Journal of Chromatography, 328 (1985) 362–366
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 709

Note

Analysis of volatile nitrosamines by microbore high-performance liquid chromatography and thermal energy analyser detection

CHRISTIAN RÜHL
Max von Pettenkofer-Institut des Bundesgesundheitsamtes, Berlin (F.R.G.)
and
JOACHIM REUSCH*
Wissenschaftlicher Geraetebau Dr. Ing. H. Knauer GmbH, Hegauer Weg 38, Berlin 37 (F.R.G.)
(First received November 9th, 1984; revised manuscript received March 11th, 1985)

Numerous laboratory experiments with animals have shown that the N-nitroso compounds are among the strongest chemical carcinogens presently known. Because they are readily formed they are ubiquitous[1], and their elimination, or at least the reduction of their concentration, is of considerable interest for health reasons.

Thus, through the "Nitrosamin-Bedarfsgegenstaende-Verordnung" (nitrosamine-commodities-regulation) of 1 January 1982, the migration of N-nitrosamines and nitrosatable substances (identified as nitrosamines) in pacifier and bottle nipples made of elastomers is restricted to minimal amounts[2].

N-nitrosamines are normally detected with the aid of a thermal energy analyser[3]. Even though this detector exhibits a high specificity for these compounds, positive results must be confirmed[3,4], *e.g.* by use of parallel gas chromatography (GC) and high-performance liquid chromatography (HPLC) with thermal energy analysis (TEA).

In contrast to GC–TEA, the extensive use of a TEA as a nitrosamine-specific detector is subject to certain limitations in HPLC: it cannot be used with aqueous systems or inorganic buffers, and it cannot be operated continuously[3]. Pastial solutions to the first two problems have recently been reported[5]. The third problem can be traced back to the necessary removal of the eluent and the by-products of pyrolysis by cold traps prior to the detection of N-nitrosamines. Whereas only a minimal amount of substances is condensed in the cold-trap system in a GC operation, the considerable amounts of solvent in HPLC mean that the cold traps must be constantly cleared, which causes repeated interruption of the analysis. Continuous operation is made possible by use of a secondary vacuum system for emptying the cold traps[6] or by employing microbore columns.

This paper describes a process that allows results obtained by GC–TEA analyses of pacifiers and bottle nipples to be reproduced by a microbore HPLC–TEA technique under continuous operation.

0021-9673/85/$03.30

EXPERIMENTAL

Apparatus

Two HPLC pumps were used alternately, a Knauer Model 64.00 with a micropumphead and a Jasco Model BIP-1 with a Knauer 4-μl loop injector and a Knauer microbore column (filled with Spherisorb 3 SW) coupled by a microvolume three-way valve (Knauer) to a TEA 502 A detector (Thermo Electron Corp. Analytical Instruments). The cold trapping system of the thermal energy analyser is shown in Fig. 1. The TEA conditions were: vacuum without flow, 0.05 Torr; vacuum with oxygen flow, 0.3 Torr; vacuum with oxygen and carrier gas (helium), 0.55 Torr; furnace temperature, 500°C; attenuation, 256. A Hewlett-Packard integrator 3390 was used for quantification. The chromatographic conditions are shown in the figures; the eluents were continually stirred after degassing in an ultrasonic bath.

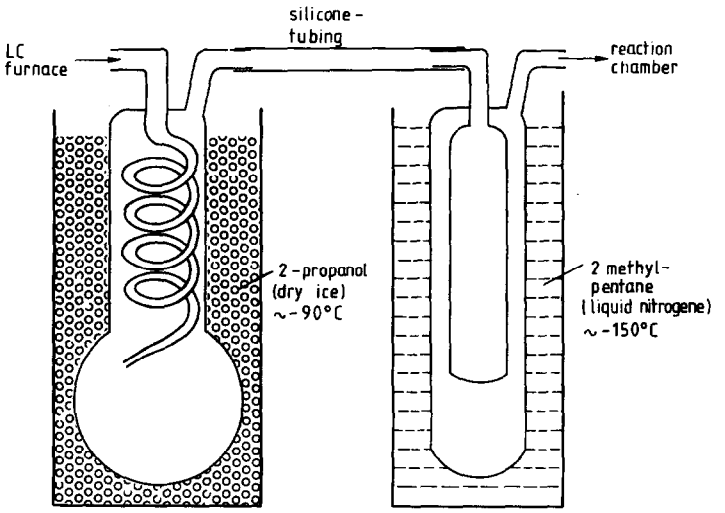

Fig. 1. Schematic diagram of the cold trap (total volume, 300 ml).

Procedure

The nitrosamines and nitrosatable compounds in the rubber nipples were estimated as described elsewhere[4]: sliced rubber material was incubated in a nitrite-containing saliva test solution for 24 h at 40°C. The migrated nitrosamines were extracted with dichloromethane from a part of this solution. For the determination of the migrated nitrosatable compounds as nitrosamines, the other part of solution was acidified for 30 min. After addition of alkali, the solution was extracted by dichloromethane to screen the formed nitrosamines. N-Nitrosodiisopropylamine (NDiPA) was added to the migration solution as internal standard before processing. The recovery of NDiPA was typically between 70 and 100%.

RESULTS AND DISCUSSION

Microbore HPLC–TEA can be performed with very low flow-rates, so it is possible to operate the system continuously. The cold-trap system shown in Fig. 1

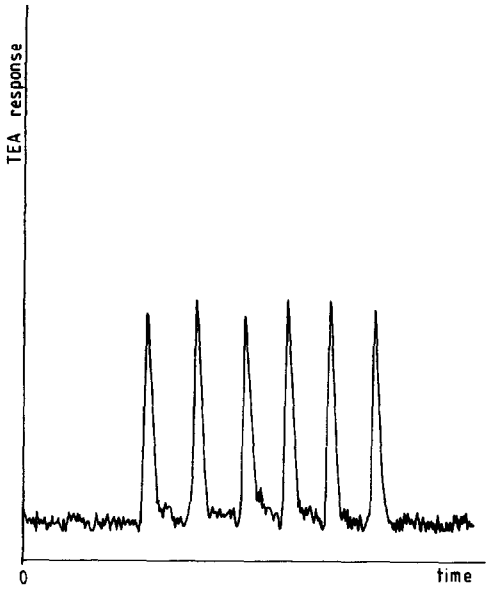

Fig. 2. Reproducibility of six 4-μl replicate injections of a NDEA sample: integrator attenuation, 4; solvent system, 2-propanol–*n*-hexane (2.5:97.5, v/v); flow-rate, 80 μl/min; mean (x), 28.1 mm peak height; S.D., 1.01; n = 6.

is very efficient. Even at a flow-rate of 150 μl/min, the HPLC–TEA system can be operated all day without interruption and without problems.

This method makes possible the detection of trace amounts of volatile N-nitrosamines. Fig. 2 shows that the reproducibility of replicate injections of 1.0 ng of

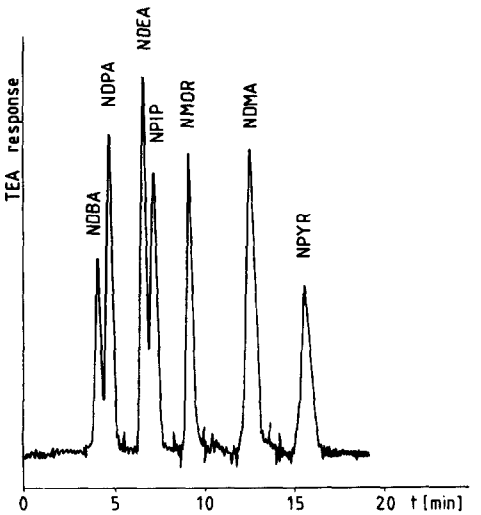

Fig. 3. Separation of a mixture of NDMA, NDEA, N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine(NDBA),N-nitrosopiperidine(NPIP),N-nitrosopyrrolidine(NPYR),N-nitrosomorpholine(NMOR). Integrator attenuation, 8; solvent system, 2-propanol–*n*-hexane (2.5:97.5, v/v); flow-rate, 60 μl/min.

TABLE I

CONTENT OF NITROSATABLE COMPOUNDS (IDENTIFIED AS N-NITROSAMINES) IN RUBBER NIPPLES AS DETERMINED BY MICROBORE HPLC–TEA AND GC–TEA

Mean of two determinations, calculated for 100% recovery related to added NDiPA.

Nipple	*Concentration (μg/kg)*					
	HPLC–TEA			*GC–TEA*		
	NDMA	*NDEA*	*NEPhA*	*NDMA*	*NDEA*	*NEPhA*
A	35	198	–	31	206	–
B	–	–	377	–	–	365

N-nitrosodiethylamine (NDEA) is very good [mean (x) = 28.1 mm peak height; standard deviation = 1.01; n = 6].

Complex mixtures of N-nitrosamines can also be separated. Fig. 3 is a chromatogram of a standard nitrosamine mixture (sample sizes, 3–4 ng each) is a compromise between resolution and analysis time.

Extracts from two different nipples (types A and B), obtained according to the process described in the Experimental section, were tested for nitrosatable substances (identified as N-nitrosamines) with the microbore HPLC–TEA technique. These data were compared with the results obtained by GC–TEA (Table I), all values were calculated for 100% recovery related to added NDiPA[4]. The table indicates good agreement in respect of the nitrosamines themselves —NDEA and N-nitrosodimethylamine (NDMA) in nipples A, N-nitrosoethylphenylamine (NEPhA) in nipple

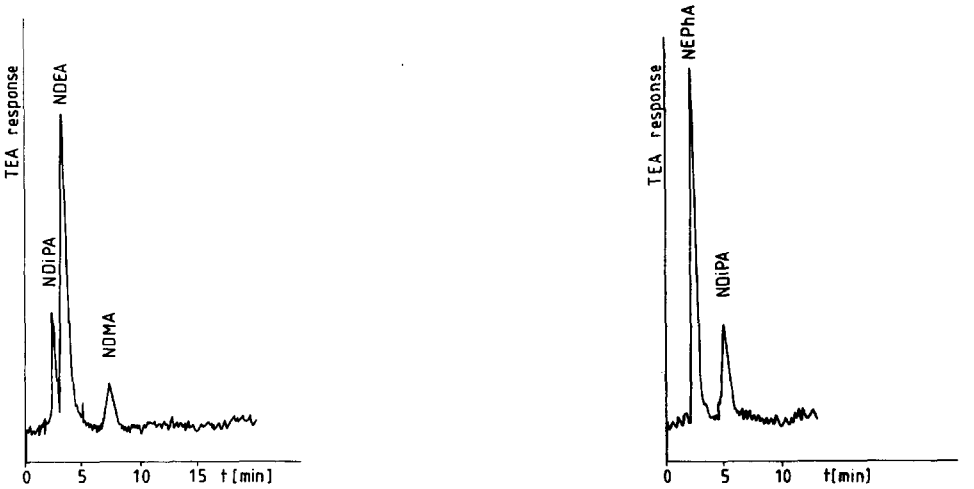

Fig. 4. Examination of rubber nipple A for nitrosatable substances (identified as nitrosamines). Integrator attenuation, 4; solvent system, 2-propanol–*n*-hexane (2.5:97.5, v/v); flow-rate, 80 μl/min. NDiPA was added as internal standard for recovery.

Fig. 5. Examination of rubber nipple B for nitrosatable substances (identified as nitrosamines). Integrator attenuation, 4; solvent system, 2-propanol–*n*-hexane (0.5:99.5, v/v); flow-rate, 80 μl/min. NDiPA was added as internal standard for recovery.

B— and their concentrations. As in the GC technique[4], the conditions for HPLC depend on the type of N-nitrosamine. A more polar eluent has to be used for the determination of alkylphenylnitrosamines than for that of dialkylnitrosamines.

Figs. 4 and 5 show the HPLC chromatograms of the nipples examined: note the good baseline stability found in these natural samples.

Even though the HPLC–TEA method is less sensitive to trace amounts than the GC–TEA method[3], it does reproduce the results of the latter technique[3,4], and can be used on a routine basis.

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