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HIGH-RESOLUTION GAS CHROMATOGRAPHIC DETERMINATION OF NITROGEN AND PHOSPHORUS COMPOUNDS IN COMPLEX ORGANIC MIXTURES BY A TUNABLE SELECTIVE THERMIONIC DETECTOR

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SUMMARY

A flame thermionic detector, allowing unambiguous distinction between nitrogen compounds and phosphorus compounds in complex organic matrices, is described. If necessary, the interfering carbon compounds can be almost totally suppressed or identified as negative peaks in the chromatogram. The different selectivity profiles are achieved by actuating specific detector configurations without any modification of the operating conditions, such as gas flow, alkali-source temperature or electrode-polarizing voltage. The selective determination of nitrogen and phosphorus compounds in complex samples of natural origin, performed by capillary gas chromatography is reported.

INTRODUCTION

Flame thermionic detectors have been widely used since 1964 in the gas chromatographic (GC) determination of organic compounds containing phosphorus or nitrogen. Different and improved versions of such detectors have become commercially available^{2,4}, and different theories on the detection mechanism have been proposed^{2,5,6}, even though much still remains contradictory and unexplained. Nevertheless, the fields of application of nitrogen-phosphorus detection (NPD) are continuously increasing, particularly in combination with capillary columns for the analysis of complex mixtures.

The selectivity of this mode of detection allows extracts of natural origin to be processed easily without necessarily requiring complex clean-up procedures. Generally, the discrimination factor between nitrogen/phosphorus and carbon depends on the composition of the gas surrounding the alkali source, the source temperature, the hydrogen flow and the electrode polarization voltage^{4,7,8}.

Operating conditions are often adjusted experimentally so as to attain the best possible compromise between selectivity and sensitivity⁸. However, when NPD is used by itself, it is difficult to distinguish between small amounts of nitrogen/phosphorus-containing compounds and a large quantity of a purely organic compound⁹.

The unambiguous identification of nitrogen and phosphorus molecules, together with carbon-based compounds cannot easily be performed without an extra flame ionization detector.

The flame thermionic detector presented in this paper (NPD-40), in addition to featuring the ability to discriminate between the nitrogen and phosphorus responses, allows the carbon response to be adjusted in both positive and in negative directions on the chromatographic tracing. No modifications of the existing pneumatic system of the gas chromatograph are needed and no extra modules are required besides the normal electrometer.

DESCRIPTION OF THE DETECTOR

The NPD-40 is assembled directly on the gas chromatograph, using the conventional base body of a flame ionization detector. As shown in Fig. 1 the alkali source is located between the polarized jet and the cylindrical collector electrode. It consists of a rubidium-silica salt tip that can easily be replaced after an average working life of 800 h. The position of the thermionic source on the flame can be adjusted to regulate the response selectivity by tuning the built-in calibration trimmer. For capillary column operations, helium or hydrogen can be used as carrier gas, while an extra flow of helium or nitrogen is recommended as make-up gas.

A three-way switch system with mercury-wetted "reed switches" achieves three different electrical configurations inside the detector, corresponding to the nitrogen-phosphorus, phosphorus and nitrogen modes (Fig. 2). The first mode is normally used for detection of either nitrogen- or phosphorus-containing compounds; the last two modes enhance the response to phosphorus and nitrogen respectively, relative to the other element, and thus make the identification easy and positive.

When switching to the phosphorus mode, the carbon peak reverses, if the hydrogen flow has been correctly set, allowing large amounts of other organic materials in the extract that may also be present to be immediately identified.

In any case, in all three modes of operation the carbon response can be regulated in both directions by means of the built-in trimmer.

As far as the detection mechanism is concerned, it can be hypothesized that the alkali metal ions, formed during the gas-phase ionization process, interact with the

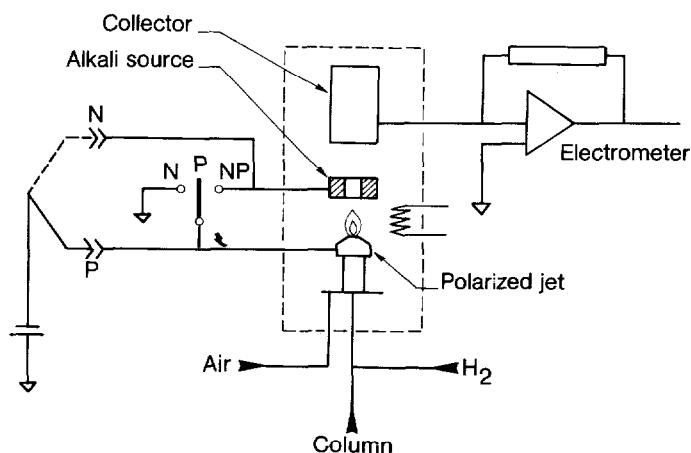


Fig. 1. Schematic diagram of the nitrogen-phosphorus detector (NPD-40).

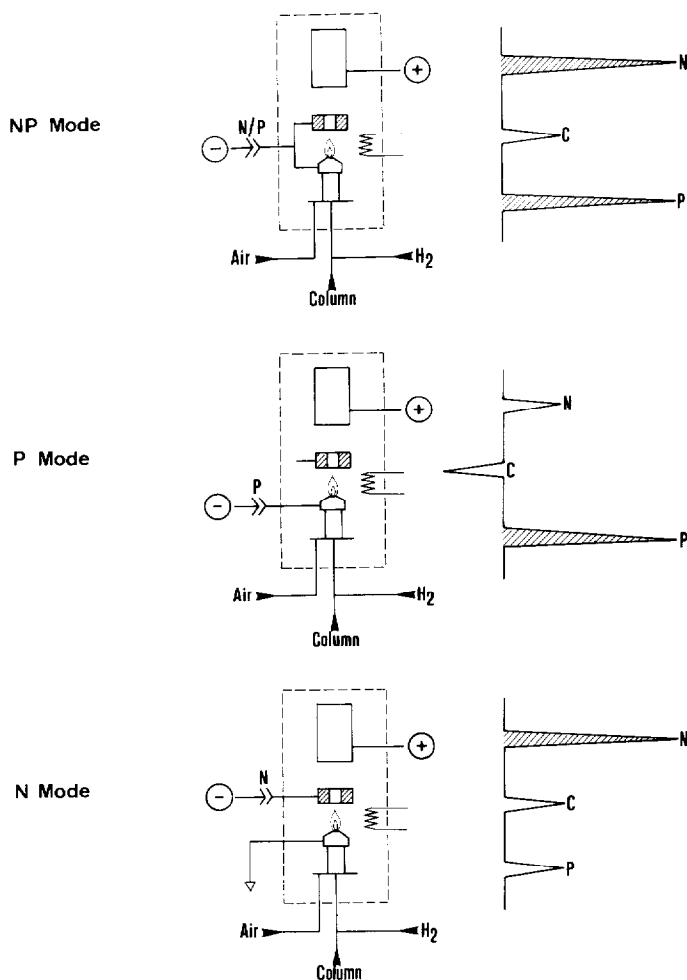


Fig. 2. Operational modes of the nitrogen-phosphorus detector.

radicals containing phosphorus or nitrogen to form complex ions of increased lifetime, which are selectively collected. The resulting ion current is measured by the normal electrometer used with the flame ionization detector.

Taking into consideration that the zones of maximum signal collection for P, N, and C are spatially separated^{10,11}, different selectivity effects are obtained by altering the electrical configuration of the collecting zone and varying the ion concentration. The detector selectivity appears to be related to the standing current level and to the flame temperature, *i.e.*, to the gas composition. Having correctly set the flow-rates of carrier (helium is suggested for packed column operations), hydrogen and air, it is possible to vary the standing current by slightly shifting the alkali source vertically on the flame. When the regulation trimmer, located on the detector, is turned counter-clockwise, the source is lowered towards the flame, the standing current increases and the carbon response decreases to give a negative tracing in the chromatogram. Nitrogen and phosphorus compounds do not generate negative peaks in any operative mode.

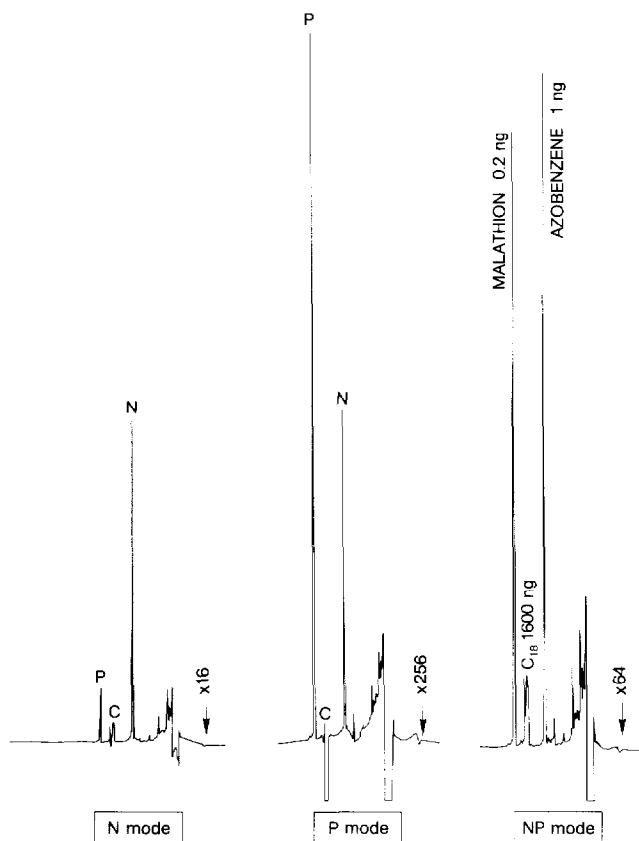


Fig. 3. Analysis of a test sample in the NPD-40 working modes. Column: 15-m glass capillary, OV-1. Carrier gas: hydrogen, 2 ml/min. Make-up gas: helium, 40 ml/min. On-column injection: Fractovap Model 4160. Column temperature: 60 ballistically to 220°C. Sample: 1 ng/ μ l azobenzene; 0.2 ng/ μ l malathion and 1600 ng/ μ l octadecane in *n*-hexane.

Whatever the mechanism of the response, which is incompletely understood, the detector capabilities in terms of sensitivity and N,P,C selectivity are clearly illustrated in Fig. 3. The GC tracings are shown for a test sample containing 1 ng/ μ l azobenzene (N), 0.2 ng/ μ l malathion (P) and 1600 ng/ μ l octadecane (C) in *n*-hexane, processed in the three operating modes. The different selectivity profiles allow the nitrogen compound and the phosphorus compound to be easily recognized; the large hydrocarbon peak, showing an overloading effect on the capillary column, can also be identified as negative on the "P mode" tracing.

APPLICATIONS

The nitrogen-phosphorus detector, coupled to capillary columns, represents an extremely useful tool for the detection of nitrogen-containing drugs in complex samples of biological origin in different areas of clinical chemistry.

The determination of clonidine, 2-(2,6-dichloroanilino)-2-imidazoline, in blood

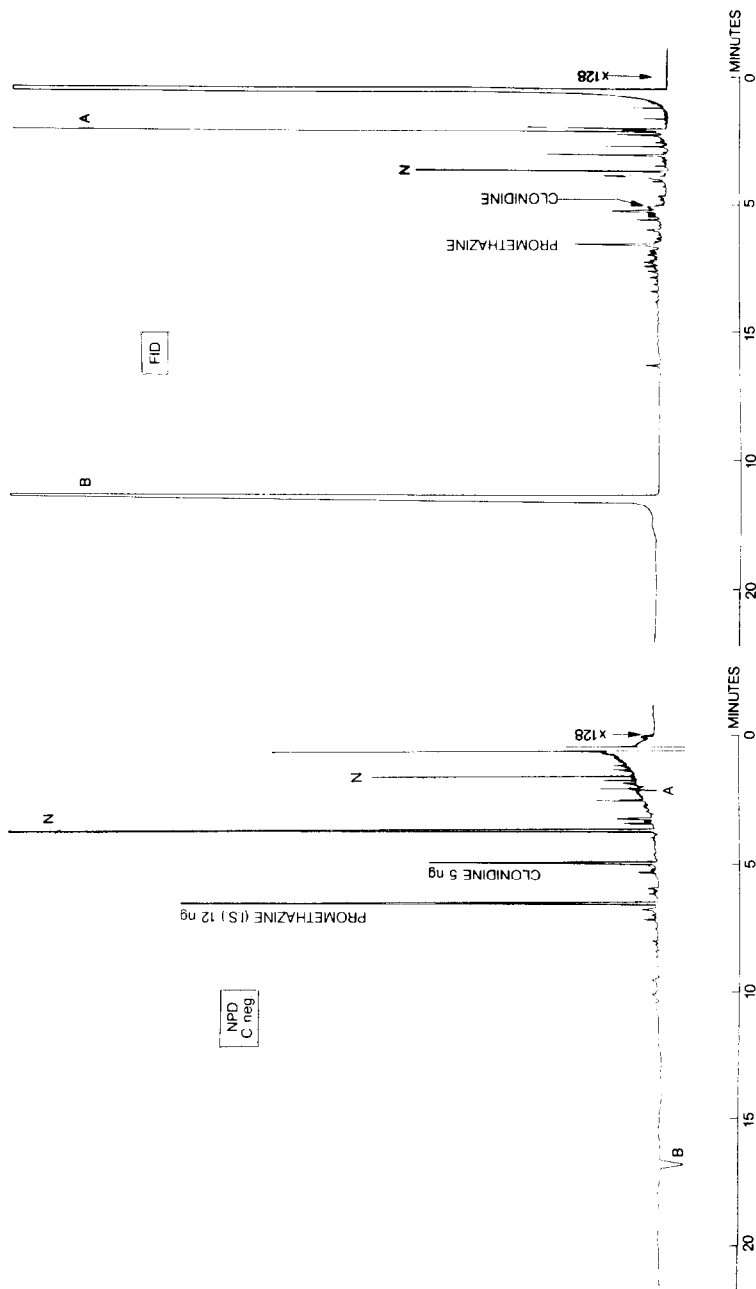


Fig. 4. Analysis of clonidine in liver extract (ethanol solution). Conditions: Carlo Erba gas chromatograph, Model 4160, with cold on-column injector; glass capillary column $15\text{ m} \times 0.32\text{ mm I.D.}$, SE-54, $0.4\text{-}\mu\text{m}$ film; carrier gas, hydrogen, 2 ml/min ; make-up gas, He, 30 ml/min ; column temperature, 80°C , ballistically to 190°C , programmed to 270°C at 8°C/min .

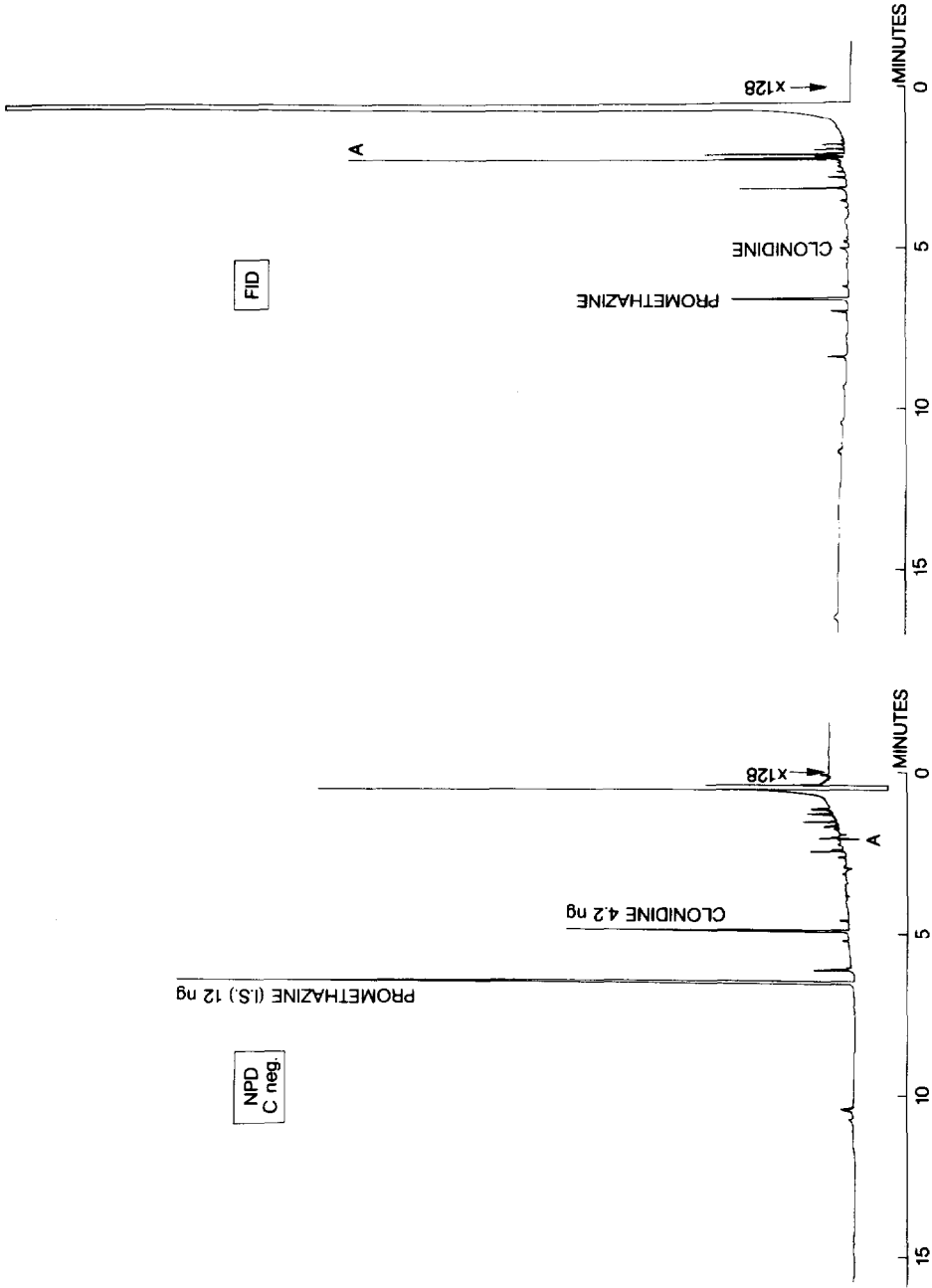


Fig. 5. Analysis of clonidine in blood extract (ethanol solution). Conditions as in Fig. 4.

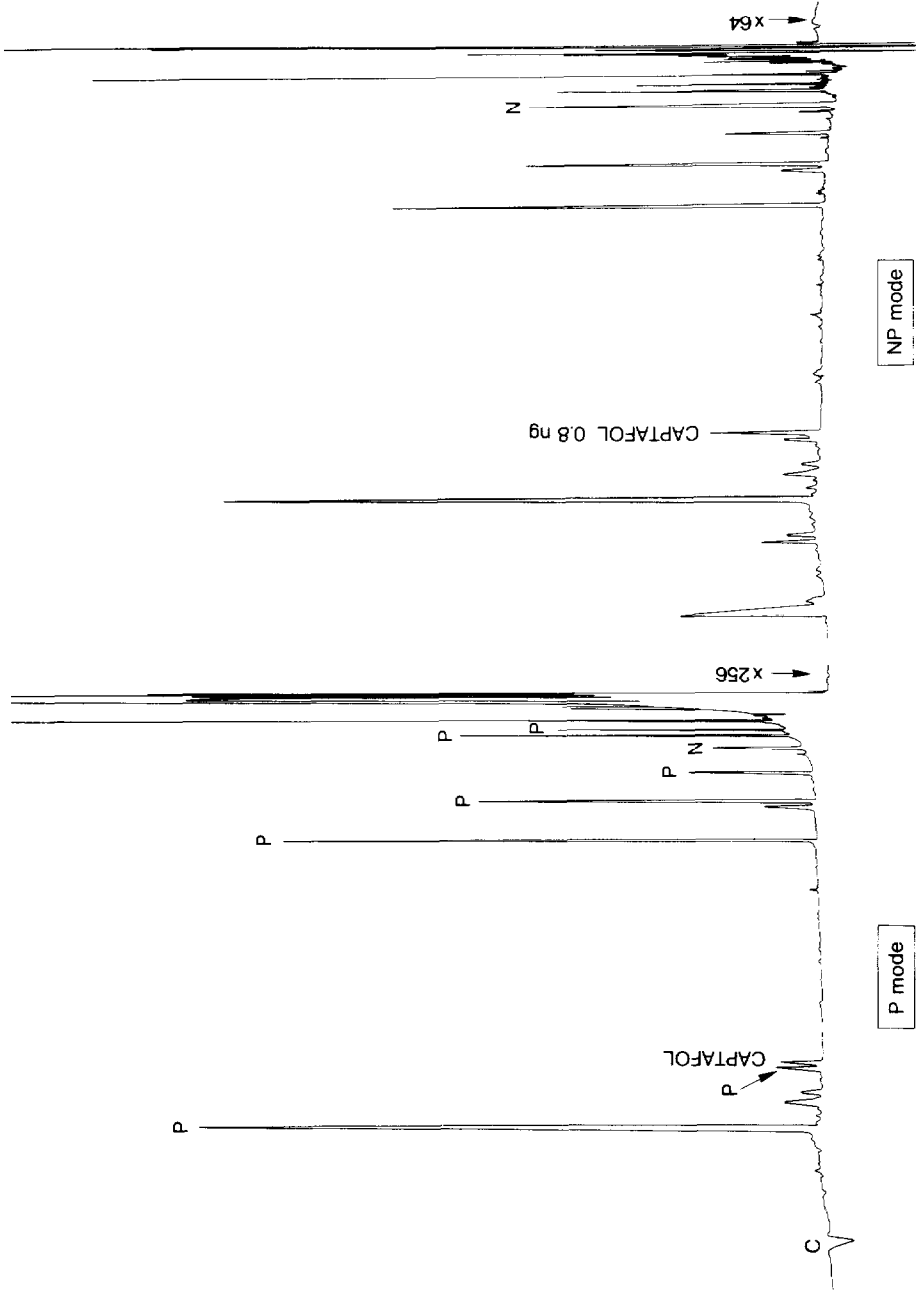


Fig. 6. Analysis of apple extract (benzene solution). Conditions: Carlo Erba gas chromatograph, Model 4160, with cold on-column injector and NPD-40 detector; glass capillary column, 20 m x 0.32 mm I.D., OV-1, 0.15- μ m film; carrier gas, hydrogen 2 ml/min.; make-up gas, N₂, 10 ml/min; column temperature, 60°C ballistically to 130°C, programmed to 270°C at 10°C/min.

and liver may be used as an example of application to forensic toxicology (poisoning by overdose). The extraction was performed by the Toxicology Laboratory of the Istituto di Medicina Legale, University of Milan (Italy), and the final ethanol solutions were analyzed with a glass capillary column of SE-54, 15 m \times 0.32 mm I.D., film thickness 0.4 μ m (MEGA). For both samples a Carlo Erba gas chromatograph, Fractovap Model 4160, equipped with an NPD-40 detector and cold on-column injector was used. The analyses were also repeated, under the same operating conditions, with a conventional flame ionization detector.

It is evident that the identification and quantitation of clonidine, and promethazine as an internal standard (I.S.) is easily accomplished with the NPD-40 in both samples: 2.08 μ g/g were found in liver (Fig. 4) and 1.67 μ g/ml in blood (Fig. 5). In contrast, it would be impossible to obtain quantitative result with flame ionization detection (FID) especially in liver extract, without additional sample clean-up. The FID response confirms the presence of large amounts of other organic materials in the extracts, identified as negative peaks on the nitrogen-phosphorus detector tracings (indicated by A and B on the chromatograms).

Comparing the two chromatograms in Fig. 4, it is also possible to identify an unknown peak in the liver extract as a nitrogen compound (indicated by N on the tracings).

The ability of the NPD-40 to discriminate between nitrogen and phosphorus compounds has wide applications in the fields of pesticide residue determinations. Fig. 6 shows the analysis of a nitrogen-containing fungicide (captan) in an apple sample, performed with a glass capillary column of OV-1, 20 m \times 0.32 mm I.D., film thickness 0.15 μ m and cold on-column injection (Carlo Erba Fractovap 4160 gas chromatograph). It is clear that the detector identifies unexpected phosphorus compounds and other compounds present, in addition to the fungicide to be determined.

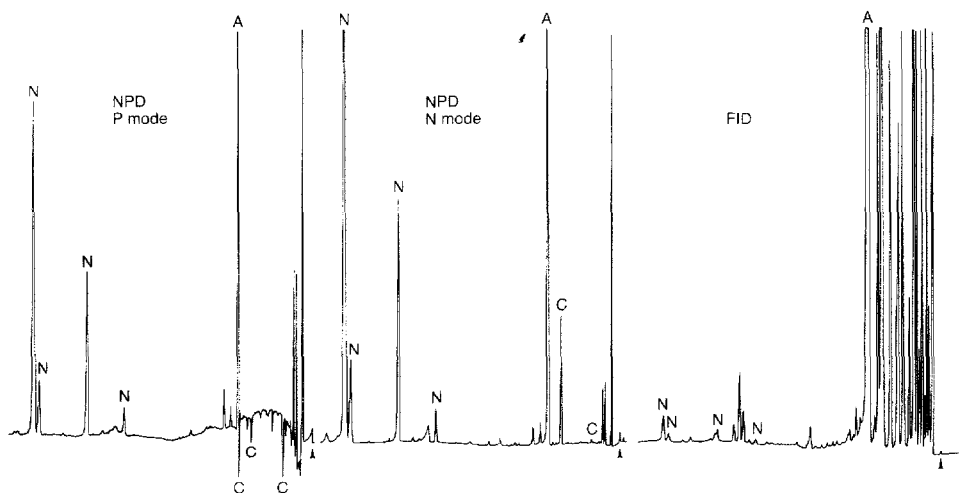


Fig. 7. Analysis of tobacco aroma by headspace technique. Conditions: Carlo Erba gas chromatograph, Model 2900; glass capillary column, 40 m \times 0.6 mm I.D., Carbowax 400; carrier gas, hydrogen 4ml/min; make-up gas, He, 30 ml/min; column temperature, 20°C to 75°C, at 3.5°C/min. Headspace gas volume injected, 2 ml (splitter 1:3). A is an unidentified peak used as reference.

The NPD-40 detector has been used for the determination of various volatile nitrogen or phosphorus compounds in connection with the headspace technique. The chromatograms shown in Fig. 7 were in fact, obtained by headspace analysis of a tobacco sample with flame ionization and in nitrogen-phosphorus detectors parallel. The nitrogen-phosphorus detector tracing clearly shows some peaks (N) which are virtually negligible on the FID tracing. Comparing the nitrogen and phosphorus modes, where carbon gives a negative response, the peaks of nitrogen-containing compounds can readily be identified, and others can be seen which are carbon-based organics (C).

CONCLUSIONS

The flame thermionic detector described here can be advantageously used for the determination of nitrogen and phosphorus compounds in complex samples by high-resolution gas chromatography.

The tunable selectivity allows easy distinction to be made that may also be between hetero compounds and carbon-based compounds present in large amounts. This is of great interest in the analysis of samples of biological or natural origin and simplifies the extraction procedures.

The high resolution of capillary columns, coupled with the high detector sensitivity for nitrogen and phosphorus compounds, furnishes an essential tool for different applications.

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