High-resolution Assays Combined with HPLC-HRMS-SPE-ttNMR for Identification of Antidiabetic Compounds in root of Scutellaria Baicalensis

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**Abstract**
This work describes an analytical platform based on high-resolution radical scavenging and high-resolution α-glucosidase inhibition assays in combination with hyphenation of high-performance liquid chromatography, high-resolution mass spectrometry, solid-phase extraction, and tube-transfer nuclear magnetic resonance spectroscopy, i.e., HPLC-HRMS-SPE-ttNMR/high-resolution radical scavenging and high-resolution α-glucosidase assays. The platform enables fast screening of complex matrices for individual analytes with α-glucosidase inhibitory and radical scavenging activity, followed by structural identification targeted the active analytes only.

**Introduction**
Type 2 diabetes is one of the most prevalent diseases affecting 246 million worldwide and its incidence and serious complications continue to grow rapidly. Patients with type 2 diabetes suffer from a series of micro- and macrovascular complications such as visual impairment, blindness, neuropath, kidney failure and cardiovascular diseases. *Radix Scutellaria* is the dried root of the medicinal plant *Scutellaria baicalensis*. *Radix Scutellaria* is officially listed in the Chinese Pharmacopeia and Japanese Pharmacopoeia, and exhibits a variety of therapeutic effects and has long history of application in traditional formulation and in modern herbal medication – and is also used as a food additive.

**Method**
**Figure 1.** Schematic representation of HPLC-HRMS-SPE-ttNMR analysis of *Radix Scutellaria* extract guided by high-resolution ABTS⁺⁻ reduction and α-glucosidase inhibition profiles. **Path 1:** microfractionation into six 96-well microplates followed by ABTS⁺⁻ reduction assay of each microfraction to produce high-resolution ABTS⁺⁻ reduction profile. **Path 2:** microfractionation into six 96-well microplates followed by α-glucosidase inhibition assay to produce high-resolution α-glucosidase inhibition profile. **Path 3:** HPLC-HRMS-SPE-ttNMR analysis targeting antioxidants and α-glucosidase inhibitory metabolites identified in the preceding procedures.

**Results**
**Figure 2.** High-resolution α-glucosidase inhibition and radical scavenging profiles of *Radix Scutellaria* with overlaid HPLC chromatogram at 280 nm. Peaks are numbered sequentially with increasing elution order. The radical scavenging and α-glucosidase inhibition profiles provide good resolution that allows disclosure of the individual metabolites responsible for the observed activities, that is, correlating peaks in the biochromatogram with individual peaks in the overlaid HPLC chromatograms.

**Concluding Remarks**
This work describes the development of a bioassay-coupled HPLC-HRMS-SPE-ttNMR platform for identification of radical scavengers and α-glucosidase inhibitors in an extract of *Radix Scutellaria*. The biochromatogram can be used to pinpoint HPLC-analytes with bioactivity.

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