Analytical Performance Evaluation of a New Turbidimetric Immunoassay for Valproic Acid on the ADVIA® 1650 Analyzer: Effect of Gross Hemolysis and High Bilirubin

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Valproic acid is an anticonvulsant that requires careful therapeutic drug monitoring. Valproic acid is also used in psychiatric patients. Bayer Diagnostics (Tarrytown, NY) recently marketed a turbidimetric immunoassay for monitoring valproic acid concentrations in serum or plasma using the ADVIA® 1650 analyzer. We evaluated the performance of this new assay by comparing it with a widely used fluorescence polarization immunoassay (FPIA) on the AxSYM® analyzer (Abbott Laboratories, Abbott Park, IL). The total coefficient of variation (CV) for the low control of this new assay was 6.8% (mean = 30.7, SD = 2.1 µg/mL, n = 44) while the corresponding CVs for the medium and high controls were 3.3% (mean = 81.0, SD = 2.7 µg/mL, n = 44) and 5.9% (mean = 142.9, SD = 8.4 µg/mL, n = 44), respectively. The assay is linear up to a serum valproic acid concentration of 170 µg/mL, and the

detection limit is 4.4 µg/mL. We observed an excellent correlation between the FPIA of valproic acid and the turbidimetric assay using specimens from 52 different patients who were receiving valproic acid. Using the valproic acid concentrations obtained by the FPIA as the x-axis, and the corresponding valproic acid concentrations obtained by the turbidimetric assay as the y-axis, we developed the following regression equation: $y = 1.03x + 1.55$ ($r = 0.98$). With this new assay, high concentrations of bilirubin (unconjugated 30 mg/dL and conjugated 30 mg/dL) and gross hemolysis (4+, hemoglobin: 1,500 mg/dL) have no effect on measurements of valproic acid concentration. We conclude that the new turbidimetric assay for valproic acid can be used for routine therapeutic drug monitoring of valproic acid in clinical laboratories. *J. Clin. Lab. Anal.* 19:31–35, 2005. © 2005 Wiley-Liss, Inc.

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Valproic acid is an antiepileptic drug that is structurally unrelated to phenytoin, phenobarbital, or carbamazepine. The chemical name of valproic acid is 2-propylpentanoic acid. It was first synthesized in 1881, but the antiepileptic property of valproic acid was not discovered until 1963. Valproic acid is the primary drug of choice for treating absence seizures (1,2) and controlling generalized tonic-clonic, myoclonic, and partial seizures (3,4). Valproic acid along with carbamazepine is still considered the first-line antiepileptic therapy for children with partial seizures (5). Valproic acid in the form of divalproex sodium is used as a migraine prophylaxis, except in patients with epilepsy (6). Valproic acid is also used to treat a variety of psychiatric disorders, and may be effective in treating posttraumatic stress disorder (7). Valproic acid leads to a decrease in positive symptoms as well

as hostility in patients with schizophrenia (8). Although lithium is the first choice for treating bipolar disorder, the second-choice prophylactic treatment is carbamazepine plus valproic acid (9).

It is important to therapeutically monitor valproic acid in order to avoid toxicity and determine effective dosages (10). El Desoky et al. (11) reported that when therapeutic drug monitoring of digoxin and valproic acid was instituted in Egypt and Saudi Arabia, a

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significant decrease in potentially toxic levels was observed (11). Although valproic acid concentrations in serum can be measured by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (12), gas chromatography, or high-performance liquid chromatography (HPLC) (13), immunoassays are widely used for routine monitoring of valproic acid in clinical laboratories. Liu et al. (14) demonstrated a satisfactory correlation between results obtained by HPLC and immunoassays. Steijns et al. (15) also demonstrated an excellent correlation between the fluorescence polarization immunoassay (FPIA) for valproic acid and an HPLC method. Bayer Diagnostics (Tarrytown, NY) recently marketed a turbidimetric immunoassay for valproic acid on the ADVIA[®] 1650 analyzer. We evaluated the analytical performance of this new assay by comparing it with the widely used FPIA for valproic acid. In a recent trial of therapeutic drug assays conducted by the College of American Pathologists (Z-series, first quarter of 2004), 672 out of 2,834 participating laboratories used the FPIA assay for valproic acid using the AxSYM analyzer, and 117 laboratories used the same FPIA assay for valproic acid using the TDx analyzer. The FPIA method was the most frequently used method in all of the participating laboratories.

MATERIALS AND METHODS

We purchased the FPIA kits for valproic acid along with calibrators and controls from Abbott Laboratories (Abbott Park, IL), and ran the assay using an AxSYM analyzer (also obtained from Abbott Laboratories). The turbidimetric assay for valproic acid was run on an ADVIA 1650 analyzer (Bayer Diagnostics, Tarrytown, NY). Triglyceride concentrate was purchased from Pentex (Kankakee, IL). Intralipid, unconjugated bilirubin (catalog #B4126), and a hemoglobin determination kit were purchased from Sigma Chemical Company (St. Louis, MO). Conjugated bilirubin (catalog #201102) was purchased from Calbiochem (San Diego, CA). We used 52 different specimens from patients receiving valproic acid to compare the two assays. These specimens are routinely submitted to our clinical laboratory for therapeutic drug monitoring. In this study we used specimens that were left over after all tests requested by the respective clinicians were performed and the results were reported. These specimens would otherwise have been discarded.

The ADVIA 1650 turbidimetric valproic acid assay is a latex-enhanced turbidimetric immunoassay based on the principle of measuring absorbance changes

when immune complexes are formed. The latex particles are coated with the analyte, which in the presence of valproic acid antibody (mouse monoclonal) solution rapidly agglutinates. When a sample containing valproic acid is introduced, the agglutination reaction is partially inhibited, slowing down the agglutination process. The rate of agglutination is inversely dependent on the concentration of valproic acid in the sample. In this assay, the sample is first prediluted (1:4). The system then pipettes the prediluted sample into a reaction cuvette, adds the valproic acid-specific antibody, and then (after 5 min of incubation at 37°C) adds the latex-valproic acid conjugate. The resulting turbidimetry is measured at 596 nm, and the signal is converted to the equivalent valproic acid concentration using a six-level calibration curve. The analytical range of the assay is 4–170 µg/mL.

We calculated the total CV of the turbidimetric assay using three different levels of controls (also supplied by Bayer Diagnostics). We also calibrated the new assay using the calibrators supplied by Bayer Diagnostics. Linearity was verified by means of a serial dilution of a high standard with saline. Potential interference of hemolysis was evaluated with blood hemolysate prepared in-house and hemoglobin measured in the specimen. The hemolysate was made by repeated freeze-thawing of red blood cells (twice, at –20°C), and was centrifuged to obtain clear hemolysate supernatant. The supernatant was then aliquoted and stored frozen at –20°C until it was used. Similarly, we studied the effect of high triglycerides by adding a triglyceride concentrate or intralipid (achieving a maximum triglyceride concentration of 1,000 mg/dL). We also supplemented aliquots of serum pool containing valproic acid with various concentrations of conjugated and unconjugated bilirubin in order to study the potential interference of bilirubin in this assay.

The FPIA was run on an AxSYM analyzer. This assay uses a polyclonal (sheep) antibody against valproic acid. The assay uses a six-point calibration curve and is linear up to a valproic acid concentration of 150 µg/mL. The detection limit of this assay is 0.7 µg/mL. The lowest measurable concentration is defined as the concentration that can be distinguished from zero concentration at a 95% confidence level.

The statistical analysis included calculation of the CV, regression analysis, and Student's *t*-test to determine the statistical significance of the difference in valproic acid concentrations obtained in the presence of various interfering substances. For the *t*-test, we considered a difference significant at a 95% confidence level, using a two-tailed analysis ($P < 0.05$).

RESULTS AND DISCUSSION

The total CV for the low control of this new turbidimetric assay was 6.8% (mean = 30.7, SD = 2.1 µg/mL, n = 44), while the corresponding CVs for the medium and high controls were 3.3% (mean = 81.0, SD = 2.7 µg/mL, n = 44) and 5.9% (mean = 142.9, SD = 8.4 µg/mL, n = 44), respectively. We calculated the total CV by running each control in duplicate twice daily for 11 days (n = 44).

The assay is linear up to a serum valproic acid concentration of 170 µg/mL, and the detection limit is 4.4 µg/mL. We determined the dilution recovery of the assay by diluting a high standard (170 µg/mL) with saline and then comparing the observed valproic acid concentrations with the target concentrations. We observed excellent dilution recoveries (103.8–91.1%), with an average recovery of 99.1% (Table 1).

We also studied the recovery of samples spiked with known concentrations of valproic acid. When drug-free serum was supplemented with valproic acid to achieve a final concentration of 69.5 µg/mL, the observed valproic acid concentration was 69.1 µg/mL (recovery: 99.4%). Similarly, in another specimen with a target valproic acid concentration of 89.4 µg/mL, the observed valproic acid concentration was 91.4 µg/mL (recovery: 102.2%). In another spiked sample, the target concentration was 119.2 µg/mL, and the observed concentration was 117.0 µg/mL (recovery: 98.2%).

We observed an excellent correlation between the valproic acid concentrations obtained by the reference FPIA assay for valproic acid and the turbidimetric assay. With the valproic acid concentrations obtained by the FPIA as the x-axis, and the corresponding concentrations obtained by the turbidimetric assay as the y-axis, we developed the following regression equation using a traditional least-squares approach (Fig. 1): $y = 1.03x + 1.55$ ($r = 0.98$); $n = 52$.

TABLE 1. Dilution recovery of a high standard in the new turbidimetric assay of valproic acid

Specimen	Valproic acid concentration, µg/mL		
	Target	Observed, mean (SD), n = 3	Recovery
Original standard	170.0	170.1 (10.7)	100%
Diluted standard	148.8	150.4 (11.8)	101.1%
Diluted standard	127.6	124.4 (2.1)	97.5%
Diluted standard	106.3	106.0 (5.8)	99.7%
Diluted standard	85.0	85.3 (1.6)	100.4%
Diluted standard	63.8	63.9 (3.4)	100.2%
Diluted standard	42.5	44.1 (4.7)	103.8%
Diluted standard	21.3	19.4 (0.7)	91.1%

Fig. 1. Regression analysis using valproic acid concentrations observed by the FPIA reference method and the new turbidimetric assay as the x- and y-axes, respectively.

To study the effect of high concentrations of triglycerides on the turbidimetric assay for valproic acid, we supplemented an aliquot of serum pool containing valproic acid with triglyceride concentrate or Intralipid to achieve a triglyceride concentration of 1,000 mg/dL. The interference index for the ADVIA analyzer is (++++) for such a high concentration of triglyceride. We observed no interference from triglyceride in this assay because the observed valproic acid concentration of 36.2 µg/mL (SD = 1.5 µg/mL) in the presence of high triglycerides (1,000 mg/dL, achieved by adding triglyceride concentrate) was not statistically different from the original valproic acid concentration of 34.4 µg/mL (SD = 4.3 µg/mL). Similarly, when another aliquot of this serum pool was further supplemented with Intralipid to achieve a triglyceride concentration of 1,000 mg/dL, the observed valproic acid concentration was 36.0 µg/mL (SD = 2.5 µg/mL), a value that is not statistically significant from the concentration of valproic acid observed in the original pool. We observed no interference of triglycerides in two other pools (valproic acid concentrations = 82.4 and 72.7 µg/mL, respectively).

We compared the potential interference of hemolysis on this new turbidimetric assay with the reference FPIA method by adding various amounts of hemolysate in specimens containing valproic acid. We observed the degree of hemolysis manually, and measured hemoglobin concentrations in the respective specimens using the ADVIA analyzer. The ADVIA analyzer also indicates the interference index (ranging from + to +++++). If the concentration of hemoglobin is ≤ 50 mg/dL, the interference index is +. For a hemoglobin concentration of 150 mg/dL the index is ++. For a concentration of 250 mg/dL, the interference index is +++. For a hemoglobin concentration of ≥ 500 mg/dL, the interference index is +++++. We observed no significant

interference of hemoglobin with the new turbidimetric assay or the reference method. For example, in one valproic acid-containing serum pool supplemented with 1,500 mg/dL of hemoglobin (ADVIA interference index + + + +), the observed mean valproic acid concentration using the turbidimetric assay was 52.7 $\mu\text{g/mL}$, which was not significantly different from the mean valproic acid concentration of 50.9 $\mu\text{g/mL}$ in the original pool (Student's *t*-test, two-tailed). Similarly, the concentration of valproic acid in the original pool as measured by the FPIA (reference method) was 54.9 $\mu\text{g/mL}$. In the presence of 1,500 mg/dL of hemoglobin, the observed mean valproic acid concentration by the FPIA was 54.2 $\mu\text{g/mL}$, which was not significantly different from the original value. We also observed similar results with another serum pool containing a larger amount of valproic acid (Table 2). According to the package insert of the FPIA assay for valproic acid, the error was less than 10% in the presence of 1,000 mg/dL of hemoglobin. Our study indicates that both the FPIA assay and the new turbidimetric assay for valproic acid are free from interference at hemoglobin concentrations up to 1,500 mg/dL.

We also studied the potential interference of both unconjugated and conjugated bilirubin in the turbidimetric assay by comparing the results with those from the FPIA assay. The ADVIA instrument also indicates an interference index that depends on bilirubin concen-

trations. For a bilirubin concentration of 1.7 mg/dL, the index is +. For bilirubin concentrations of 6.6 mg/dL, 16.0 mg/dL, and 30.0 mg/dL, the corresponding interference indexes are + +, + + +, and + + + +, respectively. For example, when an aliquot of a valproic

TABLE 2. Effect of hemoglobin on the measurement of valproic acid by the new turbidimetric assay and the FPIA assay for valproic acid

Specimen	Hemoglobin added mg/dL	ADVIA interference index	Valproic, $\mu\text{g/mL}$, mean (SD), n = 3	
			Turbidimetric	FPIA
Valproic pool 1	0.0	None	50.9 (1.8)	54.9 (2.1)
+ Hemoglobin	100.0	+	51.1 (1.2)	52.8 (1.6)
+ Hemoglobin	250.0	+ + +	52.1 (0.7)	52.9 (1.4)
+ Hemoglobin	500.0	+ + + +	51.2 (1.6)	54.7 (0.8)
+ Hemoglobin	750.0	+ + + +	52.6 (0.8)	53.1 (1.1)
+ Hemoglobin	1000.0	+ + + +	49.0 (1.6)	54.7 (0.9)
+ Hemoglobin	1500.0	+ + + +	52.7 (0.9)	54.2 (0.7)
Valproic pool 2	0.0	None	99.4 (1.6)	103.9 (1.3)
+ Hemoglobin	100.0	+	99.5 (1.4)	103.3 (0.8)
+ Hemoglobin	250.0	+ + +	96.3 (1.9)	99.4 (1.9)
+ Hemoglobin	500.0	+ + + +	101.0 (1.1)	100.4 (0.4)
+ Hemoglobin	750.0	+ + + +	103.1 (1.0)	102.1 (0.9)
+ Hemoglobin	1000.0	+ + + +	100.8 (0.9)	96.9 (0.8)
+ Hemoglobin	1500.0	+ + + +	106.2 (0.7)	98.8 (1.5)

TABLE 3. Effect of unconjugated and conjugated bilirubin on the turbidimetric and FPIA assay for valproic acid

Specimen	Bilirubin added mg/L	ADVIA interference index	Valproic, $\mu\text{g/mL}$ mean (SD), n = 3	
			Turbidimetric	FPIA
Valproic pool 1	0.0	None	51.0 (1.3)	57.1 (1.2)
+ Conjugated bilirubin	10.0	+ +	50.8 (1.4)	60.1 (1.2)
+ Conjugated bilirubin	15.0	+ +	50.6 (1.8)	58.2 (0.7)
+ Conjugated bilirubin	20.0	+ + +	49.2 (1.1)	54.8 (1.1)
+ Conjugated bilirubin	25.0	+ + +	49.4 (1.4)	61.4 (0.8)
+ Conjugated bilirubin	30.0	+ + + +	52.4 (0.9)	59.5 (1.4)
+ Unconjugated bilirubin	10.0	+ +	51.1 (1.0)	60.1 (0.9)
+ Unconjugated bilirubin	15.0	+ +	52.1 (1.4)	58.7 (1.0)
+ Unconjugated bilirubin	20.0	+ + +	51.2 (1.3)	57.1 (0.5)
+ Unconjugated bilirubin	25.0	+ + +	52.6 (0.8)	56.7 (0.7)
+ Unconjugated bilirubin	30.0	+ + + +	50.1 (1.8)	57.0 (0.9)
Valproic pool 2	0.0	None	97.4 (1.6)	106.3 (1.1)
+ Conjugated bilirubin	10.0	+ +	101.0 (2.3)	106.6 (0.9)
+ Conjugated bilirubin	15.0	+ +	96.7 (1.5)	105.1 (1.2)
+ Conjugated bilirubin	20.0	+ + +	98.2 (0.7)	106.4 (0.8)
+ Conjugated bilirubin	25.0	+ + +	97.4 (1.1)	106.6 (0.8)
+ Conjugated bilirubin	30.0	+ + + +	99.7 (1.7)	103.8 (1.2)
+ Unconjugated bilirubin	10.0	+ +	96.8 (3.6)	100.1 (0.7)
+ Unconjugated bilirubin	15.0	+ +	100.4 (0.6)	106.0 (1.2)
+ Unconjugated bilirubin	20.0	+ + +	98.3 (0.9)	105.6 (0.5)
+ Unconjugated bilirubin	25.0	+ + +	98.9 (0.9)	104.3 (1.1)
+ Unconjugated bilirubin	30.0	+ + + +	97.0 (0.8)	102.0 (1.7)

acid-containing serum pool was supplemented with 30 mg/dL of conjugated bilirubin, the observed mean valproic acid concentration was 52.4 µg/mL, which was not significantly different from the mean valproic acid concentration of 51.0 µg/mL in the original pool as measured by the turbidimetric assay. When another aliquot of the serum pool was supplemented with 30 mg/dL of unconjugated bilirubin, the observed mean valproic acid concentration was 50.1 µg/mL, which was again not significantly different from the original concentration of valproic acid. We observed similar results with another valproic acid-containing serum pool (valproic acid: 97.4 µg/mL) when aliquots of the pool were supplemented with conjugated or unconjugated bilirubin. Although the package insert of the FPIA assay states that the assay is not affected by a bilirubin concentration of 20 mg/dL, our study indicates that FPIA, like the new turbidimetric assay, is not affected by either conjugated or unconjugated bilirubin up to a concentration of 30 mg/dL (Table 3).

We conclude that the new turbidimetric assay for valproic acid is comparable to the widely used FPIA assay for valproic acid. Moreover, this new assay is not affected by gross hemolysis of a specimen or the presence of high bilirubin concentrations. Therefore, this new assay can be used in clinical laboratories for routine therapeutic drug monitoring of valproic acid.

REFERENCES

1. Sato S, Wright DG, Penry JK, Dreifuss FE, Sackellares JC, Kupferberg HJ. Valproic acid versus ethosuximide in the treatment of absence seizure. *Neurology* 1982;32:157–163.
2. Henriksen O, Johannessen SI. Clinical and pharmacokinetic observations on sodium valproate: a five-year follow up study on 100 children with epilepsy. *Acta Neurol Scand* 1982;65:504–523.
3. Bruni J, Albright P. Valproic acid therapy for complex partial seizures: its efficacy and toxic effects. *Arch Neurol* 1983;40:135–137.
4. Lee K, Melchior JC. Sodium valproate versus phenobarbital in the prophylactic treatment of febrile convulsions in childhood. *Eur J Pediatr* 1983;137:151–153.
5. Coppola G. Treatment of partial seizures in childhood: an overview. *CNS Drugs* 2004;18:133–156.
6. Spina E, Perugi G. Antiepileptic drugs: indications other than epilepsy. *Epileptic Disord* 2004;6:57–75.
7. Asnis GM, Kohn SR, Henderson M, Brown NL. SSRIs versus non-SSRIs in post-traumatic stress disorders: an update with recommendations. *Drugs* 2004;64:383–404.
8. Hosak L, Libiger J. Antiepileptic drugs in schizophrenia: a review. *Eur Psychiatry* 2002;17:371–378.
9. Muller-Oerlinghausen B, Berghofer A, Bauer M. Bipolar disorder. *Lancet* 2002;359:241–247.
10. Irshaid YM, Hamadi AA, Al Homrany M. Evaluation of therapeutic drug monitoring of antiepileptic drugs. *Int J Clin Pharmacol Ther* 2003;41:126–131.
11. El Desoky ES, Al-Ghamdi HA, Halaby FH, Al-Beshri M. Therapeutic monitoring of digoxin and antiepileptic drugs in Egypt and Saudi Arabia. *Ther Drug Monit* 2003;25:211–214.
12. Mino T, Nakajima M, Wakabayashi H, Yamato S, Shimada K. Determination of valproic acid in serum by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *Anal Sci* 2001;17:999–1001.
13. Darius J, Meyer FP. Sensitive capillary gas chromatography-mass spectrometric method for therapeutic drug monitoring of valproic acid and seven of its metabolites in human serum: applications of the assay for a group of pediatric epileptics. *J Chromatogr B Biomed Appl* 1994;656:343–351.
14. Liu H, Forman LJ, Montoya J, Eggers C, Barham C, Delgado M. Determination of valproic acid by high performance liquid chromatography with photodiode array and fluorescence detection. *J Chromatogr* 1992;576:163–169.
15. Steijns LS, Bouw J, van der Weide J. Evaluation of fluorescence polarization assay for measuring valproic acid, phenytoin, carbamazepine and phenobarbital in serum. *Ther Drug Monit* 2002;24:432–435.