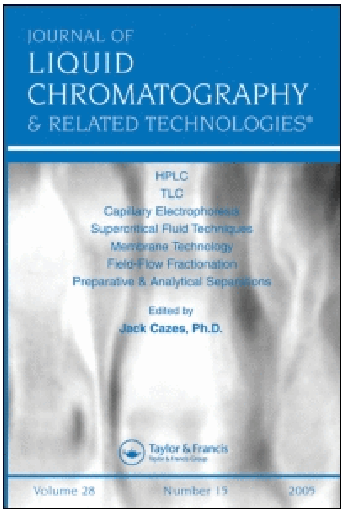

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Brown, Phyllis R. and Beauchemin Jr., Bernard T.(1988) 'Thin-Layer Chromatographic-Diffuse Reflectance Infrared Fourier Transform Spectroscopic Analysis', Journal of Liquid Chromatography & Related Technologies, 11: 5, 1001 — 1014

To link to this Article: DOI: 10.1080/01483918808068361

URL: http://dx.doi.org/10.1080/01483918808068361

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(5), 1001-1014 (1988)

THIN-LAYER CHROMATOGRAPHIC-DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM SPECTROSCOPIC ANALYSIS

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ABSTRACT

Diffuse reflectance Fourier transform spectroscopy (DRIFTS) is used to gain abundant structural information about thin layer chromatographically (TLC) separated materials. Quantitative and qualitative in situ DRIFTS analysis can be accomplished considering the effect of the TLC chromatographic support on the optical measurement of the TLC spot. Methods for in situ FTIR analysis of TLC spots are reviewed along with techniques proposed to overcome spectral interferences associated with the in situ measurement.

INTRODUCTION

Thin layer chromatography (TLC) is a simple and rapid separation method for the analysis of non-volatile compounds. TLC's unique capability of simultaneous sample analysis enables its application as a rapid screening technique, particularily suitable for the analysis of drugs. It is useful for monitoring synthetic reactions, screening drug metabolites in biological samples and analyzing antibiotics in fermentation media (1).

TLC is a chromatographic technique which is presently enjoying a resurgence of interest (2). The successful development of high performance liquid chromatography (HPLC) initially slowed the interest in TLC, however, technology improvements in HPLC have fueled advances in TLC. Improvements in TLC plate coating materials, developing techniques , and sample application, have led to the development of high performance thin layer chromatography (HPTLC) and improved efficiency and precision of the technique (3,4). These developments have led to high quality commercially available TLC plates, particularly suitable for quantitative reflectance methods (5). Precoated TLC plates are available with a wide range of chromatographic supports such as normal phase silica gel, alumina, or cellulose; reversed-phase diphenyl,C-2,C-8,or C-18; cyano and amino ; ion exchange and chiral supports.

TLC is used to separate a wide range of compounds. After spotting, development and plate drying, the solutes are identified by comparing the distance they migrate to the distance standard materials migrate. Detection of separated sample spots may be achieved through exposure to iodine vapors, charring with sulfuric acid, or visualization under UV light if the TLC plate contains a UV fluorescent indicator. The use of spray reagents also gives information about the identity of the chromatographed material. Often, spectral measurement is desired to obtain structural information and possibly identify an unknown spot.

Spectral identification of a TLC spot is usually time consuming and problematic. To obtain spectral information often requires the removal of the separated material from the chromatographic support and further sample preparation prior to spectroscopic measurement. Such treatment may lead to contamination , degradation or loss of the species of interest.

Alternatively, spectroscopic information about TLC separated materials can be obtained in situ . In situ quantitative measurement of TLC spots can be accomplished by densitometry, either in reflectance or fluorescence (6). Typically, fixed wavelength detection is used, usually in the ultraviolet spectral region, and therefore limited qualitative information about the sample is derived from the detector during the quantitative measurement. In situ ultraviolet-visible or fluorescence spectra may be obtained using scanning densitometry (7).

Monitoring the infrared spectral region by in situ diffuse reflectance Fourier transform spectroscopy (DRIFTS) enables both quantitative and qualitative characterization of a TLC spot with one measurement. This quantitative/qualitative aspect is particularily useful when TLC-DRIFTS is used to follow a labile compounds such as diazonaphthoquinones used in the microelectronics industry (8).

Optical measurement of infrared spectra of by use of an FTIR spectrometer requires measurement of background and sample (9). In situ TLC spot measurement requires the background measurement of a portion of the TLC support exposed to all chromatographic conditions, but free of any sample. The final infrared spectrum of a TLC spot is obtained by either ratioing sample and background measurements or subracting background spectrum from sample spectrum. Often, strong infrared absorbances of the chromatographic support inhibits obtaining accurate infrared spectral information about the sample in these spectral regions. A number of approaches to the FTIR measurement of infrared spectra of TLC spots have evolved which attempt to deal with the problem of background TLC plate interferences.

This review will concentrate on recent developments of infrared reflectance spectrometry of TLC separated spots. Emphasis will be placed on diffuse reflectance infrared Fourier transform spectrometry of TLC spots (TLC-DRIFTS). Comparison of analysis of TLC spots by TLC-PAS (photoacoustic infrared Fourier transform spectroscopy) and TLC-FTIR (transmission infrared Fourier transform spectroscopy) will be presented where appropriate.

IN SITU MEASUREMENTS

Depending on the nature of the isolated TLC spot and the goal of the analysis, the choice of infrared measurement of a TLC spot can be either in situ or after transfer to an infrared-transparent substrate. If the TLC separated material is thermally or photochemically labile, then an in situ technique is desirable. In quantitative applications,an in situ measurement approach is necessary to minimize sample loss, where as for qualitative applications, spot transfer to a non-infrared absorbing substrate allows measurement of spectra free of stationary phase interferences and more amenable to computer searching of spectral data bases.

The first demonstration of in situ measurement of TLC spots was done by Percival & Griffiths using direct transmission measurement of spots separated by silica gel on silver chloride TLC plates (10). A thin adsorbent coating and infrared transparency of the AgCl support permitted enough energy throughput to acquire spectra of the adsorbed species at 1 to 10 microgram levels of material. Several amino acids, including glycine , along with a few dyes were studied. Their approach produced useful infrared spectra in the 2200 to 1300 cm-1 region ,however, their work was limited to non-commercially availble AgCl specialty plates.

Gomez-Taylor and Griffiths also used infrared transparent AgCl plates coated with silica gel to obtain in situ qualitative spectra of pesticides (11). They proposed to avoid infrared scattering, which reduces the energy throughput at high infrared frequencies ,by coating the adsorbent plate with fluorolube prior to measurement. Later , the detection limits of this transmission technique were reduced through the use of programmed multiple development (PMD) which increased the chromatographic resolution and four-fold concentrated each sample spot on the TLC plate compared to conventional TLC. Spectra in the 2000 to 1200 cm-1 infrared region of 10 nanograms of a strong infrared absorber (mthylene blue) were measured by transmission after PMD treatment of the alumina on silver chloride plate (12).

Griffiths and Fuller were first to demonstrate in situ diffuse reflectance infrared Fourier transform spectrometric (DRIFTs) analysis of TLC spots (13). TLC spots were cut from aluminum backed silica gel plates by means of a punch and placed at the focus of the DRIFT spectrometer. In another reflectance approach by Griffiths and Fuller (14), the isolated spot ,together with the silica gel, were scraped from the TLC plate and measured by DRIFTs. A portion of the neighboring silica gel was used as background and similarily treated. Sample preparation contributed to the difficulty in obtaining spectra and the compensation of absorbance due to background silica gel was difficult to achieve even with strongly absorbing dyes.

Zuber et al. demonstrated the applicability of direct in situ analysis of TLC spots by DRIFTS using variety of commercially available plates (15). In Zuber's method, two plates were used, one for sampling and one for background correction. The procedure requires spotting of one of two identical plates. Both plates are simultaneously developed and dried. The TLC plates are directly inserted into the spectrometer sample chamber and measured at the same place on the plate. Plate contributions to background are subtracted from resultant IR spectra through use of the FTIR data system. DRIFTS analysis required approximately 30 minutes. This method was applied to caffeine pharmaceutical formulations at the 10 microgram per spot level with detection limits estimated at about 1 microgram.

Zuber et al. studied various plates including silica gel GF; Avicel (microcrystalline cellulose); Florisil (activated magnesium silicate); aluminum oxide; and acetylated cellulose. Plates generally had a 250 micron thick chromatographic layer.Zuber et al. demonstrated infrared regions were found to exist where the plate material absorbed so greatly only minimal information remained after subtraction. Therefore, only areas having minimal absorption were of use. Silica gel TLC plates were found to be of low sensitivity in the IR region of 3700 to 3100 cm-1 and 1650 to 800 cm-1 due to the absorptivity of background silica gel. Avicel has low IR absorption from 4000 to 3500 cm-1 and 2700 to 1500 cm-1, and , as a result had better limits of detection for carbonyl containing materials (IR region 1800 to 1600 cm-1) than did Silica gel GF.

Zuber et al. showed that in situ infrared reflectance spectra from TLC spots differed from transmittance spectra of the same compounds in a non IR absorbing matrix. In situ reflectance spectra were less detailed than transmittance spectra, however spectra did possess infrared absorbances bands which were both assignable to structure and similar to known characteristic infrared functional group frequencies.

In situ DRIFTs analysis of TLC spots was used to analyze reversed-phase HPTLC separated photochemically labile compounds (8). In this DRIFTs method,the dried TLC plate is directly inserted into the spectrometer chamber, and the same plate is used for spot measurement and for backqround correction. The procedure minimizes background differences such as plate thickness,adsorbed residual developing solvents or water, or particle size that may exist when two plates are used. A reference laser aids in spot alignment and the location of the background and sample spot can be found quickly. Plate contributions to background are compensated for quickly and accurately without spectral data manipulation. DRIFTs analysis time for one microgram TLC spots was less than one minute. Precision of 2 to 4 % RSD for diazo peak absorbance measurements was acheived for diazonaphthoquinones separated by reversed-phase HPTLC.

Quantitative in situ analysis of diazonaphthoquinones by reversed-phase HPTLC-DRIFTs was shown using an octadecylsilane bonded to a silica gel support (16). Areas of the spectral region where silica gel absorbs strongly in the infrared (3400 to 3100 cm-1 and 1650 to 800 cm-1) have little reflectivity and minimal information could gained about the species adsorbed on the surface. The area of greatest reflectivity is the region of 3100 to 1650 cm-1 and coincided with the strong absorbance of the diazo group, C=N=N, of the diazonaphthoquinone in the 2160-2100 cm-1 region. Therefore , quantitation by diffuse reflectance infrared spectroscopy of diazonaphthoquinones in this region was suitable and free from spectral interference from the stationary phase. Although application of the Kubelka-Munk function for components in absorbing matrices may vary in a non-linear manner with concentration (17) , good linearity of Kubelka-Munk data with concentration was found in the 1 to 10 microgram range.

Although the spectral window of DRIFTs using reverse phase TLC is narrow, abundant spectral information was obtained on the nature of the diazonaphthoquinone material. The strongest absorbing functional group (diazo) is also the group which is photochemically labile; thus under appropriate conditions the diazo group was used to follow the integrity of the diazonaphthoquinone compound (18).

PHOTOACOUSTIC MEASUREMENTS

Photoacoustic spectroscopy (PAS) has been suggested as the method of choice over DRIFTs analysis of high infrared absorbing sample matrices (19). Childers et al. indicated that a major problem encountered with in situ DRIFTs analysis of TLC separated materials is the appearance of reststrahlen bands,or reflectance maxima, particularly with the use of silica gel (19). Childers has shown reststrahlen bands are not present in infrared analysis by PAS . The presence of reststrahlen bands in DRIFTs have been attributed to highly absorbing species and to the design of the reflectance accessory (20,21).

Analysis of TLC spots by photoacoustic spectrometry (TLC-PAS) is less subject to interferences from the stationary phase, however signal intensities are low (22). One reported detection limit for PAS was 70 times higher than for DRIFTs (22). PAS accessories are not as widely used as diffuse reflectance accessories, but some work has been reported in the area of TLC adsorbate analysis (22,23). Unlike the in situ approaches, PAS requires the sample spot be physically removed from the TLC plate , either by removing it with a hole punch or scraping the adsorbant from the backing. The prepared sample is placed in the photoacoustic cell for sample measurement.Lloyd et al. showed the quantitative nature of the TLC-PAS technique for tetraphenylcyclopentadienone in the 300 microgram range with the detection limits estimated at 90 micrograms (ug/cm2) (22) .White demonstrated a one microgram detection limit for the TLC-PAS measurement of caffeine (23). Analysis time for sample preparation and photoacoustic analysis was estimated at 5 minutes .

SAMPLE SPOT TRANSFER TECHNIQUES

Recently, two novel techniques involving sample spot transfer to a non-infrared absorbing matrix have been proposed to avoid interferences by the stationary phase in DRIFTs in situ measurements of TLC spots.

In the first method , Shafer suggests a DRIFTS method using a sample transfer accessory attached to the TLC plate which allows direct transfer of the isolated TLC spot to a cup of a non-infrared absorbing glass (24). Transfer is accomplished through solvent extraction of the spot and the DRIFT spectrum is taken after the transfer solvent is evaporated from the sample cup. Qualitative spectra were obtained , but, efficiency of sample transfer may limit quantitative applications of this approach. Recently, a TLC-FTIR sampling accessory was commercially introduced based on this principle (25). A one microgram quantity of acenaphthenequinone spotted onto a TLC plate was sufficient for measurement of an identifiable infrared reflectance spectrum.

In the second method, Chambers et al. proposed transfer of the TLC eluate into a pressed KCl pellet for measurement of its DRIFT spectrum (26) . As with the first method, the TLC eluate is transferred via solvent extraction from the plate. Applicability of this procedure was shown for polypropylene antioxidants additives separated by normal phase TLC (silica gel on aluminum) at the 50 microgram level.

CONCLUSIONS

Choice of chromatographic support must be considered when in situ DRIFTS analysis is undertaken. Prominent infrared absorbance frequencies of the analyte , should , ideally, be matched with areas of the chromatographic support which weakly absorb in the infrared spectral region.Proper choice of TLC support and analyte absorbance band can lead to precise quantitative infrared reflectance measurements. The effect of strongly infrared absorbing matrices, such as silica gel, on diffuse reflectance infrared spectra of TLC spots requires consideration for both qualitative and quantitative analysis.

TLC-DRIFTs can be used , under appropriate conditions , for the in situ quantitative analysis of photochemically labile compounds.

TLC separated spots which are not labile and not sample limited, may be transferred to non-infrared absorbing matices for DRIFTS analysis with minimal spectral interferences.

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