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Analytical agreement between CEDIA immunoassay and HPLC for therapeutic drug monitoring of carbamazepine: a narrative review

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ABSTRACT

Background: Carbamazepine (CBZ) is a first-generation antiseizure medication with a narrow therapeutic index requiring routine therapeutic drug monitoring (TDM). High-performance liquid chromatography (HPLC) remains the reference standard due to its accuracy and capacity to quantify CBZ metabolites; however, its operational demands limit its use in resource-constrained settings. The Cloned Enzyme Donor Immunoassay (CEDIA) offers practical advantages in speed, simplicity, and cost, but the extent of its agreement with HPLC across diverse clinical contexts requires careful evaluation.

Method: This narrative review synthesizes evidence from nine studies identified through Scopus and Google Scholar to assess the analytical agreement and clinical validity of CEDIA and related immunoassay platforms compared to HPLC and advanced chromatographic methods.

Results: The reviewed evidence demonstrates strong inter-method correlation, although systematic positive bias in immunoassay results is consistently observed, particularly in patients receiving enzyme-inducing co-medications where carbamazepine-10,11-epoxide accumulation affects antibody cross-reactivity. CEDIA is analytically acceptable for routine CBZ TDM in stable patients on monotherapy. HPLC and LC-MS/MS remain preferable when metabolite quantification, polytherapy monitoring, or precise measurement near therapeutic boundaries is clinically required.

Conclusion: Method selection should be guided by the clinical question, patient population characteristics, and available laboratory resources.

Keywords: Carbamazepine, CEDIA immunoassay, high-performance liquid chromatography, method comparison, therapeutic drug monitoring, clinical validation

Introduction

Carbamazepine (CBZ) is a first-generation antiseizure medication (ASM) widely used in the management of partial and tonic-clonic seizures, trigeminal neuralgia, and bipolar disorder [1,2]. Despite its long-standing clinical use, CBZ presents considerable pharmacokinetic challenges. Its narrow therapeutic index, with a serum therapeutic range of 4–12 µg/mL (approximately 17–51 µmol/L), means that small fluctuations in blood concentration can result in therapeutic failure at subtherapeutic levels or dose-dependent toxicity at supratherapeutic levels [1,3]. These characteristics make therapeutic drug monitoring (TDM) an essential component of clinical management, enabling individualized dose optimization, safety assessment, and monitoring of patient adherence [3,4].

The clinical complexity of CBZ TDM is further compounded by its pharmacokinetic variability. CBZ undergoes extensive hepatic metabolism, primarily via CYP3A4, to its pharmacologically active and potentially toxic metabolite carbamazepine-10,11-epoxide (CBZ-E) [5]. The ratio of CBZ-E to the parent drug can vary substantially among patients, particularly those receiving enzyme-inducing co-medications such as phenytoin, phenobarbital, primidone, and valproic

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acid [5]. In patients on polytherapy, a method capable of measuring only the parent drug may therefore provide incomplete clinical information, and the choice of analytical platform becomes a clinically consequential decision [5,6].

High-performance liquid chromatography (HPLC) has long been regarded as the reference standard for CBZ measurement. Its principal advantage lies in its capacity to separate CBZ from endogenous matrix components and co-administered drugs, with the ability to simultaneously quantify CBZ and its metabolites across clinically relevant concentration ranges [7,8]. However, HPLC entails significant operational demands, including the need for trained personnel, expensive instrumentation, longer turnaround times, and complex sample preparation, limiting its routine application in resource-constrained healthcare settings [4,9].

In response to these practical limitations, immunoassay-based platforms have been widely adopted for routine CBZ TDM. The Cloned Enzyme Donor Immunoassay (CEDIA), along with related platforms such as the Enzyme-Multiplied Immunoassay Technique (EMIT), Particle-Enhanced Turbidimetric Inhibition Immunoassay (PETINIA), and Chemiluminescent Microparticle Immunoassay (CMIA), offers substantially faster turnaround times, simpler operation, and lower per-sample costs [10,11]. These advantages have made immunoassay methods the predominant choice in high-throughput hospital laboratories worldwide.

Nevertheless, the analytical validity of immunoassay methods relative to chromatographic reference methods remains an area of active investigation. Immunoassays rely on antibody-antigen interactions that are susceptible to cross-reactivity with CBZ-E and to matrix effects from endogenous substances, concurrent medications, and pathological alterations in protein binding [6,11]. These factors can introduce systematic bias, with reported immunoassay overestimation of CBZ ranging from approximately 5.6% compared to UHPLC-MS/MS [12] to as high as 29.5% compared to LC-MS [13]. Whether such differences are clinically meaningful depends on the patient population and the clinical context in which TDM results are interpreted.

Despite an increasing number of comparative studies, no narrative review has systematically examined the agreement between CEDIA and HPLC across diverse patient populations and analytical conditions or evaluated the clinical implications of inter-method differences in routine practice. This review therefore aims to evaluate the analytical agreement and clinical validity of CEDIA compared to HPLC for measuring CBZ serum concentrations, to assess the practical suitability of CEDIA as a routine TDM tool, and to identify the conditions under which HPLC or more advanced chromatographic platforms remain the preferred analytical choice.

Methods

A narrative review of the literature was conducted to evaluate the analytical agreement and clinical validity of the CEDIA immunoassay compared to HPLC for measuring CBZ serum concentrations. Literature searches were performed across two databases: Scopus and Google Scholar, covering publications from January 2015 to June 2025.

The Scopus search employed the following Boolean string: TITLE-ABS-KEY (carbamazepine AND ("therapeutic drug monitoring" OR TDM) AND (CEDIA OR "cloned enzyme donor immunoassay" OR immunoassay) AND (HPLC OR "high-performance liquid chromatography" OR "liquid chromatography"))

The Google Scholar search used the keywords "TDM Carbamazepine," "CEDIA Carbamazepine Immunoassay," and "HPLC Carbamazepine."

Inclusion criteria were: (1) original research articles available in full text; (2) studies comparing at least two analytical methods for CBZ measurement in human biological matrices (serum or plasma); (3) studies reporting analytical validation parameters such as precision, accuracy, linearity, or inter-method agreement statistics; and (4) publications in English or Indonesian. Review articles, editorials, letters to the editor, and conference abstracts without full text were excluded, as were studies in which CBZ measurement was not a primary outcome.

The Scopus search yielded 19 articles, of which one was excluded as it was a review article, resulting in 18 original research articles. Combined with articles identified through Google Scholar, a total of nine studies were selected for inclusion based on their direct relevance to the comparison or validation of analytical methods for CBZ TDM. The selected studies are summarized in Table 1.

As this is a narrative review, article selection was purposive rather than exhaustive. The included studies were evaluated descriptively for methodological quality,

focusing on study design, sample size, analytical parameters reported, and statistical methods used for inter-method comparison. No formal risk of bias assessment tool was applied. Data synthesis was conducted narratively, comparing and contrasting findings across studies rather than performing quantitative meta-analysis.

Results

The literature search identified nine studies meeting the inclusion criteria, comprising studies that directly compared immunoassay and chromatographic methods for CBZ measurement, as well as studies validating individual analytical methods with full parameter reporting. The key characteristics and findings of the included studies are summarized in Table 1.

Analytical agreement between immunoassay and chromatographic methods

Across the studies included in this review, a consistent pattern emerges: immunoassay platforms for CBZ TDM demonstrate good to strong correlation with chromatographic reference methods, yet systematic positive bias is a recurrent finding. Dasgupta et al. reported a strong correlation between CEDIA and CMIA (r = 0.98), and both methods showed acceptable agreement with LC-MS/MS, confirming that CEDIA performs comparably to other immunoassay platforms when cross-reactivity with CBZ-E is low [11]. Similarly, Sakaguchi et al. found a good correlation between SR-HPLC and chemiluminescent immunoassay for CBZ (r = 0.969), with a slope of 1.072 indicating only minor proportional deviation [8]. Negrini et al. further confirmed that a routine immunoassay (Cobas CARB4) showed no statistically significant difference from LC-MS/MS for the parent drug measurement, supporting the clinical acceptability of immunoassay for routine CBZ quantification [6].

However, correlation coefficients alone can be misleading as indicators of method agreement. Bland-Altman analyses across several studies revealed clinically relevant systematic bias that correlation statistics would not detect. Bi et al. reported a mean overestimation of 29.5% by homogeneous enzyme immunoassay compared to LC-MS, with Passing-Bablok regression confirming non-equivalence between the two methods [13]. Jiang et al. similarly found that immunoassay overestimated CBZ by 5.6% relative to UHPLC-MS/MS [12]. While 5.6% may appear modest within the 4–12 µg/mL therapeutic range, a systematic upward bias of this magnitude could result in underestimation of true exposure and inappropriate dose reduction in patients near the lower therapeutic boundary. The substantially larger bias reported by Bi et al. underscores that the magnitude of overestimation is not uniform across immunoassay platforms and warrants careful consideration when selecting a method for clinical use.

Cross-reactivity with CBZ-E as a source of immunoassay bias

A critical determinant of immunoassay accuracy for CBZ is the degree of antibody cross-reactivity with CBZ-E. Dasgupta et al. reported cross-reactivity values of greater than 90% for PETINIA, compared to only 7.3% for CEDIA and 5.1% for CMIA [11]. This difference has direct clinical consequences: in the same study, 15% of specimens showed carbamazepine values more than 30% higher by PETINIA than by CMIA, and when PETINIA results were compared against the combined CBZ and CBZ-E concentration measured by LC-MS/MS, bias was effectively eliminated, confirming that PETINIA measures the sum of both compounds rather than the parent drug alone [11]. CEDIA and CMIA are therefore substantially more reliable than PETINIA in patients where CBZ-E accumulation is expected.

CBZ-E accumulation is particularly relevant in polytherapy. Burianová and Bořecká demonstrated that the CBZ-E/CBZ ratio increases significantly in patients receiving enzyme-inducing co-medications including phenytoin, phenobarbital, primidone, and valproic acid, with serum CBZ-E levels ranging from 1.38 to 27.79 µmol/L [5]. Negrini et al. similarly reported a wide CBZ-E/CBZ ratio of 3.37 to 12.55, and notably found only a weak correlation between CBZ and CBZ-E concentrations measured by LC-MS/MS (r = 0.639), directly confirming that parent drug levels alone are insufficient to predict metabolite burden [6]. In such patients, even immunoassay platforms with low CBZ-E cross-reactivity should be interpreted with caution, and chromatographic confirmation considered.

Performance of HPLC as the reference standard

The HPLC methods evaluated in the included studies consistently demonstrated the analytical characteristics expected of a reference standard. Milosheska and Roškar validated an HPLC-UV method for simultaneous quantification of 12 AEDs including

Table 1. Summary of studies on analytical method comparison and validation for carbamazepine therapeutic drug monitoring

No	Methods compared	Matrix and sample size	Concentration range	Precision (CV%)	Statistical analysis	Key findings	Ref
1	Routine immunoassay (Cobas CARB4) vs LC-MS/MS	Plasma; n = 30	CBZ: 11.9–49.5 µmol/L; CBZ-E: 2.1–11.9 µmol/L (LC-MS/MS results)	LC-MS/MS CV%: CBZ 2.2–4.0%; CBZ-E 6.5–7.7% (CV% of immunoassay not characterized)	Passing-Bablok regression, Bland-Altman plot	No significant difference between immunoassay and LC-MS/MS (absolute bias 0.400 µmol/L; 95% CI: -2.450% to 2.773%). CBZ-E/CBZ ratio showed wide variability (3.37–12.55; mean 6.52), confirming metabolite levels are unpredictable from parent drug concentration alone.	[6]
2	CEDIA vs PETINIA vs CMIA vs LC-MS/MS	Serum; n = 40 (3-immunoassay comparison); n = 15 (vs LC-MS/MS)	CMIA: 0.5–15.0 µg/mL; CEDIA & PETINIA: 0.5–20.0 µg/mL	CMIA within-run: 1.80–2.01%; between-run: 1.60–2.60% (precision of CEDIA not characterized)	Linear regression, correlation coefficient	Strong correlation between CEDIA and CMIA (r = 0.98). PETINIA showed significant positive bias (10-20% higher) due to high cross-reactivity with CBZ-E (>90%). CEDIA and CMIA showed comparable, lower cross-reactivity with CBZ-E (7.3% and 5.1% respectively), and both correlated well with LC-MS/MS.	[11]
3	LC/MS vs EMIT	Plasma; n = 93	LC/MS: 5–1000 ng/mL; EMIT: 2–20 µg/mL	Intra- and inter-day CV < 3% (LC/MS)	Bland-Altman plot, Deming regression	Good correlation between methods (R^2 = 0.971). EMIT yielded slightly higher CBZ concentrations than LC/MS. EMIT is faster and more cost-effective, supporting its use as a routine alternative	[14]
4	HPLC vs CMIA	Serum; n = 151 (51 monotherapy, 56 polytherapy with pharmacokinetic interactions, 44 other polytherapy)	Not reported	Not reported	Passing-Bablok regression	Close correlation between CMIA and HPLC. CMIA results were lower than HPLC (mean difference 3.8 µmol/L). CBZ-E/CBZ ratio increased significantly in patients receiving enzyme-inducing co-medications	[5]

No	Methods compared	Matrix and sample size	Concentration range	Precision (CV%)	Statistical analysis	Key findings	Ref
5	Homogeneous enzyme immunoassay (HEIA) vs LC-MS	Serum; n = 110	Not reported	Not reported	Spearman correlation, Bland-Altman plot, Passing-Bablok regression	High correlation (r = 0.982) but HEIA consistently overestimated CBZ compared to LC-MS (mean difference 29.5%). The two methods were not equivalent; reference ranges should be adjusted when switching between methods	[13]
6	UHPLC-MS/MS vs Chemiluminescent immunoassay (Siemens ADVIA Centaur)	Plasma; n = 118 (CBZ samples)	Not specifically reported for CBZ	< 6.70% inter-day (all analytes)	Bland-Altman plot	Immunoassay overestimated CBZ by 5.6% compared to UHPLC-MS/MS. Greater bias was observed for valproic acid (16.5%) and phenobarbital (40.3%), suggesting analyte-specific differences in immunoassay accuracy	[12]
7	SR-HPLC-UV vs Chemiluminescent immunoassay	Plasma; sample size not reported	CBZ: 0.40–50 µg/mL	RSD < 5.1%	Correlation coefficient, linear regression	Good correlation for CBZ (r = 0.969, slope = 1.072). SR-HPLC requires no external CBZ reference standard, offering an efficient and accurate option for routine clinical TDM	[8]
8	HPLC-UV method validation (single method; no direct immunoassay comparison)	Plasma; epilepsy patients on combination AED therapy	CBZ: 0.5–50 mg/L; CBZ-E: 0.2–10 mg/L	Met FDA guideline requirements	FDA bioanalytical guideline validation	Fully validated HPLC-UV method for simultaneous quantification of 12 AEDs and their main metabolites including CBZ and CBZ-E. Selective and suitable for routine TDM in polytherapy patients	[7]
9	HPLC-PDA method validation (single method; applied in monotherapy and polytherapy patients)	Plasma; epilepsy patients	0.5–16 µg/mL	Intra-day: 3.4–4.13%; Inter-day: 3.39–4.38%	Linearity, accuracy, precision, selectivity (EMEA guideline)	Valid HPLC-PDA method for CBZ plasma measurement. Meets EMEA guideline requirements. LOQ 0.5 µg/mL, LOD 0.2 µg/mL. Selective in polytherapy patients	[15]

CBZ and its metabolites CBZ-E and 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine, with precision and accuracy meeting FDA bioanalytical guideline requirements [7]. The ability to quantify both the parent drug and its metabolites in a single analytical run represents a clear advantage over immunoassay platforms, which are designed to detect only the parent compound. Budikayanti et al. further validated an HPLC-PDA method for CBZ plasma measurement across a clinically relevant concentration range of 0.5–16 µg/mL, with intra-day and inter-day precision below 4.4% and selectivity confirmed in polytherapy patients [15]. Sakaguchi et al. introduced a single-reference HPLC approach that eliminates the need for external CBZ reference standards while maintaining good correlation with immunoassay results, offering a practical modification that reduces the logistical burden of routine HPLC-based TDM [8].

Taken together, the HPLC studies included in this review confirm that HPLC provides superior absolute accuracy and metabolite detection capabilities. Its validated performance across diverse patient populations, including those on monotherapy and polytherapy, makes it the preferred method when comprehensive CBZ pharmacokinetic assessment is clinically required.

Practical considerations for method selection in clinical settings

The choice between immunoassay and HPLC for routine CBZ TDM ultimately depends on the clinical context, laboratory resources, and the patient population being monitored. For most outpatient settings requiring rapid turnaround and high-throughput processing, immunoassay platforms such as CEDIA offer a practical and analytically acceptable alternative to HPLC. The studies reviewed here consistently support the use of CEDIA and equivalent immunoassay platforms for stable patients on monotherapy whose CBZ concentrations are well within the therapeutic range. In these cases, the systematic bias observed between immunoassay and chromatographic methods is unlikely to influence clinical decisions materially.

Conversely, several clinical scenarios warrant the preferential use of HPLC or LC-MS/MS over immunoassay. These include patients on polytherapy with enzyme-inducing co-medications, where CBZ-E accumulation may inflate immunoassay results; patients with uncontrolled seizures or unexplained adverse effects where discrepancies between clinical presentation and laboratory results are suspected; patients with hepatic or renal impairment where altered protein binding and metabolite accumulation may affect immunoassay accuracy; and patients undergoing dose titration near the toxicity threshold where small concentration differences carry clinical significance. In addition, the comparison by Shaikh et al. of LC/MS against EMIT in 93 epileptic patients demonstrated that while overall correlation was good (R^2 = 0.971), EMIT consistently yielded slightly higher concentrations than LC/MS, reinforcing the recommendation that clinical decisions near the therapeutic boundaries should be supported by chromatographic confirmation [14].

From a laboratory management perspective, the shift toward LC-MS/MS as an increasingly accessible and cost-effective platform in tertiary laboratories also warrants consideration. Jiang et al. demonstrated that a single validated UHPLC-MS/MS method can simultaneously quantify 24 AEDs and their metabolites with inter-day precision below 6.7%, offering a comprehensive solution for institutions managing patients on complex antiepileptic regimens [12]. As this technology becomes more widely available, the gap in operational complexity between immunoassay and advanced chromatographic methods may progressively narrow.

Limitations and future directions

This review has several limitations that should be acknowledged. First, the number of studies directly evaluating CEDIA specifically, as opposed to other immunoassay platforms, remains limited. Several included studies employed CMIA, EMIT, or HEIA rather than CEDIA, and while these platforms share similar operational principles, their antibody characteristics and cross-reactivity profiles differ. Direct head-to-head comparisons of CEDIA against HPLC using standardized patient populations and analytical protocols are still needed to fully characterize the agreement between these two methods. Second, the included studies varied considerably in sample size, patient demographics, co-medication profiles, and the chromatographic comparator method used, limiting the generalizability of pooled conclusions. Third, as a narrative review, the study selection process was purposive rather than exhaustive, and the possibility of selection bias cannot be excluded.

Future research should prioritize prospective comparative studies that evaluate CEDIA performance specifically in high-risk subgroups, including pediatric patients, the elderly, pregnant women, and those with organ impairment, where inter-method differences are most clinically consequential. Studies examining the impact of specific drug-drug interactions on immunoassay accuracy, beyond the enzyme-inducing AEDs already investigated, would also strengthen the evidence base. The expanding availability of LC-MS/MS platforms in clinical laboratories further warrants investigation of standardized multi-analyte methods that could replace both immunoassay and single-drug HPLC methods in high-volume TDM programs.

Conclusion

This narrative review demonstrates that immunoassay methods, including CEDIA, provide clinically acceptable agreement with HPLC for routine CBZ TDM in most patient populations and clinical contexts. Strong correlations between immunoassay and chromatographic methods are consistently reported across the included studies, and the systematic bias observed is generally within acceptable clinical limits for stable patients on monotherapy. The operational advantages of immunoassay platforms, namely speed, simplicity, and cost efficiency, support their continued use in high-throughput hospital laboratories.

Nevertheless, HPLC and advanced chromatographic platforms such as LC-MS/MS remain indispensable in clinical scenarios requiring metabolite quantification, polytherapy monitoring with enzyme-inducing co-medications, or precise measurement near the therapeutic boundaries. The choice of analytical method for CBZ TDM should therefore be guided not by a universal preference for one platform over another, but by the specific clinical question being addressed, the characteristics of the patient population, and the analytical capabilities available in each laboratory setting.

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Conflict of interest

The authors declare no conflict of interest related to this study.

Author contributions

MAM conceptualized and designed the review. NF, YFI, and MRU conducted data collection and analysis. MAM and MRU wrote and revised the manuscript. All authors reviewed and approved the final manuscript for publication.

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