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Letter to the Editor

Analysis of anthocyanins in red wines by high-performance liquid chromatography using butylamines in the mobile phase

Sir,

We are studying the optimization of the separation of anthocyanins in red wine by high-performance liquid chromatography (HPLC) by using alkylamines in the mobile phase with gradient elution. In the present experiments we used a Varian 8500 chromatograph, a Variscan 634 D UV–VIS spectrophotometric detector, an A-25 recorder and a high-pressure stop-flow injection system. The column (15 cm × 0.32 cm I.D.) was packed with CGX C_{18} silica gel chemically modified with octadecyl groups (particle 5 μm) (Tessek, Prague, Czechoslovakia). The wavelength of detection was 520 nm; the pressure was 14 MPa[1,2].

The wine sample selected was Alibernet (Komplexný Výskumný Ústav Vinohradnický a Vinársky, Bratislava, Czechoslavakia) as it was found to exhibit the most uniform content of anthocyanins among those investigated, the other wines being Svätovavrinecké, Frankovka Modrá, Cabernet Sauvignon and André).

Butylamine, diethylamine, triethylamine, hexylamine and octylamine were examined as mobile phase additives and the best results were obtained with butylamine. We then optimized the gradient elution by means of solution A containing 10% (v/v) of methanol, perchloric acid (0.16 mol dm^{-3}) and butylamine (0.122 mol dm^{-3}) and solution B containing 90% (v/v) of methanol, perchloric acid (0.16 mol dm^{-3}) and butylamine (0.122 mol dm^{-3}). The pH of the solutions was 1.45. Similar pH conditions have been used by other workers[3–5].

The gradient was optimized in two steps involving the initial composition of the mobile phase and the form and slope of the gradient. As the optimization of gradient elution has not yet been mathematically processed, we tried to find out the most convenient gradient by experiment. The following gradient proved to be the best: start 25% B, finish 51% B, 0–8 min 25% B isocratic, 8–12 min from 25 to 41% B at 4% B min^{-1}, 12–22 min from 41 to 51% B at 1% B min^{-1} and 22–34 min 51% B isocratic.

The chromatographic separation of the anthocyanin pigments from Alibernet wine using the above gradient is shown in Fig. 1. The following peaks of anthocyanins were identified: (1) delfidin-3-glucoside, (2) cyanidin-3-glucoside, (3) petunidin-3-glucoside, (4) peonidin-3-glucoside, (5) malvidin-3-glucoside, (6) unidentified, (7) delfidin-3-glucoside acetate, (8) cyanidin-3-glucoside acetate, (9) petunidin-3-glucoside acetate, (10) peonidin-3-glucoside acetate, (11) malvidin-3-glucoside acetate, (12) delfidin-3-glucoside *p*-coumarate, (13) peonidin-3-glucoside *p*-coumarate and (14) malvidin-3-glucoside *p*-coumarate.

For comparison of gradient programmes with and without butylamine in the mobile phase, the time of analysis, separation efficiency and gradient reproducibility

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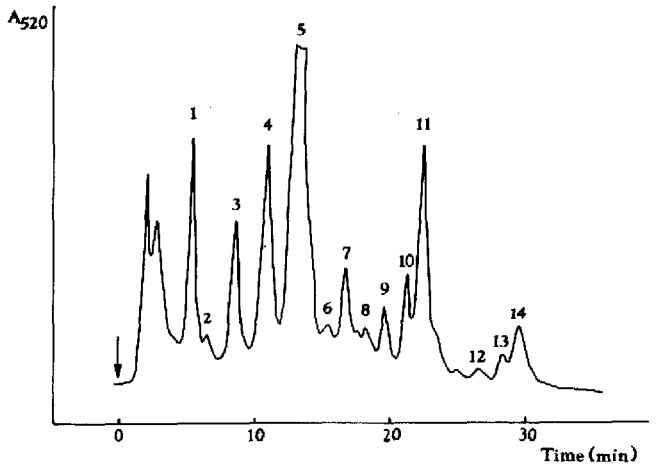

Fig. 1. Chromatogram for an Alibernet wine sample. Injection volume, 8 μl; sensitivity, 20 mV. For chromatographic conditions and peak identification, see text.

were considered. The gradient with butylamine requires 6 min less than that without butylamine. Also, the peaks are much more regularly located when butylamine is present. The gradients were compared with respect to selectivity (α), resolving power (R_S) and capacity factors (k'). In calculating the capacity factors given in Table I, the elution dead time $t_0 = 120$ s was taken into account. Table I demonstrates that better results and better resolution of peaks 4,5 and 10,11 in particular were obtained by using the gradient with butylamine. To evaluate the reproducibility of measurements

TABLE I

COMPARISON OF GRADIENTS FROM THE VIEWPOINT OF SELECTIVITY

Peak No.	*Gradient without butylamine*			*Gradient with butylamine*		
	k'	α	R_S	k'	α	R_S
1	3.37	1.33	16.33	1.71	1.43	17.29
2	4.48	1.20	11.26	2.45	1.39	17.41
3	5.37	1.22	12.78	3.40	1.35	17.05
4	6.57	1.10	6.37	4.58	1.24	13.18
5	7.22	1.41	21.35	5.67	1.19	11.06
6	—	—	—	6.73	1.10	6.61
7	10.18	1.14	9.14	7.42	1.11	6.83
8	11.61	1.08	5.26	8.20	1.08	5.55
9	12.49	1.10	6.79	8.88	1.09	5.97
10	13.73	1.02	1.82	9.67	1.05	3.68
11	14.07	1.05	3.58	10.18	1.14	9.26
12	14.77	1.05	3.57	11.63	1.06	4.35
13	15.50	1.04	2.60	12.35	1.07	5.09
14	16.05	1.05	3.57	13.25	1.05	3.52

[a] See text for identification of peaks.

we performed repeated analyses on nine different samples of Frankovka wine and calculated the mean capacity factor, standard deviation and scatter. The results showed that the reproducibility of the measurements made with the gradient in the presence of butylamine is much better and the system fulfils the high criteria demanded for the separation of anthocyanins by HPLC.

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