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In situ green analytical methods for the rapid and sensitive determination of a newly launched orphan anticancer drug; Tigecycline in infusion bags: comparative study

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Abstract

Background: Tigecycline (TIG), an antimicrobial agent indicated for complex bacterial infections, is now approved by FDA as an orphan chemotherapeutic agent for the treatment of acute myeloid leukemia due to its inhibitory effects on pathways of activating, signaling and abnormal mitochondrial function in cancer cells. TIG is mainly administered as intravenous infusion through centralized unit of oncology centers. This necessitates the continuous analytical quality control of the prepared solution in order to identify and quantify TIG for safe intravenous administration to patients. Moreover, the clinical staff exposure risk to toxic drugs during daily handling must be considered. Such concerns require a fast, cost-effective and green analytical procedure for sensitive determination of TIG directly in infusion bags. In this work, we propose a simple, rapid and green capillary zone electrophoretic (CZE) method for the sensitive assay of TIG directly in infusion bags, in addition to three simple and green spectrophotometric methods.

Results: TIG solutions corresponding to clinical ranges were detected in 5%glucose. Validation of all the proposed methods was according to ICH guidelines. Greenness assessment was performed depending on Green Analytical Procedure Index (GAPI) and the Eco-scale approach which showed that the proposed methods are better eco-friendly methods than reported ones. It also revealed the superiority of our proposed methods in terms of simplicity and sensitivity for TIG determination in infusion bags. Quantification limits obtained were significantly lower than the administered range of TIG in infusion bags and lower than its maximum serum concentration (C_{max}). This promotes the application of the proposed methods for the pharmacokinetics and bioavailability studies of TIG in various biological fluids.

Conclusions: This work reports, for the first time, CZE method for the direct and rapid determination of TIG and its separation from other components in intravenous infusion solution. The developed CZE method has several advantages over current chromatographic methods such as higher efficiency of separation within short analysis time, consumption of fewer quantities of chemicals and offering better resolution than HPLC. Moreover, three green spectrophotometric methods are also proposed for TIG determination that offer many advantages such as accuracy, precision, simplicity, specificity and facility of quantification and separation of the selected drug in infusion bags and pharmaceutical preparations without any techniques for extraction.

Keywords: Tigecycline, Capillary electrophoresis, Spectrophotometric methods, Infusion bags

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1 Background

Tigecycline (TIG) is a member in glycylcycline antibiotics (Fig. 1a) [1]. It was approved by FDA in 2005. Its structure is related to minocycline. In comparison with other tetracyclines, TIG has activity with a broad spectrum and lower susceptibility to resistance development. It is active against drug-resistant gram-positive bacteria such as methicillin-resistant *Staphylococcus Aureus* (MRSA), penicillin-resistant *Streptococcus pneumonia* and vancomycin-resistant *Enterococcus* (VRE). Studies showed that TIG is effective for treatment of complicated infections in skin or intra-abdominal infections. So TIG has an advantage of treating infections caused by resistant microorganisms without sacrificing safety [2].

Recently, many studies have presented the anticancer activity of TIG [3–5]. It has been recently approved by the FDA as an orphan chemotherapeutic agent for acute myeloid leukemia (AML). In AML cases, leukemia cells fulfill the high energy demands by enhancing the biogenesis and protein translation in mitochondria. Therefore,

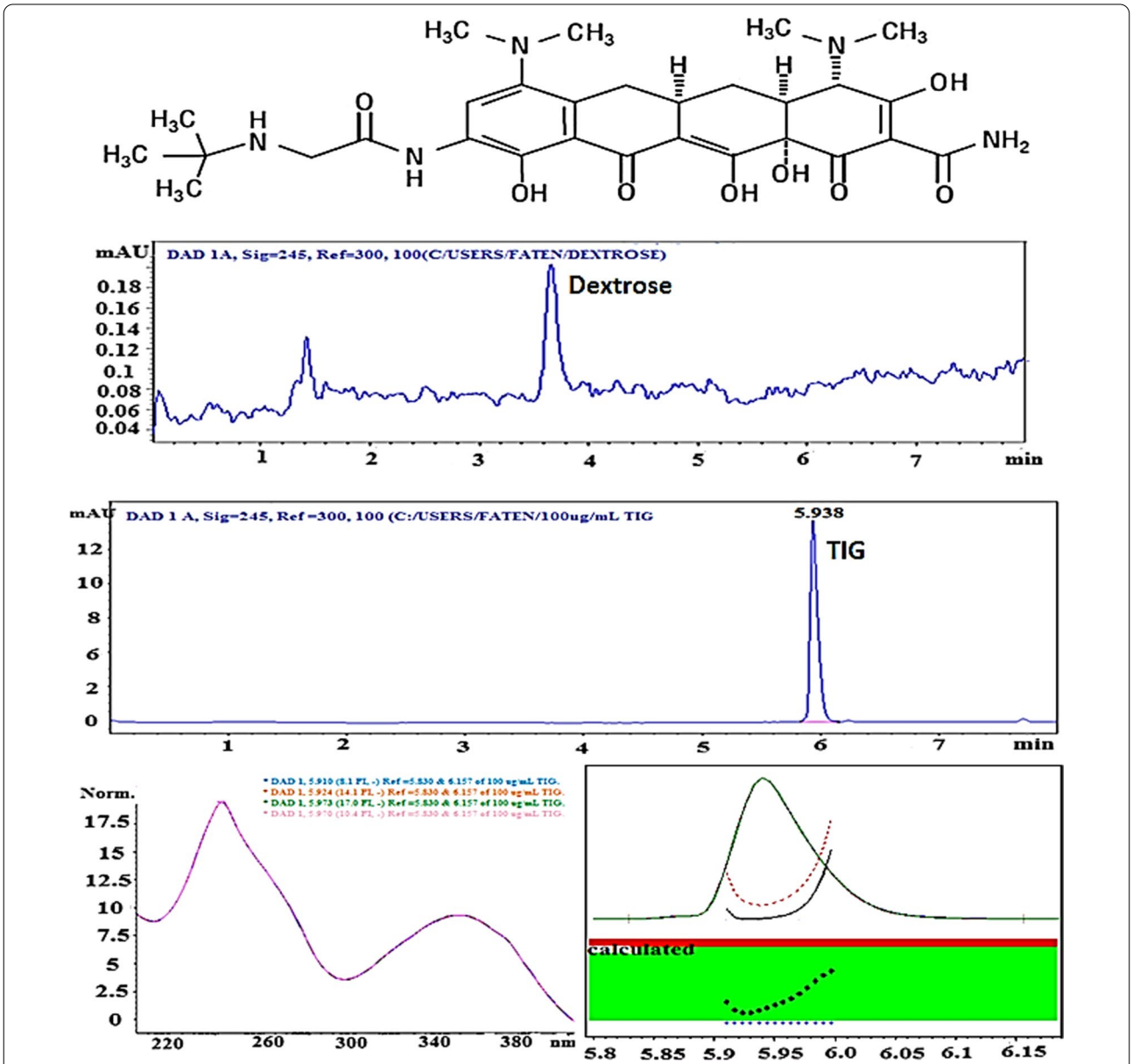

Fig. 1 (**a**) Chemical structure of TIG, (**b**) electropherogram of the 5% dextrose blank solution, (**c**) electropherogram of 100 µg/mL TIG in 5% dextrose injection solution using 50 µm internal diameter capillary of 56 cm effective length, (**d**) absorption spectra of TIG measured at 5 different time intervals across the peak and (**e**) its purity plot

TIG acts as an anticancer agent by blocking the mitochondrial protein synthesis that damages human AML cells at various stages of development [3–5].

TIG is mainly administered as intravenous infusion therapy. The antibiotic intravenous infusion therapy is a common therapy in hospitals, and it has become more popular in recent years, in addition to, TIG use in oncology centers for AML patients. Maximum precautions must be approached for each dose preparation to assure safe intravenous administration to patients and minimize the clinical staff exposure risk to dangerous chemicals. For this reason, sensitive and rapid analytical procedures are essential for the quality control of such dose preparations [6].

On literature survey, TIG has been assayed by only few analytical methods. These methods have been applied on the selected drug either in pharmaceutical preparations and biological fluids such as chromatographic methods including RP-HPLC [7, 8] and UPLC [9], spectrophotometric methods [10, 11] and spectrofluorometric methods [12, 13]. It is worth mentioning that TIG has not been determined previously using capillary zone electrophoresis (CZE) which represents an important green alternative analytical technique. Besides its high sensitivity, CZE has many advantages, such as short analysis time and low consumption of reagents and solvents. Thus, the aim of work is to develop green, cost-effective and selective analytical methods for determination of TIG directly in infusion bags without any prior sample preparation steps.

Moreover, the availability, simplicity and low cost of spectrophotometric methods are the basis to develop three green spectrophotometric methods for assay of TIG in both pharmaceutical preparations and intravenous infusion bags. Method I involves measuring amplitude of peak to peak for first derivative spectrophotometric analysis of TIG in methanolic solution at 334–372 nm. Method II depends on measuring the difference spectrophotometry (ΔA) and its first derivative (ΔD_1). The method depends on measurement of analytical responses from peak to peak at 342–401 nm and 279–311 nm for ΔA and ΔD_1, respectively, for TIG in acidic solution against its alkaline solution. Method III is based on reaction with 3-Methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) which is oxidative coupling reaction. This reaction is performed in the presence of ceric ammonium sulfate, Ce (IV), which is oxidizing agent to provide colored product with maximum absorbance at 409 nm after 35 min in a thermostated water bath at 40 °C.

The proposed work provides simple and green methods for determination of TIG either in their bulk or dosage form as intravenous infusion solution without interfering of other components. On the proposed methods, ICH guidelines were applied to guarantee validation of the methods either in routine work or in quality control units. The developed methods proved to be simple, highly reproducible with high selectivity and sensitivity for the determination of TIG. The comparison between the reported and developed methods and assessment of their greenness depend on two approaches: Green Analytical Procedure Index (GAPI) and the Eco-scale penalty points approach (PPs) showed that the proposed methods are the most eco-friendly methods.

2 Material and methods

2.1 Instrumentations and materials

Measurements were performed on Agilent Capillary Electrophoresis Instrument 7100 series (Agilent Technologies Deutschland, GmbH, Hewlett-Packard-Str. 8 Waldbronn, Germany) with diode array detector. A deactivated fused silica capillary from Agilent (Waldbronn, German with the following dimensions: 56 cm effective length and 50 μm id) was used. Measurements were performed at ambient temperature. Samples were injected with hydrodynamic mode by applying pressure equal to 50 mbar for 20 s with applied voltage of 30 kV. The data were measured and manipulated using ChemStation software of Agilent. Adjusting pH of solutions is performed using JENWAY pH meter (model 3505). For spectrophotometry, analysis is applied using Helios alpha UV–Vis spectrometer from England in 1-cm quartz cuvettes. The responses were obtained from vision32 software and were manipulated on Excel™ software.

Authentic TIG was purchased from Pfizer Company, Giza, Egypt. Tygacil® vials, orange powder, with 50 mg of TIG were obtained from local market. Sodium dihydrogen orthophosphate was obtained from Oxford Lab Chem, Mumbai, India. Ortho-Phosphoric acid 85% was obtained from El-Nasr Chemical Industry Company. Sodium hydroxide was purchased from Union Drug & Chemical Company, respectively. Dextrose intravenous infusion 5% w/v was obtained from El Fath for Drug and Cosmetics Industry (FIPCO). HPLC and analar methanol were obtained from SD Fine-Chem Limited (SDFCL) and Sigma-Aldrich Chemie GmbH (Germany), respectively. Dimethylformamide (DMF) was a product of Pharmachem & Company, Kolkata. 3-Methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH, 99.0%) and ceric ammonium sulfate were products of Sigma-Aldrich Company and of Qualitech Fine Chemicals, India, respectively.

2.2 Capillary zone electrophoretic method

On every working day, the capillary was flushed for 10 min with 0.5 M NaOH and then for 10 min with

water, followed by flushing for 5 min with 0.1 M NaOH. To guarantee activation of the inner capillary wall, waiting step for 2.5 min was applied followed by washing the capillary for another 5 min with water. Lastly, we equilibrated it with phosphate buffer (running buffer) pH 2.0 for 10 min. The capillary was washed for 2 min with the selected phosphate buffer between injections. Phosphate buffer pH 2.0 was always freshly prepared before each analysis.

The separation was performed on a deactivated fused silica capillary (56 cm effective length and 50 µm id) by assessing peak area at 245 nm. Measurements were performed at ambient temperature. Samples were injected with applying pressure equal to 50 mbar as hydrodynamic mode for 20 s at 30 kV as applied voltage.

For the CZE, 2 mg/mL standard stock solution of TIG was prepared in 5% dextrose. Working solutions were prepared by transferring various aliquots of stock solution into a set of 10 mL-volumetric flasks to obtain concentration ranges of 5–300 µg/mL. Such volumes were completed to 2 mL with 5% dextrose solution. Finally, solutions were diluted with distilled water to final volume (10 mL). Each solution was measured three times according to the optimal conditions by using phosphate buffer (running buffer), pH 2.0. Peak areas of TIG peak were plotted against the corresponding concentrations to construct calibration graphs.

2.3 Spectrophotometric methods

2 mg/mL stock solution of TIG was prepared in 5% dextrose in methods I and II, while for method III, aliquot from the stock solution was transferred to prepare standard solution containing 500 µg/mL in 0.1 M HCl.

2.3.1 Method I: First derivative spectrophotometric method

Solutions with concentration range 20–120 µg/mL were prepared from stock solution by accurately introduced into a set of 10-mL volumetric flasks followed by addition of dextrose to 1.0 mL as shown in Additional file 1. Then, dilution to final volume was performed by methanol. Working solutions were measured from 200 to 600 nm against blank. Manipulation of data was performed to obtain D_1 spectrum ($\Delta\lambda = 5$ nm) of each solution. D_1 amplitudes from peak to peak at 334–372 nm were measured and plotted against the concentrations of TIG to construct the calibration graph.

2.3.2 Method II: Difference spectrophotometric analysis (ΔA) and its first derivative (ΔD_1)

Equal portions from the standard stock solution, with 40–140 µg/mL as concentration range, were accurately transferred into two sets of 10-mL volumetric flasks followed by addition of 5% dextrose to 1.0 mL as shown in Additional file 1. The first set was diluted to volume with 0.1 M HCl, while 0.1 M NaOH was the diluent for the second set. The solutions were measured from 200 to 600 nm. The ΔA and ΔD_1 spectra of drug solutions in 0.1 M HCl were recorded against the corresponding drug solutions in 0.1 M NaOH. The amplitudes of ΔA and ΔD_1 ($\Delta\lambda = 5$ nm) from peak to peak at (342–401) and (279–311) nm were measured, respectively, and were plotted against concentrations of TIG in order to obtain the calibration graphs.

2.3.3 Method III: Colorimetric spectrophotometry

Different volumes from the standard working solution of TIG (500 µg/mL), within the linearity range 20–140 µg/mL, were accurately put into a set of 10-mL volumetric flasks. 1.0 mL of 0.01 M MBTH was added. Then, 1.5 mL of 1% Ce (IV) solution was added to each flask as shown in Additional file 1. The solutions were left for 25 min before dilution to final volume with DMF solvent. A value of each solution was scanned from 200–600 nm against a similarly prepared blank after 35 min in a thermostated water bath at 40 °C. The absorbance values at λ_{max} 409 nm were plotted against corresponding concentrations to get the calibration graph.

2.4 Sample preparation (Tygacil®)

1 mg/mL stock solution was prepared by reconstitution of Tygacil® vial (50 mg) in 50 mL 5% dextrose. According to each proposed method, repeated measurements were performed within the specified ranges in Table 1 to ensure that the method is repeatable and reproducible.

3 Results

3.1 Validation parameters

The proposed methods were validated depending on guidelines of International Conference on Harmonization (ICH) guidelines [14].

3.1.1 System suitability parameters

According to ICH Guidelines, studying system suitability parameters is an integral part in electrophoretic methods. The system suitability parameters were performed to confirm peak symmetry as tailing factor,

Table 1 System suitability parameters for TIG analysis in intravenous infusion solution for CZE method

Parameters	CZE Method
tR ± SD (min)	5.95 ± 0.04
Retention factors (k′)	3.58
Theoretical plates (N)	79,140
USP tailing factor	1.25

separation efficiency as theoretical plate number and reproducibility of migration as %R.S.D of migration time. Values of these parameters and their accepted criteria are presented in Table 1.

3.1.2 Linearity

Calibration graphs are obtained by plotting responses of each method at the selected wavelengths against TIG concentrations. Regression parameters are demonstrated in Table 2. The high values of correlation coefficient ($R \geq 0.999$) with small intercepts indicate good linearity with slight scattering of the experimental points from the regression line.

3.1.3 Detection and quantification limits

These limits were calculated depending on ICH guidelines equations [14]. In electrophoretic method, estimation of detection limit (DL) was obtained from the signal-to-noise ratio (S/N), where DL = 3.3 S/N. But for quantification limit (QL), the equation is QL = 10 S/N, while for the three spectrophotometric methods, DL was calculated as 3.3 σ/S and QL calculated as 10 σ/S. In these equations, σ refers to standard deviation of responses, while S refers to calibration curve's slope. Low values of these limits were obtained for each drug as presented in Table 2. It is worth mentioning that the DL and QL obtained by the proposed methods were significantly lower than the administered range of TIG in infusion solutions and lower than its reported C_{max} [3]. This demonstrates the sensitivity of the proposed methods and the feasibility to apply such methods to determine TIG in its infusion bags and in patients' biological fluids.

3.1.4 Accuracy

Different concentrations of TIG, within the linearity range, were prepared, and each solution was measured three times ($n = 3$). Results of measurements were manipulated to obtain values of percentage relative error (E_r %) which is not more than 2% and accepted percentage recoveries indicating high accuracy of the proposed methods (Table 3).

3.1.5 Precision

Intra-day precision is achieved through measuring three different concentrations of TIG three times on the same day. But inter-day precision is performed using similar procedure but on three consecutive days. The precision is presented as relative standard deviations (RSD %) as demonstrated in Table 3. The obtained results for precision studies show that RSD % is not more than 2% which means that the proposed methods are highly precise.

3.1.6 Stability of solutions

The stability of solutions was tested on 2 mg/mL TIG stock solution at approximately 4 °C for a week. Solutions prepared from this stock were measured indicating high stability of the selected drug under selected conditions with no significant changes in responses at maximum wavelengths. The values of %RSD were within the accepted limit.

3.1.7 Selectivity

For CZE, under the optimized conditions, TIG peak was well separated from matrix peaks of 5% dextrose

Table 2 Analytical parameters for the quantification of TIG in intravenous infusion solution

Parameters	CZE method	Spectrophotometric methods			
		Method I	Method II		Method III
			ΔA	ΔD_1	
Linearity range (µg/mL)	5–300	20–120	40–140	40–140	20–140
QL (µg/mL)	3.25	11.46	17.02	15.58	24.41
DL (µg/mL)	0.98	3.78	5.62	5.14	8.06
Intercept (a)	6.34×10^{-2}	1.33×10^{-2}	4.38×10^{-2}	5.56×10^{-2}	2.78×10^{-1}
Slope (b)	5.01×10^{-1}	3.43×10^{-3}	9.13×10^{-3}	4.71×10^{-3}	2.91×10^{-3}
Correlation coefficient (r)	0.9999	0.9997	0.9995	0.9994	0.9990
S_a	3.00×10^{-1}	3.84×10^{-3}	2.06×10^{-2}	9.73×10^{-3}	6.91×10^{-3}
S_b	2.18×10^{-3}	4.74×10^{-5}	2.02×10^{-4}	9.55×10^{-5}	7.37×10^{-5}
S_b^2	4.74×10^{-6}	2.25×10^{-9}	4.08×10^{-8}	9.12×10^{-9}	5.43×10^{-9}
$S_{y/x}$	6.25×10^{-1}	4.00×10^{-3}	1.55×10^{-2}	7.34×10^{-3}	7.10×10^{-3}
F	52,936.46	5237.07	2042.96	2437.61	1556.59
Significance F	7.73×10^{-15}	5.81×10^{-6}	2.38×10^{-5}	1.83×10^{-5}	3.58×10^{-5}

Table 3 Intra-day and inter-day precision and accuracy for the determination of TIG in intravenous infusion solution using the proposed methods

Method	Conc. (µg/mL)	Average % recovery ± SD[a]	% RSD[b]	% E_r[c]
(a) Accuracy and intra-day precision				
CZE method	20	99.85 ± 1.52	1.52	− 0.15
	100	99.23 ± 1.79	1.80	− 0.77
	300	99.47 ± 0.34	0.34	− 0.53
Spectrophotometric methods				
Method I	40	101.37 ± 0.42	0.42	1.37
	100	98.60 ± 0.37	0.37	− 1.39
	200	101.84 ± 0.37	0.37	1.84
Method IIA	40	100.85 ± 1.44	1.43	0.85
	80	101.06 ± 1.55	1.53	1.06
	140	98.61 ± 0.33	0.33	− 1.39
Method IIB	40	101.44 ± 0.85	0.83	1.44
	80	101.82 ± 0.76	0.75	1.82
	140	100.18 ± 0.61	0.61	0.18
Method III	20	100.38 ± 1.55	1.54	0.38
	80	100.67 ± 0.46	0.46	0.67
	140	99.38 ± 0.05	0.05	− 0.62
(b) Accuracy and inter-day precision				
CZE method	20	101.18 ± 0.99	0.98	1.18
	100	99.03 ± 1.97	1.99	− 0.97
	300	98.78 ± 0.99	1.01	− 1.22
Spectrophotometric methods				
Method I	40	101.86 ± 0.42	0.41	1.86
	100	98.35 ± 0.56	0.57	− 1.65
	200	101.60 ± 0.28	0.27	1.60
Method IIA	40	98.01 ± 1.93	1.96	− 1.99
	80	100.92 ± 1.96	1.94	0.92
	140	98.65 ± 0.19	0.19	− 1.35
Method IIB	40	99.58 ± 0.90	0.91	− 0.42
	80	98.75 ± 1.41	1.43	− 1.25
	140	100.14 ± 0.18	0.18	0.14
Method III	20	98.63 ± 0.57	0.58	− 1.37
	80	99.41 ± 1.41	1.42	− 0.59
	140	98.49 ± 1.18	1.19	− 1.51

[a] Average percentage recovery ± standard deviation for three determinations

[b] % Relative standard deviation

[c] % Relative error

intravenous infusion solution as dextrose has small peak area value with migration time 3.65 s, while TIG appears at 5.94 s as shown in Fig. 1b and c. Moreover, the peak purity of TIG was assessed using photo-DAD (Fig. 1e). For the spectrophotometric methods, application of difference spectrophotometry (ΔA) has an advantage of increasing the selectivity by eliminating the foreign light absorbance effect or foreign substances on the absorbance of the analyte. Also, method III relies on using MBTH as analytical reagent which is selective to aromatic amines drugs.

3.1.8 Robustness

The method was found to be robust after applying slight variation in selected parameters (Table 4). These parameters involved buffer pH and concentration, wavelength and injection time for electrophoretic method. For spectrophotometric methods, the examined parameters were the wavelength, reaction time, volume of reagent and temperature.

3.2 Analysis of pharmaceutical formulation (Tygacil®)

Assay of TIG antibiotic in its infusion solution is achieved depending on our proposed methods. Analyses are performed for each method with its specification at selected wavelength. The obtained values of recovery% and RSD% ensure accuracy and precision of these methods as shown in Table 5. Thus, these methods are applicable for determination of the investigated drug in intravenous infusion solution with acceptable accuracy and precision. Student's t test and the variance ratio F-test are suitable tools for statistical comparison between the proposed methods and reported one [7]. The results of comparison show that there is no significant difference between the methods either in accuracy or in precision (Table 5).

3.3 Comparison with reported methods

Literature review involved few analytical procedures for assay of TIG, such as chromatographic [7–9], spectrophotometric [10, 11] and spectrofluorometric methods [12]. Table 6 demonstrates a comparison between our methods and six reported methods for analysis of TIG. It was obvious that our proposed methods have better sensitivities than most of reported methods with lower LOD and LOQ values. Also, our reported methods have larger linearity ranges than those of the reported methods that allow a wider range of applications for the proposed methods. Furthermore, the advantages of the proposed methods are being simple and inexpensive without any pre-treatment steps that may lead to loss of sample. Also, using few quantities of solvents is another advantage where dextrose and water are used as solvents (not hazardous solvents). These solvents enhance the greenness of the proposed methods against other chromatographic methods which involve the use of expensive organic solvents. CZE has many advantages including high separation efficiency with short migration times, small quantities of solvents and reagents and the possibility to analyze even untreated biological samples with good resolution and simple development of sample.

Table 4 Robustness evaluation for the analysis of TIG in intravenous infusion solution using the proposed methods

Parameters	Recovery ± RSD %	
	CZE method	Method III
(1) pH of phosphate buffer (2 ± 0.1)	98.96 ± 0.49	–
(2) Concentration of phosphate buffer (20 ± 0.5 mM)	100.27 ± 1.01	–
(3) Injection time (20 ± 1 min)	99.76 ± 0.69	–
(4) Maximum wavelength (λ_{max} ± 2 nm)	99.36 ± 0.74	101.21 ± 0.52
(5) Volume of reagents		
(a) Volume of 0.01 M MBTH (1 ± 0.05 mL)	–	100.47 ± 0.24
(b) Volume of 1% W/V Ce (IV) (1.5 ± 0.1 mL)	–	100.51 ± 0.58
(6)Variation in time (35 ± 1 min)	–	99.90 ± 1.05

Table 5 Application of the proposed methods for determination of TIG in intravenous infusion solution for five determinations

Pharmaceutical preparation	Recovery %[a] ± % RSD[b]				
	CZE method	Spectrophotometric methods			Reported HPLC method [3]
		Method I	Method II	Method III	
Tygacil® vials	100.89 ± 0.52	100.69 ± 0.73	100.67 ± 0.54	100.50 ± 0.54	101.01 ± 0.46
t^c	0.38	0.83	1.08	1.60	
F^d	1.24	2.43	1.32	1.32	

[a] Average of three determinations

bRelative standard deviation

[c] Represents calculated values of *t*

d Represents calculated values of *F*

Thus, the developed methods proved to be simple, highly reproducible with high selectivity and sensitivity for the determination of TIG.

3.4 Greenness of the methods

Assessing the environmental effect of various analytical procedures is necessary before development of analytical procedures. The greenness of the analytical procedures was assessed by using different tools. Green Analytical Procedure Index (GAPI) is an important tool to evaluate the greenness in analytical protocols. This is performed depending on information about the analytical procedures from collection to determination of the sample. GAPI performs pictograms to allow comparison between the greenness of the analytical methods using three colored scales. The most eco-friendly stage takes green color, medium one is yellow, and red color represents less eco-friendly stages.

According to GAPI pictograms (Fig. 2), the difference between the proposed and reference methods is more visible. Spectrophotometric methods I and II are the most eco-friendly and similar in greenness followed by method III. CZE method has less green nature than proposed spectrophotometric methods. This is due to the requirements of CZE method to filtration and energy consuming (Table 7). But reference method is the worst green method. GAPI cannot be used as quantitative tool. To get more information, Eco-scale penalty points (PPs) are calculated where the proposed spectrophotometric method II is the most eco-friendly method, reference RF-HPLC method is the worst one and the other methods are in between as shown in Table 7 [15]. All evaluated procedures have the advantage of no need of more extraction steps.

It was obvious after comparison that the proposed methods are the most eco-friendly method with high. eco-scale score, higher than 75. This indicates that such methods are excellent eco-friendly methods, while the RP-HPLC reference method is acceptable owing to consumption of large amount of organic solvents and more energy consumption [16].

CZE analysis provides a greener alternative and complementary method to the more frequently used chromatographic methods. It has several advantages such as high separation efficiency with short migration time, small quantities of solvents and reagents and the possibility to analyze even untreated biological samples with good resolution. Also, it is a fast and green technique for sample

Table 6 Comparison of the proposed methods to other reported methods for TIG determination in intravenous infusion solution

Parameters	Proposed methods	Reported methods
Analytical reagent	CE method: –––	RP-HPLC [7]: 1-Hexane sulfonic acid sodium monohydrate salt
	Spectrophotometric method	Stability-Indicating RP-LC [8]:–––
	Method I: –––	UPLC-PDA [9]: –––
	Method II: –––	AUC Spectrophotometric method [10]: –––
	Method III: MBTH (chromogenic reagent)	Thermal analysis Spectrophotometric method [11]: Copper acetate reagent for visible method
		Fluorimetric method [12]: –––
λ_{max}	CE method: 245 nm	RP-HPLC [7]: 247 nm
	Spectrophotometric methods	Stability-Indicating RP-LC(8): 280 nm
	Method I: from peak to peak at 334–372 nm	UPLC-PDA [9]: 350 nm
	Method II ΔA: from peak to peak at 342–401 nm	AUC Spectrophotometric method [10]:between 240 to 256 nm
	Method II D_1: from peak to peak at 279–311 nm	Thermal analysis Spectrophotometric method [11]: 245 nm for UV method and 378 nm for visible method
	Method III: 409 nm	Fluorimetric method [12]: $\lambda_{ex}=333$ nm and $\lambda_{em}=453$ nm
Linearity range	CE method: 5–300 µg/mL	RP-HPLC [7]:40–60 µg/mL
	Spectrophotometric methods	Stability-Indicating RP-LC [8]: 40–100 µg/mL
	Method I: 20–200 µg/mL	UPLC-PDA [9]: 0.024 to 6 µg/mL
	Method II ΔA and D_1: 40–140 µg/mL	AUC Spectrophotometric method [10]: 4–20 µg/mL
	Method III: 20–140 µg/mL	Thermal analysis Spectrophotometric method [11]: 10–22 µg/mL for UV method and 10–34 µg/mL for visible method
		Fluorimetric method [12]: 0.1–2.0 µg/mL
LOQ	CE method: 3.25 µg/mL	RP-HPLC [7]: 5.42 µg/mL
	Spectrophotometric methods	Stability-Indicating RP-LC [8]: 5.05 µg/mL
	Method I: 11.46 µg/mL	UPLC-PDA [9]: 0.024 µg/mL
	Method II ΔA: 17.02 µg/mL	AUC Spectrophotometric method [10]: 0.08 µg/mL
	Method II D_1: 15.58 µg/mL	Thermal analysis Spectrophotometric method [11]:1.00 µg/mL for UV method and 0.89 µg/mL for visible method
	Method III: 20.00 µg/mL	Fluorimetric method [12]: 0.1 µg/mL
LOD	CE method: 0.98 µg/mL	RP-HPLC [7]: 1.8 µg/mL
	Spectrophotometric methods	Stability-Indicating RP-LC [8]: 1.67 µg/mL
	Method I: 3.78 µg/mL	UPLC-PDA [9]: 0.006 µg/mL
	Method II ΔA: 5.62 µg/mL	AUC Spectrophotometric method [10]: 0.02 µg/mL
	Method II D_1: 5.14 µg/mL	Thermal analysis Spectrophotometric method [11]:0.33 µg/mL for UV method and 0.29 µg/mL for visible method
	Method III: 8.06 µg/mL	Fluorimetric method [12]: 0.033 µg/mL
Correlation coefficient	CE method: 0.9999	RP-HPLC [7]: 0.9999
	Spectrophotometricmethods	Stability-Indicating RP-LC [8]: 0.9997
	Method I: 0.9997	UPLC-PDA [9]: > 0.995
	Method II ΔA: 0.9995	AUC Spectrophotometric method [10]: 0.9991
	Method II D_1: 0.9994	Thermal analysis Spectrophotometric method [11]: 0.9998 for UV method and 0.9997 for visible method
	Method III: 0.9990	Fluorimetric method [12]: 0.9997

development [17–21]. Thus, it was valuable to develop a CZE method for rapid and effective separation and determination of TIG in intravenous infusion solutions.

4 Discussion

4.1 Capillary Zone Electrophoretic method

4.1.1 Optimization of CZE conditions

Different factors were applied to obtain optimal conditions for separation of TIG in intravenous infusion solution such as type, pH and concentration of the buffer, time of injection, applied voltage and detection wavelengths (Table 8).

4.1.1.1 Buffer pH TIG has basic pK_a of 8.97. It is specified to present in the positively ionized form for lower pH [22]. Buffer pH was examined within the pH_s from 2 to 9 using 20 mM of phosphate buffer in CZE. The best peak shape and baseline shape were observed at pH 2. Higher pH showed distorted peak and baseline shape.

4.1.1.2 Buffer type By comparing 20 mM phosphate buffer, and 20 mM acetate buffer and 20 mM citrate buffer at pH 2, the obtained result is that phosphate buffer showed accepted peak and baseline shape. Therefore, phosphate buffer pH 2 was the selected one.

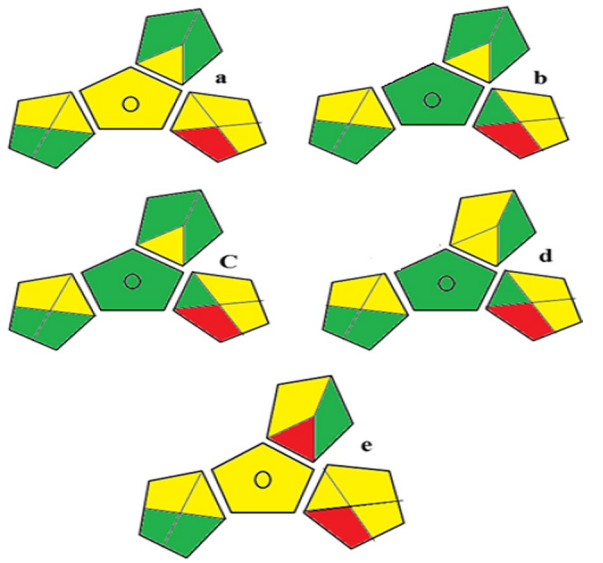

Fig. 2 GAPI assessment of the green profile of the evaluated procedures for determination of TIG in intravenous infusion solution by the proposed CZE method (**a**), spectrophotometric methods, method I (**b**), method II (**c**), method III (**d**) and reference RP-HPLC method (**e**)

4.1.1.3 Buffer concentration The impact of concentration of selected buffer was confirmed using phosphate buffer with different concentrations (between 20 and 100 mM) as running buffer pH 2 with applying 30 kV. It was obtained that decreasing buffer concentration significantly affected the intensity of response (AUC) of the analyzed drug. 20 mM phosphate buffer showed symmetric peaks for TIG with the highest response (AUC).

4.1.1.4 Detection wavelength Diode array detectors (DADs) have the capability to detect several peaks in the electropherogram at different wavelengths on the same run. This improves sensitivity and selectivity of the proposed method where the investigated drug could be determined specifically at its maximum wavelength in the presence of other eluting peaks. Moreover, DAD has an advantage of confirming peak purity of the separated drug. The UV spectrum of the TIG was measured at 245 nm. Also, purity of the separated peak of TIG was demonstrated as absorption spectra at different intervals of time across the peak that was superimposed (Fig. 1d) and peak purity spectrum (Fig. 1e). The peak purity evaluation was assessed by Agilent ChemStation Software [23] where all spectra at different regions of each peak are compared with one or more spectra, for example, an apex or an average spectrum. The degree of match or spectral similarity is plotted over time during elution of the peak to obtain the similarity curve. A threshold curve is a similarity curve with the contribution of back-ground noise. The chromatographic peak, similarity and threshold curves are demonstrated in the upper part of Fig. 1e for TIG. Moreover, the purity ratio is an additional parameter to measure the peak purity. It is plotted in the lower part of Fig. 1e, and it is the purity value of each single spectrum. For a spectral pure peak, the purity ratio appears in the green band as shown in Fig. 1e, while, for spectral impure peaks, the purity ratio is in the red band. Therefore, the selected wavelength allowed the pure separation of each drug in bulk powder and drugs stability in pharmaceutical formulation.

4.1.1.5 Injection time In hydrodynamic injection, sample solutions were injected at 50 mbar. Different injection times (3–25 s) were applied. Increasing injection time led to increase in peak height; but additional increase in injection time more than 20 s led to deviation from linearity and peak shape distortion. 20 s was the optimum injection time which provided linearity between peak height and injection time and good peak symmetry (Fig. 3).

4.1.1.6 Applied voltage Applied voltage was tested by variation in its values from 15 to 30 kV. It was observed that slight change in voltage led to significant effect on migration time which decreased when applied voltage increased. This is owing to increase in electroosmotic flow (EOF) producing fast and effective separation. Best peak shape with short migration time was achieved at 30 kV.

4.2 Spectrophotometric methods

4.2.1 Method I

This method requires measuring D_1 amplitudes from peak to peak for TIG in methanol at 334–372 nm (Fig. 4a and b). The first derivative spectrophotometric method allows elimination of spectral interference and as consequence leads to increased selectivity of the assay.

4.2.2 Method II

The application of difference spectrophotometry has many advantages in qualitative and quantitative analysis. The aim of this method is to increase the selectivity by eliminating the foreign light absorbance effect or foreign substances on the absorbance of the analyte.

Measuring of analytical signal values of TIG using difference spectrophotometry is the principle of this method. It involves measuring acidic solution of the drug against its alkaline one from peak to peak at 342–401 nm for ΔA and 279–311 nm for ΔD_1. Several values of $\Delta\lambda$ were performed, and $\Delta\lambda = 5$ nm was selected to obtain good linearity. Figure 4 c shows absorption spectrum of

Table 7 The penalty points (a) and Green Analytical Procedure Index (b) for the determination of TIG in intravenous infusion solution

(a) Analytical Eco-scale

Reagents/instruments	Penalty points				
	CZE method	Spectrophotometric methods			Reported RP-HPLC method (3)
		Method I	Method II	Method III	
Acetonitrile	–	–	–	–	12
1-Hexane sulfonic acid sodium salt	–	–	–	–	3
Potassium hydroxide	–	–	–	–	4
Phosphoric acid	2	–	–	–	2
Potassium dihydrogen orthophosphate	Not hazardous	–	–	–	Not hazardous
Dextrose 5%	Not hazardous	Not hazardous	Not hazardous	Not hazardous	–
Methanol	–	6	–	–	–
0.1 N HCl	–	–	1	1	–
0.1 N NaOH	–	–	2	–	–
DMF	–	–	–	6	–
MBTH	–	–	–	2	–
Ceric ammonium sulfate	–	–	–	1	–
Heating < 1 h	–	–	–	2	–
UV–Vis Spectrophotometry	–	0	0	0	–
HPLC	–	–	–	–	1
CE-DAD	2	–	–	–	–
Occupational hazard	0	0	0	0	0
Waste > 10 Ml	5	5	5	5	5
Total penalty points	9	11	8	17	27
Analytical Eco-Scale total score	91	89	92	83	73

(b) Green Analytical Procedure Index

Reagents/instruments	Proposed CZE method	Reported spectrophotometric methods			Reported RP-HPLC method (3)
		Method I	Method II	Method III	
Sample preparation					
Collection (1)	At-line	At-line	At-line	At-line	At-line
Preservation (2)	None	None	None	None	None
Transport (3)	None	None	None	None	None
Storage (4)	Under normal conditions	Under normal conditions	Under normal conditions	Under normal conditions	Under normal conditions
Type of method: direct or indirect (5)	Filtration	No sample preparation	No sample preparation	No sample preparation	Filtration and degasing
Scale of extraction (6)	Micro-extraction	Micro-extraction	Micro-extraction	Micro-extraction	Micro-extraction
Solvents/reagents used (7)	Green solvents/reagents used	Non-green solvents/reagents used	Non-green solvents/reagents used	Non-green solvents/reagents used	Non-green solvents/reagents used
Additional treatments (8)	None	None	None	None	None
Reagent and solvents					
Amount (9)	10–100 mL	10–100 Ml	10–100 mL	10–100 mL	> 100 mL
Health hazard (10) (NFPA health hazard score)	0	1	1	1 and 2	1, 2 and 3

Table 7 (continued)

(b) Green Analytical Procedure Index

Reagents/ instruments	Proposed CZE method	Reported spectrophotometric methods			Reported RP-HPLC method (3)
		Method I	Method II	Method III	
Safety hazard (11) (instability score)	0	0	0	0	0
Instrumentation					
Energy (12)	≤ 1.5 kWh per sample	≤ 0.1 kWh per sample	≤ 0.1 kWh per sample	≤ 0.1 kWh per sample	≤ 1.5 kWh per sample
Occupational hazard (13)	–	–	–	–	–
Waste (14)	1–10 mL	1–10 mL	1–10 mL	1–10 mL	1–10 mL
Waste treatment (15)	No treatment	No treatment	No treatment	No treatment	No treatment
Quantification	Yes	Yes	Yes	Yes	Yes

Table 8 Effect of the applied voltage on migration time for the analysis of TIG in intravenous infusion solution using the proposed CZE method

Applied voltage (kV)	Migration time (min)
15	15.2
20	11.2
25	9.1
30	5.9

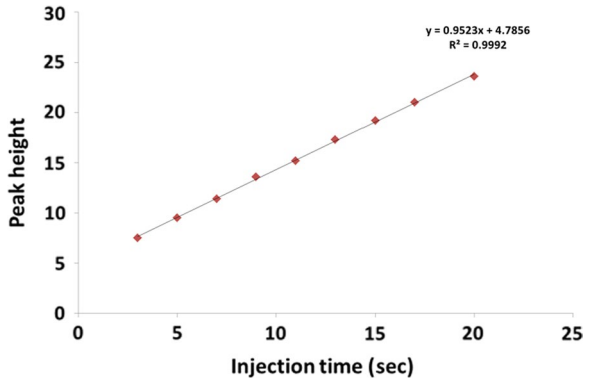

Fig. 3 Effect of injection time on the linearity of TIG in intravenous infusion solution at 245 nm

TIG in 0.1 M HCl and in 0.1 M NaOH, while Figs. 4d and 2e show the difference absorbance (ΔA) and its corresponding first derivative (ΔD_1), respectively.

4.2.3 Method III

MBTH is a chromogenic analytical reagent used for colorimetric estimation of drugs containing phenolic group, active methylene group and aromatic amines. This reagent interacts with the investigated drug and exhibits band at 409 nm which represents a bathochromic shift as demonstrated in Fig. 5a and b. Also, the expected mechanism of this colorimetric reaction is shown in Fig. 5c.

4.2.3.1 Optimization of method III Different factors were studied to ensure complete oxidation and to affect intensity of the oxidative colored product such as MBTH volume, Ce (IV) volume, reaction time, temperature, order of addition of reagents and solvent types.

4.2.3.1.1 Volume of 0.01 M MBTH

Different volumes of 0.01 M MBTH were used from 0.5 to 5 mL depending on the above-mentioned procedure. Maximum intensity of color with reproducible values of λ_{max} was obtained with 1 mL MBTH as shown in Fig. 6a. This indicates complete oxidative reaction.

4.2.3.1.2 Volume of 1 g % Ce (IV)

Different volumes of 1 g % Ce (IV) were tested from 0.5 to 3.5 mL using the above-mentioned procedure. Maximum color intensity with reproducible values of λ_{max} was obtained with 1.5 mL as shown in Fig. 6a.

4.2.3.1.3 Reaction time

This method was performed by leaving the solutions at different time intervals from 5 to 60 min. It is obvious that optimum absorbance was given after 25 min (Fig. 6b).

4.2.3.1.4 Order of addition of reagents

Different orders were examined. The results showed that addition order of TIG-MBTH-Ce (IV) solutions gave maximum absorbance values but other orders of addition produced lower values (Fig. 6c).

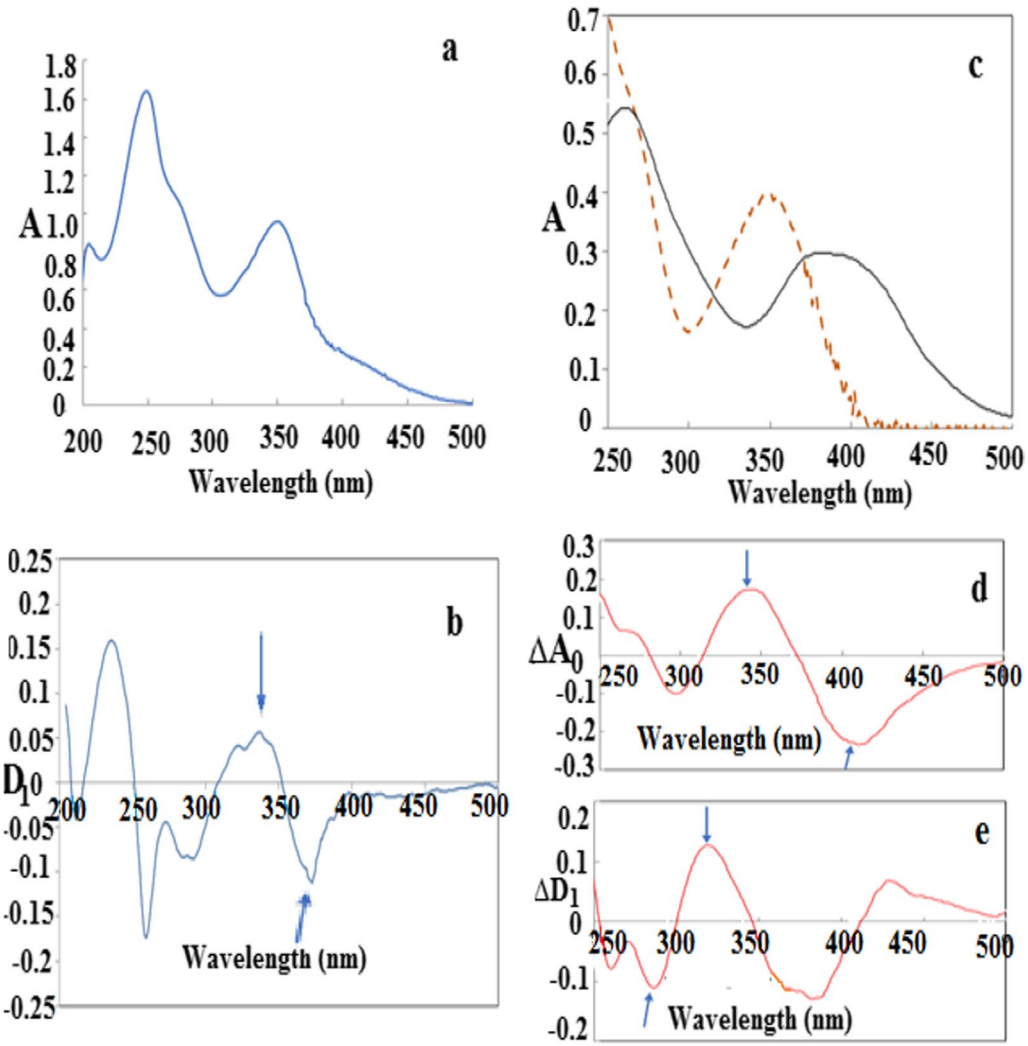

Fig. 4 Absorption (**a**) and its first derivative (**b**) spectra of 100 µg/ mL TIG in methanol, (**c**) Absorption spectra of 40 µg/mL TIG in 0.1 M HCl (---) and in 0.1 M NaOH (___), (**d**) the difference absorbance spectrum and (**e**) its corresponding first derivative spectra in 0.1 M HCl against its solution in 0.1 M NaOH

4.2.3.1.5 Temperature effect

The reaction was performed at different temperature values. As shown in Fig. 6d, maximum absorbance was obtained in 40 °C.

4.2.3.1.6 Diluting solvent

Different solvents were used to perform this reaction such as DMF, methanol, water and acetone. Low absorbance values were obtained with methanol and water, but acetone gave a turbid solution. DMF produced maximum absorbance and was used as the solvent of choice.

4.2.3.1.7 Time after dilution

This procedure was applied by keeping the solutions after dilution at different time intervals (10–60 min) in thermostated water bath at 40 °C. The maximum intensity was obtained after 35 min.

4.2.3.1.8 The method of continuous variation (Job's method)

This method is valuable to determine drug-to-reagent ratio. In this method, the mole fraction of either TIG or MBTH is plotted against the absorbance. Maximum absorbance was obtained when mole fraction was equal to 0.5 (Fig. 7). This proves that the ratio between TIG and MBTH is 1:1.

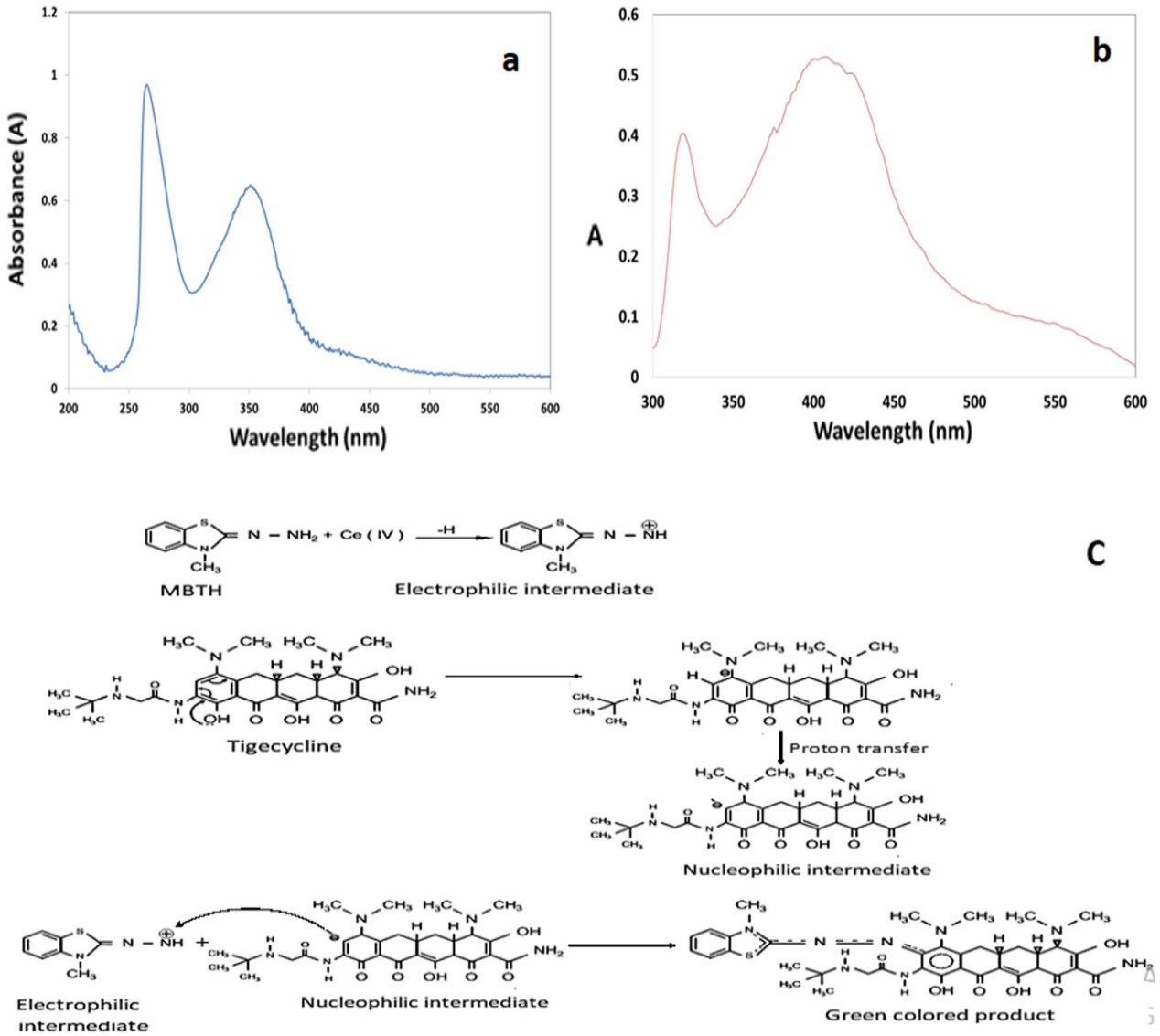

Fig. 5 (**a**) Absorption spectrum for 100 µg/mL TIG in DMF, (**b**) absorption spectrum of reaction product of 100 µg /mL TIG with 0.01 M MBTH in the presence of 1% Ce (IV) as oxidant and (**c**) mechanism of TIG—MBTH reaction

5 Conclusions

This work reports, for the first time, CZE method for the direct and rapid determination of TIG and its separation from other components in intravenous infusion solution. Fast and direct determination of TIG is essential for quality control assessment in centralized units in various oncology centers in order to confirm the correct concentration of TIG administered to cancer patients and assure the safe intravenous administration to patients and minimize the clinical staff exposure risk to dangerous chemicals. Moreover, three green spectrophotometric methods were also proposed for TIG determination that offer many advantages such as accuracy, precision, simplicity, specificity and facility of quantification and separation of the selected drug in infusion bags and pharmaceutical preparations without any techniques for extraction. Furthermore, the developed CZE method has several advantages over current chromatographic methods such as higher efficiency of separation within short analysis time, consumption of fewer quantities of chemicals and offering better resolution than HPLC. On top of that, the quantification limits obtained by the proposed methods were significantly lower than the administered range of TIG in infusion solutions and lower than its C_{max}. This promotes the applying of the proposed methods for the

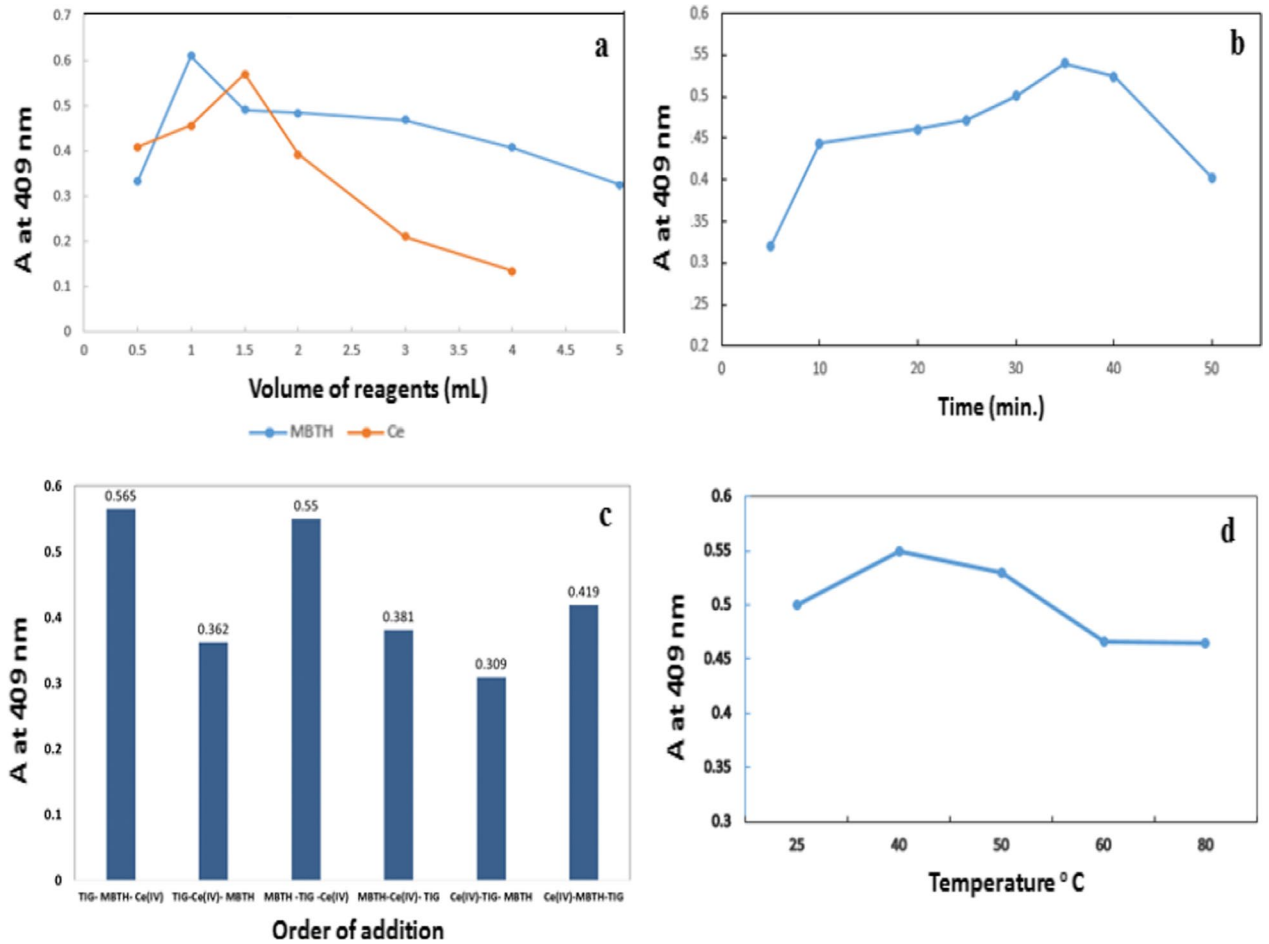

Fig. 6 (**a**) Studying the effect of volume of the reagents, (**b**) the effect of reaction time, (**c**) the effect of order of addition, (**d**) temperature on the oxidation reaction product of 100 µg/mL of TIG

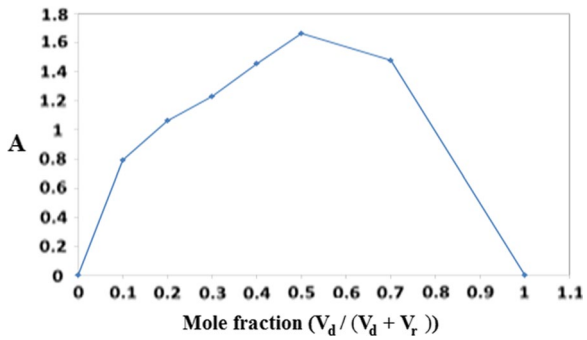

Fig. 7 Job's method for the reaction between MBTH and TIG in the presence of 1%Ce (IV) as oxidant

pharmacokinetics and bioavailability studies of TIG in various biological fluids.

Abbreviations

AML: Acute myeloid leukemia; AUC: Area under curve; Ce: Ceric ammonium sulfate; C_{max}: Maximum concentration; CZE: Capillary zone electrophoresis; DAD: Diode array detector; DL: Detection limit; DMF: Dimethylformamide; ΔA: Difference absorbance; ΔD_1: Difference first derivative; EOF: Electroosmotic flow; GAPI: Green Analytical Procedure Index; HPLC: High-performance liquid chromatography; kV: Kilo-voltage; MBTH: 3-Methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate; $\Delta\lambda$: Maximum wavelength; E_r%: Percentage error; QL: Quantification limit; RSD: Relative standard deviation; s: Second; S: Slope of calibration curve; σ: Standard deviation of responses; TIG: Tigecycline; UPLC: Ultra-performance liquid chromatography.

Supplementary Information

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Additional file 1. Volumes and concentration used for each method.

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Author contributions

All authors read and approved the final manuscript. AFE-Y contributed to supervision, conceptualization, methodology, data analysis, data curation and writing—original draft preparation, reviewing and editing. FMA was involved in methodology, data analysis, data validation and writing—original draft

preparation. EFK contributed to supervision, reviewing and editing. RMY was involved in supervision, conceptualization, methodology, data curation, investigation and writing—original draft preparation, reviewing and editing. MAE-S contributed to supervision, reviewing and editing.

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