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Note

High-performance liquid chromatographic analysis of eugenol in pimento using ultraviolet and electrochemical detection

ROGER M. SMITH* and STEVEN BECK
Department of Chemistry, University of Technology, Loughborough, Leics. LE11 3TU (U.K.)
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The berries of the pimento tree (*Pimenta dioica* L.) are the source of the important spice Allspice and a number of workers have examined their chemical composition[1]. Most of the recent work has concentrated on the gas–liquid chromatographic separation of the major components eugenol (approx. 70%) and methyl eugenol (approx. 9%) and the identification of the numerous minor constituents in the essential oils from the berries and the leaves[2–4].

A preliminary examination of the use of high-performance liquid chromatography (HPLC) to analyse eugenol in pimento has been reported using ultraviolet (UV) spectrometric detection but few details of the quantitative measurements were given[5]. In earlier studies it has been shown that liquid chromatography with electrochemical detection (ED) could be applied to the analysis of phenolic constituents in the spices ginger[6] and turmeric[7]. This paper extends this work and reports the results of a comparative study of the use of UV and ED for the determination of eugenol in pimento.

EXPERIMENTAL

Materials

Samples of pimento berries were of whole berries. Eugenol, methyleugenol, and test compounds were laboratory grade and methanol was Fisons (Loughborough, U.K.) HPLC grade.

Method

Samples of the berries (0.5 g) were ground to a fine powder and extracted with methanol (50 ml) overnight at room temperature. Samples of 20-μl were analysed by HPLC. The eugenol was determined by comparison with standard solutions made up in methanol–0.05 *M* phosphate buffer pH 7.0 (50:50).

High-performance liquid chromatography

Liquid chromatographic separations were carried out using a Pye Unicam XPS pump, a Kipp 9205 electrochemical detector and Pye Unicam UV detector PU 4020. Samples of 20-μl were injected using a Rheodyne 7125 valve on to a Shandon Southern column (10 cm × 5 mm I.D.) packed with 5-μm ODS-Hypersil and were eluted

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TABLE I

DETERMINATION OF EUGENOL ON HPLC USING UV AND ELECTROCHEMICAL DETECTION

Eugenol concentration (% v/v)	*Peak heights (mm)*	
	*UV**	*ED***
0.0	0	0
0.0001	5	39
0.0002	9	77
0.0003	13	112
0.0004	18	162
Correlation	0.9989	0.9982
Slope	44000	397000
Intercept	0.2	−1.4

* 0.04 a.u.f.s.
** 2 μA, 0.7 V *versus* Ag/AgCl.

with methanol–0.05 *M* phosphate buffer pH 7.0 (75:25) at 1.0 ml/min. The components were detected using UV detection at 278 nm and ED at 0.7 V relative to Ag/AgCl. Retention indexes were determined as described[8].

RESULTS AND DISCUSSION

Methanolic extracts of ground pimento berries were examined by reversed-phase HPLC using an ODS-Hypersil column. The chromatograms from both the UV and electrochemical detectors contained only one significant peak ($k' = 2.50$), which was identified as eugenol by comparison with an authentic sample. No peak was observed in the chromatogram from the UV detector for methyl eugenol ($k' = 4.71$) and it is electrochemically inactive.

Quantitative comparison of the two detectors showed that both gave a linear response for eugenol (Table I) but at the concentrations used the UV detector was near its limit of detection, whereas the electrochemical detector was much more sensitive. At higher concentrations the electrochemical detector has been found to be irreproducible because of electrode contamination[5]. The calibration curves were used

TABLE II

COMPARISON OF DETERMINATIONS OF EUGENOL IN DRIED PIMENTO BERRIES WITH DIFFERENT DETECTORS

Samples	*Concentration of eugenol (% v/m)**	
	UV detector	*Electrochemical detector*
Belize 9	2.93	2.83
Belize 22	1.25	1.21
Jamaican	1.26	1.21

* Means of duplicate extractions.

TABLE III

CAPACITY FACTORS AND RETENTION INDEXES OF EUGENOL AND COLUMN SELECTIVITY TEST COMPOUNDS

Eluent, methanol–0.05 *M* phosphate buffer pH 7.0 (75:25).

Sample	*k'*	*Retention index*
Eugenol	2.50	932
Methyl eugenol	4.71	1031
Selectivity test compounds		
Toluene	4.68	1030
Nitrobenzene	1.48	850
2-Phenylethanol	0.97	783
p-Cresol	1.09	803
N-Methylaniline	1.13	809

to determine the concentration of eugenol in three samples of stored pimento berries (Table II). The results from the two detectors were very similar and agreed with the concentrations found using gas–liquid chromatography and results for similar samples reported by other workers[2–4].

It has been suggested that capacity factors are an unsuitable method for reporting retentions for comparison purposes because they are susceptible to the uncertainty in the measurement of the column dead volume (t_0) and to small changes in the composition of the mobile phase. It has been proposed that a preferable method is to use retention index values relative to a homologous series of standards, such as the alkylarylketones, as it has been shown that retention index values are largely independent of t_0 and eluent composition[8]. In addition by measuring the indexes of a series of appropriately chosen test compounds the selectivity properties of the column–eluent combination can be quantified and thus comparison with other separation systems can be made[9]. By using the alkylarylketones acetophenone–valerophenone as retention index standards the retention indexes of a set of test compounds, toluene, nitrobenzene, *p*-cresol, 2-phenylethanol and N-methylaniline and the analytes eugenol and methyl eugenol were determined (Table III). These could then be compared with the conditions used for related assays or to test the effect of changing the organic modifier or column material.

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