

# High-Throughput Lead Discovery with Agilent RapidFire/MS Systems: Analysis of Stearoyl-Coenzyme A Desaturase (SCD)

## Application Note

### Authors

William Lamarr, Lauren Frick,  
and Peter Rye  
Agilent Technologies, Inc.  
Wakefield, MA USA

### Introduction

The RapidFire High-throughput Mass Spectrometry System provides drug discovery researchers with mass spectrometry-based, high-throughput screening solutions for targets that have proven challenging to screen using conventional approaches. These intractable targets have substrates and products that are either too small to label or undergo modifications that are difficult to detect. RapidFire technology provides the most relevant data, with label-free native analyte detection that eliminates the need for cumbersome and costly labeling methods. RapidFire technology enables traditionally low-throughput, intractable assays to be converted into high-throughput assays processed at speeds approaching plate-based optical methods. In this application note, a stearyl-coenzyme A desaturase (SCD) assay is used to illustrate the power of Agilent RapidFire/MS Systems for screening intractable targets.



**Agilent Technologies**

## Using RapidFire High-throughput Mass Spectrometry to Analyze SCD Assay Samples

The enzyme SCD catalyzes the conversion of stearoyl-coenzyme A (SCoA) to oleoyl-coenzyme A (OCoA) as shown in Figure 1. This enzyme plays a critical role in the desaturation of fatty acids and is an important therapeutic target for a range of disease states.

However, the reaction results in only the desaturation of a single carbon-carbon bond. This conversion is an extremely subtle change which presents a number of significant challenges during the screening process. In addition, the use of a radiometric assay for these challenging lipophilic analytes is typically a barrier to efficient high-throughput screening of targets like SCD. In the case of SCD, the radiometric assay is a tritiated

water release assay that has been employed for the determination of enzyme activity.

Here we present an example of a RapidFire high-throughput mass spectrometry assay developed for SCD that overcomes the need for radioactive labeling, making this target class a candidate for a high-throughput screening approach.

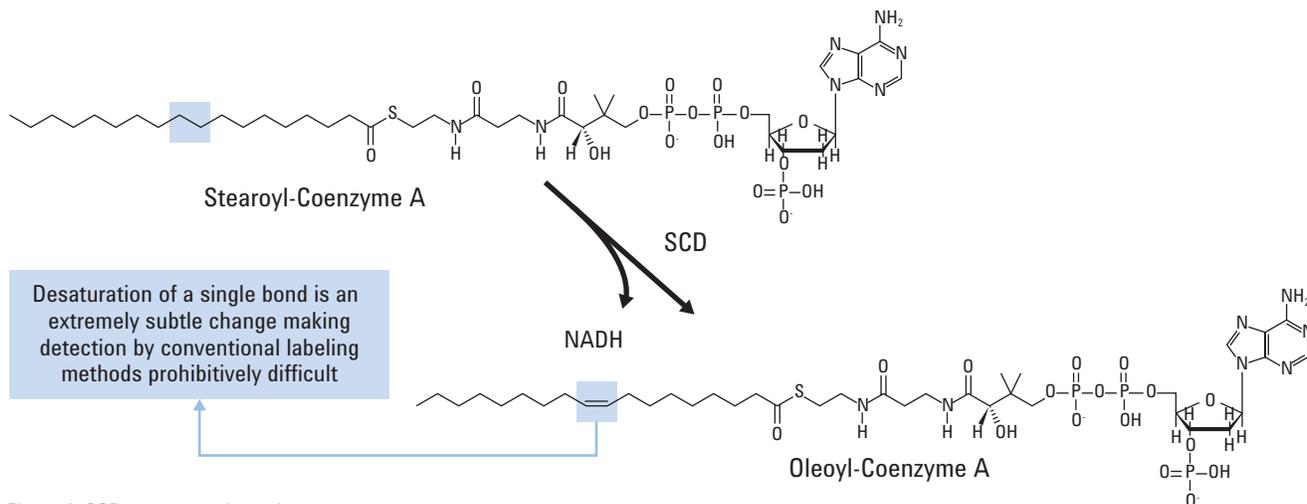


Figure 1. SCD assay reaction scheme.

Mass spectrometry is a highly sensitive method for detecting the small changes in mass and is well suited for detecting the single desaturation that occurs with SCD conversion. Both SCD substrate and product can be directly and accurately measured by mass spectrometry at sub-micromolar concentrations. The RapidFire method employs a solid phase extraction (SPE) sample cleanup step directly coupled to MS detection. Figure 2 shows standard measures of assay quality – linearity with respect to enzyme concentration for the indicated reaction time and initial reaction velocity within the tested range.

Furthermore, the RapidFire system yields highly repeatable results. Figure 3 demonstrates that the assay was reproducible for a set of 518 plates with an average Z' score of 0.597 and a median Z' score of 0.60.

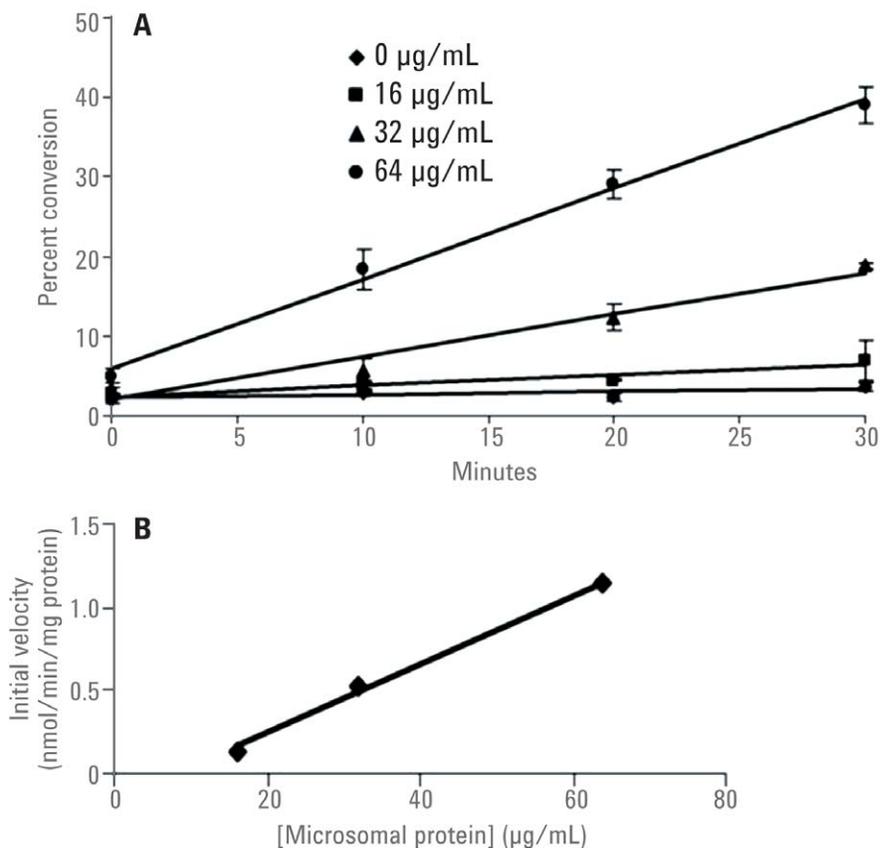


Figure 2. SCD1 assay linearity with respect to time (A) and microsomal protein concentration (B).

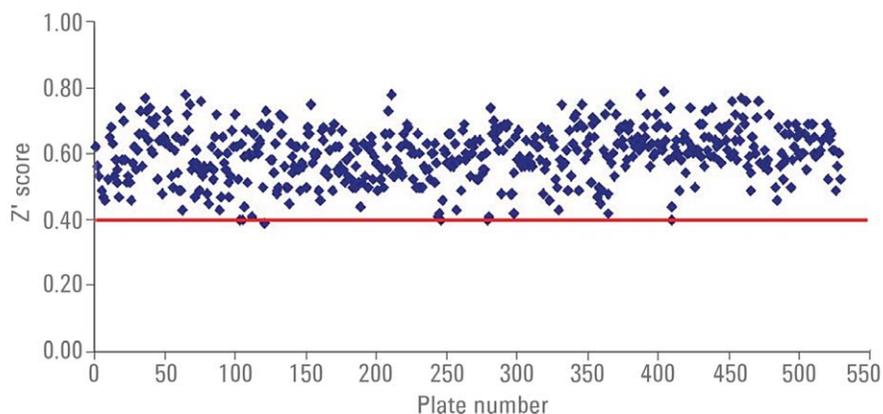


Figure 3. SCD1 assay quality as determined by Z' values for 518 plates (384-well).

The RapidFire screen yielded a number of potent and specific inhibitors, with 346 confirmed as active inhibitors of SCD activity. The RapidFire SCD assay effectively differentiates IC<sub>50</sub> potencies during hit to lead expansion (Figure 4).<sup>2</sup>

The SCD example illustrates that RapidFire/MS delivers a high throughput alternative, with integrated sample preparation and sensitive mass spectrometry detection that streamlines the drug discovery process for even the most difficult assays.

## Conclusions

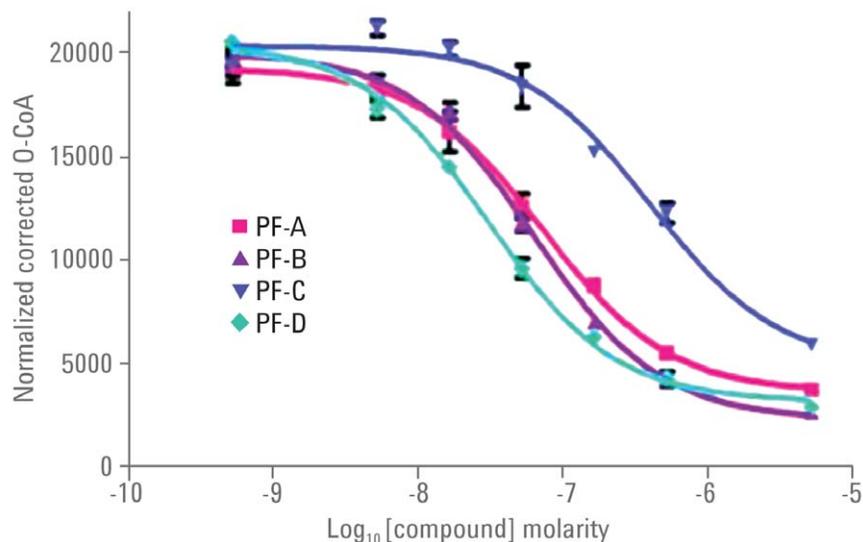
The Agilent RapidFire High-throughput Mass Spectrometry System demonstrated a number of key benefits for the high-throughput screening of stearyl-coenzyme A desaturase, an intractable target traditionally requiring extremely laborious labeling methods. RapidFire provides sample processing speeds of 6-10 seconds, increasing throughput over conventional methods by more than 10-fold.

RapidFire/MS enables sensitive and reliable analysis of challenging drug target classes with label-free, native molecule detection. RapidFire/MS

can be used to efficiently screen chemical libraries with results comparable to optical methods. As a result, incorporation of RapidFire/MS systems into the lead discovery phase of the drug discovery process delivers efficiency and productivity advances unrivaled by other technologies.

## References

1. Soulard, P., *et al.* Development of a high-throughput screening assay for stearyl-CoA desaturase using rat liver microsomes, deuterium labeled stearyl-CoA and mass spectrometry. *Anal Chim Acta.*, **2008**, 627(1):105-11.
2. Schilling, R., *et al.* The use of high-throughput mass spectrometry (HTMS) in drug discovery. *Presented at the SBS 14<sup>th</sup> Annual Conference, 2008*, St. Louis, USA.



	EC <sub>50</sub>
PF-A	7.030e-008
PF-B	6.080e-008
PF-C	3.884e-007
PF-D	2.964e-008

Figure 4. Demonstration of secondary characterization HTS screen for SCD inhibitors.

[www.agilent.com/chem/rapidfire](http://www.agilent.com/chem/rapidfire)

DE.478275463

Information, descriptions, and specifications in this publication are subject to change without notice.

©Agilent Technologies Inc., 2011, 2020  
Published in the USA, September 29, 2020  
5990-9357EN



**Agilent Technologies**