



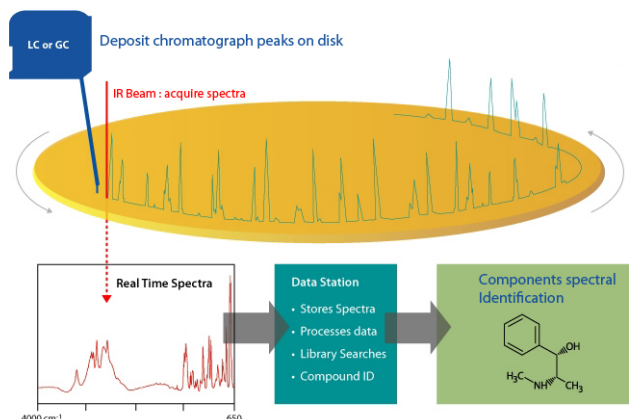
DiscovIR-LCTM

Deposition and Detection System

Application Note 026
May 2008

READING TEA LEAVES

The DiscovIR-LC is a powerful new tool for materials analysis. When connected to the outlet of an LC column, the DiscovIR deposits LC eluants as a continuous track on an infrared transparent substrate. The built-in interferometer simultaneously captures a set of time-ordered infrared spectra from the deposited track. The result is a map of molecular structure of all sample components.



SUMMARY

Natural food/beverage products contain a complex array of substances. Frequently these materials are quite similar species or isomers. Described here is a fast technique for isolation and identification of individual species based on the power of vibrational spectroscopy to characterize materials based on their unique structure.

INTRODUCTION

There is a strong interest in food and beverage products that contain health promoting ingredients. Products such as red wine, chocolate, and tea contain various natural substances correlated with beneficial health effects. Polyphenols, collectively known as flavonoids, are ubiquitous in the plant kingdom, where they function as a defense against invading pathogens. Their antioxidant properties are reported to have beneficial effects against cancers, heart disease, cerebral damage, suppression of inflammatory response, viral inhibition, and allergenic afflictions. Food producers and consumers alike are increasingly interested in these substances, and their potential in the prevention of cancer and heart disease. Inasmuch as many flavonoids are natural protectants, the pharmaceutical industry is exploring their therapeutic applications.



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Tea contains significant quantities and variety of these substances. Catechins, native to tea leaves, form the base compound for the formation of various polyphenols during the enzymatic oxidation (“fermentation” in the parlance of tea manufacture) of tea leaves.

This application note describes the analysis and identification of flavonoids and alkaloids contained in tea. A simple extract of tea was prepared and injected onto a reverse-phase column. Column eluate was directed to the DiscovIR-LC, where it was deposited as a track on the sample collection disk and scanned with the built-in FTIR interferometer. The time-ordered spectral data set was observed, and eluted materials directly identified. Several identified extract components are described here.

EXPERIMENTAL

Sample preparation

One gram of green tea was placed in 10 ml of distilled water and heated for five minutes at ca. 85°C. The extract was allowed to cool to room temperature and then filtered (first through a Whatman 41 filter paper, then through a 0.45 µm membrane filter) prior to HPLC analysis.

LC-IR analysis

Chromatography

Tea extract components were separated on a reverse-phase column set, operating in a gradient mode. A 30 µl aliquot of extract was injected for analysis.

Columns: The column set employed was an Upchurch C18 guard column plus an Eclipse C18 column, 150mm X 4.6 mm.

Mobile Phase: 1 ml/minute water/methanol. Gradient used was 20% -100% organic over a 20 minute linear gradient.

FY-IR spectrometry

Eluant from the chromatography separation passed through a UV detector and then deposited as a solid phase track on the Zn-selenide collection disk of the DiscovIR. Operating conditions for the DiscovIR were as follows:

Nebulizer power	14W
Gas flow	350 cc/min
Disk speed	3 mm/min
Disk temperature	0°C
Condenser Temperature	4°C
Chamber/Condenser pressure	4 torr
Cyclone temperature	220 – 210 °C



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RESULTS

The infrared chromatogram (IR Peaks Chromatogram) in Figure 1 shows a number of elution peaks representing discrete extract components. The alkaloids, theobromine and caffeine have very similar structures, and consequently share some principal infrared bands. A chromatogram based on the 1695 cm⁻¹ band displays only two components.

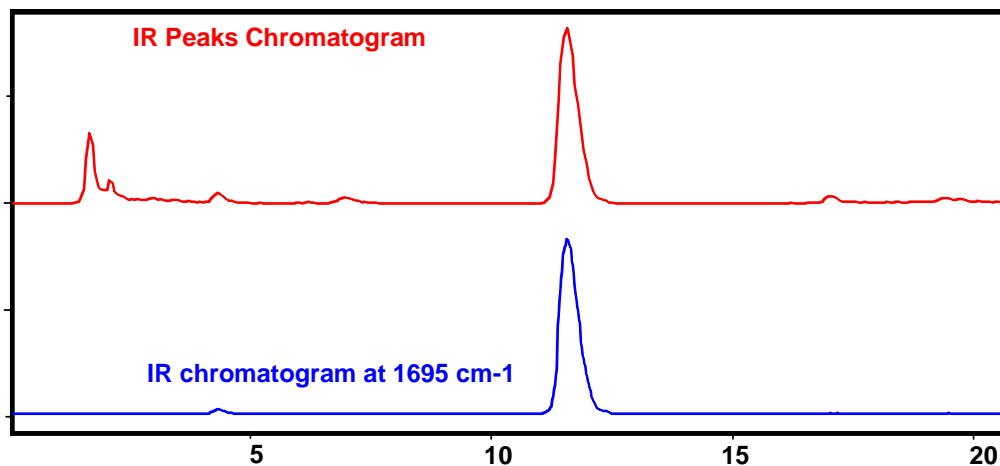


Figure 1 Infrared chromatograms of tea extract

Their spectra reveal them to be theobromine and caffeine respectively, as determined from a standard library search.

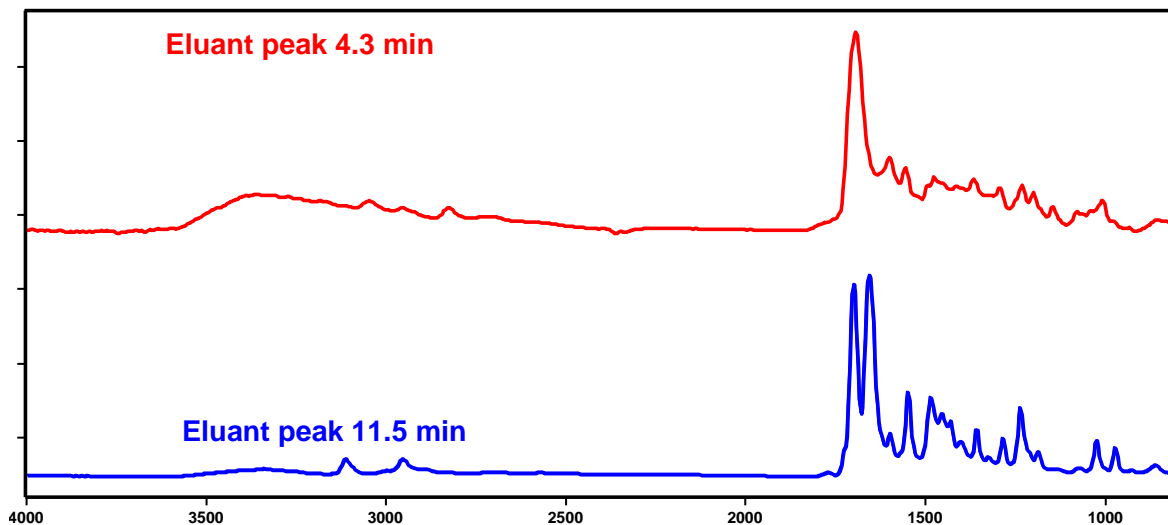
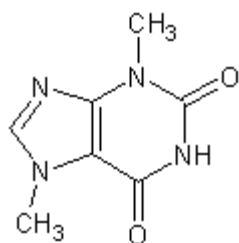


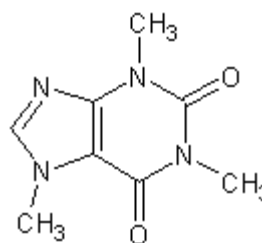
Figure 2 Spectra of chromatography peaks at 4.3 and 11.5 minutes



Figure 2 displays the spectra from the two peaks. When spectra were compared in a spectral library, they were readily identified as theobromine and caffeine. These two substances differ only in a methyl group on the urea nitrogen, present in caffeine but absent in theobromine.



Theobromine



Caffeine

The peaks eluting at 6.9 minutes and 17 minutes are the isomers catechin and epicatechin.

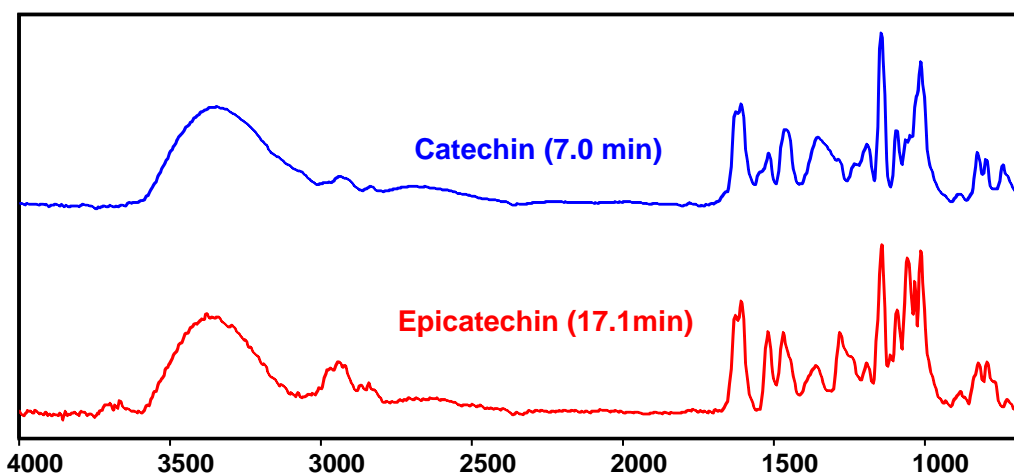
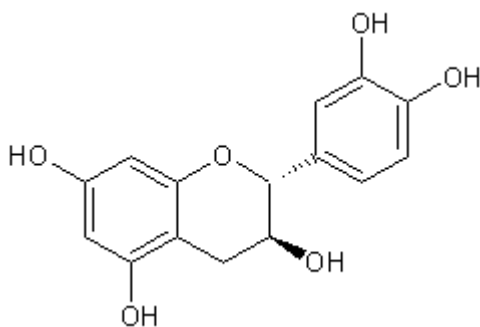
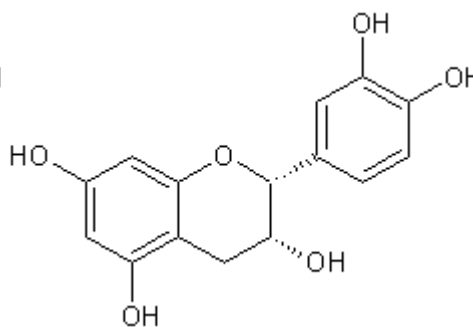


Figure 3 Catechin and Epicatechin peaks



(+)-Catechin



(-)-Epicatechin



Observe the structure diagram of these two diastereomers. Molecular weight and configuration are identical for the two molecules Catechin and Epicatechin. Their structures differ only in the orientation of the hydroxyl group on the aliphatic ring. Nonetheless, these two molecules are readily differentiated and identified by vibrational spectroscopy through comparison to a spectral library. Note the differences in the 1040 cm⁻¹ region that differentiates these two isomers. The different configuration of the hydroxyl group causes the sympathetic vibration of the other atoms within the molecule to alter vibrational intensities and resonant frequencies.

“The IR absorption spectrum of a compound is probably its most unique physical property. Except for optical isomers, no two compounds having different structures have the same IR spectrum. In most instances the IR spectrum is a unique molecular fingerprint that is easily distinguished from the absorption patterns of other molecules.”¹

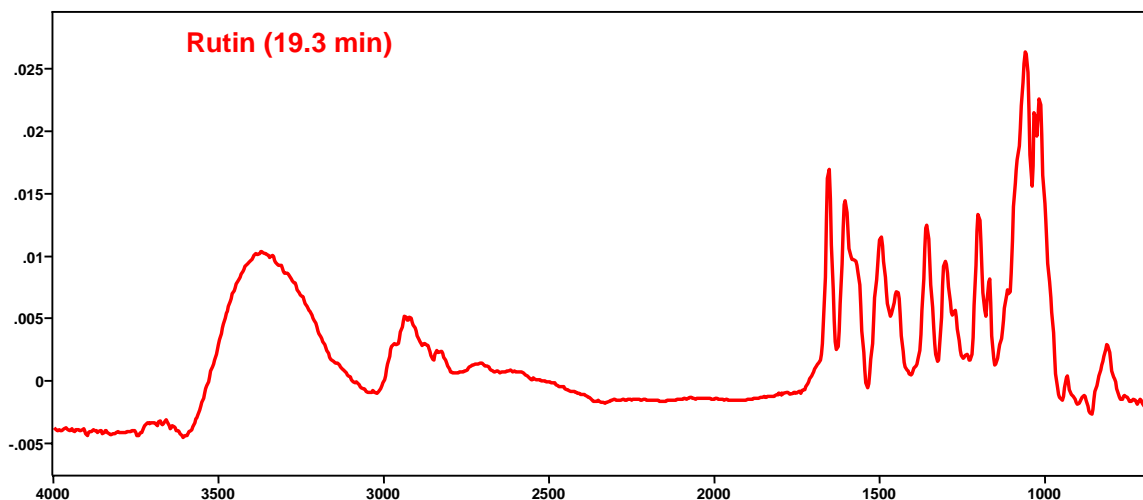


Figure 4 Rutin

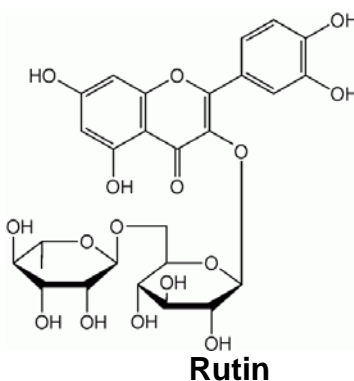


Figure 4 is the spectrum of the final elution peak of the separation. It is identified as Rutin. Rutin is found in many fruits, vegetables, and other plants. It is an antioxidant, and is a factor in inhibition of some cancers. Rutin strengthens the blood capillaries, and can reduce the symptoms of haemophilia. Rutin, in the form of ferulic acid, reduces the cytotoxicity of oxidized LDL cholesterol and lowers the risk of heart disease.

CONCLUSION

Natural food products contain a host of substances, many of which have highly similar chemical structures. These materials have been shown to provide various beneficial health effects. Chromatography combined with vibrational spectroscopy of separated components is a fast, discriminating way to obtain information regarding these components.

¹ Smith, A.L. Appl. Spectrosc. 41 (1987) 1101

