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300 *Current Analytical Chemistry*, 2015, *11*, 300-306

A Derivative Method with Free Radical Oxidation to Predict Resveratrol Metabolites by Tandem Mass Spectrometry

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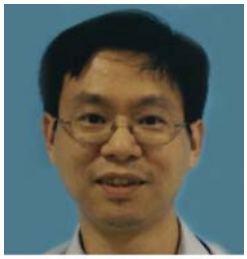

Shih-Shin Liang

Abstract: In this study, we demonstrated an oxidative method with free radical to generate 3,5,4′-trihydroxy-*trans*-stilbene (*trans*-resveratrol) metabolites and detect sequentially by an autosampler coupling with liquid chromatography electrospray ionization tandem mass spectrometer (LC-ESI–MS/MS). In this oxidative method, the free radical initiator, ammonium persulfate (APS), was placed in a sample bottle containing resveratrol to produce oxidative derivatives, and the reaction progress was tracked by autosampler sequencing. Resveratrol, a natural product with purported cancer preventative qualities, produces metabolites including dihydroresveratrol, 3,4′-dihydroxy-*trans*-stilbene, lunularin, resveratrol monosulfate, and dihydroresveratrol monosulfate by free radical oxidation. Using APS free radical, the concentrations of resveratrol derivatives differ as a function of time. Besides simple, convenient and time- and labor saving, the advantages of free radical oxidative method of its *in situ* generation of oxidative derivatives followed by LC-ESI–MS/MS can be utilized to evaluate different metabolites in various conditions.

Keywords: Free radical, metabolite, resveratrol, ammonium persulfate (APS), liquid chromatography tandem mass spectrometry (LC–MS/MS), multiple reaction monitoring (MRM).

1. INTRODUCTION

In the last decades, metabolomics has developed at an amazing rate in the –omics field. At an early stage of development in the research of metabolic derivatives, biological generation method can be used; specifically, human and rat liver microsomes (HLMs & RLMs) were processed to investigate metabolites with specific cytochrome P450 (CYP450) activity [1-4]. However, metabolites studied by HLM and RLM methods are expensive, time consuming, and labor intensive. Besides, under different conditions and extraction times, the results of HLM or RLM treatments will display different profiles in HLM and RLM metabolites [1].

In addition, electrochemical methods for producing metabolites, such as cyclic voltammetry (CV) use various buffer solutions, probes, and voltage values to generate oxidative and reductive derivatives. For example, metabolic or oxidative products of uric acid were detected by C-60-modified glassy carbon electrodes [5], and multi-walled carbon-nanotube-modified carbon-ceramic electrodes [6]. In another studies, electrochemical oxidation of adenosine and guanosine-5′-triphosphate was investigated by glassy carbon and pyrolytic graphite electrodes [7, 8]. Additionally, DNA and DNA-related biological researches including DNA damage were demonstrated [9-11]. However, CV is an off-line technique and is incompatible with tandem MS. Furthermore, researchers have to contend with electrode aging, probes' activity and stability loss over time. The results of CV technique cannot directly show compound antioxidant capacities [12]. Consequently, there was a novel method which was integrated Fenton reaction to generate free radical and CV to demonstrate mimic drug metabolites in phase I period [13, 14].

Nevertheless, for studying well-known metabolites, it is convenient to utilize the detection mode multiple reaction monitoring (MRM) by tandem mass spectrometry. In the previous studies, electrochemical cells (EC) coupled with electrospray ionization–tandem mass spectrometer (EC-ESI–MS/MS) can be used as powerful online instruments for oxidative derivative detection and prediction of metabolites [15-19]. In recent years, the application of EC-ESI–MS/MS systems has been extended to include separation apparatus such as liquid chromatograph (LC) to generate EC/LC-ESI–MS/MS systems [13, 14]. Using this type of instrument, tetrazepam metabolism has been investigated by comparing *in vivo* and *in vitro* methods [18, 19]. Similar EC/LC–

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