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Letter to the Editor

Comments on enhanced peak response due to solvent interaction

Sir,

There has been a series of claims[1,2] and counter claims[3] recently concerning the change in liquid chromatographic (LC) peak response due to solvent interactions. This effect, if it in fact exists, has far ranging implications to quantitation in LC in general. We present here measurements in our laboratory on the same compounds. We thus show that the "anomalous" peak responses can be readily explained based on well-known behaviors in LC columns and LC detectors.

All eluents used are reagent grade material without further purification. A conventional chromatographic system was used. It consisted of a reciprocating pump (Milton Roy, Riviera Beach, FL, U.S.A., Model 196-0066), a 25 cm × 4.6 mm, 5-μm C_{18} column (Alltech, Deerfield, IL, U.S.A.), a 20-μl sample loop at a conventional injection value (Rheodyne, Berkeley, CA, U.S.A., Model 7010) and a commercial UV variable-wavelength detector (ISCO, Lincoln, NE, U.S.A., Model V[4]) without a reference cell. A flow-rate of 1.0 ml/min is used throughout. The output of the UV detector is connected to an IBM PC/AT computer with an analog interface (Data Translation, Marlboro, MA, U.S.A., Model DT2827 with ATLAB software). The computer takes readings every 0.1 s. Typically at least 100 of these data points define an analyte peak. The area is determined by locating and determining the peak start/-stop points manually, interpolating a baseline, and summing all intermediate points after baseline correction. The test compounds were captopril (Squibb, Rolling Meadows, IL, U.S.A.), nadolol and bendroflumethiazide (U.S. PC, Rockville, MD, U.S.A.). The eluents used were methanol–water–phosphoric acid (40:60:0.04) for captopril, methanol–0.1 *M* acetate buffer (pH 5) (35:65) for nadolol, and methanol–0.1 *M* acetate buffer (pH 5) (50:50) for bendroflumethiazide. The wavelengths used were 214 nm, 270 nm and 270 nm respectively. Care was taken to use freshly prepared solutions. The amounts injected were in the 1 μg range, which minimize column saturation effects. The detector was operated within its linear range, typically using the 0.05 a.u. full scale.

The results of our investigation are shown in Table I. Since the determinations of peak areas and peak heights are greatly influenced by the interpolated chromatographic baseline, the trials included in Table I are only those for which a stable baseline is achieved throughout the entire chromatogram, *i.e.* detector drift or baseline shifts are not present. The nadolol peak exhibits substantial tailing, and this resulted in poorer statistics in determining the peak areas. This is particularly true when ethanol is used as the solvent, when the signal does not return to the original baseline until after 2–3 peak widths. All of the peaks are well resolved from the refractive index disturbance at the void volume, and the injected quantities are small, so that artifacts of the detector are not expected to be important. Several conclusions can be drawn:

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(1) There is no statistically significant change in the areas of the peaks when the solvent is changed. This is in agreement with Berridge[3] but in variance with Perlman and Kirschbaum[1,2]. In fact it will be truly surprising if the areas did change. For retained peaks, one can infer that the *environment* of the species at the detector is constant regardless of its original solvent. Assuming there are no instrumental artifacts, Beer's law should hold and the integrated response (area) should match the total amount of material injected in the limit of low concentrations. In conventional spectrophotometry, we try to reproduce the final solvent (environment) when measuring unknowns and standards. The *history* of the sample, however, is never known to be a factor provided chemical reaction did not take place. The types of interactions suggested in ref. 2, *i.e.* intramolecular hydrogen bonding, are not permanent, and should be invariant once the species is put back into the environment of the chromatographic eluent. It is possible that for certain systems[4], the column itself promotes chemical changes, but then the effect cannot be classified as an enhanced peak response. In fact, if chemical changes lead to spectroscopic changes, it is highly likely that one can observe a second peak in the chromatogram, such as Fig. 7 in ref. 5.

(2) We have measured the molar absorptivities of captopril at 214 nm in 100% water, methanol–water (90:10), and ethanol–water (90:10), and found that the ratios are 1.0, 0.81, and 0.63 respectively. This indicates a substantial solvent effect is present, which can be explained by a hypothesis such as intramolecular hydrogen bonding. We believe that the results in ref. 2 (Fig. 1) can be explained in this way. The aztreonam peak is *not* retained, so that the environments of the measurements reflect those of the original solvents, and not the constant environment of the eluent. However, these variations in the areas are not related to the relative areas in the chromatographic runs in Table I, since the solvent there is of constant composition. Measurements of peak responses at the void volume are also subject to known interferences from the changing refractive index created by the solvent injected.

(3) There is no significant change in retention times of the solutes on changing solvents. We note that because of the asymmetric peak shapes, the peak maximum cannot be used directly to determine the retention time. For these compounds, errors of up to 5% can be introduced if the peak maximum rather than the peak centroid is used. The slight variations in retention times reported in ref. 3 are thus not conclusive.

TABLE I

DETECTOR RESPONSES FOR CAPTOPRIL, NADOLOL AND BENDROFLUMETHIAZIDE

Solute	*Solvent*	*No. of trials*	*Area*	*Height*
Captopril	Water	3	6410 ± 30	101 ± 0.5
	Methanol	8	6480 ± 50	62 ± 1.0
	Ethanol	5	6390 ± 20	43 ± 0.3
Nadolol	Water	5	6774 ± 117	108 ± 2.3
	Methanol	4	6696 ± 135	90 ± 2.8
	Ethanol	2	6398 ± 220	79 ± 2.0
Bendroflumethiazide	Methanol	5	3400 ± 30	51 ± 0.8
	Acetonitrile	5	3400 ± 30	54 ± 0.7
	Ethanol	4	3410 ± 40	40 ± 1.0

(4) Table I shows that there is a clear trend in the observed peak heights as a function of the initial solvents. As the eluting strength of the solvent increases, the peak height decreases because the peak becomes broader. This can be readily explained as being the result of dynamic gradient·elution caused by the injected solvent[6,7]. In going from water to methanol to ethanol in the injected plug, the elution strength increases and the solute is spread out more and more in the beginning section of the column. This is analogous to using larger and larger injection loops for the same injected amount, and band broadening eventually becomes important. The eluent quickly equilibrates once again and the whole (broadened) band moves down the column as before. This accounts for the invariance in the retention times for the solutes in different solvents. This is also why the peak heights are almost constant in ref. 3, where a much smaller sample loop is used. The effect of the solvent plug is then minimized.

In summary, we can conclude that there is no anomalous behavior in response for this group of compounds in LC, unless the detector is used in its non-linear range (Fig. 1 of ref. 3), chemical reaction or decomposition is present[5], or the eluent environment is not maintained when the solute elutes[2]. All of the unusual responses reported so far can be explained based on well-known behaviors in LC.

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