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Analytical Performance Evaluation of ADVIA Chemistry Carbamazepine_2 Assay: Minimal Cross-Reactivity With Carbamazepine 10, 11-Epoxide and None With Hydroxyzine or Cetirizine

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Carbamazepine is an anticonvulsant requiring routine therapeutic drug monitoring. Recently, Siemens Healthcare Diagnostic Division released a new carbamazepine assay: ADVIA Chemistry Carbamazepine_2 (Carbamazepine_2) for application on ADVIA analyzers. We evaluated the analytical performance of this assay as well as its potential cross-reactivities with carbamazepine 10, 11-epoxide, hydroxyzine, and cetirizine. The within-run and between-run precisions of the Carbamzepine-2 assay were $<6\%$ and limit of detection was 0.5 μg/ml using ADVIA 1800 analyzer. The assay was linear up to a carbamazepine concentration of 20.0 μg/ml. The new method compared well with a widely used carbamazepine EMIT 2000 assay on the Hitachi 917 analyzer. Using 75 patients' specimens (where carbamazepine concentrations varied from 0.5 to 21.7 μg/ml) and carbamazepine EMIT 2000 as the reference method (*x*-axis), we observed the following regression equation: $y = 1.04x + 0.32$ ($r = 0.99$). The new carbazepine_2 method was not affected by a hemoglobin concentration of 1,000 mg/dl, conjugated or unconjugated bilirubin concentration of 60 mg/dl, and triglyceride concentration of 1,000 mg/dl. In addition, this assay showed no cross-reactivity with hydroxyzine or cetirizine and demonstrated minimal cross-reactivity with carbamazepine 10, 11-epoxide. We conclude that the ADVIA Chemistry carbamazepine_2 assay has adequate precision and accuracy for routine therapeutic drug monitoring of carbamazepine in clinical laboratories. J. Clin. Lab. Anal. 24:278–282, 2010. © 2010 Wiley-Liss, Inc.

Key words: carbamazepine; immunoassays; carbamazepine 10, 11-epoxide; interference

Carbamazepine is an iminostilbene derivative structurally similar to the tricyclic antidepressant imipramine. It was approved in the United States in 1974 as an anti-epileptic for management of seizure disorders in adults and in 1979 for use in children over 6 years of age. The current uses of carbamazepine include treating partial seizure with complex symptomatology, generalized tonic–clonic seizure, and mixed seizure. Carbamazepine along with phenytoin is considered as the drug of choice for treating these seizure disorders (1). Carbamazepine is sometimes added to the existing tricyclic antidepressant therapy (2). Carbamazepine may help some individuals with episodic behavioral lack of control and aggression even in the absence of epileptic,

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affective, or organic features (3,4). Tricyclic antidepressants and anticonvulsants such as carbamazepine are also used in the treatment of pain in polyneuropathy (5). Similar to other classical antiepileptics, the therapeutic or toxic effects of carbamazepine are better correlated with plasma concentrations rather than the dosage.

Immunoassays are commercially available for therapeutic drug monitoring of carbamazepine. Recently, Siemens Healthcare Diagnostics Division has marketed a new carbamazepine assay: ADVIA Chemistry Carbamazepine_2 (Carbamazepine_2) for application on ADVIA analyzers. We evaluated the analytical performance of this assay and also evaluated the potential interference of hemoglobin, bilirubin, and triglycerides with this assay as well as cross-reactivity of carbamazepine 10, 11-epoxide, hydroxyzine, and cetirizine. The cross-reactivity of carbamazepine 10, 11-epoxide with different immunoassays for carbamazepine may vary between 0 (Vitros) and 94% (Dade Dimension) (6). Hydroxyzine and cetirizine, which are widely prescribed antihistamine drugs, also known to cross-react with the Dade Dimension particle-enhanced turbidimetric inhibition immunoassay (PETINIA) carbamazepine assay (7,8). In this study, we report our findings.

MATERIALS AND METHODS

Carbamazepine 10, 11-epoxide and hydroxyzine hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). Cetirizine was extracted from Zyrtec tablet (cetirizine hydrochloride 10 mg; Pfizer, New York, NY), as described by Parant et al (7). Standard solution of carbamazepine 10, 11-epoxide (1 mg/ml) was prepared in absolute ethanol. Standard solution of hydroxyzine and cetirizine (1 mg/ml) was also prepared in absolute ethanol. Then, two working solutions of hydroxyzine (0.1 and 0.01 mg/ml) were prepared by diluting working solution with absolute ethanol. We also prepared two working solutions of cetirizine (0.1 and 0.01 mg/ml).

ADVIA Chemistry Carbamazepine_2 is a new method to measure human serum or plasma carbamazepine concentration to be used on the automated random access auto-analyzers, ADVIA analyzer. The method uses the EMIT® technology with a murine monoclonal antibody to the analyte in Reagent 1 and the label enzyme glucose-6-phosphatase (bacterial) coupled to carbamazepine in Reagent 2. The enzyme activity is inhibited when the enzyme conjugate binds with the antibody. The carbamazepine in a sample competes with the analyte–conjugate for limited number of binding sites of the antibody in Reagent 1, thus increasing concentration of the active enzyme. Hence, the enzyme activity is directly proportional to the analyte concentration in the sample. The enzyme activity is measured in the assay using glucose-6-phosphate as substrate and nicotinamide adenine dinucleotide (NAD) as the cofactor; during the enzyme reaction, NAD is reduced to NADH with an increase in 340 nm absorbance. The new method uses a serum-based 5-level calibrator, ADVIA Chemistry Drug I Calibrator. The calibration curve is stable for 30 days. The on-system stability of the reagent is also 30 days.

Within-run and between-run precisions of the carbamazepine_2 assay were investigated using low, medium, and high control and ADVIA 1800 analyzer. For within-run precision, each control was assayed 20 times in a single run, whereas for between-run precision, each control was run once a day for 20 consecutive days. Sensitivity of the assay was determined by running a blank specimen containing no carbamazepine. Linearity was established by using low- and high- serum pools containing carbamazepine. Method comparison was carried out using 75 patients' sera assaying parallel using EMIT 2000 carbamazepine assay (reference method) on Hitachi 917 analyzer and carbamazepine_2 assay on ADVIA 1800 analyzer. Cross-reactivity studies were performed using hemolysate, bilirubin, and intralipid. For studying the cross-reactivity of carbamazepine 10, 11-epoxide, hydroxyzine, and cetirizine, we compared results obtained by using carbamazepine_2 assay with PETINIA assay using Dimension Vista analyzer (Siemens, Deerfield, IL). We selected PETINIA assay for its known high cross-reactivity with carbamazepine 10, 11-epoxide, hydroxyzine, and cetirizine. For this study, we supplemented aliquots of drug-free serum pool with various amounts of carbamazepine 10, 11-epoxide, hydroxyzine, or cetirizine. To ensure ethanol in which standard solutions of these compounds were prepared should not have any interference with carbamazepine assay, we added microliter quantity of a standard or working solution in a test tube followed by evaporating ethanol under a gentle stream of nitrogen. Then the dry residue was reconstituted with drug-free serum. We run all specimens using the carbamazepine_2 Assay on the ADVIA 1800 analyzer. For comparison, these specimens were also analyzed using the PETINIA carbamazepine assay on the Dimension Vista 1500 analyzer. As cross-reactivity should be assessed in the presence of the primary analyte (9), we prepared a carbamazepine serum pool from patients receiving carbamazepine and then aliquots of the pool were further supplemented with carbamazepine 10, 11-epoxide, hydroxyzine, or cetirizine and carbamazepine values were measured again using both Carbamazepine-2 assay and PETINIA assay for comparison with carbamazepine values of the original pool obtained by both assays.

RESULTS

Both within-run and between-run precisions of the carbamazepine_2 assay were evaluated using low, medium and high controls and the values were <6% (Table 1). The detection limit of the assay was established by running a blank specimen containing no carbamazepine for 20 times and then mean and standard deviations were calculated. Mean+2 standard deviation was considered as the detection limit (sensitivity of the assay), which we determined as 0.5 μg/ml for ADVIA 1800 analyzer. For the linearity study, two serum pools containing high- and low-carbamazepine concentrations were mixed in various amounts to prepare specimens containing various amounts of carbamazepine (0, 2.7, 5.4, 8.1, 11.0, 13.7, 16.1, 19.2, and 21.9 μg/ml). Then these nine specimens were analyzed using the carbamazepine_2 assay on ADVIA 1800 analyzer and percent recoveries were calculated based on the target and observed concentration. We observed recoveries between 92.6 and 105.6%. As we observed good recoveries and these values cover the range of calibrators from low to the high end with the highest calibrator being 20 μg/ml, we established that the carbamzepin-2 assay is linear up to a serum carbamazepine concentration of 20 μg/ml.

The carbamazepine_2 method on ADVIA 1800 platform correlated well with EMIT 2000 carbamazepine assay on the Hitachi 917 platform. We used 75 patients' specimens for comparison (values ranged from 0.5 to 21.7 μg/ml). Using x-axis as the results obtained by using EMIT 2000 assay (reference method) and y-axis as the results obtained by using carbamazepine_2 assay on the ADVIA 1800 analyzers, we observed the following regression equation (Fig. 1):

$$y = 1.04\,x + 0.32\ (r = 0.99), \quad n = 75.$$

Interference studies were performed using hemolysate for hemoglobin, conjugated as well as unconjugated bilirubin, and Intralipid for triglyceride interference. The new carbazepine_2 method was not affected by a hemoglobin concentration of 1,000 mg/dl, conjugated or unconjugated bilirubin concentration up to 60 mg/dl, and triglyceride concentration up to 1,000 mg/dl. In addition, this assay is free from cross-reactivity of hydroxyzine and cetirizine and is minimally affected by carbamazepine 10, 11-epoxide. In contrast, the PETINIA carbamazepine assay in the Dimension Vista analyzer demonstrated significant cross-reactivity (approximately 94%) with the carbamazepine 10, 11-epoxide, and both hydroxyzine as well as cetirizine in high concentrations affect PETINIA carbamazepine assay (Table 2). As expected when aliquots of a carbamazepine serum pool prepared from patients were supplemented with various concentrations of carbamazepine 10, 11-epoxide, hydroxyzine, and cetirizine, we observed minimal effect on carbamazepine measurement (10% false increase in carbamazepine value with 30 μg/ml of carbamazepine 10, 11-epoxide supplementation). However, such high epoxide concentration is clinically unlikely. In addition, we observed no effect of hydroxyzine or cetirizine on carbamazepine measurement using the new Carbamzepine_2 assay. In contrast, we observed significant false increases in carbamazepine values using the PETINIA assay (Table 3).

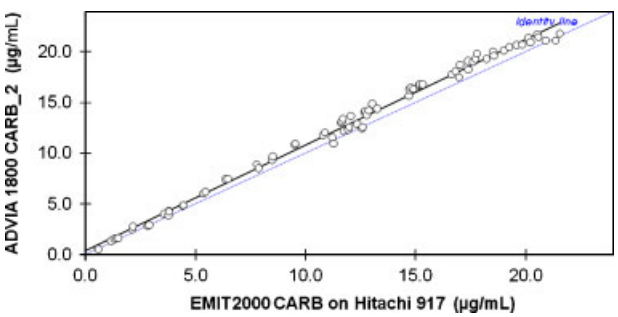

Fig. 1. Regression equation showing values obtained in 75 patient's serum using EMIT 2000 carbamazepine assay as the reference method (x-axis) and carbamazepine _2 on ADVIA 1800 analyzer as the method evaluated (y-axis).

TABLE 1. Precision of Carbamazepine_2 Assay on ADVIA 1800 Analyzer

		Within-run ($n = 20$ for each control)			Between-run ($n = 20$ for each control)		
Platform	Specimen	Mean (μg/ml)	SD	CV (%)	Mean (μg/ml)	SD	CV (%)
ADVIA 1800	Low control	4.0	0.19	4.7	3.9	0.22	5.7
	Medium control	9.7	0.32	3.3	9.8	0.41	4.2
	High control	15.4	0.30	2.0	15.5	0.51	3.3

TABLE 2. Effect of Supplementing Aliquots of Drug-Free Serum Pool With Carbamazepine 10, 11-Epoxide, Hydroxyzine or Cetirizine on Carbamazepine_2 and PETINIA Assay

		Apparent carbamazepine, μg/ml, mean value[a]	
Compound	Concentration, μg/ml	Carbamazepine_2	PETINIA
Carbamazepine	1.0	ND	0.97
10, 11-epoxide	2.5	ND	2.4
	5.0	ND	4.8
	7.5	ND	7.2
	10.0	ND	10.1
	15.0	ND	14.7
	20.0	1.0	18.6
	30.0	1.2	27.9
Hydroxyzine	1.0	ND	0.7
	2.5	ND	1.2
	5.0	ND	4.3
	10.0	ND	8.6
	20.0	ND	14.2
	39.0	ND	23.9
	103.0	ND	59.9
Cetirizine	1.0	ND	1.1
	2.5	ND	2.4
	5.0	ND	4.9
	10.0	ND	10.4
	20.0	ND	19.3

ND, none detected.
[a]Value represents the mean of three replicates.

TABLE 3. Effect of Supplementing Aliquots of a Carbamazepine Pool (prepared from patients receiving carbamazepine) Further With Carbamazepine 10, 11-Epoxide, Hydroxyzine or Cetirizine on Serum Carbamazepine Measurement Using New Carbamazepine_2 Assay and PETINIA Assay

	Apparent carbamazepine, μg/ml, mean value[a]	
Specimen (Concentration,μg/ml)	Carbamazepine_2	PETINIA
Carbamazepine serum pool	7.7	7.9
+1.0 μg/ml Carbamazepine 10, 11-epoxide	7.8	8.8
+2.5 μg/ml Carbamazepine 10, 11-epoxide	7.6	10.1
+5.0 μg/ml Carbamazepine 10, 11-epoxide	7.7	12.1
+7.5 μg/ml Carbamazepine 10, 11-epoxide	7.8	14.7
+10.0 μg/ml Carbamazepine 10, 11-epoxide	7.9	18.4
+15.0 μg/ml Carbamazepine 10, 11-epoxide	7.8	22.1
+20.0 μg/ml Carbamazepine 10, 11-epoxide	8.3	28.9
+30.0 μg/ml Carbamazepine 10, 11-epoxide	8.5	34.1
+1.0 μg/ml Hydroxyzine	7.7	8.7
+2.5 μg/ml Hydroxyzine	7.6	9.3
+5.0 μg/ml Hydroxyzine	7.7	10.6
+10.0 μg/ml Hydroxyzine	7.6	14.7
+20.0 μg/ml Hydroxyzine	7.7	23.1
+39.0 μg/ml Hydroxyzine	7.8	38.7
+103.0 μg/ml Hydroxyzine	7.9	74.4
+1.0 μg/ml Cetirizine	7.7	9.3
+2.5 μg/ml Cetirizine	7.8	10.2
+5.0 μg/ml Cetirizine	7.6	12.6
+10.0 μg/ml Cetirizine	7.8	17.0
+20.0 μg/ml Cetirizine	7.7	24.2

[a]Value represents the mean of three replicates.

DISCUSSION

Carbamazepine, a commonly used classical anticonvulsant, which is also used in treating certain types of psychiatric illness, requires therapeutic drug monitoring. A therapeutic range of 4–12 μg/ml is recommended for carbamazepine to optimize dose, but its active metabolite carbamazepine, carbamazepine 10, 11-epoxide, is not routinely monitored and is not reflected in the therapeutic range. Although specific analytical methods such as gas chromatography, gas chromatography combined with mass spectrometry or liquid chromatography combined with mass spectrometry can provide accurate results for determination of carbamazepine concentrations in serum, these techniques are rarely used in clinical laboratories except for large national reference laboratories where simultaneous determination of carbamazepine and carbamazepine 10, 11-epoxide is warranted. Immunoassays are used in most clinical laboratories for routine therapeutic drug monitoring of carbamazepine. Chen et al. showed excellent correlation between carbamazepine concentrations obtained by gas chromatography and a widely used fluorescence polarization immunoassay due to minimal cross-reactivity with the epoxide metabolite (10). An ideal immunoassay for carbamazepine should have minimal cross-reactivity with the epoxide metabolite. Our study shows that carbamazepine_2 assay has minimal cross-reactivity with carbamazepine 10, 11-epoxide.

In addition to metabolite cross-reactivity, an immunoassay should also be free from interference of other drugs. Hydroxyzine and cetirizine are widely prescribed antihistamine drugs. The expected in vivo concentration of hydroxyzine after a standard dose is 0.10 μg/ml, while toxic concentration is more than 1.0 μg/ml. Johnson et al. reported a woman who committed suicide by taking hydroxyzine had a serum hydroxyzine level of 39 μg/ml (11), while a 13-month-old girl with a serum hydroxyzine concentration of 102.7 μg/ml, who survived the life-threatening hydroxyzine overdose (12). In one patient with cetirizine overdose, the serum cetirizine level was 2.4 μg/ml (13). Our investigation showed that hydroxyzine as well as cetirizine has no cross-reactivity with carbamazepine_2 assay, but PETINIA assay may show a false-positive carbamazepine level in a patient severely overdosed with hydroxyzine due to significant cross-reactivity. In addition, PETINIA may also show falsely elevated carbamazepine value in a patient with renal insufficiency where accumulation of epoxide metabolite is likely.

We conclude that the carbamazepine_2 assay has excellent precision, sensitivity, and specificity. This method also correlates well with a commercially available carbamazepine assay and is free from cross-reactivity of hydroxyzine and cetirizine. In addition, carbamazepine 10, 11 epoxide has only minimal cross-reactivity with this assay. Therefore, this assay is suitable for routine therapeutic drug monitoring of carbamazepine in clinical laboratories.

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