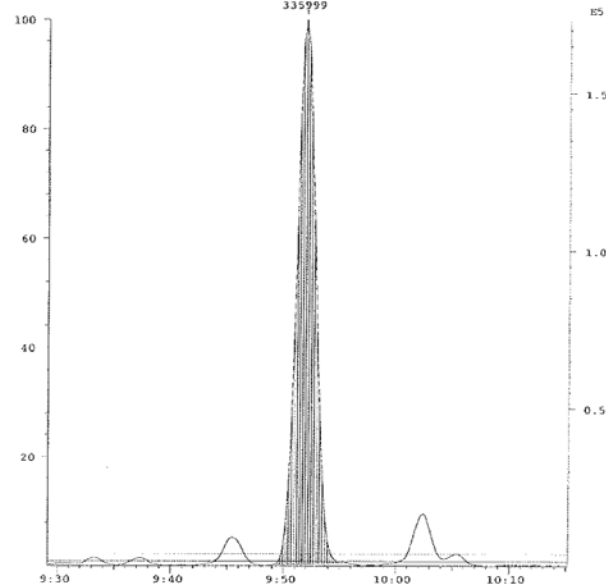
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359617
684171



1048BHC
M/Z 714.4 +/- 500.0 mmu

SMTH 3

313
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172654
335999



Date: Fri May 31 08:54:45 2013 ICIS: 8.3.0 SP2 for OSF1 (V4.0) build 98-238 from 26-Aug-98



Demonstrating Specificity—PK Studies

- We can often achieve that with MS/MS and little or no chromatography.
- We can't prove specificity, but we can come close.
- Most drugs are more compatible with LC than with GC, and LC provides all of the separation that we need.
- High resolution gas chromatography adds little or no value.



The Dominance of LC-MS/MS for PK

How did LC-MS/MS gain such complete dominance?

As a single tool it meets virtually every need.



The Advantages of a Single Platform

- The ruggedness and relative ease of LC-MS/MS helped to commoditize bioanalysis.
- This helped keep down the cost.
- It also facilitated the meeting of timelines.



Biomarkers

- Biomarkers present fundamentally different challenges.
- First and foremost it is not possible to demonstrate specificity.



Typical Matrix Blank

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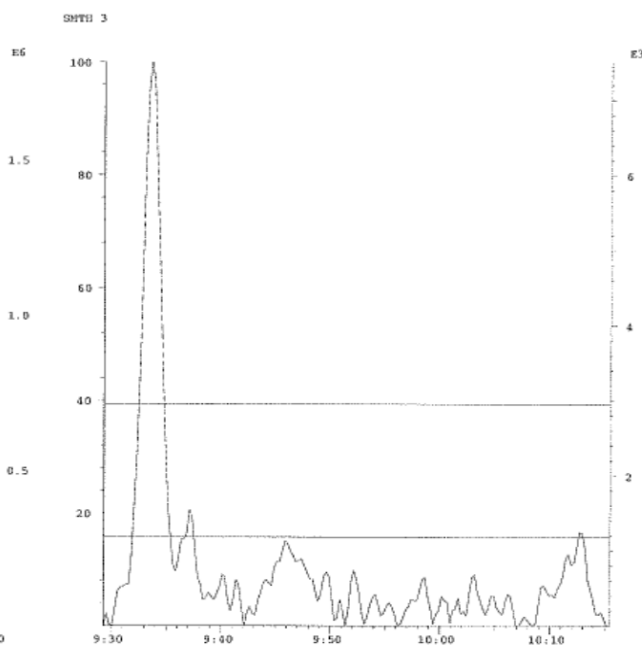
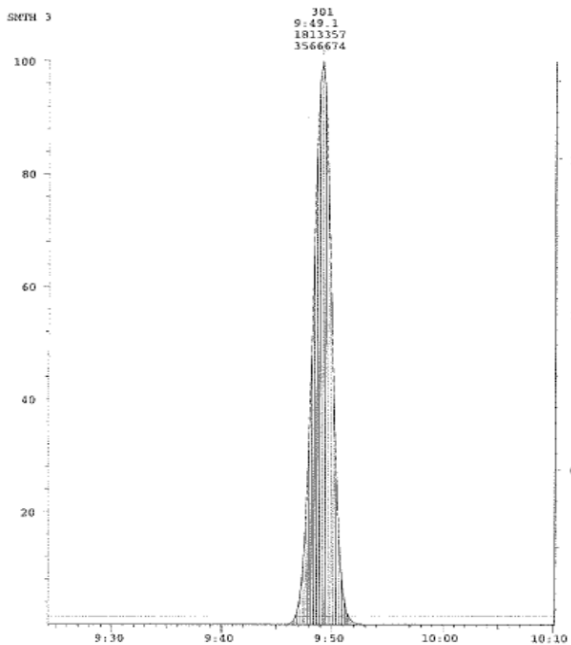
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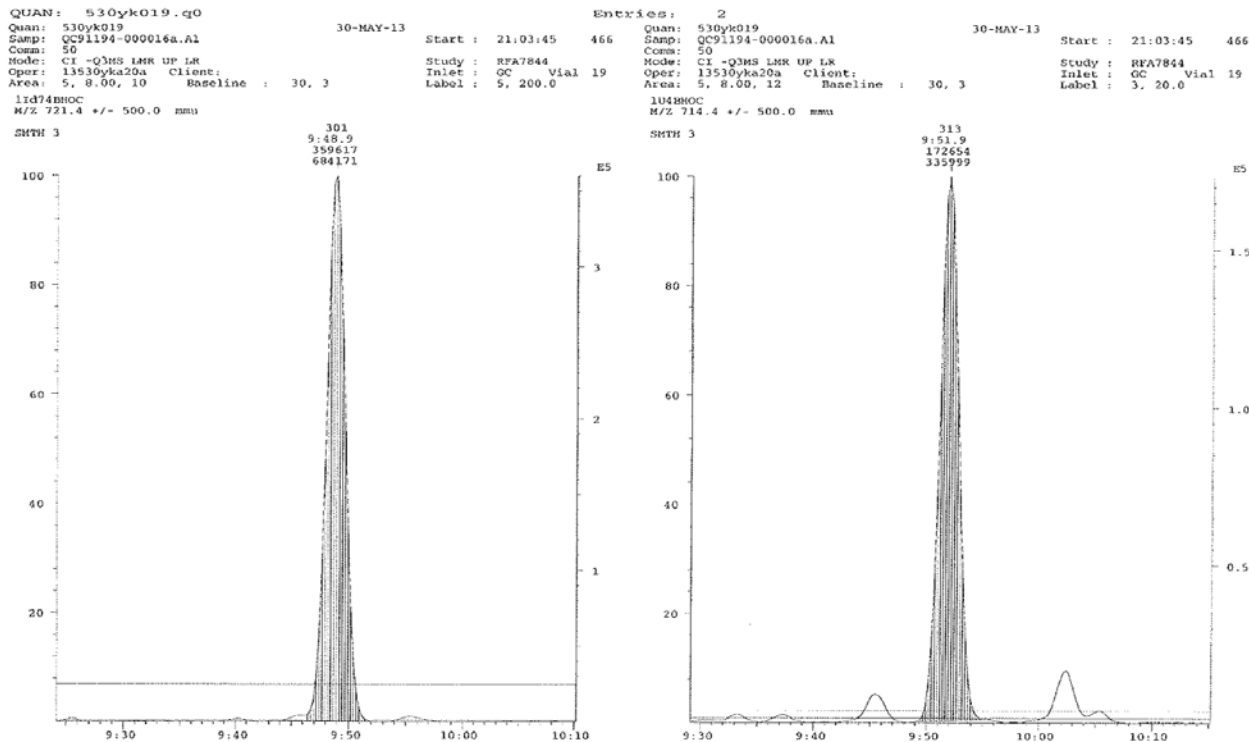
1U4BHOC
M/Z 714.4 +/- 500.0 m/z



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Typical Biomarker "Blank"



Date: Fri May 31 08:54:45 2013 ICIS: 8.3.0 SP2 for OSF1 (V4.0) build 98-238 from 26-Aug-98

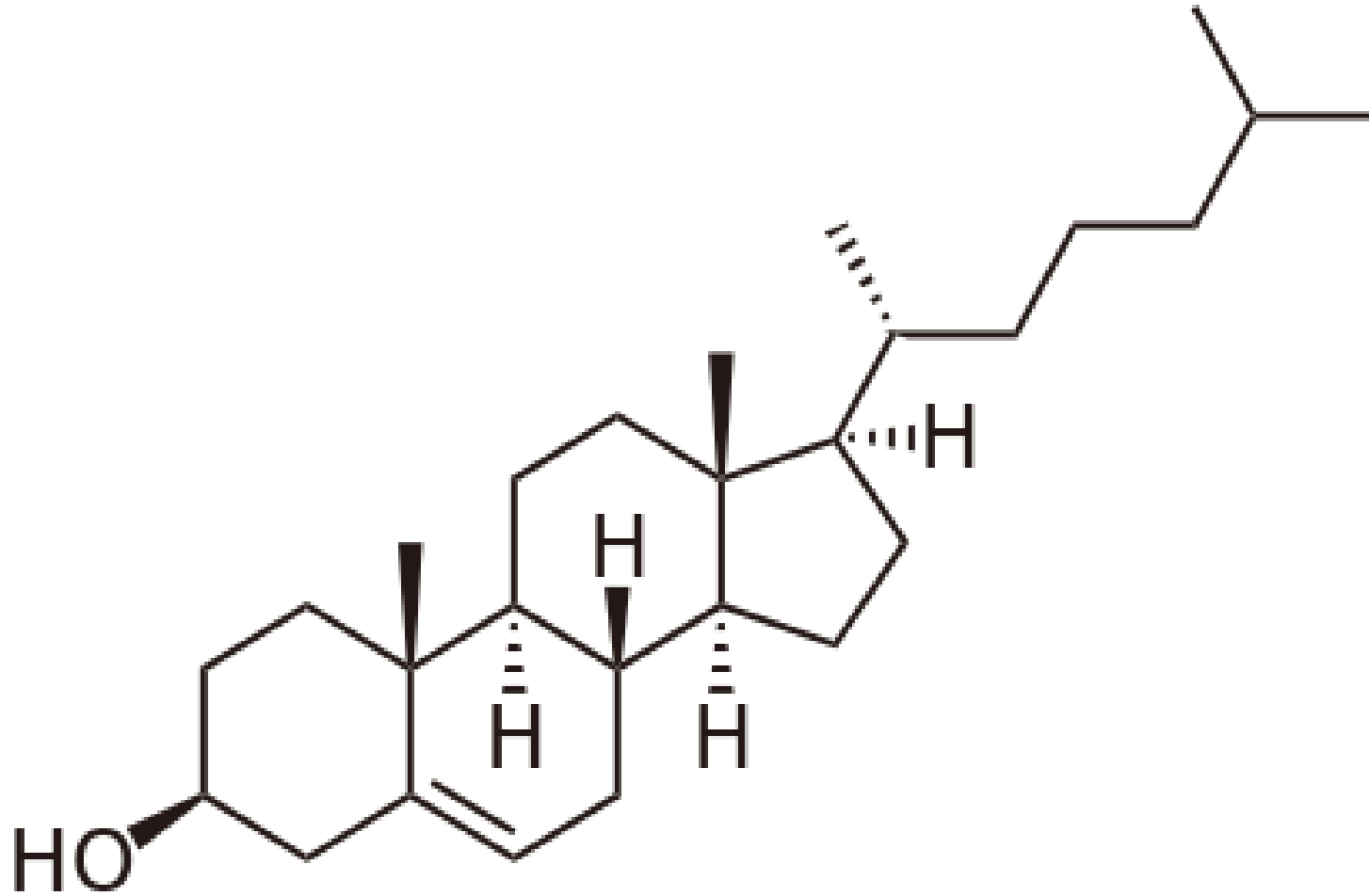


Biomarkers

- Not only is it impossible to demonstrate specificity....
-**it is less likely to be achieved.**
- 4-beta hydroxy cholesterol serves well to illustrate this point.

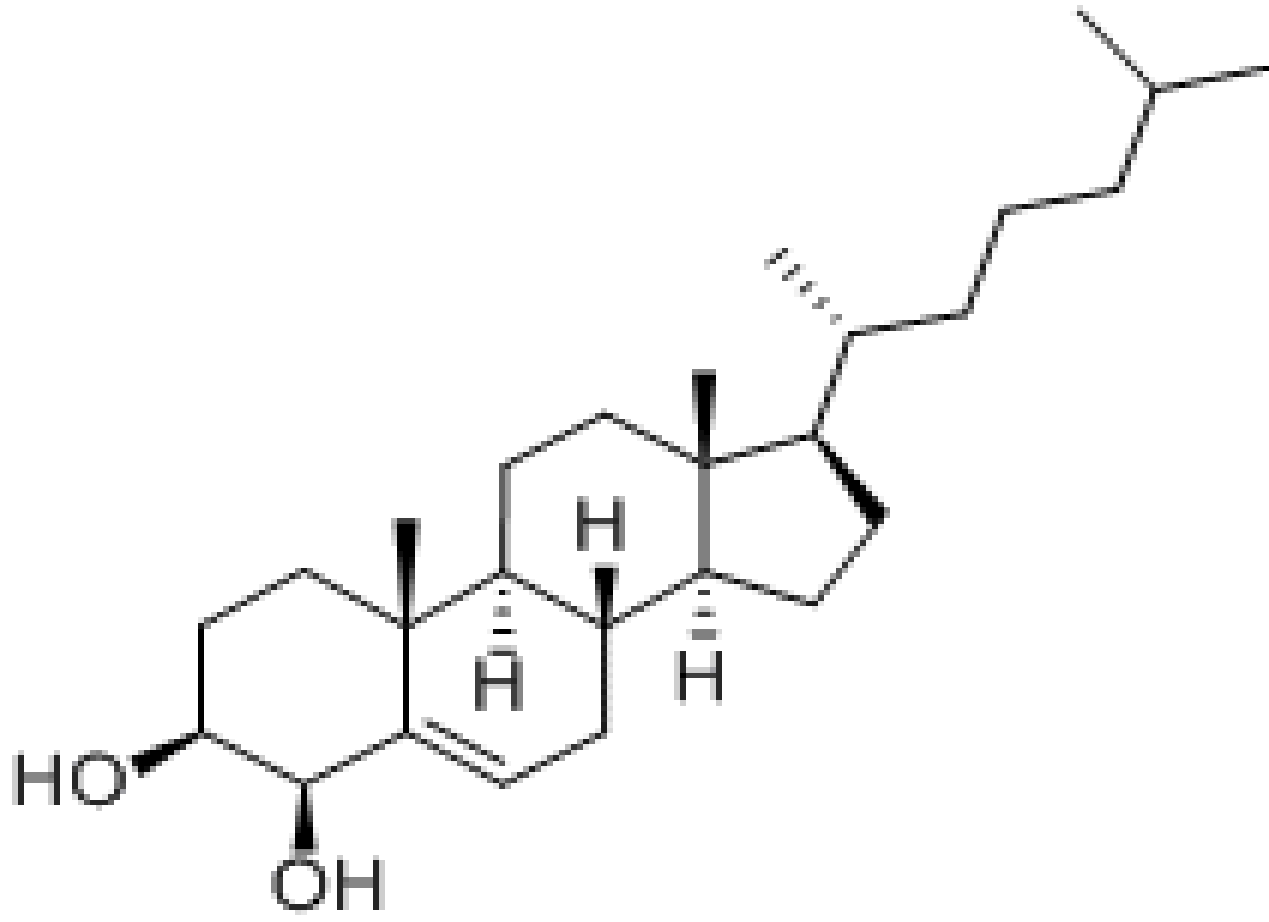


Cholesterol



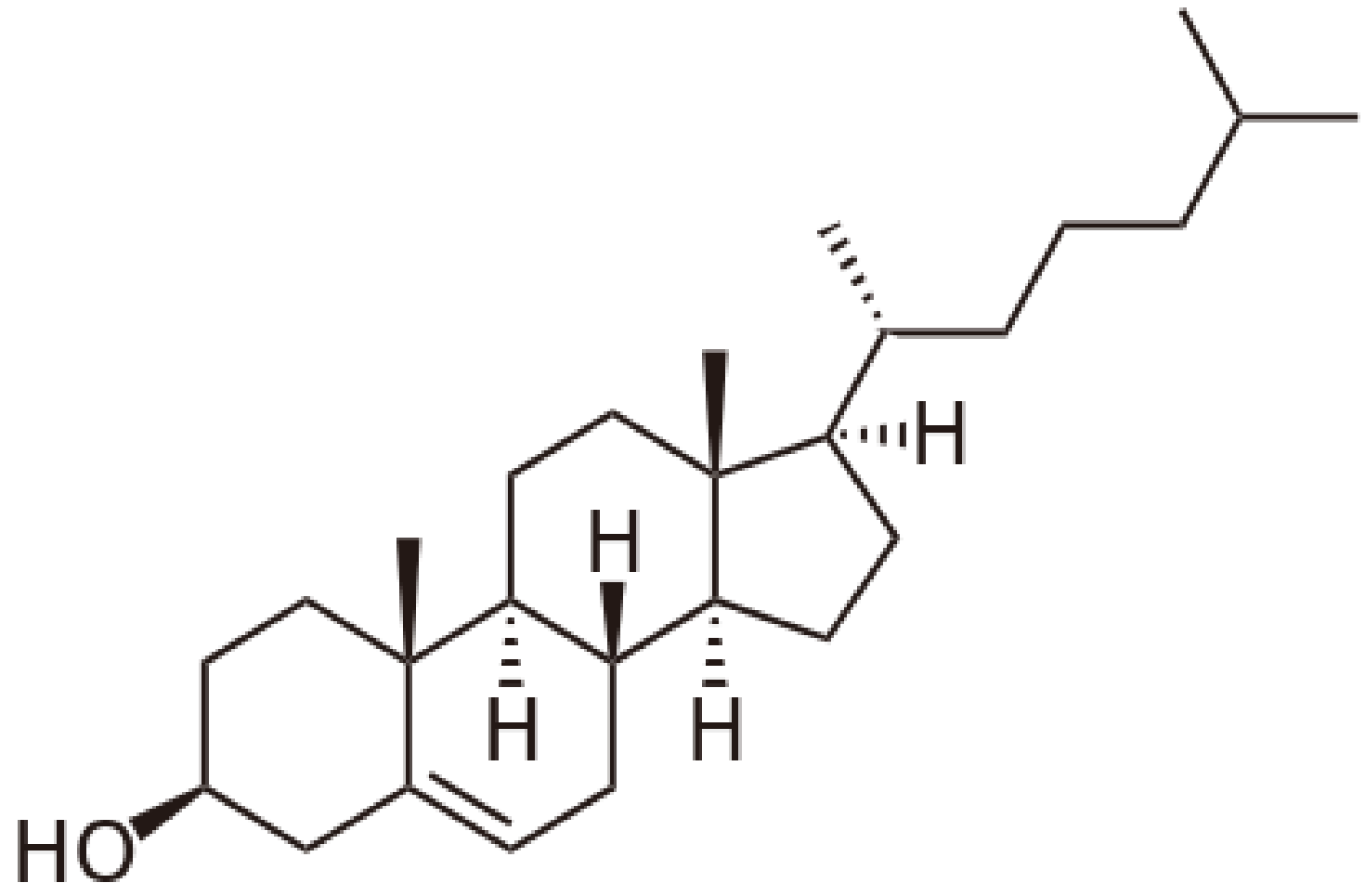


4-beta hydroxycholesterol





Cholesterol





Specificity for Biomarkers

- Biomarkers tend to be members of a class of molecules with similar structure and often identical mass.
- This is true of all of the isoforms of hydroxycholesterol.
- If even one isoform of 4-beta hydroxycholesterol co-elutes with the biomarker it could bias the results unless there is enough selectivity to isolate the analyte of interest.
- Because of the huge relative abundance of cholesterol even a small amount of oxidation could be problematic.



Specificity for Biomarkers

- If all isoforms have the same mass.....
-no selectivity from Q1 MS.
- Isoforms also tend to form the same daughter so.....
-no selectivity from MS/MS.



No additional selectivity from MS/MS?

- This conjecture can be put to a test.
- Analyze a sample using an MS/MS method.
- Record the peak area ratio.
- Analyze it again with MS/MS turned off (Q1 MS).
- If the peak area ratio is the same, MS/MS has not provided any additional selectivity.



Our Selectivity Tool Box

- Protein precipitation, liquid-liquid or solid phase extraction
- Column chromatography
- Mass selective detection
- MS/MS



MS/MS and Biomarkers

- The tremendous selectivity of MS/MS for drugs and metabolites falls short for biomarkers.
- But because of the many similar molecular structures of biomarkers even more selectivity is often needed.
- This places a great deal of importance on chromatographic separation.

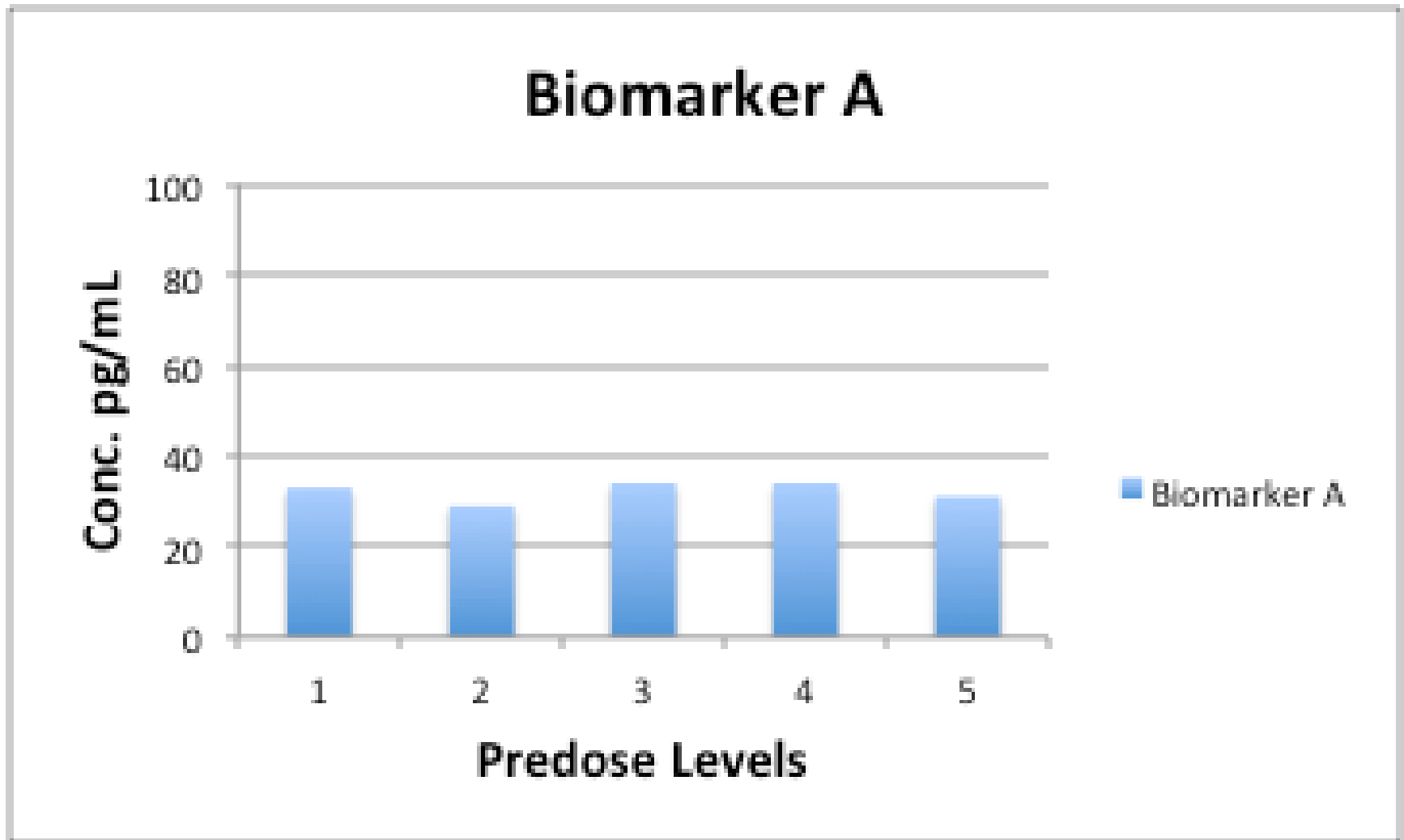


Our Selectivity Tool Box

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- Mass selective detection
- MS/MS



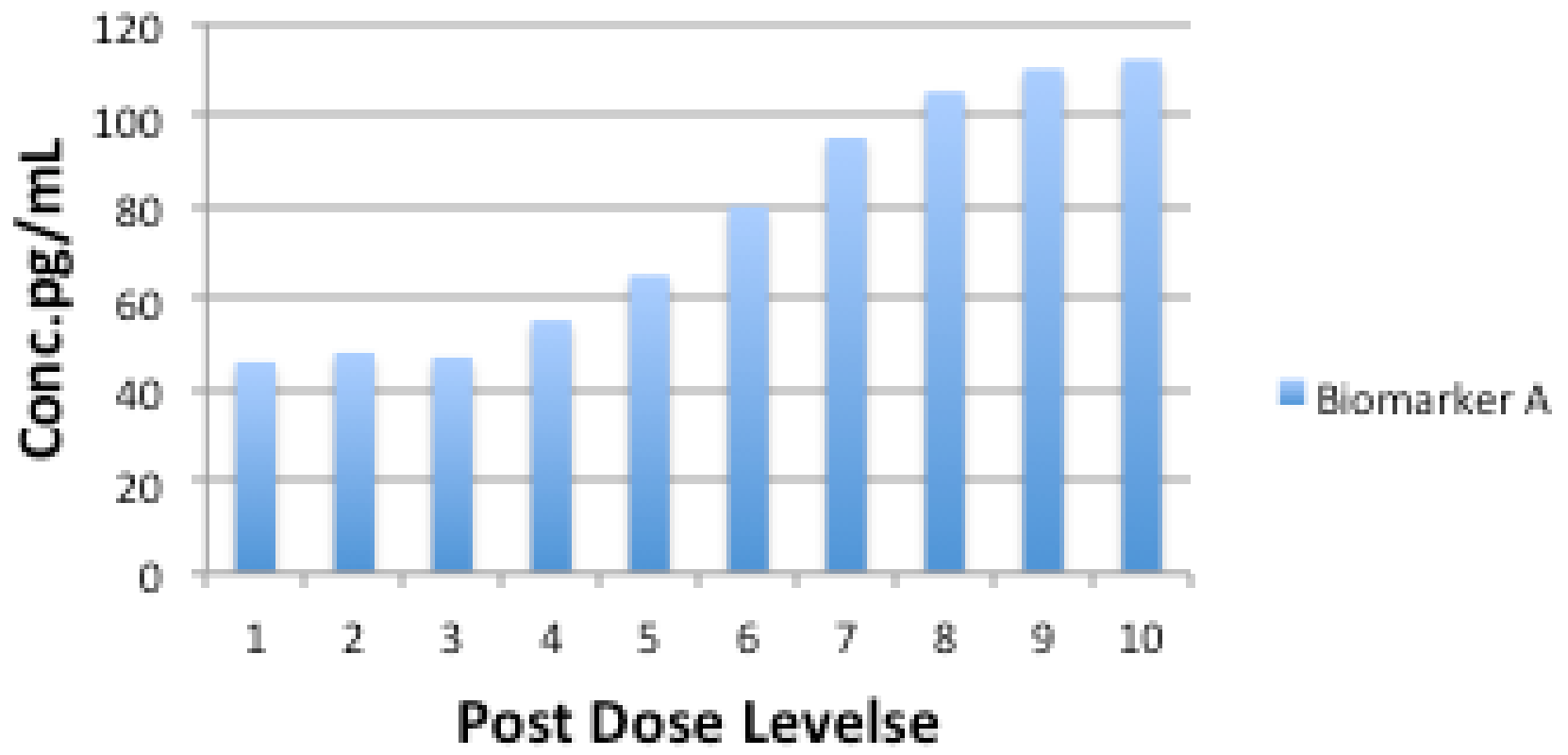
Test Data for a Biomarker Assay





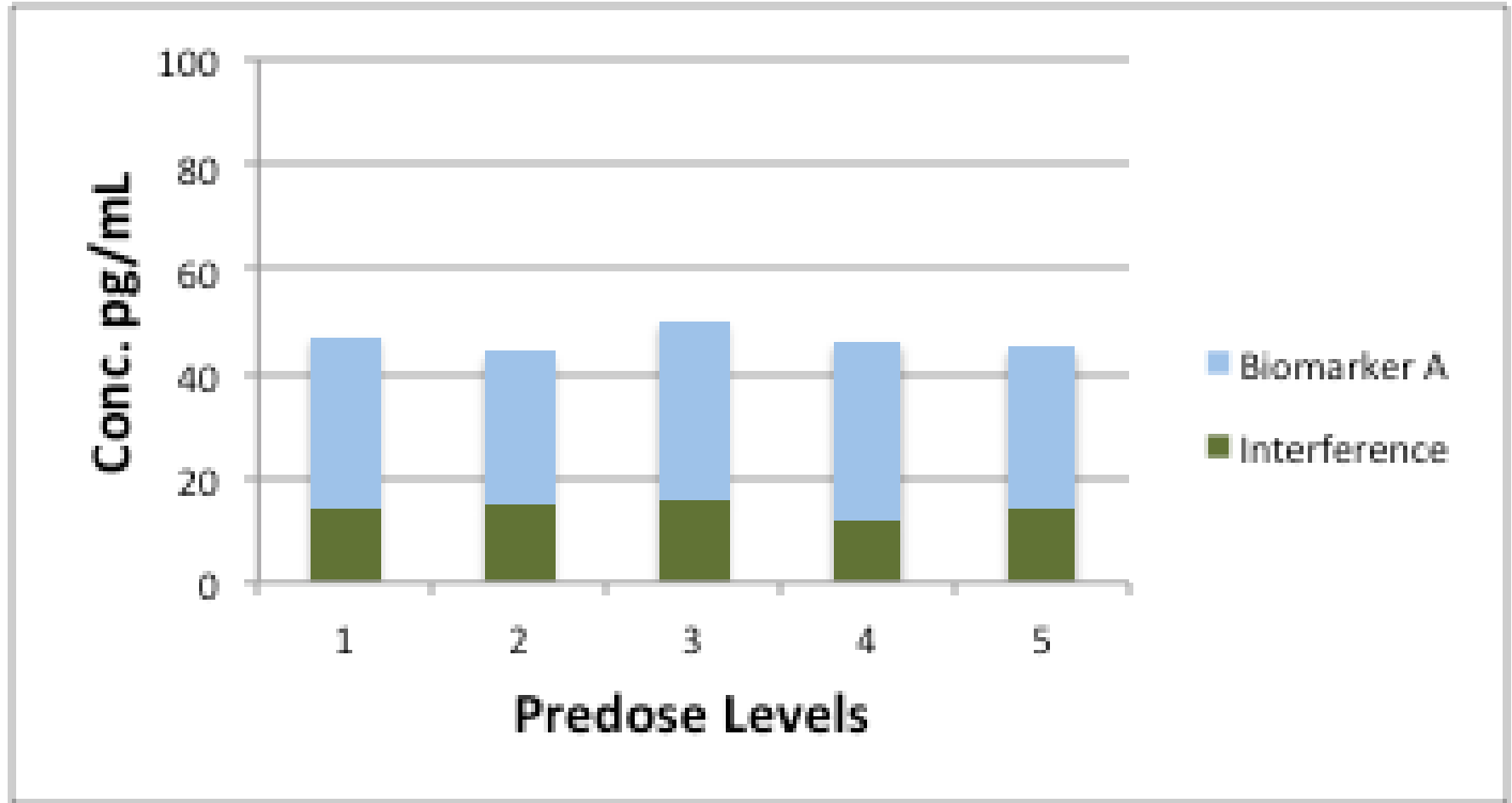
Data after Dosing

Biomarker A



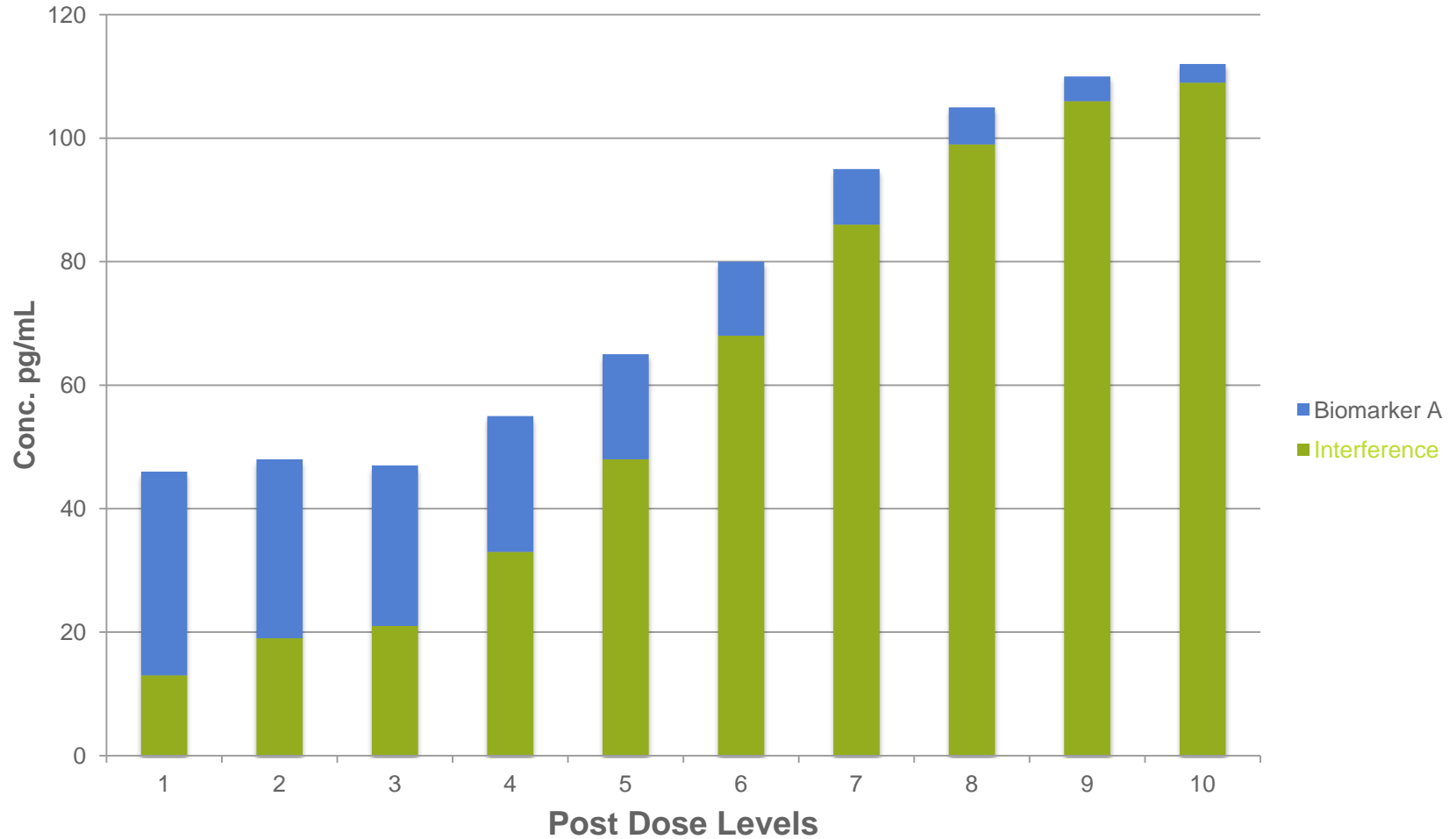


What if.....



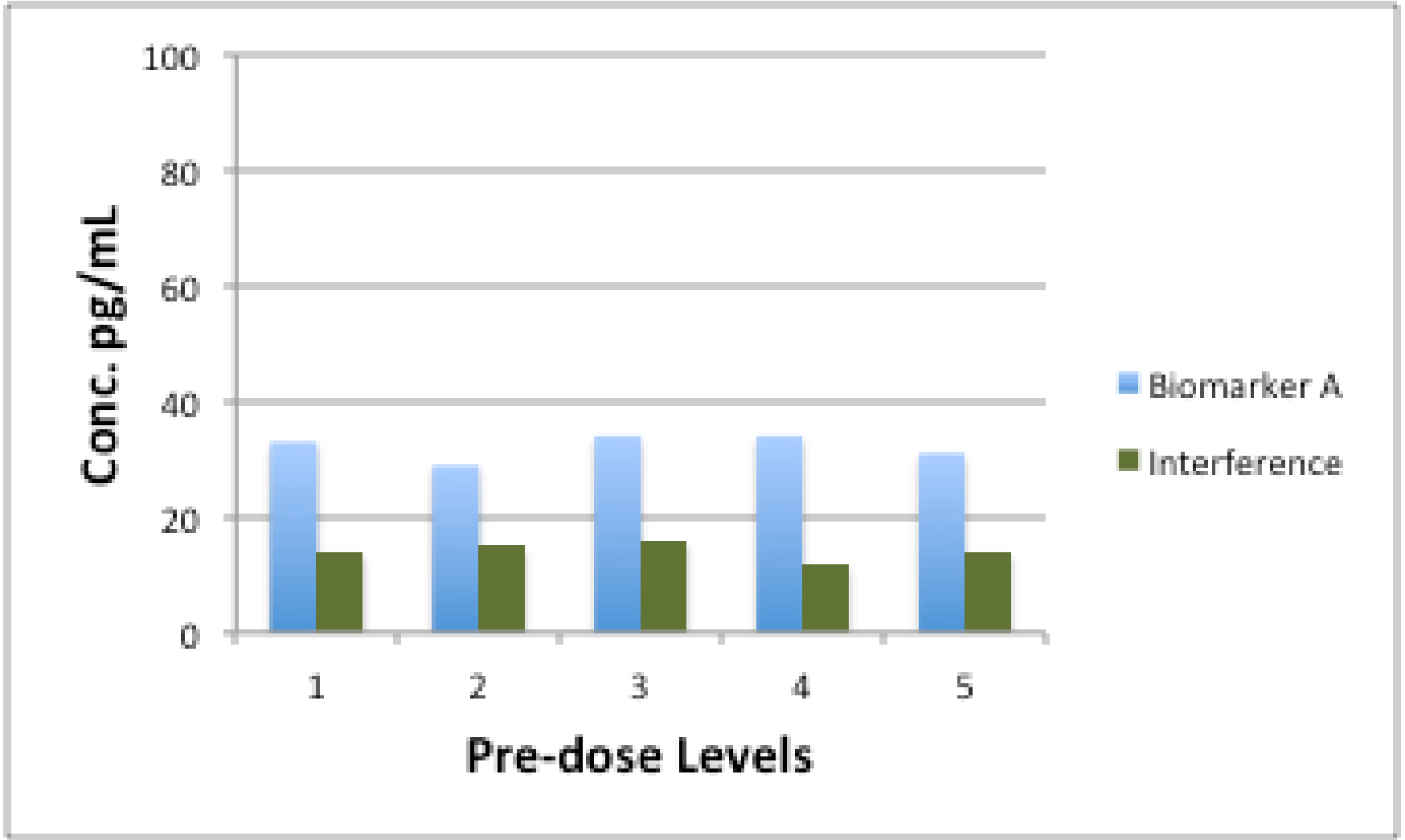


Could this be happening?



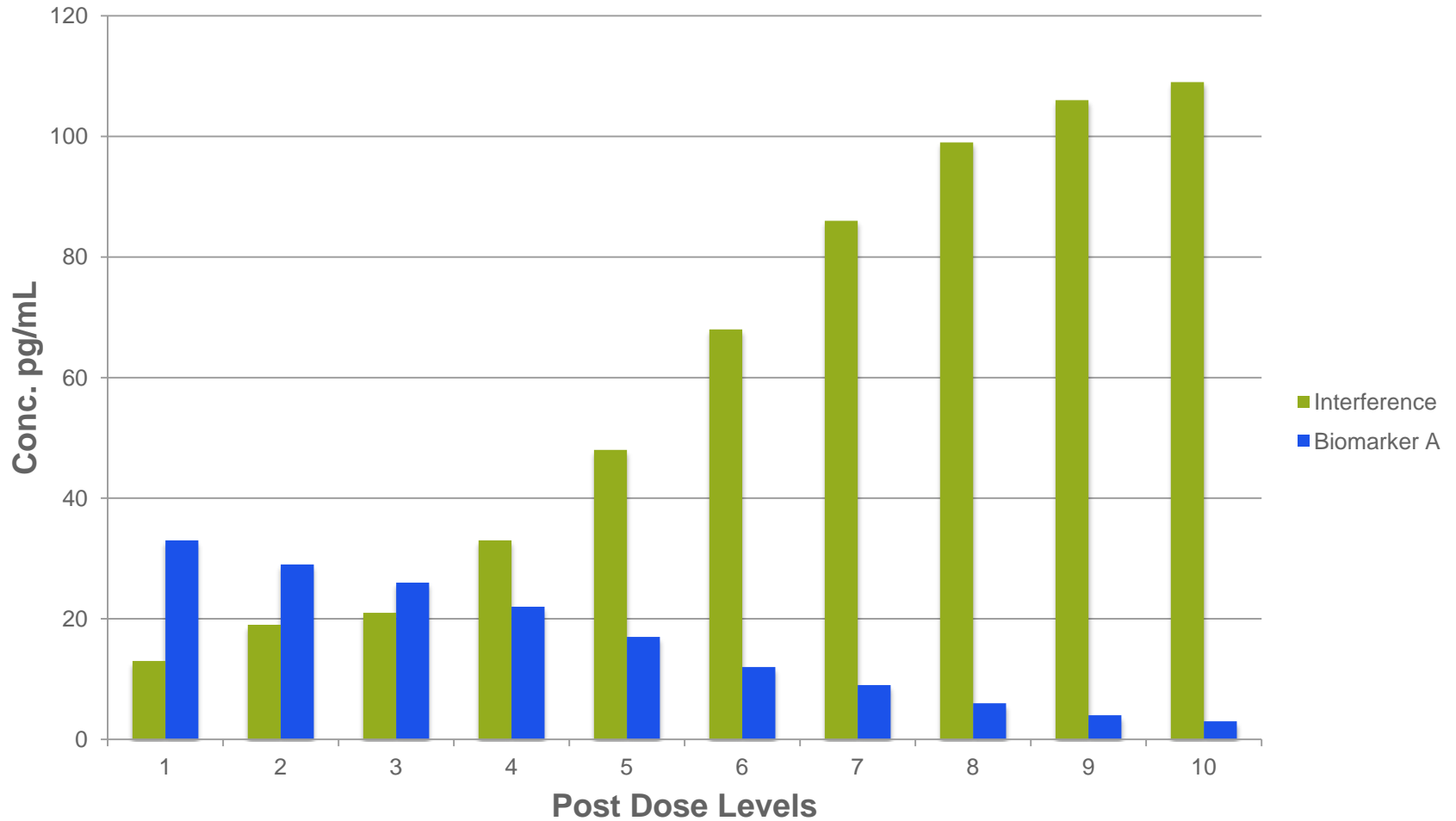


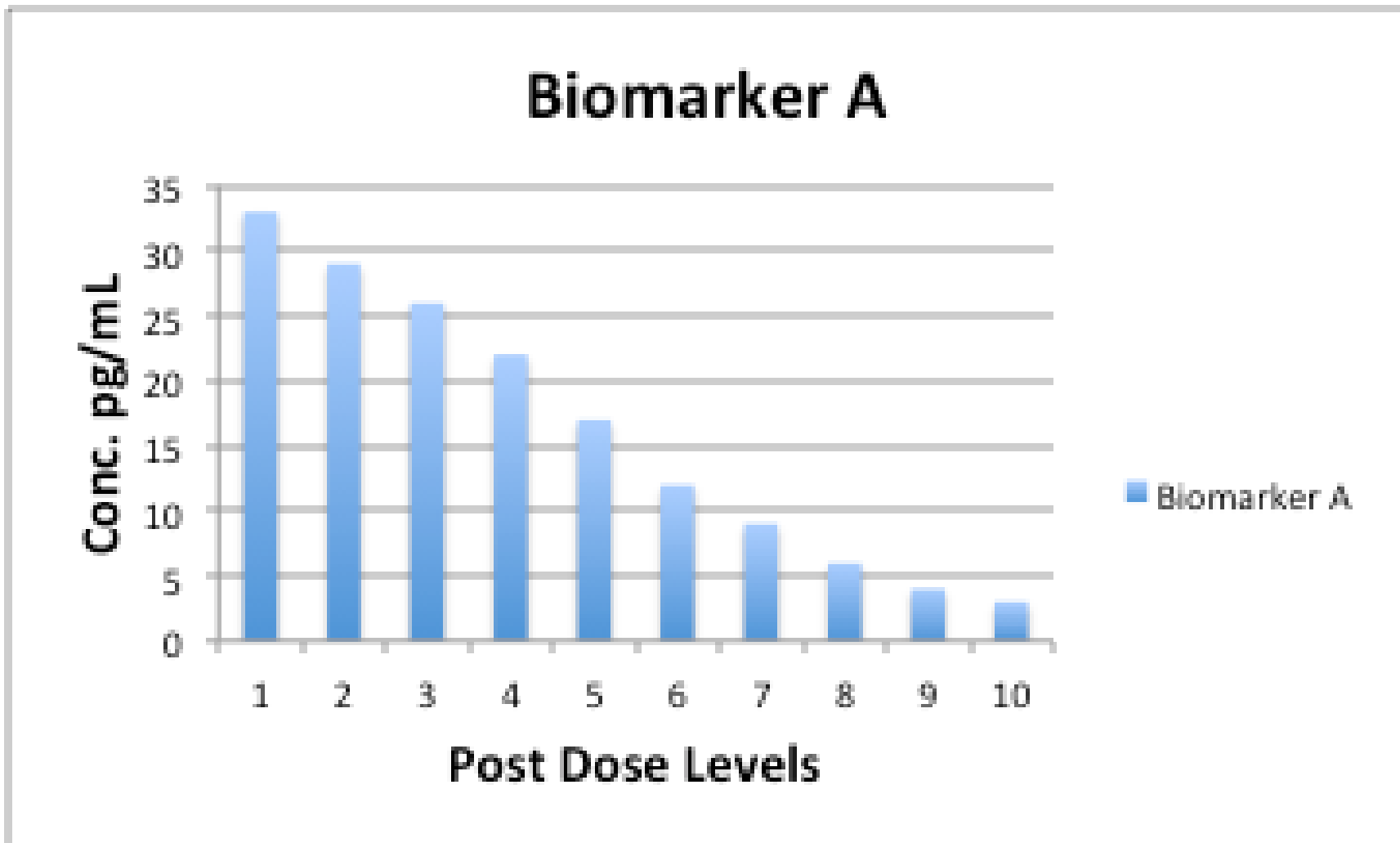
What if.....





After Chromatographic Isolation

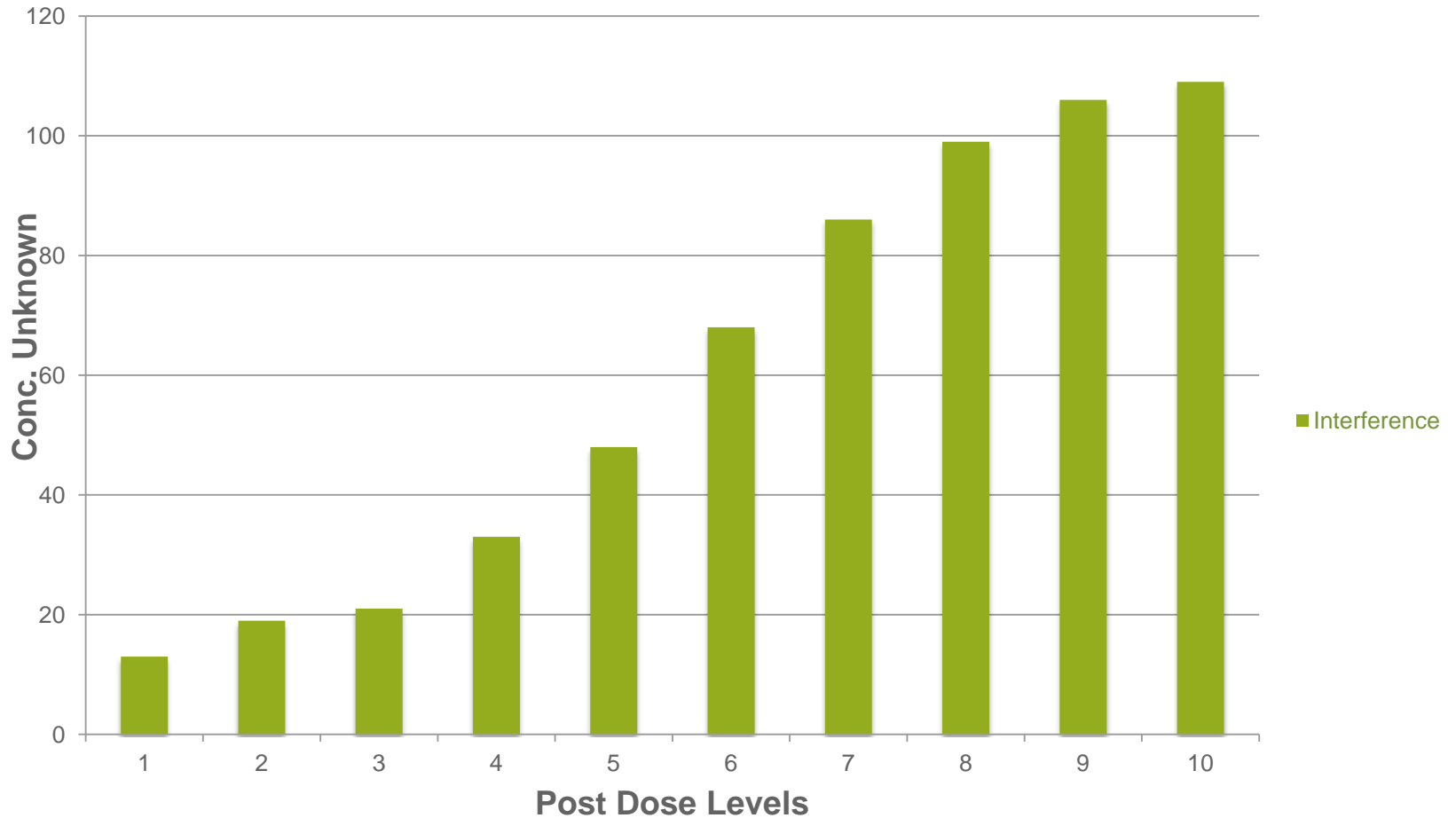






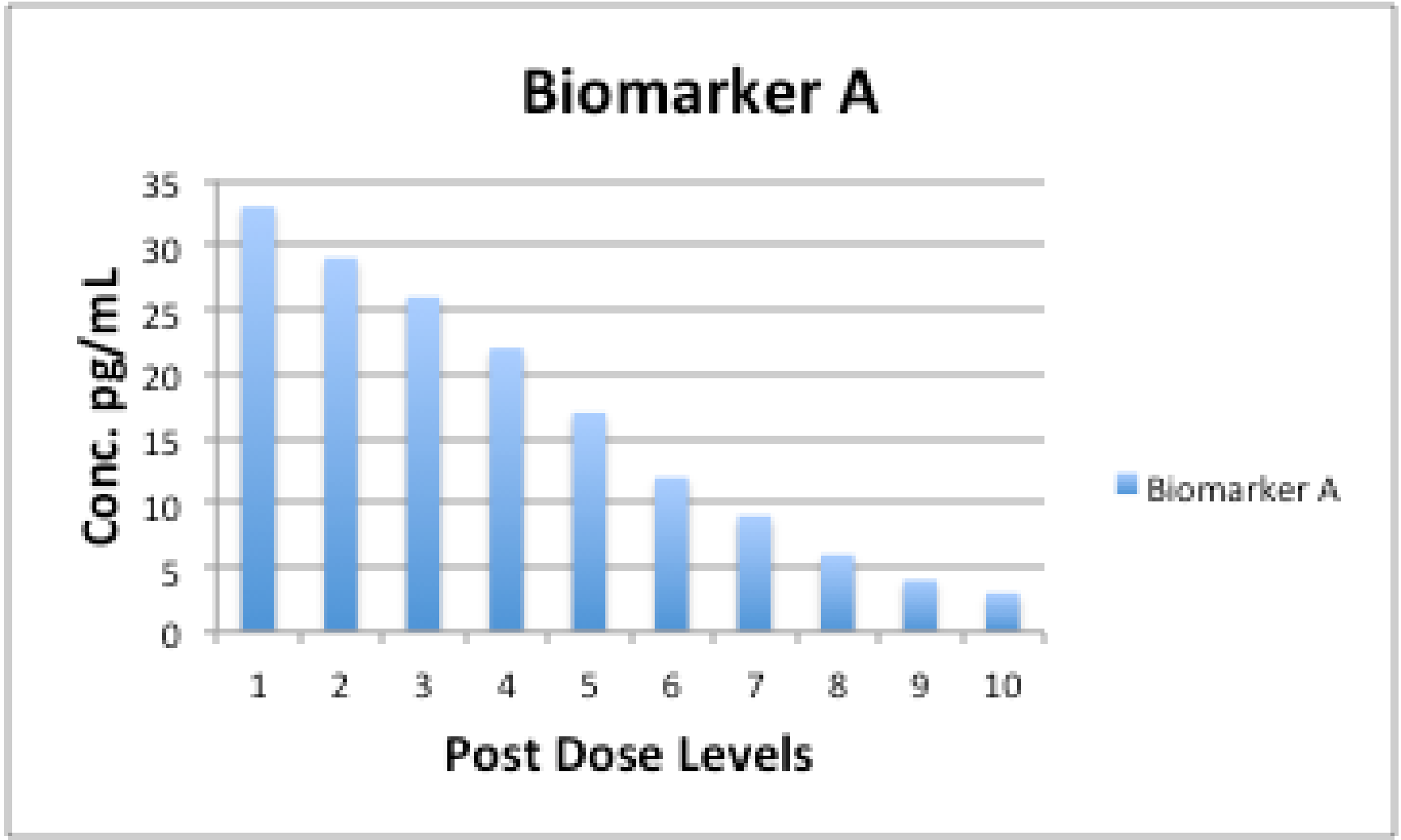
Interference now isolated

Interference



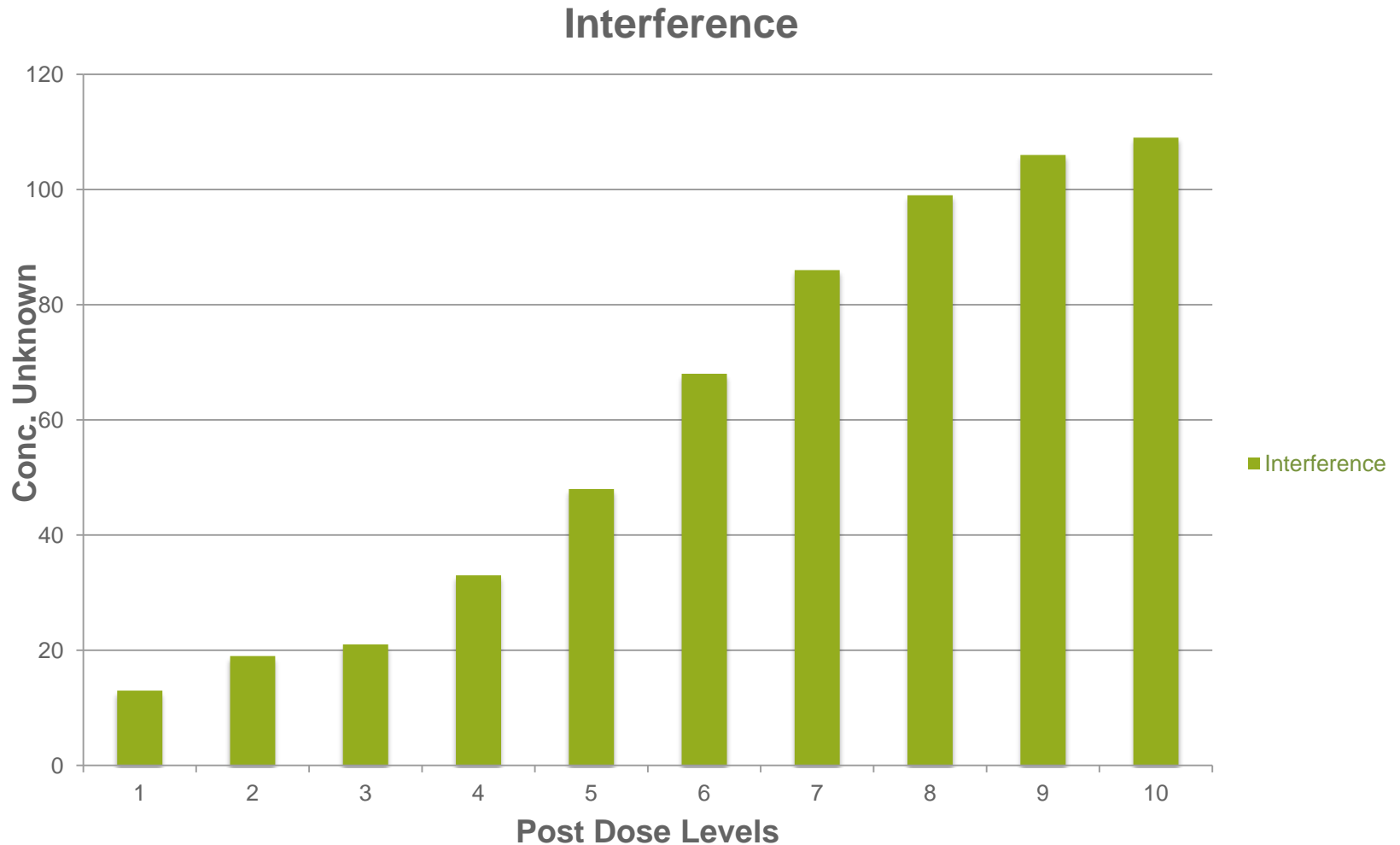


If these data support efficacy...





...might these data present a safety concern?





What is the message from this?

- Biomarkers are confusing enough because of the biological unknowns.
- We must try not to add to the confusion by introducing analytical artifacts.
- Use all of the selectivity at our disposal.



Total Testosterone Data from Seven Labs¹

Interlaboratory Total Testosterone

Bias Data

		over all mean bias	mean bias at ≤ 1 ng/mL	mean bias at > 1 ng/mL
	Method			
Lab 2	LC-MS/MS	-14.1	-17.8	-10.5
Lab 3	LC-MS/MS	9.9	18.2	1.7
Lab 4	LC-MS/MS	10.1	10.7	9.6
Lab 5	LC-MS/MS	-7.4	-12.6	-2.3
Lab 6	LC-MS/MS	19.2	21.6	16.8
Lab 7	LC-MS/MS	17.2	25.3	9.2
inVentiv	GC-MS/MS	-2.1	-2.0	-2.2



Barriers to the Acceptance of GC-MS/MS

- “LC-MS can do anything that GC-MS can do.”
- “LC-MS can be as sensitive as GC-MS.”
- “GC-MS is too expensive.”



Name a biomarker that is best measured with a hammer.





Name a biomarker that is best measured with a hammer.





Biomarker Toolbox

Our biomarker toolbox needs to contain a full set of tools.

One of those tools needs to be GC-MS/MS.



References

1. Vesper et al. Steroids 74 (2009) 498-503 Interlaboratory comparison of serum total testosterone measurements performed by mass spectrometry methods.



Thank you for your attention.

