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Note

Determination of phenols and cresols in aluminium-backed paper by high-performance liquid chromatography

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The use of aluminium-backed paper for food packaging has risen above that of all other packaging materials. Incorrect levels of some phenols and cresols, which are added as preservatives to the glue used in production of this paper, can alter the packed food. Hence, a rapid and easy-to-perform method for the analysis of these substances is called for.

In the past, paper chromatography[1–5], thin-layer chromatography[6–10] and gas chromatography[11–15] were used to analyse many phenols and their derivatives. High-performance liquid chromatography (HPLC) has replaced these methods because of its greater resolution, efficiency and speed[16–26]. Burtscher *et al.*[27] succeeded in separating different phenols by using a reversed-phase C_{18} column and a gradient formed by a phosphate–acetonitrile buffer at pH 2. McLeod and Laver[28] described a method which uses a phosphate–acetonitrile mixture at a pH between 7.4 and 12. Though very effective, the pH of these methods is not compatible with the reversed-phase C_{18} columns commonly used. More recently[29–36] some methods have been described for the HPLC determination of phenols using previous derivatization reactions.

The method now described has the advantage of being rapid, easy to perform and worklable under pH conditions more suitable to reversed-phase C_{18} columns, moreover no derivatization was required.

EXPERIMENTAL

Chemicals

The solvents used were of analytical grade (LiChrosolv, E. Merck, Darmstadt, F.R.G.). The standards used were supplied by Carlo Erba (RP, Milan, Italy).

Standard solutions

A stock solution was prepared by dissolving a known weight of phenols and

cresols in methanol at a concentration of 1 mg/ml. This stock solution was diluted in methanol to concentrations in the range of 200 to 12.5 μg/ml to give a series of standards for analysis. A *p*-cresol methanolic solution (100 μg/ml) was used as the internal standard.

Adsorption and elution of standards by aluminium-backed paper

A 1-ml volume of internal standard solution and 1 ml of each standard solution were adsorbed on ten strips (10 cm × 1 cm) of aluminium-backed paper. After drying each strip was eluted using 8 ml of ethanol in about 30 min, performed by dropping the ethanol through a burette in a corked test-tube to avoid evaporation of the solvent. The eluates from each strip were pooled and evaporated under vacuum (15 mmHg at 25°C) in a 100-ml flask. The residue was dissolved in 1 ml of methanol, vortexed for 2 min and filtered through 0.45-μm OE67 membranes (Schleicher and Schull) before injecting in the chromatograph.

HPLC

HPLC analyses were conducted with a Beckman Model 420 chromatograph equipped with a Model 165 variable-wavelength UV detector operating at 284 nm, an Altex Model 210 syringe-loading sample injector with a 20-μl loop and a 25 cm × 4.6 mm I.D. Altex Ultrasphere C_{18} (5 μm) reversed-phase column. The wavelength of the chromatograph detector was selected from an examination of the UV spectra of each compound tested in methanol–0.011 *M* phosphate buffer (pH 7.5)–acetonitrile (50:47:3). Elution was carried out at a flow-rate of 1.0 ml/min and a pressure of 3.20 kp.s.i. using the complex gradient described in Fig. 1.

RESULTS AND DISCUSSION

The chromatographic separation of standard cresols and phenols is shown in Fig. 1. Controls were carried out using aluminium-backed paper free from cresols and phenols, to observe the eventual interference of the other peaks (Fig. 2A). Fig. 2B shows a chromatogram of a standard mixture of cresols and phenols. The results obtained show a good repeatability and linear response in the concentration range of 12.5–100 μg/dm² of *o*-phenylphenol and in the concentration range of 25–200 μg/dm² of the other cresols and phenols in aluminium-backed paper (Fig. 3).

Table I shows the efficiency of the extraction and recovery of cresols and phenols from 1.0 dm² of aluminium-backed paper. The quantities of the compounds tested ranged from 12.5 to 200 μg/dm². Methanolic solutions of cresols and phenols in the same concentration range were used as references. As shown in Table I, the percentage of compound recovered was on average 85.25%. Additionally, the recovery of 100 μg *p*-cresol as internal standard was 90 ± 2.3% S.D.

In Table II the capacity factors, k', and separation coefficients, α, of cresols and phenols are reported. The capacity factor is defined as $k' = (V' - V_0)/V_0$, where V is the retention volume. The α values were calculated for each compound by dividing the k' values for that compound by the corresponding value for *o*-phenylphenol.

The proposed method permits the determination of the presence and quantity of the tested cresols and phenols in aluminium-backed paper with good sensitivity, excellent repeatability and relative rapidity. Moreover we analysed different alumin-

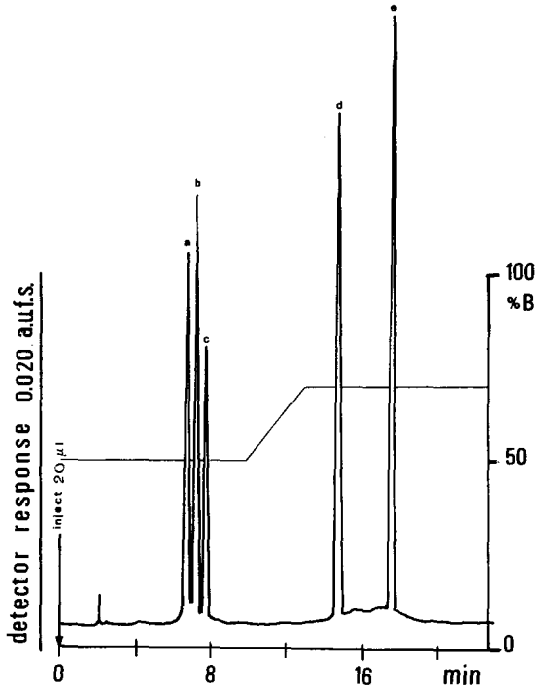

Fig. 1. Chromatogram of a standard mixture containing 50 µg/ml of 2,4,6-trichlorophenol and 100 µg/ml of each other compound. Peaks: a = 2,4,6-trichlorophenol; b = *p*-cresol; c = *o*-cresol; d = *p*-chloro-*m*-cresol; e = *o*-phenylphenol. Solvents: A = 0.011 *M* phosphate buffer (pH 7.5)–acetonitrile (94:6); B = methanol.

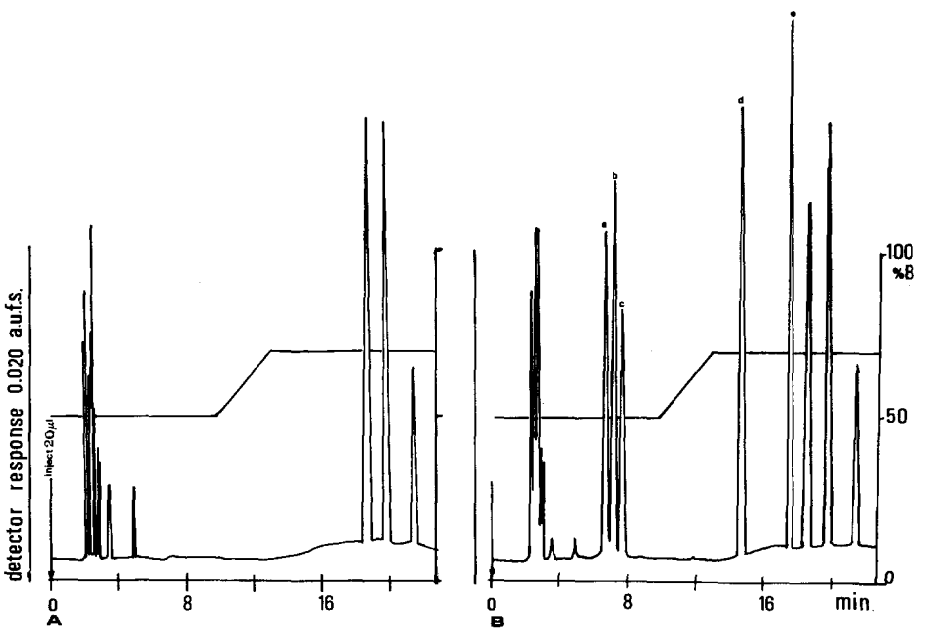

Fig. 2. Chromatograms of (A) blank control aluminium-backed paper and (B) aluminium-backed paper containing 50 µg/dm² of 2,4,6-trichlorophenol and 100 µg/dm² of each other compound.

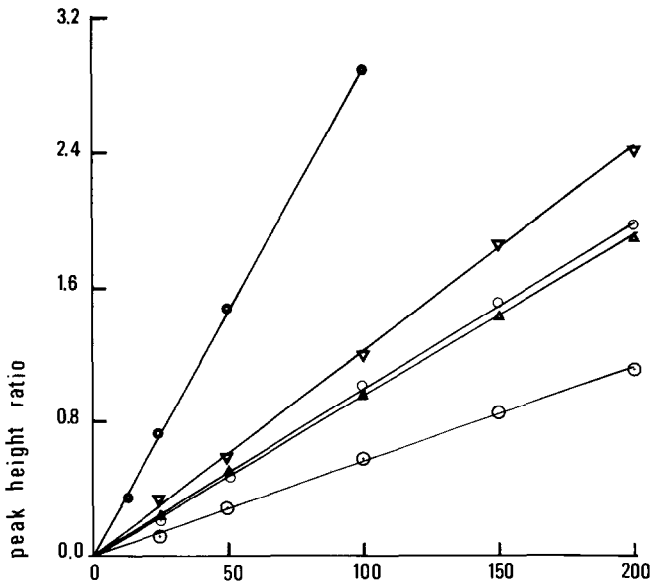

Fig. 3. Recovery–concentration curves for phenols and cresols. Abscissa: initial concentration of compound in aluminium-backed paper ($\mu g/dm^2$). Ordinate: response expressed as the peak-height ratio of each compound *vs.* *p*-cresol (100 $\mu g/dm^2$) as the external standard. ●—●, *o*-Phenylphenol, $y = 0.029x - 0.018$; ▽—▽, *p*-chloro-*m*-cresol, $y = 0.012x - 0.037$; ○—○, *p*-cresol, $y = 0.010x + 0.011$; △—△, 2,4,6-trichlorophenol, $y = 0.009x - 0.004$; ⊙—⊙, *o*-cresol, $y = 0.006x - 0.023$. Correlation coefficient for each slope was >0.997.

ium-backed paper samples containing the compounds tested. The average amount of cresols and phenols found in these samples was 89 ± 31 $\mu g/dm^2$. Fig. 4 shows the chromatogram of one sample containing *p*-chloro-*m*-cresol (68 $\mu g/dm^2$).

Italian law sets a limit of 200 $\mu g/dm^2$ phenols for double-foil sheets. However, at this concentration some phenols can cause alterations of the organoleptic characteristics of the packed food due to slow transmigration. At this point the food would become inedible. Hence, these phenols, and in particular *p*-chloro-*m*-cresol

TABLE I

PHENOLS AND CRESOLS RECOVERED FROM ALUMINIUM-BACKED PAPER

Values are given as % ± S.D. ($n = 4$).

Compound	*Phenols and cresols ($\mu g/dm^2$) added to aluminium-backed paper*					
	200	*150*	*100*	*50*	*25*	*12.5*
2,4,6-Trichlorophenol	88 ± 4.6	88 ± 5.2	88 ± 5.1	86 ± 4.8	84 ± 4.9	–
p-Cresol	89 ± 4.2	89 ± 4.6	89 ± 4.3	89 ± 3.5	87 ± 4.1	–
o-Cresol	89 ± 4.0	85 ± 3.6	83 ± 4.8	83 ± 4.8	83 ± 5.0	–
p-Chloro-*m*-cresol	86 ± 4.7	86 ± 4.6	86 ± 4.7	80 ± 4.1	80 ± 4.5	–
o-Phenylphenol	–	–	84 ± 4.3	84 ± 4.0	82 ± 4.8	82 ± 5.3

TABLE II

CAPACITY FACTORS AND SEPARATION COEFFICIENTS OF SEPARATED PHENOLS AND CRESOLS

Compound	*Retention time (min) ± S.D.*	*Capacity factor, k'*	*α*
2,4,6-Trichlorophenol	6.6 ± 0.009	2.3	0.29
p-Cresol	7.2 ± 0.010	2.6	0.33
o-Cresol	7.6 ± 0.009	2.8	0.36
p-Chloro-*m*-cresol	15.0 ± 0.009	6.5	0.81
o-Phenylphenol	17.6 ± 0.010	7.8	1.00

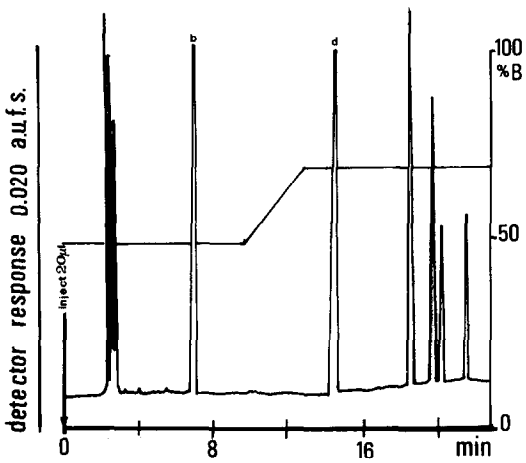

Fig. 4. Chromatogram of aluminium-backed paper sample containing 68 µg/dm² of *p*-chloro-*m*-cresol (d) and 100 µg/dm² of *p*-cresol as the internal standard (b).

which is mainly responsible for the alterations, should be used in even lower concentrations than those permitted by law.

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