Bull. Environm. Contam. Toxicol. 24, 746-751 (1980)

Determination of Diphenylamine Residues in Apples

B. G. Luke and S. A. Cossens

Department of Science and the Environment, Australian Government Analytical Laboratories, 11 William St., Melbourne, Victoria, Australia 3000

Superficial scald is a physiological skin disorder which affects some varieties of apples and pears held in storage. PADFIELD (1969) and SMOCK (1955) reported on the use of diphenylamine to control scald, and investigations into the efficacy and distribution of diphenylamine in apples (HARVEY & CLARK 1959, HALL et al.1961) led to the commercial use of this treatment in New Zealand.

The Codex maximum residue limit for diphenylamine in apples (fresh fruit basis) is 10 mg/kg, although this level is currently under review, and consideration is being given to lowering the limit.

BRUCE et al. (1958) and HANEKOM et al. (1976) used spectrophotometric methods for residue determination, whilst GUTENMANN & LISK (1963) carried out bromine derivatization followed by gas-liquid chromatography (GLC) with electron capture detection. These detection methods are relatively non-specific and were developed prior to the introduction of GLC element sensitive detectors.

The method described involves extraction of diphenylamine residues in peel with acetonitrile, clean up by hydrochloride formation, and finally GLC determination using a Hall electroconductivity detector.

MATERIALS AND METHOD

Extraction. Peel several apples to a depth of 2-4 mm. Weigh a 25 g aliquot of the chopped peel and macerate in a Waring blender with 100 mL of acetonitrile for 2 min. Filter the bulk of the pulp on a 9- cm Buchner funnel which is fitted with a glass fibre filter pad. Return the majority of the pulp to the Waring blender, and regrind for 1 min with a further 100 mL of acetonitrile. Filter on the same filter pad, and wash the pulp with 50 mL of acetonitrile.

0007-4861/80/0024-0746 $01.20

Divide the acetonitrile extract into two equal portions, and reserve one portion for later confirmation analysis if necessary.

Clean up. Dilute the remaining acetonitrile extract (approx. 125 mL) with 600 mL of 2% aqueous sodium sulphate in a 1-L separating funnel. Add 100 mL of redistilled diethyl ether, shake, and allow the layers to separate. Transfer the lower layer to a second 1-L separating funnel and repeat the extraction with two further 50 mL portions of diethyl ether. Combine the ether layers in a 250-mL round bottom flask and concentrate the solution to about 20 mL using a rotary evaporator.

Transfer the diethyl ether solution to a 100 mL separating funnel using 5-10 mL of diethyl ether to rinse the flask. Add 5 mL of concentrated HCl to the separating funnel, shake gently for 5 min, and allow the layers to separate. Run the lower acid layer into a 500 mL separating funnel containing 300 mL of distilled water. Repeat the extraction with two further 5 mL aliquots of HCl, and combine the acid layers.

Add 100 mL of petroleum ether to the 500-mL separating funnel, shake for 1 min, and transfer the aqueous layer into a second 500-mL separating funnel. Pass the petroleum ether layer through a sodium sulphate drying column which has been pre-washed with 25 mL of petroleum ether, and collect the eluate in a Kuderna-Danish (K.D.) evaporator fitted with a 2.0-mL graduated tube. Repeat the extraction of the aqueous acid layer with two further 25 mL portions of petroleum ether, dry through sodium sulphate as before, and combine the extracts in the K.D. apparatus. Attach a Vigreaux column and evaporate the solvent. Add two separate 10 mL portions of petroleum ether and re-evaporate to facilitate removal of traces of acetonitrile. Finally adjust the volume to 2.0 mL with a fine nitrogen stream.

Gas Chromatography. Inject 2-5 µL of the petroleum ether extract into a gas chromatograph fitted with a Hall electroconductivity detector operating in the nitrogen mode. The retention time for diphenylamine on 3% OV-1 (1.8 m x 4 mm i.d., 80-100 mesh Gas Chrom Q) at 200°C with helium carrier gas flow of 50 mL/min is 1.5 min. Hall detector conditions include a furnace temperature of 840°C, hydrogen flow rate of 50 mL/min, and a 50% aqueous n-propanol electrolyte flow rate of 0.4 mL/min. The amount of diphenylamine in the injection volume is read from a calibration graph prepared by plotting peak height in mm for 10-60 ng injections of standard diphenylamine.

RESULTS AND DISCUSSION

Recoveries of diphenylamine in fortified apple peel pulp at the 1.0 and 4.0 mg/kg level were 83 and 84%, respectively. (Table 1). When applied to analysis of whole fruit at the 1.6 mg/kg level , recoveries were 91%. Analysis of untreated apples (both peel and whole fruit) showed no interference by co-extractives in the region where diphenylamine eluted (Fig 1). Detector sensitivity, and linearity (Fig 2) were such that detection, based on 12.5 g peel, of 0.05 mg/kg was readily obtained.

TABLE 1. Recoveries of diphenylamine in peel[a] and whole fruit.[b]

Variety	Fortification (mg/kg)	% Recovery
Granny Smith[a]	1.0	83, 82, 83
Granny Smith[a]	4.0	83, 85, 84, 83
Delicious[a]	4.0	88, 81
Granny Smith[b]	1.6	93, 88

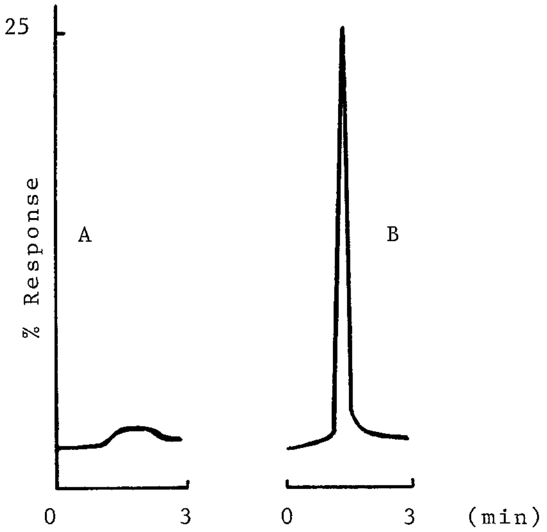

Fig 1. Chromatograms from Granny Smith peel: A, untreated sample; B, diphenylamine treated sample, peak represents approx. 20 ng at attenuation 10 x 8.

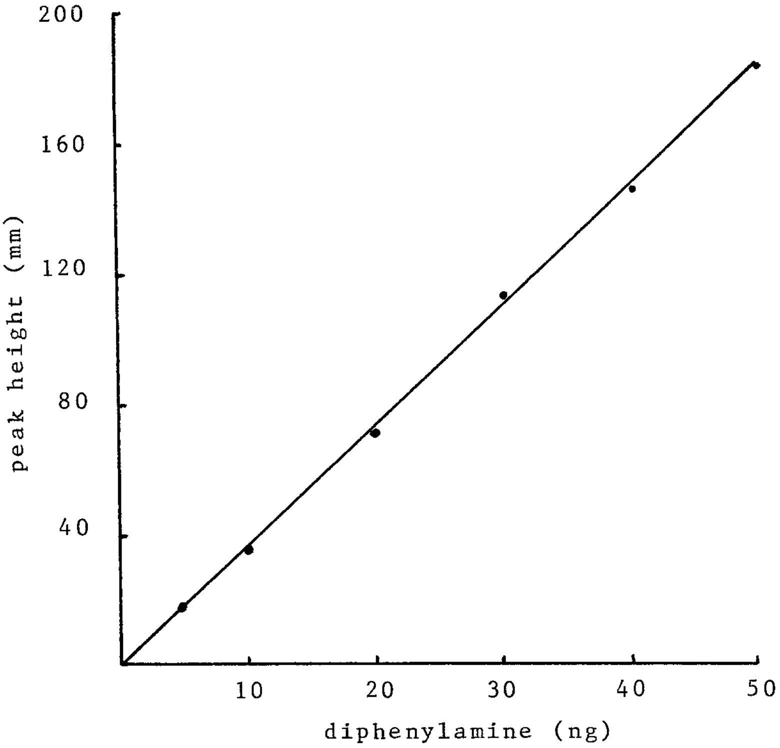

Fig 2. Linearity graph for diphenylamine using Hall electroconductivity detector.

The final extract is coloured and usually contains some sediment. Because of the specificity of the detector up to 50 sample injections can be made before these co-extractives begin to adversely affect the quality of the chromatograms. Replacement of the glass wool plug and the first 3-5 cm of the GC column packing is then recommended, and this restores the column to its original performance. Our observations in regard to time saving to be gained from the use of highly selective GC detectors are similar to those reported by LUKE et al. (1975) in their work on the clean up of fruit and vegetable extracts.

The presence of sediment and coloured co-extractives can be almost completely eliminated if petroleum ether is used instead of diethyl ether for the initial extraction of the aqueous acetonitrile layer. However, with the former solvent 5-10% lower recoveries were obtained. This is probably due to increased emulsion formation in the concentrated HCl partition step when petroleum ether, rather than diethyl ether, is used.

TABLE 2

Diphenylamine (DPA) residues in peel resulting from commercial treatments.

Variety	Treatment	Storage Time (weeks)	No. Samples Analysed[a]	DPA Residues (mg/kg)	
				Range	Mean
Granny Smith	Dip (0.1%)	10	7	3.1-8.7	6.1
Granny Smith	Impregnated paper wraps	30	10	1.1-7-7	3.3
Delicious	Dip (0.1%)	10	11	3.3-9.5	5.2

[a] Each sample represents a separate consignment of apples.

Because diethyl ether is relatively soluble in concentrated HCl a miscible solution may be formed at the extraction stage. To overcome this problem it is often necessary to add a further 10-20 mL of diethyl ether to the 250-mL separating funnel in the initial acid extraction.

The method has been used for Granny Smith and Delicious apple varieties which have been subject to commercial post harvest treatment (Table 2). In both dip and impregnated paper wrap applications our results confirm previous reports that negligible amounts (less than 0.1 mg/kg) of diphenylamine penetrates beyond 4 mm depth. Remembering that analysis was carried out on peel, residues resulting from normal commercial treatments are well below the recommended Australian and Codex maximum residue limits (fresh fruit) of 7 and 10 mg/kg, respectively.

ACKNOWLEDGMENT

The authors thank R. Symons and M. Sheen for their assistance.

REFERENCES

BRUCE, R.B., H.W. HOWARD, and B. ZINK: J. Agric. Food Chem. 6, 597 (1958).

GUTEWMANN, W.H., and D.J. LISK: J. Agric. Food Chem. 11, 468 (1963).

HALL, E.G., K.J. SCOTT, and G.G. COOTE: Aust. J. Agric. Res. 12, 834 (1961).

HANEKOM, A.N., J.L. SCHEEPERS, and J. DEVILLIERS: The Deciduous Fruit Grower: p.402, October (1976).

HARVEY, H.E., and P.J. CLARK: N.Z. J. Sci. 2, 266 (1959).

LUKE, M.A., J.E. FROBERG, and H.T. MASUMOTO: J. Assoc. Off. Anal. Chem. 58, 1020 (1975).

PADFIELD, C.A.S.: The Storage of Apples and Pears, 2 ed. N.Z. Dept. Sci. Ind. Res. Bulletin III (1969).

SMOCK, R.M.: Amer. Fruit Gr. 75, 20 (1955).