Journal of Chromatography, 447 (1988) 421–425
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 609

Note

Determination of diphenylamine residues in apples, and 4-aminobiphenyl residues in diphenylamine, by high-performance liquid chromatography and electrochemical detection

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(First received December 30th, 1987; revised manuscript received April 19th, 1988)

Diphenylamine is a post-harvest fungicide currently used on apples to prevent scald. However, its use is subject to restriction in a number of countries. These restrictions refer to (1) the amount of diphenylamine residues in treated fruit (limited to 3 mg/kg in France for example) and (2) the amount of 4-aminobiphenyl in used diphenylamine (limited to 2 mg/kg in France for example) since 4-aminobiphenyl is a very dangerous impurity as a result of its very high carcinogenic properties.

Such restrictions led us to try to develop simple and rapid methods to determine diphenylamine in apples, and residual 4-aminobiphenyl in diphenylamine.

A number of techniques have been proposed for the determination of diphenylamine involving spectrophotometry, after derivatization[1], thin-layer chromatography (TLC)[2], gas chromatography (GC)[3–7] with electron-capture detection (ECD) (after derivatization), nitrogen-phosphorus detection, or Hall's detector, and high-performance liquid chromatography (HPLC)[8,9] with UV detection.

All these methods have drawbacks. GC requires fairly lengthy sample preparation and, for ECD, a derivatization. TLC is not satisfactory for quantitative determination, and therefore needs an extraction of the spot for a later determination. Liquid chromatography with UV detection lacks sensitivity and needs a pre-concentration of the sample for satisfactory results. For all these reasons, we have turned our attention to liquid chromatography with electrochemical detection (LC–ED).

Because aromatic amines are readily oxidizable, LC–ED offers an attractive approach to their analysis. Its use for similar problems (aromatic amine residues in water, soil or skin) has given excellent results. For that reason we have applied it to the determination of diphenylamine residues in apples.

Determination of 4-aminobiphenyl residues in diphenylamine has not yet been studied. Among different methods proposed for the determination of 4-aminobiphenyl residues in other media, there are electrochemical techniques, such as polarography and coulometry[10], TLC[11], GC[12–14], and HPLC with electrochemical detection[15].

As this technique is particularly well adapted to our problems, we have also applied LC–ED to the determination of 4-aminobiphenyl residues in diphenylamine.

EXPERIMENTAL

Apparatus

The following equipment was used: a Millipore (Waters) M510 pump; a Rheodyne injection valve, equipped with a 20-μl loop; a μBondapak C_{18} column (10 μm, 30 cm × 4 mm I.D.); an amperometric detector Tacussel PRG-DEL with DEL-1 cell. The working electrode was glassy carbon, the reference electrode was silver/silver chloride/saturated potassium chloride and the auxiliary electrode was platinum. The mobile phase was acetonitrile–water (60:40) containing 2 g/l of lithium perchlorate, and run at a flow-rate of 1 ml/min. Under these conditions, the capacity factors (k') were as follows: diphenylamine, 3.75; 4-aminobiphenyl, 2.08.

Intensity potential curves for 4-aminobiphenyl and diphenylamine

To determine the optimal value of the potential to be applied, we injected a mixture (100 μg/ml) of both compounds to be studied, at different potentials, and measured the intensity of the oxidation peak corresponding to each case. The intensity potential curves observed are indicated in Fig. 1. A study of these curves shows that the limiting diffusion current is reached at + 1.2 V for 4-aminobiphenyl as well as for diphenylamine. However, in order to prevent too much residual current and background noise, the potential was fixed at + 0.9 V throughout the following procedure.

Separation of 4-aminobiphenyl from diphenylamine

For the determination of residual 4-aminobiphenyl in diphenylamine, we tested a separation method based on the difference in basicity of the two compounds: 4-aminobiphenyl is clearly more basic than diphenylamine.

Procedure. A 1-ml volume of a solution of 4-aminobiphenyl or diphenylamine

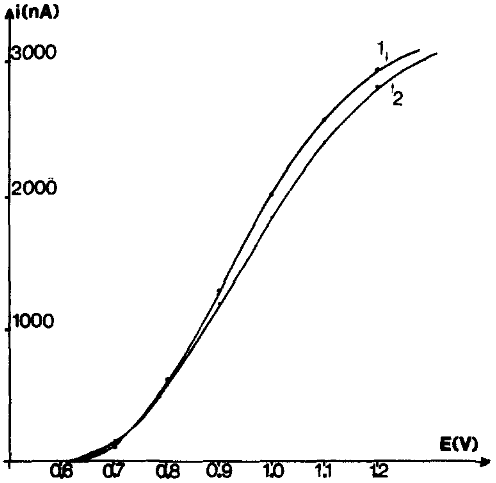

Fig. 1. Intensity–potential curves: 1 = 4-aminobiphenyl; 2 = diphenylamine.

(1 mg/ml) was placed in a 125-ml separatory funnel, and 20 ml of selected pH hydrochloric acid were added. The compounds were extracted with two 20-ml portions of hexane. The hexane phases were combined and evaporated to dryness, and the residue was dissolved into 10 ml of methanol. Then 4-aminobiphenyl or diphenylamine was injected into the chromatograph under the conditions previously described.

A study of the results shows that, although the extraction yield of diphenylamine is close to 100% at any pH, the extraction yield of 4-aminobiphenyl depends strongly on the pH. The lower the pH, the less 4-aminobiphenyl is extracted by hexane. For example, the recovery rates observed varied from 0.9% at pH 0 to 91.7% at pH 3.

Determination of 4-aminobiphenyl residues in diphenylamine

The above results led us to adopt the following procedure for the determination of 4-aminobiphenyl residues in diphenylamine. Exactly 5 g (of diphenylamine) were dissolved in 100 ml of hexane, and the solution was placed in a 250-ml separatory funnel. 4-Aminobiphenyl was extracted by four 20-ml portions of 0.1 *M* hydrochloric acid. The aqueous phases were combined and poured into another 250-ml separatory funnel; the hexane phases were discarded. The aqueous phase was washed with two 40-ml portions of hexane, and neutralized by 10 ml of 1 *M* sodium hydroxide. 4-Aminobiphenyl was extracted with two 40-ml portions of dichloromethane. The organic phases were combined, filtered over anhydrous sodium sulphate, then evaporated to dryness. The evaporation residue was dissolved into 10 ml of methanol, then injected into the liquid chromatograph under the conditions described above.

Determination of diphenylamine residues in apples

A 100-g amount of apples was weighed and peeled carefully. The peel was placed in a 250-ml flask, and 100 ml of methanol and 10 ml of 10% methanolic potassium hydroxide solution were added. The mixture was shaken for 1 h then filtered, and the filtrate was placed in a 1000-ml separatory funnel. Next, 300 ml of distilled water and 30 ml of saturated sodium chloride solution were added, and diphenylamine was extracted with two 60-ml portions of dichloromethane. The organic phases were combined, filtered over anhydrous sodium sulphate, and evaporated to dryness. The residue was dissolved into 10 ml of methanol, filtered on a 0.45-μm PTFE Millipore membrane, then injected into the chromatograph.

RESULTS AND DISCUSSION

Study of the linearity and repeatability of the detector response

In order to study the linearity and repeatability of the method, we repeatedly injected (n = 10) several mixtures of 4-aminobiphenyl and diphenylamine at 0.05, 0.5, 5 and 50 μg/ml. We measured the peak heights, and calculated the mean and the standard deviation. The results, given in Table I, show the perfect linearity and the excellent repeatability of electrochemical detection. The calculated correlation coefficients are +0.999 645 for 4-aminobiphenyl, and +0.999 968 for diphenylamine.

Detection limits of diphenylamine and 4-aminobiphenyl

In order to test the sensitivity of the method, we injected smaller and smaller

TABLE I

DETECTOR RESPONSE AND REPEATABILITY

Means and relative standard deviations obtained for ten injections (20 μl) of diphenylamine and 4-aminobiphenyl at different concentrations.

C (μg/ml)	*4-Aminobiphenyl*		*Diphenylamine*	
	I (nA)	*σ (%)*	*I (nA)*	*σ (%)*
0.05	1.2	4.5	0.67	4.1
0.5	12.6	6.6	7.25	6.5
5.0	105	3.5	73.5	2.7
50.0	816	10.6	679	4.1

amounts of each of the two compounds to determine the detection limits. For an injection of 200 pg of both compounds the signal-to-noise ratio is *ca.* 5, so we can estimate the detection limit as *ca.* 100 pg for these compounds.

Determination of 4-aminobiphenyl residues in diphenylamine

The method described has been applied to five samples of diphenylamine, spiked respectively with 0, 1, 5, 10 and 50 mg/kg of 4-aminobiphenyl. The recovery rate was determined for each sample, and the results show that it varies from 88.6% to 100%.

Determination of diphenylamine residues in apples

The recovery rate of diphenylamine has been studied in five samples of apples (Granny Smith type), spiked respectively with 0, 0.1, 0.5, 1 and 5 mg/kg of diphenylamine, according to these method described. Table II indicates that the observed recovery rates were always satisfactory, even for very low concentrations. Also, the chromatogram obtained (Fig. 2) shows that there is no interference for the determination of diphenylamine.

TABLE II

DETERMINATION OF DIPHENYLAMINE RESIDUES IN APPLES

Diphenylamine		*Yield (%)*
Added (mg/kg)	*Recovered (mg/kg)*	
0	0	–
0.1	0.115	115
0.5	0.59	118
1	0.98	98
5	4.6	92

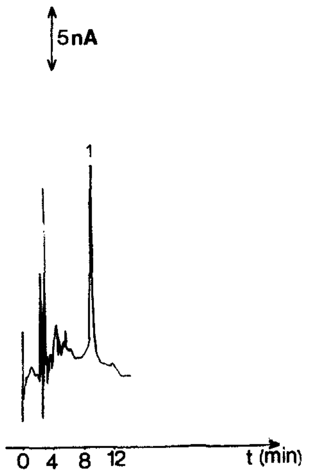

Fig. 2. Determination of diphenylamine residues in apples, obtained with a 0.1 mg/kg spiked sample. Peak: 1 = diphenylamine.

CONCLUSION

The results of this study both for 4-aminobiphenyl and diphenylamine, show that HPLC with electrochemical detection is undoubtedly one of the best methods yet proposed. Its main advantages are (1) extreme sensitivity (detection limit of 100 pg), (2) excellent selectivity, particularly for the determination of diphenylamine in apples, which requires no purifying operation (3) great simplicity and low cost.

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