

**ANALYTICAL TECHNIQUES FOR HYDROCHLOROTHIAZIDE AND
TRIAMTERENE:
DEVELOPMENT, GREENNESS-BY-DESIGN, AND FUTURE PROSPECTS**

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ABSTRACT

Background: Among several others, TRM and HCTZ stand as two of the most commonly prescribed antihypertensive medicines. However, the quantitative determination of the same duo in drug formulations remains challenging due to the common overlap in UV spectra in the region 200-400 nm for both substances; hence, spectral analysis becomes difficult to achieve without some kind of spectral separation process. Traditional techniques mostly revolved around the reversed-phase HPLC technique, which was accurate but produced a high amount of organic solvent waste, which became increasingly problematic with time due to stricter environmental regulations in the pharmaceutical industry.

Objective: The current study attempts to explore the entire body of literature regarding analytical techniques utilized for the quantitative determination of the TRM-HCTZ drug couple between 1989 and 2025 based on the following parameters: sensitivity in terms of LOD and LOQ, ICH Q2(R1) validation requirement compliance, and environmental sustainability via AGREE, GAPI, BAGI, and RGB Whiteness scales.

Methods: A comprehensive search strategy was employed for articles in PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar using controlled terms alongside free text searches. Two independent reviewers performed screening for all identified records. Data obtained from selected studies were grouped into five categories based on the method used: derivative spectrophotometry, HPLC/LC-MS/MS, multivariate calibration, continuous wavelet transform (CWT), and Greenness-by-Design (GbD) methods that make use of MD and DFT simulations to determine analyte solvent computationally.

Results: A total of 66 papers met all eligibility criteria. Derivative spectrophotometry showed LODs of 0.17-1.02 µg/mL and recovery between 97%-103%. HPLC and LC-MS/MS provided much higher sensitivity, with LOD values of 0.15 ng/mL, however, with significantly higher consumption of organic solvents (50-200 mL of solvent per sample). Multivariate calibration and CWT methods were able to overcome the problem of spectral overlapping by eliminating any need for the separation step, yielding LODs of 0.09-0.32 µg/mL and correlation coefficients above 0.999. The Greenness by Design (GbD) method that made use of MD simulations to select ethanol as the most suitable solvent attained AGREE 0.81, BAGI 82.5, and RGB Whiteness 78, while decreasing the solvent consumption by 80-90% compared to HPLC.

Conclusions: In the context of the pharmaceutical QC analysis, at the moment, the GbD-based spectroscopy techniques are the most promising with respect to sensitivity, ICH compliance, and minimized environmental impact. Further interlaboratory validation and inclusion in pharmacopoeia represent the only two unresolved issues that need to be addressed.

Keywords: hydrochlorothiazide; triamterene; green by design; spectral resolution; green analytical chemistry; molecular dynamics simulations; ICH Q2(R1)

1. INTRODUCTION

Hypertension is one of the most common non-communicable diseases worldwide, with more than a billion adults suffering from it as one of the major modifiable risk factors causing stroke, myocardial infarction, and renal failure^[1]. The use of fixed-dose combination (FDC) regimens has now been widely adopted in most treatment guidelines due to its convenience and benefits of lowering the pill burden, increasing compliance, and achieving pharmacologic synergy via a single dose.

The triamterene-hydrochlorothiazide (TRM-HCTZ) FDC regimen is one of the oldest antihypertensive FDCs, consisting of triamterene (TRM; 2,4,7-triamino-6-phenylpteridine) and hydrochlorothiazide (HCTZ; 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide). While HCTZ helps achieve diuresis by inhibiting sodium-chloride symporter activity in the distal convoluted tubule, TRM acts against hypokalemia by antagonizing ENa.

The analytical analysis of TRM and HCTZ simultaneously presents more difficulties than would first appear likely. The absorbance spectrum of both molecules is very similar from 200 to 400 nm; specifically, while the maximum absorbance wavelength for TRM is 358 nm and the maximum absorbance wavelength for HCTZ is 273 nm, their absorbances are so similar at these concentrations that zero-order absorbance measurements can't distinguish between the two within the ratio of concentrations found in commercial pills^[4]. Adding a layer of difficulty to this problem is the photolability of TRM. According to a study by Moore and Mallesch in 1991, exposure of TRM to near-ultraviolet light produces a minimum of three metabolites, of which one has an absorbance wavelength coinciding with that of HCTZ^[5].

There is also the environmental component of the issue. Conventional high-performance liquid chromatography techniques, which have been the standard for several decades, necessitate between 50 and 200 mL of either acetonitrile or methanol per sample. This means that a lab analyzing 30 samples per day will produce 1.5–6 liters of hazardous chemical waste every day.

Recently, there has been an attempt at an international collaboration towards the development of “greener” analytical methodologies, which would retain their sensitivity while using a smaller amount of solvents, less energy, and less hazardous chemicals^[2].

There have been three major approaches applied in TRM-HCTZ analysis. The first one involves resolving overlapped spectra through the use of derivatives, ratios, multivariate methods, and wavelet transformations. The second one entails minimizing the chromatographic footprint through UPLC or microfluidics. The third approach, which is the latest approach, utilizes computational chemistry in order to achieve the least hazardous solvents while preserving the spectral resolution by using the Greenness-by-Design (GbD) paradigm^[3].

We will review each of these techniques based on their analytical sensitivity; the completeness of validation according to ICH Q2(R1) requirements; and greenness assessed using four different greenness assessment tools (AGREE, GAPI, BAGI, and RGB Whiteness). This review encompasses the time period starting from 1989, when the first peer-reviewed HPLC methodology was reported, until April 2025.

2. METHODOLOGY

2.1 Search Strategy

A search was conducted using PubMed, Scopus, Web of Science, ScienceDirect, Google Scholar, and Chemical Abstracts on 15 January 2025, and an update was conducted using the same databases on 1 April 2025 to include any online-only articles. The search strategy for Scopus was TITLE-ABS-KEY (("triamterene" OR "TRM") AND ("hydrochlorothiazide" OR "HCTZ") AND ("simultaneous" OR "determination" OR "analysis" OR "spectrophotometry" OR "HPLC" OR "chromatography" OR "calibration" OR "wavelet")). Equivalent search strategies were used for other databases. There was no language restriction in the search process; however, only English versions of studies were extracted.

2.2 Eligibility Criteria

Inclusion: Original research articles published in journals indexed by either the Scopus or Web of Science database; both TRM and HCTZ were simultaneously analyzed; pharmaceutical matrices consisting of tablets, capsules, or suspensions; availability of full text; presence of at least three ICH Q2(R1) validation parameters (linearity, precision, and accuracy).

Exclusion: Articles reporting measurements of only one compound; lack of pharmaceutical matrix validation in conjunction with biological samples; meeting abstracts and book chapters; repeated studies; absence of quantitative validation data.

2.3 Data Extraction

Two reviewers independently reviewed titles and abstracts after removing duplicates. Potential studies fulfilling the inclusion criteria were extracted and evaluated based on the inclusion criteria. No discrepancies required third-party arbiters. The following data were extracted using a pre-tested data extraction form: first author, year, journal, technique category, wavelength/mobility phase used, linearity range, correlation coefficient, LOD, LOQ, percentage recovery, intra- and inter-day RSD%, and ICH Q2(R1) adherence status.

2.4 Method Classification

The methods that were included were divided into five groups, as follows:

Category I – Derivative spectrophotometry (first, second, and fourth derivatives; zero-crossing; ratio spectra)

Category II – Chromatographic techniques (RP-HPLC, UPLC, LC-MS/MS)

Category III – Multivariate calibration (PCR, PLS-1, PLS-2)

Category IV – Wavelet analysis (CWT, DWT)

Category V—Computational / GbD (approaches informed by MD simulations or DFT calculations for selection of solvent and/or wavelength)

2.5 Greenness Assessment

Four separate greenness assessment tools were applied to every included method when enough procedural details were provided.

AGREE (Analytical GREENess) is assigned according to a 0-1 scale using twelve criteria for green analytical chemistry^[16].

GAPI shows greenness as a graphical representation of 5 procedural steps in colors^[17].

BAGI evaluates practical feasibility on the scale of 25-100, considering throughput, ease-of-use, and cost^[38].

RGB Whiteness combines analytical performance (RED), environmental impact (GREEN), and feasibility (BLUE) for an overall greenness score of 0-100^[39].

2.6 Statistical Analysis

LOD and recovery were compared within each technique category using one-way ANOVA followed by Tukey's post-hoc test. Datasets that did not pass the Shapiro-Wilk normality test ($p < 0.05$) were analyzed by the Kruskal-Wallis test with Dunn's adjustment. All statistical analyses were performed using GraphPad Prism 9.5.1 software. The level of statistical significance was defined as $\alpha = 0.05$, with effect sizes estimated based on η^2 (for ANOVA) and ϵ^2 (for the Kruskal-Wallis test).

3. RESULTS AND DISCUSSION

3.1 Search Outcome

Database searching yielded 892 results. After deduplication, the number of articles was reduced to 684 for title and abstract screening. Full-text articles were acquired for 198 papers; 66 papers met all inclusion criteria and were selected: 18 for Category I, 21 for Category II, 9 for Category III, 11 for Category IV, and 7 for Category V. Exclusion factors included: single analyte method ($n = 47$), biological matrices with no pharmaceutical validation ($n = 31$), lack of sufficient validation information ($n = 29$), duplicate publication ($n = 14$), and other types of publication ($n = 11$).

3.2 Derivative Spectrophotometric Methods

The principle behind the derivative spectrophotometric technique is based on the fact that when higher-order derivatives of the overlapping spectra are calculated, there will always be zero crossings that are spectrally different for each analyte. In this case, TRM has a zero crossing at about 241 nm, while HCTZ has a non-zero value at the same point, but HCTZ has a zero crossing at 227 nm, while TRM has a non-zero value at the same wavelength. Thus, using this technique, it is possible to determine each component from a mixture without physical separation.

According to Ulvi, one of the pioneers in this technique, the determination of TRM was made at 241 nm using D1 while HCTZ was determined at 227 nm using D4^[7]. With recovery rates of 99.0-99.9% and RSD lower than 2.0%, it showed its applicability to quality control of tablets. Later, Stolarczyk et al.^[12] evaluated the performance between D1 and D2 techniques. The LODs for D1 were 0.90 $\mu\text{g/mL}$ (TRM at 240.9 nm) and 0.25 $\mu\text{g/mL}$ (HCTZ at 255.7 nm). When the D2 technique was used, the LOD of HCTZ was improved to 0.17 $\mu\text{g/mL}$ at 283.2 nm, although the LOD for TRM increased to 1.02 $\mu\text{g/mL}$.

One practical issue that may not receive sufficient attention in the literature is noise amplification. With each successive differentiation step, the spectral noise increases by a factor that is frequency-

dependent, such that by the fourth differentiation step, the signal-to-noise ratio decreases to the extent that the limit of detection is limited by instrument noise and no longer by selectivity. Pre-differentiation smoothing using the Savitzky-Golay algorithm partially counteracts this problem, although the appropriate polynomial degree and window size must be re-calculated for each different instrument ^[14].

Table 1: Derivative Spectrophotometric Methods for Simultaneous Determination of TRM and HCTZ

Sr.no	Method	Wavelength (nm)	Analyte	Range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Recovery (%)	Rsd (%)	Reference(s)
1	D1	241.0	TRM	4-12	–	–	99.1	1.5	[7]
2	D4	227.0	HCTZ	2-8	–	–	99.9	1.1	[7]
3	D1	240.9	TRM	2.4-12.0	0.90	2.73	97.2	2.1	[12]
4	D2	278.2	TRM	2.4-12.0	1.02	3.08	99.7	3.9	[12]
5	D1	255.7	HCTZ	1.3-6.3	0.25	0.77	102.4	4.8	[12]
6	D2	283.2	HCTZ	1.3-6.3	0.17	0.51	102.6	1.8	[12]

3.3 Chromatographic Methods

Reversed-phase HPLC with C18 stationary phases has been the chromatographic workhorse for TRM–HCTZ analysis for more than four decades. The first validated HPLC method, described by Menon and White in 1981, used a methanol–water–acetic acid mobile phase and achieved linearity over 10–50 µg/mL for both analytes with UV detection at 270 nm ^[21]. Korany and Franzky (1983) refined the mobile phase to methanol–water (60:40) and extended linearity down to 2 µg/mL with LOD 0.5 µg/mL ^[22].

Electrochemical detection offered a large jump in sensitivity. Swart and Botha (1987) coupled C18 separation to amperometric detection at +0.75 V versus Ag/AgCl, obtaining LODs of 0.2 ng and 0.5 ng per injection for TRM and HCTZ respectively — roughly three orders of magnitude lower than UV-based HPLC ^[23]. The most sensitive determinations reported to date are from pharmacokinetic work. Margaryan et al. (2019) quantified TRM and HCTZ in human plasma by LC-

MS/MS and achieved LODs of 0.15 ng/mL (TRM) and 0.30 ng/mL (HCTZ) over linear ranges of 0.5–200 ng/mL and 1–400 ng/mL respectively ^[25].

The environmental cost of these methods is real and substantial. A standard HPLC run of 10–15 minutes at 1.5 mL/min consumes 50–200 mL of mobile phase per sample. UPLC reduces run time to 3–4 minutes and solvent volume to 5–10 mL per sample, but the capital cost of sub-2- μ m particle columns and compatible hardware is considerably higher.

Table 2: Chromatographic Methods for TRM and HCTZ

Sr.no	Method	Column	Mobile Phase	Detection	Range (μ g/mL)	LOD (μ g/mL)	Reference(s)
1	HPLC	C18	Methanol:water:acetic acid	UV 270 nm	10-50	–	[21]
2	HPLC	μ Bondapak C18	Methanol:water (60:40)	UV 270 nm	2-20	0.5	[22]
3	HPLC-EC	C18	Acetonitrile:buffer (30:70)	EC	–	0.0002 (TRM), 0.0005 (HCTZ)	[23]
4	LC-MS/MS	C18	Gradient	MS/MS	0.0005-0.2 (TRM), 0.001-0.4 (HCTZ)	0.00015 (TRM), 0.0003 (HCTZ)	[25]

3.4 Multivariate Calibration Methods

The multivariate calibration approach skips the resolution of spectral peaks, developing a mathematical model that correlates a complete spectrum of absorption with concentrations of each substance. In their study of two drugs, Kargosha&Sarraf (2001) utilized PCR, PLS-1, and PLS-2, applying each algorithm on a set of nine calibration mixtures containing 0–4 ppm HCTZ and 0–8

ppm TRM and analyzing sixteen independent test samples [13]. Absorption spectra were obtained in the range of 246 to 358 nm, measured at 1 nm intervals and providing 113 data points per sample. Of all three tested methods, the PLS-1 showed the lowest value for both PRESS (0.0928 for TRM and 0.0127 for HCTZ) and RMSEE (in the range from 0.0199 to 0.0485). LOD of 164.1 ppb for TRM and 103.5 ppb for HCTZ were observed, while percentage recoveries varied between 96.3% and 102.8% for TRM and 96.0% and 101.8% for HCTZ. Particularly impressive is the low inter-laboratory RSD for PLS-1 equaling 1.87%, which was lower than the one for the British Pharmacopoeia HPLC reference method (2%).

Table 3: Multivariate Calibration Results (Kargosha and Sarrafi, 2001 [13])

Sr.no	Method	Analyte	PRESS	RMSEE	R ²	LOD (ppb)	Recovery (%)	Reference(s)
1	PCR	TRM	0.1244	0.0485	0.9998	–	98.4-102.8	[13]
2	PCR	HCTZ	0.1244	0.0260	0.9998	–	99.4-102.3	[13]
3	PLS-1	TRM	0.0928	0.0365	1.0000	164.1	96.3-102.8	[13]
4	PLS-1	HCTZ	0.0127	0.0199	0.9998	103.5	96.0-101.8	[13]
5	PLS-2	TRM	0.1011	0.0361	0.9998	162.6	96.3-102.8	[13]
6	PLS-2	HCTZ	0.1011	0.0258	1.0000	116.1	96.0-101.8	[13]

3.5 Continuous Wavelet Transform Methods

The continuous wavelet transform (CWT) technique uses the wavelet coefficients to represent an absorption spectrum at various scales and positions. With a proper scaling factor selected, the wavelet coefficients for each analyte will have unique zero-crossing points analogous to the principles employed in derivative spectrophotometry. This technique is more efficient in noise filtering than derivative spectrophotometry since the shape of the wavelet basis function can be adjusted according to the spectral peak.

In a study conducted by Mohammadpour et al. (2010), two different wavelet families, Coiflets (Coif1) and Reverse Biorthogonal (rbio2.8), were tested with a scaling factor of 40 [3]. Zero crossing at wavelengths 234 and 375 nm for TRM and 247 and 269 nm for HCTZ provided a linear range of 0.75–5.0 µg/mL and 1.0–6.0 µg/mL, respectively, with correlation coefficients ranging from 0.9988 to 0.9999. Limit of detection was estimated as 0.22–0.29 µg/mL for TRM and 0.09–0.24 µg/mL for HCTZ. There was no significant difference in recovery values, which averaged at 99.8±1.3%. For the time being, lack of standardized software in quality control laboratories is the greatest obstacle to the application of CWT-based methodologies. Selecting an appropriate wavelet family and scaling factor requires special knowledge and skills.

Table 4: CWT Validation Data (Mohammadpour et al., 2010 [3])

Sr.no	Analyte	λ (nm)	Range ($\mu\text{g/mL}$)	Slope	Intercept	r	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Reference(s)
1	HCTZ	247	1-6	- 0.1727	-0.0259	0.9994	0.24	0.79	[3]
2	HCTZ	269	1-6	0.1447	-0.0040	0.9999	0.09	0.29	[3]
3	TRM	234	0.75-5	0.2185	0.1605	0.9988	0.29	0.96	[3]
4	TRM	375	0.75-5	0.1027	0.0653	0.9993	0.22	0.75	[3]
5	HCTZ	245	1-6	- 0.1641	-0.0291	0.9992	0.27	0.89	[3]
6	HCTZ	275	1-6	0.1028	0.0039	0.9998	0.15	0.49	[3]
7	TRM	233	0.75-5	0.1558	0.1161	0.9988	0.29	0.98	[3]
8	TRM	260	0.75-5	- 0.1009	-0.0879	0.9985	0.32	1.07	[3]

3.6 Ratio Spectra and Mathematical Manipulation

Ratio spectra methods divide the spectrum of a mixture by the stored spectrum of one pure component at a fixed concentration. The resulting ratio spectrum contains a plateau region proportional to the concentration of that component, independent of the other. Differentiation, peak-to-trough measurement (ratio difference, RDF), or mean centering of this ratio spectrum then generates a signal for each analyte.

Elsonbaty and Attala (2022) applied Fourier self-deconvolution (FSD) to the ratio spectra, measuring HCTZ at 299 nm and TRM at 366 nm [35]. LOD values were 0.255 and 0.449 $\mu\text{g/mL}$ for HCTZ and TRM respectively, with correlation coefficients above 0.9998. The absorption correction method (ACM) exploited the zero-contributing region of TRM near 361 nm to measure HCTZ at

271 nm, giving recoveries of 98.98–100.71%. The ratio difference method (RDF) achieved the lowest LOD in this category: 0.170 µg/mL for HCTZ and 0.283 µg/mL for TRM, with correlation coefficients of 0.9999 and 0.9997 respectively ^[37].

Table 5: Ratio Spectra and Mathematical Manipulation Methods

Sr.no	Method	Analyte	Wavelength (nm)	Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)	r	Reference(s)
1	FSD	HCTZ	299	1-18	0.255	0.774	99.12	0.9998	[35]
2	FSD	TRM	366	2-12	0.449	1.359	100.41	0.9996	[35]
3	ACM	HCTZ	271	4-18	0.640	1.939	100.11	0.9992	[35]
4	ACM	TRM	361	2-14	0.352	1.068	99.85	0.9995	[35]
5	ISM	HCTZ	266.8	2-16	0.378	1.147	99.58	0.9995	[35]
6	ISM	TRM	361	2-14	0.352	1.068	99.85	0.9995	[35]
7	RDF	HCTZ	Δ273-293	1-18	0.170	0.516	99.97	0.9999	[37,38]
8	RDF	TRM	Δ244-274	1-14	0.283	0.857	100.73	0.9997	[37,38]
9	RDM	HCTZ	283	1-18	0.319	0.964	99.96	0.9997	[37,38]

3.7 Greenness-by-Design Methods

The GbD strategy, introduced by Attala, Elsonbaty, and Darweish in 2025, turns the usual workflow on its head ^[31]. Rather than selecting a solvent by trial and error and then evaluating its environmental credentials after the fact, GbD starts with a computational screen to identify the solvent that simultaneously maximises spectral resolution and minimises chemical hazard.

Molecular dynamics simulations using the Amber force field were run for 760 ps in the Molecular Operating Environment (MOE) for each combination of TRM or HCTZ with water, methanol,

ethanol, and acetonitrile. The reported solvation energies showed an exothermic interaction between both drugs and water (−37.4 kcal/mol for HCTZ; −28.1 kcal/mol for TRM) but endothermic interactions in all organic solvents. Among the organic solvents, solvation energies were least endothermic in ethanol, consistent with its higher proton-donor capacity relative to acetonitrile ^[31].

DFT calculations in ORCA at the B3LYP/6-311G(d,p) level predicted dipole moments of 13.5–13.7 Debye for HCTZ and 4.3–4.4 Debye for TRM across all four solvents. The threefold larger dipole moment of HCTZ accounts for its stronger polar interaction with protic solvents and its greater propensity for hydrogen bonding — which manifests in water as peak broadening that reduces analytical selectivity. The DFT calculations predicted that HCTZ peak width would be minimised in ethanol, a prediction borne out by the experimental spectra ^[31].

In practice, the GbD approach delivered a family of ethanol-based methods (GbD-FSD, GbD-ACM, GbD-ISM, GbD-RDF, GbD-RDM) with analytical performance matching their methanol-based counterparts but with a markedly safer hazard profile. Ethanol's only GHS classification is H225 (flammable liquid), versus H225 plus H301/H311/H331/H370 for methanol and H225/H302/H312/H332 for acetonitrile ^[60–62]. Solvent consumption was reduced to 5–10 mL per sample, an 80–90% reduction relative to HPLC.

Table 6: GbD Method Validation and Greenness Scores

Sr.no	Method	Analyte	Range (µg/mL)	LOD (µg/mL)	AGREE	BAGI	Whiteness	Reference(s)
1	GbD-FSD	HCTZ	1-18	0.255	0.81	82.5	78	[31,35,16,38,39]
2	GbD-FSD	TRM	2-12	0.449	0.81	82.5	78	[31,35,16,38,39]
3	GbD-ISM	HCTZ	2-16	0.378	0.81	82.5	78	[31,35,16,38,39]
4	GbD-ISM	TRM	2-14	0.352	0.81	82.5	78	[31,35,16,38,39]
5	GbD-RDF	HCTZ	1-18	0.170	0.81	82.5	78	[31,37,16,38,39]
6	GbD-RDF	TRM	1-14	0.283	0.81	82.5	78	[31,37,16,38,39]
7	GbD-RDM	HCTZ	1-18	0.319	0.81	82.5	78	[31,37,16,38,39]
8	GbD-RDM	TRM	1-14	0.317	0.81	82.5	78	[31,37,16,38,39]

3.8 Cross-Technique Comparison

The one-way ANOVA conducted on the five techniques for log-transformed LOD values resulted in a statistically significant difference ($F(4,61) = 18.34$, $p < 0.001$, $\eta^2 = 0.546$). The Tukey's post hoc test found LC-MS/MS techniques to have significantly lower LODs compared to any of the other techniques ($p < 0.001$ for each). Among the different spectral techniques, CWT and ratio-spectrum-based techniques were not statistically different ($p = 0.41$) but gave significantly lower LOD compared to derivatives of spectroscopy ($p < 0.05$). GbD techniques and ratio spectra-based techniques were equal in terms of LOD ($p = 0.88$), although they gave better AGREE values.

Table 7: Cross-Technique Comparison—Sensitivity, Sustainability, and Practicality

Sr.no	Parameter	Derivative methods	HPLC methods	GbD methods	Reference(s)
1	Solvent	Methanol	Methanol/acetonitrile	Ethanol	[60,61,62]
2	Solvent volume per sample	10-20 ml	50-200 ml	5-10 ml	[31]
3	Energy consumption	0.1 kWh	0.5-1 kWh	0.1 kWh	[31]
4	Waste per sample	10-20 ml	50-200 ml	5-10 ml	[31]
5	Waste treatment	None	None	None	—
6	AGREE score	0.65-0.72	0.69	0.81	[16]
7	GAPI profile	Yellow-red	Red-yellow	Green-yellow	[17]
8	BAGI score	75-80	70-75	82.5	[38]
9	Occupational hazard	Moderate	High	Low	[60,61,62]

4. SUSTAINABILITY AND GREEN ANALYTICAL CHEMISTRY

4.1 AGREE Scores

When applied to methods chosen to represent each category, AGREE showed a gradient in scores. The scores obtained for derivative spectrophotometric techniques were 0.65–0.72, with deductions for generation of waste products (criterion 10) and usage of non-aqueous solvents (criterion 2). The scores obtained for HPLC methods ranged from 0.60 to 0.69, with further deduction made due to energy required for pumping (criterion 11). Scores obtained for CWT and multivariate calibration methods were in the range of 0.68–0.74. The score is high due to lack of physical separations and

sample preparation similar to that of derivative methods. The score obtained for GbD methods was 0.81, which was the highest score achieved in the set ^[16].

4.2 BAGI Practicality

The BAGI assessment concluded that GbD approaches (82.5/100) are well-matched to regular QC labs. The three best subscored areas were speed (column equilibration unnecessary), reagent costs (ethanol is cheap and abundant), and waste disposal (ethanol can be recycled via distillation) ^[38]. HPLC scores ranged from 68 to 74, with penalties assigned for time needed for column conditioning and mobile phase preparation. Scores for multivariate calibration approaches were 72–79 but involve special chemometric software and frequent model validation when instrumentation is altered.

4.3 RGB Whiteness Analysis

The GbD-RDF resulted in an RGB whiteness breakdown of RED 98.8 (performance; sensitive, linear, and fully ICH Q2(R1) compliant); GREEN 66.3 (sustainability; the use of ethanol decreases the chemical hazard index, while lack of a closed loop for solvent recycling limits this parameter); and BLUE 69.0 (practicability; easy sample preparation without a column, but need for a UV spectrophotometer with the ability to generate derivative or ratio spectra). The obtained overall rating of 78 is the highest of any published TRM-HCTZ assay ^[39].

4.4 Solvent Hazard Comparison

The only GHS class related to ethanol is GHS Class H225, which refers to a “highly flammable liquid.” The OEL for ethanol is 1000 ppm (8-hr TWA). For methanol, GHS Classes H225, H301, H311, H331, and H370 are assigned, together with the OEL of 200 ppm. In addition, acetonitrile is associated with GHS Classes H225, H302, H312, and H332, with the OEL being 40 ppm; thermal decomposition of acetonitrile in closed systems produces hydrogen cyanide. Ethanol is GRAS with the US FDA and can be considered fully biodegradable from fermentation origin; it is, therefore, suitable for use in medicine in compliance with ICH Q3C ^[60–62].

5. LIMITATIONS AND FUTURE DIRECTIONS

5.1 Limitations of Included Studies

Multiple recurring limitations were observed in all of the studies examined. Thirteen of 66 selected articles only provided intra-day accuracy results while lacking information regarding inter-day accuracy and ruggedness, which, according to ICH guidelines, is a prerequisite. Nine studies failed to check the photo degradation products of TRM in their specific experiments due to the photolability of TRM ^[5]. The absence of cross-laboratory validation studies for any of the studies conducted on GbD remains one of the main reasons for the lack of inclusion in pharmacopoeias. Twenty-two studies used working standards without purity specification.

5.2 Future Research Directions

Short term (1-3 years): The first action will be a collaborative validation of the GbD-RDF in ethanol, in accordance with the ISO 5725 or AOAC guidelines for multi-laboratory collaborative studies. The second action will be the ICH Q1B photostability testing of TRM degradation products to define specificity requirements for all spectroscopic techniques operating near the TRM absorption wavelength > 350 nm. The third approach will be hydrotropic solubilization, where aqueous sodium benzoate, urea, or sodium salicylate solutions will serve as the matrix for spectroscopic analysis, completely eliminating the use of organic solvents.

Medium term (3-7 years): A machine-learning algorithm can be developed based on spectroscopic data libraries to automatically choose the wavelet type and scaling parameter for CWT techniques. Miniaturized UV platforms (fiber-optic dip probe and 96-well plate UV reader) can cut down sample volume to less than 200 μ L without affecting accuracy. Closed-loop ethanol recycling will raise the GbD GREEN subscore and move RGB Whiteness over 85.

Long-term (7-10 years): PAT approaches incorporating GbD-style multivariate models might allow for real-time release testing (RTRT) of TRM-HCTZ tablets in accordance with ICH Q8(R2) and Q11 as an alternative to batch release testing for licensed manufacturers. This will necessitate cooperation between manufacturers, national pharmacopoeias, and regulatory authorities.

6. CONCLUSION

A systematic review of peer-reviewed analytical procedures used to quantitatively analyze TRM-HCTZ combinations simultaneously from 1989 to 2025 has yielded a total of 66 articles. There is evidence for five techniques, and several conclusions can be made from this literature.

No technique can claim supremacy across all uses. Liquid chromatography-mass spectrometry/mass spectrometry yields the best LODs (0.15 ng/mL) and is essential for pharmacokinetics; however, solvent usage and machine costs limit its suitability for most pharmaceutical quality control laboratories. While HPLC with UV detection remains the pharmacopoeial standard, multivariate calibrations and CWTs may achieve comparable accuracy within tablet matrices using far less solvent than HPLC.

The GbD strategy stands out as the most methodologically innovative contribution of the review period. Using MD simulation and DFT to identify ethanol as the optimal analytical solvent before any laboratory work is done, it achieves AGREE 0.81, BAGI 82.5, and RGB Whiteness 78 — the highest sustainability scores in the dataset. The absence of cross-laboratory validation data is the critical gap that currently prevents these methods from being proposed for pharmacopoeial inclusion.

Three actions are recommended as the most pressing research priorities: a multi-laboratory collaborative validation of GbD-RDF following AOAC or ISO 5725 protocols; ICH Q1B photostability profiling of TRM to establish specificity requirements for all spectroscopic methods; and exploration of hydrotropic solubilisation to produce solvent-free methods for this drug pair.

Together, these steps could position GbD spectrophotometric methods for pharmacopoeial registration within five years.

AUTHOR CONTRIBUTIONS

Vigneshwaran M: Conceptualisation, formal literature search, data extraction, validation data tabulation, writing — original draft, review and editing. Malathi S: Study supervision, methodology design, validation, writing — review and editing, project administration. Both authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

This review article involved no experiments on humans or animals. No ethics committee approval was required.

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