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SIMPLE CHEMILUMINESCENT DETECTOR FOR THE SCREENING OF FOODSTUFFS FOR THE PRESENCE OF VOLATILE NITROSAMINES

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SUMMARY

The construction and subsequent evaluation of an apparatus for the detection of trace amounts of nitrosamines is described. The apparatus consists of a gas chromatograph, a catalytic chamber to generate nitric oxide from eluted nitrosamines, and a chemiluminescent detector to measure the infra-red emission resulting from the interaction of this gas with ozone. Examples of the use of the system for determining the nitrosamine concentration in food extracts and other materials are given.

INTRODUCTION

The observation by Magee and Barnes[1] that simple volatile nitrosamines are carcinogenic toward many animal species, necessitated the development of analytical methods for the detection of these compounds in the human environment. Studies have been directed toward the examination of foodstuffs in which interaction between nitrite and amines could result in the formation of nitrosamines[2,3]. Nitrosamines have been reported in a variety of foods up to mg/kg levels, but many of the analytical techniques employed have subsequently been shown to be unreliable. The use of polarographic and colorimetric procedures and of other methods not involving any separation of the constituents of extracts of food is unsatisfactory. Gas chromatographic (GC) separation of constituents after a preliminary distillation or extraction forms the basis of all methods in current use. The presence of nitrosamines in GC effluent may be observed using nitrogen-selective detectors, and alkali-flame ionisation (thermionic)[4–6] and electrolytic conductivity detectors[7–11] are used extensively for this purpose.

It has, however, been demonstrated that even with such detectors a response coincident with the retention of a nitrosamine is insufficient evidence for its unambiguous detection. Such a response is often attributable to other nitrogen-containing compounds. Electrolytic conductivity detectors are more reliable than thermionic detectors, but even these give rise to many positive results which are subsequently shown to be false by mass spectrometry (MS)[11]. It remains mandatory to confirm the presence of suspected nitrosamines by combined GC and high-resolution MS[12–14].

Nitrogen-selective detection cannot be regarded as satisfactory even for screening purposes at least for complex samples such as food extracts, and an alternative method of screening is desirable.

Fine and Rufeh[15] have proposed the use of chemiluminescence to detect nitrosamines as nitric oxide after catalytic cleavage of the N–NO bond. Detectors for oxides of nitrogen based on chemiluminescence have become commercially available in recent years[16] and are both reliable and extremely sensitive instruments. The incorporation of a device to generate nitric oxide form a nitrosamine would thus offer in principle a very selective and sensitive nitrosamine detector. Subsequent work by Fine *et al.*[17,18] has resulted in the development of such a system, which is commercially available. This particular apparatus, known as a thermal energy analyser (TEA), whilst simple in concept is far more expensive than a conventional GC detector. Although is has a much better detection limit than existing detectors for nitrosamines, it cannot be regarded as a replacement for relatively cheap but unsatisfactory detectors for screening, nor at the present time at least, as an alternative to MS for confirmation.

The present paper describes the construction of an inexpensive detector based on the chemiluminescent principle, and its evaluation and use for the screening of foodstuffs and other materials for the presence of volatile nitrosamines. The detector can be built in the laboratory and requires the minimum of workshop and other services for its construction. It has been built mainly from commercially available units and components, most of which are commonly used in analytical laboratories. The cost is the same order as that of a conventional non-selective detector such as flame ionisation, with its associated amplifier and control units.

The apparatus consists of a gas chromatograph connected to a catalytic chamber for the conversion of nitrosamines to nitric oxide. Effluent from this chamber passes into a cold trap to remove the majority of organic compounds and then enters the detector unit. The latter consists of a reaction chamber under vacuum and situated in front of a red sensitive photomultiplier tube. A mixture of oxygen and ozone from a generator also enters this chamber and light emission from the interaction of nitric oxide and ozone is detected by the photomultiplier tube, amplified and displayed on a potentiometric recorder. The most difficult part of the detector development lies in the choice of an appropriate catalyst. The requirement is for a catalyst which will quantitatively cleave the N–NO bond of a variety of nitrosamines, and whose performance will not deteriorate by the passage of other volatile organic compounds present in the extract under analysis. It is desirable but not essential that the catalyst is specific only toward N–NO cleavage and is otherwise inactive. However, this is not a major consideration for screening, particularly when some selectivity toward nitric oxide can be introduced in other parts of the detection system. For example by incorporation of the cold trap which removes most organic compounds, and an optical cut-off filter which prevents detection of emissions below 610 nm. Selectivity is also introduced by the nitric oxide–ozone interaction itself[19,20]. Several catalysts have been evaluated in this laboratory and data on a palladium–silver alloy tube for use with the chemiluminescent detector have been published[21]. Poisoning of this catalyst after only a few analyses of food extracts has resulted in a search for catalysts of longer life. Those used by Fine include a tungsten–molybdenum–chromium alloy on a silica base[15], tungsten oxides and nickel oxides[17]. Catalysts examined during the present study included palladium on celite,

or tungsten(VI) oxide on celite, contained in stainless-steel tubing and operated at temperatures between 150° and 400°. Whilst functioning satisfactorily for a limited number of samples, the activity of these catalysts rapidly deteriorates and reproducible conversion to nitric oxide cannot be guaranteed. Experiments to isolate the cause of the difficulties established, using MS, that even for catalysts which give rise to nitric oxide, some at least is absorbed by the catalyst support and by the walls of the stainless-steel tubing containing it. In order to minimize re-absorption, a catalyst was prepared in which the support itself formed the walls of the tubing, and this has resulted in the development of a catalyst with a satisfactory performance.

EXPERIMENTAL

The gas chromatograph is a Pye 104 containing a 4 m × 1.8 mm I.D. stainless-steel column packed with 5% Carbowax 20 M on Diatomite C AW DMCS. The carrier gas is helium at a flow-rate of 11 ml/min. The column, which is operated at 150°, is connected by 0.5 mm I.D. stainless-steel tubing to a Coulson electrolytic conductivity detector furnace. The quartz tube normally inside the furnace is replaced by a porous ceramic tube on to which the catalyst has been absorbed. The tube (Steatite and Porcelain Products, Stourport-on-Severn, Great Britain), which is fully fired but unglazed is 250 mm × 3 mm I.D. and 1.5 mm wall thickness. The catalyst is tungsten(VI) oxide which is absorbed into the walls of the tube as a solution of ammonium paratungstate (ICN Pharmaceuticals) in aqueous hydrogen peroxide[22]. Tungstate (41 g) is dissolved in 100 ml of 30% hydrogen peroxide and diluted to 200 ml with water. The porous tube is immersed in this solution for 1 h and then dried in air to constant weight at 100°. The tube is then heated in air at 550° overnight and finally placed in the detector furnace which is also operated at 550°. The catalyst chamber temperature is not critical, but operation at too low a temperature necessitates more frequent regeneration of the catalyst. Regeneration, which may become necessary after a few weeks operation, can be carried out *in situ* by disconnecting the gas chromatograph and allowing oxygen from the reaction chamber to pass through the catalyst. The ozoniser should be switched off during this operation.

The outlet from the catalyst tube is connected by 4 mm I.D. steel tubing to a U-shaped trap of the same material which can be immersed in a freezing mixture of solid carbon dioxide and alcohol. The outlet from the U-tube passes directly into the reaction chamber of the detector. However when the detector is not in use and the U-tube is at ambient temperature, the outlet is diverted directly to a rotary pump (Edwards ES150) fitted with a charcoal trap. This prevents contamination of the reaction chamber by organic compounds eluting from the U-tube. The detector chamber consists of two cylindrical sections, each 56.5 mm I.D., one being the reaction chamber and the other the photomultiplier tube housing. The reaction chamber which is 40 mm long is coated with white reflectance paint (Eastman-Kodak). The two chambers which screw together are separated internally by a 610-nm cut-off filter (Ilford 204 or Corning 2-60), held against viton O-rings to effect a vacuum and light-tight seal. The gas chromatograph effluent enters the chamber through a flange fitted with Swagelok unions, on the end of the reaction chamber cylinder, and is fed by 4 mm tubing so as to impinge on the cut-off filter. The oxygen–ozone mixture enters the reaction chamber at 5.5 ml/min by the same means and also impinges on the filter. The outlet from

the chamber is connected to a charcoal trap to remove ozone and thence to the rotary pump to maintain a pressure of 0.15 torr. Ozone is generated by subjecting oxygen dried over molecular sieve to a 7 kV discharge in a laboratory-built ozoniser. A small commercial unit (British Oxygen Company) has also been used. Such units, however, do not work satisfactorily below about 500 torr and a needle valve must be placed between the ozoniser gas flow outlet and the reaction chamber. Selection of a photomultiplier tube of appropriate characteristics follows the same criteria as used for conventional nitric oxide detectors, and is well documented[20]. In the present work a shielded Centronic tube (type P4283TIR) with extended red sensitivity was used. The high voltage supply is from a Brandenburg power unit 472R, operated at 1.3 kV. The output from the tube is fed to a Pye wide range amplifier normally used in conjunction with a flame ionisation detector. The output signal is displayed on a Leeds & Northrup Model W 1-mV potentiometric recorder fitted with a 50-Hertz filter.

RESULTS AND DISCUSSION

The characteristics of this system were studied using a series of dilute solutions each containing six volatile nitrosamines in hexane, and covering the range 1–1000 μl/l. The nitrosamines used were N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR). For each concentration and injected volume (1, 5 and 10 μl), 3 replicate injections were made and linearity plots constructed. An example is shown in Fig. 1, from which it can be seen that at least up to 1000 μl/l the detector response is linear. With large injected volumes a tail-off in response was observed for high concentrations of nitrosamines and this was attributed to saturation of the photomultiplier. Since under normal conditions it is unlikely that concentrations of nitrosamines over 100 μl/l will be encountered this effect is not regarded as a disadvantage and can in any case be overcome by lowering the tube voltage or by dilution of the sample.

The detection limit of the apparatus is the same order as that obtained by high-resolution MS, namely 0.1 μl/l in the extracts, which represents 0.1 μg/kg in the original material. The detection limit is governed predominantly by the photomultiplier tube dark current which can be reduced by operating at sub-ambient temperatures. The incorporation of a cooling unit would substantially increase the cost of the detector and in the present system the tube was operated at +20°. Detector noise can also be reduced by the incorporation of an annular magnet at the face of the tube although this gives a decrease in active tube area. Experiments with such a magnet (EMI type C122/12) showed that the noise was reduced by a factor of four, but the diffuse nature of the chemiluminescent emission resulted in an even greater loss of sensitivity, and the use of the magnet was abandoned.

Reproducibility of response over a normal working day was measured by repeatedly injecting standard solutions, and some results for 10 replicate analyses of a 10-μl/l mixture are given in Table I. Responses are based on peak height measurements, normalised with respect to NDMA. For comparison the repeatability of response, as the coefficient of variation, is also given in Table I for the Coulson electrolytic conductivity detector[11]. These data were generated for similar solutions over the same period of time.

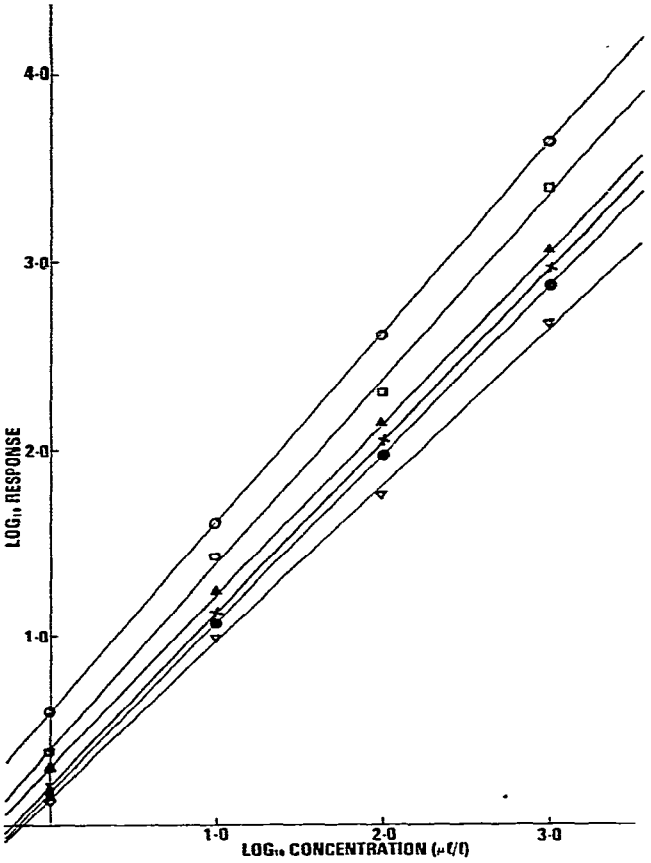

Fig. 1. Linearity of chemiluminescent detector response to 5-μl injections of standard nitrosamine solutions covering the range 1–1000 μl/l in hexane. ○ = NDMA; □ = NDEA; ▲ = NDPA; × = NPIP; ● = NPYR; ▽ = NDBA.

TABLE I

REPEATABILITY OF RESPONSE OF DETECTOR: SHORT TERM

$\bar{x}$ = Mean of 10 determinations, normalised with respect to NDMA; σ = standard deviation; v = coefficient of variation.

Compound	*Response with respect to NDMA*			
	Chemiluminescent detector			*Conductivity detector*
	$\bar{x}$	σ	v	v
NDMA	100.00	3.9	3.9	10.9
NDEA	87.6	3.1	3.5	5.2
NDPA	48.5	2.0	4.1	2.8
NDBA	20.8	2.3	10.9	4.7
NPIP	37.8	2.6	6.8	6.8
NPYR	28.7	1.9	6.6	22.8

TABLE II

REPEATABILITY OF RESPONSE OF DETECTOR: LONG TERM

$\bar{x}$ = Mean of 10 determinations normalized with respect to NDMA; σ = standard deviation; v = coefficient of variation.

Compound	*Response with respect to NDMA*		
	$\bar{x}$	σ	v
NDMA	100.0	36.3	36.3
NDEA	68.9	23.8	34.5
NDPA	42.2	11.1	26.3
NDBA	16.7	4.5	26.9
NPIP	31.4	7.6	24.2
NPYR	27.7	8.7	31.4

Long term repeatability of response was followed over a period of 1 month and involved 2 determinations per day. Variation of response is far higher (see Table II), but the detector was still functioning adequately at the end of this period. Provided the detector is calibrated each day with a standard nitrosamine solution, as is normal with any detector, then the variations over an extended period are of no consequence. The variations are similar to those observed using the thermionic[4] and conductivity detectors[11] for the same compounds.

The efficiency of conversion of each of the nitrosamines into nitric oxide was measured by using the catalytic chamber in a combined GC–MS system[12]. No nitrosamines were detected, and there was no evidence that unchanged nitrosamines were absorbed within the system, based on comparative results obtained in the absence of the catalyst. Further evidence for complete conversion was obtained by comparing the integrated response of the chemiluminescent detector during the elution of each nitrosamine, with the response expected on the basis of the proportion of NO in each compound. Results normalised with respect to NDMA are given in Table III, from which it is seen that the response on this basis is the same for all the nitrosamines.

The selectivity of the detector and hence its value for screening was assessed by examining a variety of extracts of food, vegetation and biological fluids, some of which had been shown by MS to contain nitrosamines. A qualitative examination of the chromatograms was made and in no case did the detector respond to any material other than at the retention times of the specified nitrosamines, between the elution of NDMA and NPYR. In addition the detector only responded in instances where

TABLE III

RESPONSE OF DETECTOR ON BASIS OF NITRIC OXIDE

Compound	*Integrated response (arbitrary units)*	*NO in molecule (%)*	*Response based in NO content*
NDMA	157	40.5	40.5
NDEA	108	29.4	27.9
NDPA	96	23.0	24.8
NDBA	56	19.0	14.4
NPIP	106	26.3	27.3
NPYR	119	30.0	30.7

nitrosamines had been confirmed by MS. This is a great improvement on the situation pertaining to the use of nitrogen selective detectors which usually give rise to a host of peaks few of which are nitrosamines even at the appropriate retention times. A comparison of the chromatograms obtained from a fish extract using a thermionic and a Coulson conductivity detector is shown in Fig. 2. The same extract run on the chemiluminescent detector was found to contain only NDMA and this was confirmed by high-resolution MS. Fig. 3 shows the chemiluminescent chromatogram of this extract and a standard 10-μl/l solution of six volatile nitrosamines. On Fig. 2 and 3 the attenuation is such that 10 detector response units represent 0.2 μl/l of NDMA.

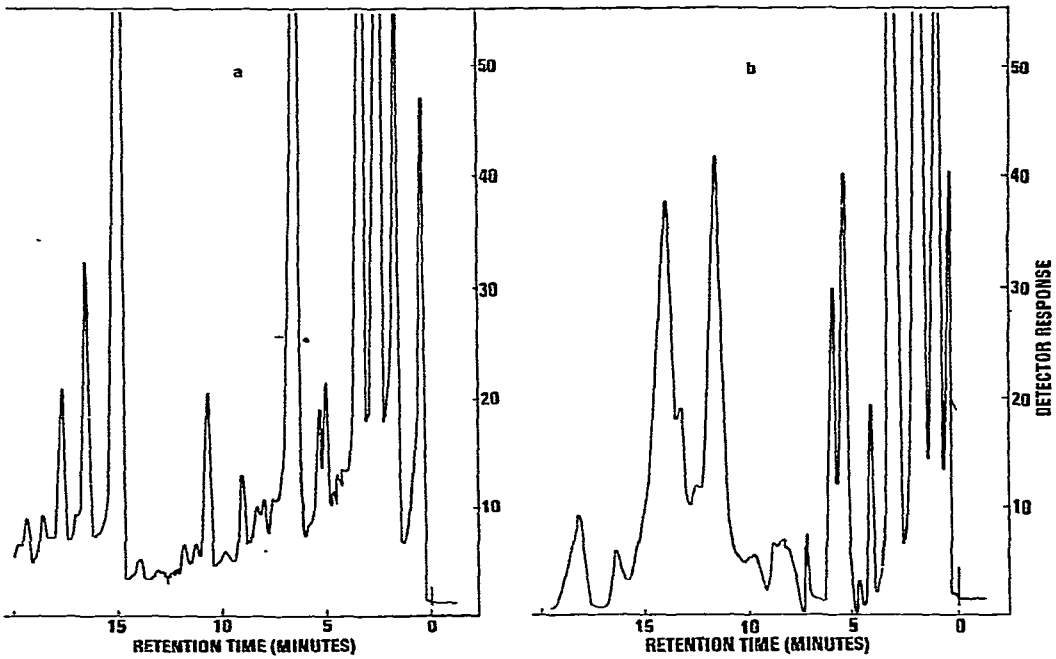

Fig. 2. Chromatograms of fish extract. (a) Coulson conductivity detector response to 5-μl injections of an extract containing NDMA; (b) thermionic detector response to 5-μl injections of an extract containing NDMA.

Replicate quantitative measurements of nitrosamines detected in some of the samples were made to determine whether the efficiency of the catalyst was impaired by the passage of extraneous organic material. Table IV gives the results of analysing a bacon sample 10 times during a working day. There was no deterioration of the detector response over this period, and analysis of the same sample at weekly intervals for 4 weeks did not result in any significant change in the quantitative results. Although the level for NPIP was near the detection limit, reliable results were still obtained. Calibration of the detector against a standard solution was carried out prior to, and after each determination. During the same period of 4 weeks, the system was in continual use and over 100 injections of other extracts were made.

A comparison of the quantitative data obtained using the chemiluminescent detector and well established GC–MS techniques was made. A variety of different

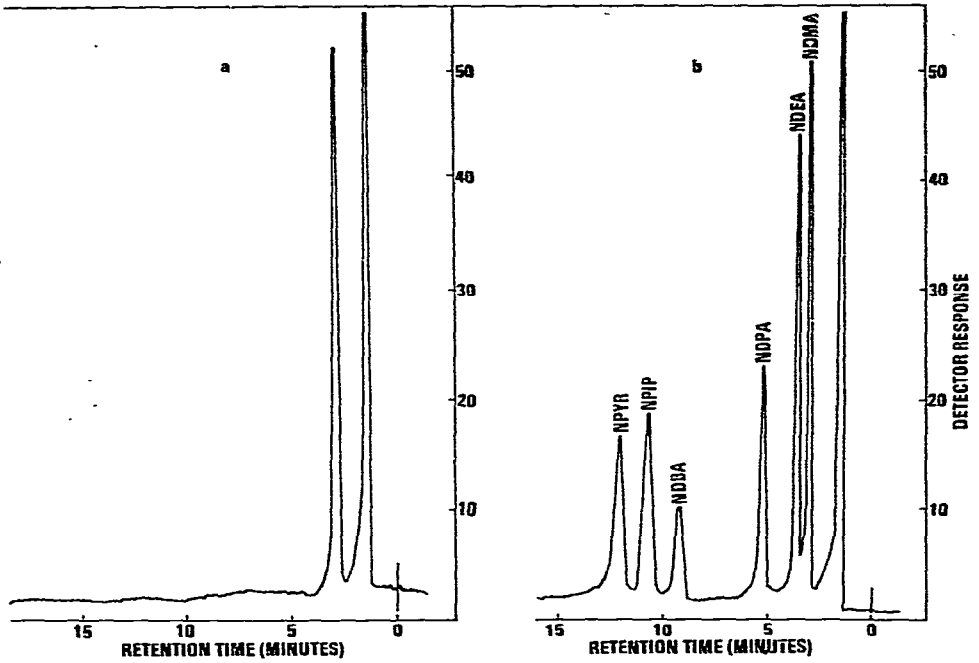

Fig. 3. Chemiluminescent detector response (a) to 5-μl injection of a fish extract containing NDMA and (b) to 5-μl injections of a 10-μl/l standard nitrosamine solution.

commodities was examined by both techniques on the same day and Tables V and VI show the results. The GC–MS system, which has been previously described, consists of combined packed and open tubular columns connected to micro-volume switching valves for solvent venting and effluent path selection purposes[23]. Nitrosamines are detected by parent-ion monitoring with peak matching against a suitable fragment of a fluorinated hydrocarbon[24]. A similar exercise in which the data was obtained using several published MS procedures and TEA has recently been carried out[14]. Table V shows that the chemiluminescent detector gives in all cases values for nitrosamine content of the extracts close to those obtained by high-resolution MS, and no false negative or false positive results were observed. Results using this detector can therefore be regarded as more reliable than all MS techniques except that of high-resolution peak matching, which still remains the best method for confirmation purposes.

TABLE IV

DETERMINATION OF NITROSAMINES IN BACON EXTRACT

Compound	*10 Analyses over 8 h*		*4 Analyses over 4 weeks*
	$\bar{x}$ *(µl/l)*	σ	$\bar{x}$ *(µl/l)*
NDMA	0.9	0.2	1.0
NDPA	3.2	0.4	3.9
NPIP	0.5	0.2	0.6
NPYR	11	1.2	12

TABLE V

COMPARISON OF DIALKYL NITROSAMINE CONCENTRATIONS IN EXTRACTS USING CHEMILUMINESCENCE (CL) AND MASS SPECTROMETRY (MS)

— = Not detected.

*Commodity**	*Extract no.*	*NDMA (mg/l in extract)*		*Other dialkyl nitrosamines*		
		CL	*MS*		*mg/l in extract*	
					CL	*MS*
Luncheon meat (6)	1	0.3	0.2	None	—	—
	2	0.2	0.5	None	—	—
	3	—	—	None	—	—
	4	3.7	4.3	NDEA	2.5	2.6
				NDPA	4.6	4.0
				NDBA	4.3	4.1
	5	14	15	NDEA	3.8	6.8
				NDPA	9.4	12
				NDBA	7.1	7.7
	6	11	15	NDEA	3.9	6.6
				NDPA	7.6	11
				NDBA	5.7	6.8
Fried bacon (14)	7	1.1	1.3	None	—	—
	8	1.4	1.4	None	—	—
	9	0.9	1.4	None	—	—
	10	1.0	1.4	None	—	—
	11	21	21	None	—	—
	12	30	33	None	—	—
	13	31	35	None	—	—
	14	16	18	None	—	—
	15	23	25	None	—	—
	16	4.3	3.6	None	—	—
	17	17	25	None	—	—
	18	5.4	4.0	None	—	—
	19	9.4	13	None	—	—
	20	3.1	2.9	None	—	—
Fish (5)	21	0.1	0.1	None	—	—
	22–25	—	—	None	—	—
Cheese (5)	26–30	—	—	None	—	—
Vegetables (10)	31	0.5	0.5	None	—	—
	32	0.2	0.3	None	—	—
	33	0.1	0.1	None	—	—
	34–40	—	—	None	—	—
Urine (6)	41	42	48	None	—	—
	42	0.3	0.1	None	—	—
	43	19	25	None	—	—
	44–46	—	—	None	—	—

* Figures in parentheses indicate number of samples.

CONCLUSIONS

A chemiluminescent detector can be built in the laboratory with the minimum of cost and will respond selectively to trace amounts of nitrosamines. The detection

TABLE VI

COMPARISON OF HETEROCYCLIC NITROSAMINE CONCENTRATIONS IN EXTRACTS USING CHEMILUMINESCENCE (CL) AND MASS SPECTROMETRY (MS)

— = Not detected.

Commodity	*Extract no.*	*NPYR (mg/l in extract)*		*NPIP (mg/l in extract)*	
		CL	*MS*	*CL*	*MS*
Luncheon meat	1	—	—	1.5	1.6
	2	—	—	1.0	1.0
	3	—	—	1.0	1.1
	4	3.5	3.8	4.3	3.5
	5	7.3	8.8	8.0	7.7
	6	5.6	6.7	5.8	7.6
Fried bacon	7	16	12	0.9	1.0
	8	11	8.2	0.9	1.0
	9	10	10	—	—
	10	18	12	—	—
	11	82	83	—	—
	12	104	110	—	—
	13	45	47	—	—
	14	105	101	—	—
	15	216	208	—	—
	16	25	21	—	—
	17	49	51	—	—
	18	54	58	—	—
	19	23	19	—	—
	20	10	9.6	—	—
Fish	21–25	—	—	—	—
Cheese	26–30	—	—	—	—
Vegetables	31–40	—	—	—	—
Urine	41	1.0	1.1	2.3	2.2
	42–46	—	—	—	—

limit is the same as that obtained using GC–MS. The detector is suitable for the screening of extracts of foodstuffs and other complex mixtures prior to confirmation of the presence of nitrosamines by MS. The detector will work reliably for extended periods of time and requires very little maintenance. No example has been found in which the detector gives rise to a false positive or false negative result for any of the nitrosamines studied in this work. The detector gives quantitative data for the nitrosamine content of food and other extracts in excellent agreement with MS measurements.

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