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Enhancement of Drug Detection and Identification by Use of Various Derivatizing Reagents on GC-FTIR Analysis

AUG 24 1992

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July 1992

Final Report

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Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-92/25		

4. Title and Subtitle	5. Report Date
Enhancement of Drug Detection and Identification by Use of Various Derivatizing Reagents on GC-FTIR Analysis	July 1992
	6. Performing Organization Code
7. Author(s)	8. Performing Organization Report No.
Edwin F. Huffine and Dennis V. Canfield	
9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
FAA Civil Aeromedical Institute P.O. Box 25082 Oklahoma City, Oklahoma 73125	11. Contract or Grant No.
	13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address Office of Aviation Medicine Federal Aviation Administration 800 Independence Avenue, S.W. Washington, D.C. 20591	14. Sponsoring Agency Code

15. Supplementary Notes

Work was accomplished under approved task AM-B-91-TOX-66

16. Abstract

Phenylpropanolamine (PPA) is a relatively common non-prescription sympathomimetic amine. As such, it is frequently detected during forensic analysis. The presence of phenylpropanolamine can be confirmed by using Gas Chromatograph-Fourier Transform Infrared (GC-FTIR) spectrophotometry. One constraint of the GC-FTIR is the quantity of material required to obtain a suitable IR spectrum. If a drug is a relatively weak infrared absorber, several micrograms may be required in order to obtain a clear, reliable spectrum. While this amount of material may be readily available for some types of analysis, it can easily exceed the quantity of material available in the forensic toxicology setting. One method that can be used to increase a drug's infrared absorption is to derivatize the drug with a polyfluorinated acid anhydride. Since carbonyl and carbon-fluorine bonds are strong infrared absorbers, molecules that possess such bonds have a heightened sensitivity to GC-FTIR analysis. Polyfluorinated acid anhydrides are capable of adding both carbonyl and carbon-fluorine bonds to drugs that possess either a hydroxyl, primary or secondary amine, or primary or secondary amide functional groups. Several derivatizing reagents were used and the extent to which they enhanced the identification of phenylpropanolamine were compared. Of the reagents studied, heptafluorobutyric acid anhydride (HFAA) produced the greatest increase in the phenylpropanolamine's sensitivity to GC-FTIR identification. Prior to derivatization, 1.8 micrograms of phenylpropanolamine was required for identification on the GC-FTIR, while only 0.032 micrograms of phenylpropanolamine was required after derivatization with HFAA.

17. Key Words	18. Distribution Statement
GC-FTIR, Drugs, Phenylpropanolamine	Document is available to the public through the National Technical Information Service Springfield, Virginia 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	9	

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Enhancement of Drug Detection and Identification by Use of Various Derivatizing Reagents on GC-FTIR Analysis

INTRODUCTION

With only a few exceptions, the infrared (IR) spectra of molecules are unique. This often permits accurate identification of a molecule based solely on its IR spectrum (1). Even in the case of D-pseudoephedrine and L-ephedrine, which are stereoisomers of each other, infrared spectroscopy produces unique spectra (Fig. 1 & Fig. 2). When infrared spectroscopy is combined with gas chromatography, a powerful analytical methodology is created for the identification of drugs in a complex biological matrix.

A weakness of GC-FTIR analysis is the amount of sample required for identification. It may require up to several micrograms of material to produce a valid IR spectrum. The quantity of drug required for GC-FTIR analysis is directly related to the drug's ability to absorb infrared radiation. As the number of carbonyl and/or carbon-fluorine bonds in a drug increases, the absorption of infrared light increases, consequently requiring less drug for identification. This is caused by the strong absorption properties that carbon-fluorine and carbonyl bonds produce in the infrared region of the electromagnetic spectrum.

Phenylpropanolamine (Fig. 3) is a relatively common non-prescription sympathomimetic amine. As such, it is frequently detected during forensic analysis. We have studied the effects of five different phenylpropanolamine derivatives (Fig. 4) on GC-FTIR sensitivity. The five derivatizing agents used are: N,O,-bis-(trimethylsilyl) trifluoroacetamide (BSTFA), acetic anhydride, trifluoroacteic anhydride (TFAA), pentafluoropropanoic anhydride (PFPA), and heptafluorobutanoic acid anhydride (HFAA). The BSTFA does not add any carbonyl or carbon-fluorine bonds to phenylpropanolamine. Its main effect is to improve column chromatography. The acetic anhydride derivative adds two carbonyl functional groups but no carbon-fluorine bonds to phenylpropanolamine. TFAA, PFPA, and HFAA each add one carbonyl functional group as well as three, five, and seven carbon-fluorine bonds per derivatization, respectively. The addition of these various functional groups will provide a quantitative way of comparing the effects that carbonyl and carbon-fluorine functional groups have on the sensitivity of GC-FTIR to phenylpropanolamine.

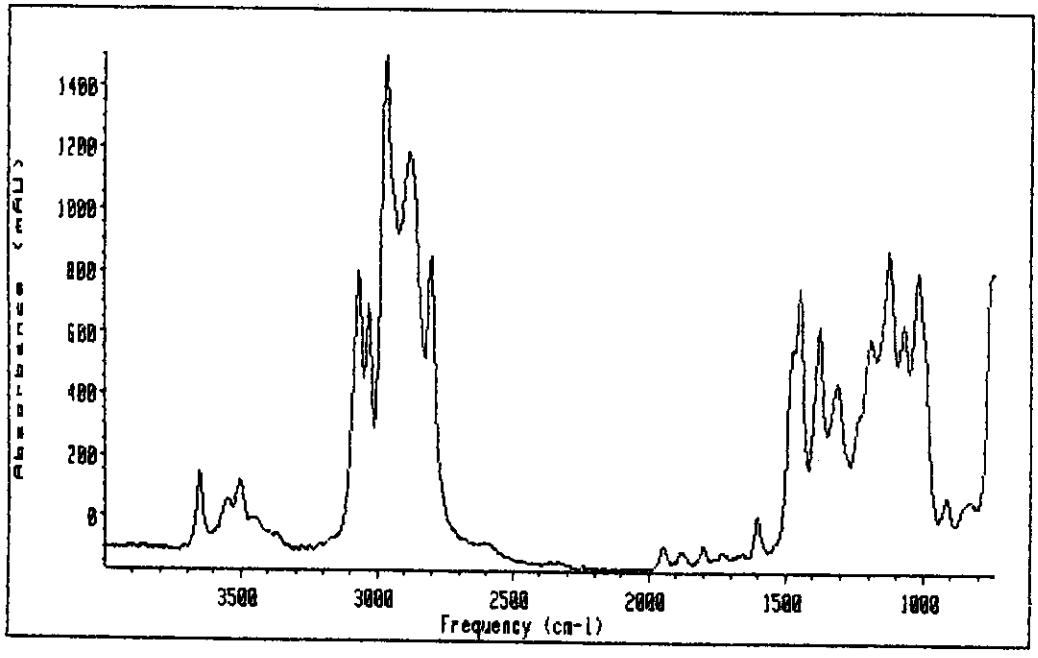


FIGURE 1. IR spectrum of L-ephedrine.

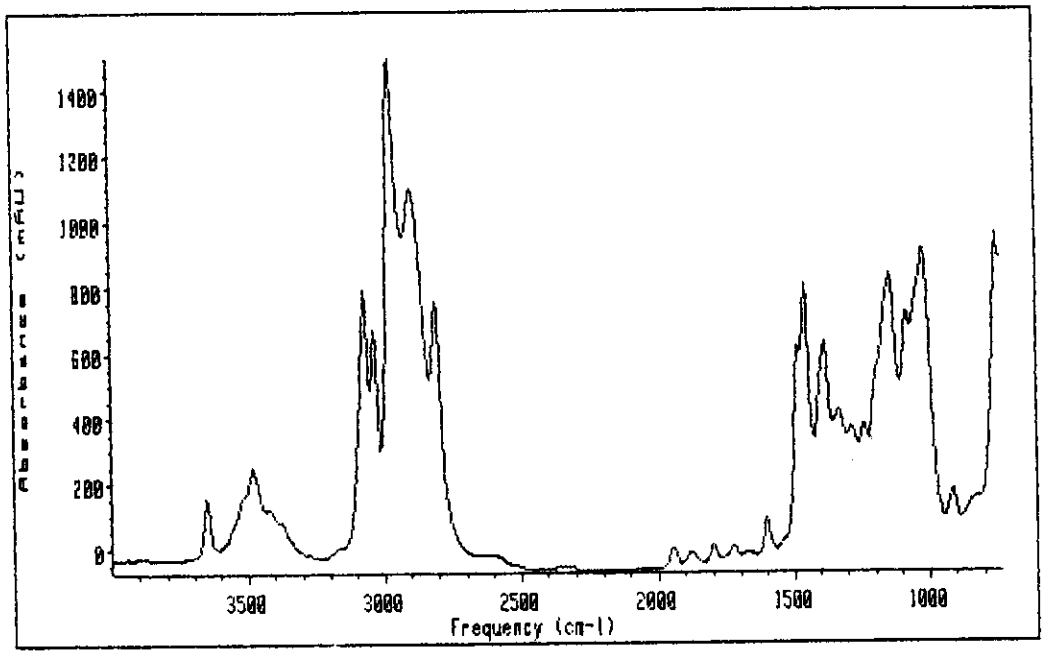


FIGURE 2. IR spectrum of D-pseudoephedrine.

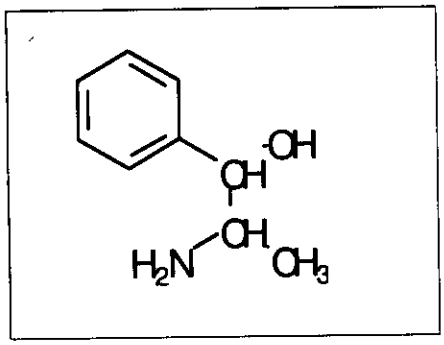


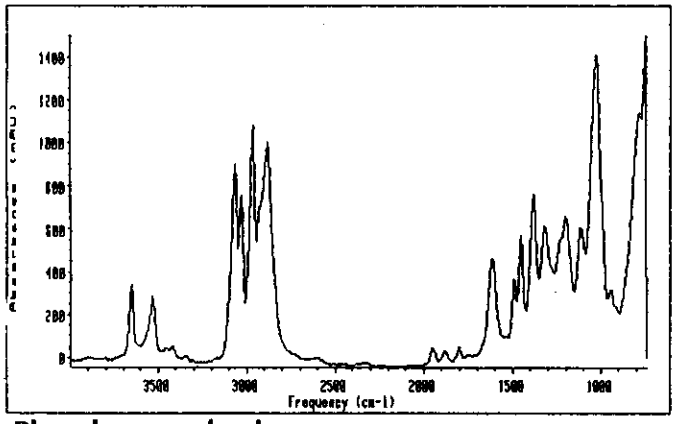
FIGURE 3. Structure of phenylpropanolamine.

METHOD

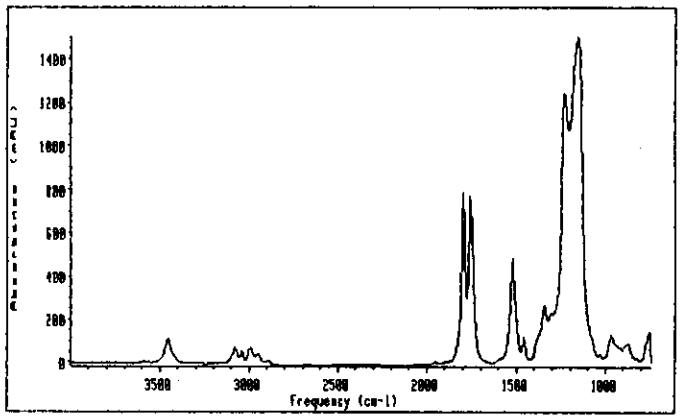
A phenylpropanolamine hydrochloride solution was prepared by placing 0.3144 grams of phenylpropanolamine hydrochloride into a 50 mL volumetric flask and filling to the mark with methanol. This results in a phenylpropanolamine concentration of 5.08 mg/mL.

Next, a doxylamine succinate solution was prepared by placing 0.2134 grams of doxylamine succinate into a 50 mL volumetric flask and filling to the mark with methanol. Doxylamine was chosen as an internal standard because it does not derivatize with any of the derivatizing agents chosen for this study and it has a retention time within 4 minutes of phenylpropanolamine under the conditions used. The resulting doxylamine solution has a concentration of 2.97 mg/mL. A standard mixture was prepared by adding 7.50 mL of the phenylpropanolamine solution to 7.50 mL of the doxylamine internal standard. The standard solution was then vortexed. Six aliquots of 100 uL of the standard mix were withdrawn and placed into six different conical vials. The vials were placed into an N-Evap and the methanol evaporated to dryness. After the evaporation of the methanol, one of the vials had 50 uL of methanol added to it. Each of the remaining vials had 100 uL of one of the following derivatizing reagents added to it: acetic anhydride, BSTFA, TFAA, PFPA, or HFAA. After the addition of the derivatizing reagent, the conical vial was heated at 70°C for 30 minutes.

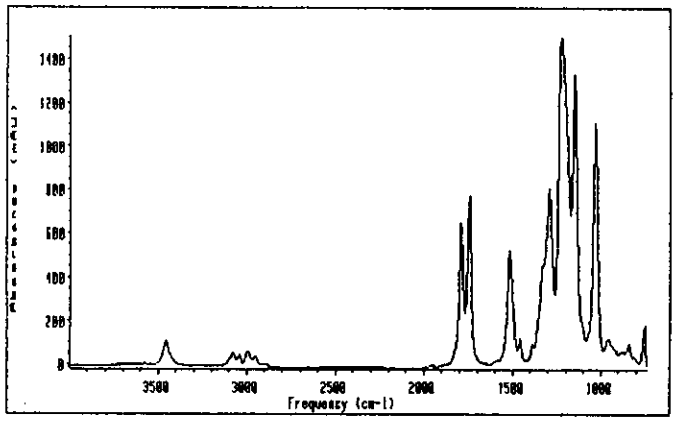
After heating for 30 minutes, the remaining derivatizing reagent was evaporated from the sample by using the N-Evap. A 50 uL sample of methanol was added to the vial and the sample was vortexed. A 1 uL sample of this solution was then injected into the GC-FTIR and the sample was chromatographed using the method described above. While the GC-FTIR analysis was in progress, a 1 uL sample of the derivatized solution was injected into a Hewlett-Packard 5989A mass spectrometer. A positive CI run was made in order to



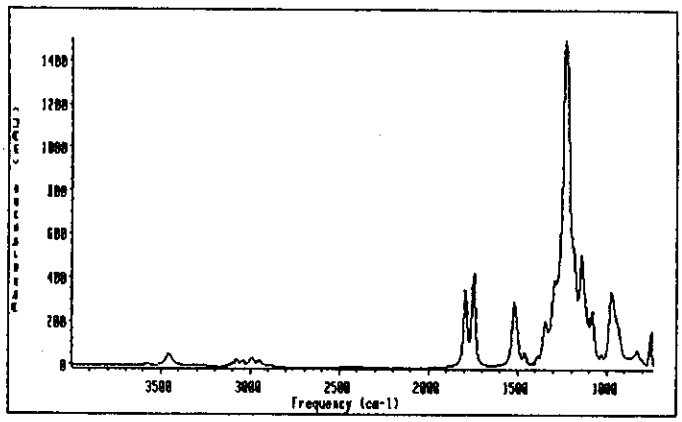
Phenylpropanolamine



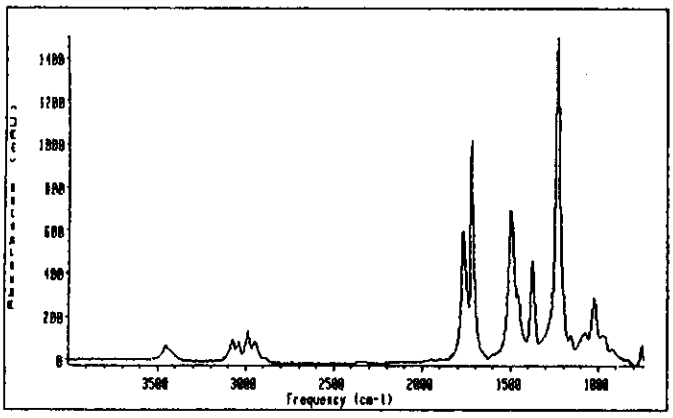
TFAA Derivative



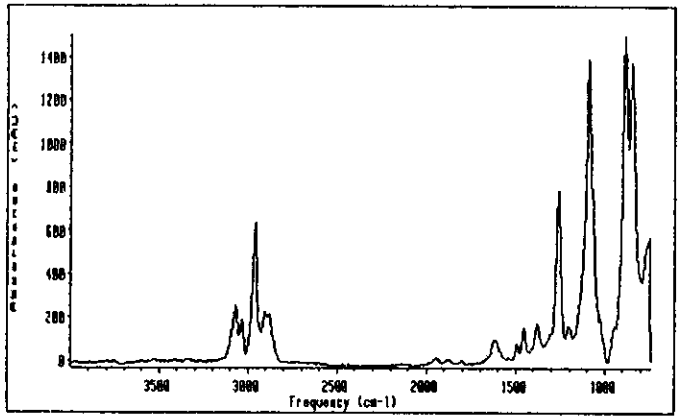
PFPA Derivative



HFAA Derivative



Acetic Anhydride Derivative



BSFTA Derivative

FIGURE 4. IR spectrum of phenylpropanolamine and its derivatives.

determine the mass of each of the derivatized molecules. This entire procedure was repeated three times for each sample. Only after one derivatized sample was completely analyzed was the next sample prepared and analyzed in the same manner. The ratio of the peak areas for phenylpropanolamine or its derivative to doxylamine was calculated and recorded in Table I.

The lower limit of detection and identification was established for each derivative by pipetting 1.0 mL of the phenylpropanolamine and doxylamine standards into six conical vials and evaporating to dryness. The appropriate derivatizing reagents were added to the vials and the vials were then heated at 70°C for 30 minutes. After the derivatizing reagents were evaporated from the samples, they were reconstituted with 2.0 mL of methanol. A 1.0 uL sample was then injected into the GC-FTIR. A serial dilution was then performed until identification was no longer possible.

Two factors other than an increased IR absorption, could result in a change in the peak ratios. These are: incomplete reactions or differential loss of sample during the evaporation process. In order to determine if either of these potential problems actually affected the results of the experiment, the following procedures were employed. After the three injections of the derivatized samples had been made, the remaining samples were returned to the N-Evap and evaporated to dryness. Once the methanol had been evaporated off, the samples were left in the N-Evap for 15 additional minutes. The samples were reconstituted after the 15 minutes of evaporation with 100 uL of methanol and a 1 uL injection was made into the GC-FTIR. If differential evaporation of the samples did occur, the peak area ratio between the phenylpropanolamine or its derivative to the doxylamine would change. To test for incomplete reactions, 1 mL of the original phenylpropanolamine and doxylamine mixture was placed into a conical vial and evaporated to dryness. Derivatization was performed by using the same procedure as before. After derivatization, 50 uL of methanol was added to the conical vial and a 1 uL injection was made on the GC-FTIR. This resulted in a one-hundred fold increase in the amount of material injected. If underivatized phenylpropanolamine did remain in significant quantities, the IR would be able to detect it. (Additional confirmation of incomplete reactions was obtained from mass spectroscopy data).

The GC used was a Hewlett Packard 5890 Series II equipped with a SUPELCO 0.25mm i.d. 15 meter SPB-1 column. The FTIR used was a Hewlett Packard 5965B Infrared detector. All runs were 150°C isothermal.

RESULTS AND CONCLUSION

The primary tabulations of test results are found in Table I. It is apparent that derivatizing the sample caused an increase in the peak area of phenylpropanolamine with respect to the doxylamine internal standard (Fig. 5).

As the number of fluorines added to the phenylpropanolamine increased, so did the relative peak area. This resulted in an increase in the peak area ratio. The response of GC-FTIR to the drug was increased in all cases where the samples were derivatized.

Derivative Used	PPA Derivative/INTS	Relative Sensitivity
None	0.116	8
BSTFA	2.85	185
Acetic Anhyd.	7.05	458
TFAA	9.13	593
PFPA	12.1	786
HFAA	15.4	1000

TABLE I. The sensitivity of GC-FTIR to different derivatizing agents.

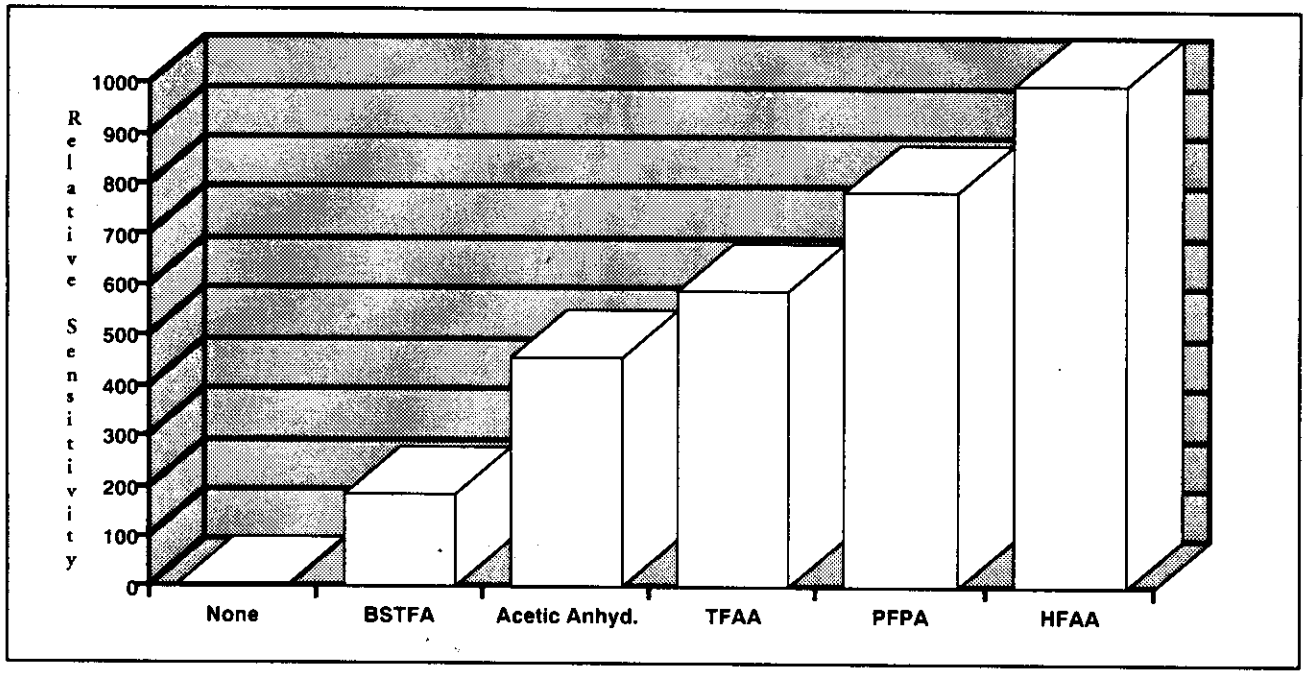


FIGURE 5. Relative change in FTIR sensitivity.

Mass spectroscopy data indicated that the BSTFA derivatized both of the primary amine hydrogens as well as the hydroxyl hydrogen. This can be confirmed from the IR spectrum. The IR spectrum shows that the N-H and O-H stretch are no longer present after derivatization with BSTFA. Therefore, BFSTA derivatizes both of the amine hydrogens as well as the hydroxyl hydrogen. The polyfluorinated acid anhydrides each showed a mass gain consistent with a monosubstitution. IR data of each polyfluorinated derivative indicates that the O-H stretching is no longer present while the N-H stretching is still occurring. Therefore, it appears that only the hydroxyl group derivatized with the polyfluorinated acid anhydrides. The acetic anhydride shows a mass gain indicative of two substitutions. IR data of the acetic anhydride derivative indicates that the O-H stretch is lacking while the N-H stretch is reduced.

An increased number of carbonyl or carbon-fluorine bonds in a molecule caused an increase in the response of the FTIR detector to the derivative. The one derivative that did not add any carbonyl or carbon-fluorine bonds to the phenylpropanolamine was BSTFA. This particular derivatizing reagent adds three trimethyl silyl groups to phenylpropanolamine. A trimethyl silyl functional group adds carbon-silicon bonds and carbon hydrogen bonds. Carbon-silicon bonds have absorption intensities relatively similar to the carbon-carbon bonds already present within phenylpropanolamine. Because of this the increase in sensitivity to GC-FTIR detection will mostly be from the deactivation of the primary amine and the hydroxyl groups, resulting in improved chromatography and not from a gain in the FTIR sensitivity.

Derivatizing the specimen with acetic anhydride adds two carbonyl groups to phenylpropanolamine. Addition of these carbonyl functional groups to phenylpropanolamine will result in improved chromatography and will simultaneously increase the absorption of infrared radiation. The remaining polyfluorinated derivatives will also improve chromatography and add a carbonyl functional group. The difference between these derivatives and the ones already mentioned is the number of carbon-fluorine bonds added to phenylpropanolamine. The TFAA adds three carbon-fluorine bonds to phenylpropanolamine, PFPA adds five, and HFAA adds seven. This is due to the mono-substitution of each of these derivatives. As the number of carbon-fluorine bonds added to phenylpropanolamine increases, so does the sensitivity of the FTIR to the derivative.

Differential evaporation rates or incomplete reactions have been demonstrated to have no effect on the results. No phenylpropanolamine was detected in any of the concentrated derivatized samples. Additionally, the ratio of the peak areas of doxylamine to the phenylpropanolamine or its derivative did not change significantly when placed in the N-Evap for 15 extra minutes.

REFERENCES

1. Moffat AC, ed. Clarke's isolation and identification of drugs. 2nd ed. London: The Pharmaceutical Press. 1986;895-6.

2. Morrison R, Morrison B, Boyd R. Morrison and Boyd organic chemistry. 4th ed. Boston: Allen and Bacon, Inc.1983;680.

*U.S. GOVERNMENT PRINTING OFFICE: 1992-661-063/60022