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Applications of a simultaneous atomic emission mass spectral gas chromatography detector in drug analysis*

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Abstract: For gas chromatographic eluents a microwave induced plasma (MIP) emission detector has two important features for a wide range of nonmetals. These features are (1) elemental selectivity and (2) the ability to determine elemental composition. These capabilities, used individually or in combination, can provide important information which is largely complementary to mass spectral data. Simultaneous determination of the MIP emission and mass spectral data for individual chromatographic peaks can be very useful in resolving a variety of problems encountered in pharmaceutical analysis. Several of these possible applications are illustrated with specific examples.

Keywords: *Gas chromatography; microwave induced plasma emission; elemental analysis.*

Introduction

The need for chromatographic analysis arises in many areas of pharmaceutical research and development. These analyses often involve finding, characterizing or identifying materials present in small amounts in complex matrices. The rapid rise in the use of mass spectrometers in chromatography demonstrates how important these powerful detectors can be in resolving difficult analytical problems. The purpose of this work is to give an indication of how the information obtained from the analysis of microwave induced plasma (MIP) emission can be used to complement the data obtained from gas chromatography mass spectrometry (GC–MS).

The MIP emission technique is especially interesting for pharmaceutical applications because the technique can provide useful information on a wide variety of important elements including carbon, hydrogen, deuterium, fluorine, chlorine, sulphur and phosphorus [1]. Since the use of MIP emission as a gas chromatographic detector was first reported [2], several investigators have demonstrated its utility both as an elementally selective detector and as a means of determining elemental compositions [3–5]. Presented below are examples of how the simultaneous generation of mass spectral data and elemental composition data can be applied to resolving problems

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encountered in pharmaceutical analysis. These examples include the location and characterization of metabolites in complex media and the identification of low level impurities in bulk chemicals. The metabolite examples illustrate the elemental selectivity and the ability to correlate emission and mass spectral data obtained in complex mixtures which is made possible by the combined detection scheme. The identification of impurities illustrates the important complementary information obtained for structure determination.

Experimental

The gas chromatographic work reported here was conducted using a Hewlett–Packard model 5890 capillary column chromatograph with a 15 m (0.25 mm ID) DB-5 (J & W) fused silica column. The output of this column was split inside the GC oven using an SGE splitter (Model VSOS, Anspec Company, Inc., Ann Arbor, Michigan, USA) with the arrangement shown in Fig. 1. The tubing dimensions and lengths were determined empirically to deliver appropriate sample levels to the two different vacuum systems. The reducing unions were drilled out to fit the particular capillary tubing with the capillary tubes butted to each other in the centre. All unclad vitrous capillary tubing was obtained from the Anspec Company, Inc. (Ann Arbor, Michigan, USA). The aluminium clad vitrous tubing (No. 061220) was obtained from Scientific Glass Engineering (Austin, Texas, USA).

The combined MIP–MS detector used was based on two commercial units, a benchtop mass selective detector and an emission spectrometer. The mass selective detector used was a Hewlett–Packard model 5970B which incorporated model 59970A Hewlett–Packard workstation. This instrument allows for the collection of total ion current (TIC) chromatograms and mass spectra of each peak.

The emission spectrometer used was a model MPD-850 (Applied Chromatography Systems, Luton, UK). This instrument incorporated a low pressure (5–10 torr) ¼ wavelength Evenson type cavity [6] to sustain the plasma and a ¾ m (dispersion = 1.39 nm/mm) monochrometer. The outputs from up to eight fixed exit slits were monitored simultaneously using a pair of four channel interface boxes in a MAXIMA 820 chromatography workstation. The microwave plasma was sustained in a matrix of helium which contained 0.2% oxygen. A forward microwave power of 100 W was used with minimal reflected power.

The emission wavelengths monitored for several common elements and the sensitivities and selectivities obtained under these conditions are presented in Table 1. The sensitivities reported reflect the weights "on column" before splitting which are required to produce a signal twice the size of the peak to peak background noise. The selectivities reported give an indication of the magnitude of the desired atomic emission signal relative to the undesired carbon background or ghost which occurs whenever a material

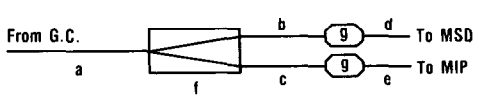


Figure 1
Sample splitting arrangement inside the GC oven where a is the DB-5 column, b is 17 inches of 0.15 mm OD by 0.025 mm ID vitrous silica capillary tube, c is 8 inches of 0.22 mm OD × 0.15 mm ID vitrous silica tube, d is 25 inches of 0.30 mm OD × 0.20 ID vitrous silica tube, e is 72 inches of 0.48 mm OD by 0.22 mm ID aluminium clad vitrous silica tube, f is the SGE splitter and g are reducing unions.

Table 1
Emission wavelengths, sensitivities and selectivities obtained using the combined MIP–MS detector

Element	Wavelength (nm)	Sensitivity (ng s^{-1})	Molar selectivity (mole mole carbon^{-1})
Carbon	247.9	0.082	—
Hydrogen	486.1	0.043	—
Fluorine	479.5	0.40	0.003
Chlorine	545.4	0.36	0.005
Sulphur	488.0	0.55	0.006

is introduced into the plasma. For example, if a molecule contains 10 carbons for each fluorine, selectivity of 0.003 indicates the response observed at 685.6 nm arises from 3% ghost and 97% atomic emission.

Results and Discussion

Elemental selectivity

In situations where the compound or family of compounds of interest are known to contain an element which is not commonly found in the matrices being studied, the MIP emission detector can be very useful in locating the materials of interest. In biological samples, for example, compounds containing chlorine, fluorine, sulphur, bromine or phosphorus are effectively labelled and can often be easily picked out of complex matrices. If mass spectra are also obtained, chemical structures may often be associated with the peaks. This is particularly true in cases where new materials are generated from a parent compound of known structure.

The capabilities of a combined MIP–MS detector are illustrated in Figs 2 and 3. In Fig. 2 the responses obtained by total ion current (TIC), the carbon emission and the chloride emission from the same sample injection are shown. The sample in this case was an ethyl acetate extract of tissue taken from a mouse which had been orally dosed with a chlorine containing drug substance.

The TIC carbon responses shown in the chromatogram in Fig. 2 are nearly identical with the major differences being slight variations in relative peak intensity and differences arising from data and plotting systems. In all important respects, such as retention time and peak width, the responses are very similar. This demonstrates that even though the sample is split in the GC oven and the two fractions travel different paths to different detectors, the responses obtained from the MIP and MS may be unambiguously correlated even in complex media. This correlation could be extremely difficult if the MIP and MS data were generated separately.

The response obtained in the chlorine channel for the chromatogram is also shown in Fig. 2. As discussed earlier, response obtained from materials containing chlorine will arise from the sum of two independent components, the desired chlorine atomic emission and the non-selective background emission or ghost. Response observed at 479.5 nm from materials which do not contain chlorine arise completely from non-selective background emission. Because some non-chlorine containing species may be present at very high levels it is completely possible that the ghost signals produced by these materials may give rise to a larger ghost response than the chlorine emission signal from a

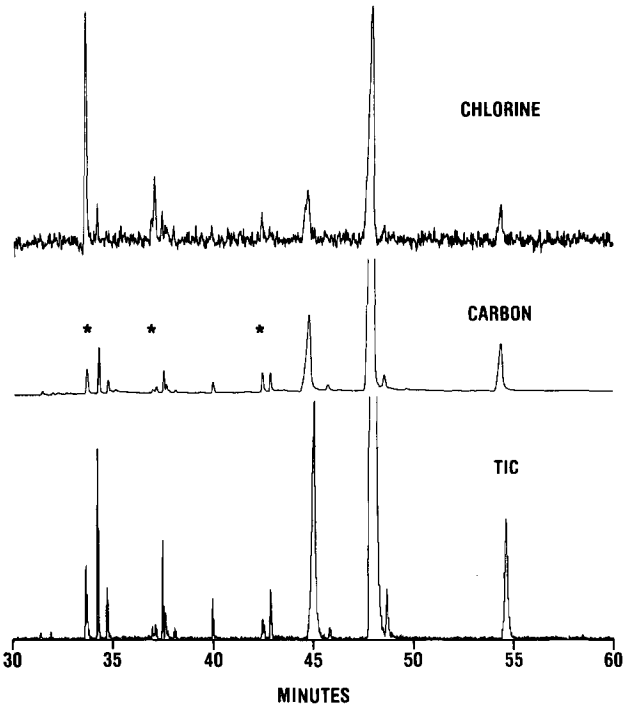


Figure 2
The total ion current, carbon emission and chlorine emission responses obtained simultaneously from the same sample injection. The sample was obtained by extracting the brain of a mouse which had been dosed with a chlorine containing drug substance. The starred (★) peaks indicate materials containing chlorine.

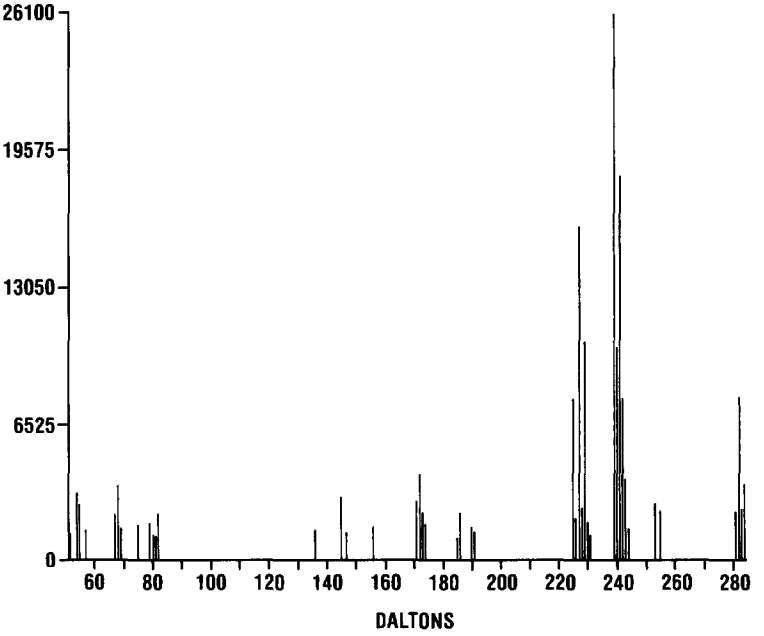


Figure 3
The mass spectrum measured for the first starred material in Fig. 2. This mass spectrum was obtained from the same injection which produced the data in Fig. 2.

minor, chlorine containing component. In spite of this apparent interference the chlorine containing materials may be readily identified in Fig. 2 because the chlorine response observed for a material with a 20:1 carbon to chlorine molar ratio would be about an order of magnitude larger than the expected ghost response. Thus by comparing the carbon and chlorine responses in Fig. 2, four chlorine containing peaks may be readily identified by their disproportionately large chlorine response. These peaks are indicated in the figure.

Of these peaks, the one with a 42.5 min retention time arises from the parent material and was present in the tissue at less than 0.1 m g^{-1}. The other chlorine containing peaks represent metabolites of the parent. The mass spectrum obtained for the first (33.7 min) chlorine containing peak is given in Fig. 3. This mass spectrum was obtained simultaneously from the same sample injection which generated the chromatogram in Fig. 2. This mass spectrum is of high quality and clearly shows the [M + 2] isotope peak expected for a material containing chlorine.

Although the presence of certain elements provides a natural label for the MIP emission detector in many compounds of interest, many interesting materials do not contain a unique atom. Because incorporating halogens, sulphur, etc., into small molecules greatly alters their behaviour, synthetically incorporating these elements just to serve as labels is not a reasonable approach. To apply this selective detector to a wider range of problems then would require either a precolumn derivatization of samples to incorporate an appropriate element or the use of an isotope of a common element.

The carbon and deuterium responses (emission at 656.1 nm) obtained from a blood serum extract are presented in Fig. 4. The serum used was obtained from a rat after IV dosing with benzodiazepine (with deuterated *N*-methyls). Figure 4 clearly shows the presence of two deuterated materials and illustrates the selectivity attainable with deuterium and its utility as a label at realistic concentrations (submicrogram per ml) in complex media.

Elemental composition

In cases, like the metabolite examples discussed earlier, where the family of materials of interest arise from a single parent material of known structure, the structure

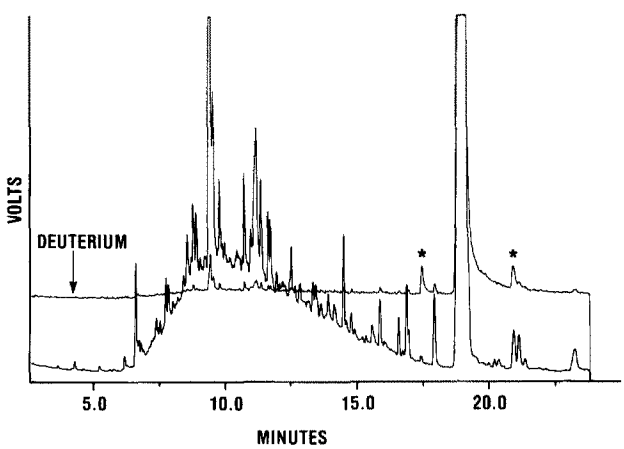
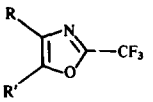
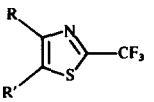
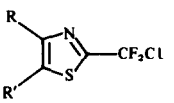


Figure 4
The deuterium and carbon responses obtained from a serum sample. The serum was obtained from a rat after IV dosing with a deuterium labelled material.

Table 2
Elemental response ratios, molecular weights and proposed structures for components A, B and C in Fig. 5

Peak	Elemental response ratios F/C	S/C	Cl/C	Molecular weight	Structure
A	0.77 (0.12)	0.006 (0.005)	0.06 (0.004)	355	
B	0.83 (0.05)	0.11 (0.01)	0.05 (0.001)	371	
C	0.43 (0.04)	0.13 (0.00)	0.69 (0.06)	387	

The ratios reported are the average of five runs. The standard deviations are given in parentheses.

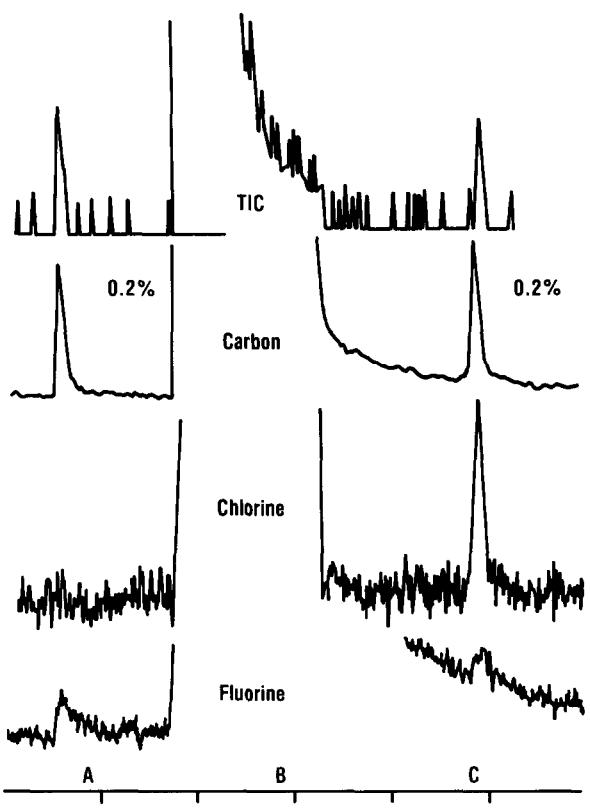


Figure 5
The TIC and carbon, fluorine and chlorine emission responses obtained simultaneously from a methanol solution of a bulk chemical (peak B). The carbon response indicates the presence of two impurities (peaks A and C) at the 0.2% level.

associated with each peak can often be established (except for isomerism) from the mass spectrum observed and the MIP emission data is needed only for its selectivity. However, in situations where the mass spectrum obtained is not sufficient to establish the structure of the material, MIP emission data can provide additional complementary data on elemental composition. This additional information comes in the form of elemental response ratios. If the individual elemental responses are calibrated relative to each other, the experimentally derived elemental response ratios can be used to determine empirical formulae [5].

Although the determination of empirical formulae using MIP emission is an established procedure, it requires calibration of the elemental responses. This calibration can be difficult and time consuming. In many cases encountered in pharmaceutical research, however, the responses obtained may be sufficiently calibrated without requiring any additional samples or chromatograms. This is true in cases where the sample of interest contains a molecule of known structure which is similar in elemental composition to the materials which must be identified. A case of this type is illustrated by the data shown in Fig. 5, which were obtained on a single injection of a bulk chemical which had been produced using a new synthetic procedure. Both the TIC and carbon responses indicate the presence of two small impurities (labelled A and C in Fig. 5). Integration of the carbon responses indicates that each impurity constitutes 0.2% of the material.

The area response ratios of fluorine, chlorine and sulphur to carbon for each of the peaks shown in Fig. 5 are given in Table 2. Even though absolute magnitude of the individual responses vary considerably, the ratios of various elemental responses within a peak should indicate the relative level of the various elements in the three peaks. Also given in Table 2 are the molecular ions for the three peaks which were determined simultaneously and the structure of the desired compound (peak B). In this structure neither R or R′ contain S, Cl or F.

For peak A, the absence of sulphur and the loss of 16 mass units in the molecular weight combined with the same level of fluorine and very similar chromatographic behaviour strongly support the oxazole type of structure for compound A. For compound C, in addition to similar chromatographic behaviour and an increase of 16 in the molecular ion, sulphur was found at the same level as in compound B, the fluorine content was diminished and a chlorine response was observed. These observations indicate that C is the difluoro-chloro analogue of B.

In this case the combination of molecular weight and elemental composition data generated simultaneously allowed a problem to be completely resolved within a couple of hours. This was accomplished without extensive calibration and required only that the sample size used produced a measurable response for all components but was not off scale for any major component.

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