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Note

Analysis of a model ionic nitrosamine by microbore high-performance liquid chromatography using a thermal energy analyser chemiluminescence detector

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Trace levels of volatile nitrosamines have been reported in a number of different types of foodstuffs and beverages including cured meat[1], cheese[2] and beer[3]. The occurrence of these compounds is of concern because of the carcinogenic properties many of them exhibit in animal feeding studies[4]. The analysis of trace levels of volatile nitrosamines in complex samples of biological origin has been considerably facilitated by the introduction of the nitrosamine-specific thermal energy analyser (TEA) chromatographic detector[5]. The TEA comprises a catalytic pyrolyser which cleaves the nitrosamine N–NO bond and a cold trap for removing organic matter; the nitric oxide is then detected by a chemiluminescent reaction with ozone. Due to the high sensitivity and selectivity of the TEA in comparison with most other gas chromatographic (GC) detectors[6], GC–TEA is now the standard method for trace analysis of volatile nitrosamines[7,8].

The TEA may also be used as a liquid chromatography detector permitting, in principle, the analysis of non-volatile nitrosamines. However, in contrast to volatile nitrosamines the field of non-volatile nitrosamines is still largely unexplored due to the constraints the TEA places on the choice of high-performance liquid chromatographic (HPLC) solvent systems. Inorganic buffers and ion-pair reagents cannot be used with the TEA pyrolyser; additionally, the detector output is highly unstable in the presence of aqueous HPLC mobile phases and only trace levels of water may be used. As a result HPLC–TEA has been used only for the analysis of a limited number of nitrosamine classes, *i.e.* non-polar nitrosamines[9], polar nitrosamines such as nitrosodiethanolamine[10] and only those ionizable nitrosamines (*e.g.* nitrosoamino acids) where the ionization can be suppressed by the HPLC solvent[11]. There are no reports concerning the analysis of ionic nitrosamines such as zwitterions, quaternary nitrogen-compounds and macromolecular peptides and this is principally due to the incompatibility of the TEA with the amount of water introduced into the detector under HPLC conditions required for the chromatography of ionic compounds. However, the recent advent of microbore HPLC with its very low flow-rates may permit the use of these chromatographic conditions with the TEA. To assess the applicability of this technique we have synthesized a model ionic nitrosamine N-nitroso N^1,N^1-dimethylpiperazinium iodide. In this note we report for the first time the TEA analysis of an ionic nitrosamine using microbore HPLC.

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EXPERIMENTAL

The analysis was performed by reversed-phase ion-pair chromatography using a Chrompack 50 cm × 1 mm microbore ODS column coupled directly to a 0.5-μl internal-loop Valco valve. The mobile phase of 0.1 *M* ammonium heptane-sulphonate in methanol–water (70:30) was pumped at 20 μl/min using a Waters 6000A pump modified for microbore flow-rates. The column eluent was mixed with acetone (2 ml/min) and introduced into the TEA pyrolyser operating at 650°C. Two sets of TEA cold traps were used in series (solid carbon dioxide–isopropanol and liquid nitrogen).

N-Nitroso N^1,N^1-dimethylpiperazinium iodide was prepared by reacting N-nitroso N^1-methylpiperazine with methyl iodide in ethanol at 0°C. The results of elemental analysis (found: C, 26.80; H, 5.14; N, 15.49; I, 46.38%. $C_6H_{14}N_3OI$ requires C, 26.58; H, 5.20; N, 15.50; I, 46.81%) and ^1H nuclear magnetic resonance (NMR) spectroscopy on the resulting white solid were consistent with the expected structure $[ON \cdot N \cdot (CH_2 \cdot CH_2)_2 \cdot N(CH_3)_2]^+ I^-$.

RESULTS AND DISCUSSION

The microbore HPLC–TEA chromatogram of N-nitroso N^1,N^1-dimethylpiperazinium iodide is shown in Fig. 1. Under the reversed-phase ion-pair conditions employed the ionic nitrosamine eluted as a sharp symmetrical peak after 20.5 min (capacity factor, $k' = 1.3$). Due to the very low flow-rate involved the highly

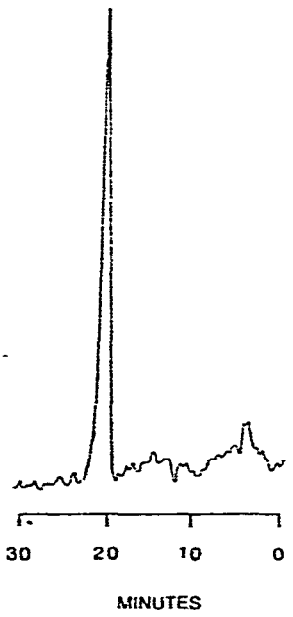

Fig. 1. Reversed-phase ion-pair HPLC–TEA chromatogram of N-nitroso N^1,N^1-dimethylpiperazinium iodide (105 ng); TEA attenuation, × 16.

polar nature of the microbore HPLC mobile phase had little adverse effect on the stability of the detector signal.

As far as we are aware microbore HPLC coupled with the highly sensitive and nitrosamine-specific TEA detector is presently the only chromatographic technique suitable for the trace analysis of ionic nitrosamines. The procedure may be of considerable use for the unexplored field of ionic, zwitterionic and macromolecular nitrosamines in foodstuffs.

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