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Quantitative analysis and validation of naproxen tablets by using transmission raman spectroscopy

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Abstract: A transmission Raman spectroscopy-based quantitative model, which can analyze the content of a drug product containing naproxen sodium as its active pharmaceutical ingredient (API), was developed. Compared with the existing analytical method, i.e., high-performance liquid chromatography (HPLC), Raman spectroscopy exhibits high test efficiency owing to its shorter sample pre-treatment and measurement time. Raman spectroscopy is environmentally friendly since samples can be tested rapidly via a nondestructive method without sample preparation using solvent. Through this analysis method, rapid on-site analysis was possible and it could prevent the production of defective tablets with potency problems. The developed method was applied to the assays of the naproxen sodium of coated tablets that were manufactured in commercial scale and the content of naproxen sodium was accurately predicted by Raman spectroscopy and compared with the reference analytical method such as HPLC. The method validation of the new approach was also performed. Further, the specificity, linearity, accuracy, precision, and robustness tests were conducted, and all the results were within the criteria. The standard error of cross-validation and standard error of prediction values were determined as 0.949 % and 0.724 %, respectively.

Key words: naproxen sodium, coated tablet, transmission raman spectroscopy, quantitative model, method validation

1. Introduction

Spectroscopic analysis is generally applied to quality control tests in pharmaceutical manufacturing processes. In addition to the drying and coating processes, the assay of semi-finished drug products is performed rapidly and accurately before packaging to improve drug quality.[1-5] However, the content of commercial-scale-produced drugs is measured via high-performance liquid chromatography (HPLC), which represents a standard analytical procedure. HPLC requires the dissolution of the sample in a solvent or its pretreatment via shaking, extraction, etc. Additionally, the cost of the consumables increases because the solvents and columns are utilized in every test. Owing to the complication of pretreating the sample, the accuracy

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of the test is generally affected depending on the analyst's skill; moreover, economic losses might occur because of the destruction of the sample. Thus, Raman spectroscopy is being developed to replace the existing analytical method for analyzing the active pharmaceutical ingredient (API) content of a drug.[6-15]

Compared with HPLC, Raman spectroscopy does not require complicated preprocessing because it directly irradiates the sample through a laser and does not depend on the analyst's skill. Kim developed a model for analyzing the API content of a capsule via wide-area Raman irradiation.[12] Further, ambroxol and lactose were employed as the API and excipient, respectively, to establish a prediction model for the API contents of drugs. Dissimilar to the composition ratio of the pharmaceuticals, it consisted only of two components, which differed from the commercial-scale-produced samples. Further, Eliasson performed content analysis via transmission Raman spectroscopy, which transmits and measures the sample with a laser to increase the representativeness of the capsule sample.[13] After producing 15 standard samples by mixing four components, including the API, in a certain ratio, one standard sample was added into 10 capsules (a total of 150 capsule samples were analyzed). The effects of the capsule size and color on the accuracy of measuring API were studied, confirming that the capsule type did not significantly affect the API content analysis when transmission Raman spectroscopy was employed. Griffen established a model for analyzing the contents of five components, including their API and excipients, via transmission Raman spectroscopy.[14] Furthermore, Mazurek quantitatively analyzed the APIs and excipients of ointments and gel formulations. Accordingly, the development of Raman-based analytical methods for analyzing different pharmaceutical samples has been studied at the level of evaluating the applicability of laboratory-scale, rather than commercial-sale Raman.[15] In this study, for commercial-scale quality control tests, the API content of the drug product was analyzed, and its accuracy was compared with those of the existing HPLC method. A standard sample was prepared with naproxen sodium (API); this API underwent the granulation process and was tableted into the same size and shape as that of the actual tablet. Raman spectroscopy was also performed employing a tray, which was dedicated to naproxen sodium, to analyze the standard samples, as well as the product tablets. After establishing a quantitative model employing the measured Raman spectra, the method validation (MV) was performed to verify the analytical method. The quantitative model employed a multivariate regression method, namely the partial least square method from which the standard error of cross-validation (SECV) and standard error of prediction (SEP).[16-17] Finally, MV parameters, such as specificity, linearity, accuracy, precision, and robustness, were verified.

2. Experimental

2.1. Raw material and the composition ratio

Naproxen sodium was employed as the API of the tablets in this study. The API content was 275 mg per tablet and represents a proportion of 68.75%. The microcrystalline cellulose (MCC), a disintegrant, content of the tablet was 80 mg per tablet, accounting for 20 % of the total content. Another excipient (lactose hydrate) and a lubricant (magnesium stearate) were present in 9.25 % and 2.00 % proportions, respectively.

2.2. Preparation of the standard samples

Standard samples were prepared to establish a quantitative model for measuring the API content. The standard samples comprised naproxen sodium, MCC, lactose hydrate, and magnesium stearate, as obtainable in the commercial-scale-produced tablets. Among these constituents, naproxen sodium was employed as the API, which had undergone granulation and sizing processes in the actual process. It was manufactured via a similar process as that of the commercial-scale-produced tablet.[14] The standard samples exhibited the same physical and chemical properties as those of the commercial-scale products. The standard sample was manufactured at 415 mg at

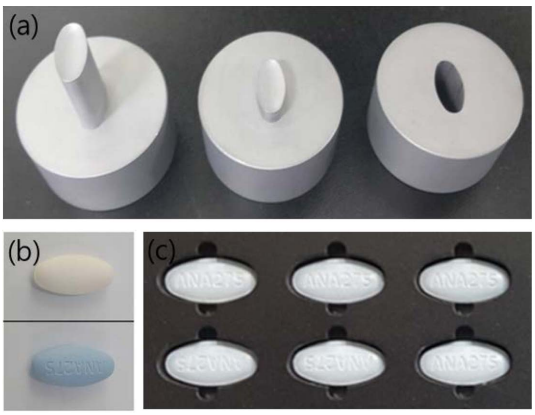

Fig. 1. Comparison of the standard sample tableting of the lab-produced and commercial-scale-produced tablets. (a) Tableting parts for producing the standard samples. (b) Comparison of the manufactured standard sample tablets and commercial-scale-produced tablets. (c) Dedicated tray for the Raman measurement.

a pressure of 2 tons, which is the same as the actual process. The API contents of 30 standard samples were randomly prepared in the 80.8 %–119.3 % range. Four raw materials, including the API per one standard sample, were weighed and prepared to obtain a total of 50 g. The standard samples were prepared with the same shapes, weights (415 mg), and methods as those of the commercial-scale-produced tablet employing a tableting machine (*Fig.* 1(a)). *Fig.* 1(b) shows the standard tablet sample, which was compared with the commercial-scale-produced tablet. The standard samples and commercial-scale-produced tablets were placed in a dedicated measuring tray (*Fig.* 1(c)) and analyzed in the same position and via the same method.

2.3. Raman spectroscopy

A transmission Raman spectrometer, TRS 100 (Agilent, USA), was employed to analyze the standard samples and commercial-scale-produced tablets. The wavelength of the laser was 830 nm, and the diameter of the laser that was irradiated to the sample surface was 4 mm, ensuring that a highly representative spectrum was obtainable. Additionally, the detection signal was collected by applying the TE-cooled 1024 CCD detector. For the spectral region, 1800–165 cm^{-1} corresponding to the entire region was selected so that the chemical information of the API that was distributed in the entire region was reflected in the quantitative model. The optimum parameters for measuring the naproxen sodium samples via Raman spectroscopy were, as follows: measurement was repeated three times per sample, exposure time was 0.1 sec, and accumulation was set to 50.

2.4. Quantitative model establishment method

Twenty-three of the 30 standard samples were set as the calibration set to establish the quantitative model, and the remaining seven were set as the validation set to verify the model. The range of the API content of the calibration set was 80.8 %–119.3 %, and that of the validation set was selected from the seven standard samples in the 92.4 %–109.8 % range. Additionally, to analyze the commercial-scale-produced tablets (100 tablets per batch), 10 batches were compared with the HPLC result, and the accuracy was evaluated.[15] To establish the quantitative model, baseline correction and standard normal variate (SNV) spectral preprocessing were performed on the Raman spectra of the standard samples. In the case of the pharmaceutical samples, nonspecific scattering occurred based on their physicochemical properties, such as their particle sizes, densities, and chemical compositions, and this phenomenon can be corrected by spectral pretreatment to remove the quantitative interference.

2.5. MV parameters and criteria

MV was performed to verify the quantitative model. The parameters comprised five items, namely specificity, standard error of the model, and linearity, accuracy, precision, and robustness.[15]

2.5.1. Specificity

The API, tablet, and raw material were measured, after which their Raman spectra were compared. This comparison confirmed that a minimum of one unique peak of the API is present in the tablet.

2.5.2. Standard error of the model and linearity

The 23 standard samples with different API contents

were measured via Raman spectroscopy, and the quantitative model was established via the partial least squares (PLS) method employing the obtained spectra. The regression coefficient (R2) and SECV of the quantitative model must be ≥0.9 and ≤3 %, respectively.

2.5.3 Accuracy

The seven standard samples corresponding to the validation set were subjected to three measurements per sample. Additionally, 100 commercial-scale-produced tablets (10 per batch) produced from 10 batches of drug products were analyzed and compared with the reference value (the accuracy and standard error of prediction [SEP] must be 100 % ± 5 % and <5 %, respectively).

$$SEP = \left(\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N-1} \right)^{1/2}$$

y_i = Reference value, $\hat{y}_i$ = Measured value, N = Number of samples

2.5.4. Precision

The repeatability of measuring one tablet 10 times, the intermediate precision in which three analysts analyzed 10 tablets, respectively, and the precision test during the measurement day, in which 10 tablets were analyzed every day for 10 days, were performed. The relative standard deviation (RSD) of the content value of the API in each analyzed test must be <1 %.

2.5.5. Robustness

The robustness test was performed by selecting 10 tablets per batch and a total of 100 tablets corresponding to 10 batches. To evaluate the robustness, the exposure times for irradiating the sample were set to 3 and 7 s based on the existing 5 s to evaluate the accuracy owing to the change in the laser irradiation time. Thus, the exposure time was set to 3, 5, and 7 s, and it was confirmed whether the analyzed accuracy result satisfied the criterion of ≤5 % for the SEP of each condition.

3. Results and Discussion

3.1. Raman spectrum of the raw materials

Fig. 2 shows the API and excipients of the naproxen sodium-containing tablet. The spectrum of naproxen sodium (the API, *Fig.* 2(b)) was present in a high proportion (68%) of the total component; thus, it exhibited a shape that was similar to that of the spectrum of the commercial-scale-produced tablet (*Fig.* 2(a)). The Raman spectrum of lactose hydrate exhibited a strong peak in the entire region, whereas only a relatively small peak appeared in MCC, and

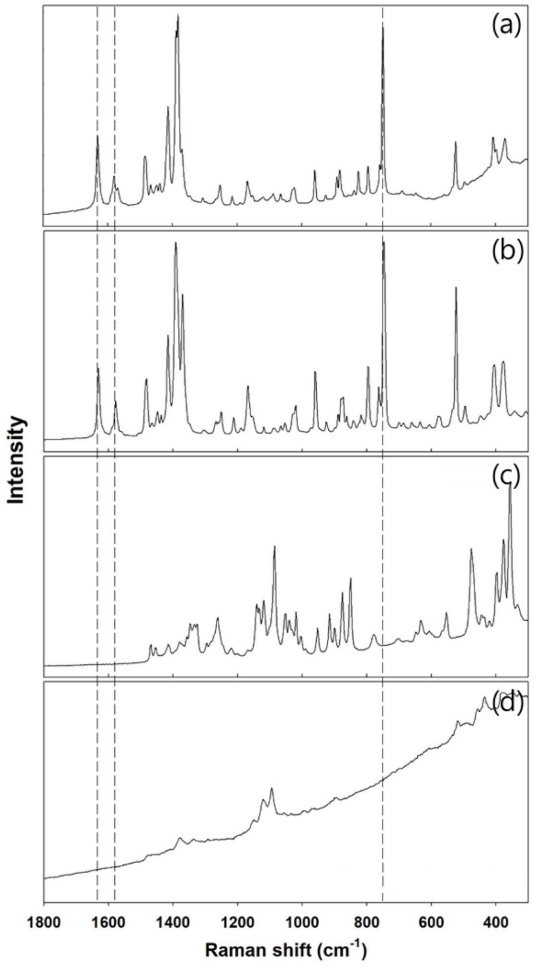

Fig. 2. Comparison of the Raman spectra of the commercial-scale-produced tablet, API, and excipients. (a) commercial-scale-produced tablet, (b) naproxen sodium (API), (c) lactose hydrate (excipient), (d) MCC (excipient).

the baseline increased rapidly in the short-wavelength region under fluorescence. Under the fluorescence condition, the baseline intensity of the spectrum of naproxen sodium, which is the API, was low in the 600–300 cm^{-1} region, whereas the baseline intensity of the spectrum of the commercial-scale-produced tablet was high. Further, the main peak of naproxen sodium was observed at 1631, 1580, and 748 cm^{-1}, as indicated by the dotted line. This main peak did not overlap with those of lactose hydrate and MCC, which were excipients. To quantitatively analyze the API content, the main additive peaks acted as a quantitative hindrance factor if they overlapped with the API peaks. Thus, since the API and additive peaks did not overlap more than one, they represented an ideal specificity parameter.

3.2. Spectral range

Fig. 3 shows the spectra, following the pretreatment of the 23 standard samples corresponding to the calibration set. For the preprocessing methods, the baseline correction and SNV were applied. The intensity corresponding to the concentration of the API content appeared in the entire 1800–200 cm^{-1} region. Particularly, the peak intensity was proportional to the concentration in the 1392–1380 cm^{-1} region corresponding to the main peak region of naproxen sodium. In the 1383 cm^{-1} peak, the black spectrum displaying the highest intensity exhibited an API content (119.3 %), and the red spectrum displaying the lowest exhibited the lowest concentration (80.8 %). Further, the Raman spectrum with a 100.0 % content of the commercial-scale-produced tablet was observed in the middle of all the peak intensities. Thus, when the standard samples with different contents were measured, a spectrum reflecting the API content appeared. The concentration of the API content was reflected in the spectrum of the main area of the API spectrum and the entire area. The possibility of establishing a quantitative model for measuring the API content was confirmed employing the spectrum reflecting such a quantification.

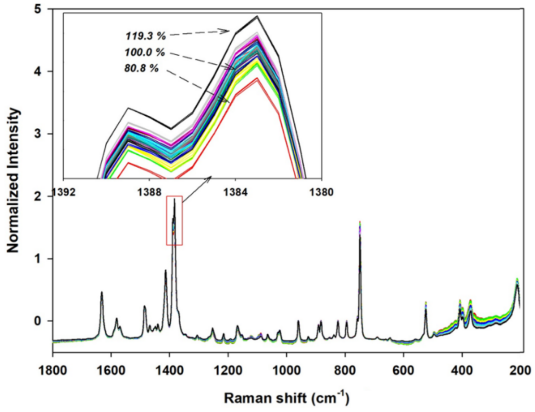

Fig. 3. Raman spectra after the baseline correction and SNV (standard normal variate) pretreatment of the calibration set.

3.3. Influence of tablet coating

The standard sample tablets, which were prepared for the establishment of the quantitative model for measuring the API content, were not coated. After establishing the quantitative model employing an

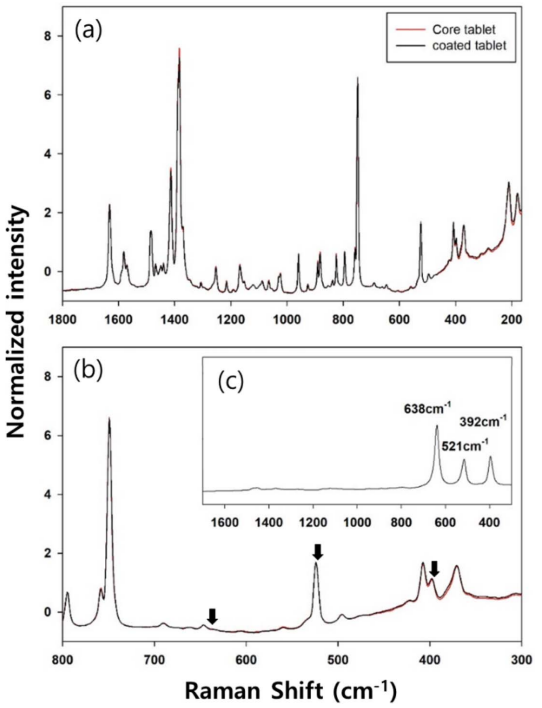

Fig. 4. Comparison of the spectra of the core and coated tablets with 100 % API content. (a) is a comparison in the entire area of the normalized spectra, (b) is a comparison through the magnification of an area of 800–300 cm^{-1}, and (c) is a coating solution component of the Raman spectrum.

uncoated standard sample, the coating component must not affect the model to ensure the prediction of the API content of the coated tablet. To evaluate the effect of coating, the difference was confirmed by comparing the spectra of the core and coated tablets. *Fig.* 4(a) shows the normalized spectra of the core and coated tablets. The two spectra appeared almost similarly in the entire area. *Fig.* 4(b) enlarges the 800–300 cm^{-1} area. Since the spectrum of the pure coating solution appeared at 638, 521, and 392 cm^{-1} (*Fig.* 4(c)), this area was enlarged for comparison. The result demonstrated that the spectra of the core and coated tablets were exactly the same; moreover, one of the three unique peaks of the coating solution did not appear in the coated tablet. Further, the coating solution, which was thinly applied to the surface, did not affect the Raman spectrum representing the components and content of the entire tablet since the Raman laser completely penetrated and measured the sample. These results demonstrated that coating did not exert any effect when the content of the coated tablets was analyzed employing the uncoated standard sample.

3.4. Quantitative model

Fig. 5 shows the quantitative model of naproxen sodium. The model was established via the multivariate regression method, PLS, employing the Raman spectra that were measured from the 23 standard samples.

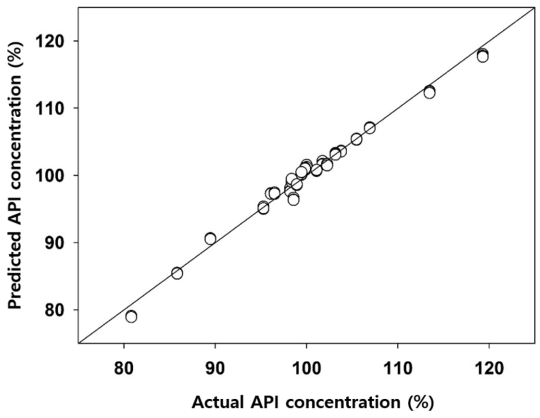

Fig. 5. Optimal quantitative model of naproxen sodium (API) employing the calibration set.

The standard error value of the established quantitative model was 0.949 %, which was very low, and the risk of overfitting was minimized by applying three factors. To establish the quantitative model, a quantitative model that could analyze API was developed by changing the following five conditions: spectrum area, preprocessing method, laser exposure time, number of measurement repetitions, and number of factors.

3.5. MV results

3.5.1. Specificity

Fig. 2 shows that the specificity was confirmed since the main peaks of the API, naproxen sodium, (1631, 1580, and 748 cm^{-1}) did not overlap with the Raman peaks of the other excipients (lactose hydrate and MCC).

3.5.2. Standard error of the model and linearity

SECV of the quantitative model, which was established with the calibration set was 0.949 %, which was lower than the criterion value, 3 %, and the regression coefficient was also 0.985, confirming a suitable result above the criterion value, 0.9. Thus, the standard error and linearity of the quantitative model were suitable for the criteria.

3.5.3. Accuracy

Table 1 presents the results of the analysis of the API content of the validation set and the comparison with the reference value to verify the quantitative model. When the 92.4 % sample with the lowest API

Table 1. Analysis results of the API contents of the validation set sample

Validation set	Reference value (%)	Predicted value (%)	Accuracy (%)
Sample 1	97.8	99.1	101.3
Sample 2	104.8	103.8	99.0
Sample 3	100.9	100.3	99.4
Sample 4	100.3	99.7	99.4
Sample 5	94.2	94.5	100.4
Sample 6	92.4	92.8	100.4
Sample 7	109.8	109.6	99.8

Table 2. Results of API contents of the commercial-scale-produced tablets

Batches	Reference value (%)	Predicted value (%)	Accuracy (%)
#1	97.5	100.3	102.9
#2	98.8	99.5	100.7
#3	99.3	100.4	101.1
#4	99.7	99.9	100.2
#5	97.9	99.9	102.1
#6	97.6	99.7	102.1
#7	99.7	100.2	100.5
#8	99.4	99.2	99.8
#9	99.7	99.4	99.7
#10	100.3	99.7	99.4

content was analyzed, it exhibited a Raman prediction result of 92.8 %, and when the sample with the highest content (104.8 %) was analyzed, it obtained a prediction result of 103.8 %. Each accuracy was 100.4 % and 99.0 %, and all the seven validation sets were analyzed within the criteria value of 100 ± 5 %. Furthermore, SEP, a prediction error, was 0.724 %, which was <5 % (this is the criterion value). By measuring the validation set with different API contents and comparing the values with the reference ones, SEP and accuracy obtained results that were ideal for the criteria. *Table* 2 presents the results of measuring the API contents of the commercial-scale-produced tablets. After measuring 10 tablets per batch three times, the average was compared with the value analyzed via the reference HPLC method to evaluate the accuracy. When the API of Batch 4 was analyzed via HPLC, it was 99.7 %, and the result of the API content analyzed via the quantitative model was 99.9 %, demonstrating accuracy of 100.2 %. The measurement result of the API content of Batch 1 was 100.3 %, which was higher than the reference value, 97.5 %, indicating accuracy of 102.9 %. This result demonstrated the lowest accuracy value among all the 10 batches. All the 10 batches of the commercial-scale-produced tablets were analyzed within 100 ± 5 % of the accuracy criteria with suitable results. *Fig.* 6 shows the measurement results of the API contents of the 10 batches via Raman spectroscopy. All the 300 API content measurements obtained results of 97 %–103 %, confirming

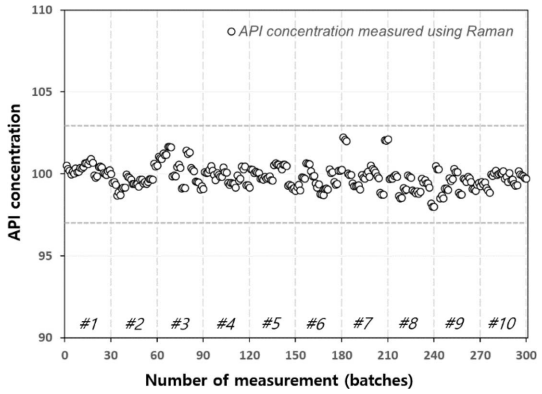

Fig. 6. Results of the determination of the API content of 100 tablets corresponding to 10 batches of commercial-scale -produced tablets (10 tablets per batch, 3three measurements per tablet) using employing Raman spectroscopy.

the good reproducibility.

3.5.4. Precision

Each tablet was measured 10 times, and the averaged API content value was 98.9 %, and each RSD was 0.323 %. In the intermediate precision test in which three testers measured 10 tablets, the RSD value of each analyzed tablet was in the 0.283 %–0.505 % range. When 10 tablets were measured daily for 10 days, the RSD ranged from 0.626 % to 0.807 %. Thus, all the results obtained from the three tests analyzed by the precision parameter obtained results that met the criteria, with an RSD of ≤1 %.

3.5.5. Robustness

When the laser exposure time was set to 5 s to analyze the API content of the commercial-scale-produced tablet, an SEP value of 2.054 % was obtained. The average SEP value of 10 tablets per batch (set to 3 s and measured in 10 batches) was 1.993 %, and the average SEP result of the API content, which was analyzed by applying the spectrum obtained by irradiating the sample for 7 s to the quantitative model, was 1.943 %. All the three exposure time conditions were <5 %, confirming that the analyzed API content was suitable for the criterion when the laser irradiation time was changed.

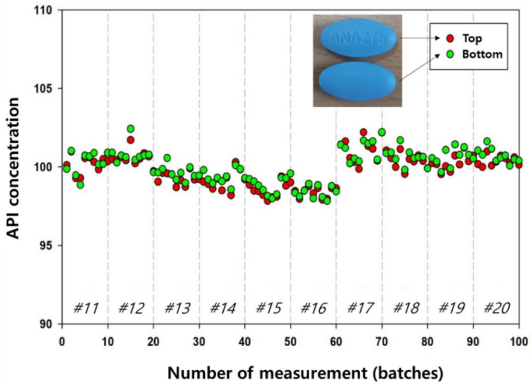

Fig. 7. Comparison of the API contents measured by setting the engraved top and non-engraved bottom positions of 100 tablets corresponding to 10 batches of the commercial-scale-produced tablets (10 tablets per batch, one measurement per tablet).

3.5.6. Influence of tablet engraving

There was an engraving of "ANA275" on the front side of the tablet, and there is none on the back. To evaluate the effect on the measurement of the API content according to the direction with and without the engraving, both sides of 100 tablets (10 per batch) in 10 batches (#11–#20) of the commercial-scale-produced tablets were separately analyzed once, after which the API contents were compared. *Fig.* 7 shows the results of measuring 10 batches of the samples (#11–#20). The red and blue circles indicate the API contents of the front and back, respectively, and all the 100 samples corresponding to the 10 batches exhibited similar API contents on the front and back sides. Additionally, *Table* 2 shows the average values analyzed on the front and back sides of 10 batch samples. In all the 10 batches, the difference between the API contents of the front and back sides was very small (~0.4 %). Further, statistical analysis was employed to confirm that there was no difference in the analyzed values of the API contents according to the measurement of the front and back sides of the same sample. First, the normal distributions of the front and back measurements were confirmed, after which the correlation between them was evaluated through the Mann–Whitney test. The measurement of each side demonstrated a normal distribution, and the p-value of the measurement method was 0.1014, confirming that there was no significance between both methods. Through this statistical evaluation, it was verified that the measurement result of the tablet did not depend on whether it was engraved or not.

4. Conclusions

Raman spectroscopy was employed to analyze the API (naproxen sodium) contents of tablets. Compared with HPLC, one of the existing measurement methods, transmission Raman spectroscopy shortened the sample pretreatment and measurement time and enabled nondestructive analysis, thereby increasing the test efficiency. Through this study, a quantitative model was established for the analysis of an API (naproxen sodium), and the accuracy of the model was confirmed. Further, this model measured the API content of the commercial-scale-produced samples corresponding to 10 batches and compared the result with the reference HPLC results. The Raman and HPLC results exhibited similar accuracy values. The specificity, linearity, accuracy, precision, and robustness were verified as the MV parameters, and the ideal results of each criterion were obtained. The study revealed that the analytical method for analyzing naproxen sodium-containing tablets via Raman spectroscopy can replace the existing HPLC method, which exhibits several limitations.

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