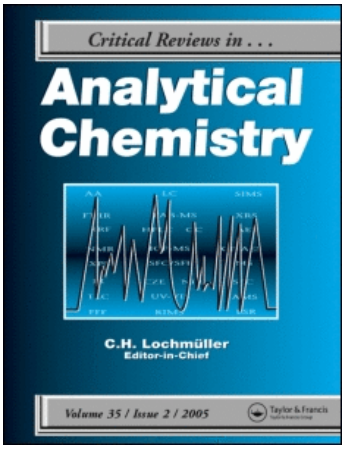

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Comparison of Infrared and Mass Spectroscopies for Drug Analysis*

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* The opinions or assertions contained herein are the private views of the authors and are not to be construed as those of the Department of the Army or Department of Defense.

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ABSTRACT: Forensic drug testing requires high confidence of identification and low limits of detection. Mass spectroscopy combined with gas chromatography (GC/MS) has typically been the analytical method of choice. To increase the detection capabilities, selected ion monitoring (SIM) has been employed with electron impact (EI) quadrupole MS instruments. This leads to a lower confidence of identification, inasmuch as the full scan is lost. Due to these limitations, alternate mass spectroscopy methods and infrared methods have been explored. Ion trap mass spectrometry reportedly provides the full scan information needed for identification at lower detection limits than full scan quadrupole MS. Methods employing gas chromatography Fourier transform infrared (GC/FT-IR) spectroscopy provide absolute identification through infrared fingerprinting with routine detection in the parts per billion range. New developments in cryogenic sample deposition for GC/FT-IR have increased the sensitivity of the technique 100 to 1000-fold to match or surpass that of GC/MS. Vapor phase GC/FT-IR has been configured serially with quadrupole MS providing both identification processes with one GC injection, GC/IR/MS. GC/FT-IR methods for vapor phase and cryogenic deposition techniques have been developed for certain drugs and development is continuing for others.

KEY WORDS: IR, MS, drug analysis, spectroscopic comparisons.

I. INTRODUCTION

A. Drug Testing Requirements

One of the most fundamental concepts in forensic toxicology is the requirement that each substance be identified by at least two analytical techniques.[1] The analytical methods chosen for the initial and confirmation testing in forensic toxicology must be based on differing chemical or physical properties, and the confirmation method must be sensitive and specific for the analyte in question. These guidelines have been published by the National Institute on Drug Abuse (NIDA) of the Department of Health and Human Services for drug testing and for the Nuclear Regulatory Commission, Department of Transportation, and other federal government agencies.[1] In both urine drug testing and post-mortem cases, relevant clinical information is often not available, and interpretation of results is based solely on the analytical data. This heavy reliance on the toxicologic result adds to the importance of ensuring that confirmation methods are legally and scientifically defensible. The legal literature em-

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phasizes the need to confirm drug analyses with the most specific and sensitive tests possible.[2]

B. Amphetamine Problems

The term "amphetamines" refers to many drugs and structurally related compounds possessing similar pharmacological and toxicological effects. Many of these analogues are sold in over-the-counter medications for nasal decongestants and weight control. Other designer drug derivatives of amphetamine, such as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), are Drug Enforcement Administration (DEA) Schedule I psychotropic compounds, which are chemically related to mescaline.[3] MDMA and other related compounds have been associated with fatal intoxication and potential neurotoxicity.[4] Amphetamine abuse can be found worldwide to such an extent that the World Health Organization has recommended world control of various amphetamine-like compounds.[5] Thus, it is not surprising that drug testing programs are becoming increasingly concerned with the analysis of amphetamines and related analogues.

It is common in post-mortem analyses to get a false-positive immunoassay screen for amphetamines due to amines produced from degradation of the specimen.[6] This places the burden of distinguishing amphetamines from biogenic amines on the confirmation method. In the case of amphetamines, when GC/MS is employed, care must be taken because false-positive methamphetamine results have been reported for urine samples containing only ephedrine.[7]

II. BACKGROUND

A. GC/MS as a Confirmatory Technique

GC/MS is considered the definitive confirmatory method for urine drugs of abuse testing. The majority of Department of Defense (DoD) and NIDA certified laboratories are using quadrupole electron ionization GC/MS and selected-ion monitoring (SIM) for quantitation.[8] Selective scanning allows the mass analyzer to dwell for a longer period on selective masses, which optimizes the limits of detection and limits of quantitation, but loses specificity.[9,10]. Alternatively, full scan mass spectrometry may yield more relevant spectral or chromatographic information and more specific identification,[11] but with a possible loss in quantitative sensitivity and accuracy when compared to quadrupole SIM GC/MS.

GC/MS is a common confirmation method and generally accepted as the method of choice for positive identification of drugs. However, two widespread misconceptions about MS are that GC/MS is a specific method and that GC/MS is 100% accurate.[12] In 1989, the American Association for Clinical Chemistry/College of American Pathologists (AACC/CAP) Forensic Urine Drug Confirmation Survey, in which 150 to 200 laboratories participated, reported 94 false-positive and 304 false-negative results. Most of these laboratories used GC/MS as the confirmation method with EI in the selected ion monitoring mode. Full scan GC/MS with EI probably provides the best identification data for mass spectroscopy. A quadrupole GC/MS in the full scan mode, however, has a Limit of Detection (LOD) of 25 to 50 ng/ml, hence SIM mode with a much lower LOD is used for forensic urinalysis.

B. Specificity vs. Sensitivity in Analysis

The apparent lack of specificity and sensitivity in the common techniques for drug testing, which are recognized by the 1989 CAP survey, has spurred the exploration into alternate techniques. Ion trap mass spectrometry can provide the needed full scan for specificity with sensitivity near that of SIM.[13] The DoD Drug Detection Quality Control Laboratory has a study in place to evaluate the technique of ion trap mass spectrometry to that of the commonly used technique of quadrupole SIM GC/MS for confirmations of drug metabolites in urine.

Infrared spectroscopy has long been noted as the analytical technique with the highest selectivity due to its "fingerprinting" ability for molecular compounds.[14] The superiority of IR over MS

for specificity in forensic testing has been demonstrated.[15,16] Vapor phase GC/FT-IR has been shown to be a viable alternative or complement to GC/MS,[9] and when matched run for run, the IR spectrometer identifies more materials accurately than the mass spectrometer.[17] This infrared selectivity is what is needed in identification of drug metabolites and the various other compound derivatives that may be present.[15] A study on the specificity of GC/FT-IR for forensic drug analysis demonstrates this point.[18] In the past the biggest drawback of IR was sensitivity, but new developments in instrumentation have increased detection limits to match or surpass that of MS. Court cases have suggested that there is a need to have an instrumental method that can yield high confidence data for qualitative identification and quantitation.[19]

III. INSTRUMENTATION

A. Mass Spectrometry (MS)

Quadrupole mass spectrometers are the most prevalent and well known of the mass spectrometers in the field, and the most common instrument used for drug analysis. Their limited full scan sensitivity for urine drug testing has prompted the exploration into other possible techniques. The ion trap mass spectrometer is reported to have the sensitivity of quadrupole MS in SIM mode with the selectivity of quadrupole MS in full scan mode.[12] The third generation ion trap mass spectrometers, introduced in 1990, have the reliability necessary for drug and environmental analysis and are currently being evaluated by several different laboratories.

B. Infrared Spectroscopy

Two basic types of GC/FT-IR instruments exist: vapor phase and cryogenic deposition. Recent developments in both areas have improved the sensitivity of the techniques to the degree that they are now applicable to the low level determinations needed for forensic testing of biological fluids.[20,21]

1. Vapor Phase GC/FT-IR

The developments in vapor phase GC/FT-IR have concentrated on minimizing the noise sources so that lower level signals are detectable. This is an application of the Hirschfeld advantage.[22] The basic design of a vapor phase GC/FT-IR consists of a heated fused silica transfer line that directs the GC effluent to flow through a long narrow infrared gas cell (12 cm × 1 mm) known as a "lightpipe". The IR beam transmits through the lightpipe which is sealed on each end with IR transparent windows. IR data is continually collected as the effluent flows from the GC.

2. Cryogenic Deposition GC/FT-IR

The developments in cryogenic deposition GC/FT-IR have focused on cold trap depositing the eluents into a small spot (100 μm), hence concentrating the sample and increasing the signal. This is a simple relationship of Beer's Law and can be shown to give over a 100 fold enhancement in the sensitivity of the data.[23] For this instrumentation, the GC is interfaced to the FT-IR via a heated fused silica capillary connected to the GC column in the oven with a butt-connector. The transfer line enters a vacuum chamber (held at 10^{-5} torr) and ends with a fused silica restrictor (50 μm i.d.) positioned <50 μm above a zinc selenide (ZnSe) IR transparent plate. The effluent exits the transfer line and is deposited onto the ZnSe plate held at liquid nitrogen temperatures by a cold block. The plate with the frozen line of eluent continually translates into the IR beam where the sample is analyzed in real time. Schwartzchild microscope objectives focus the IR beam onto a high sensitivity Mercury Cadmium Telluride (MCT) IR detector.

IV. ANALYTICAL METHODS

In general, the drugs must be separated from the biological specimens before being analyzed for identification. The most common separation method is liquid-liquid extraction. This technique uses differences in pH and solubility characteris-

tics of the various analytes. A less time consuming but sometimes less consistent separation technique is solid phase extraction.

A. Sample Preparation

The samples in the comparison studies were all urine matrix and guidelines for drugs of abuse testing were followed.

1. Extraction

All of the studies used in comparison of the analytical techniques employed liquid-liquid extractions for sample preparation. For amphetamines these procedures were performed under alkaline conditions into organic solvents. Each extraction set included a negative urine matrix standard and a standard curve of spiked urines at various levels corresponding to values above and below the NIDA cutoff limits for positive detection.

2. Derivatization

Typically, following the extraction, the samples are derivatized. Derivatizing the samples aids the chromatography so that sharper, more easily defined peaks are present for better detection. The derivatives also have higher absorptivity than the underivatized samples increasing the IR sensitivity for detection. The same is true of derivatized samples for mass spectral detector response. The ions of the derivatized samples are more easily discerned because they are in a higher mass range than those of the underivatized samples.

Although the extraction methods were similar in each study, the derivatizing agents varied. Heptafluorobutyric anhydride (HFBA) was used as the derivatizing agent in the IR methods due to its high IR absorptivity. An alternate IR study found chlorodifluoroacetic anhydride to yield more consistent results although not quite as strong an IR absorber. Menthyl chloroformate was the derivatizing agent for some MS determinations and HFBA derivative for others, with each having similar LODs. The menthyl chloroformate derivative assay for amphetamines in mass spectrometry was developed to help discriminate over-the-counter medications from illicit preparations.[24]

3. Recovery

The extraction efficiencies of the various methods all fell between 80% and 90%. However, as typically found with amphetamines, an additional 20% to 40% is lost during the dry down step in derivatization.

4. Concentration

Bringing the sample back up in differing solvent volumes before analysis can alter the concentration of analyte in a single analysis, but will not affect the ultimate ng/ml concentration of the determination. This variability allows the lesser sensitive techniques to boost their single analysis concentration without affecting the overall determination. This factor is demonstrated in Table I which tallies and compares the volumes all relating to a 200 ng/ml amphetamine sample.

B. Operational Parameters

Gas chromatography is used as the sample introduction technique for each of the differing instrumental analyses. Typically, for drugs of abuse analysis, a 5% phenyl methyl silicone capillary column is used with a temperature program ramp of 10 to 20°/min from approximately 100 to 200°C. The variations of this configuration depended on the optimization desired.

1. MS Features

The quadrupole electron ionization mass spectrometers were operated in the selected ion monitoring mode for greater sensitivity and quantitation. Reference quantitative peaks and qualitative iden-

TABLE 1
Comparative Results using Three Techniques for a Sample of Amphetamine/ Methamphetamine and Determined Instrumental Limits

Parameter	GC/MS (SIM)	GC/FT-IR (Vapor phase)	GC/FT-IR (Cryogenic deposition)
		200 ng/ml Standard Sample	
Initial volume of urine	2 ml	10 ml	3 ml
Final volume	50 µl	300 µl	50 µl
Injection volume	2 µl	3 µl	2 µl
Injector split ratio	30:1	Splitless	30:1
Absolute amount detected	0.384 ng	16 ng	0.640 ng
Extraction recoveries of 80%			
		Instrumental Limits	
Limit of detection (absolute amount detected)	5 ng/ml 0.01 ng	25 ng/ml 2 ng	<10 ng/ml 0.05 ng
Limit of quantitation	10 ng/ml	100 ng/ml	20 ng/ml
Limit of linearity	10–6,000	100–25,000	20–2,500 ng/ml
Coefficient of variation (at 500 ng/ml cutoff)	8.5	10.7	12.8

tifier peaks were determined based on full scan MS analysis. Optimal quantitation was found using a dwell time of 40 to 75 msec per ion and greater than ten scans across the peak for three monitored ions.

2. IR Features

The vapor phase IR analyzer is configured serially with a quadrupole mass spectrometer, GC/IR/MS. Reconstructed chromatographic data can be obtained from both the ion abundance data and the IR absorbance data. For this instrumental design, the gas flows are critical. A complete description of optimization for the flows, interfacing transfer lines, and make-up gases has been described elsewhere.[25]

Both the vapor phase GC/FT-IR and the cryogenic deposition GC/FT-IR have the ability to reconstruct chromatographic data from the total IR spectrum or from selected group frequencies, much like the selected ion chromatograms in MS. Both IR systems typically signal average 3 to 4 full IR scans per data file. The vapor phase GC/FT-IR has a peak residence time of about 4 s where the cryogenic deposition GC/FT-IR has the advantage of having the sample frozen "in time" on the IR plate so that subsequent data acquisition with long signal averaging periods can be obtained post analysis, enhancing the already MS comparable IR sensitivities. Low resolution data collections and high sensitivity MCT infrared detectors allow rapid on-the-fly data analysis for GC/FT-IR.

As has been discussed, sensitivity was the limiting factor in routine use of GC/FT-IR for drug analysis until recent instrumental developments. IR spectral responses are dependent on the individual molecular absorptivity of each compound as related to Beer's Law. The current sensitivities for IR data are now comparable to the mass spectral data.

V. COMPARISON OF TECHNIQUES/ APPLICATIONS

Several groups are actively working on comparing GC/FT-IR methods for drug analysis in

the forensic toxicology laboratory with the existing standard GC/MS methods. One group is using the vapor phase GC/FT-IR technique in tandem with a quadrupole MS;[25,26] another group is exploring the higher sensitivity Cryogenic deposition GC/FT-IR.[27,28] The comparative results of the three techniques (standard quadrupole SIM GC/MS, vapor phase GC/FT-IR, and cryogenic deposition GC/FT-IR) for a 200 ng/ml sample of amphetamine/methamphetamine are listed in Table I along with the determined instrumental limits of each technique. The NIDA drug testing cutoff limit for these drugs of abuse is 500 ng/ml. (The concentrations listed here and throughout are for each component in solution.) The comparative instrumental limits are discussed in detail below.

Comparative spectra of the three techniques using the same sample preparation are shown in Figures 1, 2, and 3. Figure 1 is the SIM vs. full scan GC/MS data from an extract of 200 ng/ml of amphetamine/methamphetamine using HFBA as a derivatizing agent. The left portion of the figure shows the total ion chromatogram (TIC) and MS data for the SIM mode and the right portion shows the TIC and MS data for the higher confidence MS full scan data. This equipment is the standard set-up for drug lab testing and is commercially available as a complete package (Hewlett-Packard, Palo Alto, CA). Figure 2 displays the IR and MS data for the vapor phase GC/FT-IR tandem MS system for the 200 ng/ml amphetamine/methamphetamine sample. The top portion shows the total IR reconstructed chromatogram (TRC) and the IR data for the amphetamine peak. The lower portion shows the TIC and SIM MS data from the amphetamine peak. (4-phenylbutyl amine was the MS internal standard.) These data were obtained

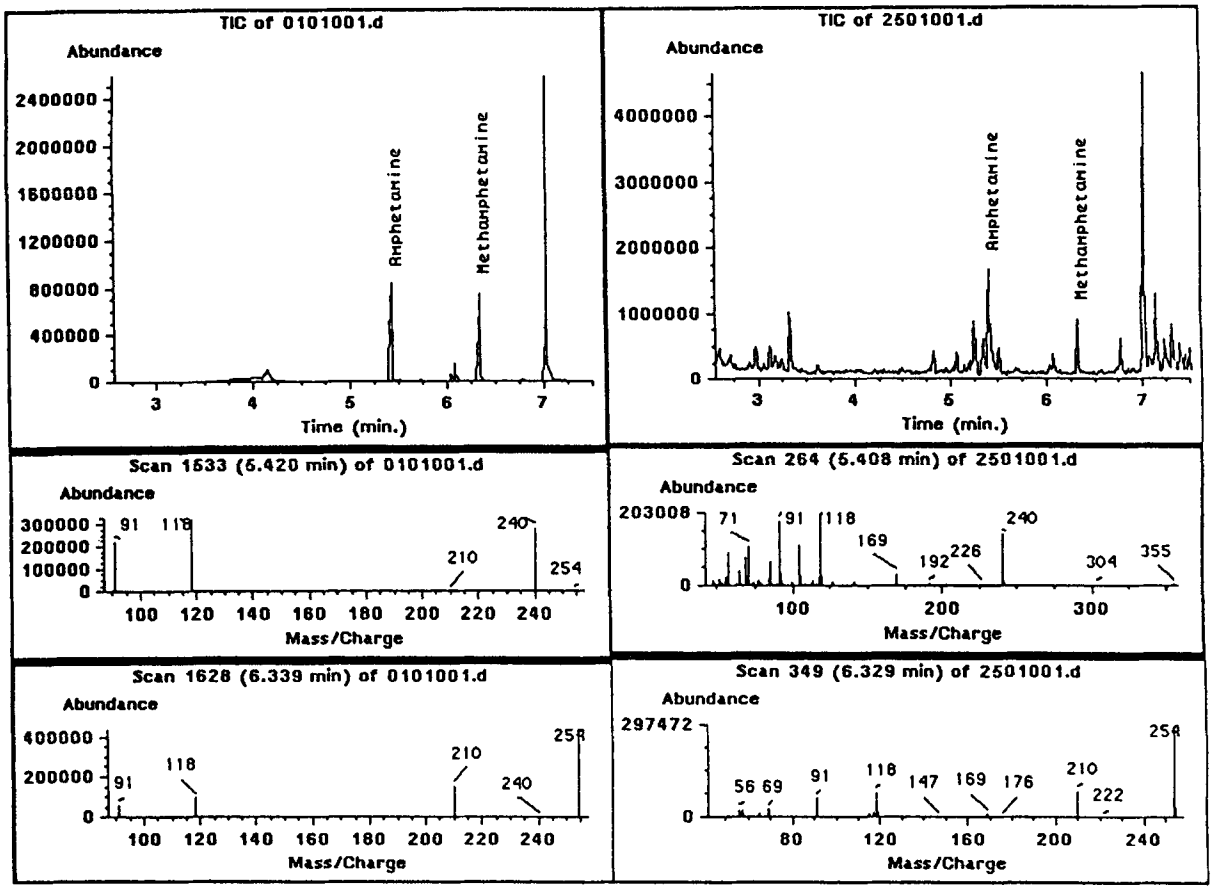

FIGURE 1. SIM vs. full scan GC/MS data of HFBA derivatives of amphetamine/methamphetamine from 200 ng/ml sample. TIC and MS data for the SIM mode (left); TIC and MS data for the MS full scan mode (right).

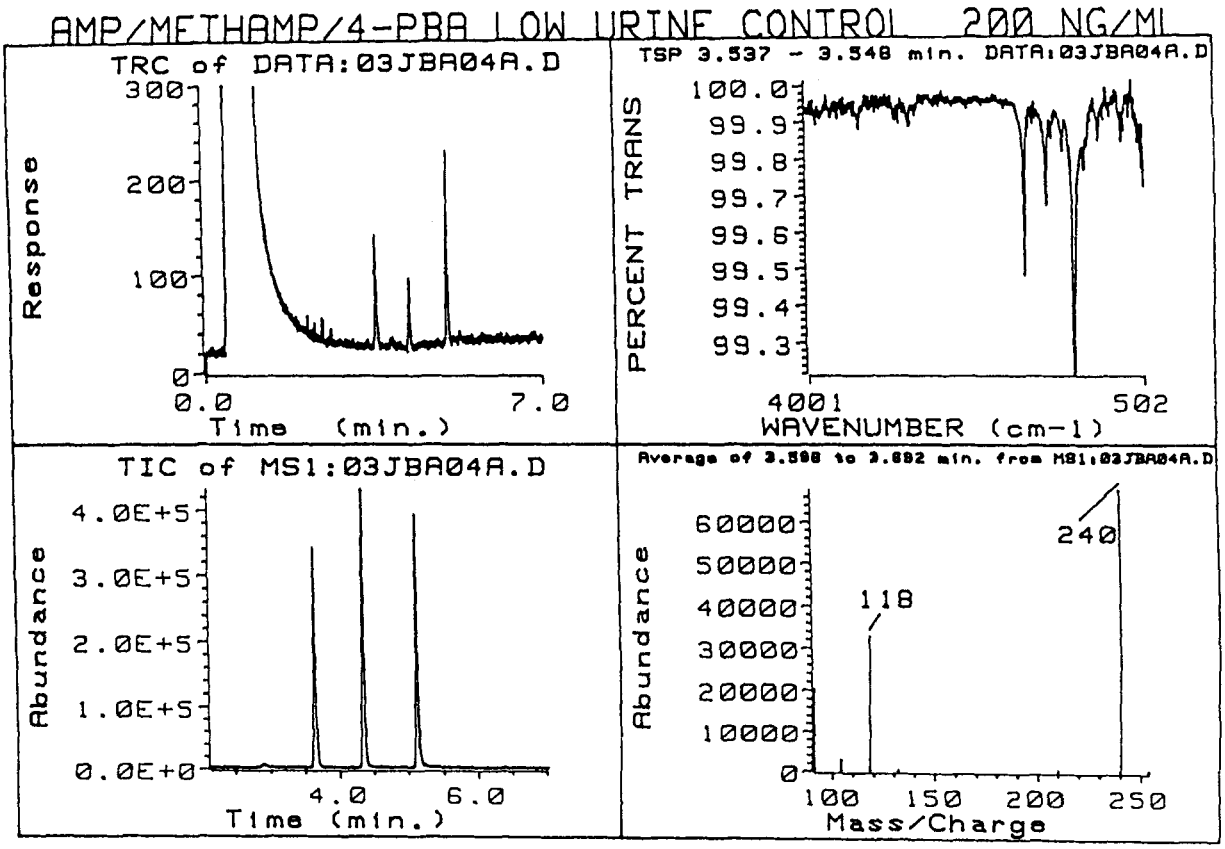

FIGURE 2. IR and MS data for the vapor phase GC/FT-IR tandem MS system for a 200 ng/ml amphetamine/methamphetamine sample. TRC and IR data for the amphetamine peak (top). TIC and SIM MS data from the amphetamine peak (bottom).

in tandem with one injection on the GC. This instrumentation is commercially available (Hewlett-Packard, Palo Alto, CA) and the method development for amphetamines and its derivatives is complete.[26] Figure 3 shows the IR data obtained on the cryogenic deposition GC/FT-IR for a 250 ng/ml sample of amphetamine/methamphetamine. The top trace is the IR spectrum of amphetamine which has a 6.6 min retention time and the lower spectrum is the methamphetamine with a 7.5 min retention time. The bottom trace is the Gram-Schmidt reconstructed chromatogram from the interferometric data. The scales are relative interferometric intensity vs. retention time. The additional peaks in the chromatogram are from the urine extracts. These peaks were also present in the GC/MS data in Figure 1. The amount of material at the detector in these IR spectra was 700 pg. This instrument (Bio-Rad Digilab Division, Cambridge, MA) is currently configured for research, however, minor custom software additions to the system would make it useful for routine service work and a compact instrument configuration could be designated for drug lab work.

A. Qualitative Identification

When monitoring single ions with mass spectral response, multiple samples yield response at the same mass. Such is the case with amphetamine and phenylpropanolamine (PPA), as well as methamphetamine and pseudoephedrine. Full spectral scan is necessary to differentiate these pairs in MS. The IR spectra are uniquely different. Although the derivatization yields similar IR major peaks, the IR fingerprints are distinctly different from each of the other sympathomimetic

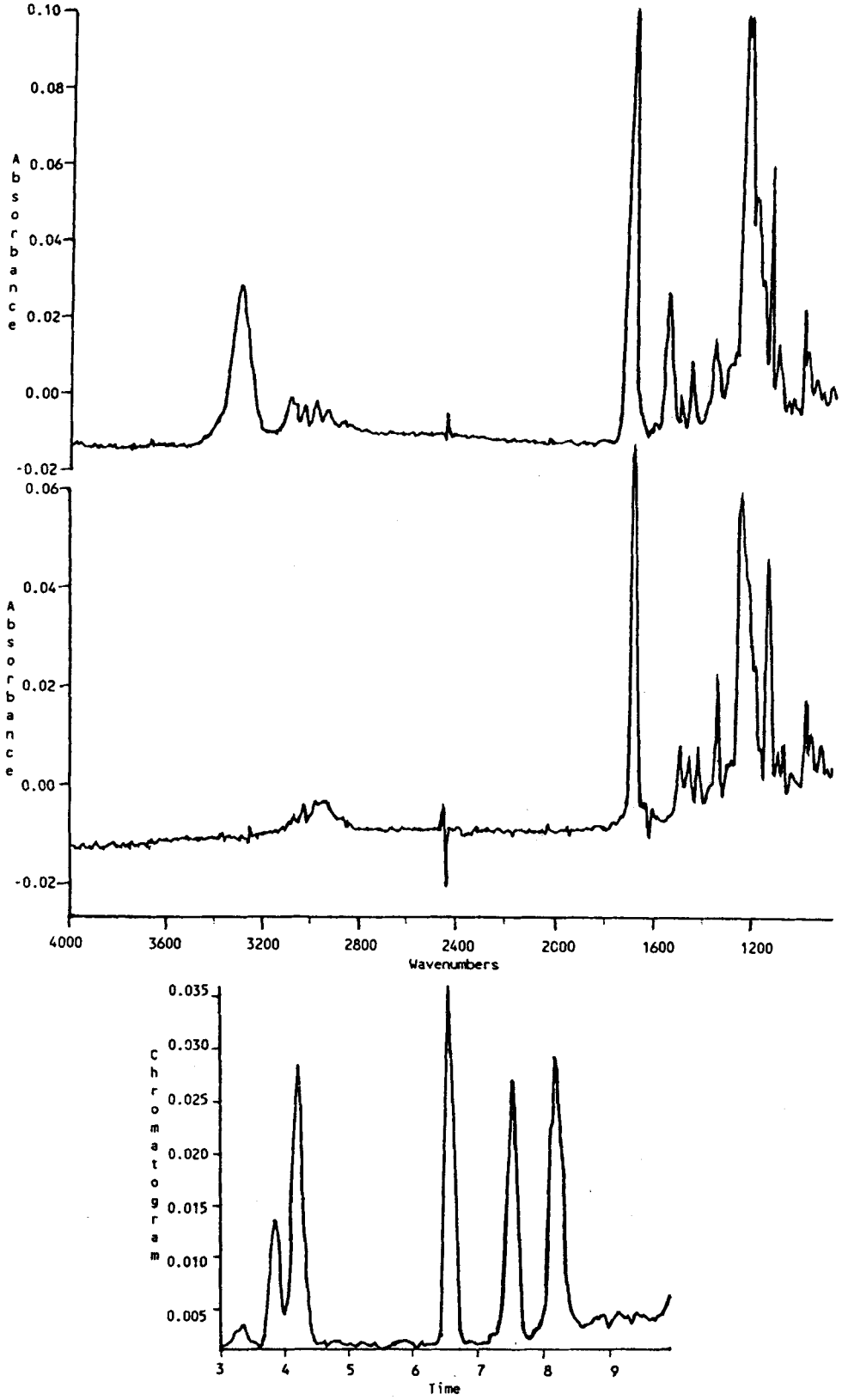

FIGURE 3. IR data for the cryogenic deposition GC/FT-IR for 250 ng/ml sample of amphetamine/methamphetamine. IR spectrum of amphetamine, retention time 6.6 min (top). IR spectrum of methamphetamine, retention time 7.5 min (middle). Gram-Schmidt reconstructed chromatogram from the interferometric data (bottom).

amines. IR spectral searches can yield a positive match on these materials at the limit of detection. The library data of both the IR and mass spectral data must be of the derivatized reference compounds with the derivative used in the sample analysis.

Figure 4 shows a Gram-Schmidt reconstructed IR chromatogram of amphetamine, methamphetamine, and related analogues. The total reconstructed ion chromatogram is similar for MS. The IR to MS retention time varies between 5 to 11 s for the serially configured vapor phase GC/IR/MS. The optical isomer pairs (d-amphetamine & l-amphetamine, and d-methamphetamine & l-methamphetamine) cannot be differentiated by either system without using a chiral derivative or chiral GC column for differentiation. The cryogenic deposition IR spectra obtained from the reconstructed chromatogram in Figure 4 are shown in Figures 5 and 6. These samples were derivatized with chlorodifluoroacetic anhydride. All of the analogues were uniquely identified by spectral searches of the appropriate reference library data base. Vapor phase IR and full scan mass spectral data from the tandem GC/IR/MS system for a similar standard mixture of analogues are shown in Figures 7 and 8. These samples were derivatized with HFBA. Differentiation can be seen in the IR spectra by observing the C=O stretch region (1700 to 1750 cm^{-1}), N–H bend regions (1650 to 1850 cm^{-1}), and the fingerprint region (800 to 1200 cm^{-1}). Routinely in a laboratory analysis situation only SIM MS data are obtained. If this

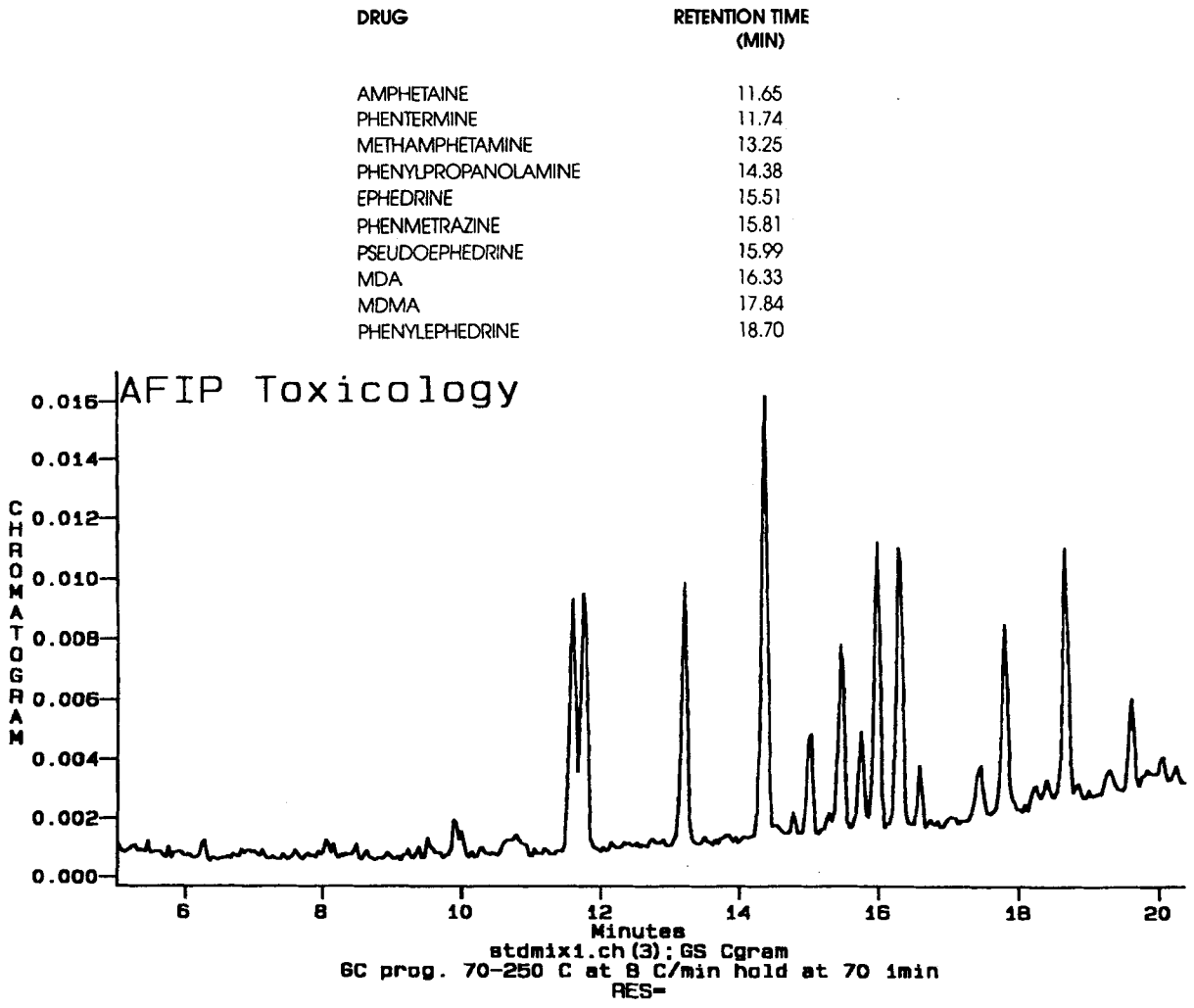

FIGURE 4. Gram-Schmidt reconstructed chromatogram of the IR data of amphetamine and its analogues derivatized with chlorodifluoroacetic anhydride obtained with the cryogenic deposition GC/FT-IR.

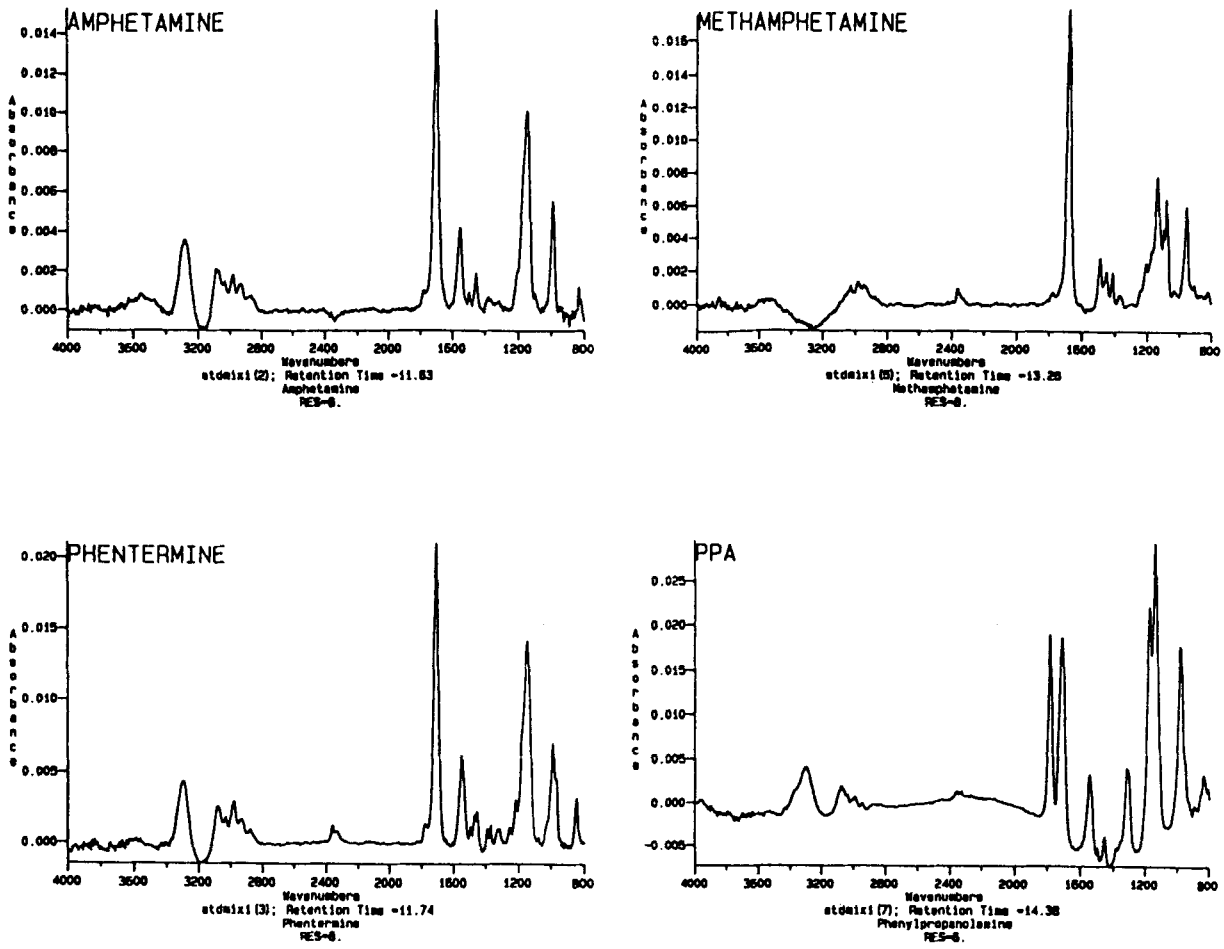

FIGURE 5. IR spectra of derivatized amphetamine, methamphetamine, phentermine and PPA as detected by the cryogenic deposition GC/FT-IR from the chromatogram in Figure 4.

does not conclusively identify the sample, a second injection employing full scan is needed with a full scan mass spectral library of derivatized samples for comparison.

B. Quantitative Analysis and Linearity

Quantitation and linearity are based on Beer's Law, and the methods for quantitating IR data are well established.[29] In GC/FT-IR, quantitation can be derived from the spectral data or from the reconstructed chromatographic data.[30] Deviation from Beer's Law and linearity do not occur until high concentrations of sample are encountered or, in the case of vapor phase GC/FT-IR, when column overloading occurs. Actual data obtained for drugs have shown that the limits of linearity for the IR technique can be greater than those of the MS technique.[18,25]

The limits of linearity (LOL) and limit of quantitation (LOQ) can be determined by observation or mathematically.[31,32] For these studies the instrumental limits were determined by observation of at least ten different extractions with replicate samples for each concentration within an extraction. The limits were established based on reproducible quantitation within 10% of the theoretical concentration.

For the IR analysis an internal standard, 4-phenylbutyl amine (4-PBA), was used. Figure 9 shows the comparative IR spectra of the two target drugs, amphetamine and methamphetamine, along with that of the internal standard. The relative areas of the isolated carbonyl functional group chromatograms between 1700 to 1750 cm^{-1} in

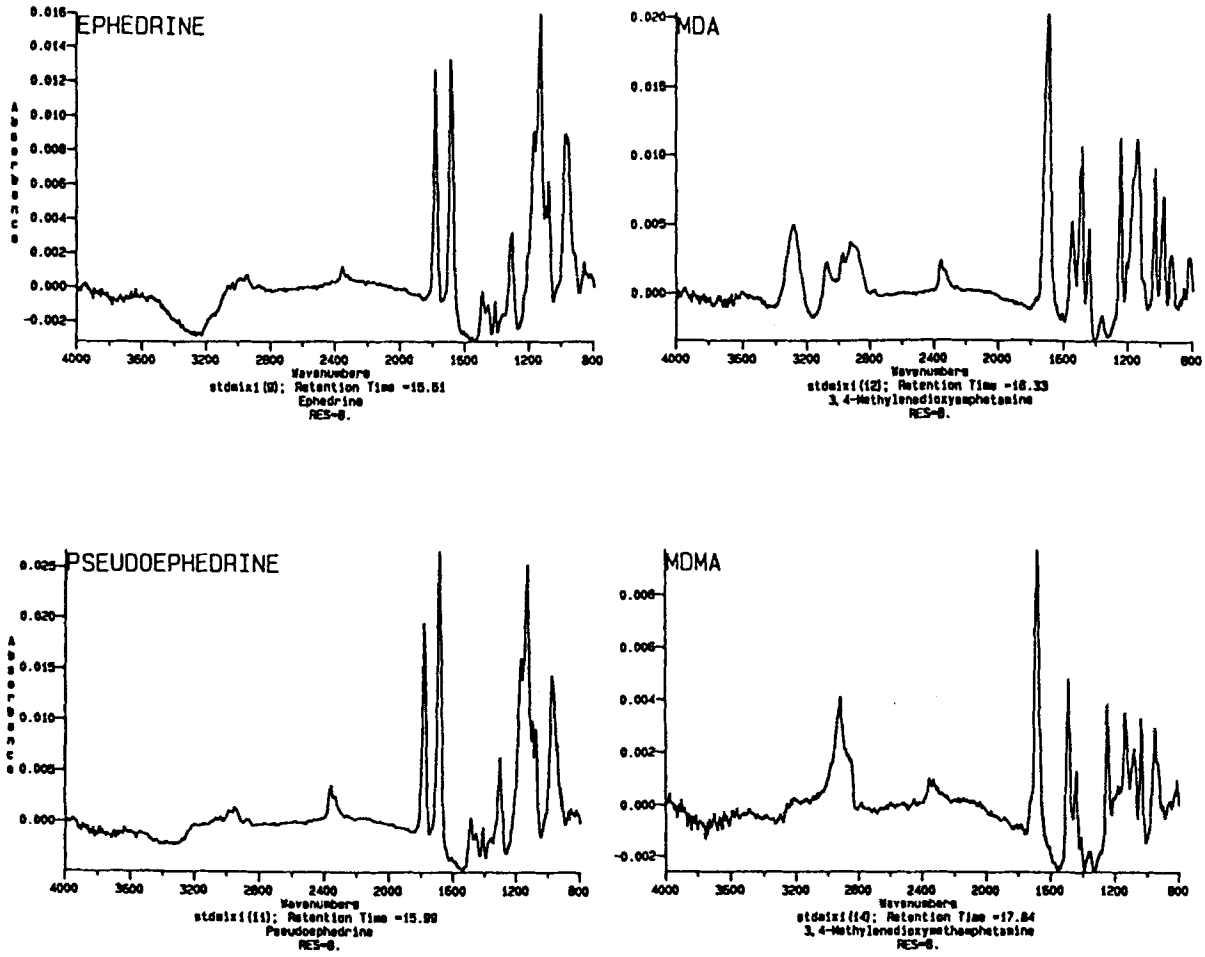

FIGURE 6. IR spectra of derivatized ephedrine, pseudoephedrine, MDA, and MDMA as detected by the cryogenic deposition GC/FT-IR from the chromatogram in Figure 4.

each of the spectra were used for the quantitative determinations. It is assumed that the spectral response of the internal standard and target drug are the same. For mass spectrometry, amphetamine-d5 is typically used as the internal standard due to the similarity of response and the ability to differentiate ions of interest with co-eluting compounds. In the case of vapor phase GC/IR/MS, it was necessary to incorporate both internal standards. For IR quantitation, the carbonyl functional group chromatogram of negative urine sample with the two internal standards was subtracted from that of the sample also containing the two internal standards, and the resultant spectra were compared to an external calibration curve for the relative peak heights for the C=O band.[25]

The final results as listed in Table I indicated that the LOQ and lower limits for LOL of each technique were close to the detection limit of each technique, with the vapor phase GC/FT-IR demonstrating the greatest difference in LOD and LOQ.

The upper limits of linearity for the vapor phase GC/FT-IR were much greater than the MS limit, due to the adherence to Beer's Law until the "lightpipe" and/or GC column became overloaded. The upper limit of linearity for the cryogenic deposition GC/FT-IR is much less than MS due to the sample deposit size (optimally 100 μm) becoming larger than the IR beam (optimized for 100 μm spot). This system was designed for very low level determinations and overloads at concentrations

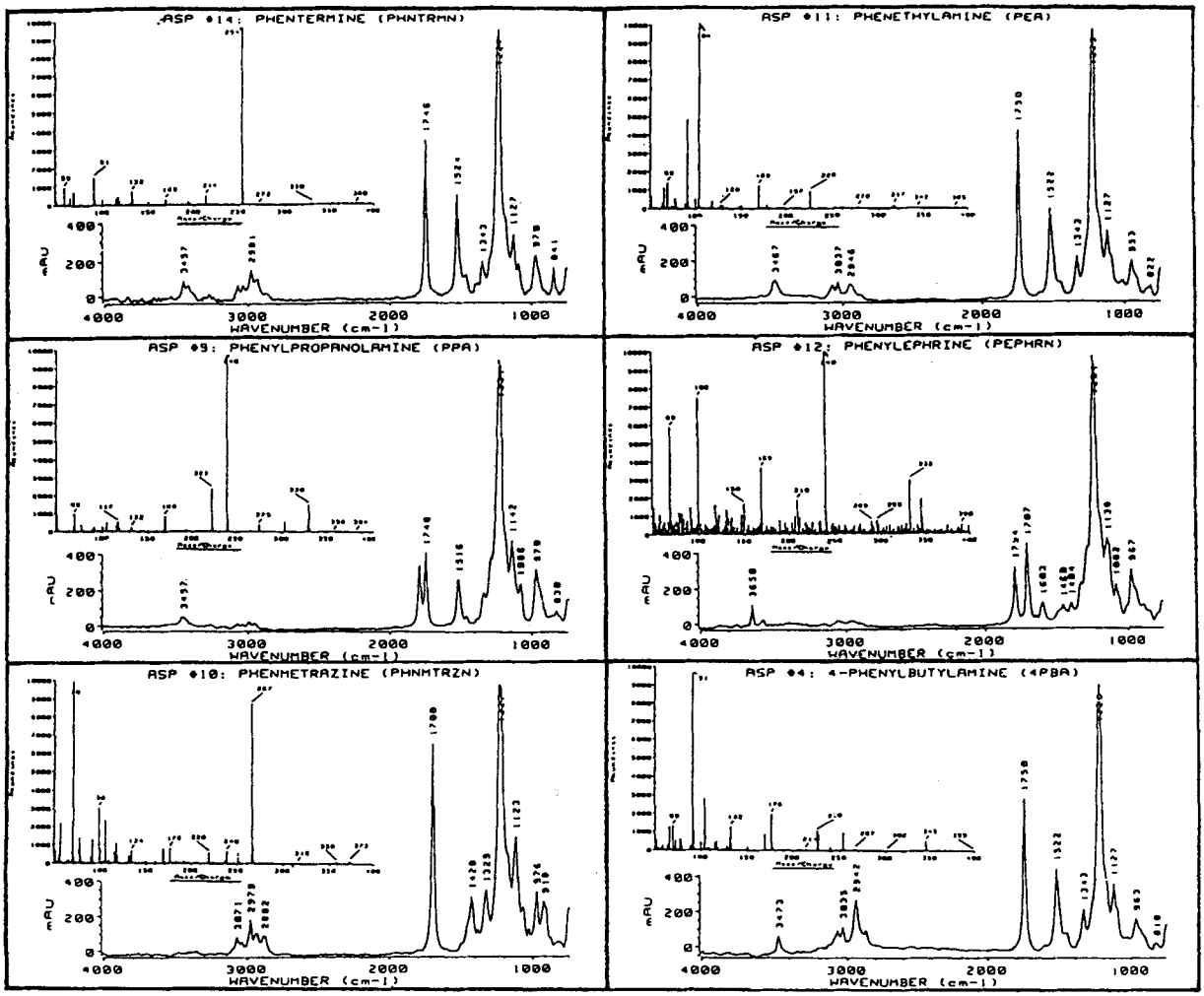

FIGURE 7. Vapor phase IR and full scan mass spectral data from the tandem GC/IR/MS system for a standard mixture of analogues. Samples derivatized with HFBA are identified as phentermine, phenethylamine, PPA, phenylephrine, phenmetrizine, and 4-PBA.

greater than a few nanograms per sample deposit.

C. Limits of Detection

The GC/MS SIM method still has the lowest detection limits, but cryogenic deposition GC/FT-IR has a similar LOD and offers identification certainty comparable to full MS scans. The IR development under investigation with cryogenic deposition GC/FT-IR indicates lower than 10 ng/ml can be detected with full scan identification confidence. The LOD was defined as the lowest reliable and reproducible (within 20%) analyte concentration.

The abundances shown in Figure 1 of the full scan MS data vs. the SIM MS data indicate a lower sensitivity for the full scan than the SIM. These differences are 5 to 10 times less sensitive when instrumental limits are determined due to the additional noise encountered in the full scan at the lower limits.

The spectra shown in Figure 3 indicate the specificity and sensitivity currently available in the IR detection field. These data were collected in 4 s in real time, but an additional option of recollecting the data after the initial run is possible for longer time averaging and

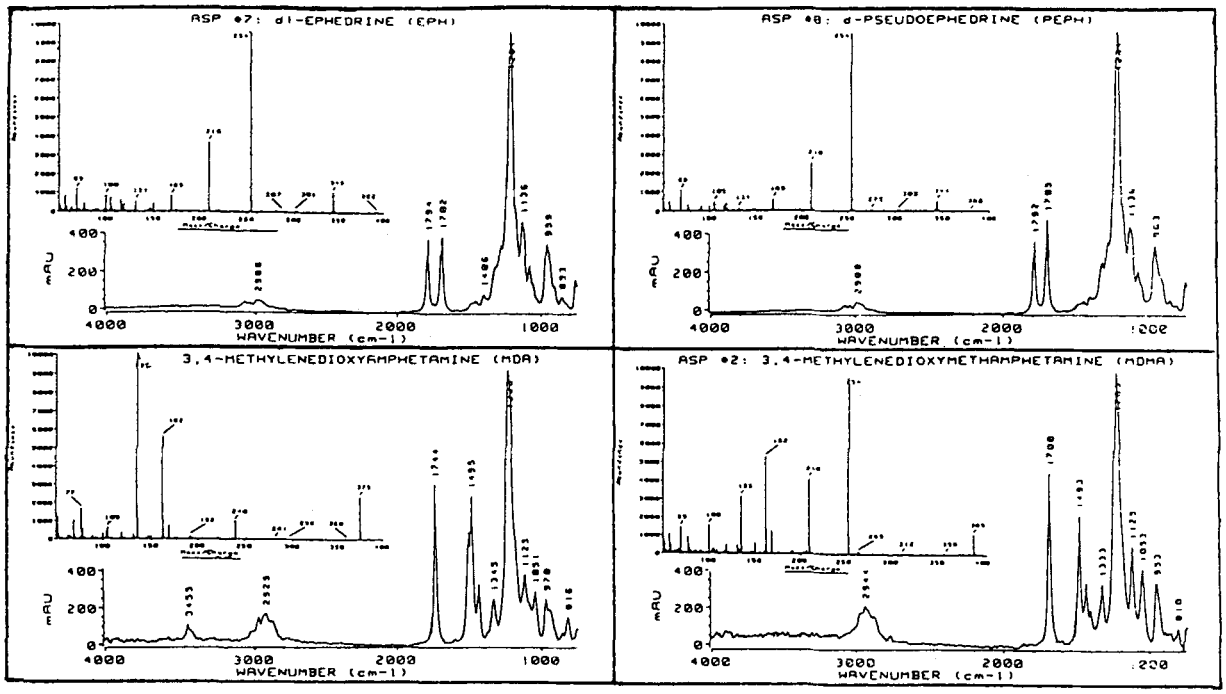

FIGURE 8. Vapor phase IR and full scan mass spectral data from the tandem GC/IR/MS system for a standard mixture of analogues. These samples derivatized with HFBA are identified as ephedrine, pseudoephedrine, MDA, and MDMA.

hence increasing the signal sensitivity. This mode of operation is available because the sample is frozen on the IR analysis plate. Increasing the collection time to 4 min would yield approximately a ten-fold increase in sensitivity. The tabulated instrumental limits listed in Table I are for data collected in real time.

Figure 10 shows the cryogenic deposition GC/FT-IR spectra of amphetamine (derivatized with chlorodifluoroacetic anhydride) collected for the detection limit study. The left column of spectra are collected in real time (four scans per data file) and the spectra in the right column are collected postrun with 256 signal-averaged scans. The spectra represent sample concentrations extracted from urine matrix of 20, 10, and 5 ng/ml from top to bottom, which correspond to an absolute amount detected of 46, 23, and 11 pg, respectively. These spectra were all identified as amphetamine by spectral searching of a data base containing cryogenic deposition GC/FT-IR spectra of amphetamine analogues derivatized with chlorodifluoroacetic anhydride.

D. Precision

The coefficient of variation (CV) was determined for between run precision. At least ten reference points were considered in each calculation. The values shown in Table I are the combined CVs for both the amphetamine and methamphetamine analyte at the NIDA cutoff concentration of 500 ng/ml. At this level the MS has the best precision followed by the vapor phase GC/FT-IR and then the cryogenic deposition GC/FT-IR. At concentrations below the cutoff, the order remains the same with greater margins between each technique, and at higher concentrations the vapor phase GC/FT-IR is superior due to the LOL as discussed previously.

E. Summary of Instrumental Parameter Limits

Throughput or turnaround time for both the GC/MS and GC/FT-IR are limited by the chromatography and not the spectroscopy. Routine procedures for automatically processing the data

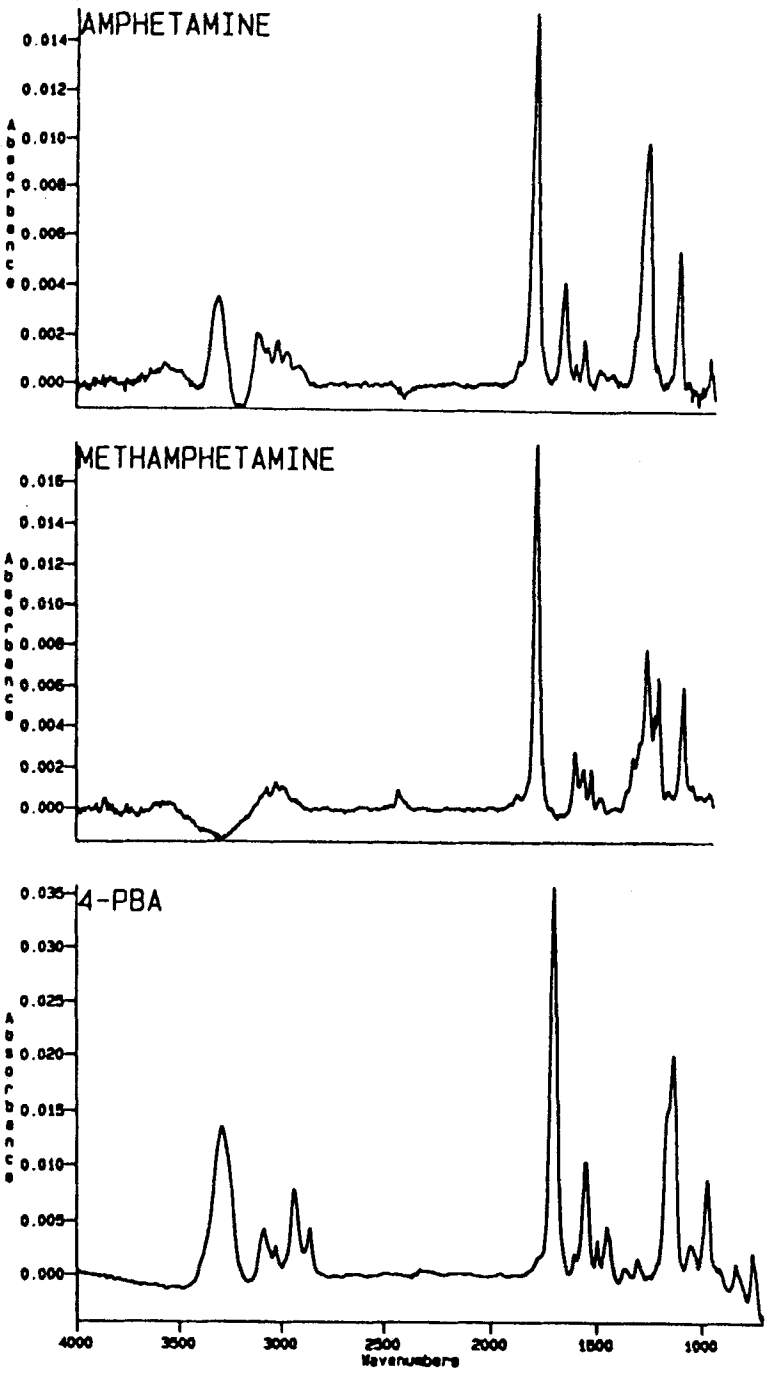

FIGURE 9. Comparative IR spectra of the two target drugs, amphetamine and methamphetamine, along with that of the internal standard, 4-PBA, from the cryogenic deposition GC/FT-IR.

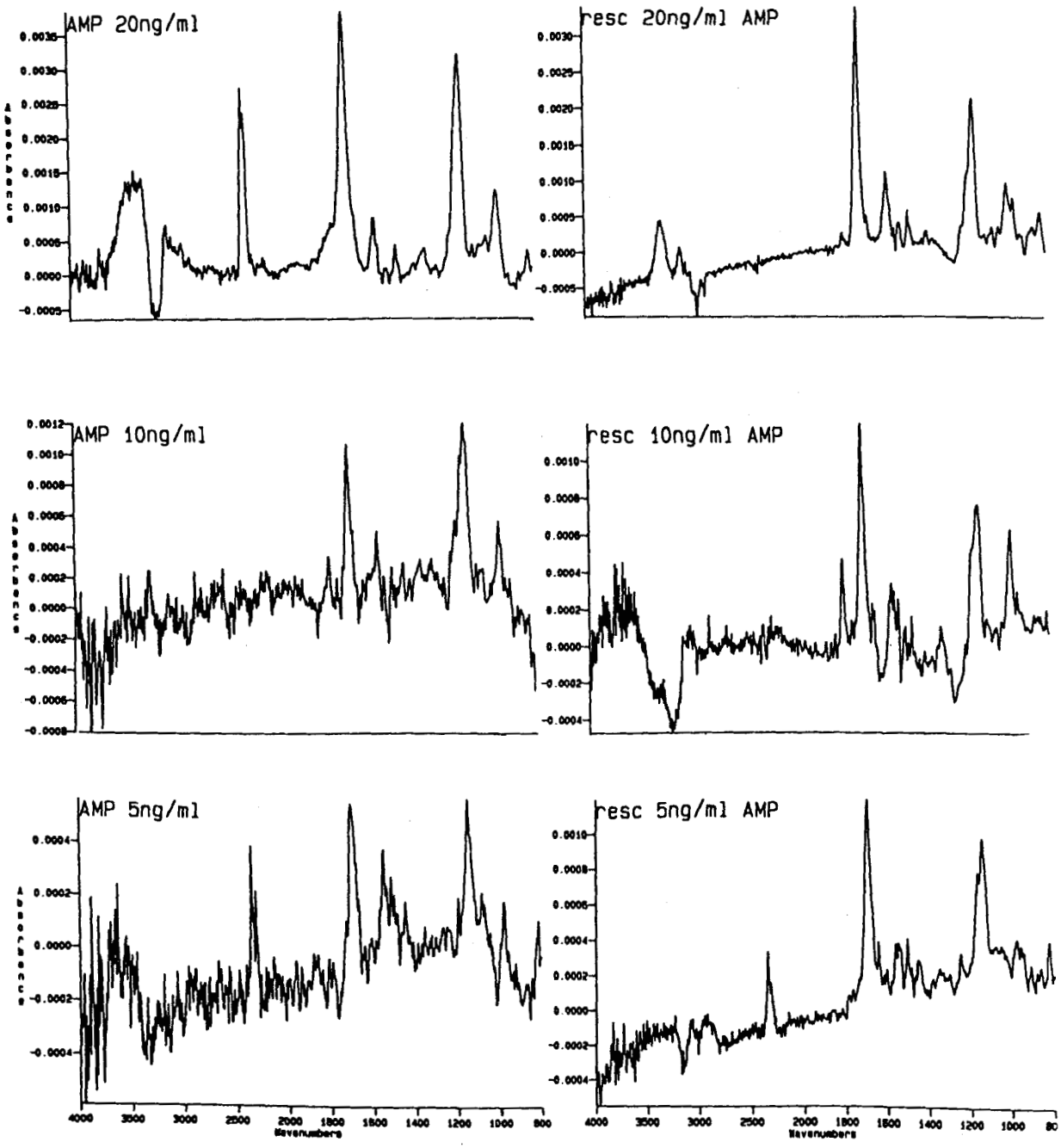

FIGURE 10. The cryogenic deposition GC/FT-IR spectra of amphetamine (derivatized with chlorodifluoroacetic anhydride) collected for the detection limit study. Left column of spectra are collected in real time (four scans per data file); spectra in right column are collected post-run with 256 signal averaged scans. The spectra represent sample concentrations extracted from urine matrix of 20, 10 and 5 ng/ml from top to bottom which correspond to an absolute amount detected of 46, 23 and 11 pg, respectively.

are in place for some GC/FT-IR manufacturers and soon to be released for others. The sample preparative methods for both the GC/MS and GC/FT-IR are the same or similar. Although GC/FT-IR has come as a second generation to GC/MS, its current state of development is not far behind.

VI. CONCLUSIONS

GC/FT-IR is beginning to break ground as a viable technique in the forensic toxicology lab. GC/FT-IR methods for alternate drugs are being developed.[33,34] IR certainly meets the criteria for analysis of drugs of abuse and can, in some instances, surpass the mass spectrometer in sensitivity limits. Future developments could make GC/FT-IR a more common tool in the drug testing laboratory.

A. IR vs. MS

The biggest advantage of using GC/FT-IR is the specificity of the data with "fingerprint" identification. The confidence of the analysis will be much greater. Isomers can be differentiated and, therefore, detection of "designer drugs" can be obtained with a routine analysis. Sensitivities are now comparable to MS limits and can be increased with off-line techniques, if the need arises. One drawback of using the GC/FT-IR technique is that the IR detector responds to everything eluting from the chromatograph, consequently, extractions need to be fairly clean. Co-eluting peaks also cause difficulty. Spectral subtraction is possible in IR for these cases, but detection by isolation of single ions, as in MS, is not readily available. Monitoring single IR group frequencies is a valid detection mode, but finding isolated group frequencies in IR is not as straightforward as finding an isolated ion fragment.

Combining IR and MS into one tandem GC/IR/MS system can be advantageous. Two pieces of confirmation data are obtainable with one injection. The two techniques complement one another, and interactive reference searches can increase the confidence of the data to greater than that of either single piece of information.[25,26] Where one unit has deficiencies, the other has strengths.

B. Alternate Techniques

Future areas of investigation will also incorporate alternate separation techniques interfaced to the tandem analytical instrumentation so that little or no sample preparation is necessary prior to sample introduction into a totally automated system. This may include separation techniques such as supercritical fluid chromatography (SFC) for an SFC/IR/MS system, or a cartridge liquid chromatographic purification and separation analyzer as the sample introduction system to a tandem IR/MS. Units such as these would be the ideal laboratory scheme allowing direct injection of biological specimens with purification, separation, identification, confirmation, and quantitation all in one.

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