Solvents for anthocyanidin chromatography

Two kinds of acidic solvents are used in anthocyanidin chromatography; the organic solvents (generally, butanolic or phenolic) and aqueous solvents (a mixture of water, mineral and organic acids). Although certain butanolic and phenolic solvents give a good "spread" of R_F values for certain anthocyanidins, the spread cannot be fully exploited in identification because the anthocyanidins often fade rapidly and small amounts of anthocyanidins are not detected.

Anthocyanidins are generally stable in aqueous acidic solvents, but there are few such solvents satisfactory for anthocyanidin identification. In Forestal solvent, it is very difficult to differentiate peonidin from pelargonidin, petunidin from cyanidin, and occasionally, cyanidin from malvidin. The formic acid solvent of HARBORNE[1] (a modification of the solvent originally proposed by ENDO, as quoted by ABE AND HAYASHI[2]), although useful, gives a poor spread between cyanidin and malvidin, and malvidin and peonidin. The acetic acid–hydrochloric acid–water (5:1:5) solvent of ABE AND HAYASHI[2] is not satisfactory with partially hydrolyzed anthocyanins because the R_F value ranges of anthocyanins and anthocyanidins overlap.

Because of the shortcomings of these solvents, new solvents were added. The composition of these and some standard solvents is listed in Table I. The range and average of R_F values of standard anthocyanidins in the solvents are listed in Table II. The first line in each column gives the range of R_F values obtained in several runs. The number of runs is given in brackets. One sample of peonidin in HARBORNE's formic acid solvent showed a pink fluorescent contaminant, probably an unknown anthocyanidin, whose R_F values are included.

The *iso*-PrOH solvent of ABE AND HAYASHI[2] is excellent for differentiating peonidin from pelargonidin. Anthocyanidins, in this solvent, fade quite rapidly. Also, it cannot be used as a general solvent for anthocyanidin chromatography because malvidin and petunidin and also cyanidin and peonidin resolve at about the same chromatographic loci.

TABLE I

COMPOSITION OF SOLVENTS USED FOR ANTHOCYANIDIN CHROMATOGRAPHY

Abbreviation	*Composition*[a]	*Development period (h)*
Forestal	HAc–HCl–H_2O (30:3:10), S	16
FA–HARBORNE	HCOOH–HCl–H_2O (5:2:3), S	7·5
FA–4 *N* HCl	HCOOH–4 *N* HCl (2:1), S	7·5
HAc–HCl[b]	HAc–HCl–H_2O (15:3:82), S	6
Propionic	PA[c]–HCOOH–HCl–H_2O (2:5:1:6), U	10
3:1:8	HAc–HCl–H_2O (3:1:8), S	8
5:1:5	HAc–HCl–H_2O (5:1:5), S	11
Iso-PrOH[d]	*i*-PrOH–5% HCl (55:45), S	20
Aq–HCl[b]	HCl–H_2O (3:97), S	4
BAW	*n*-BuOH–HAc–H_2O (4:1:5), U	13

[a] S = Single phase; U = upper phase.
[b] Anthocyanin solvent of HARBORNE[1] extended to anthocyanidins.
[c] PA = propionic acid.
[d] Isopropyl alcohol solvent of HAYASHI[3], ABE AND HAYASHI[2].

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TABLE II

RANGES AND AVERAGE OF R_F VALUES OF AUTHENTIC ANTHOCYANIDINS[a,b]

Solvent	$R_F \times 100$								
	Pelargonidin[c] *1 OH*	*Peonidin*[d] *1 OH, 1 OCH_3*	*Contaminant*[f]	*Malvidin*[d–g] *1 OH, 2 OCH_3*	*Cyanidin*[c] *2 OH*	*Petunidin*[d,g] *2 OH, 1 OCH_3*	*Delphinidin*[d] *3 OH*	*Apigenidin*[d] *1 OH*	*Luteolinidin*[d] *2 OH*[e]
Forestal	62–74 (18) 67	60–69 (13) 64		56–64 (11) 60	45–55 (14) 48	42–50 (12) 45	34–36 (4) 35	81–83 (3) 82	65–70 (4) 67
FA–Harborne	32–40 (17) 36	25–33 (12) 29	5	24–30 (11) 26	22–27 (15) 24	18–23 (11) 20	13–15 (13) 14	60–62 (2) 61	44 (2) 44
FA–4 *N* HCl	47–55 (10) 51	44–51 (10) 48	10	42–64 (10) 44	33–38 (12) 37	30–35 (12) 33	21	69	56
HAc–HCl	15–19 (15) 18	12–13 (5) 12		8–10 (10) 9	10–12 (13) 11.5	7–8.5 (10) 8	7	27–30 (3) 29	18–21 (3) 20
3:1:8	25–28 (12) 26	19–21 (7) 20	3 3	13–16 (11) 15	15–18 (15) 16	11–13 (10) 12	9 (3) 9	38	27

Propionic	45–50 (14) 46	39–43 (8) 40	8	33–38 (9) 35	28–32 (13) 30	24–29 (12) 26	20 (3) 20	65–67 (3) 65	51–53 (3) 52
Iso-PrOH	54–73 (13) 68	45–52 (6) 50		h 33	45–42 (12) 48	h 33			
5:1:5	49			38	38	31			
Aq–HCl	4.5–5 (2) 5	2.5–3 (20) 3		2–2.25 (3) 2	2.5–3 (5) 2.5	2–2.5 (3) 2	1.5[f]	7	4.25
BAW	80–82 (2) 64	61–68 (2) 64			52	60	46		

[a] In general, the R_F values of each anthocyanidin in each solvent are in three rows; in the first row, the range of R_F values; the number of runs in parentheses, in the second row; the average in the third row.

[b] The chromatography was carried out at 20 ± 1°.

[c] Provided by Dr. R. M. ACHESON, University of Oxford, England.

[d] Provided by Dr. J. B. HARBORNE, John Innes Horticultural Institution, Hertfordshire, England, (see g, h, and i).

[e] OH at 3-position absent

[f] R_F values of the fluorescent pink contaminant.

[g] The malvidin and petunidin as a mixture.

[h] Malvidin and petunidin do not separate in the *iso*-PrOH solvent.

[i] Delphinidin with a fluorescent pink contaminant at R_F 0.04 in aq. HCl.

The formic acid–4 *N* HCl (2:1) solvent developed in this laboratory is a good one for routine use and has a greater spread in R_F values than the formic acid solvent of HARBORNE[1].

The use of the HAc–HCl solvent originally proposed by HARBORNE[1] for anthocyanins was extended to anthocyanidins. In studying hydrolysis and stability of anthocyanins, anthocyanins and anthocyanidins are most usefully run in one solvent. The HAc–HCl solvent is excellent for this purpose and has been extensively used. R_F values of anthocyanins are generally above 0.20 and those of anthocyanidins below 0.20. The solvent gives sharp resolution and minimal variation in R_F values. The HAc–HCl solvent, like the *iso*-PrOH solvent, differentiates pelargonidin and peonidin.

R_F values of anthocyanidins in a given solvent are usually predictable on the basis of structure. Although the basic constituents of the HAc–HCl solvent are the same as those of Forestal solvent, the order of R_F values of malvidin and cyanidin, as found in Forestal solvent, is reversed in the HAc–HCl solvent. This is particularly useful in identifying malvidin.

The 3:1:8 solvent of ABE AND HAYASHI[2] usually gives good results. Although it is useful in characterizing anthocyanins, it is strongly acidic and may partially hydrolyze these compounds. The order of R_F values for malvidin and cyanidin in Forestal solvent is reversed in this solvent. R_F value ranges for anthocyanidins are much less in this solvent than in Forestal.

The new propionic acid solvent is an excellent solvent. There is a uniform spread of approximately 0.05 R_F value units between the common anthocyanidins and R_F values are conveniently in multiples of 5.

The 1% aqueous HCl solvent of HARBORNE[1] is very good for the purification and characterization of anthocyanins but R_F values for anthocyanidins are very low. Nonetheless, it is useful in studying the products of partial hydrolysis of anthocyanins. The colors of anthocyanins and anthocyanidins, both in visible and ultraviolet light, are very sharp in this solvent.

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