CHROM. 21 164

# INACCURACIES DUE TO SAMPLE–SOLVENT INTERACTIONS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

EUGENE L. INMAN*, ANN M. MALONEY and EUGENE C. RICKARD

*Eli Lilly and Company, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285 (U.S.A.)*

(First received June 3rd, 1988; revised manuscript received November 29th, 1988)

## SUMMARY

Inaccuracies in quantitation by high-performance liquid chromatography (HPLC) due to sample–solvent interactions are described for two compounds, vancomycin and vinblastine. Erroneously high peak areas were obtained when water was used as the sample solvent without matching the ionic strength of the comparator solutions to the ionic strength of the sample solutions. The presence and magnitude of these errors appear to be instrument related with the sample injection system identified as a key variable. These interactions demonstrate that special care is needed to match sample and standard solution matrices when HPLC is used for precise quantitative determinations.

## INTRODUCTION

A series of recent papers described the effects of sample solvent on high-performance liquid chromatography (HPLC) peak quantitation with UV detection and postulated explanations for the apparently anomalous observations[1–3]. The extent and very existence of these effects were called into question and the results were explained by well-known chromatographic behavior[4]. In this report, the authors present their observations of anomalous HPLC peak quantitation that are not readily explainable. While experimental efforts are continuing, extensive characterization of these effects demonstrate that: (1) subtle sample solvent effects can significantly influence peak quantitation; (2) these effects may be due to differences in solution ionic strengths; and (3) these effects can be traced to specific instrumental characteristics, namely the sample injection system.

Perlman and Kirschbaum[1] described quantitation differences associated with sample–solvent interactions in HPLC and concluded that only compounds which form intramolecular hydrogen bonds exhibit this effect. Their observations were made on a series of 18 pharmaceutical compounds dissolved in water, methanol or ethanol. These compounds were eluted with solvents of various polarity and detected by their UV response (214–270 nm). Their observations are similar to the classical qualitative changes observed in HPLC peak profiles when certain organic solvents are

used to aid sample solubility, the "injected-solvent effect"[5-7]. The original observations by Perlman and Kirschbaum could not be reproduced by Berridge who suggested that only peak shape was affected, possibly by an instrumental artifact[2]. In a second report by Perlman and Kirschbaum[3], an additional compound (aztreonam) prepared in either mobile phase or water is described. In this case, changes in peak areas were noted without any apparent corresponding change in peak shape. More recently, Kirschbaum[8,9] summarized the factors contributing to interlaboratory differences in quantitation including detector linearity, injection volume, and sensitivity of the analyte molar absorptivity to slight changes in environment. Finally, Chan and Yeung[4] suggested that the original observations could be readily explained by well-known behaviors in HPLC such as the injected-solvent effect and by changes in the analyte molar absorptivity with slight changes in solvent composition. However, independent observations made in the present authors' laboratories for two additional compound types clearly indicate that inaccuracies due to sample–solvent interactions occur for a number of analytes and that they are not explained by classical injected-solvent effects or by changes in the molar absorptivity of the analyte.

Refs. 2 and 4–7 focus on changes due to the organic composition of the sample solvent, *e.g.*, chromatographic peak profiles are altered by band broadening, the appearance of ghost peaks, or peak fronting. In contrast, the two examples described herein exhibit differences in peak quantitation (peak area) as a function of sample solvent composition without qualitative changes in the peak profiles. A similar behavior is observed in Fig. 1 of ref. 3. Experiments designed to characterize this phenomenon and our conclusions are described in this report.

In this work, all sample solvents were primarily aqueous and the solvent strengths were less than or equal to that of the initial mobile phase solvent. Thus, classical injected-solvent effects should not be present and were not observed. Quantitative changes were traced to differences in the ionic strength of the sample solvent and to instrumental factors. Our experiments identified conditions which can be used to estimate the "true" peak area. Our results suggest that sample–solvent interactions are more widespread than commonly believed and that inaccurate results due to this phenomenon often go undetected.

## EXPERIMENTAL

### Apparatus

*Vancomycin.* The separations for vancomycin were performed on a Varian 5560 gradient HPLC apparatus with an integrated UV-200 variable-wavelength detector (Varian, Walnut Creek, CA, U.S.A.), and a Varian 8085 autosampler with a Valco (Valco, Houston, TX, U.S.A.) sampling valve, or a Perkin-Elmer ISS-100 autosampler (Perkin-Elmer, Norwalk, CT, U.S.A.) with an attached Valco valve and temperature control capabilities. Data collection and reduction were performed on a central laboratory chromatography computer system with data storage, manipulation, and graphics capabilities. The separation was performed on a Beckman Ultrasphere™ ODS 25 cm × 4.6 mm I.D. stainless-steel column with 5-$\mu$m packing (Beckman, San Ramon, CA, U.S.A.). Sample loops of 20 $\mu$l nominal volume were used for all injections. Ultraviolet absorbance data were obtained on a Sargent-Welch Model 6-550 spectrophotometer (Sargent-Welch, Skokie, IL, U.S.A.).

*Vinblastine.* The assays for vinblastine were performed on two modular HPLC instruments. Components for the first instrument included a Waters M6000A pump (Waters Assoc., Milford, MA, U.S.A.), a Micromeritics autosampler (Micromeritics, Norcross, GA, U.S.A.), a Rheodyne 7126 sampling valve (Rheodyne, Cotati, CA, U.S.A.), and a Dupont variable-wavelength UV detector (Dupont, Wilmington, DE, U.S.A.). The second system consisted of a Varian 5000 pumping system, a Varian 8000 autosampler, a Valco valve, and a Dupont variable-wavelength UV detector. A Rheodyne 7125 manual valve was also used for one set of experiments. Data collection and reduction were performed on the same centralized laboratory chromatography computer data system. The separation was performed on an IBM $C_{18}$ 15 cm $\times$ 4.6 mm I.D. stainless steel column with 5-$\mu$m packing (IBM, Danbury, CT, U.S.A.). Sample loops of 20 $\mu$l nominal volume were used. Ultraviolet absorbance data were obtained on a Sargent-Welch Model 6-550 spectrophotometer.

## Reagents

HPLC-grade acetonitrile and methanol were purchased from Mallinckrodt (Mallinckrodt, Paris, KY, U.S.A.). Purified water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, U.S.A.). All other reagents were of analytical reagent grade. All vancomycin and vinblastine samples were prepared at Eli Lilly and Company. The solution of 0.1 $M$ phosphate buffer was prepared as a solution of sodium dihydrogen phosphate with the pH adjusted to 3.0 with phosphoric acid.

## Methods

*Vancomycin.* The mobile phases were prepared from a stock buffer solution and acetonitrile. The buffer solution was prepared by adding 4 ml triethylamine to 2 l water and adjusting the pH to 3.2 with phosphoric acid. Mobile phase A was made by adding 50 ml acetonitrile to 950 ml buffer solution. Mobile phase B was prepared by adding 400 ml acetonitrile to 600 ml buffer solution. Each mobile phase was thoroughly mixed, degassed, and covered to reduce evaporation.

The gradient program was performed as a linear ramp from 5 to 65% mobile phase B over 40 min, corresponding to a 1.5% mobile phase B change per minute. The final conditions were held for five minutes and then ramped back to initial conditions over the next minute. Equilibration was reestablished within 60 min from injection. A constant flow-rate of 0.9 ml/min was used. A detector wavelength of 254 nm was used with a setting of 0.05 a.u.f.s.

*Vinblastine.* The aqueous portion of the mobile phase was prepared by adding 14 ml diethylamine to 1 l of purified water and adjusting the pH to 7.5 with phosphoric acid. The mobile phase was a mixture of 62% of methanol–acetonitrile (4:1, v/v) with the buffer solution. It was mixed thoroughly, filtered, and degassed prior to use. The separation was performed in an isocratic mode. The flow-rate was 1.5 ml/min and the detector wavelength was 262 nm at 0.5 a.u.f.s.

## RESULTS AND DISCUSSION

## Vancomycin

The effect of sample solvent on peak quantitation for vancomycin was observed during routine HPLC characterization on the Varian HPLC with the Varian auto-

TABLE I

VANCOMYCIN HYDROCHLORIDE AND BASE SAMPLE CHARACTERIZATION PROFILES

|  | Hydrochloride | Base |
| --- | --- | --- |
| *Sample composition (%)* | | |
| Water content | 2.0 | 6.8 |
| HCl content | 2.5 | – |
| Vancomycin base content | 89.2 | 87.3 |
| Related substances | 6.3 | 5.9 |
| *Sample solutions* | | |
| Sample weight (mg) | 126.2 | 126.0 |
| Stock solution concentration as: | | |
| mg sample/ml | 2.524 | 2.520 |
| mg vancomycin base/ml | 2.251 | 2.200 |
| Diluted solution concentration as: | | |
| mg sample/ml | 0.1010 | 0.1008 |
| mg vancomycin base/ml | 0.0901 | 0.0880 |
| *HPLC peak areas* | | |
| Main peak area (arbitrary units) | 47 208 | 36 196 |
| Normalized peak area (per mg/ml base) | $5.24 \cdot 10^5$ | $4.11 \cdot 10^5$ |

sampler. Peak area differences were obtained for samples of vancomycin base and vancomycin hydrochloride at equal sample concentrations. The samples characterized in Table I illustrate typical peak area differences. The sample of vancomycin hydrochloride at a concentration of 0.1010 mg/ml (0.0900 mg vancomycin base/ml) produced a peak area count of about 47 200 (arbitrary units), whereas a sample of vancomycin base of similar relative purity at a concentration of 0.1008 mg/ml (0.0880 mg vancomycin base/ml) yielded a peak area count of about 36 200. Note that these sample concentrations are converted to vancomycin base content (*i.e.*, corrected for water, hydrochloride salt and related substance content) so that the normalized peak area responses should be equivalent whereas about a 30% difference is observed. In contrast, chromatograms of 2.5 mg/ml-solutions appeared nearly identical with no apparent differences in peak areas between the hydrochloride and base samples. These observations were repeatable within and across days. Sample preparation and instrumental parameters were studied to determine their roles in this anomalous behavior.

Sample solvent differences were investigated first. Routinely, a sample stock solution of 2.5 mg/ml vancomycin hydrochloride was prepared by dissolution of the salt in water followed by a dilution to 0.1 mg/ml with water. The pH of these solutions are about 3.5 and 4, respectively. Vancomycin base, however, has limited solubility above a pH of 4.5. To prepare the stock solution of 2.5 mg/ml of the base, concentrated phosphoric acid was added dropwise until complete dissolution of the sample was achieved. Subsequent dilutions to 0.1 mg/ml were made with water.

The effect of sample solvent was investigated further by chromatographic and spectroscopic evaluation of vancomycin solutions in numerous solvents. Stock solutions of vancomycin hydrochloride and base were prepared as described previously (data in Table I). Five different sample solvents were used for further dilution from

TABLE II

VANCOMYCIN HYDROCHLORIDE PEAK AREA AND ABSORBANCE AS A FUNCTION OF SAMPLE SOLVENT

See Table I for the sample composition.

| Sample solvent | Main peak area (relative units) | Absorbance (a.u., 254 nm) | Absorbance (a.u., 280 nm) |
|---|---|---|---|
| Water | 47 208 | 0.322 | 0.447 |
| 0.001 *M* HCl | 35 678 | 0.323 | 0.448 |
| 0.1 *M* Phosphate buffer | 35 191 | 0.324 | 0.450 |
| Mobile phase A | 35 602 | 0.317 | 0.447 |
| Mobile phase B | 4678 | 0.322 | 0.448 |

the stock solutions. The UV absorbances of these solutions were measured at 254 nm (wavelength used for HPLC detection) and at 280 nm (absorption maximum for vancomycin). Data from the solutions prepared from the vancomycin hydrochloride sample and from the solutions prepared from the vancomycin base sample are given in Tables II and III, respectively. Previous work indicates that vancomycin-related impurities have similar spectral characteristics and will contribute proportionately to the UV absorbance measurements[10]. That is, the UV absorbance will be proportional to the sum of the vancomycin base content and the related substance content whereas the HPLC peak area will include only the vancomycin base contribution.

The vancomycin hydrochloride solutions yielded equivalent absorbances for all five solvents. The five vancomycin base solutions also yielded equivalent absorbances. The vancomycin HPLC peak areas for these solutions are included in Tables II and III. The hydrochloride sample peak areas are approximately equivalent for the solutions with sample solvents of hydrochloric acid, phosphate buffer, and mobile phase A. However, the peak area for the water solution is about 33% greater, equivalent to the differences previously observed. Fig. 1 shows that no qualitative differences are observed for these chromatograms. In contrast, the peak area is reduced for the solution in mobile phase B since an injected-solvent effect is produced by a sample solvent which is stronger than the initial mobile phase conditions. This qualitative

TABLE III

VANCOMYCIN BASE PEAK AREA AND ABSORBANCE AS A FUNCTION OF SAMPLE SOLVENT

See Table I for the sample composition.

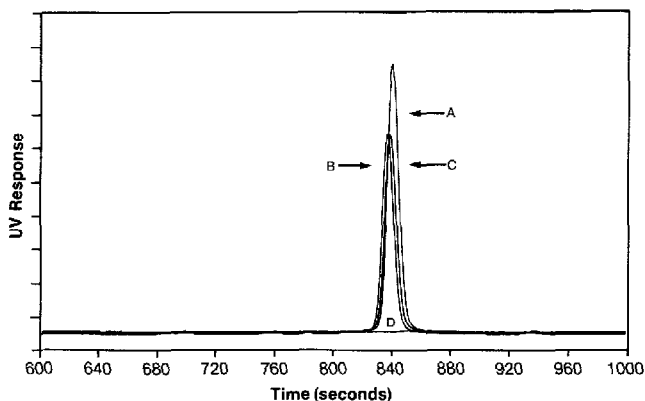| Sample solvent | Main peak area (relative units) | Absorbance (a.u., 254 nm) | Absorbance (a.u., 280 nm) |
|---|---|---|---|
| Water | 36 196 | 0.311 | 0.434 |
| 0.001 *M* HCl | 34 736 | 0.313 | 0.437 |
| 0.1 *M* Phosphate buffer | 34 920 | 0.314 | 0.438 |
| Mobile phase A | 34 812 | 0.306 | 0.432 |
| Mobile phase B | 4022 | 0.308 | 0.432 |

Fig. 1. Chromatograms from 0.1 mg/ml vancomycin hydrochloride diluted with (A) water, (B) 0.001 $M$ hydrochloric acid and (C) mobile phase A. D was obtained from a water blank.

difference is shown in the chromatogram in Fig. 2. Note that no baseline artifacts appear in the retention window for vancomycin when a water blank was injected.

The vancomycin base sample peak areas were also approximately equivalent for the solutions with sample solvents of hydrochloric acid, phosphate buffer and mobile phase A. The peak area for the water solution was greater than the others, similar to the hydrochloride samples except that the increase was now only about 4%. The peak area for the sample in mobile phase B is again reduced by an injected-solvent effect. Again, the chromatogram from the water blank shows no baseline artifacts in the vancomycin retention window. Thus, the qualitative differences for these chromatograms (Fig. 3) mirror those of the hydrochloride sample. However, there are no differences in molar absorptivity which could explain these HPLC peak area differences for either the hydrochloride or base samples.
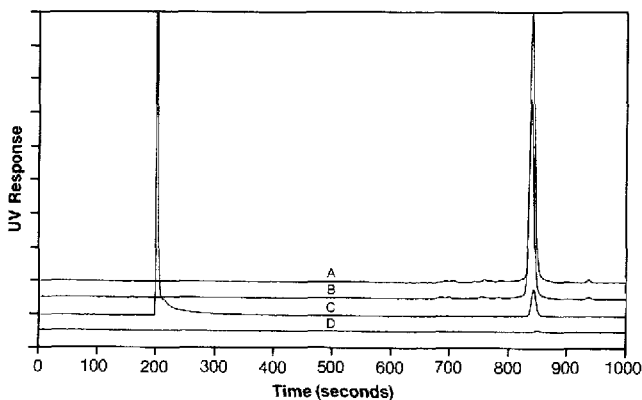


Fig. 2. Chromatograms from 0.1 mg/ml vancomycin hydrochloride diluted with (A) water, (B) 0.001 $M$ hydrochloric acid and (C) mobile phase B which demonstrates the qualitative and quantitative differences observed for these sample solvents. D was obtained from a water blank.
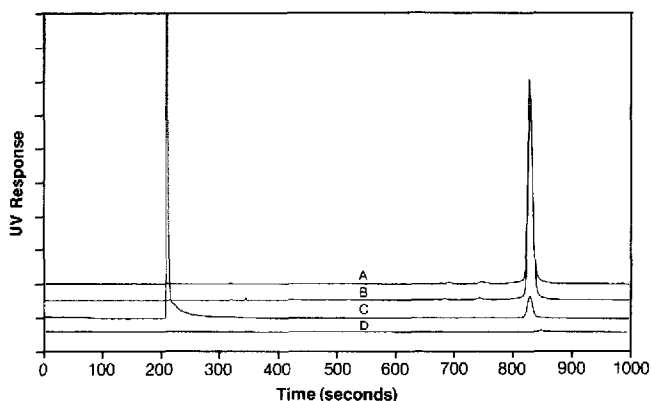
Fig. 3. Chromatograms from 0.1 mg/ml vancomycin base diluted with (A) water, (B) 0.001 *M* hydrochloric acid and (C) mobile phase B which demonstrate the qualitative and quantitative differences observed for these sample solvents. D was obtained from a water blank.

Data from either base or hydrochloride samples in the three sample solvents (0.001 *M* HCl, 0.1 *M* phosphate buffer of pH 3.0, and mobile phase A) indicate that peak areas are consistent when a minimum ionic strength is maintained for the sample solvent. In the absence of added buffer, the HPLC peak area increased without a corresponding increase in molar absorptivity. The smaller increase in peak area for the base sample in water is probably due to the increased ionic strength from the phosphoric acid required for dissolution of the original sample.

If similar peak areas were observed for the stock solutions while differences were observed for dilute solutions, the calibration curves for water and buffered sample diluents must diverge. Calibration curves for water and mobile phase A sample diluents were generated and are plotted in Figs. 4 and 5 (expanded scale). The deviation from zero of the *y*-intercept for the aqueous standards suggests that the areas for the water solutions were erroneously high and those for the mobile phase A
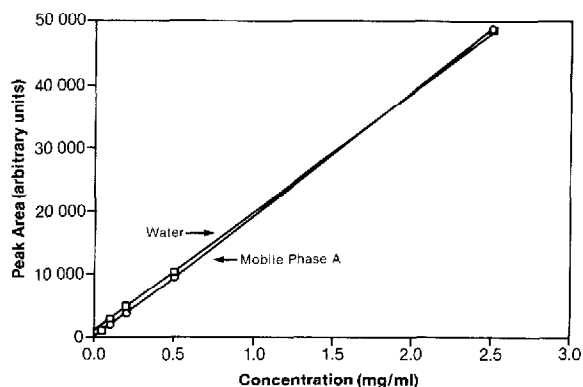


Fig. 4. Calibration curves from vancomycin hydrochloride diluted with water and mobile phase A.
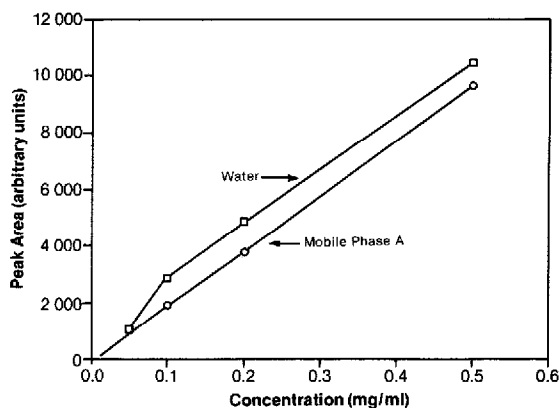
Fig. 5. An expanded view of the calibration curves from Fig. 4 which demonstrate the significant differences in *y*-intercepts.

solutions provide greater accuracy. Note that non-linearity is observed in the curve obtained from the water solutions below 0.1 mg/ml. The overlap of the two curves at 0.05 mg/ml supports the conclusion that the areas observed from water solutions are high.

Erroneously high peak areas are difficult to explain. While low peak areas are often attributable to precipitation or adsorption, high peak areas can occur for only a few reasons, *e.g.* by changes in the molar absorptivity of the analyte, a cause which has been eliminated in this example. To further characterize these observations obtained on the Varian autosampler, a second autosampler was evaluated on the same chromatographic system. The Perkin-Elmer ISS-100 autosampler yielded equivalent peak areas for hydrochloride samples dissolved in water or mobile phase A at both 5°C and ambient temperatures. These areas were approximately equivalent to the areas obtained from the Varian autosampler using mobile phase A as the sample diluent. It was noted that separate injection valves were used. Thus, the observed effect appears to be autosampler specific.

*Vinblastine*
        The effect of sample solvent on HPLC peak quantitation for vinblastine was observed during an interlaboratory collaborative study. The collaborative study was undertaken prior to transferring assay methodology for vinblastine raw material and formulations. During the course of the study in which multiple days, instruments, and analysts were evaluated, low results (lower than expected peak areas) were sometimes obtained for the vinblastine ready-to-use formulation. The formulation contains 1 mg/ml vinblastine in the excipient matrix; samples were diluted to 0.4 mg/ml with water prior to performing the assay. The vinblastine ready-to-use matrix is 100 mg/ml mannitol, 1.3 mg/ml methylparaben, 0.2 mg/ml propylparaben, 1.5 mg/ml sodium acetate (anhydrous), and 4.7 ml/l glacial acetic acid adjusted to pH 5. The chromatographic behavior (peak shapes and retention times) was unchanged except for reduced peak areas (Fig. 6). Note that baseline artifacts were not apparent in a water blank. The low results, on the order of 10% below theory, correlated with an unac-
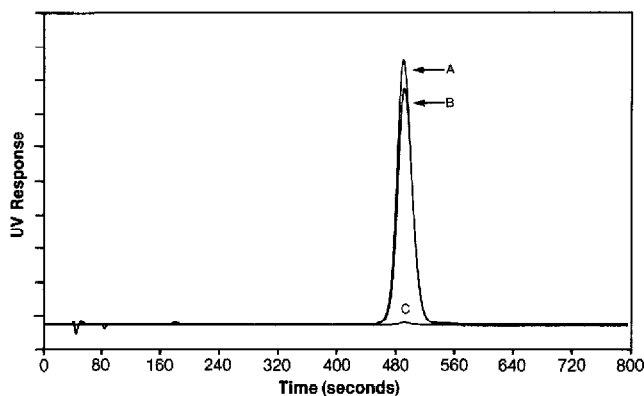
Fig. 6. Chromatograms from vinblastine samples diluted with (A) water and (B) formulation matrix. C was obtained from a water blank.

ceptably high normalized *y*-intercept-to-slope ratio for the respective standard curve. Our laboratory uses the normalized intercept-to-slope ratio as one of the parameters to evaluate standard curves. The normalized intercept-to-slope ratio (ISR) is defined as

$$\text{ISR} = (b/m) \cdot [2/(x_{\max} + x_{\min})]$$

where $b$ is the *y*-intercept, $m$ is the slope of the linear regression line fitted to the data, and $x_{\max}$ and $x_{\min}$ are the purity-corrected standard weights for the highest and lowest points on the standard curve. The ratio is deemed acceptable if it is less than 0.1, that is, the *y*-intercept is no more than 10% of the response of the midpoint of the curve.

The high intercept-to-slope ratios were determined to be instrument dependent. This led to the classification of instruments as acceptable and unacceptable for use in this assay. Arbitrarily, instruments yielding intercept-to-slope ratios of 0.1 or greater were designated as unacceptable. However, no changes in peak shapes or retention times were observed between instruments. Fig. 7 includes two standard curves obtained from an acceptable instrument and two curves obtained from an unacceptable instrument. The aqueous curve (standards prepared in water) and the matrix curve (standards prepared in the ready-to-use matrix) from the acceptable instrument both have near-zero intercepts. In contrast, the aqueous standard curve obtained on an unacceptable instrument has a very high intercept and is approximately parallel to, but offset from, its corresponding matrix curve. Using the observed response for a sample in the ready-to-use matrix, the error in the sample concentration that results from calculations *versus* the aqueous standard curve (true *vs.* apparent concentration) is approximately 14% for the unacceptable instrument.

The response on the unacceptable instrument was explored in greater detail by extending the range of the calibration curve. As expected, the curves begin to converge at low concentrations ($< 0.1$ mg/ml). Thus, the standard curves generated in the sample matrix are correct whereas those generated for standards diluted in water are
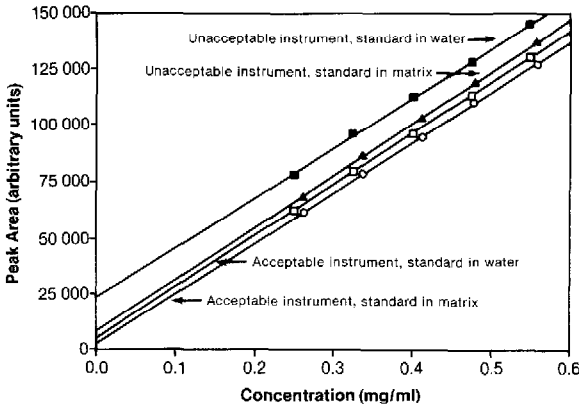
Fig. 7. Calibration curves from vinblastine standards prepared in water and in formulation matrix. Results are shown for an instrument which yields acceptable results and for an instrument which yields unacceptable results.

erroneously high. In addition to the matrix effect, there is a definite instrumental contribution to the error for aqueous vinblastine standard solutions.

Correction of the assay bias was a relatively simple matter. Calculations of the ready-to-use vinblastine formulation samples using the standard curve generated in the ready-to-use matrix eliminated the negative bias in the results. However, the underlying cause of the bias was still unknown. That is, what could produce an offset of the standard curve for the aqueous solutions?

Method conditions and the general state of the instruments involved were studied in order to eliminate sources of bias. Detector wavelength settings, sample carryover, lack of response linearity and differences in mobile phase makeup or columns were eliminated as factors contributing to bias. Absorbances of vinblastine in water, mobile phase, and acetate buffer were checked; the complete ready-to-use matrix cannot be used due to the UV absorbance of methylparaben and propylparaben. The
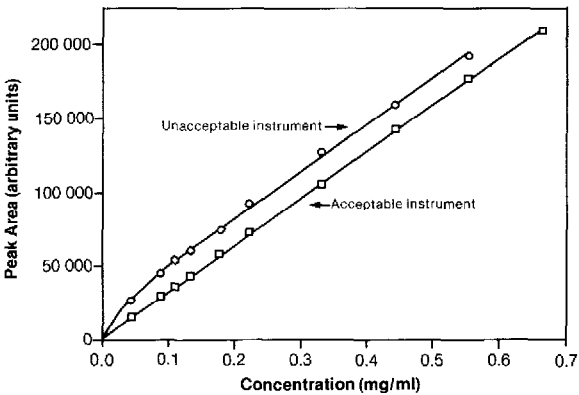


Fig. 8. Calibration curves from vinblastine standards prepared in water and run on an acceptable instrument and an unacceptable instrument.

absorptivities for vinblastine in water, pH 4 sodium acetate solution or HPLC mobile phase were equivalent whereas the absorptivity in the aqueous pH 7.5 diethylamine phosphate buffer was about 2% lower than any of the above solutions, probably because this solution is on the base side of the vinblastine $pK_a$. These minor absorptivity differences are thought to be masked in the HPLC method since all samples are eluted in mobile phase regardless of the initial sample solvent. Therefore, differences in UV absorptivity can be eliminated as the cause of the observed effect. Finally, fluorescence spectra of the mobile phase with and without vinblastine eliminated fluorescence as a factor contributing to the observed phenomena. Thus, further experiments focused on the matrix composition and instrument configuration.

The effect of mannitol concentration on vinblastine recovery was investigated on one acceptable instrument and one unacceptable instrument (Table IV). Vinblastine spiked with mannitol, ranging from 10 to 300 mg/ml, had comparable peak areas on both instruments. Thus, mannitol was eliminated as a source of the bias even though mannitol is the principal excipient and these solutions cover a wide range of viscosity.

TABLE IV

EFFECT OF MANNITOL CONCENTRATION ON VINBLASTINE RECOVERY

| Mannitol concentration (mg/ml) | Vinblastine recovery (%) | |
|---|---|---|
| | Acceptable instrument | Unacceptable instrument |
| 10 | 101 | 101 |
| 20 | 100 | 101 |
| 40 | 100 | 100 |
| 80 | 100 | 99 |
| 160 | 99 | 98 |
| 300 | 99 | 97 |

To determine the effect of the acetate, vinblastine samples at the normal acetate level were assayed *versus* aqueous standards. The acceptable instrument indicated 98% vinblastine recovery, while recovery on the unacceptable instrument was only 90%. Thus, acetate is a critical component in the matrix. However, the instrument dependence of this effect was still unknown.

The contribution of the various instrument components was investigated by sequentially interchanging the components of the acceptable instrument and the unacceptable instrument. The intercept-to-slope ratio of standard curves was used as the diagnostic parameter to detect changes in system performance. Table V is a compilation of the normalized intercept-to-slope ratios obtained for aqueous and matrix standards in this series of experiments. Changes in detectors produced no effect. However, the high intercept-to-slope ratio follows the sample injection system of the Varian instrument. Furthermore, when a Rheodyne 7125 manual injection valve was substituted, a normal intercept-to-slope ratio was obtained whereas manual injection using the Valco valve of that instrument produced a high intercept-to-slope ratio.

TABLE V

INTERCEPT-TO-SLOPE RATIOS FOR VINBLASTINE CALIBRATION CURVES AS A FUNCTION OF IN-
STRUMENT CONFIGURATION AND SAMPLE SOLVENT[a]

| Instrument configuration | | | | Sample solvent | |
|---|---|---|---|---|---|
| Pump | Autosampler | Injection valve | Detector | Aqueous | Matrix |
| Varian 5000 | Varian 8000 | Valco | Dupont (1) | **+0.18** | +0.05 |
| Varian 5000 | Varian 8000 | Valco | Dupont (2) | **+0.18** | +0.05 |
| Varian 5000 | – | Rheodyne 7125[b] | Dupont (2) | +0.00 | +0.00 |
| Waters M6000A | Micromeritics | Rheodyne 7126 | Dupont (2) | +0.04 | +0.02 |
| Waters M6000A | Micromeritics | Rheodyne 7126 | Dupont (1) | +0.01 | +0.03 |
| Waters M6000A | – | Rheodyne 7125[b] | Dupont (1) | −0.02 | −0.00 |
| Varian 5000 | – | Rheodyne 7125[b] | Dupont (1) | +0.02 | +0.02 |
| Waters M6000A | – | Rheodyne 7125[b] | Dupont (2) | −0.01 | +0.00 |
| Varian 5000 | Varian 8000 | Valco | Dupont (1) | **+0.21** | +0.06 |
| Waters M6000A | Micromeritics | Rheodyne 7126 | Dupont (2) | +0.02 | +0.07 |
| Waters M6000A | Varian 8000 | Valco | Dupont (2) | **+0.22** | – |
| Varian 5000 | Varian 8000 | Valco | Dupont (1) | **+0.28** | – |
| Varian 5000 | – | Valco[c] | Dupont (1) | **+0.16** | – |

[a] Experiments reported in approximate chronological order for data obtained on two days. The original config-
urations were repeated at intervals to insure that the original behavior was still present. **Bold** entries denote standard
curves with high intercept-to-slope ratios; +0 and −0 denote values between +0.005 and 0 or between −0.005 and 0,
respectively.
[b] The Rheodyne 7125 is a manual injection valve.
[c] Denotes valve used in a manual mode.

Thus, the injection valve from this unacceptable instrument appears to be the primary
source of high intercept-to-slope ratios.

The final experiment was designed to confirm the interaction between acetate
level and instrument configuration. Vinblastine solutions were injected at various
acetate levels using the Valco valve in an automatic and then in a manual mode on the
acceptable instrument. The relative peak areas are summarized in Table VI. The
decreased response of vinblastine as the acetate levels increased correlates with the
use of this particular valve in both modes.

It should be noted that although the detailed experiments designed to isolate

TABLE VI

EFFECT OF ACETATE CONCENTRATION ON VINBLASTINE PEAK AREAS[a]

| Solution | Relative peak area (%) | |
|---|---|---|
| | Automatic injection | Manual injection |
| No acetate | 100.0 | 100.0 |
| 1% Formulation acetate | 97.6 | 97.4 |
| 10% Formulation acetate | 92.7 | 95.2 |
| 100% Formulation acetate | 90.5 | 92.7 |

[a] Instrument is Varian 5000 pump, Dupont detector (1) and Valco valve operated in an automatic
mode (Varian 8000 autosampler) or manual mode.

the cause of the high intercept-to-slope problem focused on permutations of compo-
nents from two specific instruments, the effect has been observed with other instru-
ments. However, there is no commonality in the configuration of the unacceptable
instruments. Thus, no one component of the sample injection system can be assigned
as the cause of this effect.

CONCLUSIONS

HPLC quantitation of the two compounds vancomycin and vinblastine appears
to be highly dependent upon the sample solvent composition with some instruments.
A constant true value for the peak area from vancomycin depends on the presence of
a minimum ionic strength of the sample solvent, while the molar absorptivity was
unchanged for all solvents studied. It was concluded that stock sample solutions
diluted with water resulted in peak areas that were erroneously high. The presence
and magnitude of the error appear to be instrument dependent. Errors in quantita-
tion of vinblastine were detected by unacceptably high values for the normalized
intercept-to-slope ratios of the calibration curves. Again, standards dissolved in water
produced erroneously high peak areas on some instruments, correctable by the addi-
tion of acetate to the standard solvent. The instrument dependence of this phenom-
enon was systematically evaluated and a correlation to the sample injection system
was found.

The observed sample–solvent interactions for these two compounds appear to
be quite similar in their characteristics and their cause. However, the errors in quanti-
tation produced by these effects were not discovered until considerable experimental
evaluation was performed. Full explanations for the described observations have not
been discovered, but experimental work is continuing towards that end. These obser-
vations may be similar to those described by Perlman and Kirschbaum[3,8,9]. Certainly
these results reinforce the belief that changes in HPLC peak areas due to sample–
solvent interactions unrelated to classical injected-solvent effects are not uncommon.

REFERENCES

1 S. Perlman and J. J. Kirschbaum, *J. Chromatogr.*, 357 (1986) 39–48.
2 J. C. Berridge, *J. Chromatogr.*, 369 (1986) 265–268.
3 J. J. Kirschbaum and S. Perlman, *J. Chromatogr.*, 369 (1986) 269–270.
4 K. C. Chan and E. S. Yeung, *J. Chromatogr.*, 391 (1987) 465–467.
5 T. L. Ng and S. Ng, *J. Chromatogr.*, 329 (1985) 13–24.
6 D. J. Miner, in S. H. Y. Wong (Editor), *Therapeutic Drug Monitoring and Toxicology by Liquid
  Chromatography*, Marcel Dekker, New York, 1985, pp. 269–307.
7 J. W. Dolan, *LC, Liq. Chromatogr. HPLC*, 3 (1985) 18–21.
8 J. J. Kirschbaum, presented at the *Eastern Analytical Symposium, New York, September 13–18, 1987*,
  Paper No. 222.
9 J. J. Kirschbaum, *J. Pharm. Biomed. Anal.*, in press.
10 E. L. Inman, *J. Chromatogr.*, 410 (1987) 363–372.