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Use of Thermal Energy Analyzer in the Analysis of Nitrosamines — Volatile Nitrosamines in Samples of Italian Beers*

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Introduction

Numerous techniques have been developed and employed by analysts for the determination of N-nitroso compounds in recent years. Methods have been used which were based on thin-layer chromatography, spectrophotometry, polarography and gas chromatography.

High-resolution mass spectrometry is generally the confirmatory technique, whereas derivatization allows either GC or GC electron capture to be employed.

The latest analytical methods for N-nitroso compound determination have been recently surveyed by *Preussmann* et al. (1) but ever since 1980 the subject of N-nitroso compound analysis had been discussed and brought thoroughly up to date by *D. H. Fine* in «Advances in environmental science and technology» (2).

All of the methods mentioned above entail disadvantages of some kind or another:

- most workers have avoided TLC methods because of relatively poor sensitivity and selectivity,
- UV analysis is generally unsuitable for work at trace level in complex environmental samples due to spectral interference,
- polarographic methods are affected by a number of common natural food constituents,
- GC techniques are limited to the few N-nitroso compounds that are sufficiently volatile and stable to high temperatures inside the gas chromatogaph.

In particular, the flame ionization detector (FID) notoriously entails nonselectivity problems, while the alkali flame ionization detector (AFID) gives enhanced response to organic nitrogen compounds but its response is affected by the con-

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dition of the salt tip, by organochlorine solvents and by carbon containing compounds and, if nonnitrogen compounds are present in excess, the nitrogen enhanced response can be swamped. For the GC electron capture detector to be used, on the other hand, preventive conversion is required of N-nitroso compounds, first into hydrazines, then into 3.5-dinitrobenzaldehyde hydrazones; heptafluorobutyryl adducts and nitramines are other derivatives that can be determined by this same detector, but a number of problems have been found to be associated with methods based on the use of such a derivatization (inter alia, erratic recoveries). Finally, the combined use of both GC and MS required for conversion into methyl ethers.

An increasingly complete collection of data on human exposure to N-nitroso compounds can instead be obtained by resorting to analytical methods that call for a minimum number of analytical steps, involving the use of absolutely selective detectors and allowing extremely high sensitivity levels to be attained. The sensitivity degree required for N-nitroso compound determination stems from the order of magnitude of the exposure values involved: for quite a few food products, indeed, analytical N-nitroso compound determination is only useful when the method's sensitivity attains the order of one-hundredth of ppb.

As the chemical and physical properties of N-nitroso compounds vary widely, an optimum analytical method must enable the presence to be assessed of the =N-NO group alone — and the thermal energy analyzer (TEA) may indeed be regarded as the only detector so far that allows N-nitroso compounds to be sensitively and selectively analyzed.

Several papers (3—7) have already been published on the development and use of the TEA detector by *D. H. Fine, D. P. Rounbehler, R. Ross, F. Rufeh, D. Lieb* and other workers. Since, however, the method used for determinations by this particular detector has been developed only rather recently, scanty data have been gathered by other researchers on the N-nitroso compound contents of the majority of food products for which analyses based on TEA detection may instead yield valuable results.

Whereas the principles undelying the use of TEA detection will be outlined under «Experimental», the potential applications of such a selective method will now be discussed.

For convenience, N-nitroso compounds can be divided into four categories (fig. 1):

1 — volatile
2 — nonvolatile, low polarity
3 — nonvolatile, high polarity, nonionic
4 — nonvolatile, high polarity, ionic.

The TEA is used as a detector for either a gas chromatograph (GC) or for a high-pessure liquid chromatograph. The importance of this point will emerge from the following considerations.

The *volatile N-nitroso compounds* are generally the simplest alkyl N-nitrosamines, and include such compounds as N-nitroso dimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodi-

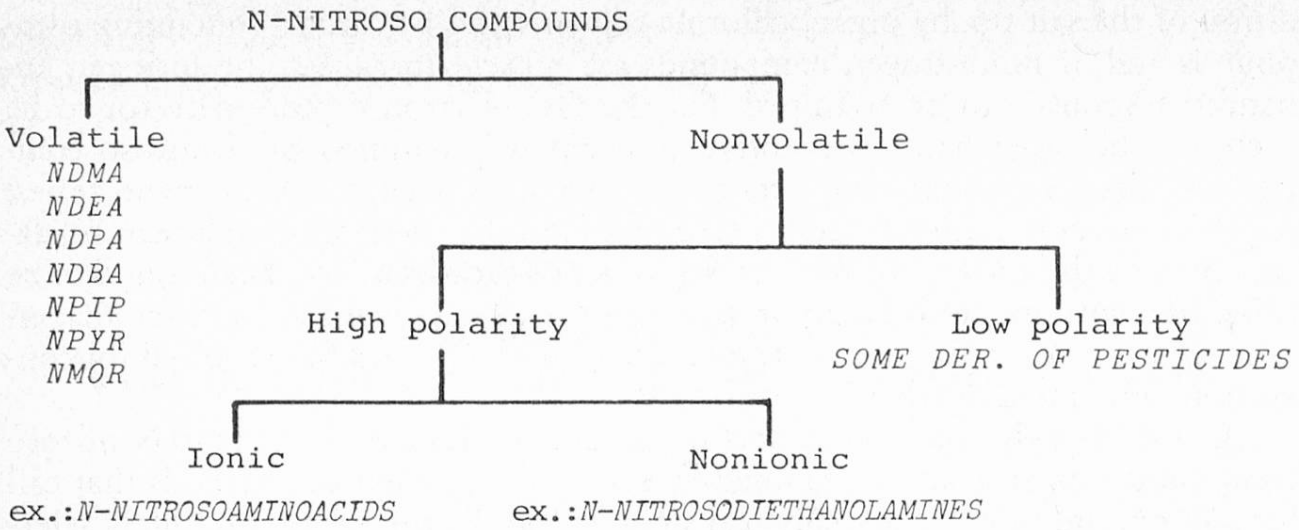


Fig. 1. Classification of N-nitroso compounds

butylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrolidine (NPYR) and N-nitrosomorpholine (NMOR). Because the compounds are volatile, they are amenable to analysis using GC techniques. There are about 25 N-nitrosamines that fall into this category: excluded are the N-nitrosoureas, N-nitrosaminoacids, N-nitrosohydroxy compounds and all compounds which decompose at elevated temperature.

The *nonvolatile, low polarity N-nitroso compounds* decompose at elevated temperature, and they are analyzed using HPLC (some N-nitroso derivatives of pesticides).

The *nonvolatile, high polarity, nonionic N-nitroso compounds* (as N-nitrosodiethanolamine) and the *nonvolatile, high polarity, ionic N-nitroso compounds* (as N-nitroso aminoacids and salts) are analyzed using HPLC: for the last two classes of compounds, GC determination is only possible after they have been converted into volatile derivatives.

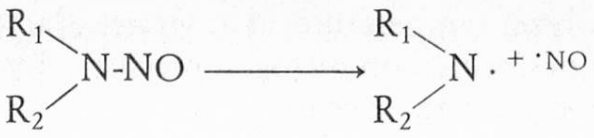
The results are reported under «Experimental» of the first TEA-detector determinations of volatile nitrosamines in samples of some of Italy's most popular beers, performed using a simplified analytical procedure, sensitive at the sub-μg/liter concentration level for all GC-amenable N-nitroso compounds. The procedure was developed in the New England Institute for Life Science (7).

Experimental

Analytical method based on TEA detector

The operational diagram of TEA detector is shown in figure 2, the individual phases of the process being summarily described hereunder.

— The effluent from the GC or HPLC is heated to about 450 °C in a catalytic pyrolysis chamber. Under vacuum, the N-NO bonds are cleaved, and the nitrosyl radicals are released:



- The nitrosyl radicals, together with other degradation products and solvent vapor, pass through a cold trap. At –150 °C, the nitrosyl radicals, the carrier gas and only the most volatile compounds survive in the vapor phase. When HPLC is used, two traps are employed, the temperature maintained in the first trap allowing the carrier solvent to be just liquefied, without freezing.
- After passing through a porous polymer (usually Tenax GC) where the organic products are detained that have escaped the cold trap, nitrosyl radicals are conveyed into a reaction chamber, where they are oxidized with ozone. At reduced pressure, excited nitrogen dioxide is formed, which relaxes to its ground state emitting radiation in the near-infrared region. The intensity of the radiation at 0.6—0.8 μ is monitored (photomultiplier and suitable red filter).

$$\cdot NO + O_3 \longrightarrow NO_2^* + O_2$$

$$NO_2^* \longrightarrow NO_2 + h$$

The intensity of the near-infrared radiation provides a direct measure of the amount of nitrosamine present. A modified TEA-detector method, on the other hand, allows amines and other nitrogen containing compounds to be determined, too. Such a modified method, called TEA (N) detector, enables all organic-N to be converted to the nitrosyl radical. The modification is effected at pyrolyzer level, where the GC effluent is oxidized by the non-catalytic metal oxide at a temperature of less than 700 °C. In this type of pyrolyzer, the metal oxide surface is regenerated by continuously passing oxygen; all nitrogen containing organic products, including solvents (though not molecular N_2) produce the nitrosyl radicals which are subsequently determined as with standard TEA detector. Obviously, when TEA(N) detector is used, the response will be proportional to the number of all nitrogen atoms contained in a molecule (e. g. with the TEA(N) detector the NDMA will give a response that will be quantitatively twice the one obtained with the standard TEA detector).

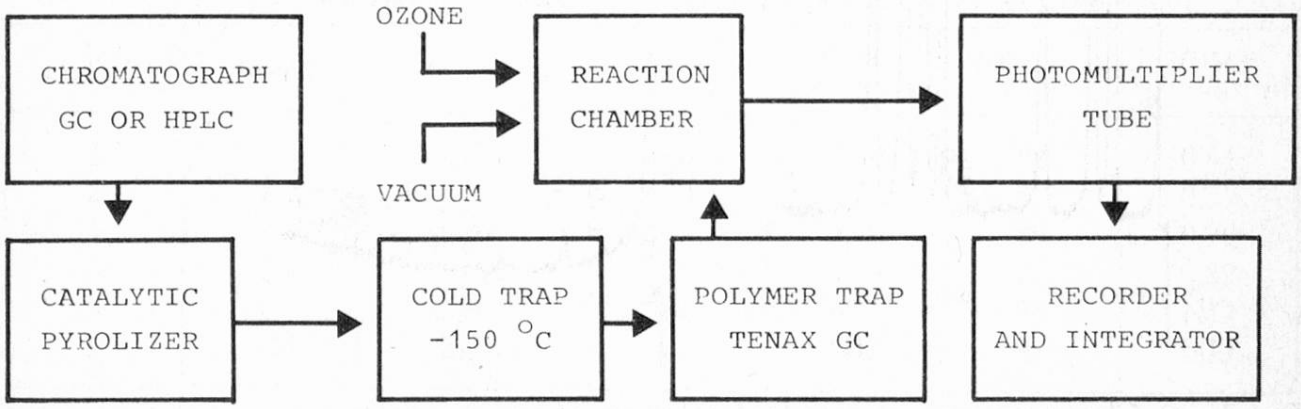


Fig. 2. TEA-Principle of operation

By varying the pyrolyzer temperature, at any rate, a large number of analytical determinations can be performed on nitrogen products, by selecting the temperature at which complete cleavage occurs.

TEA-detector determination of volatile N-nitroso compounds in beer samples

In a paper by *E. U. Goff* and *D. H. Fine* (7), data are related which concern the NDMA contents determined by GC-TEA and HPLC-TEA in 18 samples of different beer brands. Such results are reported in table 1.

The same GC-TEA method has been used to determine volatile nitrosamines in 6 samples of Italian-brewed beers, selected amongst the most popular brands and all available on the market.

In figure 3 there is shown the gas chromatogram performed for calibration, the gas chromatogram corresponding to the analytical blank appearing underneath.

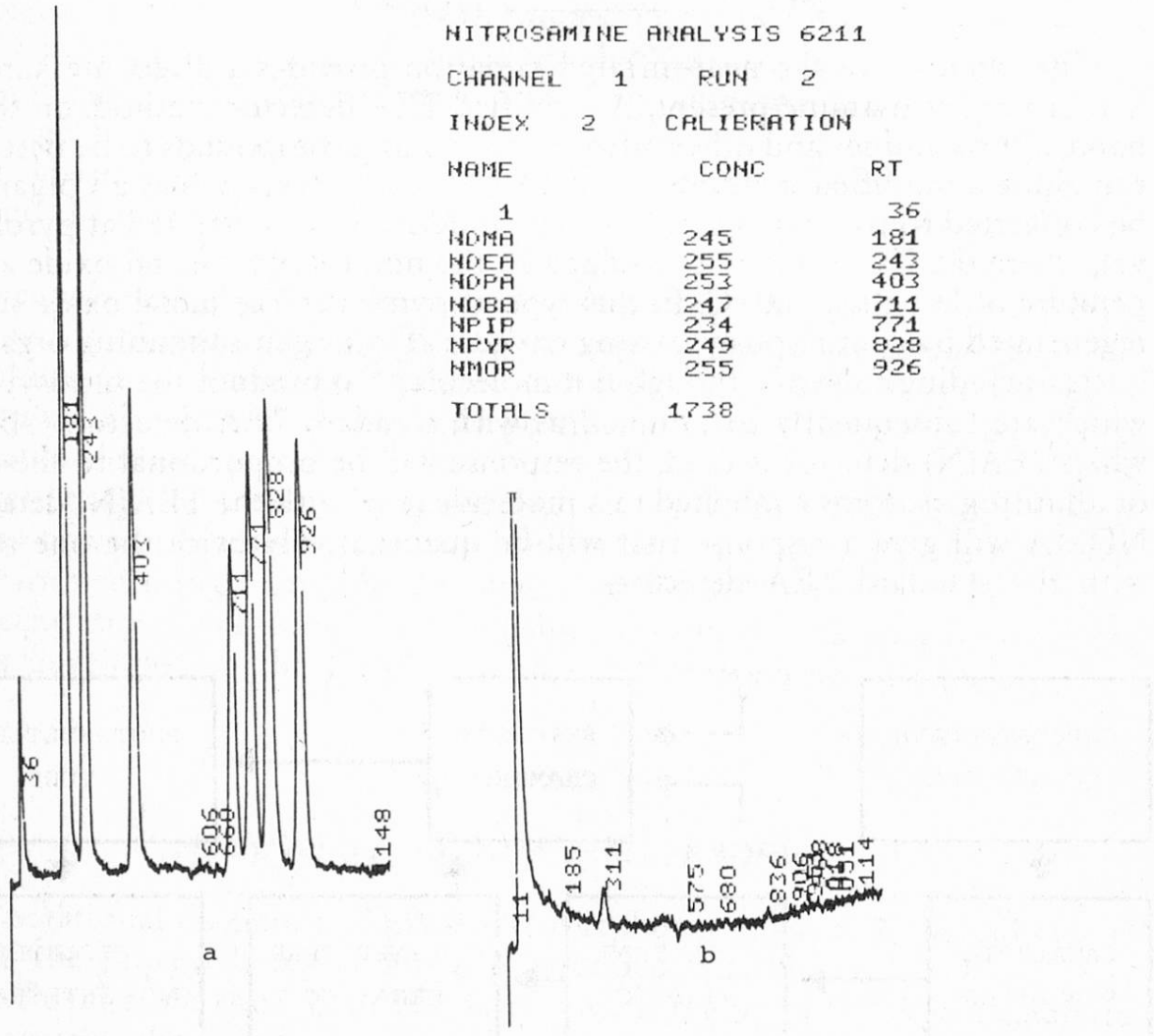


Fig. 3. a) Chromatogram of standard solution of nitrosamines
b) Analytical blank

Table 1. Analysis of volatile N-nitrosamines in samples of beer (*E. U. Goff* and *D. H. Fine)*

Type	Country of origin	(NDMA µg/litre)	
		GC-TEA	HPLC-TEA
Light	France	0.6	0.6
Light	Philippines	3.4	3.6
Light	Japan	3.7	3.7
Light	Greece	0.5	ND
Light	Holland	5.2	4.7
Light	Holland	3.1	3.2
Light	Holland	0.7	0.8
Dark	W. Germany	5.3	4.8
Dark	Mexico	0.4	ND
Dark	Ireland	0.6	1.0
Lager	Australia	1.8	1.8
Dark ale	United Kingdom	6.4	7.0
Light	USA	7.0	6.7
Light	USA	1.8	1.7
Light	USA	0.9	0.8
Light	USA	4.4	4.0
Dark	USA	1.4	0.9
Dark	USA	3.1	3.0

NDMA = N-nitrosodimethylamine
ND = less than 0.2 µg/litre

The gas chromatograms corresponding to the 6 beer samples examined are reproduced in figures 4, 5, 6, 7, 8 and 9. Each analysis was effected twice.

N-nitrosodipropylamine (NDPA) has been added as internal standard (10 ppb), and recovery values are reported in table 2, where the values are also shown of observed NDMA (ppb) and corrected NDMA (ppb).

Table 2. N-nitrosodimethylamine (NDMA) in samples of Italian beer

Sample No	Brewers	Type	Cont. (cl)	NDMA ppb observed	NDPA (i. s.) % recovery	NDMA ppb corrected
1	A	light	66	0:40	98	0.41
2	A	sp. light	33	0.34	92	0.37
3	A	sp. dark	33	0.78	99	0.79
4	B	light	33	0.79	96	0.82
5	C	light	66	ND	95	ND
6	D	light	66	ND	96	ND

ND = less than 0.3 µg/litre
NDPA = N-nitrosodipropylamine added as internal standard

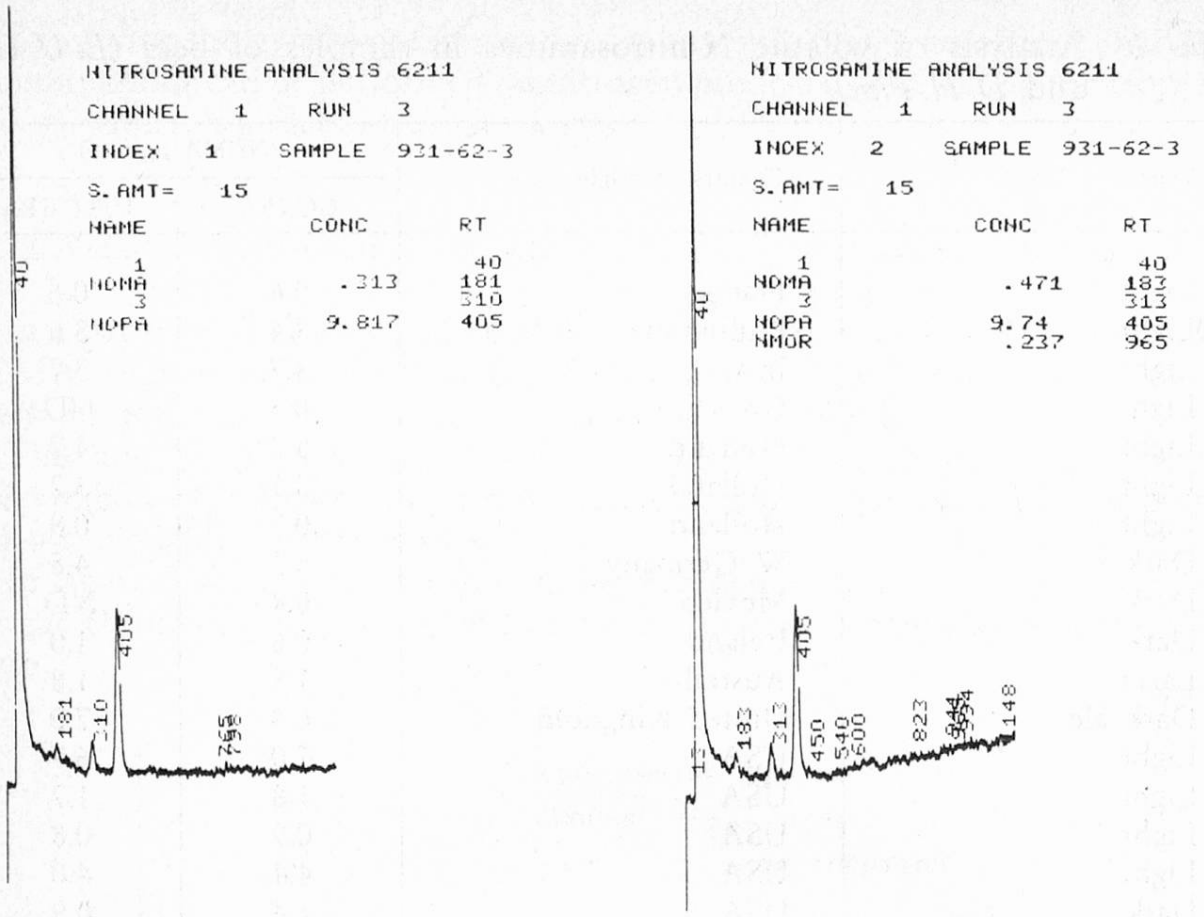


Fig. 4. Beer N° 1: GC-TEA determination of NDMA

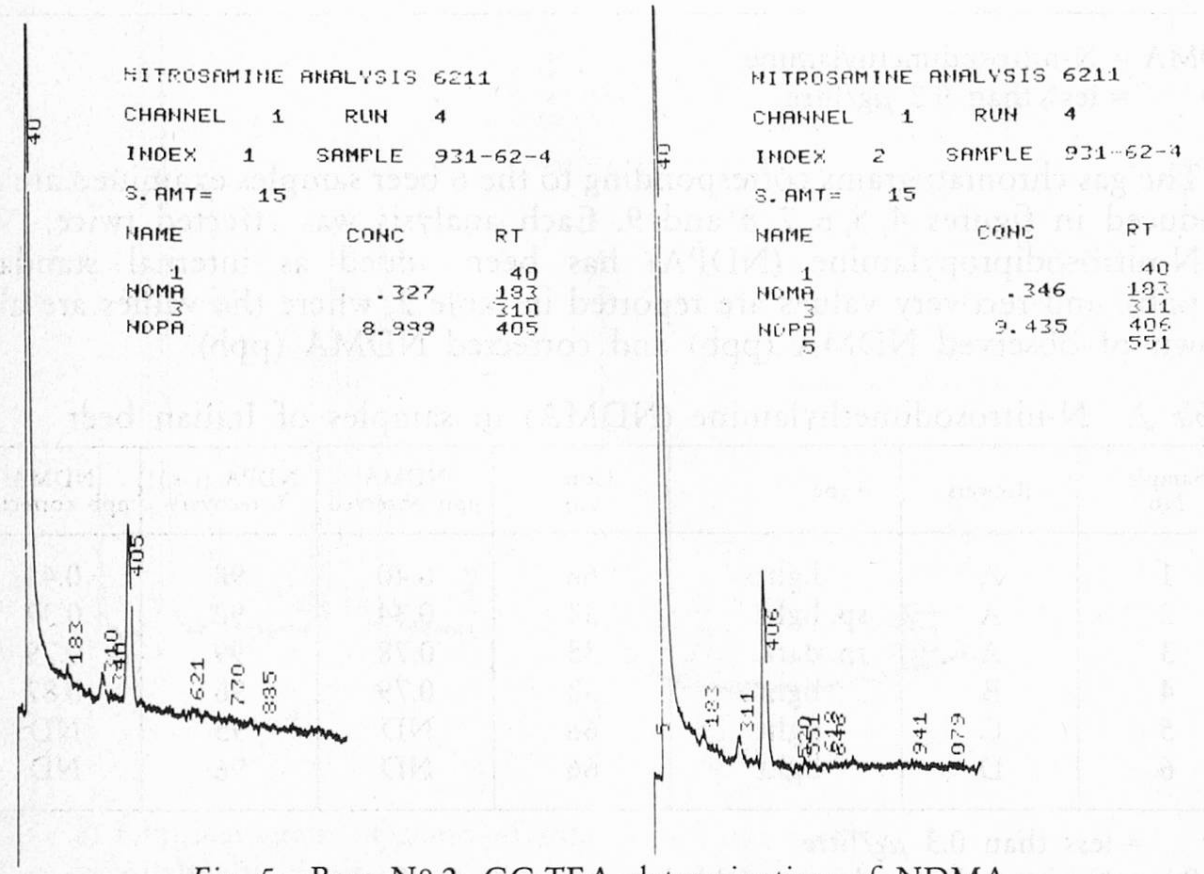


Fig. 5. Beer N° 2: GC-TEA determination of NDMA

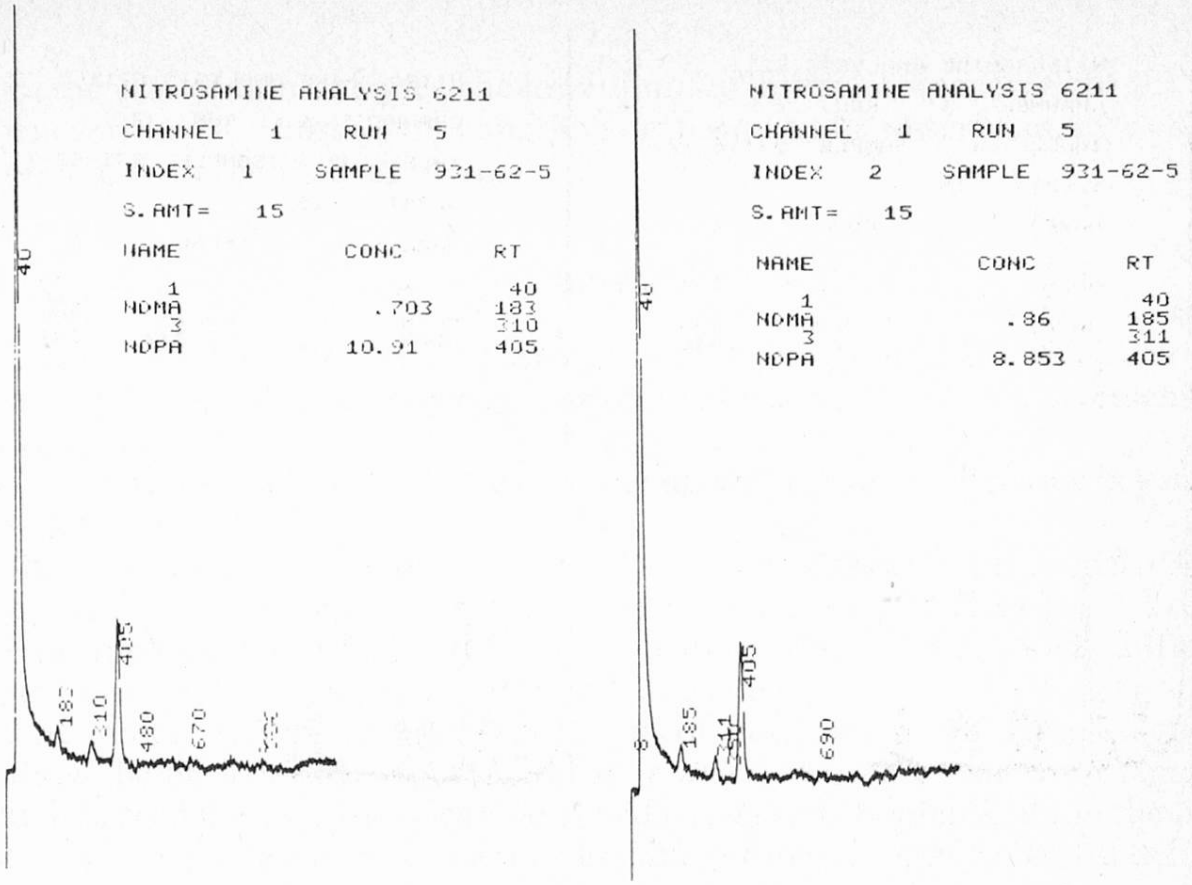


Fig. 6. Beer N° 3: GC-TEA determination of NDMA

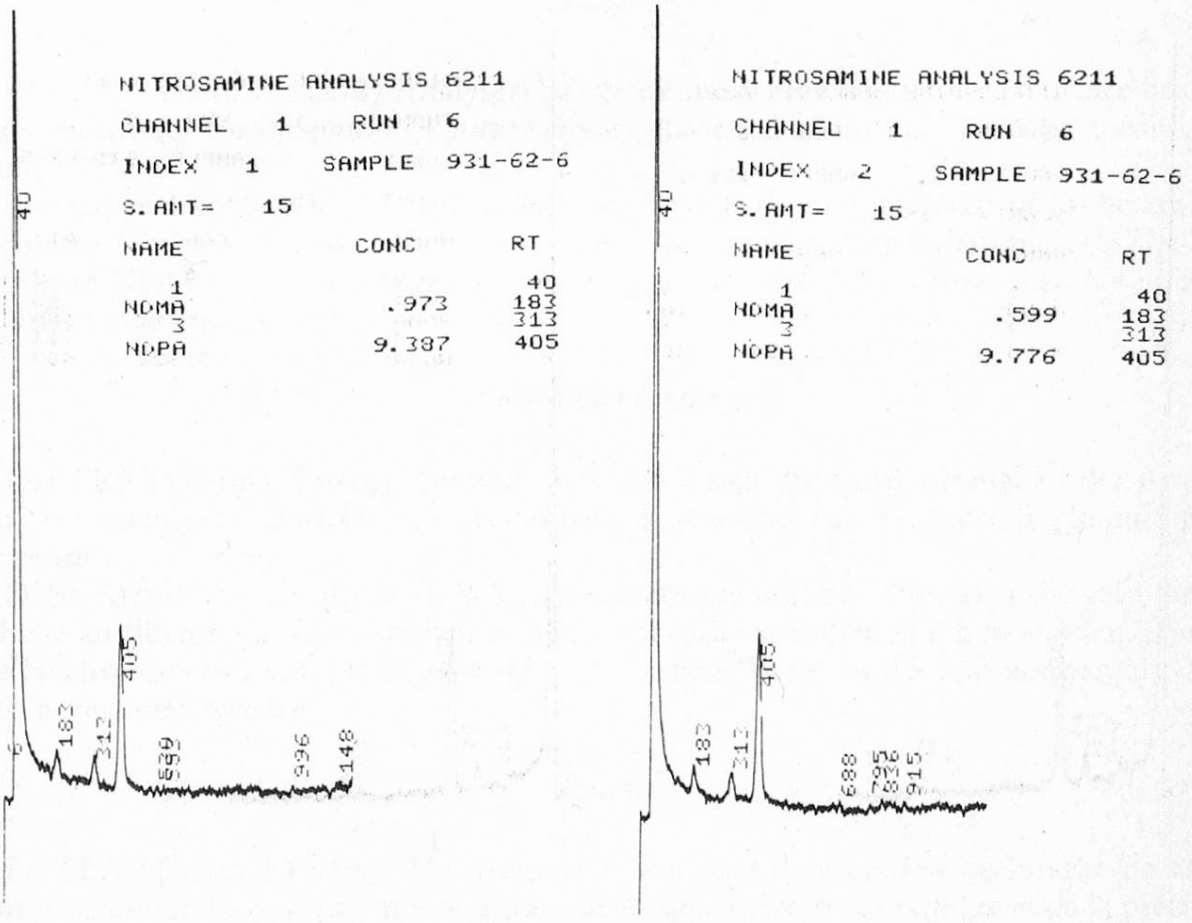


Fig. 7. Beer N° 4: GC-TEA determination of NDMA

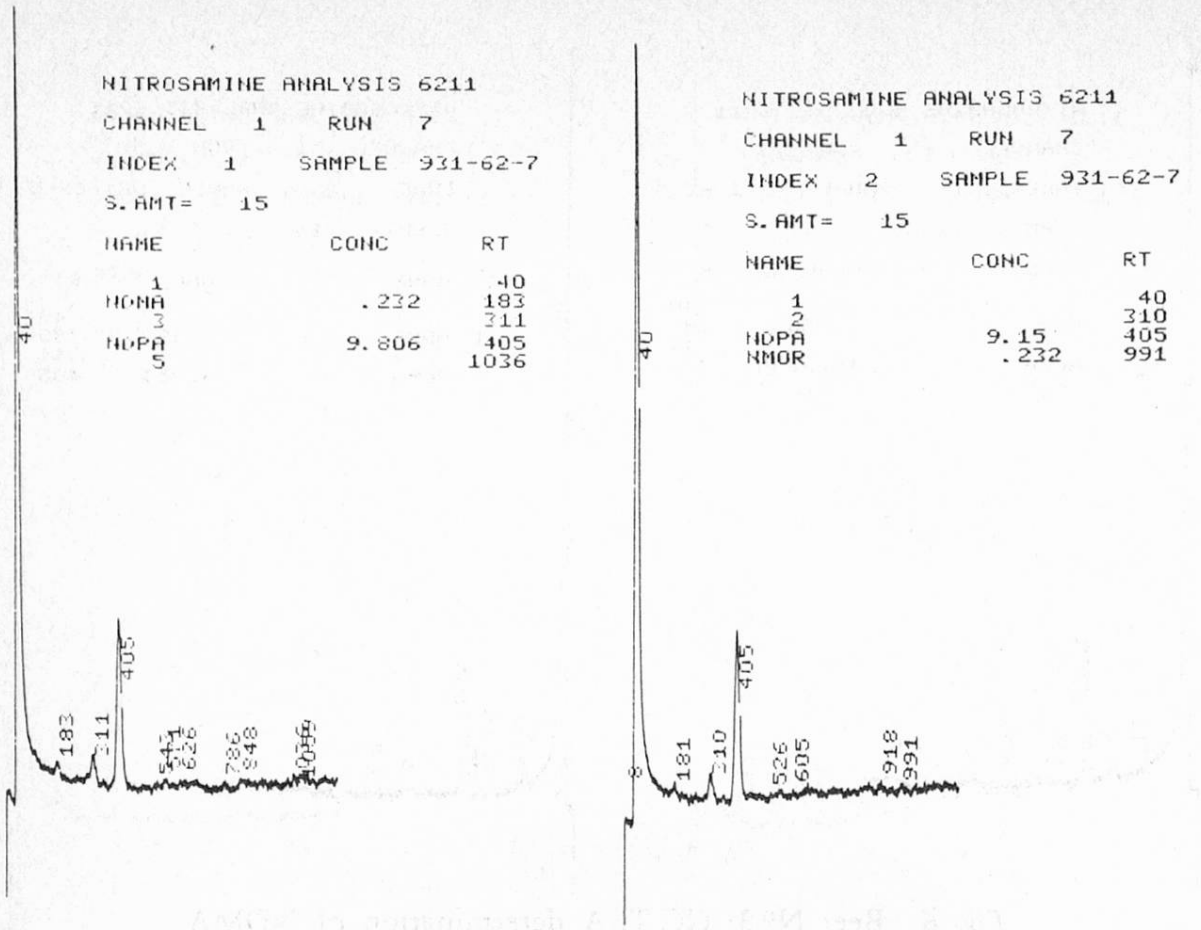


Fig. 8. Beer N° 5: GC-TEA determination of NDMA

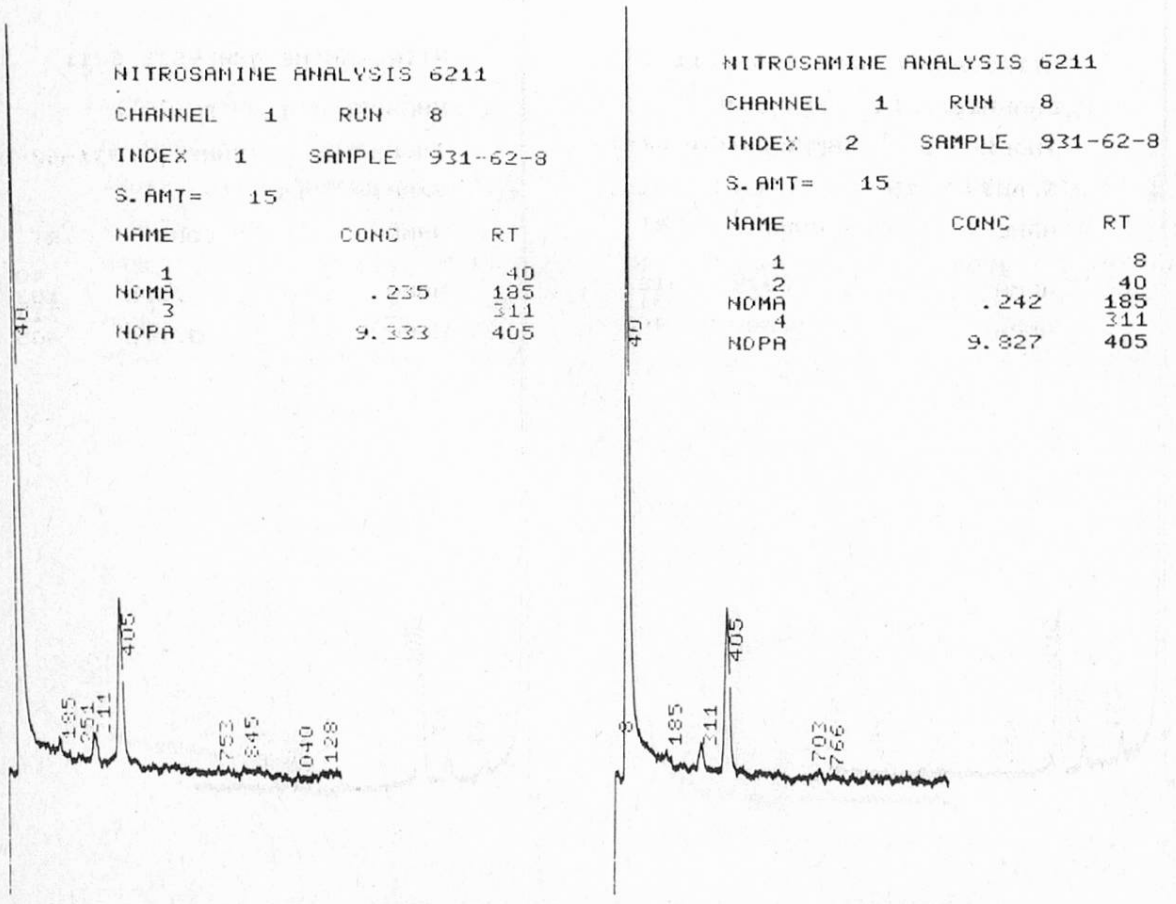


Fig. 9. Beer N° 6: GC-TEA determination of NDMA

In the case of beer analyses, the lower limit of detection appears to be 0.3 ppb (whenever the N-nitroso compound level is found to be lower than 0.9 ppb the amount can be defined as «trace»).

Conclusions

The GC-TEA method may be regarded as the most workable available so far, in terms not only of specificity and sensitivity of responses, but of convenient application as well.

The method makes volatile nitrosamine determination in alcoholic beverages remarkably easier.

In 111 German beer samples, the NDMA contents, assessed by *Spiegelhalder* et al. by TEA detector, were found to range between 0.2 and 11.2 μg per liter — whereas the findings of *Goff* and *Fine* (7), obtained from beers of widely different origins (see table 1) ranged between 0.4 and 7 μg per liter.

The earliest data on the NDMA contents of a number of beer samples (all the products being available on the Italian market) evidence that nitrosamines, though indeed present, are at least confined to amounts bordering upon the limit that may be regarded as the highest acceptable for the contents to be still defined as «trace amounts».

Summary

The TEA (Thermal Energy Analyser) has proven to be the instrument of choice because of its specificity for detection of nitrosamines, its speed of analysis and the accuracy of results.

The paper describes the TEA procedure and reports the results obtained in the analysis of volatile N-nitroso compounds in beer samples available on the Italian market. The lower limit of NDMA detection appears to be 0.3 ppb, however levels lower than 0.9 ppb can be defined as «trace».

Zusammenfassung

Der TEA (Thermal Energy Analyser) hat sich durch die Geschwindigkeit der Analyse und die Genauigkeit der Resultate als das beste Instrument zur selektiven Bestimmung der Nitrosamine erwiesen.

Diese Arbeit beschreibt die TEA-Analysenmethode und berichtet über die gefundenen Gehalte an flüchtigen Nitrosaminen in Bierproben, die in Italien erhoben wurden. Die untere Nachweisgrenze von NDMA ist 0,3 ppm; tiefere Werte als 0,9 ppb können als «Spuren» bezeichnet werden.

Résumé

Le TEA (Thermal Energy Analyser) peut être considéré comme technique de choix pour déterminer les nitrosamines en raison de sa spécificité, de sa rapidité et de la précision des résultats.

Le procédé analytique TEA est décrit et les résultats de la détermination des nitrosamines volatiles dans des échantillons de bière du marché italien sont donnés. La quantité minimum décelable de NDMA est 0,3 ppb; les teneurs inférieures à 0,9 ppb sont qualifiées de «traces».

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