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The effect of water on the retention times of alcohols and esters

The commonly used techniques for isolating volatiles from fruits and other plant products—distillation, freeze-drying, and trapping head-space vapours—yield aqueous solutions containing only traces of volatiles. It is often desirable, particularly in preliminary studies, to analyze these dilute solutions without further concentration.

We attempted to obtain tentative identifications of the volatile compounds in a dilute aqueous distillate from apples by gas chromatography, utilizing an FFAP (Wilkins-Varian Aerograph) column and the water-insensitive hydrogen flame detector. When increasing volumes (2.5 to 10 μ l) of distillate were injected onto the column, there was a noticeable variation in the chromatograms, in that some peaks appeared or disappeared and some relative peak areas were altered. Since apple volatiles consist primarily of esters and alcohols¹, we have studied the effect of injecting increasing volumes of water on the retention times of several esters and alcohols.

Table I shows typical retention data for a mixture of esters. The retention times of esters eluted before the water front (*i.e.* esters up to butyl acetate) are clearly unaffected by the presence of variable quantities of water. In contrast, esters that are eluted after the water front have a shorter retention time when water is present, and for the higher homologs at least, the effect becomes greater as the proportion of water is increased. Similar results are obtained on FFAP at 60°, on SE-30, QF-1 and Carbowax 1540 at 100°, and on Carbowax 20M at 120°, though the effect is less pronounced with the Carbowax columns. Studies with a few aldehydes and 2-alkanones

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TABLE I

EFFECT OF WATER ON ESTER RETENTION TIMES

Column: 20 ft., 3½% FFAP on Chromosorb G (60–80 mesh). Temperature: 100°. Carrier gas flow rate: 20 ml/min of nitrogen. Hydrogen gas flow rate: 20 ml/min.

Retention times given are the means of two runs.

Each injection contains 0.005 µl of each compound.

	Retention time (min)				
	Volume of water injected (µl)	0	2.5	5	10
Ethyl acetate		3.0	3.0	3.0	3.0
Propyl acetate		4.0	3.9	3.9	4.0
Butyl acetate		5.6	5.5	5.6	5.6
Pentyl acetate		8.6	7.6	7.3	7.2
Hexyl acetate		14.0	12.8	12.5	11.6
Ethyl heptanoate		19.0	17.6	17.4	16.3
Octyl acetate		24.6	23.0	22.8	21.8
Water*	—		5.4–5.9	5.4–6.5	5.4–7.6

* Water appears only at high sensitivities as a square-top peak.

indicate that these compounds behave similarly to esters on the FFAP column. PERRY² recently reported that water decreases the retention times of *n*-alkanes on polar and apolar columns. In contrast, water does not affect the retention times of primary and secondary alcohols on FFAP (Table II) or on any of the other phases tested.

The marked difference that can occur in the gas chromatogram when a mixture of alcohols and esters is injected in the presence or the absence of water is demonstrated in Fig. 1.

The effect of water on the retention times does not persist. If 10 µl of water is injected and this is followed by injections of a water-free ester mixture just as the water "peak" appears, the ester retention times will not be affected.

TABLE II

EFFECT OF WATER ON ALCOHOL RETENTION TIMES

Conditions as per Table I.

	Retention time (min)		
	Volume of water injected (µl)	0	10
Ethanol		3.4	3.4
Propanol		4.6	4.6
1-Butanol		7.0	6.9
1-Pentanol		11.5	11.4
1-Hexanol		19.2	19.1
2-Propanol		3.0	3.2
2-Butanol		4.4	4.4
2-Pentanol		6.2	6.2
2-Hexanol		9.8	9.7
2-Heptanol		16.0	16.0

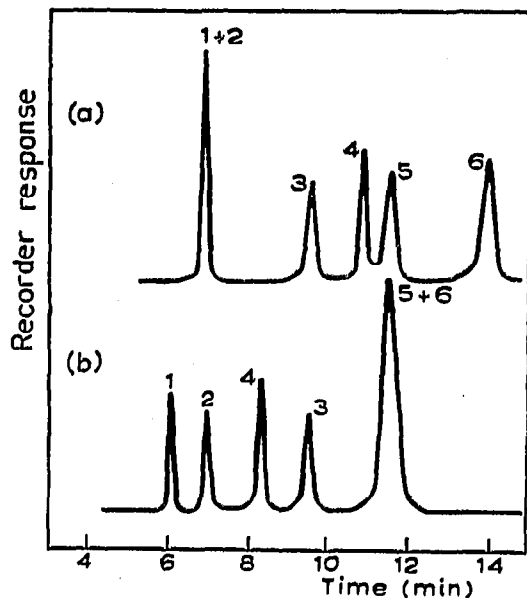


Fig. 1. Chromatograms of an ester-alcohol mixture in the presence and absence of water. Operating conditions as per Table I. (a) Sample only. (b) Sample + 10 μ l water. Sample composition: 1 = isoamyl acetate; 2 = 1-butanol; 3 = 3-methyl-1-butanol; 4 = butyl butyrate; 5 = 1-pentanol; 6 = hexyl acetate.

Discussion

Our results show that retention times for the esters eluted after water are reproducible only when the esters are injected in equal volumes of water. Alcohol retention times are not affected by up to 10 μ l of water. Once we recognized these facts, it was possible to interpret the chromatograms of the apple volatiles.

While the injection of a constant volume of solution is necessary to ensure comparable results, it is sometimes possible to achieve improved resolution of mixed alcohol-ester peaks by changing the volume of aqueous solution injected. For example, 1-butanol and isoamyl acetate normally elute simultaneously from FFAP as a single peak, but separate readily when injected along with 10 μ l of water (Fig. 1).

There is no obvious explanation for the decreased retention times. PERRY² suggests that water reduces the retention times of *n*-alkanes by a temporary modification of the stationary phase, thus decreasing the partition coefficients of these non-polar materials. If this is true, our results indicate that water also decreases the partition coefficients of the relatively polar esters, and of aldehydes and ketones, while having no effect on the partition coefficients of the highly polar alcohols.

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