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Note

Application of electrochemical detection to the determination of ethoxyquin residues by high-performance liquid chromatography

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Ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) is an antioxidant commonly used to treat apples and pears after picking to protect against scald. However, it is not unusual for fruit to be marketed with a guarantee that it is untreated, so it is important to have a highly sensitive technique for the determination of additive residues.

Methods previously described involve either gas chromatography (GC) after derivatization using heptafluorobutyric anhydride[1], or high-performance liquid chromatography (HPLC) with detection by UV absorption or fluorescence[2,3]. The GC method is highly sensitive (detection limit 0.02 ng) but requires a fairly long preparation of the test sample and the handling of highly toxic compounds (heptafluorobutyric anhydride, pyridine, toluene). HPLC methods do not have these drawbacks but have much higher detection limits (approximately 2.5 ng).

The method described here involves HPLC combined with electrochemical detection and has the advantages of high sensitivity (detection limit 0.03 ng) and simple and rapid sample preparation, with no derivatization or handling of toxic compounds. Comparison of the results obtained with those obtained by GC shows good agreement.

EXPERIMENTAL

Instrumentation

HPLC. A Gynkotek Model 600 pump, equipped with a Chromatofield EL-DEC 102 electrochemical detector and a Negretti and Zambra valve loop injector (20 μl), was used. The column (stainless steel, 150 × 4.6 mm I.D.) was packed with Nucleosil C_{18} (5 μm). A pre-column (stainless steel, 40 × 4.6 mm I.D.) was packed with Corasil C_{18} (30 μm). The mobile phase was water–methanol (30:70) containing 1 g/l of lithium perchlorate and the flow-rate was 0.8 ml/min.

Gas chromatography. Electron-capture detection was performed on a Varian 1200 chromatograph equipped with a tritium source detector. The column (2 m × 1/8 in. I.D.) contained 3% OV-17 on Gas-Chrom Q (80–100 mesh). The carrier gas was nitrogen (30 ml/min). The column temperature was 170°C and the injector and detector temperatures were 250°C.

Nitrogen–phosphorus detection was performed on a Perkin-Elmer Sigma 4

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chromatograph equipped with a flameless rubidium ball thermionic detector. The column (2 m × 1/8 in. I.D.) contained 3% OV-17 on Gas-Chrom Q (80–100 mesh). The carrier gas was nitrogen (30 ml/min). The column temperature was 180°C, the injector and detector temperatures were 275°C the rubidium ball temperature was 550°C.

Sample preparation

Extraction. A 100-g amount of apple, cut into pieces is weighed out. The apple skins are ground with 150 ml of hexane and 3 ml of sodium hydrogen bicarbonate solution (100 g/l) and then agitated for 45 min. After filtration the hexane extract is evaporated in a rotary evaporator and diluted to 10 ml with hexane. This extract will be called E_1.

Purification. Half of the above extract is placed in a separating funnel and the ethoxyquin is extracted with two volumes of 15 ml of 0.1 *M* hydrochloric acid. The aqueous phases are combined, placed in a 200-ml separating funnel and neutralized with 4 ml of 4 *M* sodium hydroxide solution, and the ethoxyquin is extracted with two volumes of 5 ml of hexane (agitation time 30 sec; standing time 1 min). The extract is then evaporated as above and diluted 10 ml with hexane. This extract will be called E_2.

Derivatization by the method of Winell[1]. A 1-ml volume of the purified extract E_2 is placed in a haemolysis tube and 50 µl of a 5% solution of pyridine in toluene and then 50 µl of heptafluorobutyric anhydride are added. The tube is agitated for a few seconds and allowed to stand for 3 min, then 5 ml of 1 *M* sodium hydroxide solution are added to stop the reaction. The extract obtained will be called E_3.

Determination of ethoxyquin content

By GC with a rubidium ball thermionic detector. A 5-µl volume of extract E_1 is injected into the apparatus. The determination is effected by external calibration, comparing the height of the peak obtained with that corresponding to the injection of 5 µl of a 10 µg/ml standard solution of ethoxyquin in hexane.

By GC with an electron-capture detector. A 5-µl volume of extract E_3 is injected into the apparatus. The determination is again effected by external calibration, by comparing the height of the peak obtained with that corresponding to the injection of 5 µl of a 6 µg/ml standard solution of ethoxyquin in hexane, already derived according to the method of Winell[1].

By HPLC with an electrochemical detector. Determination may be effected using either the raw extract E_1 or the purified extract E_2, both having been evaporated to dryness and then dissolved in and diluted to 5 or 10 ml with methanol. If the analysis is performed using E_1, it is necessary to filter using a PTFE Millipore membrane filter (pore diameter 0.5 µm) to remove deposits formed during the transfer from hexane to methanol. The determination is also effected by external calibration, by comparison with a 1 µg/ml standard solution of ethoxyquin in methanol.

RESULTS AND DISCUSSION

Setting up the HPLC system

Determination of optimal potential. In electrochemical detection, the optimal

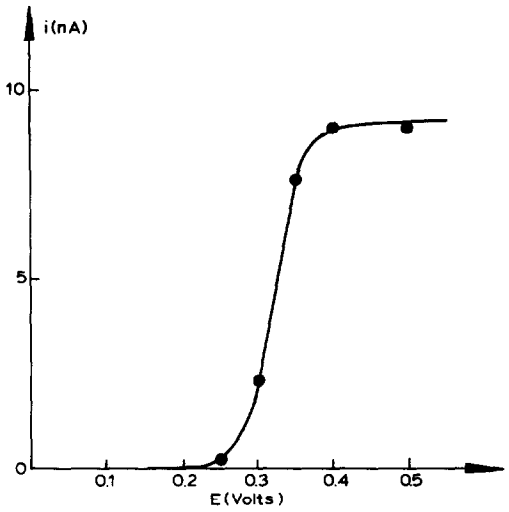

Fig. 1. Variation of oxidation current obtained for an injection of 20 ng of ethoxyquin in relation to the potential applied.

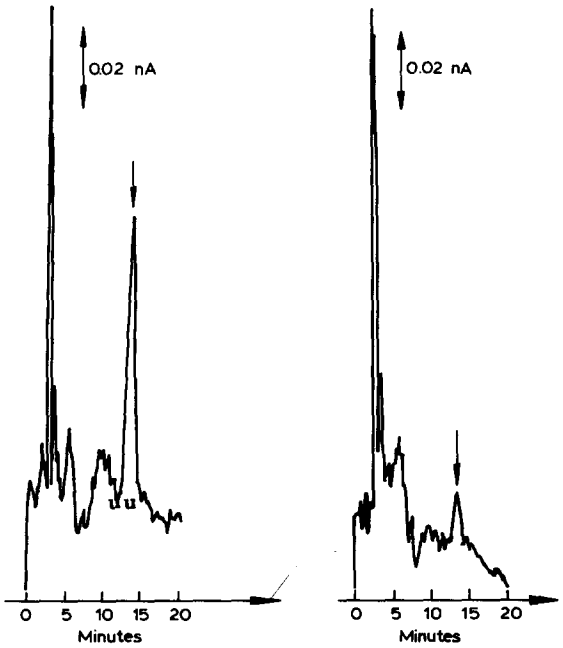

Fig. 2. Chromatograms obtained on injecting 20 μl of standard ethoxyquin solutions (0.01 and 0.002 μg/ml, *i.e.*, 0.2 and 0.04 ng of ethoxyquin injected, respectively).

TABLE I

REPRODUCIBILITY AND LINEARITY

Concentration of standard solution (μg/ml)	*Mean peak height (nA)**	*Relative standard deviation (%)**
0.1	0.2933	8.2
1	3.057	7.7
10	30.10	4.2
100	287.5	9.6

* $n = 10$.

potential lies where the diffusion limit current of the intensity–potential curve is just reached. To find this potential, we measured the heights of the peaks obtained for an injection of 20 μl of 1 μg/ml standard ethoxyquin solution at different potentials. The intensity–potential curve obtained is shown in Fig. 1.

The results show that the diffusion limit current is reached at $E \approx +0.4$ V. Henceforth, the subsequently applied potential was +0.45 V.

Detection limit. The detection limit was obtained by injecting decreasing amounts of ethoxyquin. Fig. 2 shows that the detection limit (defined as the amount

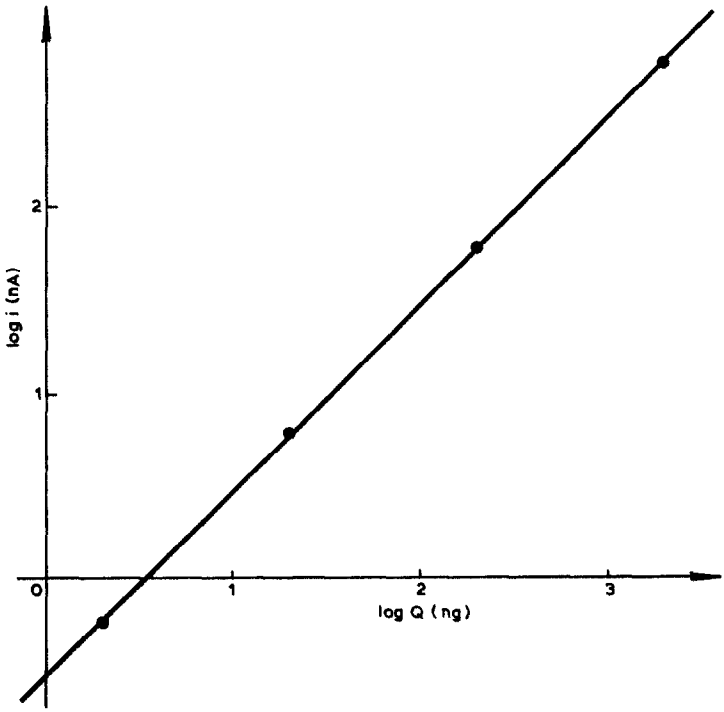

Fig. 3. Study of linearity of the response of the electrochemical detector: oxidation current *versus* amount of ethoxyquin injected (logarithmic).

TABLE II

COMPARISON OF RESULTS OBTAINED ON SIX SAMPLES OF APPLES TREATED WITH ETHOXYQUIN

Sample No.	*Ethoxyquin concentration (mg/kg)*		
	GLC, thermionic detector	*GLC, electron-capture detector*	*HPLC, electro-chemical detector*
1	0.13	0.14	0.11 (not purified)
2	0.12	0.11	0.13 (not purified)
3	Not injected	0.06	0.06 (purified)
4	Not injected	0.08	0.09 (not purified)
5	Not injected	0.09	0.11 (not purified)
6	Not injected	0.17	0.14 (not purified)

for which the peak height obtained is equal to twice the background noise) is approximately 0.03 ng of ethoxyquin injected.

Reproducibility and linearity. To test the reproducibility and linearity of the response of the electrochemical detector, we injected different standard solutions of ethoxyquin, with concentrations of 0.1, 1, 10 and 100 μg/ml, ten times.

The results in Table I show that this mode of detection has reasonable reproducibility. The calibration graph of peak current *versus* amount of ethoxyquin injected (Fig. 3) shows a linear response of the detector over the concentration range studied (2–2000 ng of ethoxyquin injected).

Application to determination of ethoxyquin residues in apples

Residues of ethoxyquin were determined in six samples of treated apples. The results obtained by the three different methods (Table II) show good agreement. However, HPLC with electrochemical detection offers several advantages over the other methods:

(1) high sensitivity (detection limit = 0.03 ng of ethoxyquin); only the GC method of Winell[1] with electron-capture detection has a comparable sensitivity;

(2) linearity of the response over a wide range (2–2000 ng);

(3) good selectivity, owing to the very low working potential used (+0.45 V);

(4) simplicity: a single extraction with hexane followed by transfer into methanol is sufficient; neither clean-up nor derivatization involving the use of toxic compounds is necessary;

(5) low cost: the electrochemical detector is the cheapest HPLC detector.

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