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Development and validation of UV Spectrophotometric method for simultaneous estimation of Efonidipine hydrochloride ethanolate and Chlorthalidone in their synthetic mixture

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Efonidipine Hydrochloride Ethanolate and Chlorthalidone is used in management of hypertension and under clinical phase 3 study. The development of quality control method is required for accurate analysis of both drugs. Two simple, precise and economical UV spectrophotometric methods have been developed for the simultaneous estimation of Efonidipine Hydrochloride Ethanolate and Chlorthalidone in their synthetic mixture. Method I is simultaneous equation method (Vierodt's Method), which is based on measurement of absorption at 251 and 227 nm i.e. λmax of Efonidipine Hydrochloride Ethanolate and Chlorthalidone, respectively. Method II is first order derivative was based on the measurement of absorbance of Efonidipine Hydrochloride Ethanolate measure at 283.2 nm (ZCP of Chlorthalidone) and absorbance of Chlorthalidone measure at 250.8 nm (ZCP of Efonidipine Hydrochloride Ethanolate). Linearity was observed in the concentration range of 6.4-38.4 µg.mL^{-1} for Efonidipine hydrochloride ethanolate and 2-12 µg.mL^{-1} for Chlorthalidone using methanol as a solvent. The accuracy of methods was assessed by recovery studies and was found to be within range of 98-102% for both the drugs. Precision of the methods was estimated by repeatability and intermediate precision studies. The % RSD values were found to be less than 2, proving methods were precise. Two methods were compared using F-test. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory.

Keywords: Efonidipine Hydrochloride Ethanolate; Chlorthalidone; simultaneous equation method; first order derivative method; recovery study; validation; F-test.

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Introduction

Efonidipine Hydrochloride Ethanolate (EFD) is a 2-(N-benzyl anilino) ethyl 5-(5,5-dimethyl-2-oxo-1,3,2λ^5-dioxaphosphinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3 carboxylate;ethanol;hydrochloride (Figure 1). It is 1,4-dihydropyridine derivative. Therapeutically categorized as Calcium channel blocker and used in treatment of hypertension and angina pectoris. It blocks L and T type of calcium channel, which leads to vasodilation and lower automaticity of heart. It exerts negative chronotropic effect by inhibiting T-type calcium channel activity of sinoatrial (SA) node, it prolongs the late phase-4 depolarization of sinoatrial action potential and decreasing heart rate (1,2).

Chlorthalidone (CTD) is a 2-chloro-5-(1-hydroxy-3-oxo-2H-isoindol-1-yl) benzenesulfonamide (Figure 2). It is benzenesulfonamide derivative. Therapeutically categorized as Thiazide diuretic and used in treatment of premenstrual tension in fluid retention and in management of hypertension. It inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segments of the ascending limb of loop of Henle by increasing the delivery of sodium to the distal renal tubule, chlorthalidone indirectly increases potassium excretion via sodium-potassium mechanism (3,4).

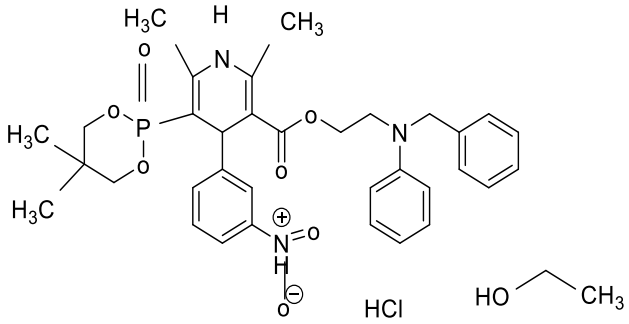


Figure 1. Chemical structure of EFD.

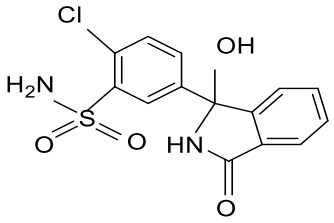


Figure 2. Chemical structure of CTD.

As consequence, this combination of EFD and CTD is an essentially use to manage hypertension and effectively regulate the blood pressure via synergistic action and CTD raise the potassium excretion by Na+/K+ exchange mechanism which results in low blood pressure (5,6). The considerable literature survey disclose that the analytical

methods reported for estimation of EFD and CTD alone and in combination with other drug, but no analytical method was reported for simultaneous estimation of EFD and CTD (7-40). So, it was thought of interest to develop simple, accurate, precise and reproducible UV-Spectrophotometric methods for simultaneous estimation of EFD and CTD in their synthetic mixture. Methods were validated as per ICH guideline [Q2 (R1)] (41).

Material and method

Instrumentation

All absorption spectra were recorded with UV-Visible double beam spectrophotometer (Simadzu-1800 Japan) with UV probe 2.33 software, with spectral band width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells. in a range of 200-400 nm. The analysis was carried by using UV Probe 2.33 software. Electronic balance (Shimadzu ATX-200) was used in the experiment.

Reagents and chemicals

Pure sample of EFD and CTD were kindly supplied as gift sample by Pure Chem Pvt. Ltd., Ankleshwar, Gujarat and CTX Life Science Pvt. Ltd., Surat, Gujarat, respectively. All reagents used were of analytical grade.

Selection of solvent

The solubility of EFD and CTD was performed using distilled water, methanol, acetonitrile, 0.1 M HCl, and 0.1 M NaOH. Both the drug was soluble in methanol so methanol was selected as a solvent.

Preparation of Standard solution

Accurately weighed quantity of 25 mg of EFD and 25 mg of CTD was transferred to separate 25 mL volumetric flask, add some methanol and sonicate for 10 min and diluted up to mark with methanol to give a stock solution having the concentration strength of 1000 µg.mL^{-1}. An aliquot of 2.5 mL from the above stock solution was pipette out in to 25 mL of volumetric flask and diluted up to mark with methanol to give a stock solution having the strength of 100 µg.mL^{-1} .

Preparation of Test Solution

Take synthetic mixture equivalent to 40 mg EFD and 12.5 mg CTD in 100 mL volumetric flask (Table 1) and add methanol up to the mark to give solution strength (400, 125 µg.mL^{-1}) sonicate for 10 min. Take 1 mL from the above solution and transfer in 10 mL volumetric flask and make the volume up to mark with methanol give solution strength (40, 12.5 µg.mL^{-1}).

Table 1: Composition of synthetic mixture.

Item	Ingredients	Quantity (mg)	Role
1	EFD	40	Anti-hypertensive
2	CTD	12.5	Anti-hypertensive
3	Micro Crystalline Cellulose	120	Disintegrant
4	Starch 5%	10	Binder
5	PVPK-30 5%	10	Binder
6	Magnesium Stearate	4	Lubricant
7	Talc	4	Glidant

Procedure for determination of wavelength for measurement

2.56 ml of stock solution of EFD (100 µg.mL^{-1}) and 0.8 mL of stock solution of CTD (100 µg.mL^{-1}) were pipette out into two separate 10 mL volumetric flasks. Volume was adjusted to the mark with methanol to get 25.6 µg.mL^{-1} of EFD and 8 µg.mL^{-1} of CTD. Each solution was scanned between 200-400 nm against methanol as a blank reagent. The spectrum of each solution was obtained. The wavelength maximums were found to be 251 nm and 227 nm for EFD and CTD, respectively (Figure 3).

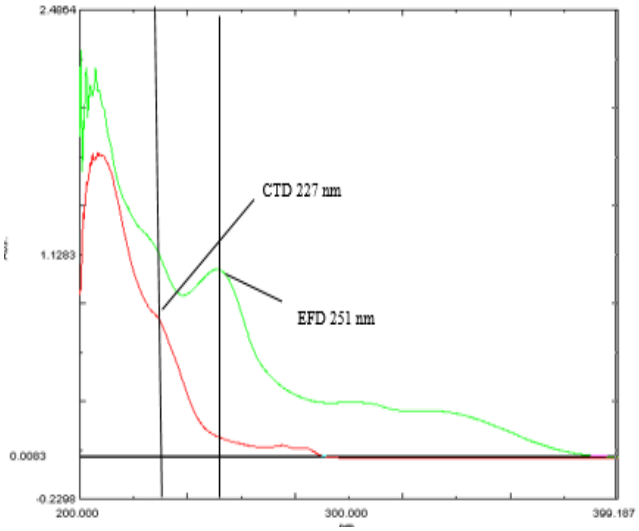


Figure 3. Overlain spectra of EFD (25.6 µg.mL^{-1}) and CTD (8 µg.mL^{-1})

Stability of Solution

The stability of the solution in methanol was done for 48 hr at room temperature. The absorbance of the solution of EFD (25.6 µg.mL^{-1}) and CTD (8 µg.mL^{-1}) was taken at 251 nm and 227 nm, respectively, at the interval of 0, 24 and 48 hr (Table 2).

Table 2. Stability of solutions.

Time (hr)	EFD at 251 nm		CTD at 227 nm	
	Absorbance ± SD (n=5)	% Degr-adation	Absorbance ± SD (n=5)	% Degr-adation
0	0.773±0.0007	-----	0.619±0.0007	-----
24	0.770±0.0008	0.38	0.617±0.001	0.32
48	0.769±0.0007	0.51	0.614±0.001	0.80

Method I: Simultaneous equation method (Vierodt's)

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 251 nm and 227 nm that are λmax of EFD and CTD, respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A1^{\%}_{1cm}$) for both the drugs at both wavelengths were determined as mean of six independent determinations. Concentrations in the sample were determined by using following equations:

$$C_{EFD} = (A_2\, a_{y1} - A_1\, a_{y2}) / (a_{x2}\, a_{y1} - a_{x1}\, a_{y2}) \quad \text{Equation 1}$$

$$C_{CTD} = (A_1\, a_{x2} - A_2\, a_{x1}) / (a_{x2}\, a_{y1} - a_{x1}\, a_{y2}) \quad \text{Equation 2}$$

where, A_1 and A_2 are absorbances of mixture at 251 nm and 227 nm, respectively, ax_1 and ax_2 are absorptivities of EFD at λ_1 (251 nm i.e. λmax of EFD) and λ_2 (227 nm i.e. λmax of CTD), and ay_1 and ay_2 are absorptivities of CTD at λ_1 and λ_2, respectively. C_{EFD} and C_{CTD} are concentrations of EFD and CTD, respectively. Figure 3 represents the overlay spectra of both the drugs in 3.2:1 ratio and criteria for obtaining maximum precision (absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1) by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the EFD and CTD.

Method II: First order derivative spectrophotometric method

For first order derivative spectrophotometric method, accurate aliquots of EFD equivalent to 6.4- 38.4 µg.mL^{-1} were transferred from its stock solution (100 µg.mL^{-1}) into a series of 10 mL volumetric flasks and diluted to mark with methanol and mixed well. Accurate aliquots of CTD equivalent to 2-12 µg.mL^{-1} were transferred from its working solution (100 µg.mL^{-1}) into a series of 10 mL volumetric flasks and diluted to mark with methanol and mixed well. Considering all the derivative order spectra of EFD and CTD from first to fourth derivative, the first order derivative spectra with δλ 2 and scaling factor 10 found suitable. From the overlain first order derivative spectra of EFD (25.6 µg.mL^{-1}) and CTD (8 µg.mL^{-1}) the zero-crossing point (ZCP) of EFD and CTD were obtained. The wavelength selected as ZCP for EFD was 250.8 nm whereas CTD give absorbance while ZCP of CTD was 283.20 nm where EFD give absorbance. These absorbances Vs concentration were plotted in the quantitative mode to obtain the working curves from which by extrapolating the value of absorbances of the sample solution, the concentration of the corresponding drugs was determined. Both the drugs obeyed Beer's Law.

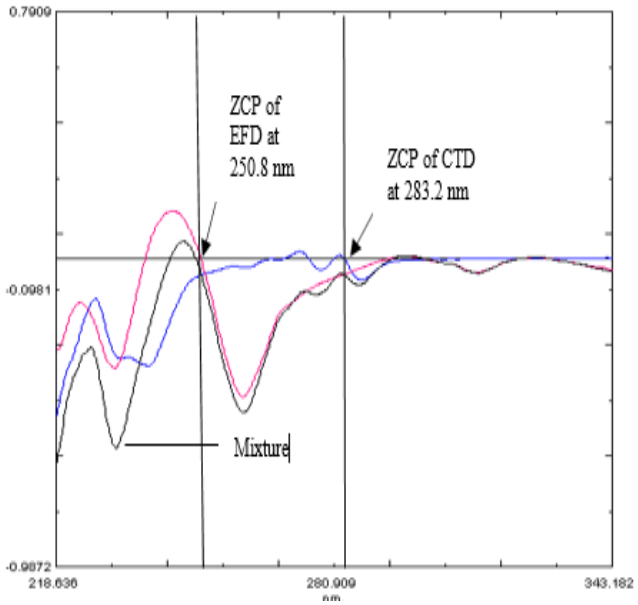


Figure 4. Overlain first order derivative spectra of EFD (25.6 µg.mL^{-1}), CTD (8 µg.mL^{-1}) and mixture (25.6 + 8 µg.mL^{-1}).

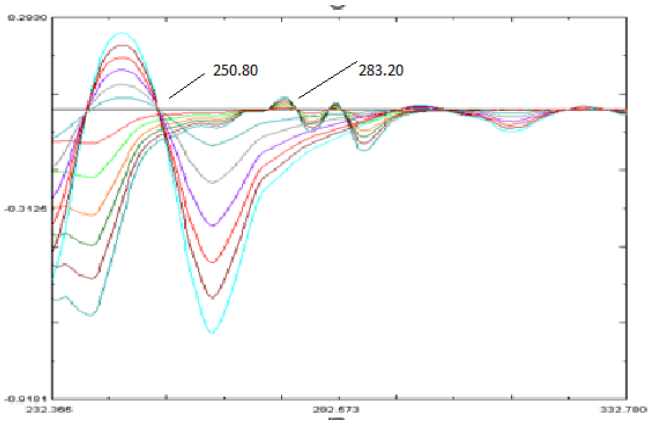


Figure 5. Overlain first order derivative spectra of EFD (6.4-38.4 µg.mL^{-1}) and CTD (2-12 µg.mL^{-1}).

Validation Parameters

Validation was carried out according to ICH guideline (ICH Q2 (R1).

Accuracy

For studying the accuracy of the proposed methods and for checking the interference from excipients used in synthetic mixtures, experiments were carried out by the standard addition method. This study was performed by addition of known amounts of EFD and CTD to a known concentration of test solution. The amounts of standard recovered were calculated in terms of mean recovery with upper and lower limit of %RSD.

Precision
Repeatability: The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for EFD and CTD without changing the parameter of the proposed spectrophotometry methods.
Intermediate Precision: Intra-day precision and inter-day precision for the developed methods were measured in terms of % RSD. The experiments were repeated three times a day for intra-day precision and on 3 different days for inter-day precision. The concentration values for both intra-day precision and inter-day precision were calculated three times separately and % RSD were calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)
Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the 3s/*m* and 10s/*m* criterions, respectively, where *s* is the standard deviation of intercept (n =6) of the sample and *m* is the slope of the corresponding calibration curve.

Statistical Analysis

Statistical analysis was performed to assess the effect of two methods in simultaneous determination of EFD and CTD using F-test.

Results and Discussion

Method I: Simultaneous equation method (Vierodt's)

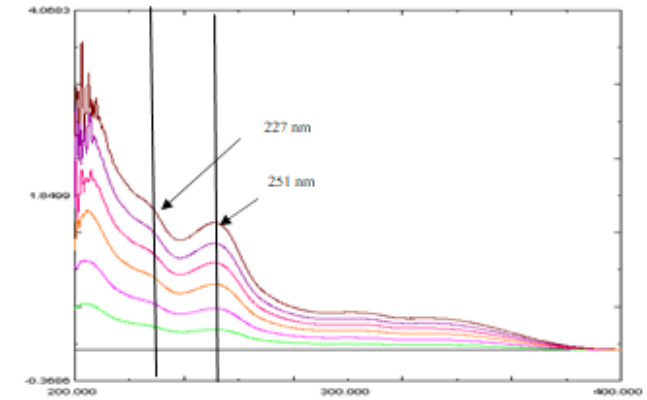
The simultaneous equation based on the absorbance of both drug EFD and CTD at their λmax. Two wavelengths selected for the method are 251 nm and 227 nm that are λmax of EFD and CTD, respectively. Figure 3 represent the overlain spectra of both the drugs in 3.2:1 ratio. The absorbances were measured at the selected wavelengths and specific absorptivities ($A1^{\%}_{1cm}$) for both the drugs at both wavelengths were determined as mean of six independent determinations (Table 3). $A1^{\%}_{1cm}$ is calculated by equation 3:

$$A = a.b.c \qquad \text{Equation 3}$$

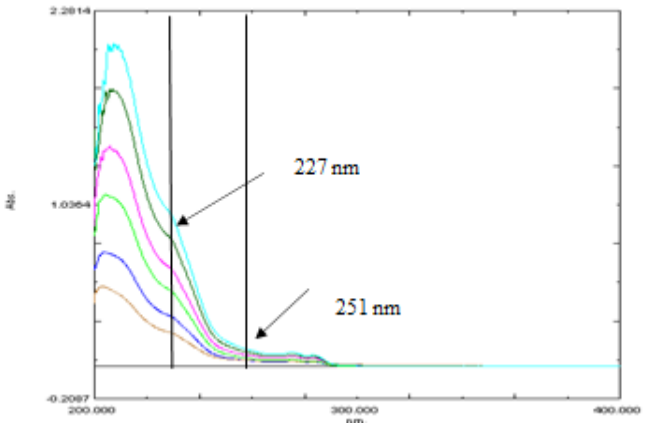
Where:
A = absorbance,
a = specific absorptivity,
b = path length 1 cm,
c = concentration of absorbing species in g.100 mL^{-1}.

Table 3. Absorptivities at 251 nm and 227 nm.

At 251 nm		At 227 nm	
a_{x1}	398.7	a_{x2}	1024.6
a_{y1}	166.2	a_{y2}	460.6



(a)



(b)

Figure 6. (a) zero-order overlain spectra of EFD (6.4 – 38.4µg.mL^{-1}); (b) zero order overlain spectra of CTD (2 – 12µg.mL^{-1}).

Method II: First order derivative Spectrophotometric method

In contrast to zero-order spectra, first derivative spectra show more resolution in terms of zero crossing points shown in Figure 4 and 5 which explain overlain first order derivative spectra for EFD and CTD. At 250.8 nm, EFD having zero crossing point and CTD can be determined. At 283.20 nm, CTD having zero crossing point and EFD can be determined.

Application of proposed methods for analysis of EFD and CTD in synthetic mixture

Method I: The zero-order spectrum of test solution was recorded and measured at absorbance of 251 and 227 nm to determine EFD and CTD, respectively. The concentrations of EFD and CTD in the synthetic mixture were determined using the simultaneous equation. The % assay values are given in Table 4.
Method II: The first-order spectrum of test solution was recorded and measured the absorbance at 283.20 nm and 250.80 nm for estimation of EFD and CTD. The concentrations of EFD and CTD in synthetic mixture were determined using the First order derivative method. The% assay values are given in Table 4.

Table 4, Table 5 and Table 6 exhibit results of assay, results of accuracy studies and summary of various validation parameters of all methods respectively.

Statistical comparison of developed simultaneous equation method and first order derivative spectrophotometric method was done by F test (Table 7). F calculated value was less than F critical 6.38 for both EFD and CTD, so it is indicating no significant difference observed in assay results among the two methods. Hence, it was concluded that both methods do not differ significantly.

Table 4. Assay results for tablets using the proposed methods.

Formulation	Proposed methods	Label Claim (mg)		Amount of drug found (mg)		% Label Claim Assay (n=5) ± SD	
		EFD	CTD	EFD	CTD	EFD	CTD
Synthetic Mixture	METHOD I	40	12.5	39.75	12.45	99.38 ± 0.21	99.60 ± 0.35
	METHOD II	40	12.5	39.87	12.46	99.68 ± 0.02	99.68 ± 0.04

Table 5. Application of the standard addition technique to the analysis of EFD and CTD in synthetic mixture by the proposed methods.

Method	Drugs	Amount present (µg.mL^{-1})	Amount added (µg.mL^{-1})	Total amount of drug (µg.mL^{-1})	Amount found (µg.mL^{-1})	%Recovery ± SD (n = 3)	%RSD
Method I	EFD	12.8	6.4	19.2	19.05	99.22 ± 0.39	0.39
			12.8	25.6	25.60	100.00 ± 0.26	0.26
			19.2	32.0	31.98	99.94 ± 0.32	0.32
	CTD	4	2	6	5.96	99.33 ± 0.22	0.22
			4	8	7.96	99.50 ± 0.14	0.14
			6	10	9.92	99.20 ± 0.29	0.29
Method II	EFD	12.8	6.4	19.2	19.10	99.48 ± 0.02	0.02
			12.8	25.6	25.57	99.88 ± 0.12	0.12
			19.2	32.0	31.90	99.69 ± 0.05	0.05
	CTD	4	2	6	5.99	99.83 ± 0.76	0.76
			4	8	8.00	100.00 ± 0.50	0.50
			6	10	9.95	99.50 ± 0.19	0.19

Table 6: Summary of validation parameter by developed method.

Parameters		Method-I		Method-II	
		EFD	CTD	EFD	CTD
Working wavelength (nm)		251 nm	227 nm	283.2	250.8
Concentration range (µg.mL^{-1})		6.4-38.4	2-12	6.4-38.4	2-12
Slope		0.031	0.078	-0.0022	-0.0524
Intercept		0.022	0.003	0.0008	-0.0023
Determination coefficient (r^2)		0.999	0.998	0.999	0.999
LOD (µg.mL^{-1})		0.148	0.012	0.15	0.04
LOQ (µg.mL^{-1})		0.449	0.036	0.47	0.14
Precision	Repeatability (n=6) %RSD	0.17	0.64	1.00	0.12
	Intraday (n=3) %RSD	0.12-0.18	0.09-0.13	0.87-1.41	0.23-0.48
	Interday (n=3) %RSD	0.10-0.27	0.12-0.64	0.87-1.41	0.13-0.66
Accuracy (%)	50%	99.22 ± 0.39	99.33 ± 0.22	99.48 ± 0.02	99.83 ± 0.76
	100%	100.00 ± 0.26	99.50 ± 0.14	99.88 ± 0.12	100.00 ± 0.50
	150%	99.94 ± 0.32	99.20 ± 0.29	99.69 ± 0.05	99.50 ± 0.19
% Label claim Assay ± SD (n=5)		99.38 ± 0.21	99.67 ± 0.35	99.60 ± 0.02	99.68 ± 0.04

Table 7: F-test for EFD and CTD.

EFD	Variable 1	Variable 2	CTD	Variable 1	Variable 2
Mean	99.38	99.60	Mean	99.67	99.68
Variance	0.00027	0.00013	Variance	0.00028	0.00013
Observations	5	5	Observations	5	5
df	4	4	df	4	4
F	2.076923		F	2.153846	
P(F<=f) one-tail	0.248219		P(F<=f) one-tail	0.237852	
F Critical one-tail	6.388233		F Critical one-tail	6.388233	

Abbreviations

EFD: Efonidipine hydrochloride ethanolate
CTD: Chlorthalidone
%RSD: % Relative standard deviation
SD: Standard deviation

Conclusions

Two spectrophotometric methods (simultaneous equation method and first order derivative method) were developed for simultaneous estimation of EFD and CTD in their synthetic mixture. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of EFD and CTD in synthetic mixture. The F-test results show that there is no significant difference between assay results obtained from these two methods. So, the proposed methods can be used in routine analysis of EFD and CTD with relatively less expensive and simple to operate instrumentation.

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Conflict of interest

The authors declare no conflicts of interest.

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