nobarbital, phenytoin, primidone, and valproate was not observed at concentrations exceeding their therapeutic ranges. A survey of library mass spectra indicated that some compounds can yield a 153 m/z or 167 m/z ion on electron impact ionization. These included allobarbital, p-aminosalicylic acid, and diphenylpyraline; however, these compounds were considered highly unlikely components of clinical specimens.

The assay demonstrated a within-run imprecision (CV) of 1.3% at a gabapentin concentration of 4.1 mg/L and a run-to-run total imprecision of 6.9% at 3.0 mg/L and 3.6% at 8.0 mg/L. Comparison of results with those of another GC/MS method (ARUP Laboratory) showed a correlation coefficient of 0.97 with a corresponding regression equation: GC/MS method (ARUP Laboratory) showed a correlation coefficient of 0.97 with a corresponding regression equation:

\[ y = 1.01x - 0.26 \] (n = 28, \( S_{yx} = 1.46 \) mg/L). Clinical values obtained over a 3-month period (n = 107) ranged from <0.05 to 17.5 mg/L with a median value of 3.0 mg/L.

We conclude that there is substantial merit in using existing GC/MS conditions as a basis for developing assays for chemically similar analytes despite unrelated clinical uses. This is particularly advantageous when the sensitivity and specificity of the mass-selective detector can be exploited relative to other analytical tools such as immunoassay or HPLC. When the productivity of a GC/MS workstation is maximized, the inherent costs related to instrument maintenance, capital depreciation, and columns are amortized over a larger test menu, and unit costs for testing are reduced. At the same time, assays with excellent performance characteristics can be established.

References


Ultrasensitive Direct Fluorescent Immunoassay for Thyroid Stimulating Hormone, Steven J. Zoha,*, Shakuntala Ramnarain, and F.C. Thomas Alhnett (Martek Biosciences Corporation, 6480 Dobbins Rd., Columbia, MD 21045; *author for correspondence: fax 410-740-2985, e-mail martek2001@aol.com)

Phycobilisomes are photosynthetic antennae complexes of red algae and cyanobacteria (1–3). They have been chemically cross-linked in such a way that they remain soluble and stable (4). These stabilized phycobilisomes (PBXL dyes) have large complex weights (between 1.0 × 106 and 1.5 × 107 Da) and Stokes shifts. They contain a large number of chromophores coordinated to efficiently transfer energy down an energy gradient and emit between 662 and 666 nm. The PBXL-1 dye, used in the thyroid-stimulating hormone (TSH) model, contains B-phycoerythrin, R-phycoerythrin, and allophycocyanin as its components phycobiliproteins.

The assay demonstrated a within-run imprecision (CV) of 1.3% at a gabapentin concentration of 4.1 mg/L and a run-to-run total imprecision of 6.9% at 3.0 mg/L and 3.6% at 8.0 mg/L. Comparison of results with those of another GC/MS method (ARUP Laboratory) showed a correlation coefficient of 0.97 with a corresponding regression equation: GC/MS method (ARUP Laboratory) showed a correlation coefficient of 0.97 with a corresponding regression equation:

\[ y = 1.01x - 0.26 \] (n = 28, \( S_{yx} = 1.46 \) mg/L). Clinical values obtained over a 3-month period (n = 107) ranged from <0.05 to 17.5 mg/L with a median value of 3.0 mg/L.

We conclude that there is substantial merit in using existing GC/MS conditions as a basis for developing assays for chemically similar analytes despite unrelated clinical uses. This is particularly advantageous when the sensitivity and specificity of the mass-selective detector can be exploited relative to other analytical tools such as immunoassay or HPLC. When the productivity of a GC/MS workstation is maximized, the inherent costs related to instrument maintenance, capital depreciation, and columns are amortized over a larger test menu, and unit costs for testing are reduced. At the same time, assays with excellent performance characteristics can be established.

References

μL/well of wash buffer. The plates were blocked with 350 μL/well blocking buffer at 37 °C for 2 h. The blocking buffer was aspirated, and the plates were washed four times with 350 μL/well wash buffer. The coated plates were then used immediately for the TSH assay.

In the assay, we added 75 μL of calibrator and 75 μL of assay buffer to each well. The plates were covered and incubated for 90 min at 37 °C. The plates were washed three times with 350 μL/well wash buffer. Fifty microliters of PBXL-1:Anti-TSH conjugate (100 mg/L in reagent dilution buffer) and 100 μL of assay buffer were added to each well. The plates were covered and incubated for 90 min at 37 °C. The plates were then washed three times with 350 μL/well wash buffer. One hundred microliters of plate-coating buffer was added to each well. The fluorescence of each well was determined on a Fluorolite 1000 plate reader (Dynex Technologies) at 10 V using 550 nm excitation and 660 nm emission filters.

The five-point calibration curve (0.0, 0.05, 1.0, 5.0, and 10.0 mIU/L) used a weighted logit fit analysis (Fig. 1).

The linearity and limit of detection were determined by assaying dilutions of the 10 mIU/L calibrator in the zero calibrator matrix (10.0, 5.0, 2.5, 1.0, 0.5, 0.2, 0.1, 0.05, 0.01, 0.005, and 0.0 mIU/L) in replicates of six. Concentrations were determined from a five-point calibration curve. Linear regression analysis of all points demonstrated a slope of 0.983 (1.010–0.956, upper and lower 95% confidence interval), a y-intercept of 0.019 mIU/L (0.110 to −0.071 mIU/L, upper and lower 95% confidence interval), standard error of the estimate of 0.334 mIU/L, and r² of 0.996. Quadratic regression analysis for curvature demonstrated a P value of 0.06, indicating lack of evidence for significance of the curvature of the line. The analytical limit of detection was 0.01 mIU/L, as calculated from two times the SD of 20 replicates of the 0 mIU/L calibrator. Similarly, 0.01 mIU/L was the lowest concentration dilution that demonstrated a statistically significant difference (P = 0.05) from the zero calibrator, as determined by a non-paired Student’s t-test analysis.

The within-run imprecision (CV) for replicates of 20 was 6.4% and 4.5% at concentrations of 0.56 and 7.2 mIU/L, respectively.

The dynamic linear assay range was 0.01–10.0 mIU/L. A 0.01 mIU/L analytical detection limit (6.2 × 10⁻¹⁴ mol/L) has been possible until now only with indirect detection technologies that involve enzymatic signal generation (e.g., chemiluminescence or chemiluminescence). PBXL pigments allowed this detection limit within the limitations of a manual assay format without enzymatic signal generation steps. It is anticipated that both detection limit and precision would improve if the assay were formatted on automated instrumentation developed for the features of the PBXL dyes.

The cost per test of PBXL pigments is competitive with traditional detection systems, and the stability of the PBXL conjugates is in excess of 1 year. PBXL technology has the combined advantages of ease of use, low cost, stability, and high sensitivity to set a new standard of performance as demonstrated by the TSH immunoassay model. This new technology has considerable potential for enhancing sensitivity in applications such as assay miniaturization, blotting technologies, and DNA arrays.

References


Charge Coupled Device Optics System for Simultaneous Measurement of Multiple Reactions in a Microplate, Brian H. Erickson, Bernard J. Van Wie, Dan M. Leatzou, Guihua Liu, Philip C. Thayer, and Thomas O. Tiffany (1 Department of Chemical Engineering, Washington State University, Pullman, WA 99164-2710; 2 DevTec, Inc., Spokane WA 99223; * author for correspondence: fax 509-335-4806, e-mail bvanwie@che.wsu.edu)

There is considerable interest in the application of Charge Coupled Device (CCD) technology to the challenges of modern analytical chemistry (1–3). CCD optical devices provide high photometric sensitivity and low optical noise and can be applied to both photometric and image analysis. These devices are of great interest in application to new state-of-the-art multiple function automated clinical analyzers. A CCD spectrophotometer has been developed by DevTec, Inc. for the simultaneous measurement of multiple reactions in a microplate format (4). The device consists of a Peltier-cooled-head Spectra Source CCD camera with a 192 × 165 pixel detector array, a 16-position interference filter wheel for wavelength selection, focusing optics for viewing a 96-well microplate, a