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Standard Practice for General Techniques of Liquid Chromatography-Infrared (LC/IR) and Size Exclusion Chromatography-Infrared (SEC/IR) Analyses[1]

This standard is issued under the fixed designation E2106; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers techniques that are of general use in qualitatively analyzing multicomponent samples by using a combination of liquid chromatography (LC) or size exclusion chromatography (SEC) with infrared (IR) spectrometric techniques. The sample mixture is separated into fractions by the chromatographic separation. These fractions are subsequently analyzed by an IR spectroscopic method.

1.2 Three different types of LC/IR techniques have been used to analyze samples (**1**,**2**).[2] These consist of eluent trapping (see Practices E334), flowcell and direct deposition. These are presented in the order that they were first used.

1.3 The values stated in SI units are to be regarded as standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E131 Terminology Relating to Molecular Spectroscopy

E168 Practices for General Techniques of Infrared Quantitative Analysis

E334 Practice for General Techniques of Infrared Microanalysis

E1421 Practice for Describing and Measuring Performance of Fourier Transform Mid-Infrared (FT-MIR) Spectrometers: Level Zero and Level One Tests

3. Terminology

3.1 *Definitions*—For definitions of terms and symbols, refer to Terminology E131.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *hit quality index (HQI), n*—the comparison of infrared spectroscopic data against a database of reference spectra of known compounds is often employed to assist in the determination of the evolved gas chemical identity. Search algorithms generate a listing of reference compounds from the database that are spectroscopically similar to the evolved gas spectrum. These reference compounds are ranked with regard to a measurement of the comparative fit of the reference spectral data to that of the spectrum of the evolved gas. This ranking is referred to as the hit quality index (HQI).

4. Significance and Use

4.1 This practice provides general guidelines for the practice of liquid chromatography or size exclusion chromatography coupled with infrared spectrometric detection and analysis (LC/IR, SEC/IR). This practice assumes that the chromatography involved is adequate to resolve a sample into discrete fractions. It is not the intention of this practice to instruct the user on how to perform liquid or size exclusion chromatography (LC or SEC).

5. General LC/IR Techniques

5.1 Three different LC/IR techniques have been used to analyze samples. These consist of eluent trapping, flowcell and direct deposition. These are presented in the order that they were first developed. Infrared detection for any of these techniques can be provided by IR monochromators, IR filter spectrometers and Fourier transform infrared spectrometers (FT-IR). These detectors yield either single absorption band or total infrared spectrum detection modes. Detection mode is dependent upon the type of IR detector employed and the acquisition time required by the LC or SEC experiment.

5.2 *Eluent Trapping Techniques*—Eluent trapping techniques, such as stopped flow and fraction collection, are the simple means for obtaining LC/IR data. In these techniques, the eluting sample is collected from the chromatograph in discrete aliquots. These aliquots are then analyzed with the

[1] This practice is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.03 on Infrared Spectroscopy.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

appropriate sampling accessory in an infrared spectrometer. In utilizing such techniques, it is essential that a suitable LC detector, such as refractive index or UV/VIS, be employed to allow definition of component elution. Since the analyte of interest is trapped physically, the spectrum can be recorded using a long integration or scan coaddition time to improve the signal-to-noise ratio (SNR). Generally, the stopped flow technique requires the use of a flow cell and the IR spectrum acquired contains both analyte and mobile phase spectral features. The fraction collection mode permits examination of the eluent as a solution of analyte and mobile phase or, with proper solvent removal, the analyte alone (provided that the analyte is nonvolatile). As such, the fraction collection mode would require either a liquid cell for solutions or a solid substrate, that is, KBr window for transmission, first surface mirror for reflection-absorption or powdered KBr for diffuse reflection measurements.

5.3 *Flowcell Detection*—With flowcell detection, the LC eluent is monitored continuously in the timeframe of the chromatography (real-time) by the IR spectrometer with the use of specially designed liquid cells (**3-9**). Liquid cells are designed to minimize dead volume and analyte mixing, to conserve chromatographic resolution, and achieve maximum optical interaction of the eluent with the infrared radiation. As the effluent is a condensed phase, several cell types have been devised to accommodate most experimental approaches for IR spectrometry, that is, transmission, reflection-absorption and attenuated total reflection (**7**). The flowcell technique typically yields submicrogram detection limits for most analytes (**1**). Typically, flowcells are mounted within the sample compartment of the spectrometer and use beam condensation optics to direct the IR beam into and out of the small volume of the cell. It is important to employ a mobile phase having low or preferably no infrared absorptions in the analytically important spectral regions for the analytes of interest. As such, the choice of mobile phase may constrain the liquid chromatographic separation. Generally, this limits the chromatographic separation to a normal phase type where nonpolar solvents like chloroform and carbon tetrachloride have sufficient solvent strength to elute components and have low infrared absorption. In contrast, flowcell detection of reversed phase separations involving aqueous mobile phases are essentially precluded as strong absorption by water occurs across the mid-infrared spectrum. If flowcell detection of reversed phase separation is to be attenuated, removal of the analytes from the aqueous mobile phase via extraction into an infrared transmissive solvent is suggested (**9**).

5.3.1 The rapidity with which spectra must be recorded during a liquid chromatographic separation typically requires a Fourier-transform infrared (FT-IR) spectrometer to capture the complete infrared spectrum. Such instruments include a computer that is capable of storing the large amount of spectroscopic data generated for subsequent evaluation. Conversely, monochromators and filter infrared spectrometers permit the monitoring of a selected absorbance band, for example, 1730 cm^{-1} for carbonyl functional groups. Data acquisition for these devices is similar to that for a typical LC detector.

5.3.2 The transfer line from the LC column to the flowcell must be made of inert, nonporous material. This normally is PTFE, PEEK or stainless steel tubing. The volume, internal diameter, and connections of the transfer line are optimized to reduce dead volume and mixing that can degrade the chromatographic separation. When performing separations at elevated temperatures, the transfer line and flowcell may require controlled heating to maintain temperatures of the eluent.

5.3.3 The flowcell is made of IR transmissive window materials to give maximum optical throughput to and from the effluent chamber. Proper selection of window material is necessary to ensure chemical inertness and IR transmissivity. The cell design and volume must maintain chromatographic resolution while maximizing optical interaction with the eluent via transmission, reflection-absorption or attentuated total reflection modes. Flowcells are typically optimized so that the sampling volume accommodates the corresponding eluent volume of a sharp chromatographic peak at the peak's full width at half height (FWHH). Typically, this volume is matched to the scale of the liquid chromatography, that is, 10 µL for analytical scale and larger volume separations and less than 10 µL for microbore separations.

5.3.3.1 The optimum infrared transmission across the full mid-infrared spectrum is obtained by using potassium bromide windows; however, this material is susceptible to damage by water and cold flows under mechanical force. As the flowcell is used, small amounts of water will etch the window surfaces, and the optical throughput of the windows will drop. Eventually, these windows will have to be changed. Users who expect to analyze mixtures containing water should consider using windows made of a water-resistant material such as zinc selenide (ZnSe). IR windows of high refractive index like ZnSe and zinc sulfide (ZnS) will result in a noticeable drop in infrared transmission due to the optical properties, that is, reflectivity, of such materials. Additionally, high refractive index materials may cause fringing, that is, create an optical interference pattern in the baseline of the IR spectrum.

NOTE 1—Fringing is due to multiple reflection optical paths created when windows are placed as parallel plates separated by a discrete pathlength. These reflection optical paths permit light, which is retarded to a greater extent than light from the transmitted optical path, to reach the detector. This reflection optical path light is out of phase with the transmitted optical path light and yields interferences fringes in the resultant spectrum. Fringing may be reduced by making the windows nonparallel or by placing the cell slightly askew, that is, 5–15°, in the optical beam of the spectrometer. Please refer to Practices E168 for additional information on fringing effects.

5.3.3.2 The optical energy throughput of the flowcell should be periodically monitored, since this is a good indicator of the overall condition of the LC/IR interface. If a Fourier transform spectrometer is used, it is recommended that records be kept of the interferogram signal strength, single-beam energy response, and the ratio of two successive single-beam curves (as appropriate to the instrument used). For more information on such tests, refer to Practice E1421. These tests will also reveal when a mercury cadmium telluride (MCT) detector is performing poorly due to loss of the Dewar vacuum and consequent buildup of ice on the detector face. As noted further in this text,

an MCT detector is commonly used with these experiments as they provide greater detectivity and fast data acquisition times.

5.3.3.3 Care must be taken to stabilize or, preferably, remove interfering spectral features resulting from atmospheric absorptions in the optical beam path of the spectrometer. Best results will be obtained by purging the complete optical path with dry nitrogen gas. Alternatively, dry air can be used for the purge gas, but has interferences in the regions of carbon dioxide IR absorption (2500 to 2200 cm^{-1} and 668 cm^{-1}). Commercially-available air scrubbers that remove water vapor and carbon dioxide also provide adequate purging of the spectrometer. In some instruments, the beam path is sealed in the presence of a desiccant, but interferences from both carbon dioxide and water vapor (1900 to 1400 cm^{-1}) may still be found. In all cases, the instrument atmosphere must be stabilized before data collection commences. Atmospheric stability inside the instrument can be judged by recording the single-beam energy response and the ratio of two successive single-beam spectra.

5.4 *Direct Deposition LC/IR*—Initial attempts at direct deposition LC/IR employed eluent deposition onto powdered KC1 (**10**). After evaporation of the mobile phase, the analysis of analytes was conducted by diffuse reflection. More recently, the direct deposition LC/IR technique is accomplished by deposition of the eluent onto a flat, moving surface to allow analysis by transmission or reflection-absorption (**11**,**12**). In these methods, the eluent is passed through a nebulizer to atomize the mobile phase, the aerosol is passed through a heated transfer zone to evaporate the mobile phase and the residue is deposited onto an appropriate optical substrate. This allows for these methods to detect as low as subnanogram amounts of material. By capturing the eluent onto a substrate, the components of the sample are effectively trapped. It is possible, therefore, to analyze the chromatographic distribution of analytes after the LC/IR experiment as well as to perform analyses in real-time.

5.4.1 For transmission spectra, the eluent is deposited directly onto an infrared transmissive plate maintained at a temperature sufficient to permit further evaporation of the mobile phase (**11**). Infrared spectra are then obtained via an infrared transmission method.

5.4.2 For reflection absorption measurements, the eluent is deposited upon a front surface mirror. The infrared beam is then transmitted through the analyte, reflected off the mirror surface and transmitted back through the analyte. A modification of this method has been introduced where the eluent is deposited upon a thin germanium wafer. The back surface of this wafer is vapor coated with aluminum to yield a reflective surface (**12**). As germanium is IR transmissive, the beam passes through the deposited analyte twice and, depending upon the angle of incidence and reflection, yields an approximate doubling of the pathlength. The advantage of this approach over that of a first surface mirror is to reduce spurious optical effects such as specular reflection which may occur as light passes through the spotted analyte.

5.4.3 Direct deposition techniques provide the advantage of post-run spectral data acquisition and possibly, decoupling the chromatographic separation from the spectrometry. Through extended co-addition of spectra, the signal-to-noise ratio (SNR) of spectral results is improved over that obtained during real-time data acquisition. It must be noted that slow sublimation of the analyte and recrystallization may occur with direct deposition techniques. It is prudent to initially obtain the spectral data with a short co-addition time to create reference data to ensure the integrity of spectra obtained with longer co-addition times after the chromatographic separation is complete.

6. Significant Parameters for LC/IR

6.1 The instrumentation used to conduct the LC/IR experiment should be properly recorded within prescribed standard operating procedures (SOPs) or laboratory notebooks as necessary to meet requirements for specific laboratory practices. If the equipment is commercially available, the manufacturers' names and model numbers for the complete LC/IR system, or the individual components, should be recorded. Additionally, various instrumental and software parameters are listed and discussed in 6.2-6.4.5 Any modifications made to a commercial instrument must be clearly noted.

6.2 *Instrumental Parameters (IR)*:

6.2.1 *Detectors*—Due to low optical throughput, most LC/IR systems typically employ MCT narrow band photoconductive detectors. It is important that the detector element be properly filled with the image of the analyte spot or image of the exit aperture of the interface to achieve the highest signal-to-noise. Additionally, care must taken to ensure the MCT detector is not operated in a light saturating condition so as to maintain linearity of the signal response. Alternatively, deuterated triglycine sulfate (DTGS) detectors may be appropriate as these extend the spectral range to 400 wavenumbers. These are slow detectors, however, and are used typically with direct deposition and discrete fraction collection methods where spectral acquisition times are not critical.

6.2.2 *Flowcell Interface*—A complete description of the flowcell interface including optical type, optical pathlength, volume, temperature and material type should be recorded.

6.2.3 *Deposition Conditions*—For direct deposition LC/IR, the temperature of the nebulizer, evaporation chamber and deposition surface should be recorded. Type of nebulization gas, its flowrate and temperature, as well as the motion of the deposition surface should be noted. Spot size of the eluent deposited is directly determined by the diameter of the restriction end (nebulizer) and the distance separating the restriction end from the deposition surface. These should be recorded.

6.3 *Instrumental Parameters (LC)*—The success of the LC/IR experiment is dependent on good chromatographic practices. It is not the purpose of this document to discuss those practices in detail, but for convenience, a list of some LC parameters of key importance to be noted is given.

6.3.1 *Chromatographic Column*—The length and internal diameter of the column including the type of stationary phase employed must be noted.

6.3.2 *Mobile Phase*—The type of mobile phase used should be noted. If binary or ternary solvent systems are employed, the gradient profile should be specified in detail and include any initial delay or final hold time.