



Continuous blending monitored and feedback controlled by machine vision-based PAT tool

Dorián László Galata, Lilla Alexandra Mészáros, Máté Ficzer, Panna Vass, Brigitta Nagy, Edina Szabó, András Domokos, Attila Farkas, István Csontos, György Marosi, Zsombor Kristóf Nagy*

Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, H-1111, Budapest, Műgyetem rakpart 3, Hungary

ARTICLE INFO

Article history:

Received 10 November 2020
Received in revised form 11 January 2021
Accepted 12 January 2021
Available online 16 January 2021

Keywords:

Continuous blending
Machine vision
PAT
API content measurement
Feedback control
Colored API

ABSTRACT

In a continuous powder blending process machine vision is utilized as a Process Analytical Technology (PAT) tool. While near-infrared (NIR) and Raman spectroscopy are reliable methods in this field, measurements become challenging when concentrations below 2 w/w% are quantified. However, an active pharmaceutical ingredient (API) with an intense color might be quantified in even lower quantities by images recorded with a digital camera. Riboflavin (RI) was used as a model API with orange color, its Limit of Detection was found to be 0.015 w/w% and the Limit of Quantification was 0.046 w/w% using a calibration based on the pixel value of images. A calibration for in-line measurement of RI concentration was prepared in the range of 0.2–0.45 w/w%, validation with UV/VIS spectrometry showed great accuracy with a relative error of 2.53 %. The developed method was then utilized for a residence time distribution (RTD) measurement in order to characterize the dynamics of the blending process. Lastly, the technique was applied in real-time feedback control of a continuous powder blending process. Machine vision based direct or indirect API concentration determination is a promising and fast method with a great potential for monitoring and control of continuous pharmaceutical processes.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Since the U. S. Food and Drug Administration (FDA)'s Process Analytical Technology (PAT) initiative [1] was released in 2004, pharmaceutical contenders in the field of pharmaceutical manufacturing have been developing analytical technologies capable of monitoring both batch and continuous manufacturing processes in real time. The emergence of fast and accurate PAT sensors capable of monitoring critical quality attributes of the processed materials was one of the prerequisites of realizing continuous pharmaceutical manufacturing. In the last decade, several drugs have been approved by the FDA which are manufactured on a continuous line [2]. Continuous production has several benefits compared to traditional batch technologies including more consistent quality of product, production lines of smaller size [3], reduced amount of waste due to more efficient control of processes and easier scale up [4]. Continuous drug product manufacturing involves several essential processes which need to be monitored by PAT tools in

order to assure that the end product is of the desired quality. These include continuous blending [5,6], dry [7,8] and wet granulation [9,10], tableting [11], film coating [12] and capsule filling [13]. Continuous blending needs to be a part of practically all production lines, as pharmaceutical products usually consist of more than one component. It is vital to ensure that the components are appropriately mixed, since variations in the concentration of the blend can influence important quality attributes of the end product, such as content uniformity of tablets [6]. So far, near-infrared (NIR) spectroscopy has been the most commonly used PAT tool in the monitoring of continuous mixing. Gao et al. used NIR spectroscopy to measure the residence time distribution (RTD) of a process where an active pharmaceutical ingredient (API) is mixed with an excipient [5]. NIR spectroscopy was utilized by Singh et al. to monitor the bulk density of the product of a continuous blender incorporated in a continuous direct compression manufacturing line [14]. Light induced fluorescence can also be used to measure the concentration of the API in powder blends [15]. Raman spectroscopy has not been used in the monitoring of continuous mixing until recently [6]. Nagy et al. created a 'Process Analytically Controlled Technology' (PACT) by utilizing Raman spectroscopy to measure the API concentration in the product of continuous twin-screw blending

* Corresponding author.
E-mail address: zsknagyo@oct.bme.hu (Z.K. Nagy).

process, this signal was used to create a feedback control of the API feeder [6]. These techniques not only enable us to examine the homogeneity of the produced blends, but also allow the construction of RTD models. These provide information about the behavior of the material inside the blender, they can be used to determine the factors influencing the residence time [7]. Residence time can be related to the efficiency of the mixing process and if a contamination occurs, the transfer function of the process can be used to predict the distribution of contaminants in the system [7].

As the API content becomes lower, the difficulty of developing a reliable mixing method becomes more challenging, as obtaining a homogeneous blend is more difficult when a component is present in a very small amount [16]. In the case of continuous manufacturing, finding the correct mixing equipment solves only a part of the problem, as these technologies (generally) also require a PAT tool that can accurately quantify the amount of API at such low concentrations. Generally, a product is considered low dose when it contains less than 2 w/w% API [17]. At such concentrations, especially below 1 w/w%, NIR and Raman spectroscopy become less accurate and chemometric models must be constructed especially carefully in order to avoid significant measurement errors [18]. Indeed, few works have been published, in which the amount of API is quantified at levels below 0.5 w/w% and even these are not necessarily suitable as a PAT tool. Alcalá et al. [18] used transmission NIR spectroscopy to measure the API content of tablets at concentrations of 0.01–5 w/w%. They found that when very low concentrations are measured, it is advantageous to use a calibration covering a narrower range around the target. Li et al. [19] utilized Raman mapping to quantify the API concentration of powders at levels around 0.1 w/w%. While a low limit of detection and good accuracy were achieved, this technique cannot be used as a PAT tool. Igne et al. [20] studied the applicability of light-induced fluorescence (LIF) spectroscopy for the monitoring of API content inside the feed frame of a tablet press. It was found that low errors can be achieved when measuring concentrations around 0.67 w/w% and after further improvement, LIF could become a reliable PAT instrument. These results imply that measuring API content in the sub 1 w/w% range is not a trivial task, this area could greatly benefit from innovative solutions. In the case of colored APIs, color measurement can potentially become a method of great sensitivity. This can be accomplished with digital cameras, which are a promising PAT tool in the pharmaceutical industry, as indicated by its various applications [21–23]. Digital cameras can acquire data in real time, they require no sample preparation, measurements are fast and non-invasive, while they are also far cheaper than spectrometers, therefore their application can be desirable in many situations. Mészáros et al. [24] used a digital camera to determine the API content of tablets containing meloxicam, a yellow drug, with a target concentration of 2 w/w%. With the developed method, it was possible to predict the API content of tablets with 2 w/w% drug with a relative error of approximately 5%. Durão et al. [25,26] and Gosselin et al. [27] compared an RGB camera with a NIR and a LIF spectrometer to evaluate their capability of measuring the concentration of various vitamins in vitamin blends inside the feed frame of a tablet press. They found that for some compounds, the RGB camera can sufficiently measure the concentration.

The analysis of relevant literature has shown that there is a scarcity of sensors capable of measuring concentrations in the sub 2 w/w% range in real time. Therefore, the purpose of this study was to develop a machine vision-based method that can measure concentrations below 0.5 w/w% with good accuracy in the case of colored APIs. The applicability of the proposed system for the in-line monitoring of a continuous blending process needed to be evaluated. Furthermore, this technique can be used in RTD measurements to characterize the system. So far most works published featuring PAT technologies were only utilized for the monitoring of the system

and the ultimate goal of feedback control is not realized. Thus, the last goal of our work is to use the machine vision-based method for feedback control, creating a real PACT tool.

2. Materials and methods

2.1. Materials

Riboflavin (RI) (vitamin B₂, an essential nutrient for humans) was obtained from Merck (Budapest, Hungary). Microcrystalline cellulose (MCC) (Vivapur 200) and anhydrous calcium hydrogen phosphate (CHP) (Anhydrous Emcompress) were purchased from JRS Pharma (Rosenberg, Germany).

2.2. Methods

2.2.1. Continuous powder blending

Powder mixtures containing low doses of RI were prepared the following way. Firstly, a mixture containing 2 w/w% of RI and 98 w/w% of MCC was prepared in batches of 100 g on a batch homogenizer (QUICK BIN, Quick 2000 Ltd., Hungary) by rotating the flask containing the powders at 60 rotations per minute (rpm) for 10 min. This blend was then passed through a twin screw continuous blender (Quick 2000 Ltd., Hungary) with a 16 mm diameter (with an L/D ratio of 25) screw operated at 100 rpm. This resulted in a homogeneous blend where RI was distributed uniformly on the MCC particles, the abbreviation RI-MCC will refer to this blend, where RI is present as a model drug. This colored powder was homogenized with CHP in the following way. RI-MCC was fed from a gravimetric feeder (MechaCAD, Dunakeszi, Hungary) operated in volumetric mode, while CHP was fed from another gravimetric feeder (Brabender Technologie, Duisburg, Germany) in volumetric or gravimetric mode depending on the experiment. The powders were fed into the same twin screw blender operated at 100 rpm. The screws consisted of conveying elements and kneading zones in a configuration shown in Figure S1.

2.2.2. Measurement of API concentration with RGB camera

The powder blend leaving the blender fell on a conveyor (Brabender Technologie, Duisburg, Germany) operated at 100 % speed (0.083 m/s). An in-house built camera stand was used to place a Basler acA4112-30uc (Basler, Germany) area scan, RGB camera above the conveyor. The camera was equipped with a Basler MEGA TS1214-MP (Basler, Germany) objective, the height of the stand was set so that the objective was 30 cm above the conveyor. A box was placed around the camera stand to exclude external light and thus protect the system from variations in ambient lighting. A ring light consisting of three rows of white light emitting diodes (Apokromat Ltd., Hungary) was attached to the objective to provide illumination. Images of 500 by 500 pixels were acquired with an exposition time of 2000 μ s with a rate of 7 frames per second (fps), at this rate, every particle is present at 7 or 8 consecutive images depending on its position on the first image. The imaged area was a square with a side length of 90 mm, therefore each pixel in the image represents an area of 0.18 by 0.18 mm. An illustration of the utilized continuous blending setup and the in-line camera is given in Fig. 1.

Images were analyzed using Matlab 9.8.0.1380330 (MathWorks, Natick, MA, USA). The first step of analysis was segmenting the pixels of the sample from the background. For this purpose an image taken from the conveyor without any material on it was used. Images were converted to CIELAB color space [28], the L component of this color space was used to determine whether a pixel belongs to the sample or the background. The average L value was calculated for the background image, and a threshold was determined by multiplying this value with 1.35. When a pixel had an L

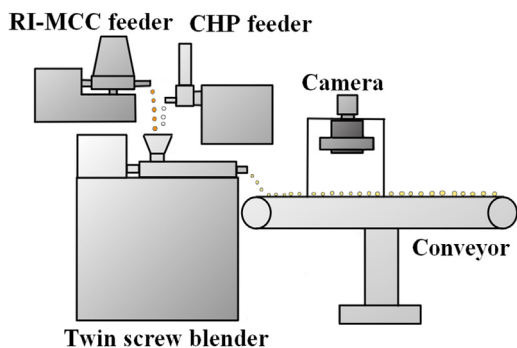


Fig. 1. In-line measurement setup of continuous blending monitored with RGB camera.

value higher than this limit, it was considered as part of the sample, otherwise it was removed from analysis. Figure S2. shows the result of this background removal process.

The API content was determined based on the b^* value of the pixels (in the CIELAB color space, this represents the position of the color on a scale between blue and yellow), for each image, the average b^* value of the sample pixels was calculated, thus the images were represented by this value. The RI content of the samples determined by UV-vis spectrometry was used as dependent variable, a second order polynomial calibration curve was fitted to the average b^* value and the measured RI content. Thereafter, the RI content of the samples was determined by calculating the average b^* value of the images and converting this value with the fitted polynomial calibration curve.

2.2.3. Measurement of API concentration with UV-vis spectrometer

UV/VIS spectrometry was chosen as a routine analytical method to determine the RI content of the samples. During experiments, samples of approximately 1 g were collected at the end of the conveyor. As the speed of the conveyor is 8.3 cm/s and the length of the imaged area is 9 cm, it takes 1.094 s for a particle to pass through the area. The total mass flow during the experiments was approximately 1200 g/h or 0.33 g/s, multiplying this with 1.094 s, 0.36 g of material is present in each image. Since 30 images were averaged for the concentration predictions, at a rate of 7 fps it requires 4.3 s to record these image, during this time approximately 1.42 g of material passes under the camera, thus the collected sample size of 1 g is similar to the sample size of the images. By recording the sample collection time and measuring how long the sample takes to travel from the camera to the end of the conveyor, the samples could be coupled with the appropriate images. The mass of the collected samples was measured, and then the whole sample was dissolved in distilled water in a volumetric flask. After stirring for 10 min, 15 mL of the solutions was filled in a centrifuge tube and the samples were centrifuged at 1000 rpm for 10 min in order to elutriate the suspended MCC and CHP particles. A 10 mm cuvette was used to measure the absorbance of the samples with an Agilent 8453 UV-vis spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) at 445 nm which was the most intense peak of RI. The calibration used to determine the RI concentration of the samples was prepared by measuring the absorbance of solutions containing RI in the following concentrations: 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15, 17.5 and 20 mg/L.

2.2.4. Characterizing the performance of the machine vision system

The Limit of Detection (LoD) and Limit of Quantification (LoQ) were calculated based on formulas given in the ICH Q2 guideline [29] (Eqs. (1) and (2)):

$$LoD = \frac{3.3\sigma}{S} \quad (1)$$

$$LoQ = \frac{10\sigma}{S} \quad (2)$$

where S is the slope of the calibration curve of the analyte and σ is the standard deviation of the response, which can be represented with various methods. In this work the standard deviation of the y-intercept of the calibration line was chosen. The slope of the calibration curve and standard deviation of y-intercept were determined by the 'slope' and 'stexy' functions in MS Excel, respectively.

In order to evaluate the capability of the method in predicting the quality attributes of test samples, the root mean squared error of prediction (RMSEP) can be calculated using the following formula (Eq. (3)):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (y_{\text{predicted}} - y_{\text{measured}})^2}{n}} \quad (3)$$

where $y_{\text{predicted}}$ is the value predicted by image analysis, y_{measured} is the value measured by UV/VIS spectrometry and n is the number of samples.

2.2.5. Residence time distribution measurement

RTD of the system was determined by adding pulses of RI-MCC to a constant flow of CHP. CHP was fed at a rate of 1 kg/h in gravimetric mode, 1 g of RI-MCC was added to this instantaneously. The obtained response curves were evaluated by fitting a tanks-in-series model to them as described by Engisch et al. [30]. Firstly, the concentration profiles were separated in a way that their timescale begins at the second the pulse was added and the baseline was subtracted so that values were close to zero before the peak. The residence time distribution $E(t)$ can be calculated by dividing the concentration profile by the area under the curve (Eq. (4)).

$$E(t) = \frac{C(t)}{\int_0^\infty C(t) dt} \quad (4)$$

In order to calculate the parameters of RTD, a function is fitted to this using two parameters, n is the number of continuous stirred tank reactors (CSTRs) in the series, while τ is the mean residence time (Eq. (5)).

$$E(t) = \frac{t^{n-1}}{(n-1)! \left(\frac{\tau}{n}\right)^n} e^{\left(-\frac{nt}{\tau}\right)} \quad (5)$$

2.2.6. Feedback control

The real-time measurement of RI concentration was utilized in feedback control experiments where the feeding rate of RI-MCC was modified in order to keep RI concentration at a set value. For this purpose, a PID controller was utilized as described by Nagy et al. [6]. The controller used a proportional and integral term, derivative term was not used. The proportional term (P) was calculated by subtracting the measured concentration ($c(n)$) from the setpoint (x_a), this was multiplied by the proportional gain, K_p (Eq. (6)):

$$P = K_p * (x_a - c(n)) \quad (6)$$

When the K_p is too small, the system responds very slowly to changes, while a too large value leads to high amplitude oscillation around the target value, thus it is vital to find the optimal value for

Table 1

RI content of calibration samples determined by UV/VIS spectroscopy.

Nominal RI-MCC content (w/w%)	Nominal RI content (w/w%)	Measured RI content (w/w%)
1	0.02	0.024 ± 0.001
2	0.04	0.043 ± 0.002
3	0.06	0.067 ± 0.003
4	0.08	0.085 ± 0.001
5	0.1	0.100 ± 0.003

this parameter. The integral term (I) is calculated as follows (Eq. (7)):

$$I = K_I \sum_{i=1}^n [t(i) - t(i-1)] * \frac{2 * x_a - c(i-1) - c(i)}{2} \quad (7)$$

where K_I is the integral gain and t is the time of the measurements. The integral term is useful in eliminating the residual error which occurs when only a proportional term is used. The control signal (x_c) is the sum of the proportional and integral terms (Eq. (8)) and it is used to calculate the output signal of the controller (x_o) that is used to determine the new feeding rate of the RI-MCC feeder (Eq. (9)).

$$x_c = P + I \quad (8)$$

$$x_o = \left(\frac{x_a - c_{min}}{c_{max} - c_{min}} \right) * 100 + x_c \quad (9)$$

where c_{min} is the RI concentration measured at the minimum feeding rate and c_{max} is the RI concentration measured at the maximum feeding rate.

3. Results and discussion

Experiments were performed with several purposes. Firstly, the ability of the machine vision technique in detecting and quantifying the colored model API, RI in very low concentrations was examined. This was followed by utilizing it in an experiment where its ability to monitor the changes in concentration around a target was validated. The method was then used to characterize the RTD of the continuous blending system to map the dynamics of the continuous blending process. Lastly, the real-time information yielded by the camera was used as a signal for feedback control, essentially creating a PACT system.

3.1. Determination of limit of detection and limit of quantification

In order to evaluate whether the proposed machine vision system is capable of measuring RI content in sub 1 w/w% ranges, an experiment was carried out to determine the LoD and LoQ of the method. Calibration samples were prepared by mixing 1-2-3-4-5 w/w% of RI-MCC with CHP in the batch homogenizer (60 rpm, 10 min). These samples were manually fed into the inlet of the twin screw blender, which was operated at 100 rpm. Images were recorded from the material falling on the conveyor. Pure CHP was also measured to represent the y-intercept of the calibration. The RI content of samples taken at the end of the conveyor was determined by UV/VIS spectroscopy, results are shown in Table 1.

For comparison, an image of the six calibration samples is presented in Fig. 2. The samples can be clearly distinguished with the naked eye, implying that the colored API gives an intense color even in these low concentrations.

After segmentation, the average b^* value of the sample pixels was calculated for all images. These values were used to create a regression model that predicts the RI content of the samples. It was found that a second order polynomial can describe the correla-

Table 2

Calculation of LoD and LoQ.

Parameter	Value
Slope of the calibration curve	0.983
Standard deviation of y-intercept	0.00448
Limit of Detection	0.015 w/w%
Limit of Quantification	0.046 w/w%

Table 3

RI content of in situ calibration samples determined by UV/VIS spectroscopy.

RI-MCC feeder rpm	Measured RI content (w/w%)
10	0.198 ± 0.005
15	0.262 ± 0.004
20	0.324 ± 0.007
25	0.388 ± 0.002
30	0.452 ± 0.014

tion with good accuracy. After fitting the polynomial, the following equation was obtained (Eq. (10)):

$$RI\% = 0.0001495b^{*2} + 0.005399b^{*} + 0.04769 \quad (10)$$

The R^2 value of the fit was 0.983. The values obtained during the calculation of LoD and LoQ are shown in Table 2.

The method has a LoD value of 0.015 w/w% and a LoQ of 0.046 w/w%. This shows that the developed machine vision method can be safely used to measure concentrations as low as 0.05 w/w%. This method can potentially be used with most of the colored substances, furthermore, the possibility of detection in such low quantities might also enable the utilization of colored compounds as tracer materials for indirect quantification of colorless APIs. A possible example of this is the case when a colorless API is coprocessed with a colored tracer material (for example spray drying, wet granulation, extrusion). If the ratio of their concentrations remains constant during the manufacturing steps, the concentration of the colored tracer allows us to indirectly determine the concentration of the API as well. Before such an implementation, careful consideration is required, as the different components have their own RTDs and the API and tracer materials do not necessarily disperse uniformly. Thus, the even distribution of the components should be proven using a reference method such as HPLC. Consequently, the tracer technique should only be applied when it can be proven that the tracer material has a similar RTD to that of the API.

3.2. Utilization of the machine vision system as a PAT tool

As the next step, a new in situ calibration was prepared in the working range of the RI-MCC feeder. In this case, CHP and RI-MCC were fed using their feeders. The mass flow of CHP was set to 1 kg/h, while the feeder of RI-MCC was operated in volumetric mode, its rpm was set to 10, 15, 20, 25 and 30. Volumetric feeding could be used in this case because during this short experiment the mass of RI-MCC in the feeder did not change significantly, thus the mass flow remained nearly constant. The twin screw blender was operated at 100 rpm. The feeding rate of RI-MCC was kept at the aforementioned values for three minutes. During this time, samples were taken at the end of the conveyor to determine the RI content by UV/VIS spectrometry. The measured RI concentration for each feeder rpm setting is shown in Table 3. Plotting these values results in an almost perfect first order linear fit (data not shown), implying that the feed factor (the weight of powder delivered by one revolution of the feeder) [31] is almost constant in this range.

Similarly to the first case, a second order polynomial was fitted to the RI concentrations measured by UV/VIS spectroscopy and the

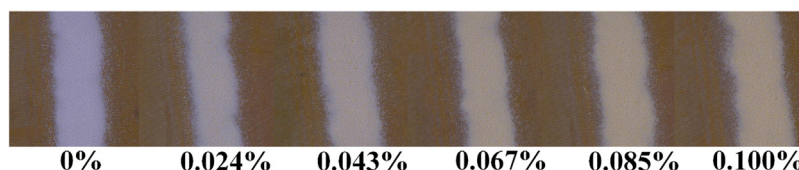


Fig. 2. Sample images of the six calibration samples used to determine the LoD and LoQ of the method.

Table 4

RI content predicted by image analysis and measured by UV/VIS spectrometry during the blending experiment.

Time (s)	Predicted RI w/w%	Measured RI w/w%	Relative error
18	0.351	0.352	-0.284%
78	0.346	0.340	1.765%
138	0.334	0.344	-2.907%
198	0.282	0.280	0.714%
258	0.279	0.276	1.087%
318	0.304	0.289	5.190%
378	0.304	0.304	0%
438	0.313	0.313	0%
498	0.314	0.303	3.630%
558	0.359	0.370	-2.973%
618	0.348	0.362	-3.867%
678	0.367	0.372	-1.344%
738	0.349	0.358	-2.514%
798	0.348	0.351	-0.855%
858	0.334	0.343	-2.624%

average b^* value of the images; in this case the equation looks as follows (Eq. (11)):

$$RI\% = 0.000488b^{*2} - 0.00267b^* + 0.0983 \quad (11)$$

An R^2 value of 0.997 was obtained. The increase compared to the previous calibration can be attributed to the absence of 0% samples, which do not fit well on the practically linear line of the samples containing RI.

This calibration was utilized in a monitoring experiment, where its predictions were validated with UV/VIS spectrometry. This was carried out during an experiment where RI-MCC was fed at constant rates and twice the rpm of the CHP feeder was changed. Both RI-MCC and CHP were fed in volumetric mode, the twin screw blender operated at 100 rpm. Images of the product on the conveyor were taken at a rate of 7 fps, the RI concentrations predicted were averaged for every 30 images to obtain a result that is more robust to variability between individual images. At 160 s, the feeding rate of CHP was increased from 52 rpm to 67 rpm and at 480 s it was decreased from 67 rpm to 52 rpm. The experiment ran for 15 min, at 30 s and every 60 s afterwards a sample was taken at the end of the conveyor for UV/VIS measurement, resulting in 15 sample points. Fig. 3. shows the predicted and measured RI concentrations during the experiment.

The concentration values predicted by images and measured by UV/VIS spectrometry were coupled by taking into account the time the sample takes to travel from the camera to the end of the conveyor, which is 12 s, thus for example the sample taken at 30 s corresponds to predictions by the camera at 18 s. To compare measured and predicted values, the values predicted by 10 images around the sampling time point were averaged with the sampling time in the middle, Table 4 shows these values.

In order to quantify the predictive capability of the image analysis method, the RMSEP was calculated resulting in a value of 0.0082 w/w%, which is a relative error of 2.53 % at the target concentration of 0.324 w/w%. This shows that the image analysis method is capable of measuring the very low concentration of RI with great accuracy. The results can be compared with other works where authors reported the measurement of API concentration with an

RGB camera. Mészáros et al. [24] measured the concentration of meloxicam in tablets with API contents between 0–2.5 %, an RMSEP value of 0.11 was obtained, which corresponds to a relative error of approximately 5%. This is a similar result, showing that this technique can also be utilized in the case of tablets with a colored API. Durão et al. [25] measured the concentration of beta-carotene, RI, ferrous fumarate, ginseng, ascorbic acid and pyridoxine in a multi-component blend. They found that the RGB camera can accurately predict the concentration of ferrous fumarate ($R^2_{\text{test}} = 0.93$), RI was predicted with an R^2_{test} value of 0.62, the other compounds resulted in values below 0.5. In a following work, Durão et al. [26] studied the specificity of PAT tools in the measurement of similar blends. Unlike the previous study, this time they introduced concentration changes according to a design of experiments, where the concentration of multiple ingredients is changed between each setting. This way it can be determined how the other components influence the measurement of a certain compound. In this case, the R^2_{test} of RI was 0.78, while a value of 0.7 was determined for ferrous fumarate and ginseng. The concentration of RI was ~1% in both works. In the present work, a higher accuracy was obtained, this shows that the measurement can be more precise when other colored substances are not interfering.

After the successful validation of the technique, the machine vision system was utilized to determine the RTD of the system. An accurately measured RTD can be very beneficial for continuous manufacturing processes, as it enables the tracing of materials, the identification of out of specification product, while understanding the reaction of the system to disturbances can also contribute to the development of a robust feedback control. The RTD measurement was done by adding pulses of RI-MCC to a flow of pure CHP. The twin screw blender was operated at 100 rpm, CHP was fed at a rate of 1 kg/h. Pulses of 1 g of RI-MCC were added instantaneously into the feeding zone of the blender at three time points (15 s, 67 s, and 125 s after starting the experiment). Images were recorded from the material on the conveyor, the developed calibration was utilized to calculate the RI content of the samples. The resulting concentration-time plot is shown in Fig. 4.

After fitting the function to the three individual measurements, it was found that a model with 104 tanks and a mean residence time of 32.12 s sufficiently fits the curves as shown in Figure 5. RI gives a rather narrow peak, about 15 s wide at the bottom. This knowledge can be used during a continuous manufacturing process to develop a more robust control and quality assurance strategy.

As RTD measurements are likely to become more common in the future of pharmaceutical manufacturing, the demand will grow for tools allowing fast and cheap execution of such measurements. For this purpose, RI can possibly be used as a tracer material in a case where the concentration of the API can not be measured directly or at least not economically.

3.3. Feedback control of the continuous blending system

In order realize an effective feedback control, an optimal value for the proportional and integral terms of the PI controller needed to be determined. The optimization of the proportional term K_p was carried out by starting at a larger value and then lowering it and

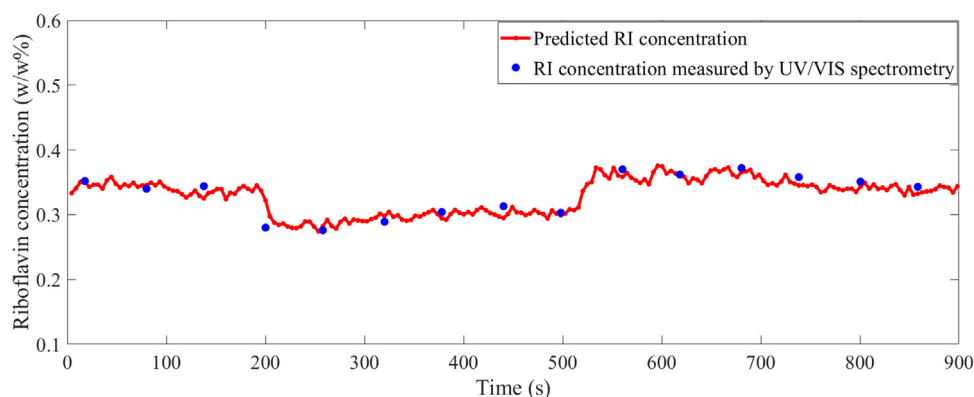


Fig. 3. Predicted and measured RI concentrations during a blending experiment. Arrows mark the step changes in the feeding rate of CHP.

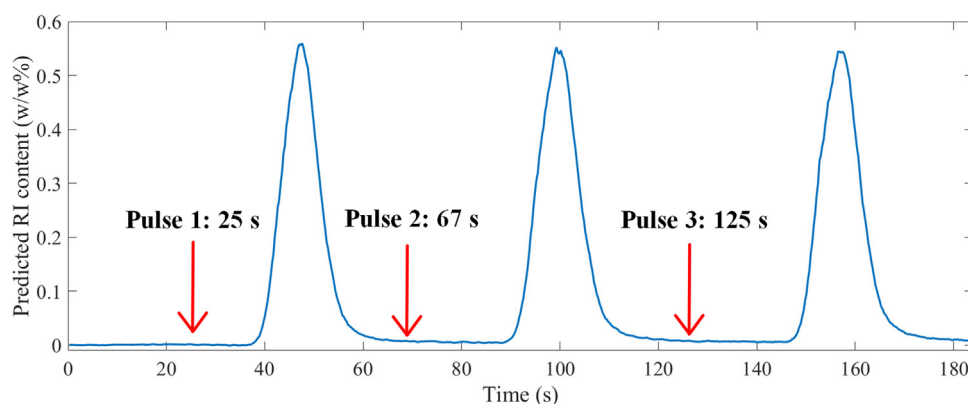


Fig. 4. Calculated RI concentration vs. time during the RTD experiment.

evaluating how the system oscillates. During the optimization of K_P , the integral term, K_I was set to 0. In the first experiment, a K_P value of 5 was tested (Fig. 5a). The results clearly show that this value is too large, as the system oscillates with high amplitude. After this the value was lowered to 3 (Fig. 5b). The performance was better in this case, however a small amount of lasting deviation could be observed, therefore in the next step, the K_I term was introduced at a value of 0.01. This setting resulted in significant improvement (Fig. 5c). In this case the oscillations are quickly dampened and after around two minutes, the concentration reaches values close to the setpoint.

This setting ($K_P = 3$, $K_I = 0.01$) was found to be reasonably good, therefore a last experiment was performed where the capability of the controller was tested by simulating a gradual decrease in the feeding of CHP which might occur during volumetric feeding of powders. (Fig. 6). This was accomplished by lowering the feeding rate of CHP by 2 rpm every 3 min during the whole experiment. In order to compare the performance of the controller with a scenario where no PAT tool is used, after 540 s the feeding rate of RI-MCC was no longer adjusted by the controller.

The controller was capable of maintaining the concentration at a level slightly above the target (from 140 to 540 s, the average concentration was 0.339 w/w%, which is a relative difference of 4.6 % compared to the target). This difference can be attributed to the fact that the controller had to constantly compensate for the reductions in the feeding rate of CHP. After feedback was no longer applied, RI concentration started to increase, highlighting the importance of a PAT tool in continuous blending. This experiment simulated a case where due to volumetric feeding, the mass flow of a powder during blending is not constant, instead it tends to decrease with time until eventually a refill is required. As the system was controlled

real-time based on the information yielded by the PAT tool, this can be considered an implementation of PACT. So far, this is not a common occurrence in the literature, as most PAT applications tend to emphasize collecting information about the system, while refraining from utilizing the data for real time control purposes.

The implementation of this machine vision-based method does not require large investments, as the utilized camera is not required to be capable of recording images at several hundred fps, nor must it have a very high resolution. In fact, a camera with performance similar to one that is found in an average smartphone is perfectly appropriate for these measurements. The main requirements are therefore the ability to establish real-time connection with a computer for the analysis of the images and a software which allows the user to save the exact settings of the camera, so that the reproducibility of the measurements can be assured. An industrial application also necessitates an apparatus which is compliant with the regulations, there are cameras which are designed to meet the standards of pharmaceutical manufacturing [32,33]. The applied illumination system must be capable of providing constant lighting, as disturbances in the brightness can be detrimental.

The proposed machine vision technique is capable of accurately measuring the concentration of RI in quantities below 0.5 %. This implies that APIs with an intense color can still be reliably measured even if its concentration is too low for spectroscopic instruments. The LoQ value of 0.046 w/w% suggests that RI concentration can be safely quantified around 0.1 w/w%. This could be the foundation of future research where RI is applied as a tracer material for APIs which do not have strong color and for some reason cannot be measured with conventional spectroscopic techniques in the case when the API and the tracer material are coprocessed. Its utilization should not cause safety concerns, as RI is vitamin essential for life

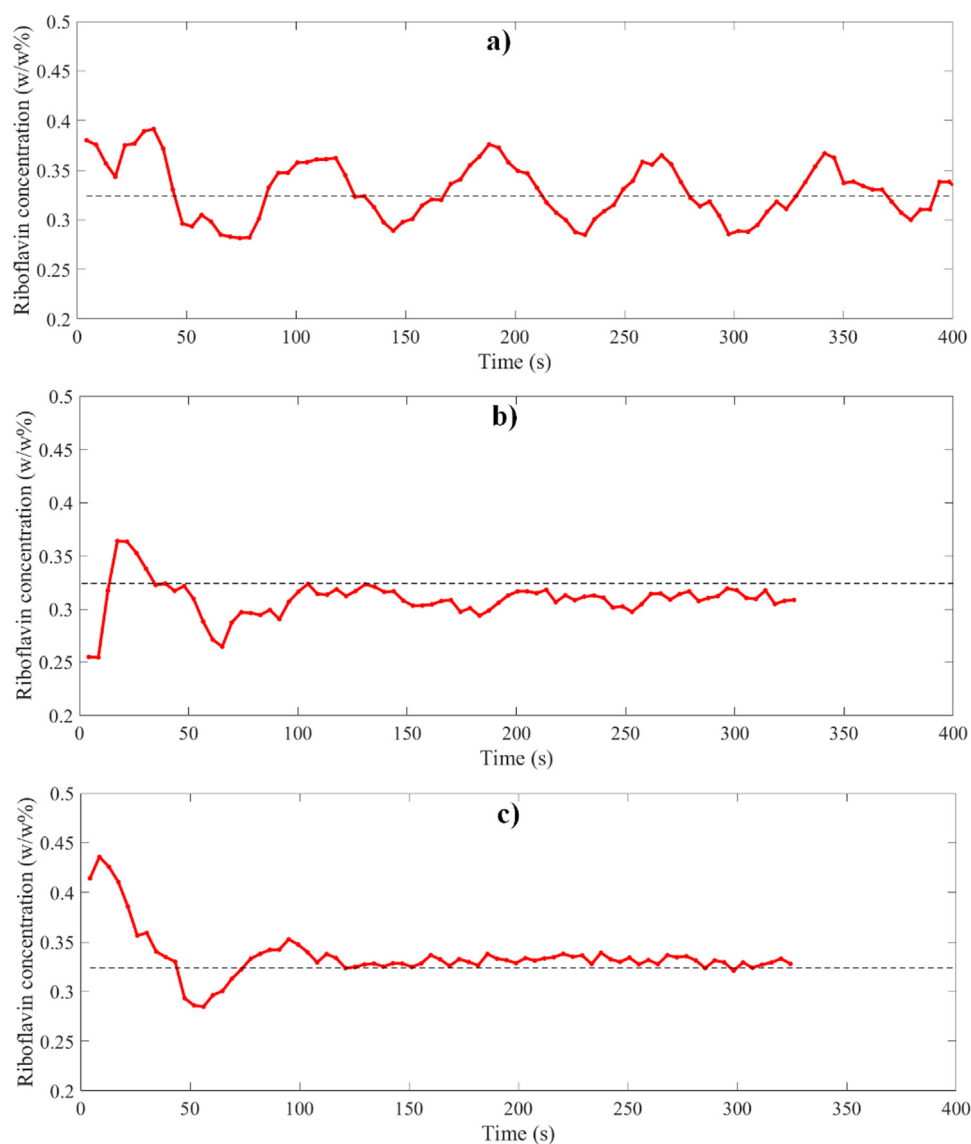


Fig. 5. Predicted RI concentration during feedback control experiments. a) $K_p = 5$, $K_i = 0$; b) $K_p = 3$, $K_i = 0$; c) $K_p = 3$, $K_i = 0.01$.

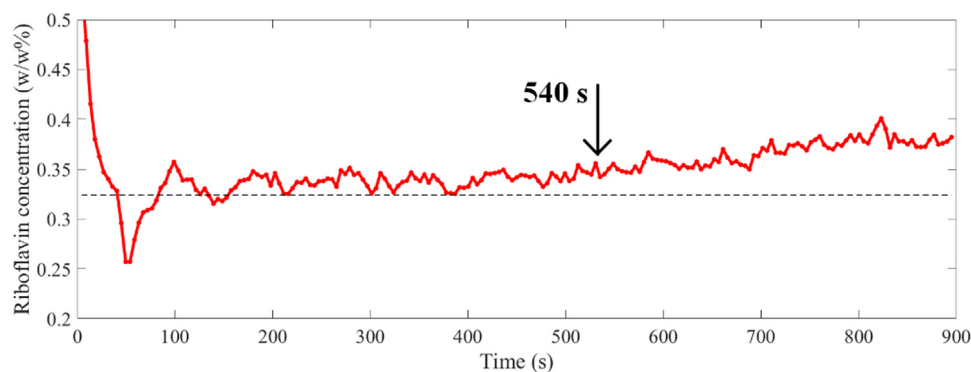


Fig. 6. Predicted RI concentration during feedback control experiments. The arrow marks where feedback control was turned off.

and as such its presence should be tolerated by patients. RI has also been suggested as an oral tracer material to be included in dosage forms in order to enable the quick determination of patient compliance by analyzing the RI content in urine [34,35]. In this case tracer refers to a material which is co-processed with the API in a low quantity but its concentration is easier to measure due to its intense

color. Such an application assumes that the API and the tracer material move together through the system, thus careful consideration and validation is required before utilization. This technique might have a great potential in the case of hormone APIs, as they are often present in such low quantities that direct in-line quantification is not possible with current methods. Continuous manufacturing of

such formulations might be especially challenging, thus a simple method of measuring their concentration in-line could turn out to be a game changer in this field.

4. Conclusions

This work proposes a novel, digital imaging-based PAT tool for the in-line monitoring of continuous blending processes where a colored API is involved. It was demonstrated that the RI can be quantified at concentrations as low as 0.05 w/w% with this method. The concentrations predicted during a continuous blending experiment were validated using UV/VIS spectrometry, showing that the technique can be used for the monitoring of blending processes. The technique can be utilized for RTD measurements, enabling a simple way of characterizing the behavior of the technology. Lastly, it was shown that with a PI controller, this machine vision system can be used to provide real time information for feedback control, essentially realizing an application of PACT.

The developed machine vision-based concentration determination should be applicable in most cases when the API is colored. In future research, the utilization of a colored tracer material in cases where the API has not got a color and is not directly detectable by the camera could also be investigated. This could be especially useful when the API concentration is too low for direct quantification by NIR, Raman or other well established techniques, hormone APIs represent such a scenario. Further research must be conducted in order to determine whether such measurements are possible. This technique might also be useful in cases where the material is moving too fast for spectroscopical measurements (e.g. falling), as with appropriate lighting, a digital camera can still capture sharp images.

CRediT authorship contribution statement

Dorián László Galata: Methodology, Investigation, Data curation, Formal analysis, Writing - original draft. **Lilla Alexandra Mészáros:** Methodology, Investigation, Data curation, Writing - review & editing. **Máté Ficzer:** Investigation. **Panna Vass:** Methodology. **Brigitta Nagy:** Writing - review & editing. **Edina Szabó:** Investigation. **András Domokos:** Methodology. **Attila Farkas:** Conceptualization. **István Csontos:** Supervision. **György Marosi:** Conceptualization, Writing - review & editing. **Zsombor Kristóf Nagy:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by OTKA grants: FK-132133 and KH-129584. The research presented in this paper was supported by the Ministry of Innovation and the National Research, Development and Innovation Office within the framework of the Artificial Intelligence National Laboratory Programme. This work was created with the financial contribution of Gedeon Richter's Talentum Foundation (1103 Budapest, Gyömrői út 19-21.).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jpba.2021.113902>.

References

- [1] Food and Drug Administration, Guidance for industry, PAT-A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance, 2004 <http://www.fda.gov/cder/guidance/published.html>.
- [2] R.C. Peters, Bio/Pharma needs ideas and incentives to advance manufacturing, *Pharm. Technol.* 43 (1) (2019) 16–23.
- [3] R.D. Worsham, V. Thomas, S.S. Farid, Potential of continuous manufacturing for liposomal drug products, *Biotechnol. J.* 14 (2) (2019) 1700740.
- [4] A.U. Vanarase, M. Alcalá, J.I.J. Roza, F.J. Muzzio, R.J. Románach, Real-time monitoring of drug concentration in a continuous powder mixing process using NIR spectroscopy, *Chem. Eng. Sci.* 65 (21) (2010) 5728–5733.
- [5] Y. Gao, A. Vanarase, F. Muzzio, M. Ierapetritou, Characterizing continuous powder mixing using residence time distribution, *Chem. Eng. Sci.* 66 (3) (2011) 417–425.
- [6] B. Nagy, A. Farkas, M. Gyürkés, S. Komaromy-Hiller, B. Démuth, B. Szabó, D. Nusser, E. Borbás, G. Marosi, Z.K. Nagy, In-line Raman spectroscopic monitoring and feedback control of a continuous twin-screw pharmaceutical powder blending and tableting process, *Int. J. Pharm.* 530 (1–2) (2017) 21–29.
- [7] M. Martinetz, A. Karttunen, S. Sacher, P. Wahl, J. Ketolainen, J. Khinast, O. Korhonen, RTD-based material tracking in a fully-continuous dry granulation tableting line, *Int. J. Pharm.* 547 (1–2) (2018) 469–479.
- [8] J. Krusz, J. Rehr, S. Sacher, I. Aigner, M. Horn, J.G. Khinast, RTD modeling of a continuous dry granulation process for process control and materials diversion, *Int. J. Pharm.* 528 (1–2) (2017) 334–344.
- [9] F. Stauffer, V. Vanhoorne, G. Pilcer, P.-F. Chavez, C. Vervae, T. De Beer, Managing API raw material variability during continuous twin-screw wet granulation, *Int. J. Pharm.* 561 (2019) 265–273.
- [10] J. Harting, P. Kleinebudde, Optimisation of an in-line Raman spectroscopic method for continuous API quantification during twin-screw wet granulation and its application for process characterisation, *Eur. J. Pharm. Biopharm.* 137 (2019) 77–85.
- [11] J.M. Vargas, S. Nielsen, V. Cárdenas, A. Gonzalez, E.Y. Aymat, E. Almodovar, G. Classe, Y. Colón, E. Sanchez, R.J. Románach, Process analytical technology in continuous manufacturing of a commercial pharmaceutical product, *Int. J. Pharm.* 538 (1–2) (2018) 167–178.
- [12] K. Korasa, F. Vrečer, Overview of PAT process analysers applicable in monitoring of film coating unit operations for manufacturing of solid oral dosage forms, *Eur. J. Pharm. Sci.* 111 (2018) 278–292.
- [13] J. Krusz, J. Rehr, E. Faulhammer, A. Witschnigg, J.G. Khinast, Material tracking in a continuous direct capsule-filling process via residence time distribution measurements, *Int. J. Pharm.* 550 (1–2) (2018) 347–358.
- [14] R. Singh, A.D. Román-Ospino, R.J. Románach, M. Ierapetritou, R. Ramachandran, Real time monitoring of powder blend bulk density for coupled feed-forward/feed-back control of a continuous direct compaction tablet manufacturing process, *Int. J. Pharm.* 495 (1) (2015) 612–625.
- [15] L. Pernenkil, Continuous Blending of Dry Pharmaceutical Powders, Massachusetts Institute of Technology, 2008.
- [16] B. Van Snick, J. Holman, V. Vanhoorne, A. Kumar, T. De Beer, J.P. Remon, C. Vervae, Development of a continuous direct compression platform for low-dose drug products, *Int. J. Pharm.* 529 (1–2) (2017) 329–346.
- [17] F. Peng, W. Wang, W. Luo, Y. Liu, H. Li, Application of near-infrared spectroscopy for monitoring the formulation process of low-dose tablets, *Anal. Methods* 6 (6) (2014) 1905–1913.
- [18] M. Alcalá, J. León, J. Ropero, M. Blanco, R.J. Románach, Analysis of low content drug tablets by transmission near infrared spectroscopy: selection of calibration ranges according to multivariate detection and quantitation limits of PLS models, *J. Pharm. Sci.* 97 (12) (2008) 5318–5327.
- [19] B. Li, A. Calvet, Y. Casamayou-Boucau, C. Morris, A.G. Ryder, Low-content quantification in powders using Raman spectroscopy: a facile chemometric approach to sub 0.1% limits of detection, *Anal. Chem.* 87 (6) (2015) 3419–3428.
- [20] B. Igne, C.N. Baldasano, C. Airiau, Feasibility of using light-induced fluorescence spectroscopy for low-dose formulations monitoring and control, *J. Pharm. Innov.* (2020) 1–7.
- [21] L. Madarász, Z.K. Nagy, I. Hoffer, B. Szabó, I. Csontos, H. Pataki, B. Démuth, B. Szabó, K. Csorba, G. Marosi, Real-time feedback control of twin-screw wet granulation based on image analysis, *Int. J. Pharm.* 547 (1–2) (2018) 360–367.
- [22] L. Madarász, Á. Köte, M. Gyürkés, A. Farkas, B. Hambalkó, H. Pataki, G. Fülöp, G. Marosi, L. Lengyel, T. Casian, K. Csorba, Z.K. Nagy, Videometric mass flow control: a new method for real-time measurement and feedback control of powder micro-feeding based on image analysis, *Int. J. Pharm.* 530 (2020), 119223.
- [23] S. García-Muñoz, D.S. Gierer, Coating uniformity assessment for colored immediate release tablets using multivariate image analysis, *Int. J. Pharm.* 395 (1–2) (2010) 104–113.
- [24] L.A. Mészáros, D.L. Galata, L. Madarász, Á. Köte, K. Csorba, Á.Z. Dávid, A. Domokos, E. Szabó, B. Nagy, G. Marosi, A. Farkas, Z.K. Nagy, Digital UV/VIS imaging: a rapid PAT tool for crushing strength, drug content and particle size distribution determination in tablets, *Int. J. Pharm.* 578 (2020) 119174.
- [25] P. Durão, C. Fauteux-Lefebvre, J.-M. Guay, N. Abatzoglou, R. Gosselin, Using multiple process analytical technology probes to monitor multivitamin blends in a tableting feed frame, *Talanta* 164 (2017) 7–15.
- [26] P. Durão, C. Fauteux-Lefebvre, J.-M. Guay, J.-S. Simard, N. Abatzoglou, R. Gosselin, Specificity of process analytical tools in the monitoring of

- multicomponent pharmaceutical powders, *Pharm. Dev. Technol.* 24 (3) (2019) 380–389.
- [27] R. Gosselin, P. Durão, N. Abatzoglou, J.-M. Guay, Monitoring the concentration of flowing pharmaceutical powders in a tableting feed frame, *Pharm. Dev. Technol.* 22 (6) (2017) 699–705.
- [28] C. Gómez-Polo, M.P. Muñoz, M.C.L. Luengo, P. Vicente, P. Galindo, A.M.M. Casado, Comparison of the CIELab and CIEDE2000 color difference formulas, *J. Prosthet. Dent.* 115 (1) (2016) 65–70.
- [29] European Medicines Agency, ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology, EMEA, London, 1995.
- [30] W. Engisch, F. Muzzio, Using residence time distributions (RTDs) to address the traceability of raw materials in continuous pharmaceutical manufacturing, *J. Pharm. Innov.* 11 (1) (2016) 64–81.
- [31] F. Tahir, J. Palmer, J. Khoo, J. Holman, I.K. Yadav, G. Reynolds, E. Meehan, A. Mitchell, G. Bajwa, Development of feed factor prediction models for loss-in-weight powder feeders, *Powder Technol.* 364 (2020) 1025–1038.
- [32] J. Chaki, N. Dey, *A Beginner's Guide to Image Preprocessing Techniques*, CRC Press, 2018.
- [33] Z. Zhang, Z. Chen, J. Shi, F. Jia, M. Dai, Surface roughness vision measurement in different ambient light conditions, *Int. J. Comput. Appl. Technol.* 39 (1-3) (2010) 53–57.
- [34] V.S. Ramanujam, K.E. Anderson, J.J. Grady, F. Nayeem, L.-J.W. Lu, Riboflavin as an oral tracer for monitoring compliance in clinical research, *Open Biomark. J.* 2011 (4) (2011) 1.
- [35] A.J. Herron, J.J. Mariani, M. Pavlicova, C.M. Parrinello, K.W. Bold, F.R. Levin, E.V. Nunes, M.A. Sullivan, W.N. Raby, A. Bisaga, Assessment of riboflavin as a tracer substance: comparison of a qualitative to a quantitative method of riboflavin measurement, *Drug Alcohol Depend.* 128 (1-2) (2013) 77–82.