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Research Article

Analytical Validation of Smartphone Spectroscopic Technic Used in an Educational Kinetic Study

S. Raissi and F. Fakhfakh

Laboratory of Material Chemistry and Catalysis, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis Campus Universitaire, Farhat Hached 2092, Tunis, Tunisia

Correspondence should be addressed to S. Raissi; sahar.raissi@fst.utm.tn

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Use of smartphone-based spectroscopy is showing a constant growth since last year. It presents the advantage of being widely available for everyone. The most important thing is that it is still a low-cost method adapted to the education context. However, as all analytical methods, it should be validated to ensure the reliability of its results. In this study, we present the steps of the validation process with its statistical tests applied to the dosage of di-iode. Shapiro–Wilk test revealed that our method has a random character. Homogeneity of variance analyses using the Cochran test confirmed the precision of the method. The Fisher test revealed the linearity of the model of correlation between I_2 concentration and the response. The relation between response and concentration is $A = 1000\,C + 0.002$. From the parameters of the linear regression of the model, we deduced the limits of quantification and $X_{Lq} = 4{\cdot}10^{-5}$ mol·L^{-1} and $X_{Ld} = 1{\cdot}10^{-5}$ mol·L^{-1}. Thanks to tightness of the sample, the method of I_2 dosage was successfully applied in iodine quantification to monitor acetone iodination during time in the context of kinetic studies with minimum system trouble. Being low cost, this method can facilitate access to physical methods in educational laboratories.

1. Introduction

Since the first edition of the EURACHEM Guide in 1998, a number of important developments in analytical quality have taken place. A growing interest is being accorded to measurement and analytical methods noticeably in relation with the development of new methods [1]. Validation of analytical methods is one of the topics requiring a sharing of practices in order to define of common guidelines for laboratories. This sharing ensures competence requirements for laboratories, proficiency testing providers, and reference material producers. As indicated in Figure 1, the life cycle of an analytical method evolves through the following:

(i) The selection of the method is a crucial step. Its selection affects directly the results.

(ii) The optimization of the method, an important step, that ensures the suitability of the method and the operation conditions of the routine.

(iii) The validation (internal or/and external) that ensures the verification of the results.

(iv) The routine use with a periodical control.

Smartphone-based spectroscopy is an emergent technic managed to quantify and describe physically human colour perception using a camera [2]. Nowadays, digitalizing images is becoming available for everyone [3]. Smartphone technology has now speared in every aspect of modern life. Since its first commercialization in 1990s, until now, smartphone use has widely expanded. In Tunisia, a North African country, in 2016, 70% of the Tunisian population possesses smartphones connected to either mobile or Wi-Fi connections according to a report of the consumer lab Ericson. The accessibility of these devices among high school students can encourage taking advantage of laboratory experiments and practical study. This type of spectroscopy is easy to handle and overcome technical problems related to material lack and damage [4–7]. It is important to be aware

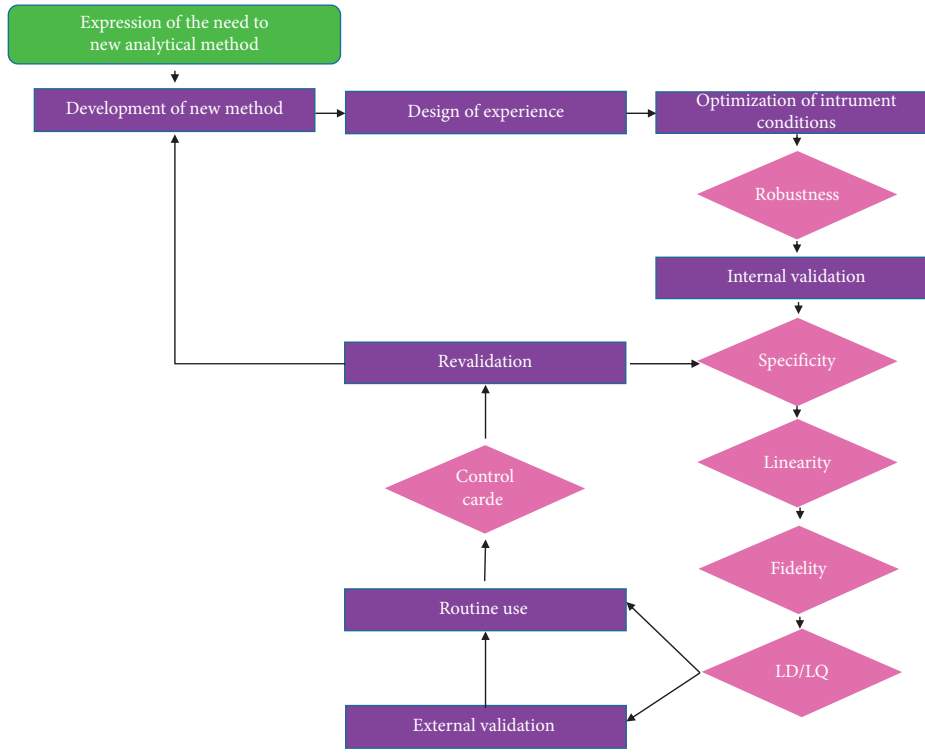

Figure 1: Process of analytical methods validation according to EURACHEM.

of the wide expansion of this type of spectroscopy which covers a lot of fields such as agriculture, biochemistry analyses, medical analyses, nanomaterial, and hazardous materials. This method can currently quantify copper [8], iron (III) [9], formaldehyde [10], water salinity [11], blood hematocrit [12], and acetazolamide [13].

The acceleration of this method spread out due to its facility and low-cost appeal to the necessity of an easy procedure establishment for its analytical validation [14]. In this study, we tried to implement a simple method using smartphone for the quantification of di-iode in order to quantify it in a kinetic lab. We describe the steps of the procedure for the analytical validation. As it is a non-normalized method, it has to be validated according to the EURACHEM Guide.

The acceleration of this method spread out due to its facility and low-cost appeals to the necessity of an easy procedure establishment for its analytical validation [14]. In this study, we tried to implement a simple method using smartphone for the quantification of di-iode in order to quantify it in a kinetic lab. Physical methods are preferred to chemical ones in reaction monitoring for kinetic studies. They are fast and they do not disturb progress. Sampling during the time is more accurate. UV-visible spectroscopy is the widely used technique mainly for coloured solutions [15]. But, this spectroscopic method is sophisticated and expensive. This apparatus cannot be afforded anywhere. Even it exists, it requires maintenance and spare parts. Use of smartphone overcomes this problem since it is affordable for all the students. So our method ensures availability at low-cost for educational institutions. However, the operation of photographing and transferring image to laptop for treatment by image *J* is still an awful operation in the method. To improve its accessibility and inclusiveness, it will be interesting to develop a smartphone application that treats images directly on the smartphone and not on a laptop. At this stage, we provide the method of smartphone use of a solution quantification, and we describe the steps of the procedure for its analytical validation. As it is a non-normalized method, it has to be validated according to the EURACHEM Guide [16]. This validation procedure has to be applied when developing smartphone new methods.

2. Materials and Methods

All reagents were handled while donning personal protection equipment (PPE), including a lab coat, gloves, and mainly eye protection under the hood. HCl is a strong acid, and skin contact should be avoided. Acetone and ethyl acetate are volatile organic solvents. They should be handled carefully. Iodine solutions must not be evacuated but stored to be correctly eliminated. Iodoacetone, a product of the reaction, is very powerful and harmful. All solutions containing this should be disposed of immediately after the experiment, and the apparatus was washed with plenty of water.

All used reagents are suitable for UV-visible spectroscopy. Acetone 99.5% and hydrogen chloride solution 1 mol·L^{-1} are delivered by Sigma-Aldrich. Iodine solution 0.5 mol·L^{-1} is provided by Merck.

In this study, samples of I_2 solutions were put in plates. This one was implanted in a carton-covered box with a small

aperture. Camera was placed in front of the aperture to photograph the plate. Image acquisition was performed by a smartphone (Samsung Galaxy A31: android version 11, 48 megapixels back camera), used with flash.

Then, Beer–Lambert relation (1) allows the calculus of solutions concentration from the intensities of solutions color measured by RGB type 3 channels solution image treatment as follows:

$$A = \log\left(\frac{I_0}{I}\right), \tag{1}$$

where I represents measurement intensity corresponding to I_2 solution and represents measurement intensity corresponding to I_2 blank.

The chosen regions of interest (ROI) were squares of 400 pixels centred on the circles of each plate wells where they were duplicated. The distribution of RGB values of every pixel was contained in histograms by applying the macro shared in the supporting information.

3. Results and Discussion

3.1. Method Validation. Reliable analytical data are a prerequisite for a correct interpretation of findings in the evaluation of scientific studies, as well as in daily routine work, analytical methods have to be validated [17]. According to the EURACHEM Guide recommendations, the required validation steps are random character, specificity, accuracy, and linearity [18]. In the case of our application, the determination of the limits of detection and quantification is also needed since the application aims to monitor the process of degradation until the total completion of the reagent. It is also important to express the results with their uncertainty. Therefore, we accomplished the process of validation by uncertainty determination of our method response.

The first parameter to evaluate in the validation process is the normal character or the random character of a series of responses to ensure the absence of bias in the results. Shapiro and Wilk [18, 19] proposed a statistic test verifying the hypothesis of normality for a random sample. Specificity traduces qualitatively the extent to which substances interfere with the determination of a substance according to a given procedure [20]. It is also an essential parameter to be verified in signal detection. It allows ensuring a negative response in the absence of measured species. Precision represents the closeness of agreement between independent test results using identical experimental procedure under stipulated conditions. It also proves the closeness between measured results and the true value of a standard sample [20]. Within a given range, the analytical responses may vary linearly with the concentration of the measured species. Linearity evaluation allows the determination of method sensitivity and method limits of detection and quantification.

Random character of the obtained measurements has to be checked and verified, it allows the confirmation of their independence and their normal distribution. Therefore, we consider 3 solutions of iodine (2.5×10^{-4} mol·L^{-1}) prepared separately from the commercial one. The dilutions and the measurements by our method were repeated for four days. Table 1 regroups the responses collected for 4 days.

To evaluate the normal character of the collected data, we use the Shapiro–Wilk test resumed in Table 1 [18]. It consists of calculating the median of responses (0.245). The number of sequences $R = 8$ is found by determining the number of values lower or higher than median. By referring to the values of $R_{\alpha/2}$ and $R_{1-(\alpha/2)}$ at the risk level $\alpha = 5\%$ given by the table, we can see that the calculated value of R is ranged between $R_{\alpha/2}$ and $R_{1-(\alpha/2)}$. Thus, we conclude that the distribution of the measurements is normal [21, 22].

We developed our method to study the kinetic of a reaction where I_2 is a reactant. Therefore, to ensure the absence of interference between the dosed species and the matrix, we evaluate the specificity of the method. We dose I_2 in a mixture composed of acetone, HCl in aqueous medium, and ethyl acetate and I_2 in water. The different solutions served to fill the wells of the same plate. Figure 2 illustrates the responses of the two series.

It shows that the two series have the same concentrations with a relative difference inferior to 5%. Specificity test prove absence of interferences by the adjunction of the kinetic blocking mixture. Our method is consequently specific and does not present a risk of interference with matrices [23].

To evaluate homogeneity of variance analyses, the Cochran test permits verification of the precision method [24]. Absorbance of iodine solution (2.5×10^{-4} mol·L^{-1}) is measured 3 times in the same plate and during 4 different days. Table 2 regroups all responses [16, 18].

We can perceive that the calculated constant C_{Cal} is less than the critical constant value at both risks of 5% and 1%. Therefore, using the Cochran test, we confirm that the variances are homogenous and there are no suspected measure [16, 18, 24]. Our method provides reliable responses with good precision.

To correlate the concentration of I_2 solutions with responses of our method, we use the Fisher test. This test allows the evaluation of relation linearity for iodine solutions in the range of concentrations: 0.5×10^{-4}; 1.0×10^{-4}; 2.0×10^{-4}; 3.0×10^{-4}; 4.0×10^{-4} mol·L^{-1} [25, 26]. Table 3 regroups all results.

From Table 3, we conclude that the model of correlation between the concentration of I_2 solutions and responses is linear. The relation between the method response and the solution concentration in I_2 is given by the following equation:

$$\begin{aligned} A &= 1000C + 0.002, \\ \text{with correlation coefficient } r^2 &= 0.9999. \end{aligned} \tag{2}$$

The detection limit is the smallest concentration that can be distinguished from the blank with a risk of 0.13%. In this case, the statistical test of comparison of the response at the value 0 becomes significant. The limit of quantification is determined with a risk of 0.05%. Their values are, respectively, calculated by equations (3) and (4) [16, 18, 24]:

Table 1: Random character of the responses of 3 iodine solutions for 4 days.

Day			Absorbance A_{ij}		
1		0.255	0.230	0.238	
2		0.255	0.219	0.252	
3		0.244	0.241	0.250	
4		0.246	0.251	0.239	
Median	R	Risk α	$(p * n)/2$	$R_{\alpha/2}$	$R_{1-(\alpha/2)}$
0.245	8	5%	6	4	10
Decision					
If $R < R_{\alpha/2}$				Monotone derive	
If $R > R_{1-(\alpha/2)}$				Fast oscillation	
If $R_{\alpha/2} < R < R_{1-(\alpha/2)}$				Random distribution	
Conclusion					
The distribution is random					

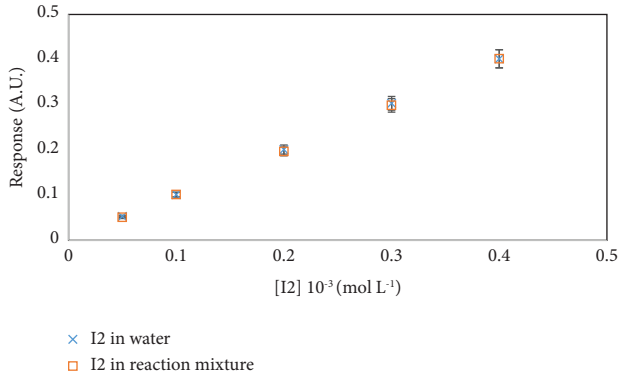

Figure 2: Responses of I_2 solutions in water and I_2 in the reaction medium for different concentrations. Error bars represent the standard deviation of measurement of each solution repeated 3 times ($n = 3$).

Table 2: Test of homogeneity of variance of iodine solution during 4 days.

Day (i)		A_{ij}		S_j^2	
1	0.255	0.230	0.238	0.163	$\sum S_j^2$=0.619
2	0.255	0.252	0.219	0.399	S_{max}^2=0.399
3	0.250	0.241	0.244	0.020	$C_{cal} = S_{max}^2/\sum S_j^2$ =
4	0.246	0.251	0.239	0.036	0.645
	C_{Cri} ($\alpha = 1\%$) = 0.864			C_{Cri} ($\alpha = 5\%$) = 0.768	
			Decision		
If $C_{Cal} < C_{Cri}$ ($\alpha = 1\%$)				Group S_{max}^2 does not contain suspected measurements	
If $C_{Cal} < C_{Cri}$ ($\alpha = 5\%$)				Group S_{max}^2 does not contain rejectable measurements	
			Conclusion		
All the values are not suspected					

S_j = standard deviation of data series J.

Table 3: Fisher test for the linearity of the method.

Concentrations (mol·L^{-1})	$A1$	$A2$	$A3$	
0.00005	0.051	0.049	0.059	Slope $a_1 = 1000$
0.00010	0.095	0.102	0.107	
0.00020	0.201	0.191	0.211	Intercept $a_0 = 0.002$
0.00030	0.302	0.310	0.298	
0.00040	0.406	0.399	0.401	Correlation coef. $r^2 = 0.9999$

Table 3: Continued.

$F_{Tab} = 3.7$	$F_{Cal} = \sum_i (\overline{Y_i} - \hat{Y}_i)^2/(n-2)/\sum_i (Y_i - \overline{Y_i})^2/(n(p-1)) = 0.03$
	Decision
If $F_{Cal} < F_{Tab}$	The model is linear
If $F_{Cal} > F_{Tab}$	The model is not linear
	Conclusion
	Calibration model is linear

Table 4: Recapitulation of validation steps.

Characteristic	Test	Description of the test
Random character	Shapiro–Wilk test conform	For the risk $\alpha = 5\%$, R, and 12 repetitions, the number of sequences $R = 8$ is between higher and lower values.
Specificity	Specific method	Response of solutions prepared in water and in reaction mixture are equal.
Precision	Cochran test conform	There is no aberrant responses in the 12 measurements; the fraction of higher variance to the sum of variances is less than Cochran critical vale at the risk $\alpha = 5\%$ and $\alpha = 5\%$.
Linearity	Fisher test conform	Fisher test showed that the fraction of calculated residuals and experimental ones is inferior to the tabulated Fisher value for 5 levels repeated 3 times.
Calibration function	$A = 1000\,C + 0.002$	Least square regression is involved to determinate the slop and the intercept of the calibration curve.
Correlation coefficient	0.9999	This value represents the fraction of the variation in one variable that may be explained by the other variable.
Limit of detection	$1\,10^{-5}$ mol·L^{-1}	Statistical test of comparison of the response at the value 0 becomes significant. The limit of detection is determined with a risk of 0.05%.
Limit of quantification	$4\,10^{-5}$ mol·L^{-1}	Statistical test of comparison of the response at the value 0 becomes significant. The limit of quantification is determined with a risk of 0.05%.
Uncertainty	$\pm\ 0.1\,10^{-4}$ mol·L^{-1}	Because of the difference between the real value and the measured one, a degree of uncertainty will pertain to measurement. Uncertainty is the absolute range in which measured value can be accepted.

$$X_{LD} = \frac{a_0 + 3S_{a0}}{a_1}, \tag{3}$$

$$X_{LQ} = \frac{a_0 + 10S_{a0}}{a_1}. \tag{4}$$

With

$$S_{a0} = \sqrt{S_e^2\left(\frac{1}{n} + \frac{\overline{X}^2}{\sum_i (X_i - \overline{X})^2}\right)}, \tag{5}$$

$$S_e^2 = \frac{\sum_{ij}\left(y_{ij} - y\right)^2 - a_1\sum_i (X_i - \overline{X})(\overline{Y_i} - Y)}{n-2}. \tag{6}$$

Expression (3) gives $X_{LD} = 1{\cdot}10^{-5}$ mol·L^{-1} and expression (4) gives $X_{LQ} = 4{\cdot}10^{-5}$ mol·L^{-1}.

The statistical precision of a response is expressed by calculating the confidence interval, which indicates the margin of error when generalizing an estimate obtained to a population of n samples. The length of the interval centred on the mean value decreases as the sample size increases. We use the following formula to calculate the uncertainty U [27]:

$$U = z * \frac{\sigma}{\sqrt{n}}, \tag{7}$$

where U: uncertainty. z = value derived from the reduced centred normal distribution, equal to 1.96 if $\alpha = 0.05$ (degree of trust); σ: standard deviation. n: the number of I_2 solutions with a concentration of 2.5×10^{-4} mol·L^{-1}.

Application of equation (7) to the results found in Table 3 indicates the $U = 0.1\ 10^{-4}$ mol·L^{-1}.

This method provides a numerical result on continuous scale from the measurement of a signal directly related to the amount of analyte. Table 4 recapitulates the steps of the validation of this method.

3.2. Monitoring of Acetone Iodination by Smartphone. Di-iode is a yellow brownish species in aqueous solution [28]. Since it has marked colour, it can be easily adapted to smartphone spectroscopy quantification as described in the supporting information, and Figure 3 describes this procedure.

From the results mentioned above, we confirm the validation of the method used for the quantification of I_2. We used our validated method to monitor I_2 concentration evolution during the reaction of acetone iodination. This method of quantification by smartphone, being easy to implement, was used to verify the mechanism and study the kinetic of acetone iodination reaction by di-iode. The equation of the reaction [29] is as follows:

$$I_2 + CH_3COCH_3 \xrightarrow{H} + \quad CH_3COCH_2I + HI. \tag{8}$$

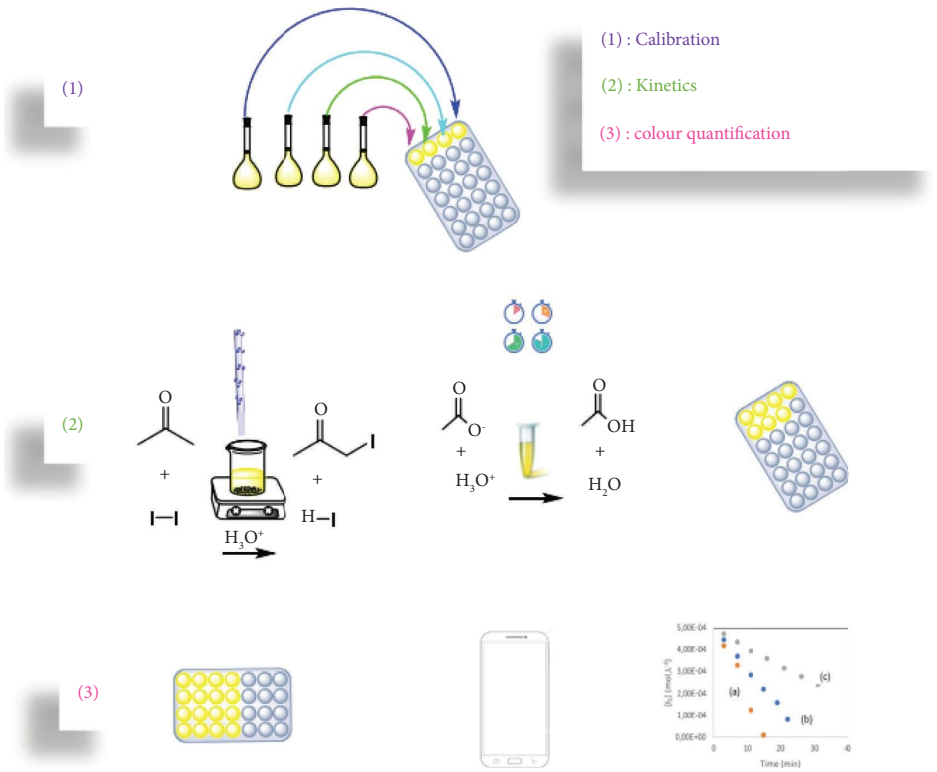

Figure 3: Graphical illustration of our smartphone analytical method and it can be used in monitoring of kinetic study.

Table 5: Composition of the experiments to evaluate the reaction order.

Experiment	V (HCl 1 M) (mL)	V (C_3H_6O) (mL)	V (I_2 0.05 M) (mL)
1	2.5	1.0	1.0
2	2.5	0.5	1.0
3	1.0	0.5	1.0

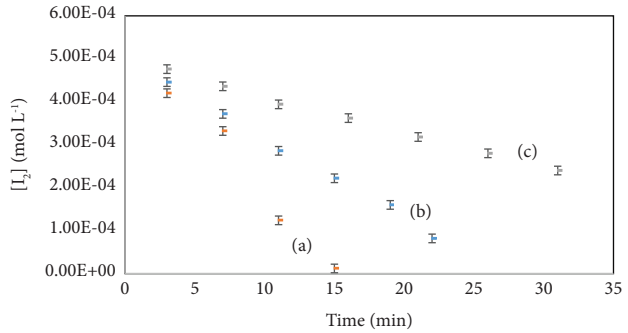

Figure 4: I_2 concentration variation versus time measured by smartphone method for 3 different concentrations of mixture. The error bars represent fixed uncertainty calculated by equation (7).

The acetone iodination mechanism in acid medium is complex. It was demonstrated that its law equation is as follows [29]:

$$r = k[H^+][CH_3COCH_3]. \quad (9)$$

Therefore, the reaction rate is of the first order toward $[H^+]$ and $[CH_3COCH_3]$ and of order 0 toward I_2 [29–31].

At an ambient lab temperature of 298 K, we prepared 3 series of experiments carried out by mixing I_2 solution with acetone in acidified aqueous medium according to the composition detailed in Table 5.

Figure 4 describes the evolution of I_2 concentration in three different initial conditions as a function of time. All variations are linear with a correlation coefficient up to 0.97, which confirms the order pseudo-zero-order to I_2. The

Table 6: Kinetic parameters of the reaction of iodination of acetone.

	Equation	Correlation coefficient
Exp (1)	$Y = -4.10^{-5}X + 0.0005$	0.9764
Exp (2)	$Y = -2.10^{-5}X + 0.0005$	0.9961
Exp (3)	$Y = -8.10^{-6}X + 0.0005$	0.9967

equation of I_2 variation versus time for each experiment is regrouped in Table 6.

Partial orders (a) and (b) toward acetone and acid can be deduced using different initial concentrations of the three experiments. The kinetic law is written for the experiments $i = 1; 2; 3$ are as follows:

$$r_i = k\left[C_3H_6O\right]_{0i}^{a}\left[H^+\right]_{0i}^{b}. \quad (10)$$

From the initial rate, we calculate the partial orders $a = 1$, $b = 1$, and the rate constant $k = 0.0035\,L^2{\cdot}mol^{-2}{\cdot}s^{-1}$. These results conform to those found before I_2 was quantified with UV-visible spectroscopy [29].

From these results, we demonstrate that using smartphone spectroscopy is reliable for the determination of I_2 concentration.

4. Conclusions

Use of smart technologies such as USB cameras or smartphones constitutes an available method that can be used for education. The quasi totality of students around the world processes such devices. Their use can facilitate the study of reaction evolution with a reduced cost. Because of the large spread of this method, we proposed in this study a procedure of validation with smartphone for I_2 quantification:

(i) We tested the random character of the method responses by the Shapiro–Wilk test.

(ii) We proved the specificity of the method. No difference was observed between the response of the method when I_2 is dissolved in water or in reaction mixture.

(iii) We verified that there is no suspected neither aberrant responses by the Cochran test.

(iv) We applied the Fisher test and we found that the method is linear. The equation of the calibration curve allowed us the determination of the limits of detection and quantification of the method.

(v) We calculated the uncertainty of the method.

The validated method of I_2 quantification is applied to the kinetic study of acetone iodation. This method can be more developed and used for other chemical reactions in laboratory. Our developed method serves the equity of studying kinetics with physical methods for the neediest institutions in sophisticated materials. Moreover, it ensures a considerable reduction of chemical quantities compared to the classical UV-visible spectrophotometer needing a minimum amount of solution to fulfil the cell. However, the operation of photographing and transferring image to laptop to be treated by image *J* is still an awful operation in the method. To improve its accessibility and inclusiveness, it will be interesting to develop a smartphone application that treats images directly on the smartphone and not on laptop.

Abbreviations

A: Absorbance
I: Colour intensity of I_2 solution
I_0: Colour intensity of water
C: Concentration of I_2 solution
r^2: Correlation coefficient
A_{ij}: Absorbance of a I_2 solution number i during the day j
R: Number of sequences in the Shapiro–Wilk test
S_j: Standard deviation of data series j
S_{max}: Maximum standard deviation of data series
C_{Cal}: Cochran-calculated constant
C_{Cri}: Cochran critical constant
a_1: Slope of linear curve
a_0: Intercept of linear curve
F_{Cal}: Fisher test calculated value
F_{Tab}: Fisher test reference value
X_{Lq}: Limit of quantification
X_{Ld}: Limit of detection
U: Uncertainty
ri: Rate of the reaction
a: Partial order toward C_3H_6O
b: Partial order toward H^+
k: Constant rate.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article's Supplementary Materials. It contains a description of the details of the kinetic experience and the image *J* macro code and can be directly used.

Additional Points

Highlights. Validation means fit for purpose. Smartphone spectroscopy for quantification. Statistical test for analytical method validation.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Sahar Raissi is responsible for conceptualization, methodology, software, formal analysis, investigation, resources, and writing original paper. Fatma Fakhfakh is responsible for validation and reviewing.

Supplementary Materials

Description of the details of the kinetic experience and the image *J* macro code are regrouped in the supporting information. (*Supplementary Materials*)

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