

# AccuTOF™

## Analysis of Tea by Using HPLC /Time-of-Flight Mass Spectrometry

### Introduction

The AccuTOF™ high-resolution time-of-flight mass spectrometer provides powerful qualitative and quantitative tools that can be applied to HPLC/MS analysis of polyphenols and other compounds in tea.

The combination of high-pressure liquid chromatography (HPLC) with high-resolution time-of-flight mass spectrometry (TOFMS) provides powerful capabilities for chemical analysis. HPLC with UV detection relies on wavelength and retention time for peak identification. Here we show how the AccuTOF™ TOFMS with its unique high dynamic range detection system can be used to determine polyphenols (such as catechins), caffeine and related compounds, amino acids, and vitamins in different teas.

### Experimental Conditions

Separations were performed by using an *Agilent* 1100 HPLC system equipped with binary pump and solvent degasser, autosampler, column compartment and diode array detector. The output of the diode array detector was introduced into the APCI source of the JEOL AccuTOF™ time-of-flight LC/MS system, operated at a resolving power of 6,000 (FWHM) in positive-ion mode with an orifice 1 potential of 28 V.

Tea samples included a high-quality green tea, a common brand of black tea, and powdered teas. The HPLC separation made use of a *Phenomenex* 2mm x 150mm LUNA 5μ C18(2) column and guard column<sup>2</sup> operated at 35 deg. C with a solvent flow rate of 0.25 ml/min. Solvent A was 0.1% TFA in water, and solvent B was acetonitrile. The solvent composition was varied from 0% B to 30% B in 30 minutes and held at 30% B until 47 minutes. The autosampler was programmed for 10 μl injections of the tea samples.

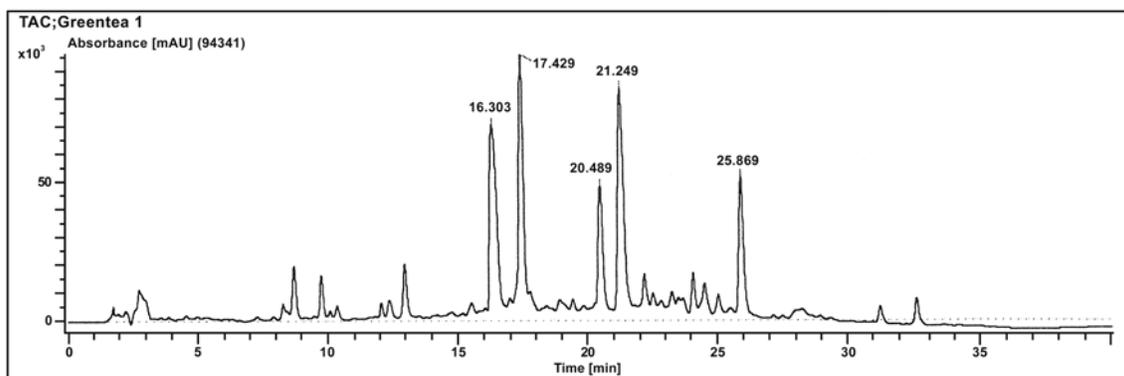
### Results and Conclusions

Suspected compounds were identified by mass chromatograms and confirmed by their exact masses. Elemental composition calculations from exact mass measurements (typically within 5 ppm) were used to make assignments for unknown compounds.

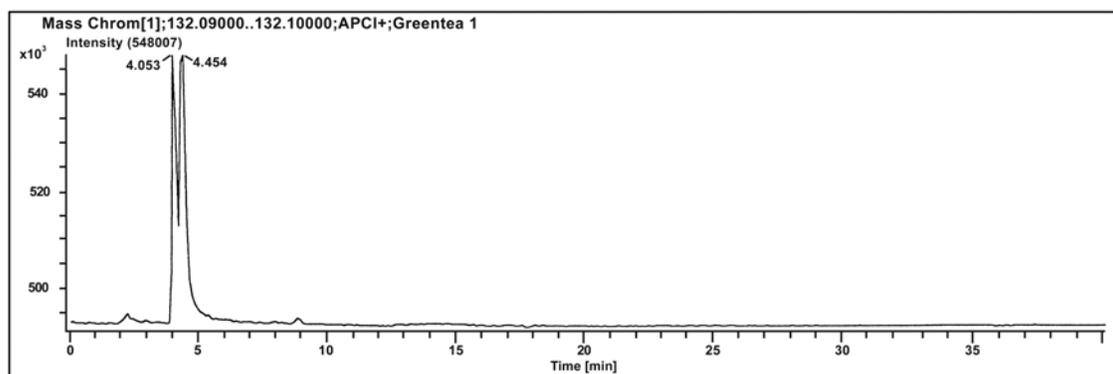
Identified compounds are shown in Table I. All of the compounds detected were found at varying levels in each of the tea samples, with caffeine being the most abundant compound in all samples. The highest levels of polyphenols were found in green tea. Significantly lower levels of polyphenols were found in black tea, and trace levels were detected in the powdered tea.

**Table I. Compounds identified in tea samples**

Caffeine	Proline	Catechin
Theobromine	Threonine	Rutin
Theophylline	Tryptophan	EGCg
Alanine	Tyrosine	ECg
Leucine	Valine	EC
Isoleucine	Histidine	EGC
Valine	Glutamic acid	Vitamin B3
Phenylalanine	Serine	



**Figure 1. DAD UV chromatogram of green tea**



**Figure 2. Mass chromatogram of leucine/isoleucine**