High-Throughput Lead Discovery with Agilent RapidFire/MS Systems: Analysis of Acetyl-Coenzyme A Carboxylase (ACC)

Application Note

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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 allows traditionally low-throughput, intractable assays to be converted into high-throughput assays that can be processed at speeds approaching plate-based optical methods. In this application note, the acetyl-coenzyme A carboxylase (ACC) assay is used to illustrate the power of Agilent RapidFire/MS Systems for screening intractable targets.

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Using RapidFire High-throughput Mass Spectrometry to Analyze ACC Assay Samples

The ACC enzyme catalyzes the conversion of acetyl-coenzyme A (ACoA) to malonyl-coenzyme A (MCoA) as shown in Figure 1. This enzyme plays a critical role in fatty acid synthesis and is an important therapeutic target for a range of metabolic disorders. However, screening this target currently requires the use of a difficult radiometric CO_2 liberation assay. This method, employing ^{14}C labeled potassium carbonate, is too cumbersome, time consuming, and costly to process the large sample sets required for screening programs. The RapidFire/MS system provides a fast, cheap, and efficient alternative to this traditional method.

Mass spectrometry is a highly sensitive method for detecting the small changes in mass that result from reactions such as carboxylation. Both the ACoA substrate and MCoA product can be directly and accurately measured by mass spectrometry at sub-micromolar concentrations.

The added carboxyl group is difficult to detect with traditional labeling methods
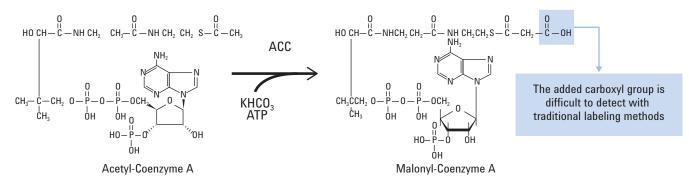
Figure 1. Acetyl-Coenzyme A Carboxylase (ACC) assay reaction scheme.

The RapidFire method employs a solid phase extraction (SPE) sample cleanup step directly coupled to MS detection. Using this method, conditions were identified in which product formation was linear with respect to both ACC enzyme concentration (Figure 2) and time. In addition, standard enzyme kinetics and reaction rates were shown to be dependent on ACoA substrate concentration (Figure 3).
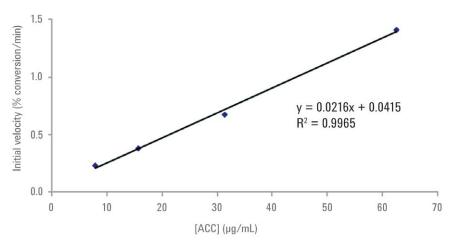

$y = 0.0216x + 0.0415$
$R^2 = 0.9965$

Figure 2. Linear dependence of product formation on ACC enzyme concentration.
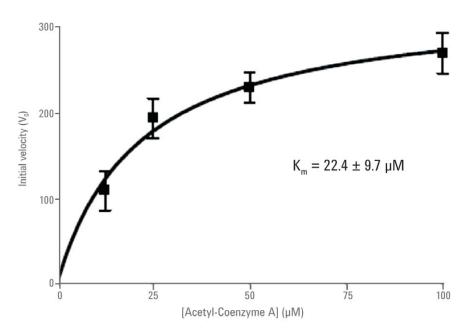

$K_m = 22.4 \pm 9.7 \ \mu M$

Figure 3. Demonstration of standard enzyme kinetics and reaction rate dependence on ACoA substrate concentration.

An *in vitro* system was also used to confirm the inhibition of ACC by long-chain acyl-coenzyme A thioesters: myristoyl-CoA, palmitoyl-CoA, and stearoyl-CoA. IC_{50} values for this experiment are shown in Figure 4. The results for myristoyl-CoA, palmitoyl-CoA, and stearoyl-CoA are in good agreement with existing literature. By eliminating cumbersome labeling methods and streamlining workflow, RapidFire reduces bottlenecks in the drug discovery process.

Conclusions

The Agilent RapidFire High-throughput Mass Spectrometry System demonstrated a number of key benefits for the high-throughput screening of acetyl-coenzyme A carboxylase as an example of an intractable target traditionally requiring laborious labeling methods. Benefits include rapid sample processing speeds, increased throughput and laboratory efficiency, and equivalent inhibition results as compared to conventional screening methods. As a result, incorporation of RapidFire/MS systems into the lead discovery phase of the drug discovery process enables efficiency and productivity advances unrivaled by other technologies.

References

1. M. Jonas, *et al*. Mass Spectrometry in High Throughput Screening: A Case Study on Acetyl-Coenzyme A Carboxylase using RapidFire – Mass Spectrometry (RF-MS). *Combinatorial Chemistry & High Throughput Screening*, **2009**, 12:752-759.
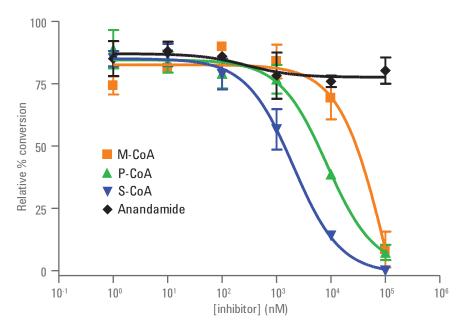
Figure 4. IC_{50} values were determined for ACC inhibitors and a control.

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