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ELECTROSPRAY FOR SURFACE DERIVATIZATIONS IN CHROMATOGRAPHY/SIMS

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INTRODUCTION

Secondary ion mass spectrometry (SIMS) has become a valuable technique for the direct imaging analysis of thin-layer chromatograms (TLC); TLC/SIMS has been demonstrated for many classes of compounds including peptides (Dunphy and Busch, 1988; Stanley and Busch, 1989), porphyrins (Dean and Busch, 1989), bile acids (Dunphy and Busch, 1990), and steroids (Brown and Busch, 1989). The developed intact chromatogram is placed directly on a platform in the source chamber of a custom-built SIMS mass spectrometer (Fiola, *et al.,* 1986) and is moved in the x and y directions by piezoelectric crystal translator stages. Because the developed spots on the chromatogram can be moved into and out of the focus of the primary ion beam, preferential or multi-spot analysis as well as chromatographic imaging is possible.

In chromatography/SIMS, as in any liquid SIMS technique, ion yields for compounds already in the ionic state are greatly increased compared to the ion yields for non-charged species. Normally, ionization of neutral molecules in liquid SIMS is achieved by protonation of the analyte to create the $(M+H)^+$ species. However, this is a relatively inefficient process; in general, only about five percent of all neutrals sputtered by the energetic primary ion beam are ionized as $(M+H)^+$ (Fenselau and Cotter, 1987). Derivatization strategies in which a localized charge is placed on a molecule of the compound of interest have been developed that increase sensitivity and lower detection limits in the chromatography/SIMS technique (Brown and Busch, 1989; Duffin and Busch, in press).

Brown and Busch (1989) have developed a derivatization strategy for post-derivatizations on TLC plates. The reaction scheme (Figure 1) involves the reaction of a carbonyl functional group from an aldehyde or a ketone with Girard's T reagent, creating a localized charge on the newly derivatized compound. The methodology for the derivatization involves suspending the developed TLC plate in a developing chamber a few cm above a solution containing the Girard's T reagent, methanol, and an acid catalyst, usually acetic acid. The developing chamber is then placed into an oven at a temperature corresponding to the boiling point of methanol (60° C). Vapors of the solution react with the analytes on the surface of the TLC plate without increasing the spot size or decreasing the resolution. However, since the derivatizations are carried out in the vapor phase, this method works only for derivatizing reagents that are relatively volatile. A method for surface derivatization involving electrospray that is capable of derivatizations using non-volatile as well as volatile reagents is described in this paper.

Reaction Scheme for Derivatizations of Aldehydes or Ketones with Girard's T Reagent

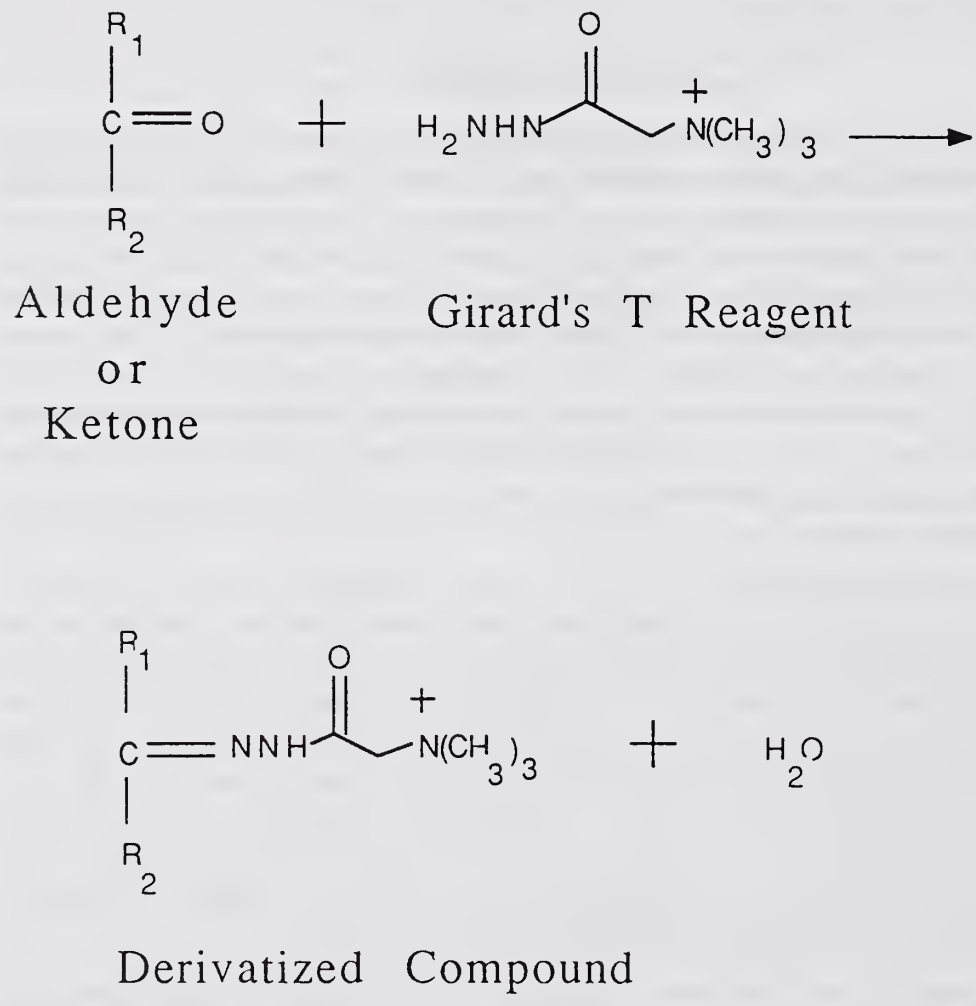

FIGURE 1. Reaction scheme for the derivatization of aldehydes or ketones with Girard's T reagent.

EXPERIMENTAL

Positive ion mass spectra were obtained with a custom built mass spectrometer (Fiola, *et al.*, 1986) based on a Extrel quadrupole mass analyzer. A 6 keV argon atom beam was used as the primary beam with a current of 4 mA. Results are the same if a cesium ion beam is used as the primary particle source. Chamber pressure was constant at 8 x 10^{-5} torr.

A suitable solvent system for the electrospray deposition was found to be methanol:water (75:25) in which 0.5 μg of Girard's T reagent was dissolved. A sample size of 5 μl of a 5 μg/μl solution of the steroid was spotted onto an aluminum-backed TLC plate. The Girard's T reagent was deposited over the spots

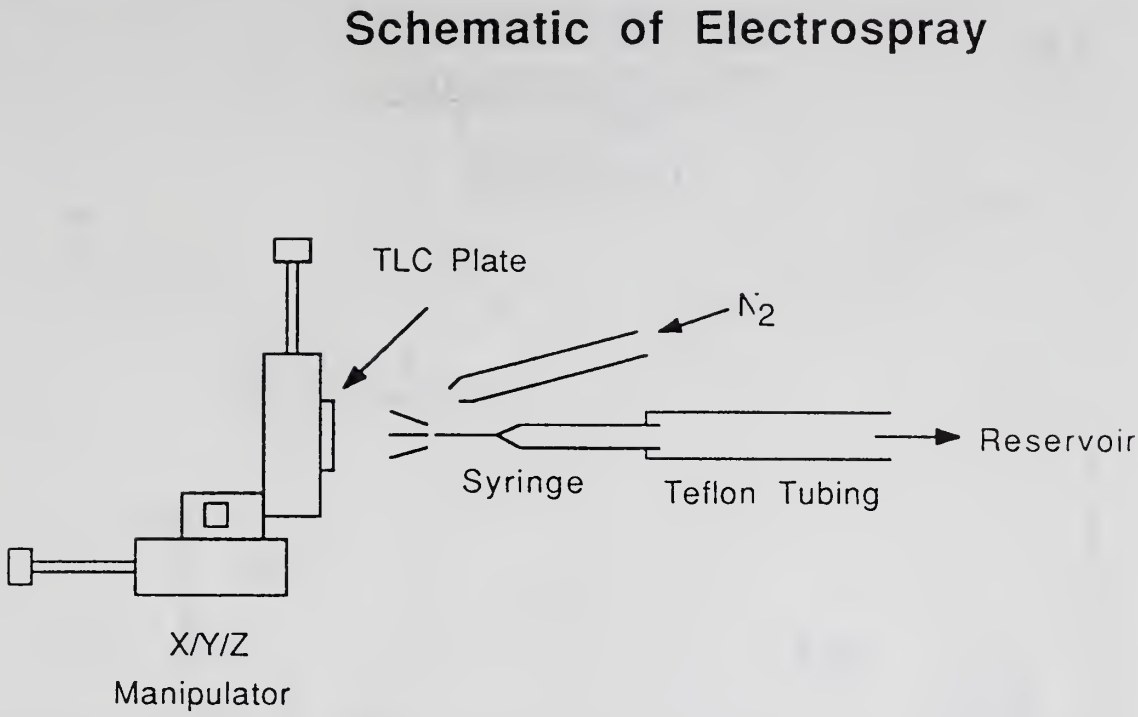

FIGURE 2. Schematic of electrospray for uniform depositions of solid materials.

by means of electrospray (see below). The plate was then suspended in a developing chamber above a solution bath containing methanol and an acetic acid catalyst. The developing chamber was then placed in an oven at 60° C so that acetic acid vapors could come into contact with the analyte and the Girard's T reagent in order to catalyze the final steps of the reaction. The plate was then placed directly in the ion source of the mass spectrometer for subsequent mass analysis.

INSTRUMENTATION

An electrospray apparatus (Figure 2) was constructed after the design developed by McNeal and Macfarlane (1979), who used the uniform deposition capabilities of electrospray to deposit samples in thin films onto foils for use in their plasma desorption mass spectrometer. The electrospray cell consists of a hypodermic needle acting as the anode or delivery electrode. The cathode, where the deposition takes place, is actually the target stage of an xyz manipulator set at ground potential. A reservoir is needed to feed the hypodermic needle, while capillary forces are responsible for the continuous flow of solvent. A high voltage power supply (0-5 kV, 3 mA) is connected to the hypodermic needle through a 50 megaohm resistor that eliminates high current and prevents voltage breakdown. The electrospray apparatus is housed in a Lucite box to minimize turbulence between the anode and the cathode, to provide a controlled environment, and to provide for safe working conditions in everyday use.

RESULTS AND DISCUSSION

Deposition of the derivatizing reagent onto the surface of the chromatogram is accomplished by placing a high voltage (3-5 kV) on the anode relative to the cathode. As a droplet forms on the tip of the needle, the high voltage overcomes the surface tension of the solvent, and the solution is dispersed into tiny charged particles. As the charged droplets travel from the anode to the cathode, the solvent

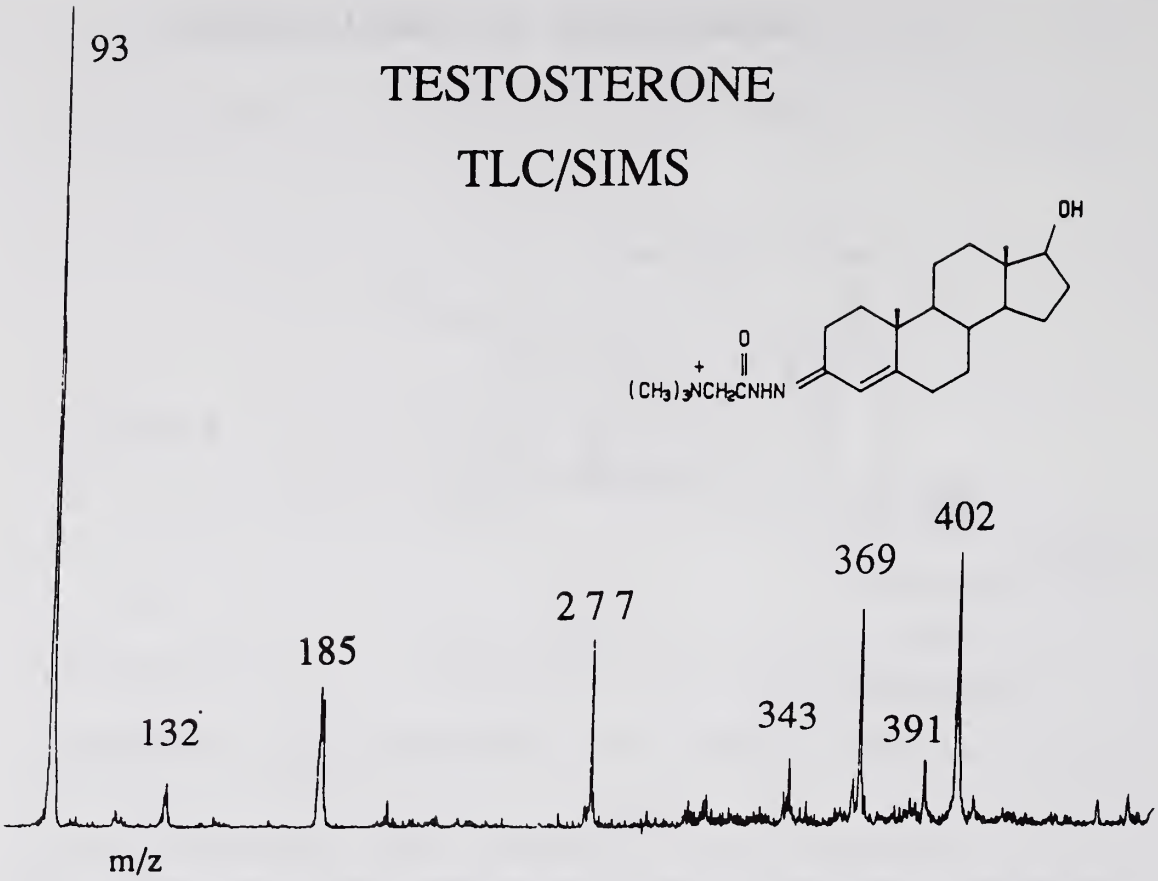

FIGURE 3. Positive ion mass spectrum of the Girard's T derivative of testosterone.

evaporates because of its high vapor pressure and by absorbing energy from collisions with atmospheric particles. The solute residue of each droplet, in this case the derivatizing reagent, is therefore deposited on the cathode.

In the method developed by Brown and Busch (1989), the derivatizing agent must be volatile for the transfer of the derivatizing reagent in the vapor phase to the TLC plate for subsequent reactions. Utilization of electrospray for transfer of the derivatizing reagent directly onto the TLC plate eliminates the need for the volatility of the reagent. Volatilization is required only to place the acetic acid, acting as the catalyst, into contact with the surface for reaction with the derivatizing reagent already on the TLC plate. If the reaction scheme demands multi-step reactions, each volatile *or* non-volatile reagent can be deposited with relative ease without harm to the reagents or analyte. With electrospray, no solvent actually comes in contact with the plate. Since the solvent evaporates in flight to the cathode, the possibility of spot spreading is eliminated. The vapor phase deposition of the acetic acid has no effect on the chromatographic resolution.

The positive ion mass spectra of derivatized testosterone and androsterone confirm the positive outcome of the derivatization strategy using electrospray. Figure 3 shows the mass spectrum of derivatized testosterone, which has a molecular weight of 402. A relatively abundant peak at 402 daltons corresponds to the derivatized testosterone/Girard's T intact cation. A characteristic fragment ion exists at m/z 343, which results from the loss of a neutral trimethylamine. Ions at m/z 93, 185, and 369 correspond to the protonated glycerol ion, the dimer of glycerol, and the trimer of glycerol, respectively. The ion at m/z 132 shows an unreacted portion of the Girard's T cation on the plate.

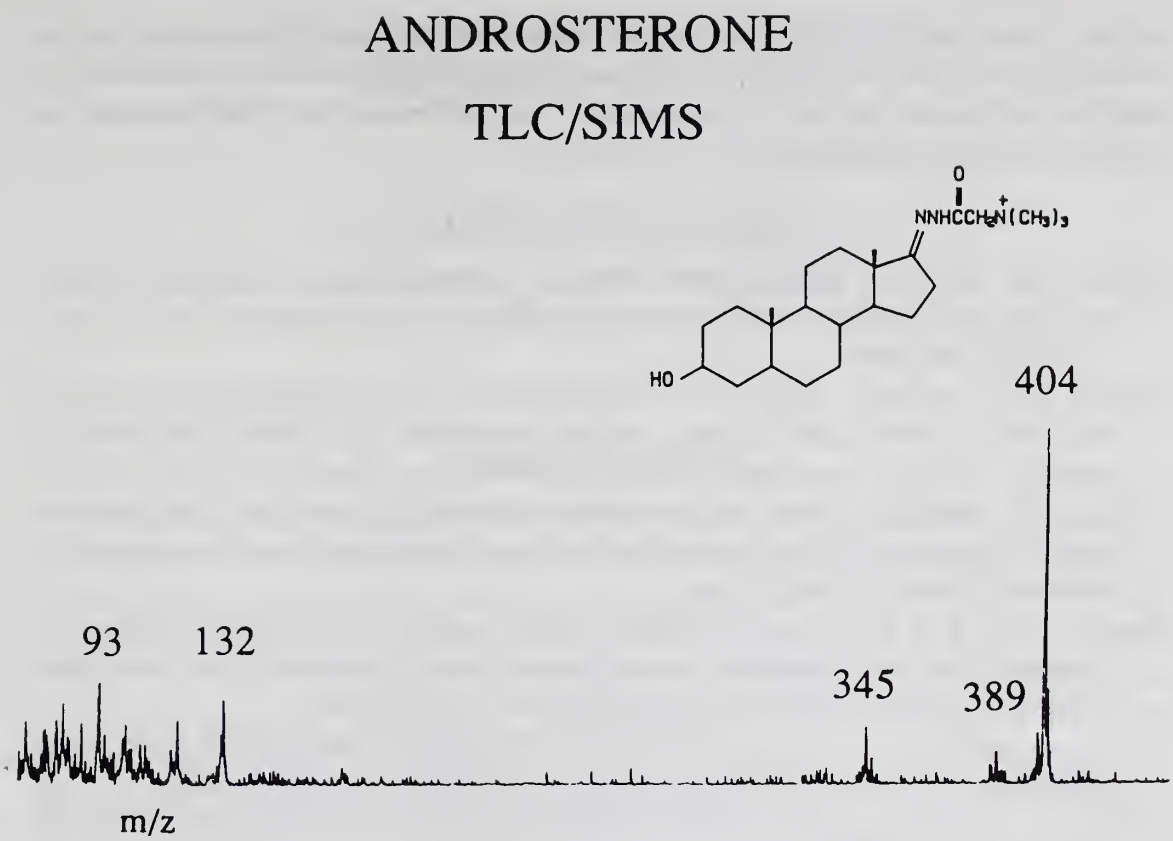

FIGURE 4. Positive ion mass spectrum of Girard's T derivative of androsterone.

Derivatized androsterone also gives a characteristic mass spectrum shown in Figure 4. The peak at 404 daltons corresponds to the intact cation of the androsterone/Girard's T derivatized compound. The loss of trimethylamine gives rise to the ion at m/z 345. Again, unreacted Girard's T is evidenced by the low abundance of the ion at m/z 132.

These initial experiments indicate the utility of the electrospray method for the derivatization of aldehydes or ketones with Girard's T reagent. However, this method has an endless supply of applications for derivatizations in planar chromatography for the purpose of detection by mass spectrometry. Many existing derivatization strategies may be modified so that deposition of the reagents onto surfaces with electrospray methods can be used. For example, analytical chemists use trypsin and cyanogen bromide in order to cleave large peptides (themselves out of the mass range of quadruple instrumentation) into characteristic and smaller fragments. These smaller compounds produce ions of lesser mass within the mass range of the instrument. The electrospray method might be particularly advantageous with multiple cleavages of biomolecules separated by TLC or electrophoresis.

CONCLUSION

Electrospray has proven to be an effective method for depositing non-volatile as well as volatile derivatizing reagents for the purpose of surface derivatizations in chromatography/SIMS. Girard's T was shown to be successful in initial experiments. The ease of the experiment leads one to believe that this method can be effective for many derivatization strategies involving planar chromatography.

Solvent evaporation prior to deposition preserves the spatial resolution of the chromatogram. The newly charged compounds provide higher ion yields lowering detection limits and bettering sensitivity, which enhances the effectiveness and utility of the chromatography/SIMS technique.

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