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Letter to the Editor

Comments on solute–solvent interactions in quantitative high-performance liquid chromatography

Sir,

We have recently become interested in the causes behind the apparent errors in quantification which have been noted with various solute–solvent combinations in high-performance liquid chromatography (HPLC)[1–5]. Inman *et al.*[1] have carefully examined this phenomenon and have identified the sample injection valve as the cause of this effect.

During the examination of the *in vitro* release characteristics of a novel anxiolytic agent in our laboratories, it was noted that there was an apparent high bias of 8–10% in the recovery of the drug from the aqueous dissolution media at levels of 6 μg/ml using standards prepared from 35% acetonitrile. This bias could be removed when standards were made up using 1% acetonitrile. Most interestingly, and in agreement with the findings of Inman *et al.*[1], the 1% acetonitrile standards gave a response which was larger than the standards prepared in 35% acetonitrile.

This problem seemed similar to those reported in the literature[1–5] and suggested that we might be experiencing the same or a related phenomenon. As sample injection valves are in wide usage, it was of general interest to further examine the problem.

Based upon our findings and the previous reports[1–5], a hypothesis was formed regarding the nature of this effect. It was thought that something in the injection valve could be acting as a site for the adsorption of the drug.

In most operations, the injection valve is overfilled several times[6] to ensure that the sample concentration in the valve is representative of the sample solution. If some component of the valve could adsorb material, then the mass of analyte in the valve would be larger than the product of the volume of the valve loop and the concentration of the sample solution. We hypothesized that the analyte may be pre-concentrated in the injection valve during the overfill process when low-solvent-strength preparations are analyzed.

The ability of the valve to adsorb a solute material would depend upon the relative free energies of adsorption and solution for the solute in the sample solvent. At low solvent strengths, the free energy of the adsorbed state might be favored over the solution state due to a lack of solubility. If the solvent strength of the sample preparation was increased, the free energies might again favor the solution state and the solute would not adsorb to the surface(s).

Confirmation of this hypothesis could explain some of the previously observed discrepancies. In the work of Perlman and Kirschbaum[2], each of the solutes exhibited the largest response in aqueous preparations as the model above suggests. Berridge[3]

did not find this phenomenon when using a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 1090 A HPLC system. Although it is not clear which injection system was used on this instrument, if the autosampler provided by the manufacturer was used, the sample is drawn by a syringe into a stainless-steel capillary of sufficient length that the sample solution does not contact the injection valve body. As there is no contact with the valve and no overfill, adsorption on potentially active sites, such as the rotor, would not be possible. (*Note:* The sample solution, bracketed by mobile phase, is passed through the injection valve at the time of injection. Pre-concentration of analyte on the rotor is not possible in this arrangement.)

If Berridge[3] used the Hewlett-Packard 1090 manual injection valve option (*i.e.*, overfilled loop and rotary injection valve), it is possible that he could not reproduce the observations of Perlman and Kirschbaum[2] due to batch-to-batch variation in the rotor material or due to the past history of this material. Chan and Yeung[4] were not able to reproduce the effect and pointed out several valid criticisms of the earlier work. Again, the past history or exact surface chemistry of the rotor may have been significantly different from that of Perlman and Kirschbaum's[2] system.

Inman *et al.*[1] also show findings consistent with this model in several areas. A relationship was found in which increases in acetate concentration could reduce the magnitude of the inaccuracy with vinblastine. This is consistent with displacement of the drug from an adsorbing surface by a competitive mechanism. The shape of the response curves for vancomycin hydrochloride and vinblastine are consistent with the model suggested above. The curves for each preparation of sample in the lowest concentration regions diverge from the origin and then become parallel. These regions may correspond to partial and complete saturation of the available adsorption sites. The variability between instruments suggests that there may be a difference in the valve surfaces both between and within manufacturers.

Several experiments were performed to test the hypothesis described above. The isocratic assay system in our examination used either a 250 × 4.6 mm I.D. Zorbax RX® column (Mac-Mod Analytical, Chadds Ford, PA, U.S.A.) which provided a capacity factor of approximately 7 with a minimum of 15 000 theoretical plates or a

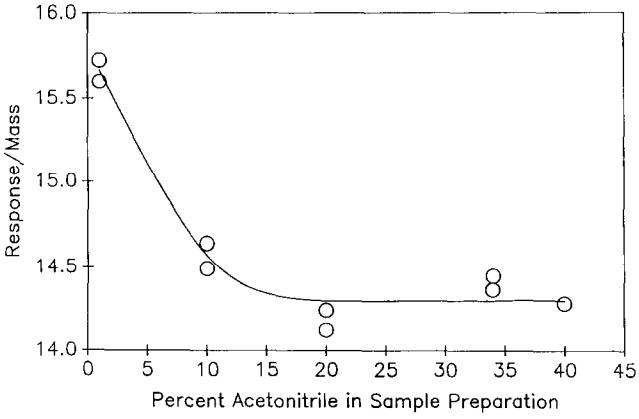

Fig. 1. Area response (arbitrary units) per mass of analyte injected as a function of percentage of acetonitrile in sample preparation. A Rheodyne injection valve was used with a 20-μl loop using a 200-μl overfill volume.

150 × 4.6 mm I.D. Zorbax C_8 column (Mac-Mod Analytical). The mobile phase was made of 35% aqueous acetonitrile and an amine modifier. The model compound in this work contains imine, amide and oxadiazole functionalities. It has a molecular weight of 335, is highly aromatic, is only slightly soluble in water and has no ionizable functions. The assay will be described in detail in a future report.

The instrumentation employed included a Perkin-Elmer (Norwalk, CT, U.S.A.) ISS-100 or a Varian Assoc. (Sunnyvale, CA, U.S.A.) 9090 autosampler each with a Rheodyne (Cotati, CA, U.S.A.) sample injection valve with a 20-μl loop and LDC UV monitor D (LDC/Milton Roy, Riviera Beach, FL, U.S.A.) or Waters-Millipore (Milford, MA, U.S.A.) 441 line source detectors at 254 or 308 nm. In one study, a Hewlett-Packard 1090 M with factory-supplied autosampler and diode array detector was used. Data were collected using a Hewlett-Packard 3392 integrator, transferred to a VAX computer (Digital Electronics, Maynard, MA, U.S.A.) and integrated using software developed in-house.

Five solutions of analyte at approximately 6 μg/ml were prepared in 1, 10, 20, 34 and 40% acetonitrile. These solutions were injected using a Rheodyne valve with a 20-μl loop using a 200-μl overfill. The resulting chromatographic response as a function of acetonitrile concentration is shown in Fig. 1. The samples prepared with the lowest eluent strength (*i.e.* 1 and 10%) had the largest area response per mass of analyte. At levels of 20% acetonitrile and above the response was essentially flat. Although peak-height data showed the same effect, some effect of sample preparation solvent composition upon peak height was noted in other work and reflected in the efficiency of the chromatography, so peak-height data are not reported further in these studies.

When these same samples were injected using a Hewlett-Packard 1090 M system with the syringe-based autosampler, each of the five samples provided essentially equivalent responses. Using the diode array detector on the Hewlett-Packard 1090, the spectra of the peaks eluted from these solutions were indistinguishable from each other as would be expected and as was suggested by Chan and Yeung[4]. This experiment supports the suggestion that there is something unique in the injection valve-based system and that solvent composition is an important parameter.

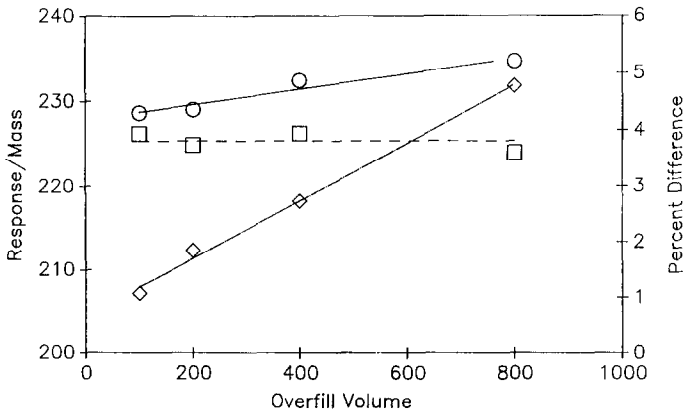

Fig. 2. Area response (arbitrary units) per mass of analyte as a function of overfill volume drawn through a 20-μl loop using samples prepared in 1% (○) and 34% (□) acetonitrile. The % difference curve (◇) shows the potential bias in the assay at each overfill volume.

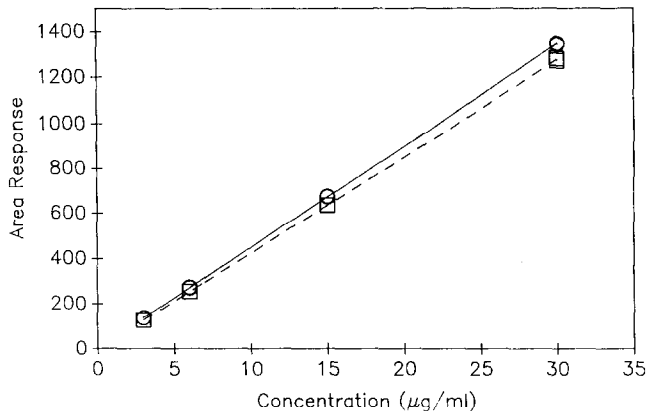

Fig. 3. Area response (arbitrary units) calibration curve for analyte in 1% (○) and 34% (□) acetonitrile sample preparations injected from a 20-μl loop with a 400-μl overfill. The slopes of these curves are statistically different.

A second experiment examined the impact of loop overfill on the proposed adsorption phenomenon. A Varian 9090 autosampler using a Rheodyne injector valve was programmed to utilize overfill volumes of 100, 200, 400 and 800 μl for the 20-μl loop. Samples at approximately 6 μg/ml were prepared in 1 and 34% acetonitrile and were analyzed with the results shown in Fig. 2. The response of these samples in 34% acetonitrile is virtually flat across the range of overfill volumes while the response of samples in 1% acetonitrile shows the tendency to increase with overfill volume. The percentage difference (or potential assay bias) between these responses is also plotted in Fig. 2. Again, these results support the hypothesis of analyte adsorption at low mobile phase strengths. No saturation of the surface(s) is observed at these concentration levels.

The effect of concentration was examined using samples at approximately 3, 6, 15 and 30 μg/ml in both 1 and 34% acetonitrile. These samples were injected using the Rheodyne valve with a 400-μl overfill volume. The response is plotted against concentration in Fig. 3 and demonstrates that there is a tendency for the 1% solution to produce a higher response than the corresponding 34% acetonitrile-based samples. Once again, no fixed amount of offset in response was observed. This suggests that the adsorption site(s) have not been saturated at these concentrations. Higher-concentration samples were not prepared due to concerns regarding the solubility of the compound in 1% acetonitrile.

These results confirm the hypothesis that the interactions and inaccuracies observed in this and other work[1–5] are due to adsorption of the analyte on surfaces of the injection system. The phenomenon only manifests itself when the eluting power of the sample solvent is below that needed to desorb the analyte from the adsorbing surface. We believe that the problem(s) noted are due to adsorption on the rotor surfaces because of the lack of effect in the Hewlett-Packard 1090 systems which have only stainless-steel contact surfaces. The variability of this phenomenon both within and between brands of autosamplers suggests that the rotor materials may be responsible.

In drug release testing and in biological or environmental analysis, the assay of aqueous samples using non-matrix matched standards is not uncommon. The poten-

tial bias could over-estimate the potency of a dosage, the drug level in a patient or the concentration of a contaminant. It is not unreasonable to expect that this phenomenon could occur in many systems, but may not be significant depending upon the difference between the potential of the injection system to adsorb the analyte and the analytical range of interest.

The compounds for which this effect have been observed seem to cover a wide range of physical and chemical properties[1–5]. The effect has been observed for large and small molecules, those with poor to excellent aqueous solubility, a wide variety of functionalities and a range of pK_a values. It is not clear that any *a priori* estimate of the tendency to adsorb may be made based on simple molecular characteristics.

It is also not unreasonable to expect that some components of mixtures may be pre-concentrated more than others resulting in biases in both relative and absolute levels. This would be expected to have the greatest impact in some assays for relative levels of impurities. The use of internal standards may also result in biases due to differential adsorption.

Fortunately, this phenomenon is relatively easily identified and corrected. By varying the amount of overfill in the injection system or by comparison of standards prepared at relevant solvent compositions, the potential for these problems can be readily found. Lack of apparent analyte adsorption in one system does not assure the lack of adsorption in all systems due to lot-to-lot variation in the rotor material as well as past history of each rotor. In our studies, the same samples resulted in a bias of 8–10% in one system while a bias of 3–5% was present in another even though both systems used the same brand of sample injection valve.

If solute adsorption on the valve is found to be significant, the analyst can avoid injector overfill provided only most assay precision is needed (*i.e.*, typically $\leqslant 0.5\%$ relative standard deviation for overfill *vs.* $\geqslant 1.0\%$ relative standard deviation for syringe-based non-overfill injectors). Matrix matching may not solve the problem if both samples and standards are prepared at low solvent strengths: the calibration curves may be non-linear if the adsorption saturation point is in the analytical range of interest. Standard addition techniques could fail for the same reasons. The problem might be reduced through the use of the new rotor materials which are commercially available. The best solution is probably to raise the solvent composition of the samples to increase eluting power during the overfill operation.

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