

Fast and Sensitive Method for the LCMS Analysis of Vitamin D

JinHee Kim and Tim Schlabach, Novilytic, Kurz Purdue Technology Center (KPTC), 1281 Win Hentschel Blvd. West Lafayette, IN 47906



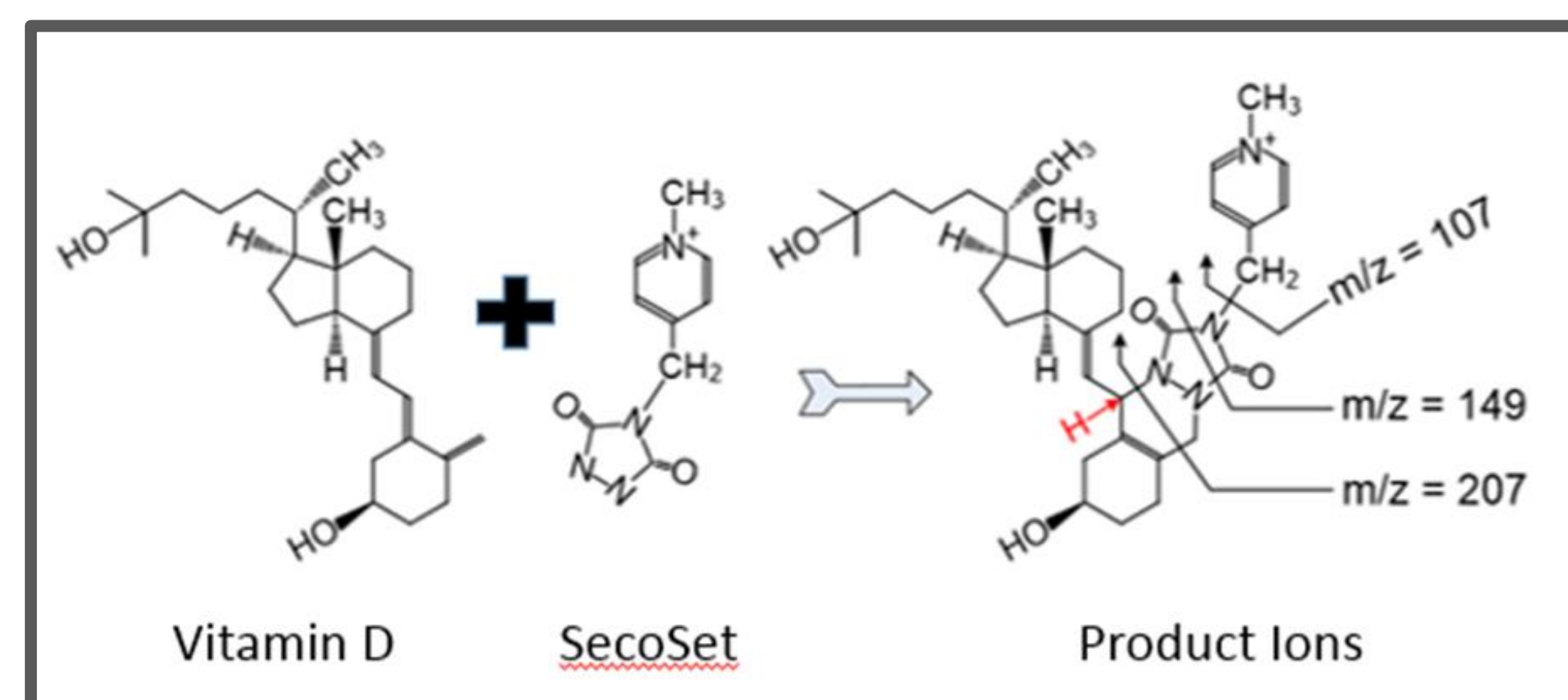
OVERVIEW

Vitamin D analysis has become a major clinical test with millions of tests performed annually. Surprisingly, most vitamin D testing is done with immunoassays. LCMS typically produces more accurate results but the LCMS methods often require lengthy derivatization which is not needed for immunoassays. Derivatization improve ionization efficiency and elevates sensitivity but requires hours of work prior to injection.

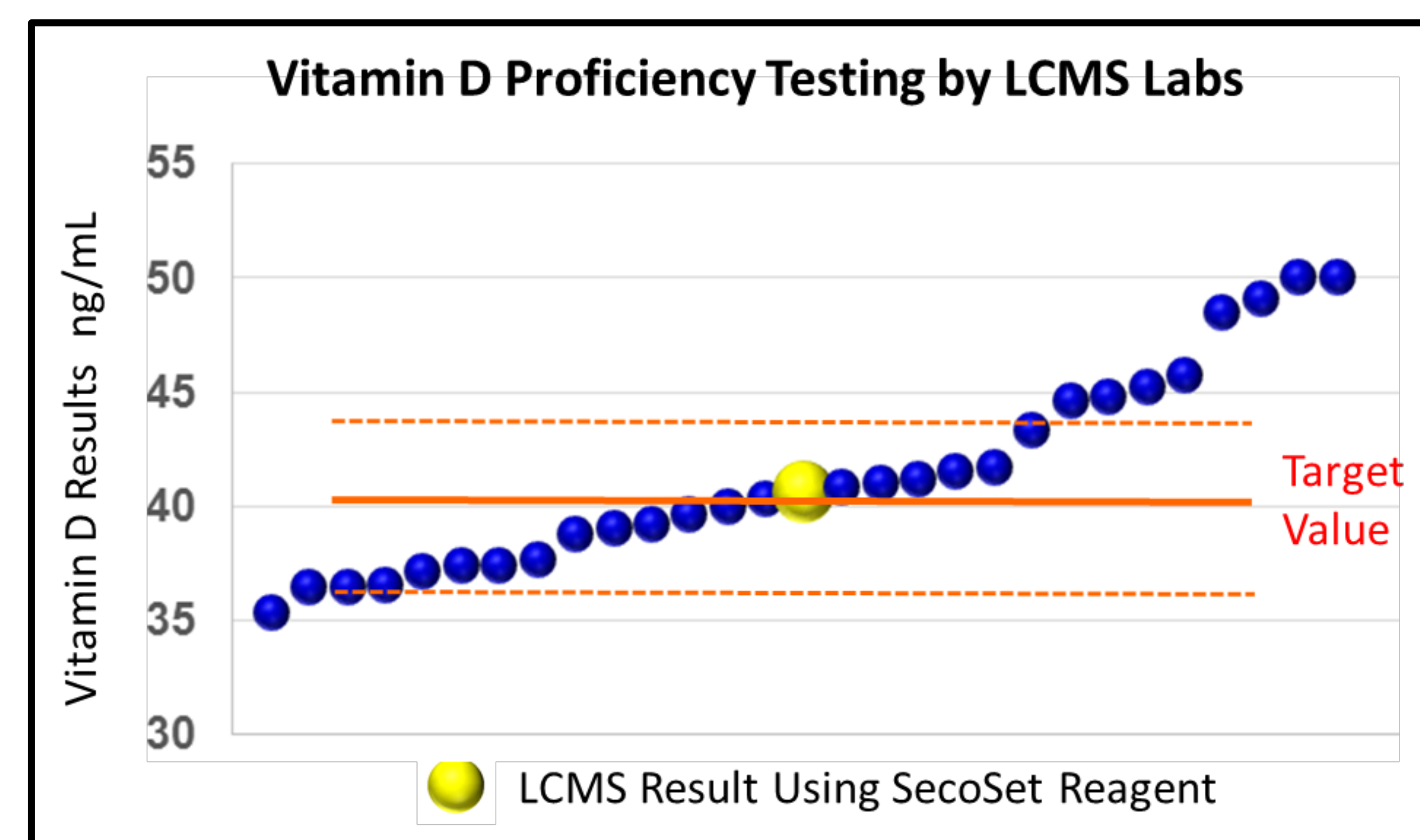
Novilytic has developed a unique, fast acting reagent that adds a permanent positive charge to secosteroids to improve sensitivity at least twenty fold. This reagent also improves extraction efficiency for blood or plasma samples collected in a dry state on commercially available dried blood spot or plasma spot cards (DBS or DPS). The derivatized vitamin D forms in 2 minutes and can be extracted in a few minutes. Then it is easily separated on a C18 column and quantified by isotope dilution, LCMS.

FAST DERIVATIZATION OF VITAMIN D

Most vitamin D derivatization reagents react slowly. SecoSet is activated in seconds with an initiation reagent and is quickly quenched with a stopping reagent. The product ions are suitable for MRM analysis resulting in signals that are about ten times stronger than underivatized vitamin D. The complete reaction as described in the SecoSet User Guide takes just 2 minutes.



SECOSET VALIDATED WITH NIST STANDARDS



NIST plasma standards were obtained and used to validate SecoSet chemistry. SecoSet derivatization was done in a few minutes, but liquid extraction of plasma took longer. SecoSet performed well among the thirty or more of LCMS labs reporting data.

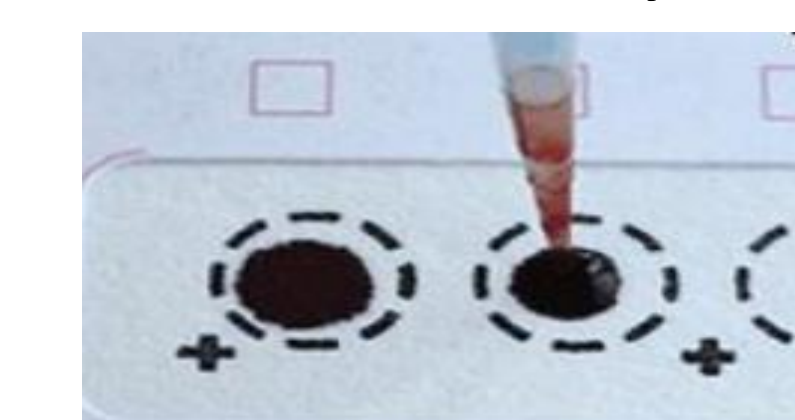
The SecoSet chemistry meets the standard clinical requirements for the determination of:

Total Vitamin D, Vitamin D₃, Vitamin D₂ and epi Vitamin D.

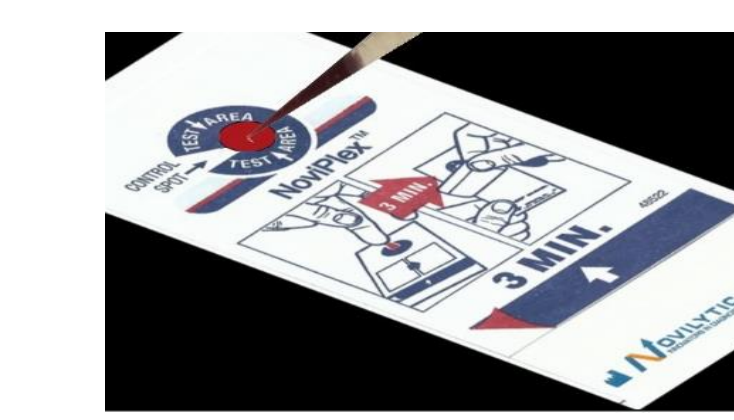
	NIST Target	SecoSet	Difference
	ng/mL	ng/mL	%
Total VitD	32.7	32.9	0.6%
	15.8	16.8	6.0%
	13.2	13.2	0.0%
Vitamin D3	39.3	40.6	3.2%
	26.2	26.9	2.6%
Vitamin D2	6.5	7.1	8.5%
	2.3	2.1	9.5%
EpiVitD	1.6	1.7	5.9%

VITAMIN D ANALYSIS WITH DBS OR DPS CARDS

The SecoSet reagent is not only fast but it also improves the extraction of derivatized Vitamin D from paper substrates. In just 3 minutes the vitamin D in paper discs from dried blood spot(DBS) cards or from dried plasma cards (DPS) can be extracted. The addition of a positive charge to vitamin D greatly improves extraction efficiency.



DBS Card

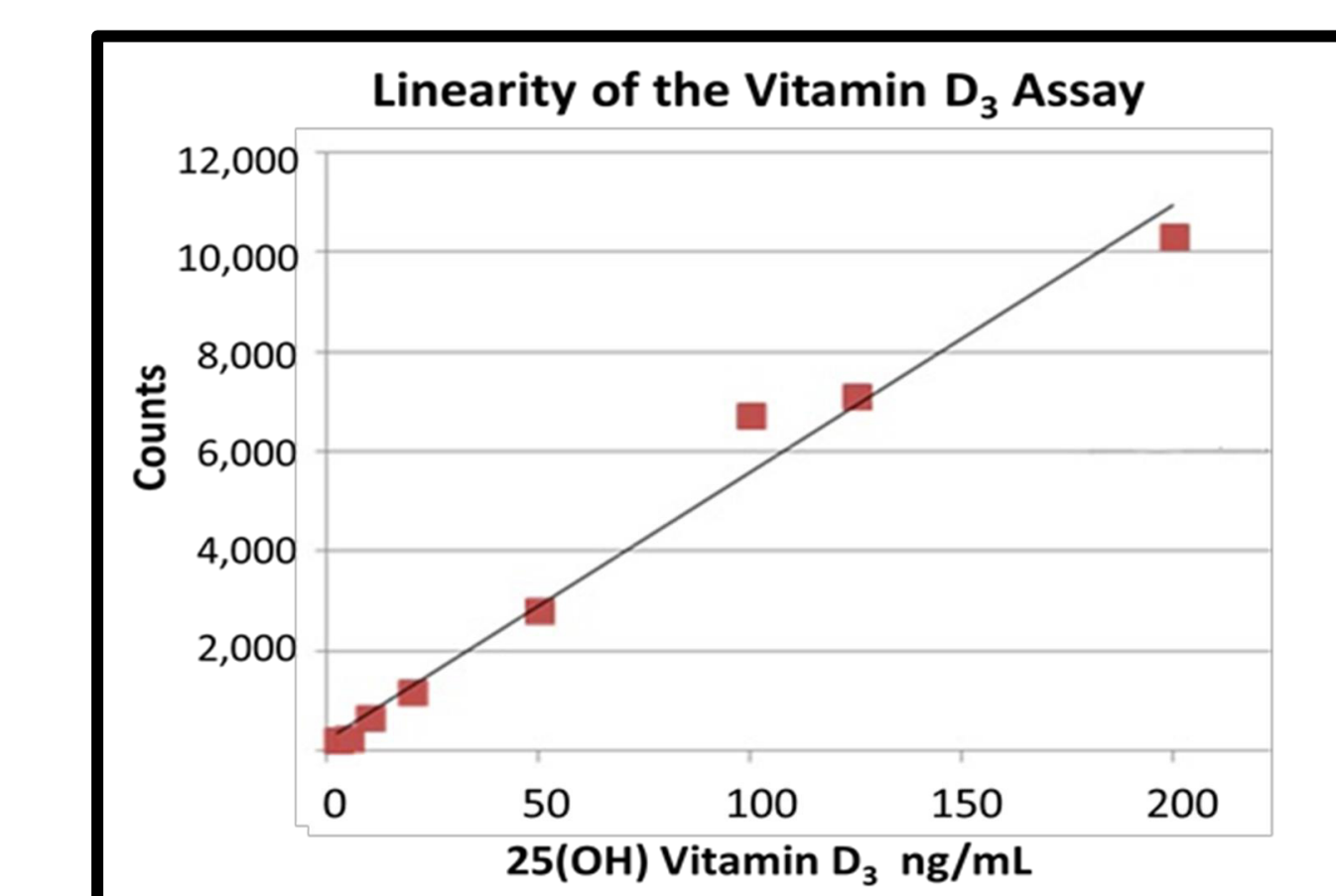


Noviplex DPS Card

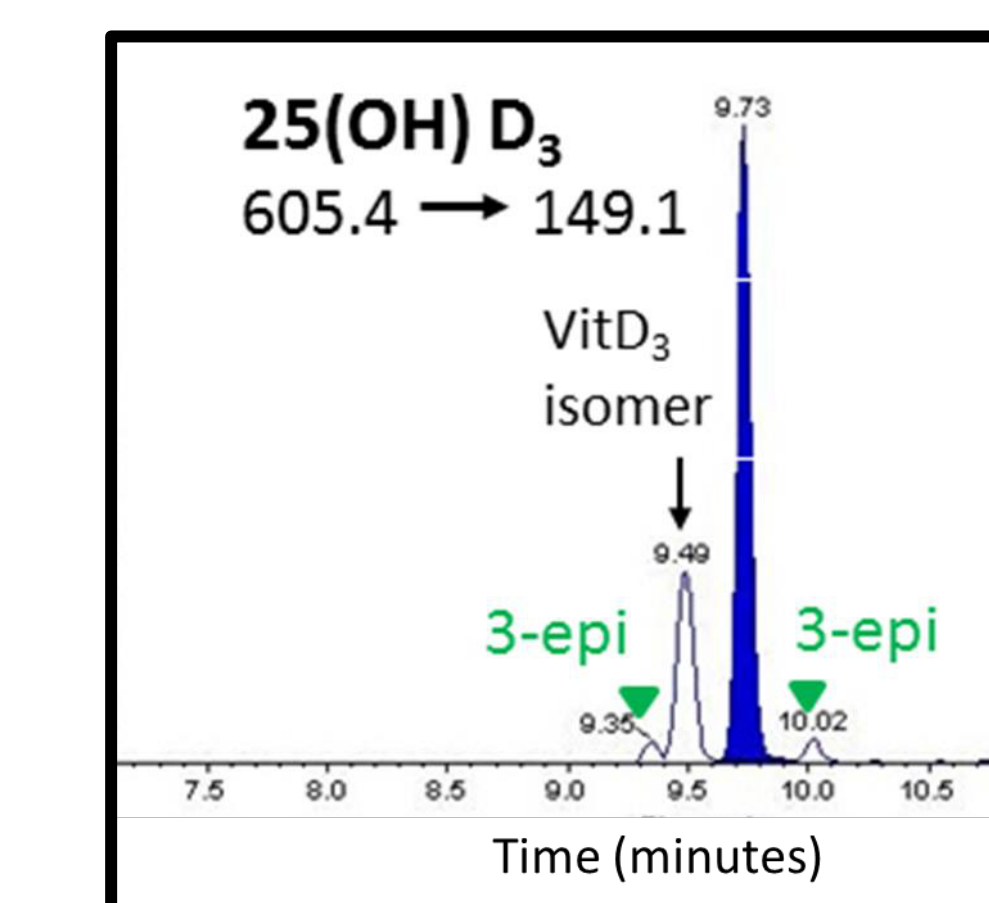
The SecoSet reagent shows good accuracy and precision with DBS or DPS cards.

Sample Amount	Collection Method	Vitamin D ₃ ng/mL	CV %	Recovery
100 μ L	Venipuncture	22.4	1.9%	NA
10 μ L	Dried Blood Spot	22.9	12.3%	102.2%
3 μ L	Noviplex Card	21.5	4.4%	96.0%

Vitamin D₃ linear range on Noviplex



MRM Transitions with SecoSet



CONCLUSIONS:

- SecoSet is a proven, LCMS derivatization reagent for vitamin D
- Linearity extends beyond 20 ng/mL, which is a clinical threshold
- Vitamin D samples are ready for **LCMS injection in 5 minutes**