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Steroid Analysis on the Ion Trap: Selective Ion Storage; Resonant and Nonresonant MS/MS

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INTRODUCTION

The potential analytical value of the quadrupole ion trap has been apparent since its decription by Paul [1-4]. Numerous technical problems, exemplified by mass "shifts" resulting from space charging, have prevented the ion trap from becoming widely accepted. Many of these technical problems can be avoided by the application of computer feedback, such as automatic gain control (AGC) to limit the number of ions in the trap and thus avoid space charging. The true power of the ion trap, however, arises from the ability to vary the waveforms applied to the ring and end cap electrodes, and thus selectively isolate, store, react, fragment, and eject ions from the device. Thus, a single mass analyzer has exhibited the ability to do selected ion storage (SIS)[5], tandem mass spectroscopy (MS/MS) [6], and high resolution mass spectroscopy (120,000 resolution [7]) by application of various wave functions to the trap.

We report here on the application of selected ion storage (SIS) and non-resonant and resonant MS/MS to the analysis of anabolic steroids.

EXPERIMENTAL

All experiments were carried out on a Varian Saturn III ion trap detector equipped with a WaveBoard. Standard Varian software (Version 5.0) and the Ion Trap MS/MS ToolKit were used to control the trap function in all experiments. Separations were performed on a Varian 3400 gas chromatograph equipped with an 8200 autosampler. The TMS-ether,TMS-enol derivatives of various steroids were prepared with the standard MSTFA/TMIS/dithierythritol mixture for 15 min. at 60°C.

RESULTS AND DISCUSSION

Ion preparation methods (IPM) can be constructed to apply a variety of RF and DC waveforms to the ring electrode and end cap electrodes of the ion trap. These waveforms can selectively store

and add kinetic energy to ions. This process can be used to selectively store reagent ions for chemical ionization or ions of interest for selective detection.

Selected ion storage (SIS) is the equivalent in the ion trap to selected ion monitoring on a quadrupole mass filter (QMF). There is little rationale to select a single ion at a time in an ion trap, since ions can be accumulated for selected time intervals and rapidly ejected. The advantage to SIS is that with selected storage only ions of interest are accumulated, thus avoiding space charging without the non-selective reduction of all ion intensities in the effluent by AGC. Figure 1 shows a standard steroid mixture analyzed with SIS. Since a range of ions can be stored simultaneously, limited spectral information can be obtained, such as the presence of the chloride isotope cluster, without the need to isolate two ions individually as is done in QMFs. The IPMs can be time programmed during the run so that groups of ions can be added and deleted from the storage function. Ions can also be selectively ejected from the trap to improve detection limits and background noise. For example, m/z 207 arising from column bleed can be selectively ejected (Figure 2). In general, a five- to ten-fold decrease in limit of detection is observed with SIS.

Tandem MS can be achieved in two ways on the ion trap. Non-resonant excitation results from application of a waveform to the trap which causes all ions to be displaced from their normal trajectories. During the relaxation of the ions to their normal secular trajectories, collisions with the helium bath gas result in collision-induced fragmentation (CID). Resonant excitation takes advantage of the fact that ions at a secular frequency which is a function of the applied RF voltage, the mass of the ion, and the trap geometry. By placing an AC waveform on the endcaps at the appropriate frequency, only the ion of interest can absorb the energy and undergo CID. Several techniques have been reported to vary the applied frequency to correct for an secular frequency shifts due to space charge or non-idealities in trap geometry. The non-resonant MS/MS spectrum of the diTMS derivative of testosterone is shown in Figure 3. The m/z 432 ion from electronic ionization was the precursor ion.

CONCLUSIONS

With the commercialization of various waveforms that can specialize the operation of the quadrupole ion trap, many of the previously reported limitations of the trap have been eliminated. Selected ion storage is comparable to selected ion monitoring in limits of detection, but has the advantage that spectral features, such as chloride isotope clusters, can be viewed without quadrupole mass filter settling times associated with single ion isolation in SIM. GC/MS/MS is

now a viable analytical approach on the ion trap. With the variety of ion excitation techniques and higher RF storage voltages available, most compounds can be fragmented.

References

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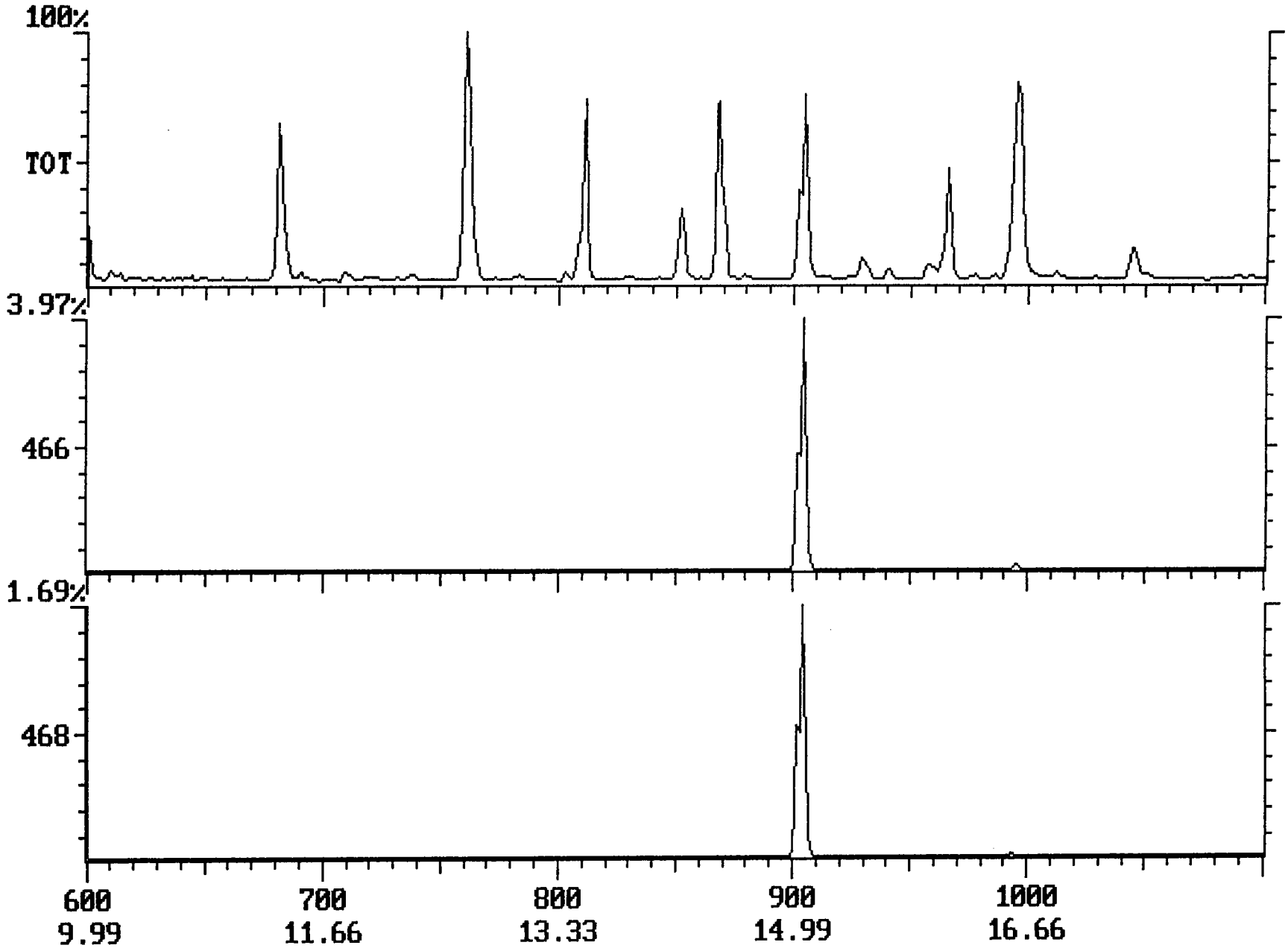
FIGURE CAPTIONS

Figure 1. Selected ion storage chromatogram of a steroid standards mixture. Ions from the chloride cluster from dehydrochlorotestosterone are shown below the TIC display.

Figure 2. Selective ejection of the m/z 207 ion from column bleed. Note in the bottom trace that the background is selectively ejected, increasing the relative intensity of the cluster of ions at m/z 222. Also note that all ions in the range m/z 205-208 are ejected simultaneously.

Figure 3. Non-resonant excitation MS/MS spectrum of the di-TMS derivative of testosterone. The precursor ion was m/z 432; excitation storage level: 90 m/z; the excitation amplitude:57 V; .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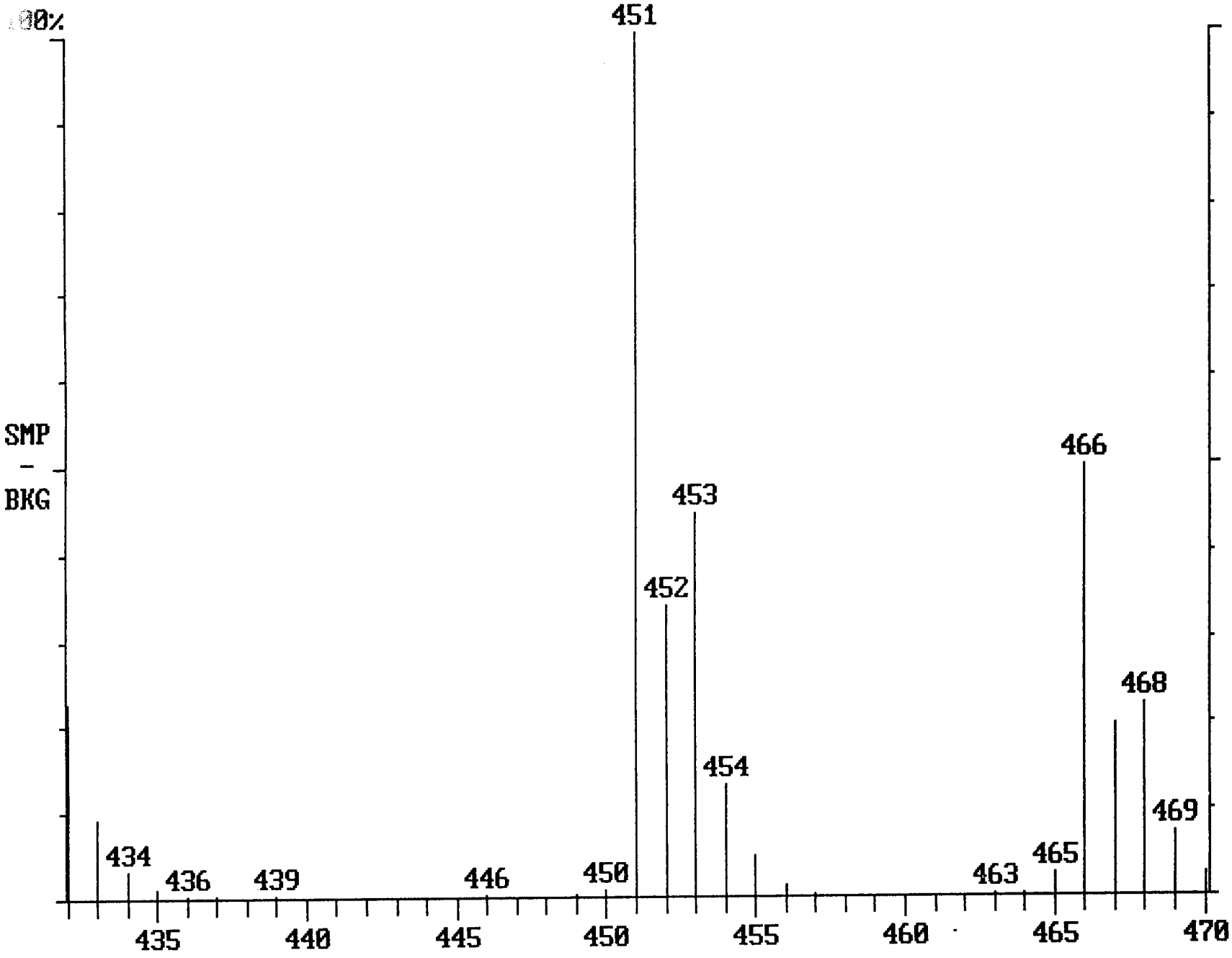
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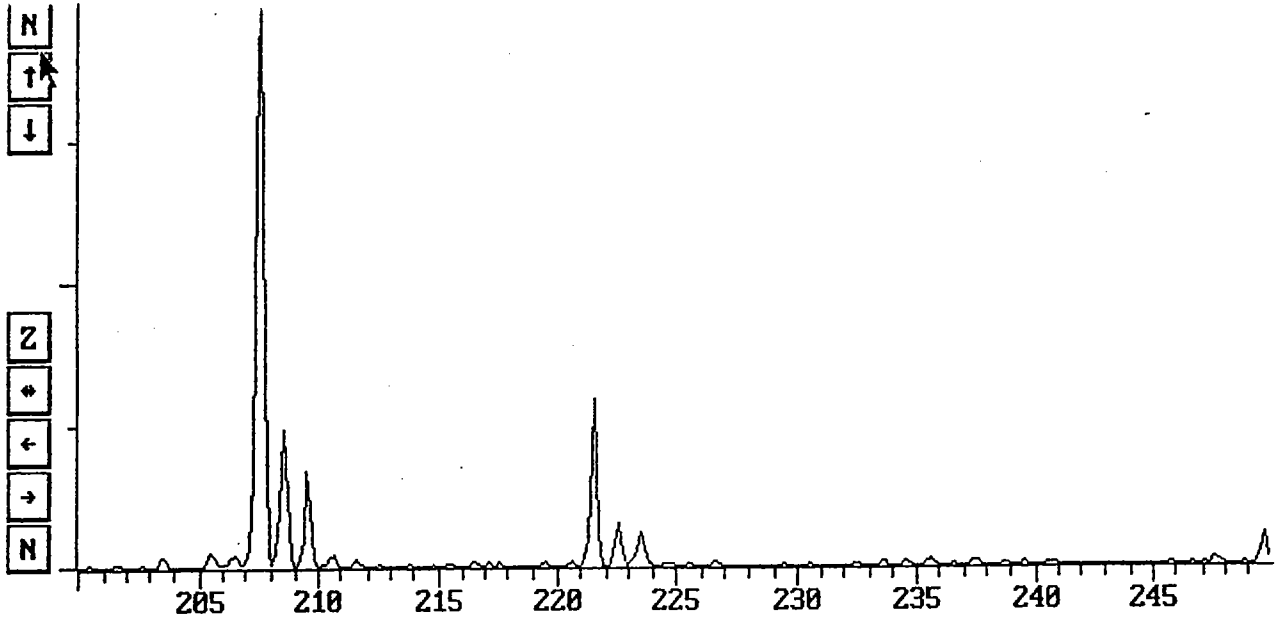
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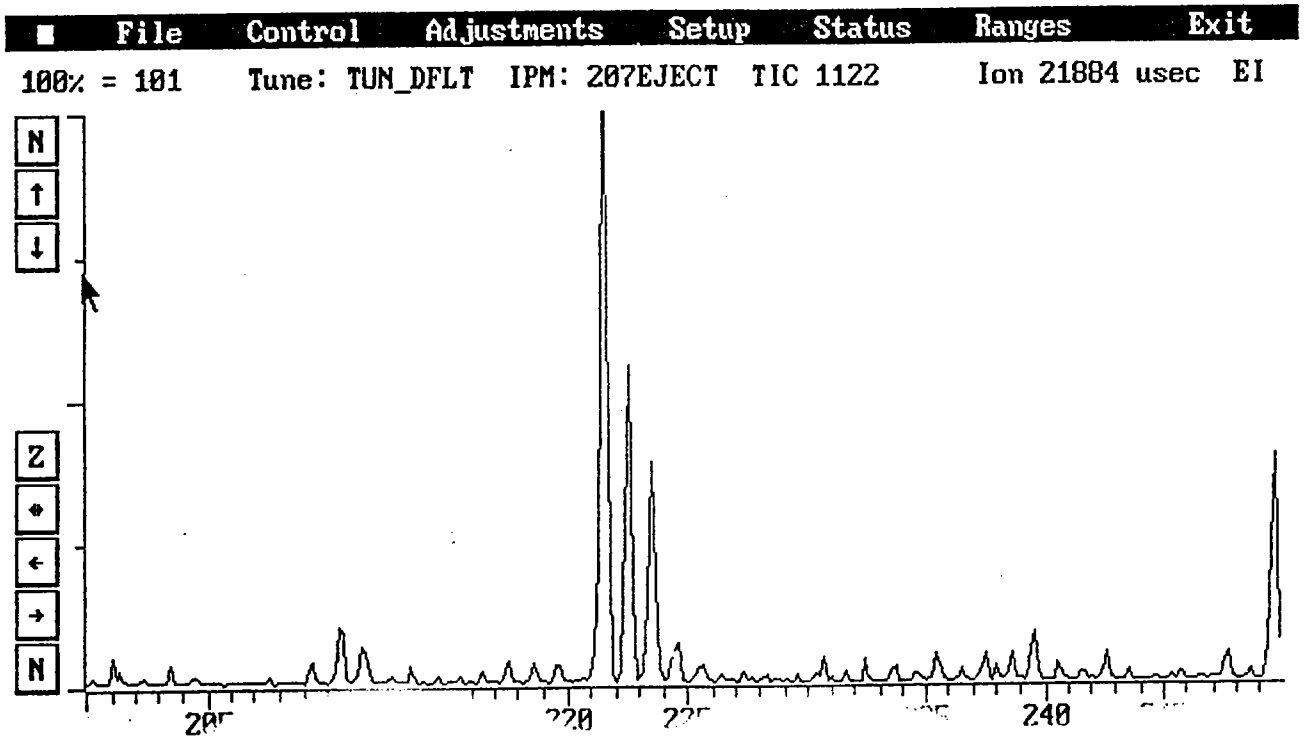
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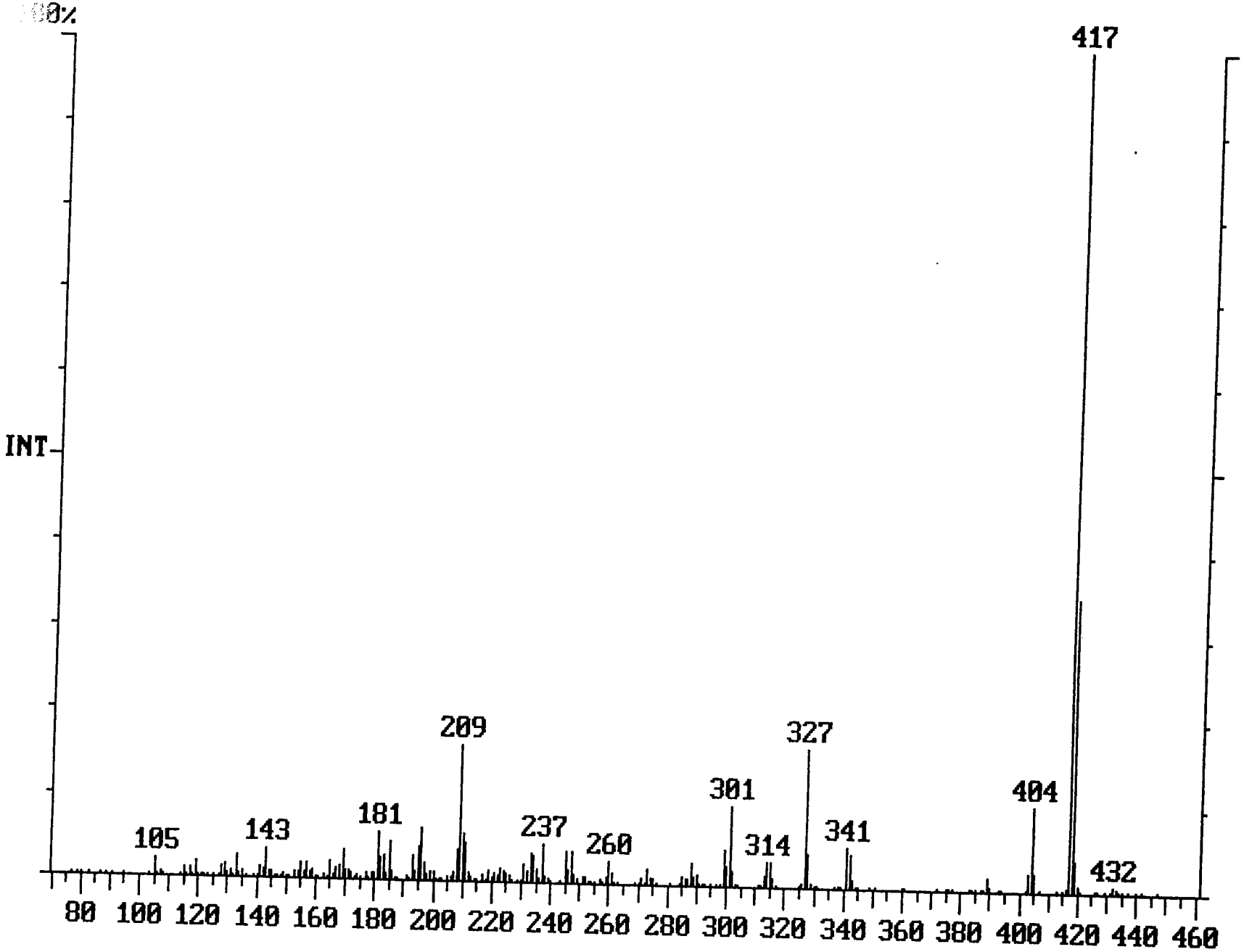
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