Quantitative separation of chloro-xylenols by thin layer chromatography

Methods for the qualitative separation of chloro-derivatives of cresols and xylenols by paper and thin layer chromatographic techniques have been described by us recently[1,2]. This paper presents a thin layer chromatographic (TLC) method for quantitative estimation of some xylenols and their chloro-derivatives using, essentially, the techniques described by BLOCK[3], ROCKLAND AND DUNN[4] and PRIVETT AND BLANK[5] for the estimation of amino acids and glycerides, respectively. An automatic Joyce Chromoscan[6] was used to measure the optical densities of the spots; these are converted by the unit into absolute areas from which the relative percentages of the components are readily calculated.

Materials and methods

The xylenols and their chloro-derivatives were obtained from commercial sources[7] and repeatedly recrystallized till chromatographically pure. The plates (20 × 2.1 cm) were coated with silica gel G and developed as described earlier[2]. Microslides (7.2 × 2.4 cm) were prepared by dipping in a well-stirred suspension of silica gel G (30 g) in 80 ml of chloroform and 20 ml of methanol[8]. Both plates and microslides were scanned by the densitometer to determine the degree and uniformity of the background density.

Known volumes of various dilutions of compounds in acetic acid were applied with an Agla micrometer syringe[9] on the TLC plates and the optimum dilution range determined. The sample was then spotted (*ca.* 50 μg) with a glass capillary or microsyringe. The chromatogram was developed in xylene saturated with formamide, sprayed with a sufficient quantity of phosphotungstomolybdic acid (Folin-Denis reagent) and the plate was then exposed to ammonia vapour. The compounds appeared as blue spots on a white background.

For scanning, the developed plate (Fig. 1) was held in the densitometer and the

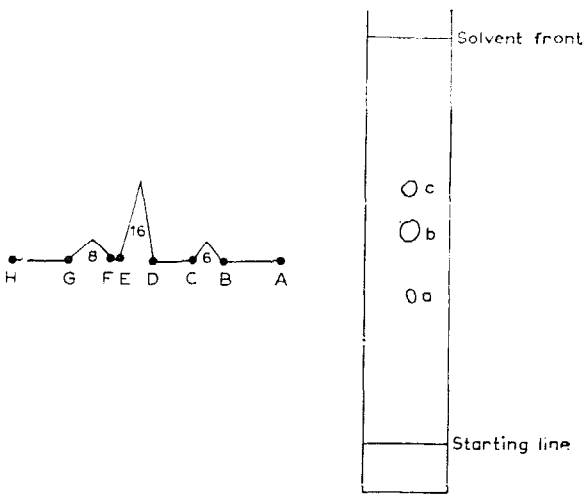

Fig. 1. Thin-layer chromatography diagram and corresponding peaks obtained on Chromoscan. B–C and a = 4-chloro-3,5-xylenol (20.0%); D–E and b = 4,6-dichloro-3,5-xylenol (53.33%); F–G and c = 2,4,6-trichloro-3,5-xylenol (26.67%).

base line adjusted for stability and maximum peak recording. The plate was then scanned with a slit opening of 1 × 10 mm when peaks were obtained for each coloured spot (Fig. 1). After this initial scanning, the process was repeated at the corners of the bases of the peaks (points B to C, D to E and F to G in Fig. 1) to obtain integrated numbers for the peak areas. The relative percentage of each component was calculated from these numbers.

Results and discussion

The optimum quantity for spotting was determined by resolving and scanning various dilutions of mixed 4-chloro-3,5-xylenol and 2,4,6-trichloro-3,5-xylenol in acetic acid on TLC plates (Table I). Though reasonable accuracy was obtained on

TABLE I

SENSITIVITY OF THE METHOD

Mixture used: (a) 4-chloro-3,5-xylenol 27.97%; (b) 2,4,6-trichloro-3,5-xylenol 72.03%

Mixture (*μl*)	*Quantity spotted* (*μg*)	*Composition found* (%)	*Difference* (%)
25.32	253.2	(a) 44.19 (b) 55.81	16.22
12.66	126.6	(a) 36.11 (b) 63.89	8.14
8.44	84.4	(a) 29.63 (b) 70.37	1.66
5.06	50.6	(a) 28.57 (b) 71.43	0.6
3.61	36.1	(a) 29.02 (b) 70.98	1.05

84.4 μg of the mixture, *ca.* 50 μg was selected for further studies. With higher quantities spots were obtained of diameter greater than 1 cm which were not fully scanned with a slit opening of this diameter.

Xylenols and chloro-xylenols taken in varying proportions were estimated by this method using 20 × 2.1 cm plates with the results shown in Table II. It is evident that this technique can be employed for the quantitative estimation of mixtures of xylenols and their chloro-derivative with a degree of accuracy of *ca.* 3%.

Overlapping or partly resolved spots, given for example by xylenols and their 4-chloro-derivatives were recorded during scanning by the densitometer as single or as incompletely resolved peaks. In such cases, the integrated peak areas of the two components together were taken. Results obtained on microslides were as good as those with larger plates (Table III). Since the scanning of a microslide takes about 2 min and entire procedure about 10 min, the technique can well be used as a control method while chlorinating unknown mixtures of phenols.

TABLE II

ACTUAL AND DETERMINED PERCENTAGE COMPOSITION OF CHLORO-XYLENOL MIXTURES

Components	*Composition (%)*		
	Taken	*Found*	*Difference*
A. 2,3-Xylenol and its chloro-derivatives			
(1) 4-Chloro-2,3-xylenol	73.89	76.40	2.51
4,6-Dichloro-2,3-xylenol	26.11	23.60	
(2) 4-Chloro-2,3-xylenol	22.98	25.37	2.39
4,6-Dichloro-2,3-xylenol	77.02	74.63	
(3) 2,3-Xylenol	24.13	60.22	1.35
4-Chloro-2,3-xylenol	34.74		
4,6-Dichloro-2,3-xylenol	41.13	39.78	
(4) 2,3-Xylenol	28.45	59.83	1.08
4-Chloro-2,3-xylenol	30.30		
4,6-Dichloro-2,3-xylenol	41.25	40.17	
(5) 2,3-Xylenol	9.3	34.61	2.01
4-Chloro-2,3-xylenol	23.30		
4,6-Dichloro-2,3-xylenol	67.40	65.39	
(6) 2,3-Xylenol	10.56	21.21	0.24
4-Chloro-2,3-xylenol	10.41		
4,6-Dichloro-2,3-xylenol	79.03	78.79	
(7) 2,3-Xylenol	11.88	29.80	1.72
4-Chloro-2,3-xylenol	16.20		
4,6-Dichloro-2,3-xylenol	71.92	70.20	
B. 2,5-Xylenol and its chloro-derivatives			
(8) 4-Chloro-2,5-xylenol	23.95	23.26	0.69
4,6-Dichloro-2,5-xylenol	76.05	76.74	
(9) 4-Chloro-2,5-xylenol	59.55	57.58	1.97
4,6-Dichloro-2,5-xylenol	40.45	42.42	
(10) 4-Chloro-2,5-xylenol	5.74	8.00	2.26
4,6-Dichloro-2,5-xylenol	94.26	92.00	
(11) 2,5-Xylenol	28.81	50.43	1.27
4-Chloro-2,5-xylenol	22.89		
4,6-Dichloro-2,5-xylenol	48.30	49.57	
(12) 2,5-Xylenol	7.97	15.00	1.36
4-Chloro-2,5-xylenol	8.39		
4,6-Dichloro-2,5-xylenol	83.64	85.00	
C. 3,5-Xylenol and its chloro-derivatives			
(13) 2,4-Dichloro-3,5-xylenol	31.16	34.38	3.22
2,4,6-Trichloro-3,5-xylenol	68.84	65.62	
(14) 2,4-Dichloro-3,5-xylenol	23.87	25.00	1.13
2,4,6-Trichloro-3,5-xylenol	76.13	75.00	
(15) 2,4-Dichloro-3,5-xylenol	77.00	76.00	1.00
2,4,6-Trichloro-3,5-xylenol	23.00	24.00	
(16) 4-Chloro-3,5-xylenol	22.06	20.00	2.06
2,4-Dichloro-3,5-xylenol	53.19	53.33	0.14
2,4,6-Trichloro-3,5-xylenol	24.75	26.67	1.92
(17) 4-Chloro-3,5-xylenol	20.92	22.22	1.30
2,4-Dichloro-3,5-xylenol	20.08	22.22	2.14
2,4,6-Trichloro-3,5-xylenol	59.00	55.56	3.44
(18) 4-Chloro-3,5-xylenol	20.83	23.37	2.54
2,4-Dichloro-3,5-xylenol	42.62	42.85	0.23
2,4,6-Trichloro-3,5-xylenol	36.55	33.78	2.77

(*continued on p. 183*)

TABLE II (*continued*)

Components	*Composition (%)*		
	Taken	*Found*	*Difference*
(19) 3,5-Xylenol	24.47	48.87	0.42
4-Chloro-3,5-xylenol	24.82		
2,4-Dichloro-3,5-xylenol	32.07	34.09	2.02
2,4,6-Trichloro-3,5-xylenol	18.64	17.04	1.60
(20) 3,5-Xylenol	22.17	38.30	2.59
4-Chloro-3,5-xylenol	13.54		
2,4-Dichloro-3,5-xylenol	32.43	34.04	1.61
2,4,6-Trichloro-3,5-xylenol	31.86	27.66	4.2
(21) 3,5-Xylenol	15.85	16.66	0.81
4-Chloro-3,5-xylenol	12.62	12.50	0.12
2,4-Dichloro-3,5-xylenol	37.36	41.67	4.31
2,4,6-Trichloro-3,5-xylenol	34.17	29.17	5.00
D. 3,4-Xylenol and its chloro-derivatives			
(22) 6-Chloro-3,4-xylenol	43.16	41.93	1.23
2,6-Dichloro-3,4-xylenol	56.84	58.07	
(23) 6.-Chloro-3,4-xylenol	13.12	16.66	3.54
2,6-Dichloro-3,4-xylenol	86.88	83.34	
(24) 6-Chloro-3,4-xylenol	60.37	57.15	3.22
2,6-Dichloro-3,4-xylenol	39.63	42.85	
(25) 3,4-Xylenol	21.76	22.21	0.45
6-Chloro-3,4-xylenol	31.86	33.34	1.48
2,6-Dichloro-3,4-xylenol	46.38	44.45	1.93
(26) 3,4-Xylenol	15.80	18.37	2.57
6-Chloro-3,4-xylenol	10.28	12.24	1.96
2,6-Dichloro-3,4-xylenol	73.92	69.39	4.53
(27) 3,4-Xylenol	26.33	24.23	2.10
6-Chloro-3,4-xylenol	13.39	13.76	0.37
2,6-Dichloro-3,4-xylenol	60.28	62.01	1.73

TABLE III

COMPARATIVE QUANTITATIVE STUDY ON 20 × 2.1 CM AND 7.2 × 2.4 CM MICROSLIDE PLATES

Components	*Taken* (%)	*Found*		*Difference* (%)
		On 20 × 2.1 cm plates (%)	*On 7.2 × 2.4 cm plates* (%)	
4-Chloro-3,5-xylenol	27.97	29.63	29.41	−0.22
2,4,6-Trichloro-3,5-xylenol	72.03	70.37	70.59	+0.22
4-Chloro-3,5-xylenol	68.82	68.42	68.75	+0.33
2,4,6-Trichloro-3,5-xylenol	31.18	31.58	31.25	−0.33
4-Chloro-2,3-xylenol	Unknown mixture obtained during chlorination of 2,3-xylenol with one mole of SO_2Cl_2	61.53	61.30	−0.23
4,6-Dichloro-2,3-xylenol		38.47	38.70	+0.23

Acknowledgement

Our thanks are due to Dr. R. VAIDYESWARAN, SHRI D. P. AGRAWAL and SHRI B. GOPINATH for the helpful discussions and also to Dr. G. S. SIDHU, Director for his keen interest.

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Received October 4th, 1965

J. Chromatog., 22 (1966) 180–184

Ultraviolet-induced isomerization of β-D-glucosyl o-hydroxycinnamic acid on filter paper*

Coumarinic acid glucoside (β-D-glucosyl *cis-o*-hydroxycinnamic acid) and *o*-coumaric acid glucoside (β-D-glucosyl *trans-o*-hydroxycinnamic acid) are readily detected as absorbing areas on filter paper chromatograms exposed to ultraviolet light at wavelengths near 260 mμ. Long wavelength ultraviolet radiation is frequently used to detect fluorescent compounds closely related to these two glucosides. The foregoing facts prompted this investigation concerning the influence of both long and short wavelength ultraviolet light on small amounts of coumarinic acid glucoside and *o*-coumaric acid glucoside, air-dried on filter paper strips. Ultraviolet-induced interconversion of these two isomers in aqueous solutions is well known[1].

Procedure

The two glucosides were isolated from hot water extracts of sweetclover leaves by paper chromatography. The solvent consisted of 2% acetic acid[2]. In this system R_F values for coumarinic acid glucoside and *o*-coumaric acid glucoside are 0.90 and 0.66, respectively. The glucosides were detected on test strips cut from chromatographic sheets; this prevented exposure of the entire chromatograms to ultraviolet light. Bands representing the two glucosides were cut out and eluted with water; eluates were assayed[3] and then diluted with water to a final concentration of 1 μmole/ml.

* Cooperative investigations of the Crops Research Division, Agricultural Research Service, Department of Agriculture, and the Nebraska Agricultural Experiment Station. Supported in part by the National Science Foundation (Grant GB1148). Published with the approval of the Director as Paper 1805, Journal Series, Nebraska Agricultural Experiment Station.