Experimental Design and Optimization for the Spectral Analysis of Two-Component Mixture

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*A chemometric experimental design was applied to obtain the optimal settings for spectrophotomeric quantitative resolution of two-component mixture containing benazepril hydrochloride (**BE**) and hydrochlorothiazide (**HCT**) in tablets. As it is known, the digital parameters for instance divisor concentration and Δλ-interval are very important concepts for the application of the ratio spectra derivative spectrophotometry to the resolution of binary mixture systems. In this manuscript, a three-level full factor design was used to optimize the effects of the divisor concentration and Δλ-interval on the quantitative analysis of the above mentioned drugs in samples. Desirable results were obtained: firstly, with the divisor concentrations and Δλ-interval, 21.20 μg/mL and Δλ=27.50 nm for BE in the ratio spectra first derivative spectrophotometry, secondly 27.20 μg/mL and Δ = 5.4 for HCT in the ratio spectra second derivative method. Under the abovementioned conditions, both first and second derivative approaches showed good precision, accuracy and linearity in the quantitative analysis of BE and HCT in two different pharmaceutical tablet products.*

Keywords: Experimental design, optimization, spectral analysis, two-component mixture

Experimental design methodology has been used for various reasons in different branches of chemistry. The basic goal of the experimental design methodology is to find the conditions giving the best analytical result. Particularly, various experimental design approaches have been applied to optimize the experimental conditions or the method in analytical chemistry [1-3].

In recent years, several chemometric experimental designs have been used to optimize the HPLC and capillary electrophorotic methods in order to obtain optimal separation of analytes in a reasonable analysis time by adjusting acceptance chromatographic and electrophorotic factors (i.e type, composition and pH of the used mobile phase) [4-8]. In some cases, a given analytical method may not give suitable results due to random experimental conditions. Therefore, the experimental design approach may apply to optimize the method depending on the effect factors of the analysis.

The method validation is a necessary step for determining the active compounds in samples. We believe that finding the optimal experimental conditions is another necessity for the application of the analytical method to the sample analysis. Thus, the experimental design approaches should not be applied only to the HPLC and capillary electrophorotic methods; therefore we may use it for the spectrophotometric determination methods to determine the optimal conditions.

The simultaneous quantitative analysis of **BE** and **HCT** in their binary mixture were performed by chemometric methods [23], spectrophotometry [24] and HPLC [24-25].

In this study, two-factor three-level full factorial design was applied to optimise the parameters of Δλ and divisor concentration in application of the ratio spectra derivative spectrophotometry to the quantitative resolution of the binary mixtutre containing HCT and BE drugs. Moreover, 21.20 μg/mL with Δλ= 27.50 nm for BE in the ratio spectra first derivative approach and 27.2 μg/mL with Δλ= 5.4 for HCT in the ratio spectra second derivative spectrophotometry were found as optimal divisor concentrations and optimal Δλ-interval, respectively. Finally, the method of finding the optimal condition was successfully applied to the synthetic mixtures and two different commercial tablet formulations of **HCT** and **BE** drugs, respectively.

Experimental part

Instruments

A Shimadzu UV-160 double beam UV-VIS spectrophotometer having a fixed slit width (2 nm) connected to a computer having Shimadzu UVPC software and a HP DeskJet 600 printer were used for the registration of the absorption spectra. The mathematical treatments were achieved by using the Microsoft *EXCEL* and Matlab 7.0 softwares.

Commercial tablet formulation

Two commercial tablet formulations, Cibadrex® Divitab 5/6.25 (**I**) and 10/12.5 (**II**) tablets (produced by Novartis Pharm., Turkey, respectively, consisting of 5 and 10 mg **BE** and 6.25 and 12.50 **HCT** were analyzed by the ratio spectra derivative method in the optimal spectral conditions.

Standard solutions

Stock solution of **BE** and **HCT** (for each drug, 100 mg/ 100 mL) were prepared in 0.1 M NaOH. The standard series in the concentration range of 12-36 μg/ mL **BE** and 10-22 μg/mL **HCT** was obtained from the stock solutions. A validation set of 14 synthetic mixture solutions in the working range of 12–36 μg/mL **BE** and 10–22 μg/mL **HCT** μg/mL was prepared from the same stock solutions.

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Tablet preparation

20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in 0.1 M NaOH in a 100 mL calibrated flask by sonication. The solution was filtered into a 100 ml calibrated flask through Whatman no.42 filter paper. This procedure was repeated for Cibadrex® Divitab 5/6.25 (**I**) and 10/12.5 (**II**) tablets. Tablet solutions (**I**) and (**II**) were diluted to the working concentration range in a 25 ml-calibrated flask with 0.1 M NaOH. The proposed method was successfully applied to the analysis of two commercial tablet formulations.

Results and discussion

As it can be seen from figure 1 the absorption spectra of **HCT** and **BE** are strongly overlapped in the spectral range of 210-300 nm. Under this condition the determination of two drugs in the same samples is not possible by using the classical analytical approaches. In order to present a viable solution to this problem a ratio-derivative spectrophotometric method was considered.

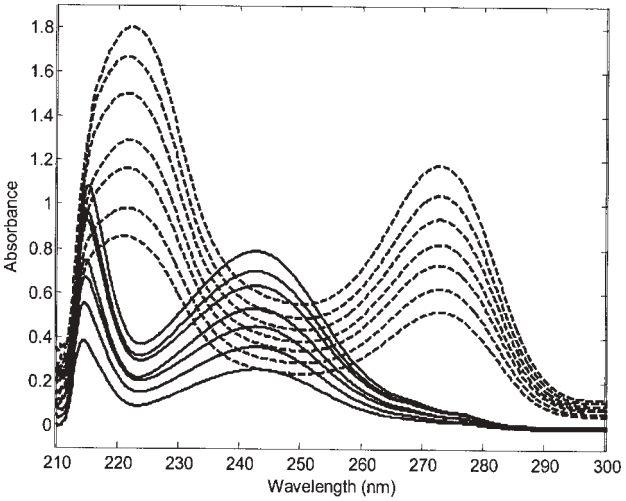

Fig. 1. Absorbance spectra of 10-22 μg/mL HCT (- - - -) and 12-36 μg/mL BE in 0.1 M HCl

Generally, the application of the ratio spectra derivative method to the quantitative resolution of binary mixture is carried out by using the random selected divisor concentration and Δλ-interval in analytical chemistry. For this reason, the method application in the optimal spectral conditions is a very important task for obtaining reliable results. In this study, instead of using the random conditions described above, we focused mainly on the identification of the optimal divisor concentration and Δλ-interval in order to apply the ratio spectra derivative method to the quantitative analysis of the binary mixtures containing **HCT** and **BE** drugs.

Design of spectral settings

As a starting point, we considered the spectral parameters (factors), which have an effect of divisor concentrations and Δλ-intervals on the analysis results in the proposed method. In our case, the values of the spectral factor settings for each **HCT** and **BE** drugs alone were presented in table 1. The main goal here is to reach the precise and the accurate results by using the optimization of the above mentioned method. Therefore, two-factor three-level full factorial design was planed by using nine runs. Standard deviations (or recovery fraction) results used as responses were calculated at different stages for divisor concentrations and Δλ–intervals. In our study, recovery fraction was not used. By using the random spectral condition the linear regression equations for each **BE** and **HCT** drugs were obtained by taking into account different divisor concentrations and Δλ-intervals as shown in figures 2 and 3, respectively. These calibration equations were used to obtain the standard deviation values as response factors by analyzing the synthetic mixtures. This obtained design was applied separately for each drug. The digated spectral settings (factors) were randomly selected as indicated in table 2.

Regression modeling

By using two-factor three-level full factorial design for the response (standard deviation) for each drug, the second degree regression model was obtained as follows

$$Y = b_o + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2, \quad (1)$$

where Y denotes the calculated response for each drug, b_0 the intercept, b_i (i= from 1 to 5) the coefficients of the above model (1) and X_i the independent spectral variables (divisor concentrations and Δλ-intervals). The regression parameters computed for each response were presented in table 3. After modeling the optimal spectral settings (divisor concentration and Δλ) for **HCT** and **BE** were calculated at scaling factor equals to 4 for **HCT** and 10 for **BE**, respectively. The optimal spectral settings for the method application were found to be 27.2 μg/mL for the divisor concentration and Δλ=5.4 nm for derivation treatment for **HCT.** The corresponding optimal settings for **BE** are 21.2 μg/mL mL for the divisor concentration Δλ=27.5 nm for derivation treatment. The recoveries and their statistical results obtained in the initial selected

Table 1
PARAMETER SETTINGS IN THE DESIGN (FOR THE DETERMINATION OF **HCT** WHEN **BE** WAS USED AS A DIVISOR)

		Factors	
		X_1	X_2
Compound	Level	Divisor con (μg/mL)	Δλ (nm)
HCT	1	12.0	3.0
	0	24.0	6.0
	-1	36.0	10.0
BE	1	10.0	3.0
	0	16.0	12.0
	-1	22.0	28.0

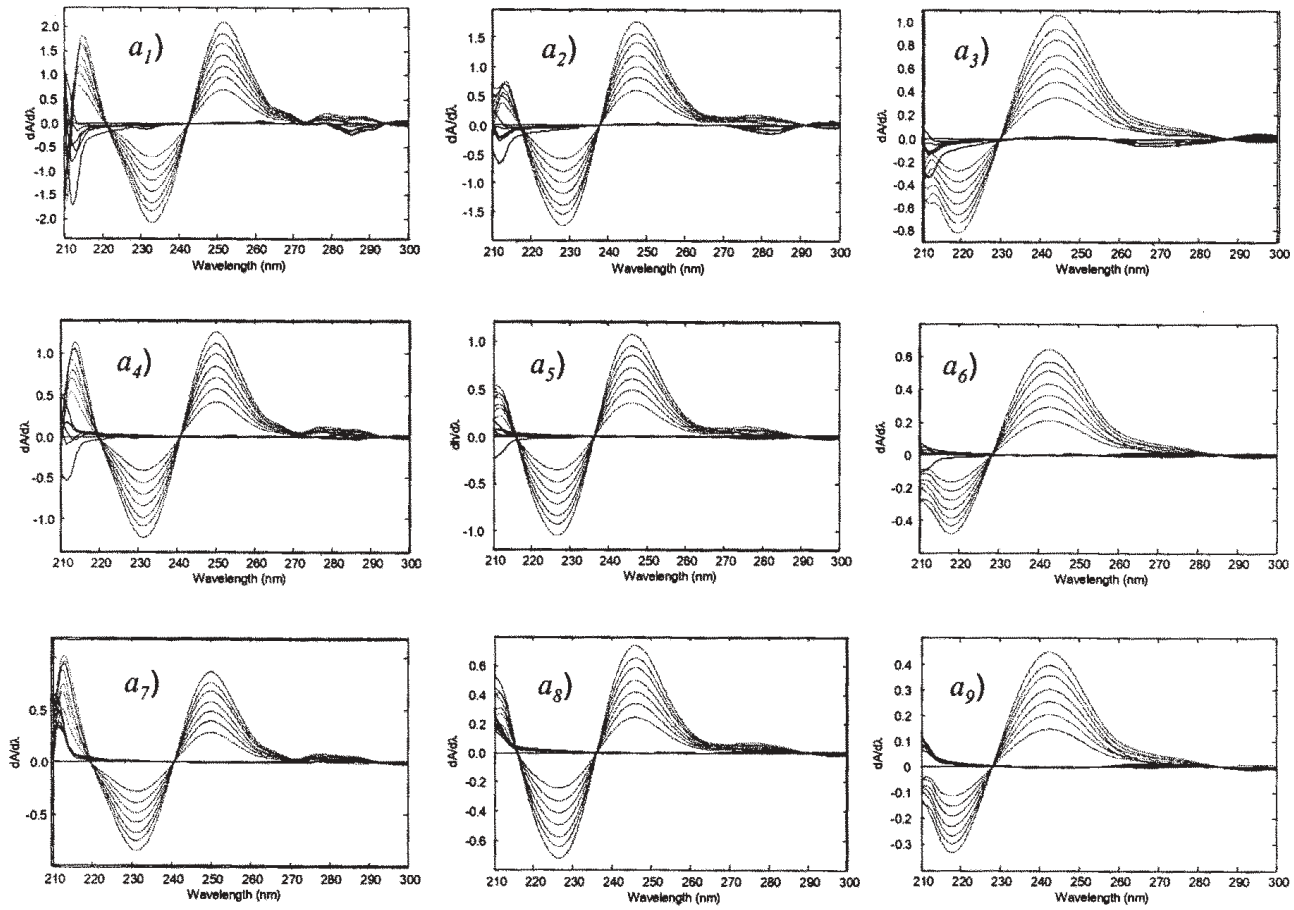

Fig. 2. First derivative of the ratio specta of BE in the concentration range of 12-36 μg/mL exsiting HCT in the range of 10-22 μg/mL - when 10 μg/mL HCT for a_1, a_2 and a_3 was used as a divisor, for each, with $\Delta\lambda$= 3, 12 and 28 nm, respectively, - when 16 μg/mL HCT for a_4, a_5 and a_6 was used as a divisor, for each, with $\Delta\lambda$= 3, 12 and 28 nm, respectively, - when 22 μg/mL HCT for a_7, a_8 and a_9 was used as a divisor, for each, with $\Delta\lambda$= 3, 12 and 28 nm, respectively

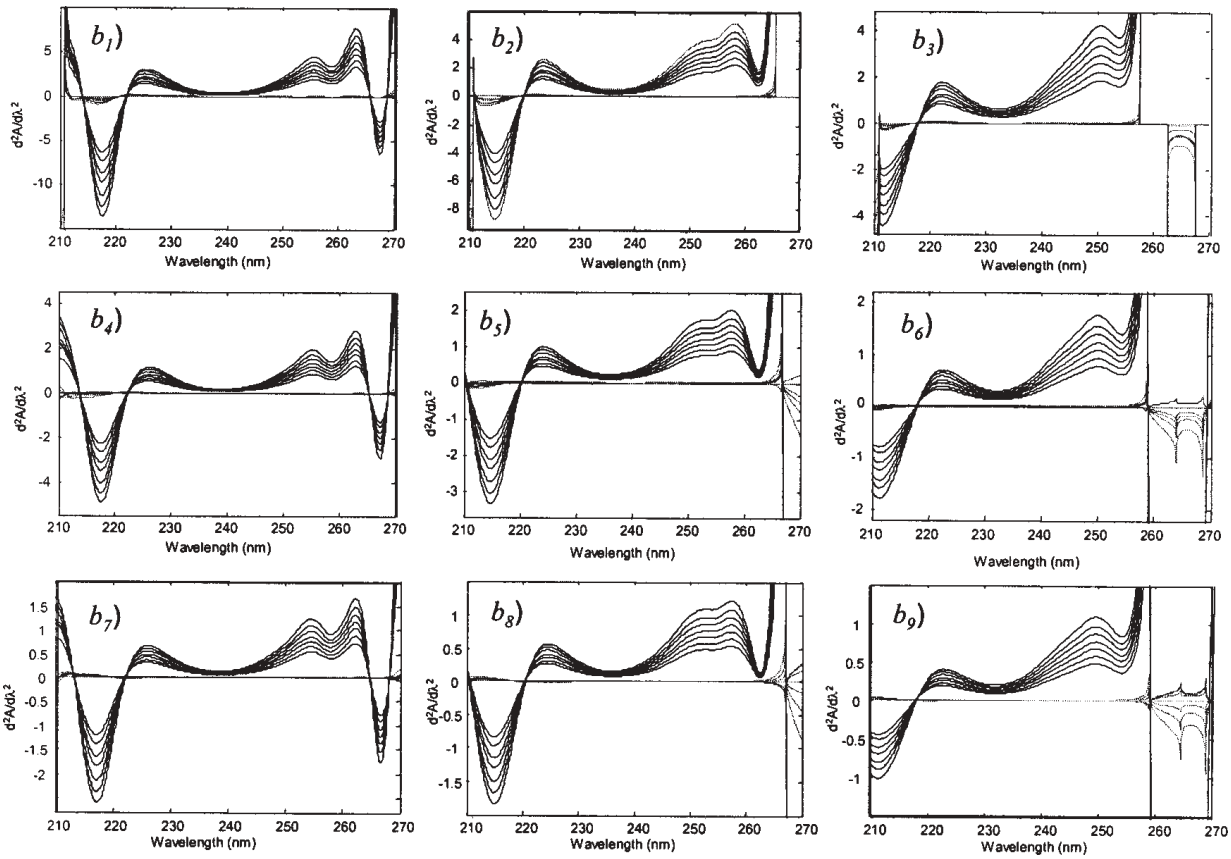

Fig. 3. First derivative of the ratio specta of HCT in the concentration range of 10-22 μg/mL exsiting BE in the range of 12-26 μg/mL - when 12 μg/mL BE for b_1, b_2 and b_3 was used as a divisor, for each, with $\Delta\lambda$= 3, 6 and 10 nm, respectively, - when 24 μg/mL BE for b_4, b_5 and b_6 was used as a divisor, for each, with $\Delta\lambda$= 3, 6 and 10 nm, respectively, - when 36 μg/mL Be for b_7, b_8 and b_9 was used as a divisor, for each, with $\Delta\lambda$= 3, 6 and 10 nm, respectively

Table 2
TWO-FACTOR THREE - LEVEL FULL FACTORIAL DESIGNS

	Factors		Responses	
Ex no.	X_1	X_2	R_{HCT}	R_{BE}
1	-1	-1	2.0718	3.1942
2	-1	0	2.1133	2.9983
3	-1	1	2.0251	3.0858
4	0	-1	2.0633	3.1259
5	0	0	2.0723	2.9959
6	0	1	2.0129	2.9343
7	1	-1	2.0739	3.1476
8	1	0	2.0761	3.0155
9	1	1	2.0071	2.9050

Table 3
ESTIMATED REGRESSION COEFFICIENTS FOR **HCT** AND **BE** IN THE MODEL

Coefficients	b_{HCT}	b_{BE}
b_0	2.0794	2.9772
b_1	-0.0089	-0.0350
b_2	-0.0273	-0.0904
b_3	0.0117	0.0390
b_4	-0.0449	0.0622
b_5	-0.0050	-0.0335

Table 4
CALCULATED **HCT** RESULTS OBTAINED FROM THE PROPOSED METHOD IN DIFFERENT SETTINGS

Parameter	Recovery (%) in the initial selected conditions									(*)
Divisor	12.0	12.0	12.0	24.0	24.0	24.0	36.0	36.0	36.0	27.2
Δλ (nm)	3.0	6.0	10.0	3.0	6.0	10.0	3.0	6.0	10.0	5.4
λ(nm)	263.8	259.8	251.2	263.5	258.2	250.7	262.8	257.9	250.3	253.1
Mean	101.02	100.60	100.11	100.52	99.76	99.88	100.48	99.64	99.69	99.63
SD	2.07	2.11	2.03	2.06	2.07	2.01	2.07	2.08	2.01	1.70
RSD	2.05	2.10	2.02	2.05	2.08	2.02	2.06	2.08	2.01	1.71

(*) stands for optimal conditions predicted by the model

Table 5
CALCULATED **BE** RESULTS OBTAINED FROM THE PROPOSED METHOD IN THE DIFFERENT SETTINGS

Parameter	Recovery (%) in the initial selected conditions									(*)
Divisor	10.0	10.0	10.0	16.0	16.0	16.0	22.0	22.0	22.0	21.2
Δλ (nm)	3.0	12.0	28.0	3.0	12.0	28.0	3.0	12.0	28.0	27.5
λ(nm)	252.3	229.5	245	251.2	247.2	243.7	251.2	246.6	243.6	245.3
Mean	101.28	102.09	103.99	100.81	101.27	102.24	99.75	100.24	101.49	101.37
SD	3.194	2.998	3.086	3.126	2.996	2.934	3.148	3.015	2.905	2.076
RSD	3.154	2.937	2.967	3.101	2.958	2.870	3.155	3.008	2.862	2.048

(*) stands for optimal conditions predicted by the model

Table 6
ANALYSIS RESULTS OF HCT IN CIBRADEX® DIVITAB (5/6.25 (I) AND 10/12.5 (II), **BE/HCT**) TABLETS

Parameter	mg/tab																			
Divisor (μg/mL)	12		12		12		24		24		24		36		36		36		27.2 (*)	
(Δλ) nm.	3		6		10		3		6		10		3		6		10		5.4 (*)	
λ (nm)	263.8		259.8		251.2		263.5		258.2		250.7		262.8		257.9		250.3		253.1(*)	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
Mean	6.28	12.53	6.21	12.40	6.18	6.18	6.27	12.52	6.14	12.17	6.16	12.19	6.25	12.48	6.13	12.14	6.15	12.15	6.26	12.51
SD	0.05	0.11	0.05	0.03	0.05	0.05	0.04	0.04	0.06	0.05	0.05	0.05	0.05	0.04	0.06	0.04	0.05	0.04	0.04	0.03
RSD	0.81	0.87	0.81	0.24	0.83	0.83	0.64	0.29	0.92	0.39	0.82	0.40	0.82	0.33	0.92	0.36	0.82	0.30	0.59	0.25
SE	0.02	0.04	0.02	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01
CL (P=0.05)	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

(*) stands for optimal conditions predicted by the model

conditions and the optimal conditions for **HCT** and **BE** were illustrated in tables 4 and 5, respectively.

Sample analysis in optimal spectral settings

In order to perform a quantitative analysis of two different commercial tablet formulations, the ratio spectra derivative method under the above optimal spectral conditions was applied and the tablet results of initial selected conditions and the optimal conditions were presented in tables 6 and 7. We observed that in the optimal conditions the following values were obtained, namely 6.26 mg/tablet and 12.51mg/tablet with relative standard deviations as 0.59% and 0.25% for **HCT**. The corresponding values for **BE** were found as

Table 7

ANALYSIS RESULTS OF BE IN CIBRADEX® DIVITAB (5/6.25 (I) AND 10/12.5 (II), **BE/HCT**) TABLETS

Parameter	mg/tab																			
Divisor	10.0		10.0		10.0		16.0		16.0		16.0		22.0		22.0		22.0		27.5 (*)	
(Δλ) nm.	3.0		12.0		28.0		3.0		12.0		28.0		3.0		12.0		28.0		21.7 (*)	
λ (nm)	252.3		229.5		245		251.2		247.2		243.7		251.2		246.6		243.6		245.3 (*)	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
Mean	4.95	10.17	5.12	10.39	5.29	11.02	4.96	10.29	4.99	10.43	5.18	10.81	4.84	10.13	4.90	10.20	5.11	10.69	5.07	10.11
SD	0.13	0.13	0.24	0.13	0.06	0.21	0.13	10.13	0.05	0.15	0.06	0.24	0.04	0.35	0.05	0.20	0.06	0.17	0.04	0.10
RSD	2.66	1.27	4.70	1.22	1.14	1.91	2.58	10.12	1.03	1.41	1.17	2.24	0.91	3.45	1.04	1.98	1.13	1.62	0.89	0.97
SE	0.05	0.05	0.09	0.04	0.02	0.07	0.05	10.08	0.02	0.05	0.02	0.09	0.02	0.12	0.02	0.07	0.02	0.06	0.02	0.03
CL (P=0.05)	0.03	0.03	0.05	0.03	0.01	0.05	0.03	10.29	0.01	0.03	0.01	0.05	0.01	0.08	0.01	0.05	0.01	0.04	0.01	0.02

(*) stands for optimal conditions predicted by the model

5.07 mg/tablet and 10.11 mg/tablet with relative standard deviations as 0.89% and 0.97%.

Conclusions

Among classical chemometric methods and graphic techniques used in the field of analytical chemistry the ratio-derivative method is applicable when strong overlapping spectra are involved. Always when some random selection is present the experimental design method offers the best solution for a given mixture.

In this manuscript we subjected to our investigation a mixture consisting of HCT and BE and we treated it by ratio derivative method. In our knowledge the optimal experimental design for this method has not yet been discussed in the literature. In our case we selected two factor three-level full factorial design and we reported the optimal values.

The experimental design for this proposed method will be very helpful for the future analysis of other compounds by using the ratio-derivative technique.

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