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Liquid Chromatographic and Thermal Energy Analyzer Detection of N-nitrosodiphenylamine in Formulations of Diphenylamine

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Diphenylamine (DPhA) is a post harvest fungicide for control of storage scald on apples. It is also a precursor of N-nitrosodiphenylamine (NDPhA) and has been used to determine the quantity of nitrogen dioxide in ambient air samples by measuring the content of NDPhA formed (Lipari 1984).

Low molecular weight volatile nitrosamines have been reported to be carcinogenic to laboratory animals (Borzsoni et al. 1978; Seiler 1977). The toxic properties of NDPhA have not been confirmed with its toxicity ranging from no (Argus et al. 1961; Rosenberg et al. 1983; Brambilla 1987) to weak carcinogenic activity (Cardy et al. 1979; Zielenska et al. 1987; Khudolei et al. 1986). However, in view of the agricultural use of DPhA, the formulated products containing DPhA were selected for the analysis of NDPhA contamination.

Methods for the analysis of NDPhA in vodka using a liquid chromatograph (LC) coupled with a thermal energy analyser (TEA) (Fine et al 1976) and in DPhA coated air sampling cartridges using LC with an ultraviolet (UV) detector (Lipari 1984) have been reported. This study reports a method for the detection of NDPhA and its levels in formulations of DPhA.

MATERIALS AND METHODS

A stock standard solution of NDPhA (1.021 mg/mL) was prepared by dissolving pure NDPhA (Sigma Chemical Company) in hexane. Working standard solutions (0.102 mg/mL or 0.04 mg/mL) were prepared by diluting this stock solution with hexane.

All solvents used were LC grade and acetone was "Baker Resi-Analyzed" grade. Cyano solid phase extraction (SPE) columns were obtained from J.T. Baker (Phillipsburg, NJ) and were pre-washed with hexane.

A 0.2 g sample of formulation containing 15% DPhA or a 0.1 g sample containing 30% DPhA was added onto a cyano SPE column

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Send reprint requests to Yuk Y. Wigfield at the above address and was eluted with hexane (8 mL). The eluate was collected in a graduated centrifuge tube, concentrated to 1.0 mL under nitrogen and filtered through a 0.45 um filter. Aliquots (50 uL) of the resulting solutions alternating with standard solutions of similar concentration were injected into a Varian Vista 5500 liquid chromatograph equipped with a Varian 8085 autosampler, a Rheodyne 7126 injector valve with a 50 uL sampling loop, a Varian 402 data acquisition system and a thermal energy analyzer (Model 502A from Thermedics, Woburn, MA). The LC column (Dupont Zorbax CN column, 4.6 mm x 25 cm) was preceded with a Brownlee Newguard guard column cartridge and followed by a two-way air actuated automatic switching valve controlled by the Vista 402 and a Cryotrol immersion cooler (Model CC-100 II from Neslab Instruments, Newington, NH). The cooler contained a 4 L Nalgene bath of 95% ethanol maintained at -70°C and into this bath was suspended a TEA glass cold trap.

The flow rate of TEA oxygen gas was 20 mL/min, that of argon carrier gas was 90 mL/min and the vacuum was maintained at 0.8 mm Hg. The LC mobile phase was 0.5% hexane in acetone and its flow rate was 1.0 mL/min. Under these conditions, the retention time of NDPhA was 7.4 min.

The levels of NDPhA in samples were calculated using the following equation: NDPhA (ppm) = A/A' x W'/W x purity of standard where A and A' = height response of sample and standard respectively; W' = weight of standard (ug) and W = weight of DPhA (g) calculated from the labels of the commercial product.

RESULTS AND DISCUSSION

In Canada, there are two formulations containing 15% or 30% DPhA. Both are used to apply on apples within 7 days of harvest to control scald. Both are emulsion formulations and thus may contain several high molecular weight and polar inert materials.

Although TEA is specific for nitroso and nitro containing chemicals, injection of hexane extracts of these formulations into the LC-TEA detection system showed an unquantifiable broad peak due to the presence of inert ingredients which suppressed the TEA response. Thus it is necessary to separate most of the other chemicals in the formulations before injection of samples. Several SPE columns were used for this purpose. Silica gel SPE columns (40-60 um from J.T. Baker) were able to retain most of the inert material when a hexane extract of sample was added onto the column. The column was eluted with hexane and then 2% acetone in hexane or 50% CH_2Cl_2 in hexane and NDPhA was eluted in 5% acetone in hexane or 100% CH_2Cl_2. However, this method also generated some TEA responding impurities which had the same LC retention time as NDPhA. The levels of these impurities appeared to be directly proportional to the length of time DPhA was left in the columns. The cyano SPE column was able to retain most of the polar inert ingredients but not able to separate NDPhA from DPhA. A DuPont Zorbax CN analytical column was able to separate NDPhA from DPhA (see Figure 1A) using 4% isopropyl alcohol (IPA) in hexane at 1 mL/min. However, IPA appeared to be incompatible with TEA detector by generating high noise levels and drifting baseline, and 0.5% acetone in hexane was used as mobile phase of LC-TEA detection system instead. To avoid contamination of the TEA detector, the column switching valve was switched off from the detector to a waste container immediately after the elution of NDPhA.

Ten samples, of which nine contained 15% and one 30% DPhA, were analysed for NDPhA using this method and the results are shown in Table 1. The identity of NDPhA was confirmed by: (1) comparison of its LC retention time with that of NDPhA standard (Figures 1B-C) and (2) disappearance of its TEA response after UV

Table 1. NDPhA (ppm) (a) in DPhA samples using LC-TEA detection

Sample	DPhA (%)	NDPhA (ppm)
1	30	0.82
2	15	0.5
3	15	0.5(b)
4	15	0.6(b)
5	15	0.5
6	15	0.5
7	15	0.5
8	15	0.5(b)
9	15	0.5
10	15	0.5

(a) Single determination; based on weight of DPhA.

(b) Average of duplicates.

Table 2. Recoveries of NDPhA from fortified sample No. 8 using LC-TEA

Analysis number	Fortification (ppm)	Found (ppm)	Recovery (%)
1	0.73	0.69	95
2	0.71	0.97	137
3	0.74	0.62	84
4	0.75	0.91	121
5	0.72	0.62	86
6	0.72	0.74	102
7	0.75	0.71	95
8	1.32	1.45	110
9	3.73	3.56	95
Mean recovery (n = 9)			103
CV (n = 9)			16.8

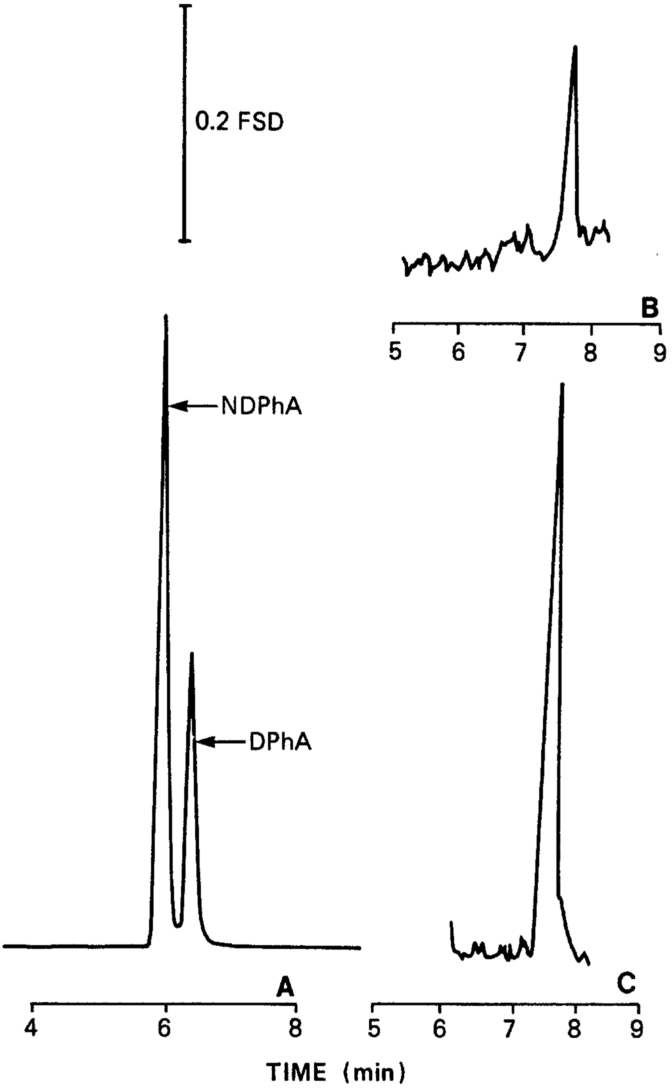

Figure 1. Typical LC chromatograms: (a) UV responses (at 243 nm) to standards of DPhA (0.124 ug) and NDPhA (0.134 ug), (b) TEA response to Sample no. 1 and (c) TEA response to NDPhA standard (5.11 ng). FSD = Full scale deflection.

irradiation. The recovery range of samples fortified at 0.7, 1.3 and 3.73 ppm was 86-137% with an average of 103% and a coefficient of variation (CV) of 16.8% (Table 2). The TEA responses to a series of sample eluates fortified with NDPhA standard were linear over a range of 0.7-4.1 ppm and to NDPhA standard of 1.0-5.1 ng. The recovery data and the first set of linearity range suggested that the TEA responses were not suppressed by impurities in the eluates injected into the detection system. The limit of detection (LOD), defined as 2 x S/N (signal to noise level), was 0.2 ppm and the limit of quantitation (LOQ), defined as 5 x S/N, was 0.5 ppm.

These data indicate that the described method is simple and adequate to analyse NDPhA in formulations containig DPhA. NDPhA was detected in all samples analysed, two at 0.82 and 0.6 ppm and all others below the LOQ.

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