



Reversed phase LC-IR collected in real time with physical solvent removal

LC-IR instrumentation has been under development for many years. Published attempts to build a viable interface to remove the LC solvent from the LC eluents and produce quality IR spectra date from the late 1970's. A robust general purpose LC-IR interface has been elusive, and although a number of techniques have shown promise, commercial instruments available to date have been quite limited in their applicability.

The new **DiscovIR-LC™** system from Spectra is able to demonstrate total solvent removal from a reverse-phase gradient run (2% to 100% organic) with no change in the operational setpoints of the system.

Figures 1-5 in this application note show the chromatogram and FTIR spectra of the eluants.

Two spectra are compared with those from traditional solid phase FTIR libraries.

Fifty microliters of a 0.5 mg/ml solution of a four component mixture was injected onto a C18 Nova-Pak 3.9mm ID x75mm column. A 10 minute gradient from 98% H₂O/CH₃OH → 100% CH₃OH at a flow rate of 1ml/minute was used to elute the compounds. Solid phase Transmission FTIR Spectra are acquired continuously throughout the run.

Figure 6 shows the spectra of GHB, a heat sensitive compound, analyzed by flow injection on the **DiscovIR-LC™** FTIR system.

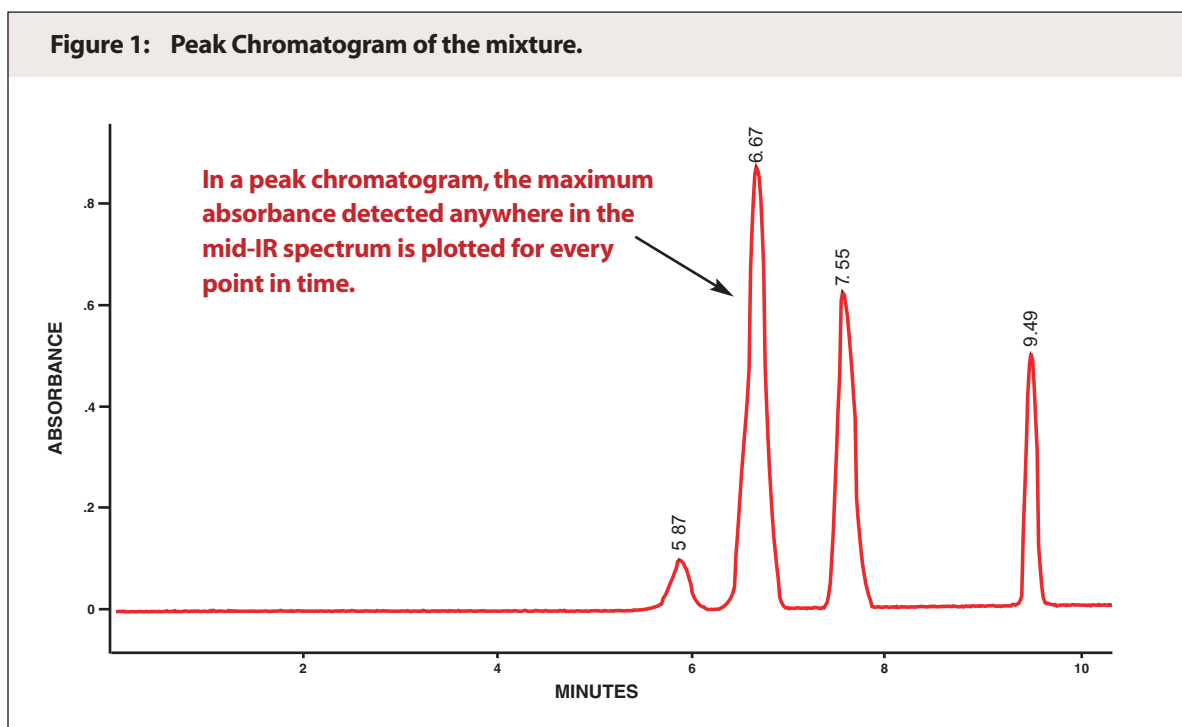




Figure 2: FTIR Spectra of Component 1: Theobromine

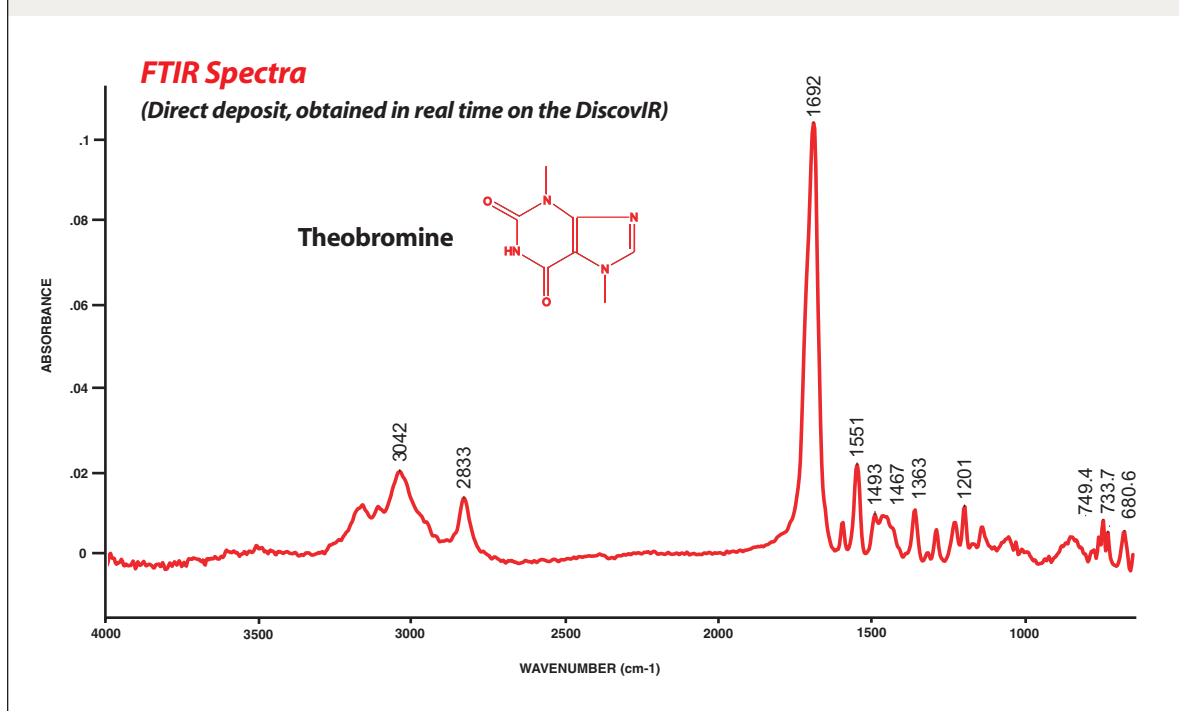


Figure 3: FTIR Spectra of Component 4: Vanillin Acetate

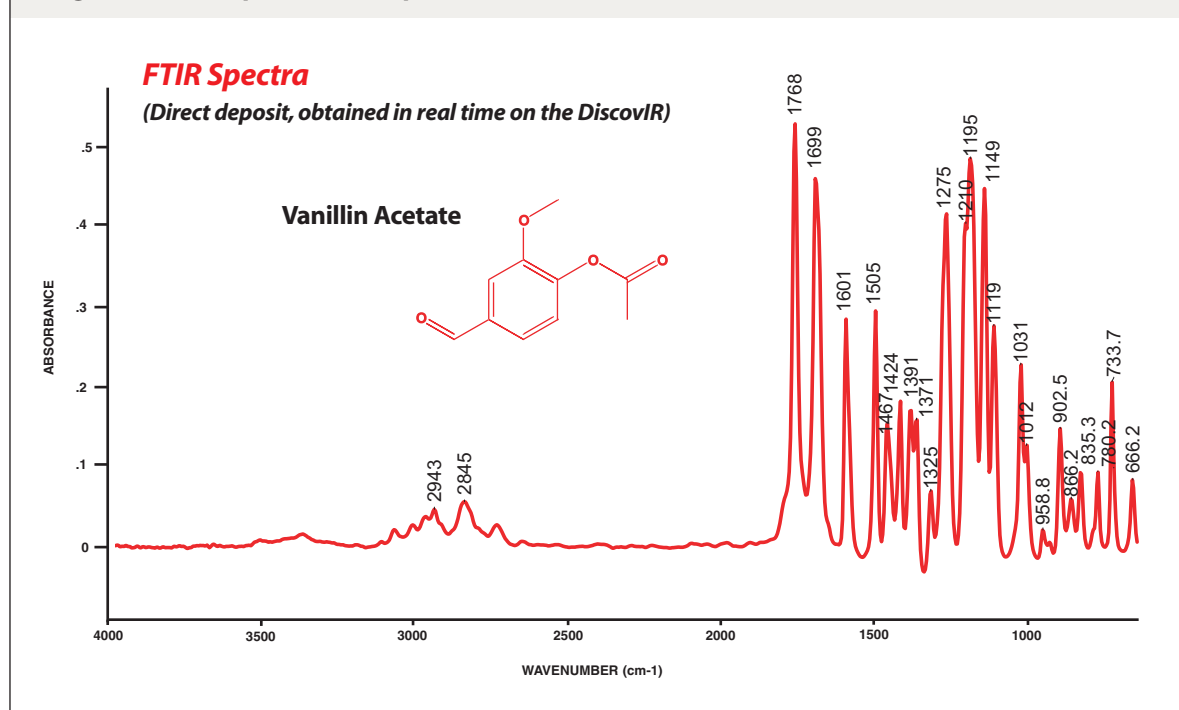
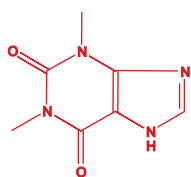




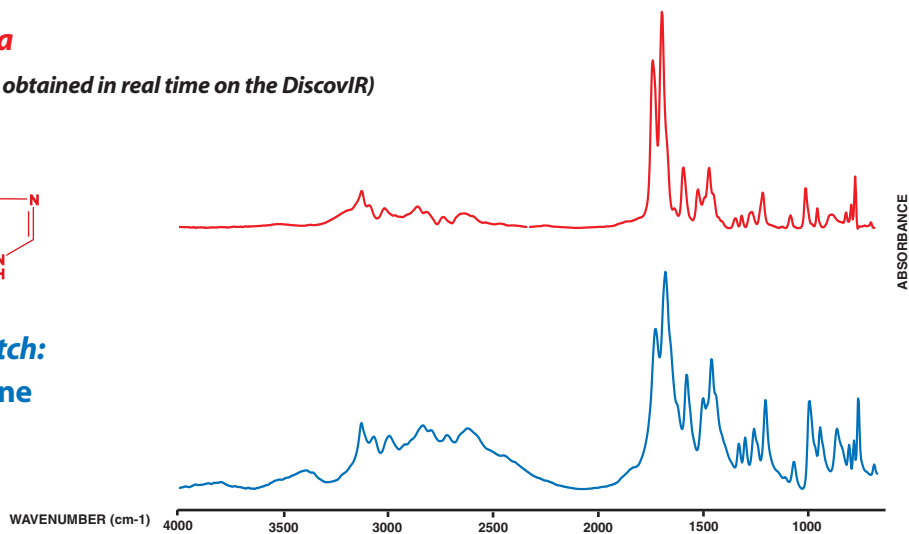
Figure 4: FTIR Spectra and Library Match for Component 2 (RT 6.67 min.)

FTIR Spectra

(Direct deposit, obtained in real time on the DiscoverIR)



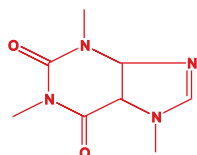
**Library Match:
Theophylline**



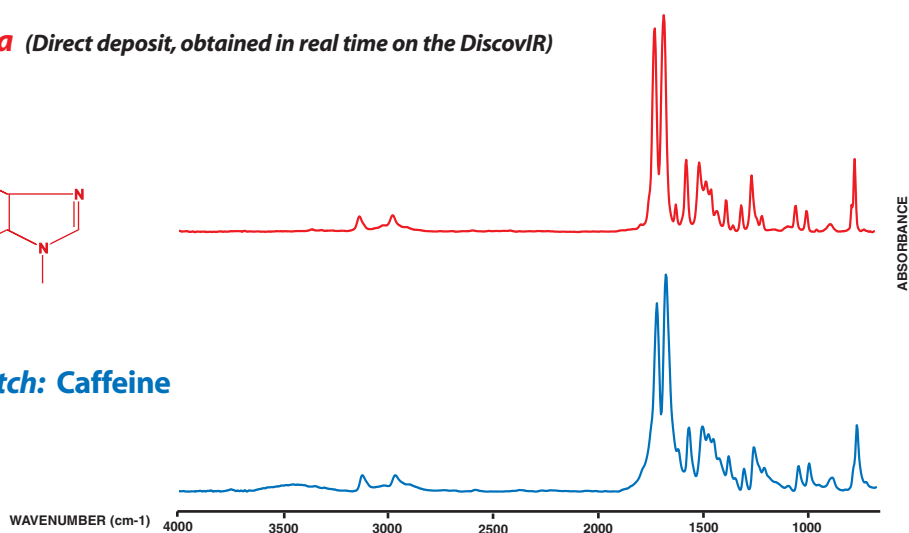
Hit	Quality	Library	Memo
01	0.456942	fdm_comprehensive_organics_05.lib	Theophylline

Figure 5: FTIR Spectra and Library Match for Component 3 (RT 7.55 min.)

FTIR Spectra (Direct deposit, obtained in real time on the DiscoverIR)



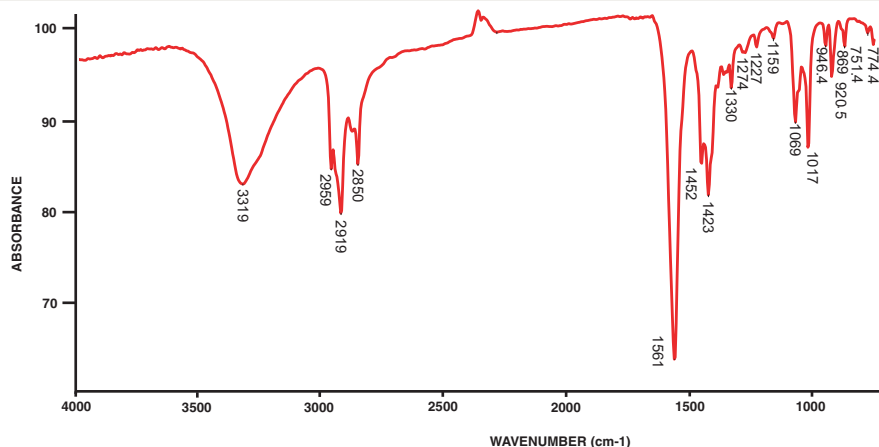
Library Match: Caffeine



Hit	Quality	Library	Memo
01	0.083686	spectra_analysis.lib	Caffeine



Figure 6: FTIR Spectra of Gamma Hydroxy Butyric Acid (GHB)



Heat sensitive compound:
The sodium salt of Gamma Hydroxy Butyric Acid (GHB) is thermally unstable and readily cyclizes to the lactone in a GC. The spectra obtained by flow injection on the DiscovIR-LC system clearly shows the uncyclized form. Note the Carboxylic group at 1561 and the OH group at 3319.

RESEARCH AND DEVELOPMENT

The spectra of the sample is independent of the solvent's FTIR spectra. There are no spectral restrictions on the solvents used as eluents. The solvents are physically removed from the sample, eliminating interference from the absorption bands of the solvent. Volatile buffers are removed with the solvent. Nonvolatile infrared transparent buffers may be deposited on the disk with the sample.

PROCESS DEVELOPMENT:

Compatible with your existing methods:

Connect the DiscovIR System to your existing chromatograph and auto sampler. Standard LC solvents, columns, injection volumes and concentrations may be used with the DiscovIR system. The solvent removal and sample analysis is completed on line during the chromatographic run.

DiscovIR-LC System Overview

Operating Principle	Desolvation followed by continuous FTIR scans of solid components on a rotating ZnSe sample disk
Detection Method	Built-in FTIR
IR Range	4000–650 cm^{-1}
Resolution	4 or 8 cm^{-1}
Data Collection	Real-time, with post-run rescan
Spectrum Type	Transmittance through disc and solid sample
Disc Capacity	50 hours of chromatography
Disc Temperature Control	-100°C to +50°C

Data Station

Platform	Dell Desktop computer w/ Windows™ XP
Standard Features	<ul style="list-style-type: none"> Real-time or post-run data collect Chromatographic & spectral data processing 30 customizable Infrared Band Chromatograms for chemical classes Alignment and tuning tools

DiscovIR-LC Configuration

LC Flow Rates Accepted	0.2 to 2.0 ml/min
Compatible Solvents	THF, TCB, ODCB Reversed phase solvents Normal phase solvents