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Comparison of direct deposition and flow-cell gas chromatography–Fourier transform infrared spectrometry of barbiturates

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Abstract

Comparison of flow-cell and direct deposition (DD) interfaces between gas chromatographs (GC) and Fourier transform infrared (FT-IR) spectrometers is reported. Seven barbiturates were separated on fused-silica GC capillary columns. Infrared spectra of the separated barbiturates were measured in real-time by either a Hewlett-Packard infrared detector (flow-cell) interface or a Digilab Division of Bio-Rad Tracer (DD) interface. Without losing chromatographic resolution, the DD GC–FT-IR interface gave both detection limits and minimum identifiable quantities nearly two orders of magnitude lower than the flow-cell GC–FT-IR interface.

1. Introduction

The combination of gas chromatography (GC), for separating mixtures into individual components, and Fourier transform infrared (FT-IR) spectrophotometry, for identifying each analyte, has been accomplished by three different approaches. The first and simplest approach is to use a light-pipe flow-cell through which the effluent from the GC column is passed while the infrared spectra are continuously measured [1–3]. The main disadvantage to this approach is its lack of sensitivity, with the typical minimum identifiable quantity (MIQ) being in the order of 10 ng. To overcome this lack of sensitivity, two other approaches were developed. The first of these is based on matrix isolation (MI) spectrometry [3–6]. In the MI approach, argon gas is mixed with the helium mobile phase. The eluites are then frozen in a track of solid argon with a width of around 300 μm which is deposited on a gold-plated metallic disk cooled to a temperature of about 13 K. After the deposition is complete, the analytes are rotated into the infrared beam where their reflection/absorption spectra are measured. This approach reduced the MIQs by about an order of magnitude with respect to the flow-cell, but was instrumentally fairly complex. The third approach was developed in our laboratory [7–10], commercialized by the Digilab Division of Bio-Rad Laboratories [11] and applied by several groups [12–18]. The eluites are directly deposited as a track with a width of about 100 μm on a moving ZnSe window cooled by liquid nitrogen. Unlike the GC–MI-FT-IR technique as currently implemented, the spectra are measured continuously a short time after deposition, as the eluites pass through the infrared beam. This

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direct deposition (DD) technique is very similar to the flow-cell technique in that the continuous measurement of infrared spectra allows for the construction of chromatograms. On the other hand, DD GC–FT-IR spectra are very different from vapor-phase spectra and have been suggested to be more similar to reference spectra of samples prepared as KBr disks [11,12,19,20].

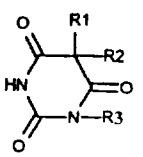
Barbiturates are an important class of pharmaceuticals that are used as sedatives and hypnotics, but have the potential to be abused. They are classified as schedule II and schedule III controlled drug substances. The similarities in the structures of the barbiturates (see Table 1) make class identification by infrared spectrophotometry relatively easy [21], but the identification of the individual compounds by this technique is difficult. The success or failure of identifying individual barbiturates by GC–FT-IR is, therefore, an excellent test for this technique. If spectral searching can successfully distinguish the barbiturates, then distinguishing less spectroscopically similar molecules from their GC–FT-IR spectra should be a relatively simple matter. The feasibility of distinguishing individual barbiturates by DD GC–FT-IR was briefly described by Bourne et al. [11] in their first report of the Digilab/Bio-Rad DD GC–FT-IR interface. In the present paper, we report a considerably more detailed investigation of the GC–FT-IR spectra of barbiturates made using both a light-pipe and a direct-deposition interface. Differences in the vapor-phase and condensed-phase spectra of barbiturates are examined and the chromatographic resolution, sensitivity and identifying power of flow-cell and DD GC–FT-IR interfaces are compared.

2. Experimental

The names, structures and elution order of the seven barbiturates investigated are shown in Table 1. All were available as 1 mg/ml solutions in methylene chloride from a ThetaKit (Chemical Research Supplies, Addison, IL, USA).

Gas chromatographic separations for the flow-cell measurements were performed on a 60 m × 250 μm I.D. capillary GC column with a 0.25 μm thick film of 5% phenyl-, 95% methyl polysiloxane (DB-5) (J&W Scientific, Folsom, CA, USA) used as the stationary phase. The column was installed in a Hewlett-Packard 5890A gas chromatograph that was attached to a Hewlett-Packard Model 5965B infrared detector (IRD). All IRD spectra were taken in real-time at a resolution of 8 cm^{-1} as the effluent passed through the light-pipe, which was maintained at 250°C. The temperature program used for the separation was: 40°C (1 min); 50°C/min to 150°C; 5°C/min to 240°C; hold at 240°C (5 min).

Table 1
Elution order, name and structure of barbiturates, where the basic structure is



Elution order	Compound name	Structure		
		R1	R2	R3
1	Barbital	C_2H_5	C_2H_5	H
2	Aprobarbital	$CH_2CH{=}CH_2$	$CH(CH_3)_2$	H
3	Butabarbital	C_2H_5	$CH(CH_3)CH_2CH_3$	H
4	Amobarbital	C_2H_5	$CH_2CH_2CH(CH_3)_2$	H
5	Secobarbital	$CH_2CH{=}CH_2$	$CH(CH_3)CH_2CH_2CH_3$	H
6	Mephobarbital	C_2H_5	C_6H_5	CH_3
7	Phenobarbital	C_2H_5	C_6H_5	H

Injections of 0.5 μl were made with a 10:1 inlet split ratio.

DD GC-FT-IR separations were performed on a 30 m × 25 μm I.D. capillary GC column with a 0.25 μm thick film of DB-5 (J&W Scientific) used as the stationary phase. The column was installed in a Hewlett-Packard 5890A gas chromatograph using the same temperature program as for the IRD data. The infrared spectra were measured using the Tracer (Bio-Rad, Digilab Division, Cambridge, MA, USA) GC–FT-IR interface attached to a Digilab Model FTS-40 infrared spectrophotometer. The eluites were deposited on a ZnSe window that was maintained at a temperature below 100 K. All DD GC–FT-IR spectra shown in this paper were taken as the eluites passed through the infrared beam (real-time) with a measurement time of about 4 s and at a resolution of 8 cm^{-1}. Injections of 1 μl were made with a 30:1 or a 60:1 inlet split ratio.

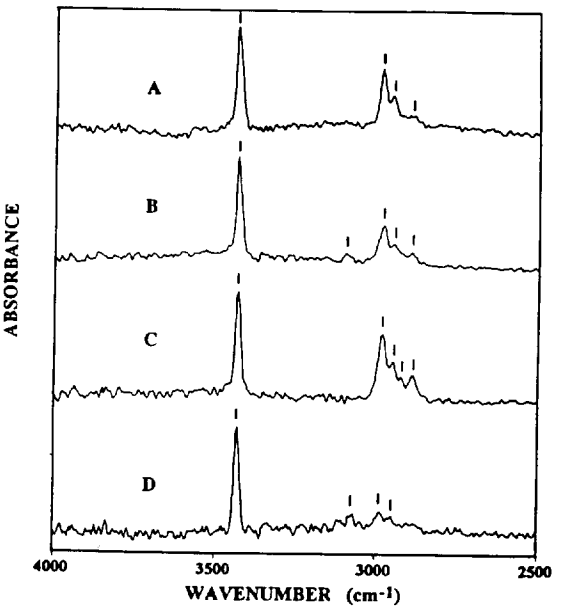


Fig. 1. Flow-cell GC–FT-IR spectra of (A) barbital, (B) aprobarbital, (C) butabarbital and (D) phenobarbital injected at a level of 12.5 ng. Tick marks correspond to the wavenumbers listed in Table 2.

3. Results and discussion

3.1. Band shifts

The spectra of polar, hydrogen-bonded compounds such as the barbiturates are more affected by their phase and local environment than spectra of non-polar compounds. The light-pipe and DD infrared spectra of four of the barbiturates in the spectral region between 4000 and 2000 cm^{-1} are shown in Figs. 1 and 2, respectively. The spectra in the lower wavenumber region of the same four barbiturates from the IRD (flow-cell) and Tracer (DD) interfaces are shown in Figs. 3 and 4. Peak positions of the stronger bands (indicated by tick marks in Figs. 1–4) were obtained using the GRAMS/386 (Galactic Industries, Salem, NH, USA) peak picking algorithm and are shown in Tables 2–4.

The difference in the spectra obtained using the two interfaces is very evident, with all bands assignable to the N–H and C=O groups exhibiting very large shifts caused by the fact that the molecules are strongly intermolecularly hydrogen-bonded in the condensed phase and completely isolated in the vapor phase. For example, in the vapor phase, the N–H stretching absorp-

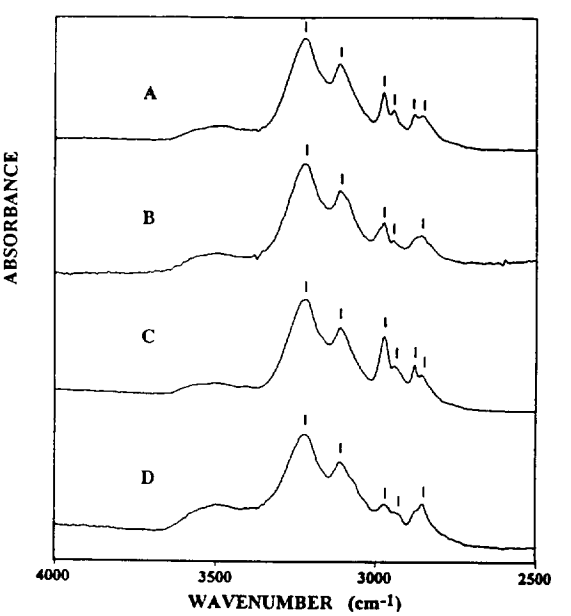


Fig. 2. DD GC–FT-IR spectra of (A) barbital, (B) aprobarbital, (C) butabarbital and (D) phenobarbital injected at a level of 375 pg. Tick marks correspond to the wavenumbers listed in Table 2.

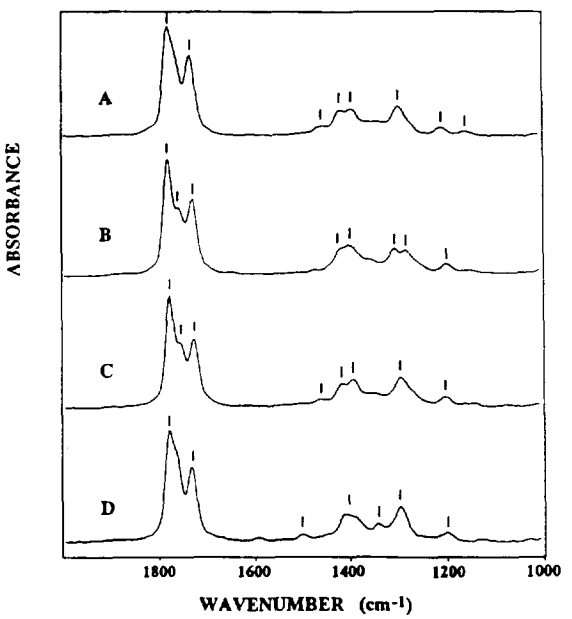


Fig. 3. Flow-cell GC–FT-IR spectra of (A) barbital, (B) aprobarbital, (C) butabarbital and (D) phenobarbital injected at a level of 12.5 ng. Tick marks correspond to the wavenumbers listed in Tables 3 and 4.

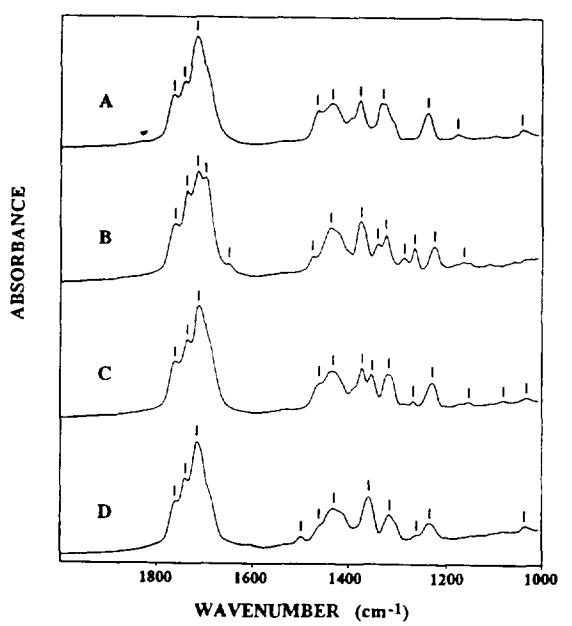


Fig. 4. DD GC–FT-IR spectra of (A) barbital, (B) aprobarbital, (C) butabarbital and (D) phenobarbital injected at a level of 375 pg. Tick marks correspond to the wavenumbers listed in Tables 3 and 4.

tion is manifested as a single sharp band at about 3430 cm^{-1}, while in the DD GC–FT-IR spectra (condensed phase with no diluent), there are two broad bands at about 3320 and 3110 cm^{-1} and one or two less intense bands at about 2960 cm^{-1} that overlap the C–H stretching modes.

There are also large differences in the spectral region below 2000 cm^{-1} (see Figs. 3 and 4). For example, the C=O stretching bands are shifted about 20–25 cm^{-1} lower in the DD spectra compared to the vapor-phase spectra. There are also dramatic differences in the relative intensities of these bands. In vapor-phase spectra, the highest wavenumber C=O band is the most intense, while in condensed-phase spectra the most intense band is the one at the lowest wavenumber of the C=O multiplet.

Examination of the spectra in the fingerprint region between 1600 and 1000 cm^{-1} shows less obvious differences, the most notable being the reduction in the number of bands in the vapor-phase spectra. The C–N stretching and N–H, C=O and C–H bending modes absorb in this region of the spectrum. Bands due to N–H, C=O and, to a lesser extent, C–N moieties should be shifted with respect to the vapor-phase spectra by the effect of hydrogen bonding, and indeed most of these bands are shifted by about 10 to 20 cm^{-1}. Conversely, the C–H bending modes would not be expected to shift dramatically when the phase of the analytes changes. In light of the weakness of the C–H stretching modes in Figs. 1 and 2 and the fact that the absorptivity of the C–H bending modes is often at least an order of magnitude less than that of the corresponding C–H stretching bands, it is probable that all C–H bending modes would fall below the noise level in these spectra, and only those bands assignable to polar functional groups that are affected by hydrogen bonding are observed strongly in the fingerprint region. The effect on spectral searching of changing from the vapor phase to the condensed phase is, therefore, significant and will be discussed later in this paper. The assignment of the absorption bands in each spectrum has not been attempted as it is not our intent to demonstrate yet again the well-known power of infrared spectrometry for structural elucidation. Instead it was our goal to

Table 2
Wavenumbers of bands in the N–H and C–H stretching region of the Tracer spectra and the IRD spectra of four barbiturates

Barbital	Aprobarbital	Butabarbital	Phenobarbital
Tracer spectra			
3222	3224	3216	3222
3111	3111	3108	3111
2973	2972	2972	2972
2942	2943	2941	(2944)
2879		2879	
2855	2857	2857	2855
IRD spectra			
3432	3431	3431	3432
	3093		3070
2982	2980	2980	2985
2953	2949	2949	2953
2898	2895	2890	

Number in parenthesis means band is an unresolved shoulder.

investigate the feasibility of using computerized spectral searching algorithms for vapor-phase and condensed-phase spectra of the type measured by light-pipe and DD GC–FT-IR instruments, respectively.

3.2. Resolution

Chromatograms were reconstructed from FT-IR data by Gram Schmidt (GS) vector orthogonalization on the interferogram [22] and by integrating the absorbance between specified wavenumber limits in the spectrum. Examination of the GS reconstructed chromatograms (see Fig. 5) from the IRD and Tracer interfaces shows that there is virtually no decrease in the chromatographic resolution with the Tracer as compared to the IRD. For example, the resolution of aprobarbital and butabarbital (peaks 2 and 3) and of mephobarbital and phenobarbital (peaks 6 and 7) were measured for both chromatograms. The resolution of the aprobarbital and butabarbital peaks was 7.0 and 5.1 for the chromatograms measured using the IRD and

Table 3
Wavenumbers of the bands in the C=O stretching region of the Tracer spectra and the IRD spectra of four barbiturates

Barbital	Aprobarbital	Butabarbital	Phenobarbital
Tracer spectra			
1759	1757	1758	(1759)
	1731	1732	1737
1711	1710	1710	1711
	1694		
	1646[a]		
IRD spectra			
1774	1776	1776	1776
	1756	1755	
1730	1725	1726	1730
	1645[a]		

Number in parenthesis means band is an unresolved shoulder.
[a] Weak band.

Table 4
Wavenumbers of bands in the fingerprint region below 1600 cm^{-1} of the Tracer spectra and the IRD spectra of four barbiturates

Barbital	Aprobarbital	Butabarbital	Phenobarbital
Tracer spectra			
			1496
1457	1470	(1458)	(1462)
1428	1432	1429	1429
1371	1370	1369	1356
1325	1335	1349	
	1318	1314	1314
	1283		
	1261	1265	(1264)
1233	1221	1226	1232
1172[a]	1162[a]	1150[a]	
		1078[a]	
1036		1028[a]	1034[a]
IRD spectra			
			1498
1452		1459	
1415	(1416)	1414	1409
1394	1398	1393	
			1341
1296	1303	1294	1296
	1282		
1208	1198	1202	1201
1160[a]			

Number in parenthesis means band is an unresolved shoulder.
[a] Weak band.

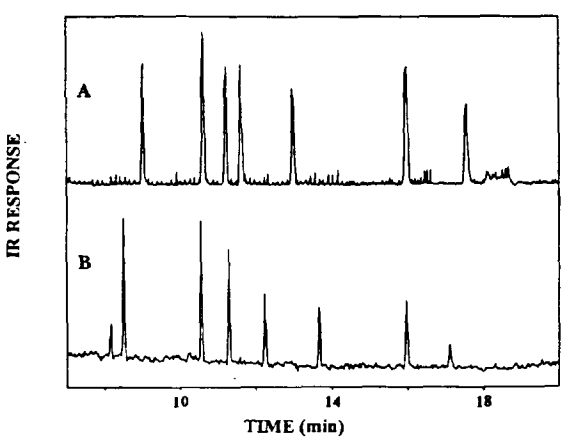


Fig. 5. Gram-Schmidt chromatograms of (A) an injection of 375 pg per component of a mixture of seven barbiturates into a DD GC–FT-IR instrument and (B) an injection of 12.5 ng per component of a mixture of seven barbiturates into a flow-cell GC–FT-IR instrument.

Tracer, respectively, while the corresponding values for the mephobarbital and phenobarbital peaks were 10.6 and 9.8. The loss in resolution for the Tracer data is less than the $\sqrt{2}$ loss expected from the column length being one-half of the length of the column used in conjunction with the IRD, presumably because of a small difference in linear velocity of the mobile phase between these runs. Thus when the operating parameters of the DD interface are optimized, it can be seen that there is no apparent loss in chromatographic resolution

3.3. Sensitivity

The chromatograms obtained by integrating the absorbance over the range of 1780 to 1680 cm^{-1} for the Tracer, called functional group

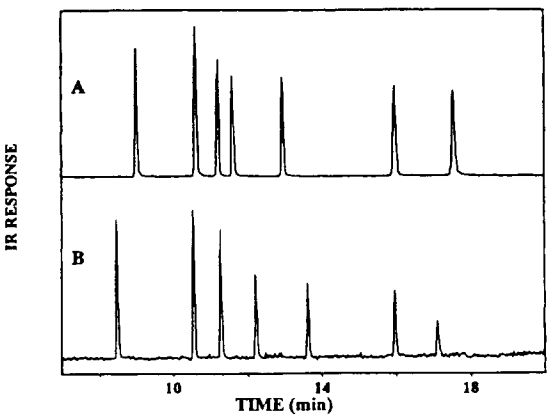


Fig. 6. Chromatograms from integrating the absorbance over the spectral region of (A) 1780–1680 cm^{-1} for the corresponding GS chromatogram shown in Fig. 5A, and (B) 1800–1600 cm^{-1} for the corresponding GS chromatogram shown in Fig. 5B.

(FG) chromatograms by Digilab, and 1800 to 1600 cm^{-1} for the IRD, called selected-wavelength (SW) chromatograms by Hewlett-Packard, are shown in Fig. 6. Note that these ranges are different because the C=O stretching modes of vapor-phase barbiturates are narrower and absorb at a significantly lower wavenumber than the same vibrational modes in condensed-phase spectra; nevertheless, both ranges that were selected encompass the entire region of the C=O stretching absorption for the appropriate measurement. Comparison of the FG and SW chromatograms measured on the IRD shows that the signal-to-noise ratio (SNR) of the FG chromatogram is better than that of the GS chromatogram. It is difficult to compare the SNR of the GS and FG chromatograms measured with the Tracer, as spikes may be seen across the GS chromatogram. The "true" noise can be seen in the region of the baseline just before peak 7 in the GS chromatogram shown in Fig. 5A. These noise-like features in the GS chromatogram in Fig. 5A probably arise from small baseline shifts in the spectra. This phenomenon is often seen in GS chromatograms, but the FG and SW chromatograms are less susceptible to such artifacts and can often be used to detect trace components (especially if the analytes have a large absorption in one region of the spectrum, e.g., the C=O stretch for barbiturates).

To compare the sensitivities of DD GC–FT-IR data to flow-cell GC–FT-IR data, the SNR was calculated for both the chromatograms and the spectra from the Tracer and the IRD interface of the peak due to amobarbital (peak 4). The absorption spectra for each chromatographic peak were obtained from both instruments by averaging all of the spectra collected between the half-height points of the peak. Injections of 25, 12.5, 5 and 2.5 ng and of 750, 375, 150 and 75 pg were made for measurements on the IRD and the Tracer, respectively. The peak-to-peak noise was determined for 1-min portions of the chromatograms where no peaks were eluting and between 2200 and 2000 cm^{-1} for the spectra. Linear plots of SNR versus amount injected were calculated with a forced y-intercept of 0 and the injected quantity required to yield a signal three times greater than the noise level was determined. The detection limit of the barbiturates was called the $3 \times$ SNR point.

For data measured using the IRD, the detection limits were 870, 870 and 750 pg using the GS chromatograms, SW chromatograms and absorbance spectra, respectively, or an average of about 800 pg. For the DD interface, the detection limit was calculated to be 30, 11 and 10 pg for the GS chromatograms (using the "true" noise), FG chromatograms and absorbance spectra, respectively, or an average of about 17 pg.

IRD spectra of amobarbital injected at levels

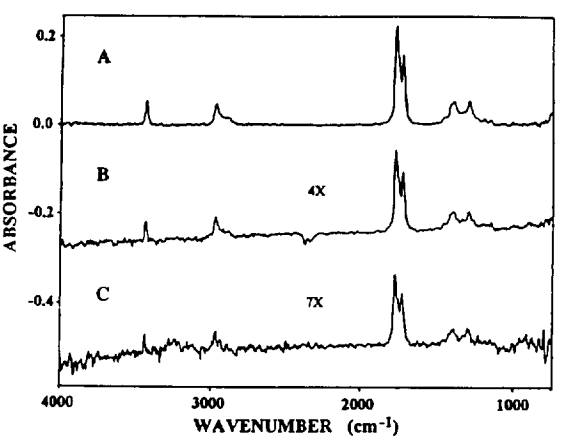


Fig. 7. IRD spectra of amobarbital injected at a level of (A) 12.5 ng, (B) 5 ng and (C) 2.5 ng.

of 12.5, 5 and 2.5 ng are shown in Fig. 7. As can be seen from this figure, the MIQ is at about 5 ng injected (which is about 5 times the detection limit). Tracer spectra of amobarbital injected at levels of 750, 150 and 75 pg are shown in Fig. 8. The spectra in this figure indicate the MIQ for DD GC–FT-IR spectra to be about 75 pg injected. Again, there is over a fifty-fold difference in the MIQ of spectra obtained by the two techniques. The DD spectra also show the evidence of icing of the detector or ice co-depositing with the eluites on the window.

The theoretical increase in sensitivity of direct deposition over light-pipe based measurements is a factor of 10, assuming that the area of the detector matches the area of the sample and neglecting reflection losses from the light pipe [11]. Except for the GS chromatograms, which are susceptible to artifactual noise spikes, the actual difference is between 40 and 70. Presumably the increased difference between theoretical and actual sensitivity is because the transmittance of light-pipes is only about 20%.

3.4. Identification

Spectral searching of the barbiturate spectra was performed using LabCalc (Galactic Industries) software, that generates a hit quality index (HQI) that is a measure of the similarity of the spectrum of the analyte and each reference spectrum in the database. Since this software uses an Euclidean distance search metric, the lower the value of the HQI calculated, the better is the spectral match. The DD spectra were searched against the Georgia State Crime Laboratory (GSCL) Library of Infrared Spectra of Commonly Abused Drugs, most of which are of samples prepared as KBr disks. The vapor-phase libraries available for this study do not contain any reference spectra of barbiturates. Since there are large differences in the vapor-phase spectra compared to the condensed-phase spectra, the flow-cell spectra were searched against a library created from the GC–FT-IR spectra measured by the IRD of 50-ng injections of the seven barbiturates.

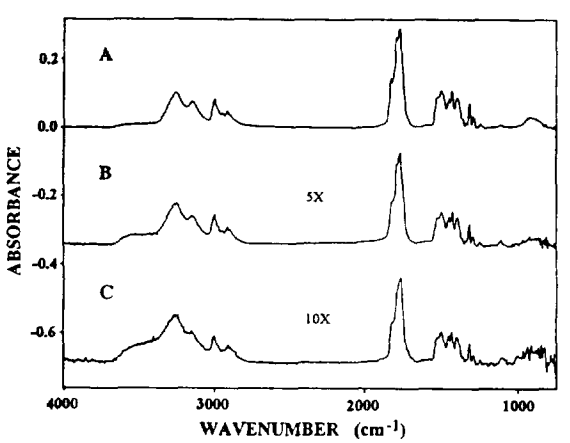


Fig. 8. Tracer spectra of amobarbital injected at a level of (A) 750 pg, (B) 150 pg and (C) 75 pg. Note the evidence of ice in the spectra at about 3400 cm $^{-1}$ (especially in B and C).

For the DD GC–FT-IR spectra, the search results usually showed the authentic compound to be the best match (hit) or in the top two "hits" found when searching the spectra from injections of 750, 375 and 150 pg. The exceptions were for phenobarbital and barbital, which had been found from a more intensive study of the factors affecting spectral searching to yield poor search results because of their polymorphism [23], i.e., it is very likely that the crystal form in which barbital is deposited in the DD GC–FT-IR interface is different from that in the GSCL library. A new KBr disk of barbital was carefully prepared and its spectrum was measured. When this spectrum was included in the GSCL library, the authentic compound was the first hit for all the DD GC–FT-IR spectra of barbital measured in this study, demonstrating the need for high-quality reference data for successful spectral searching. In all cases except those examples noted above, the authentic compound was always in the top five hits and the top twenty hits were all barbiturates. Better spectral search results were usually found on including only the region from 2000 to 715 cm $^{-1}$, i.e., the authentic compound either moved to higher position in the hit list or, if the authentic compound was already the top hit, a larger difference in its HQI as compared to the next highest hit was obtained. This observation is attributed to the N–H stretching modes being more susceptible to changes in the local environment than the rest of

the absorption bands in the DD GC–FT-IR and KBr-disk spectra. Including only the region below 2000 cm^{-1} was necessary to obtain good results when searching the spectra of the 75-pg injections. The results from these searches were then comparable to the results from the spectra of the larger injections, presumably because of lower SNR in the region of the N–H and C–H stretching bands and the interference from the absorption caused by ice (see Fig. 8C). These results show that the MIQ of the real-time DD GC–FT-IR spectra of barbiturates is about 75 pg, confirming the conclusion made somewhat intuitively from a visual examination of the spectra in Fig. 8 (vide infra). It should also be noted that because the eluites are trapped on the ZnSe window, it is possible to translate the stage to move the analytes back into the infrared beam after the chromatography is complete and perform post-run signal averaging [11,13]. Increasing the data acquisition time from a few seconds to about one minute increases the SNR by about a factor of four and should lower the MIQs by a comparable amount.

When flow-cell GC–FT-IR spectra of barbiturates were searched against the library of the vapor-phase barbiturate spectra, the spectra from the 12.5-ng injections usually yielded the authentic compound as the best match, even though some of the results showed very little difference in the HQIs of the top few hits. The results for the IRD spectra searched against the GSCL library did not even give barbiturates as the first hits. Earlier work [11] showed that it was sometimes possible to successfully search DD GC–FT-IR spectra of non-polar compounds against vapor-phase spectral libraries. If, however, the analytes are capable of hydrogen bonding (such as the barbiturates), searching vapor-phase spectra against condensed-phase spectral libraries or vice versa never permits the identification of the analytes (no matter how limited the spectral region selected).

Searching the 5-ng light-pipe GC–FT-IR spectra against the very small library of vapor-phase barbiturate reference spectra gave mixed results. The authentic compound was the top hit in some cases but was only the third or fourth hit in others. Using only the region from 2000 to 750 cm^{-1} again gave better results for most compounds. The corresponding spectra of the 2.5-ng injections were too noisy to be used for library searching, even when the region above 2000 cm^{-1} was omitted. Mephobarbital (the only compound with a tertiary amine group) was correctly identified when only 2.5 ng was injected, but the HQI was about 0.7 when the whole spectrum was searched and 0.5 for the low wavenumber region of the spectra. These values can be compared to HQIs of around 0.3 or less for the 12.5-ng spectra and the DD GC–FT-IR spectra of injections of 150 pg or greater. Since the IRD spectra are measured as the effluent passes through the light-pipe, there is no way to improve the SNR by increasing the measurement time. Therefore, the MIQ of the IRD for barbiturates, which are unusually strong infrared absorbers, is around 5 ng.

4. Conclusions

We have demonstrated that a DD GC–FT-IR interface has detection limits and MIQs that are almost two orders of magnitude lower than a flow-cell GC–FT-IR interface for real-time measurements. The DD interface achieves the same chromatographic resolution as a flow-cell interface. Compounds that can undergo strong intermolecular hydrogen-bonding have very different spectra in the vapor phase and condensed phase, and these differences are exhibited in all regions of the GC–FT-IR spectra. Thus, whereas light-pipe GC–FT-IR spectra must be searched against vapor-phase spectral libraries (which seldom contain more than 12 000 entries), it is possible to search DD GC–FT-IR spectra against much larger condensed-phase infrared spectral libraries for which the samples have been prepared as KBr disks, provided that the region above 2000 cm^{-1} is omitted.

Acknowledgement

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