

## Note

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CHROM. 6381

### **A simple and inexpensive method for the visual detection of sub-microgram amounts of halogenated substances present in gas chromatographic effluents**

Because of increasing interest in biologically important compounds containing covalently bound halogen atoms, it has become necessary to determine specifically or selectively the presence of organo-halogen substances, especially when contained in a complex mixture. Although electron capture detection is employed in the gas chromatographic detection of halogenated pesticides and polychlorinated biphenyls (PCBs), too often spurious peaks appear interfering with the analysis. Specific and selective halogen detectors have been devised and are in current use. These methods include flame photometric detectors<sup>1-7</sup>, mass spectrometry<sup>8</sup>, simple Beilstein tests<sup>9,10</sup>, "sensitized flame" or enhanced ionization responses<sup>11-13</sup>, and electrolytic conductivity<sup>14</sup>. However, the cost and sometimes complexities of these systems may negate their usage especially in a laboratory with limited facilities.

In our investigation of naturally occurring halogen-containing substances, it was imperative to utilize a detector whereby complex mixtures could be routinely screened for relatively small amounts of these interesting compounds. The approach to the problem was based on: (1) simplicity, (2) economics, (3) sensitivity, (4) specificity or selectivity, (5) applicability, and (6) rapidity of analysis. For our purposes, it was determined that a visual Beilstein test was the most suitable method of detection for volatile halogenated substances. The idea is not new and is based on the green flame emitted by the halogens (except fluorine) when burned in the presence of a copper wire.

#### *Experimental results*

A flame ionization detector (FID) fitted with a thin copper wire coil near the flame tip provided excellent results for the analysis of halogenated compounds in the sub-microgram level. The modification consists of a 24-gauge copper wire coil 6 mm × 4 mm O.D., placed around the flame tip (Fig. 1). The coil fits over the tip and extends approximately 1 mm above the tip, and approximately 1-2 mm below the collector cylinder. Care must be taken to insure that the coil does not touch the cylinder as a temporary shorting of the system will occur. If the coil is entirely below the top of the flame tip, detection is not observed. During an analysis the detector cap or lid is removed, and a visual observation is made by looking down into the detector. With the cap removed, the noise level increases but does not interfere with the qualitative analysis of the chromatograph. Generally, several hundreds of nanograms of halogenated organic compounds produced a sharp, bright and almost fluorescent green glow. At lower limits of detection the flame subsided to pale green in color. For maximum performance it is best to replace the copper coil every 4-6 h. This process involves only a 5- to 7-min delay in the analysis.

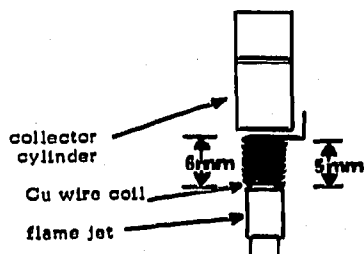


Fig. 1. Close-up view and dimensions of the copper coil set upon the flame jet.

Variations in the design of the copper wire gave less than satisfactory results. A thinner copper wire in the coil configuration was easily oxidized, producing excessive noise, and/or raised the limits of detection. A thicker wire than 24 gauge could not be satisfactorily positioned around the flame jet. Placing a copper wire inside a glass capillary support and hanging above the flame jet again led to rapid deterioration of the wire and was not suitable for more than a few experiments. Arranging the wire in a spiral fashion such that the base end was seated (like the coil) and the apex extending into, but not touching the collector cylinder, also did not offer suitable results. In addition to the above, replacement of the steel collector cylinder by a copper cylinder produced green flames which would not disappear readily and sometimes interfered with a following effluent exciting the column.

Known halogen-containing synthetics and pharmaceuticals were used to test the detector and determine its optimum conditions. A comprehensive but not necessarily extensive survey indicated that the main variable was the hydrogen flow-rate. In fact, the flow-rates of the gases, air, hydrogen and helium, were similar to those used under standard operation, *i.e.*, 300, 30, and 30 ml/min, respectively. Variation of the air and helium flow-rates had little effect on detection. Increasing or decreasing the hydrogen flow-rate generally resulted in decreased visual effectiveness.

Solutions of the known compounds tested were prepared in acetone such that  $1 \mu\text{l}$  was equivalent to  $1 \mu\text{g}$  of the organo-halogen. The amounts injected ranged from 0 to a few micrograms in 0.05- $\mu\text{g}$  portions. The results appear in Table I. As shown, the sensitivity decreases in the order  $\text{Br} > \text{I} > \text{Cl}$ . Interesting, but not readily explainable, was the fact that regardless of the number of substituent halogen atoms present in a specific halogenated series of compounds, the lower detection limits were not changed. In a more practical use of the method, the sedative and hypnotic, Somnos<sup>®</sup>, containing 100 mg/cc of chloral hydrate, was diluted with acetone, and similar to the known standard, as little as 0.3  $\mu\text{g}$  of the chlorinated substance could be detected. Although limited investigation was carried out at the microgram level, injection of as much as 10  $\mu\text{g}$  of halogenated compounds indicated the green flame dissipated upon the peak's return to baseline.

The further applicability of the method can be demonstrated from our work with natural products. Two species of red algae, *Laurencia spectabilis* (a Pacific Ocean variety) and *Laurencia papillosa* (an Atlantic Ocean type) have been found to contain halogenated organic substances utilizing the described method of detection. Column chromatographic fractions of a neutral oil from *L. spectabilis* were monitored

TABLE I

## DETECTION LIMITS OF VARIOUS HALOGENATED COMPOUNDS

Column: 6 ft.  $\times$  1/8 in. O. D. stainless steel, 3% OV-1 on 60-80 mesh Supelcoport. Detector: modified Varian 1860 FID. Conditions: flow-rates of air, helium and hydrogen 300, 30, and 30 ml/min, respectively; temperatures of detector and injector (fitted with a glass insert) 300° and 225°, respectively; attenuation 1 through  $8 \times 10^{-10}$  A.

Compound	Column temp. (°C)	Visually detectable amount ( $\mu$ g)
Bromobenzene	130	0.1
<i>o</i> -Dibromobenzene	130	0.1
Ethyl bromide	130	0.1
Iodobenzene	225	0.2
<i>o</i> -Diiodobenzene	225	0.2
Ethyl iodide	50	0.2
Chlorobenzene	130	0.3
<i>o</i> -Dichlorobenzene	130	0.3
Chlorobutanol	130	0.3
Chloral hydrate	60	0.3
Chloroform	60	0.3
Carbon tetrachloride	50	0.3

by gas-liquid chromatography (GLC) incorporating the modified Beilstein detector. In actual practice, an aliquot of the sample is run in the normal fashion (with the detector cap in place) to determine sensitivity limits and the complexity of the

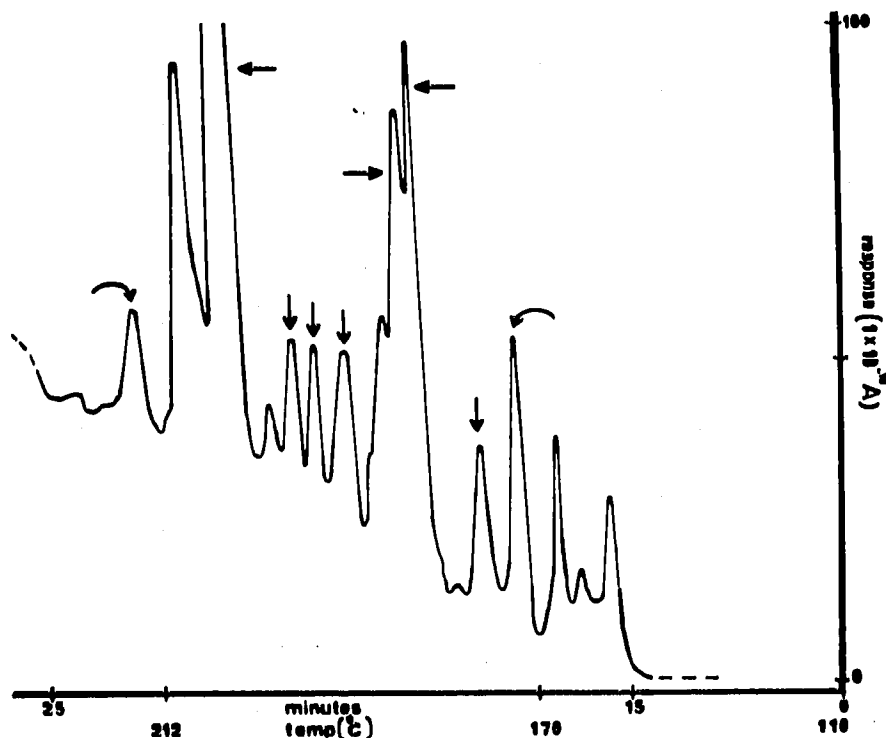


Fig. 2. Fraction 3 containing PCBs (arrowed peaks) from *L. spectabilis*.

mixture. Another portion of the same sample is then injected (with the detector cap removed). When a green flame is observed, the corresponding peak is immediately marked. The result obtained with one of the column chromatographic fractions is shown in Fig. 2. Visual observation of the effluents from this fraction produced a number of green flames. The peak corresponding to a positive test is marked by an arrow in the chromatogram. Submitting this same fraction to combination gas chromatography-mass spectrometry (GC-MS) indeed demonstrated that each of the marked peaks corresponds to a PCB. GLC of another chromatographic fraction of *L. spectabilis* is illustrated in Fig. 3. Only one peak (arrowed) gave a green flame. Again GC-MS data on this peak gave  $M^+$  312 and 310 having approximately equal intensity and indicating a monobrominated substance. Based on further detailed analysis of the mass spectral pattern<sup>16</sup> it appears that the brominated substance may be aplysinol<sup>10,17</sup>, an aromatic sesquiterpene, the mass spectrum of which apparently has not yet been published. Several other fractions have also been found to contain halogenated compounds, and this investigation is still proceeding.

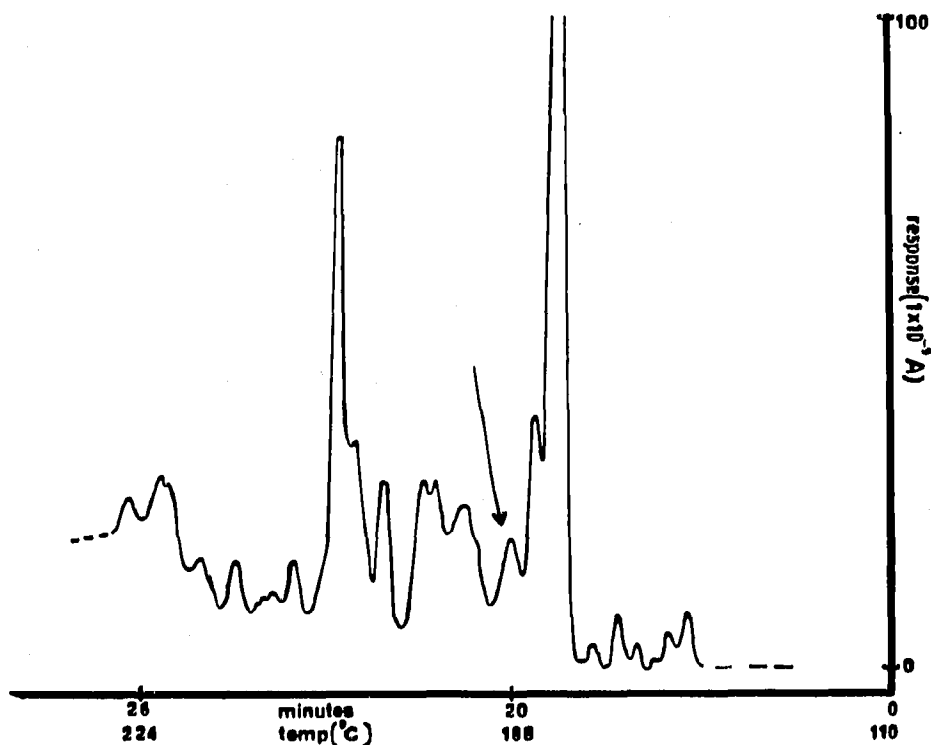


Fig. 3. Fraction 17 of *L. spectabilis* containing a bromine compound (arrowed peak).

Prior to our use of relying on the modified Beilstein test for routine detection, a fraction of *L. papillosa* subjected to GC-MS indicated the presence of a monobrominated compound ( $M^+$  296, 294)\*. In the reverse process, analysis by GLC,

\* The structure of this compound is still under consideration.

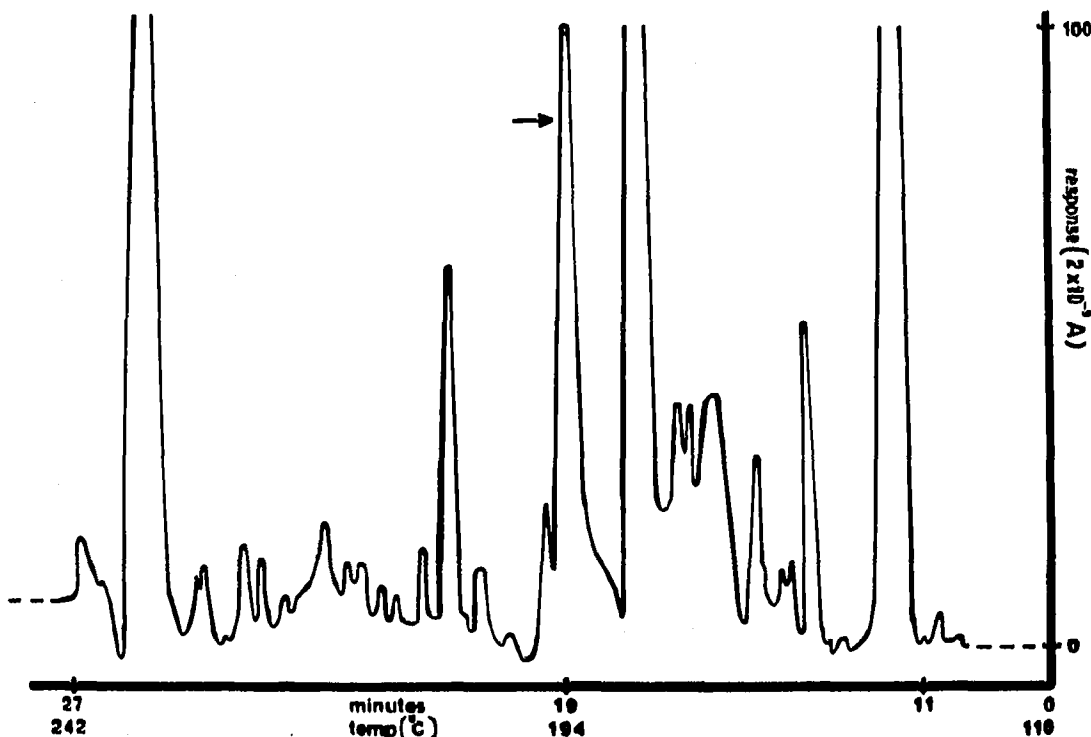


Fig. 4. Fraction 10 from *L. papillosa* containing a bromine compound (arrowed peak).

employing the modified detector, clearly demonstrated that the peak found by MS to contain a bromine atom also yielded a positive green flame test. Fig. 4 shows the arrowed peak in this complex mixture to be the halogenated effluent.

#### Discussion

Although it is our intention of specifically locating and analyzing halogen-containing organic compounds in natural products, the presence of hundreds of fractions precludes the routine analysis by GC-MS. The system described herein has satisfied our quest for a detector suitable for routine screening and enables us to at least selectively analyze those peaks which are of interest.

For our purposes, the method may be considered fairly specific since most potential interfering nitrogenous compounds may be removed by acidic ( $H_3PO_4$ ) washings of the crude extract. Nitriles which also produce a positive Beilstein test do not appear to be very common in natural systems. In order to determine the possible interference of nitriles,  $1 \mu l$  of benzonitrile was injected and observed in the Beilstein detector. Only a very pale green flame was noticed indicating this method is much more sensitive to halogens.

The modified detector appears to be at least fifty times more sensitive for halogenated compounds than similar non-photometric detectors yet devised. Surprisingly, the results with the known compounds (*cf.* Table I) show the limits of sensitivity are much lower than with a similar photometric device of HUYTEN AND

RIJNDERS<sup>7</sup>. How useful the method may be for the determination of halogenated pesticides at the sub-nanogram level is questionable. Perhaps further modification of the Beilstein detector may lower the sensitivity limits.

A Varian Model 1860 instrument was employed in this investigation. Because of the similarity of most flame ionization detectors, this simple modification could probably be incorporated in a large variety of FID instruments. The method is practical, facile, and economical and could be a suitable addition in the methods for the study of volatile halogenated compounds.

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