Journal of Chromatography, 122 (1976) 365–371

CHROM. 9130

IDENTIFICATION OF DRUGS USING A GAS CHROMATOGRAPHY–MASS SPECTROMETRY SYSTEM EQUIPPED WITH ELECTRON IMPACT–CHEMICAL IONIZATION AND ELECTRON IMPACT–FIELD IONIZATION–FIELD DESORPTION COMBINATION SOURCES

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SUMMARY

In a case of toxicological analysis by gas chromatography (GC)–mass spectrometry–data system, chemical ionization (CI), field ionization (FI) and electron impact data are shown to lead rapidly to assignments for all significant GC peaks. Valuable data include not only full spectra in the three ionization modes, but also exact mass measurements in the FI or CI mode. Such measurements, obtained by a dynamic peak-matching technique, lead to the elemental composition of the compounds of interest. This knowledge makes the assignment of key GC peaks unequivocal and provides an extremely high level of confidence regarding the identity of whole metabolite series. It is also shown that the nature of the FI information is very similar to that obtained from CI data.

INTRODUCTION

The technique of coupled gas chromatography–mass spectrometry (GC–MS) is well established in laboratories concerned with the analysis of complex mixtures[1–3]. The information contained in mass spectra obtained under electron impact (EI) provides most of the evidence for the identification of the analyzed compounds. Clearly, any alternative ionization method leading to a different set of mass spectral data[4] increases the ease and reliability of an identification. This is the case for soft ionization methods, for instance chemical ionization (CI)[5,6] or field ionization (FI)[7,8]. In CI, sample molecules are ionized by reaction with low-energy ions, whereas in FI the molecules are ionized under the effect of a high electric field. Both processes place emphasis on the molecular or quasi-molecular ion. This complements EI information where, for several classes of compounds, molecular peaks are of little abundance or are absent.

EXPERIMENTAL

Instrumentation

Samples were analyzed in the EI and CI modes using a Varian-MAT 112

double-focusing mass spectrometer equipped with a turbomolecular high-vacuum pumping system. A Varian 1400 gas chromatograph was coupled to the spectrometer via a variable-slit separator adaptable to a wide range of flow-rates. The chromatograph was fitted with a 4-ft. glass column packed with 3% OV-17 and temperature programmed at 10°/min between 120 and 320°. Isobutane was chosen as the CI reactant gas.

Analyses in FI mode were performed on a Varian-MAT 711 instrument equipped with the EI–FI–field desorption (FD) combination ion source. It was interfaced with a Varian 2700 gas chromatograph by means of a double-stage Bieman–Watson separator. A 6-ft. glass column packed with 3% OV-1 was used in this chromatograph, with temperature programming between 150 and 290° at 10°/min. Both GC–MS systems were connected to a Varian-MAT SS 100 MS data system featuring a 2.34-Mword disk memory, display unit, and a hard-copy unit.

Sample preparation

In the case of real-life samples, in order to isolate major drug components of both acidic and basic or neutral character, urine extractions were carried out at pH 6. Typically, after control of the pH, a 25-ml sample of urine was half-saturated with sodium sulphate at room temperature, then extracted with three 15-ml portions of diethyl ether. After evaporation to dryness of the combined ether extracts, the residue was taken up in 0.1 ml of methanol for instrumental analysis.

RESULTS AND DISCUSSION

Electron impact and chemical ionization data

Recently, an accidentally intoxicated child was brought to the Children's Hospital in Homburg, G.F.R., and, as often happens in such cases, little information could be obtained about the cause of the intoxication. After emergency measures had been taken, however, it was important for the physician to know the nature of the compounds involved in order to apply proper therapy. A urine sample was obtained and a GC–MS analysis was requested. Gas chromatograms were obtained in the EI (Fig. 1) and CI modes (Fig. 2), and showed the presence of eight major peaks. As all significant peaks had to be identified, EI spectra were first recorded cyclically and stored on the disk memory. The mass spectrometer was then switched to the CI mode and all CI spectra were likewise recorded.

On the basis of comparisons between the EI and CI spectra and spectral libraries[9,10], the following assignments could rapidly be made: peaks 1, 2, 3 and 4 belong to barbital, crotylbarbituric acid, butalbital and caffeine, respectively.

In order to check for the presence of crotylbarbituric acid (mol. wt. 210), an exact mass measurement of the quasi-molecular ion at *m/e* 211 was performed in the CI mode. For this measurement, obtained by the dynamic peak-matching technique, the spectrometer, operated in the CI mode, was set for a resolution of 3000 (10% valley). Experience has shown[11] that such a medium resolution is sufficient to obtain accuracies of 10 ppm or better. Allobarbital (quasi-molecular ion, *m/e* 209) was chosen as the reference compound and was introduced via the direct insertion probe. While GC peak 2 was being eluted, the ratio between the known exact mass of peak 209 and that of the unknown at *m/e* 211 was measured. On the basis of that value, the exact

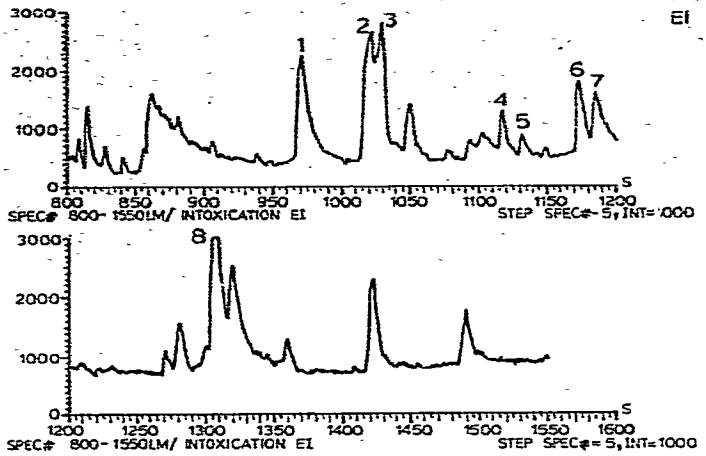

Fig. 1. Computer-reconstructed gas chromatogram of the urine extract, EI mode

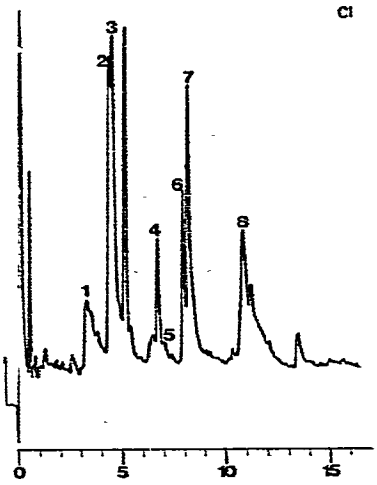

Fig. 2. Gas chromatogram of the urine extract, CI mode.

mass of *m/e* 211 was computed to be 211.1099. This value matches that corresponding to the composition $C_{10}H_{15}N_2O_3$ of $(M + H)^+$ for crotylbarbituric acid, with a deviation of 1.6 mU. Within a window of ± 2.5 mU, and with ten oxygen and ten nitrogen atoms allowed in addition to carbon and hydrogen, only two other elemental compositions were found possible.

Spectra for GC peaks 6, 7 and 8 (Figs. 3, 4 and 5) showed EI and CI data compatible with methylaminophenazone, aminophenazone and acetylaminophenazone. An exact mass measurement, described below, confirmed that peak 8 represents acetylaminophenazone and thus that peaks 6 and 7 most probably belong to the proposed aminophenazone metabolites.

This result led us to look for the presence of dimethylaminophenazone as a likely precursor. As spectra for this drug have been reported in the literature[9], it was

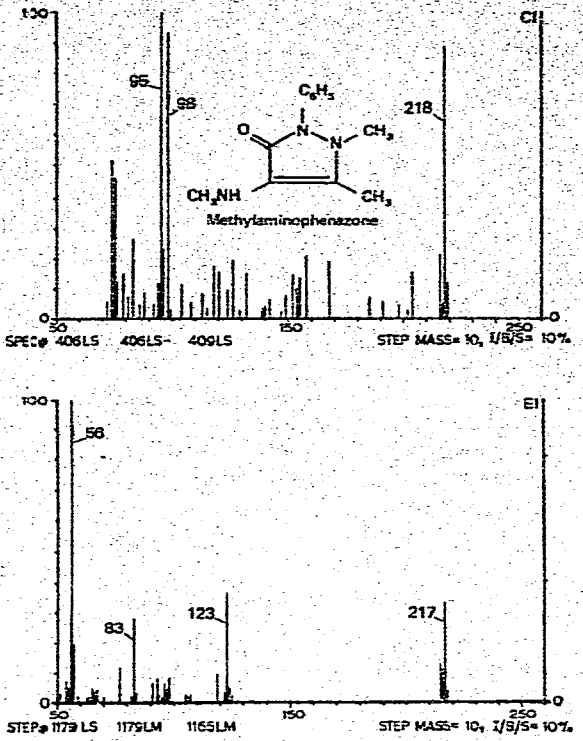

Fig. 3. EI and CI mass spectra of methylaminophenazone.

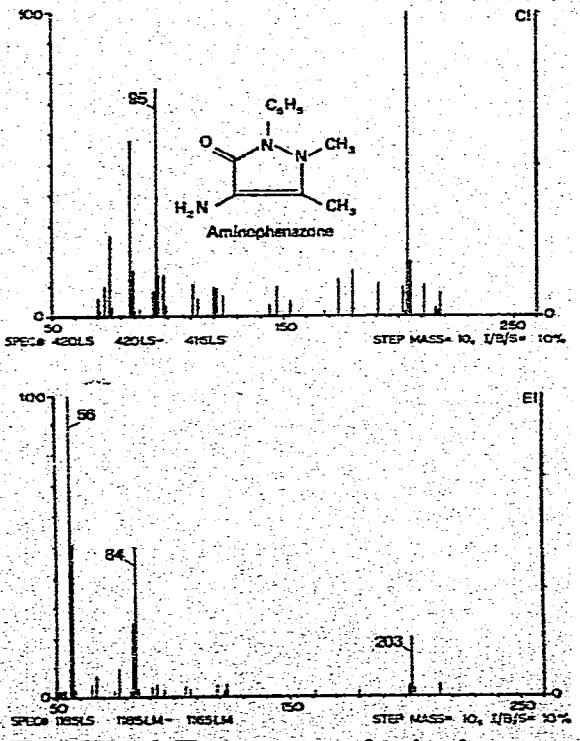

Fig. 4. EI and CI mass spectra of aminophenazone.

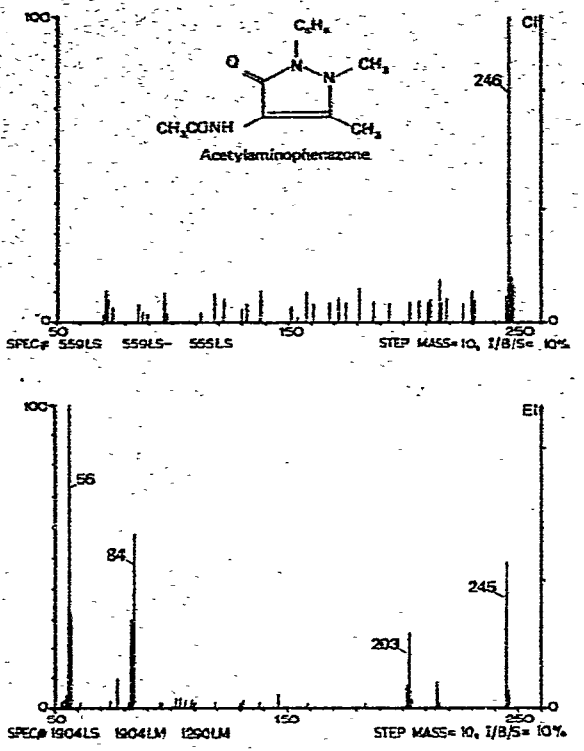

Fig. 5. EI and CI mass spectra of acetylaminophenazone.

possible to search for their most characteristic masses in the group of EI and CI data. Mass chromatograms for *m/e* 231 in the EI mode (Fig. 6) and *m/e* 232 in the CI mode were therefore requested, and indicated that GC peak 5 belongs to dimethylaminophenazone. Full spectra for this GC peak were indeed consistent with literature values[9] for this compound

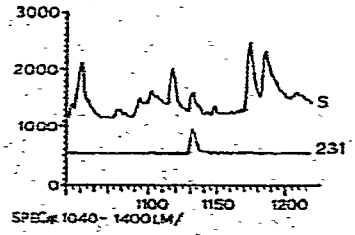

Fig. 6. Mass chromatogram for *m/e* 231 relative to EI data.

Field ionization data

In order to check the possibility of carrying out routine GC–MS measurements in the FI mode[12] as an alternative to chemical ionization, the urine extract sample was also analyzed using the EI–FI–FD combination source. Before injecting the real-life sample, however, spectra were recorded for various injected amounts of

methaqualone in order to establish the sensitivity of the method. The quality of a spectrum for a 50-ng amount injected was found satisfactory (Fig. 7). It can be seen from this typical measurement that the sensitivity of GC–MS in the FI mode is adequate for the routine analysis of samples from intoxicated patients. Indeed, in this field, the concentration of the investigated compounds is most often at the 100 ng/μl level or above.

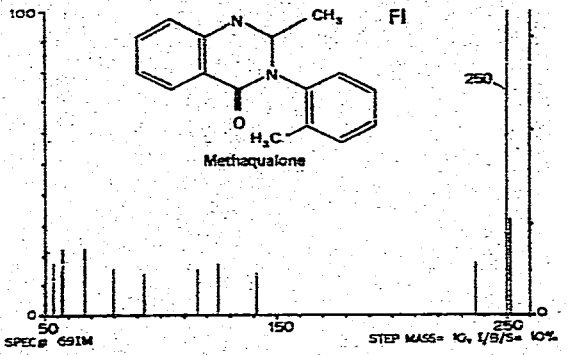

Fig. 7. FI mass spectrum relative to 50 ng of methaqualone injected.

The urine extract sample was then injected, and spectra were recorded in the cyclic scanning mode. All major peaks previously detected were found to be present in the GC trace obtained (Fig. 8). Mass spectra confirmed the previous assignments in that molecular peaks, almost exclusively present in the spectra, were found at the expected *m/e* values, as shown in the FI recording for acetylaminophenazone (Fig. 9).

The exact mass of the molecular ion relative to GC peak 8 (*m/e* 245) was then measured at a resolution of 8000 (10% valley) as the compound was being eluted. To effect this the dynamic peak-matching procedure described above was followed in the FI mode. The hydrocarbon $C_{17}H_{36}$ was used as the reference compound and was introduced via the direct insertion probe. Working in the FI mode, the reference peak at *m/e* 240 (M^+ for $C_{17}H_{36}$) was then used for the exact mass determination of the un-

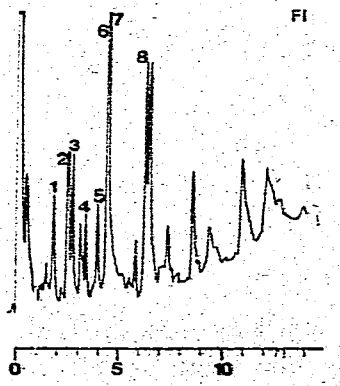

Fig. 8. Gas chromatogram of the urine extract, FI mode.

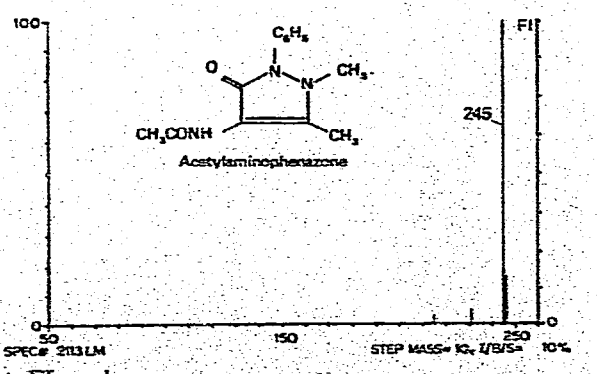

Fig. 9. FI mass spectrum of acetylaminophenazone.

known peak at *m/e* 245. The exact mass was measured to be 245.1185, matching that for the elemental composition of acetylaminophenazone within 2.1 mU. This result was taken as an unequivocal confirmation of the assignment made for GC peak 8 on the basis of spectral patterns.

CONCLUSION

In cases of accidental intoxication, GC–MS analysis in the conventional EI mode was speeded up by supplementary information obtained in the CI and FI modes. Information gained from full mass spectra helped to arrive at reasonable assignments quickly. Exact mass measurements obtained by the dynamic peak-matching procedure made these assignments unequivocal.

The analysis and interpretation of results were completed within *ca.* 2 h. It could then be stated that the patient most probably had absorbed the drugs Optalidon®, Dolo-Adamon®, and Veronal®. An evaluation of the ingested drug amounts was not possible, because the drug contents of the stomach were not determined. Besides, the drug concentration in urine depends on the nature of the compounds, and on the time elapsed after drug intake.

ACKNOWLEDGEMENTS

The sample was obtained by courtesy of Prof. Pfleger and Dr. Ehrenthal of the University of Saarland, Homburg, G.F.R.

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