Journal of the Korea Academia-Industrial cooperation Society
Vol. 22, No. 11 pp. 264-269, 2021

https://doi.org/10.5762/KAIS.2021.22.11.264
ISSN 1975-4701 / eISSN 2288-4688

Nondestructive Quantification of Acetamiophen using Near Infrared Spectroscopy

Seong-Wan Cho
Department of Pharmaceutical Engineering, Konyang University

근적외선 분광 분석법을 이용한 Acetaminophen의 비 파괴적 정량 분석 연구

조성완
건양대학교 제약생명공학과

Abstract Acetaminophen (AAP) is widely used as an antipyretic analgesic and has excellent tolerability when administered in therapeutic doses. This study introduces a non-destructive analytical method using near-infrared spectroscopy (NIR) which has the advantages of being simple, reproducible, and fast. AAP samples were analyzed by NIR and the results were compared to those obtained through the high-performance liquid chromatography (HPLC) method to validate the data and confirm the possibility of replacing HPLC with NIR. The NIR spectrum analysis method satisfied the parameters of method validation like specificity, accuracy, and linearity. In conclusion, NIR can be considered for use during pharmaceutical development, in production for process monitoring, or the validation of quality control for active pharmaceutical ingredients.

요 약 아세트아미노펜은 치료용량으로 투여시 내약성이 우수한 장점을 가진 해열진통제로 가장 널리 사용되고 있는 의약품 중의 하나이나 기존의 분석법인 HPLC 방법으로 분석하였을 때 용매 조성을 맞추어야 하며 분석시간이 상대적으로 길다는 단점이 있었다. 본 연구의 목적은 기존의 HPLC 분석방법 대신에 비 파괴적인 분석법을 가지고 있으며 단시간에 분석이 가능하며 간편함과 재현성의 장점을 가진 근적외선 분광법을 도입하여 그 결과를 상호 비교해 보고 생산 현장에서 적용 가능 여부를 확인 해 보는 것이었다. 제제학적으로 사용되는 다른 첨가제들과 함께 각 함량 별로 준비된 아세트아미노펜 시료를 근적외선으로 분석하였다. 그 결과를 HPLC 방법으로 도출된 분석법 밸리데이션의 데이터와 비교하여 근적외선 분광법으로의 대체 가능성을 확인한 결과 특이성, 정확도, 선형성 등에서 매개 변수를 만족하였으며 적합한 결과를 얻었다. 본 분석방법은 의약품 개발, 공정 모니터링을 위한 생산 또는 품질 관리 검증에서 구현될 수 있으며 향후 많은 응용이 가능할 것이다.

Keywords : NIRS, Acitive Pharmaceutical Ingredients, Quality Control, Process Analytical Technology, Validation

This paper was supported by the Konyang University Research Fund in the second half of 2020.
*Corresponding Author : Seong-Wan Cho(Konyang Univ.)
email: swcho@konyang.ac.kr
Received October 14, 2021 Revised November 3, 2021
Accepted November 5, 2021 Published November 30, 2021

1. Introduction

Acetaminophen(AAP) is a p-aminophenol derivative (Fig. 1) that is well known and widely used as an antipyretic analgesic because of the low side effects and still being well tolerated when administered in therapeutic quantities for headache, toothache, and neuralgia and has the same pain killing effect as aspirin. Therefore, it is estimated that it has an fever removal action that increases heat diffusion by dilating skin blood vessels and an analgesic action by increasing the threshold to the thalamus and cerebral cortex. Acetaminophen also has a characteristics of a white crystal or powder that is slightly soluble in water, readily soluble in methanol or ethanol, soluble in sodium hydroxide solution, and very insoluble in ether. Tablets and capsules, suppositories, oral suspensions, effervescent dry syrups are listed in the Korean and US Pharmacopoeia as a medical formulations[1,2]. The classical techniques for pharmaceutical drugs evaluation, such as chromatography and UV-Vis spectroscopy are sensitive and very specific, but these techniques are time consuming and of high costs, needing samples to undergo a preprocessing prior analysis, which may ultimately destroy the test sample.

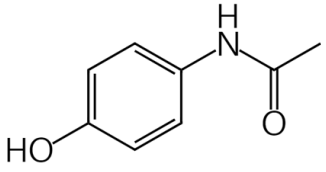

Fig. 1. Chemical structure of acetaminophen

To address this problem, optical methods have been proposed for the quantitative determination of active pharmaceutical ingredients(APIs) in drugs and medicines with advantages such as rapid APIs evaluation with minimum or even without sample destruction. Recently, in order to solve this problem, a method of quantification using near infrared spectrosocpy(NIR) has been introduced as a new technique. The NIR analysis method is a method of analyzing using the property that a material absorbs only light of a specific wavelength. In addition, the structure is analyzed by absorption of near-infrared rays derived from the vibrational motion of molecules constituting an object using near-infrared light with a wavelength band of 700 to 2,500 nm located in the middle of visible light and infrared light and hydrogen bonding. it does not require reagents and solvents compared to the conventional chemical analysis method that uses a large amount of reagents and solvents for near infrared spectroscopy. It has the advantage of being able to measure various components at the same time and measuring the same sample several times repeatedly while minimizing the error. It is also an analytical technique that can measure components quickly and reproducibility. The biggest obstacle in replacing the HPLC method with the NIR method is whether it can be verified the existing HPLC method and the newly introduced NIR method are perfectly compatible with each other[3-6].

The purpose of this study was to assess the feasibility of a quantitative evaluation of AAP based on NIR method, which is emerging as a new technology in the QC field, instead of the frequently used HPLC analysis method. AAP was prepared using the method that is actually produced in the pharmaceutical field, and the product was analyzed by NIR. We also verified the results by measuring the specificity, accuracy, and precision, which are the main parameters of method validation.

2. Material and Methods

2.1 Sample preparation method

For sample preparation, Acetaminophen, microcrystalline cellulose(MCC), low density

hydroxy propyl cellulose(L-HPC), hydrated magnesium silicate(A-Tab), Croscarmellos Na. and Magnesium Stearate were respectively weighed and mixed so that the content was 80%, 90%, 100%, 110%, and 120%. The amount and concentration (%) of each prepared sample are as follows(Table I). The formulation amount was decided by the quality target product profile with constant quality and the Critical Quality Attribute was classified into three categories like dissolution, content, and weight deviation. The design of experiment was finally carried out by setting the tablet thickness, tableting machine pressure, tableting speed, and mixing time as critical process parameters[7-9].

Table 1. Amount and concentration ratio of Acetamionphen samples

Component	Formulation amout(g)	Measurement(g)	Amount(%)
Acetamionphen	160	2400	42.11
Microcrystalline cellulose	98	1470	25.79
hydrated magnesium silicate	60	900	15.79
Low density hydroxy propyl cellulose	30	450	7.89
Croscarmellos Na.	20	300	5.26
Magnesium Stearate	12	180	3.16

2.2 The Preparation of sample and standard solution

Accurately weigh 0.1 g of AAP was added to 100mL of the mobile phase and shake for 10 minutes after taking about 20 tablets to make a powder. 5mL of this solution was filtered through a membrane filter with a pore size of 0.45 μm, discard the first 10mL of the filtrate, and take the next filtrate as the sample solution. Separately, precisely weigh about 20 mg of acetaminophen standard dried at 105°C for 2 hours, add the mobile phase, dissolve it, and take 5mL of this solution was used it as the standard solution[10].

2.3 The Preparation of Mobile phase and HPLC analysis

The mobile phase required for acetaminophen analysis was completed by mixing 250 ml of methanol, 750 ml of water. The composition ratio was Methanol:Water = 1:3. The detector was measured at a wavelength of 243 nm with an ultraviolet absorption meter, and analyzed using a C18 column with an inner diameter of 4.6mm, a length of 25 cm, and 5μm at a temperature of around 40°C. The flow rate was 1.5 ml/min, and the prepared standard solution and sample solution were analyzed by HPLC[11,12].

2.4 Method Validation of Acetaminophen

Analytical method validation was performed to verify the analytical HPLC method before NIR. Specificity was confirmed for the analytical ability to quantify the analyte in the presence of other substances in the sample, comparing the spectra of a 100% acetaminophen sample. Placebo was examined whether the two spectra do not overlap and completely separate. Accuracy indicates how close to the average test result obtained by the analytical method for the true value of the analyte. Each three field samples were taken and tested according to the content test method to confirm the content, and then the recovery rate of the predicted value was calculated by NIR, which is the average value measured three times compared to the value obtained from the standard solution analysis for this experiment. Precision refers to how close each measurement value for an analyte when repeated analysis with multiple equal sieves taken from one homogenized sample.

2.5 The sample preparation and analysis of NIR

For mutual verification with the results of HPLC analysis, we transfered the prepared sample to a petri dish and put it on the NIR

measuring unit and measured each sample 3 times accurately to obtain 3 spectrum to confirm the analysis result was derived perfectly. The particles are uniformly prepared through the process of grinding for 10 seconds using a mixer and samples were prepared in 3 lots each for validation verification, and as a control, 100% mixed powder without Acetaminophen was prepared in 3 lots each for using as a placebo. NIR analysis device was Flex N500, Solids XL for measurement cell. The number of scans were set to 32, and external standard measurement cycle was set to 60 minutes in the measurement cell. By analyzing the spectra obtained by the measurement. It was for confirmation whether method validation like linearity, specificity, and precision were compatible[13-17].

3. Results and Discussion

3.1 The verification of method validation using HPLC

As a result of the measurement, the chromatogram was analyzed cleanly without overlap or interference with the standard retention time and a mixed sample not containing AAP(placebo) and the linearity coefficient also was 0.9999 indicating excellent linearity It confirmed that the specificity and linearity of AAP established well without any interferences(Fig. 2).

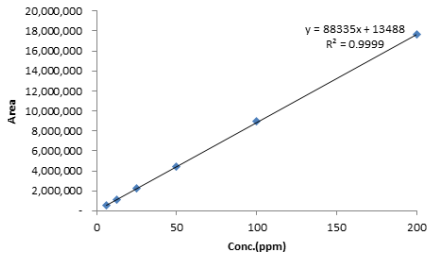

Fig. 2. The linearity graph of AAP for verifying the method validation by HPLC

As for the acceptance criteria for the accuracy and precision in the case of drug substances, the recovery rate and relative standard deviation should be 98.0~102.0%, within 1% respectively. As can be seen in Table 2, the accuracy was evaluated as the recovery rate (%), the accuracy was 98.7±1.2% and RSD was 0.78%. So, it could be confirmed that the acetaminophen analyzed by HPLC had the accuracy and precision.

Table 2. The accuracy and precision test of AAP for the method validation

Name	Recovery (%)	Relative standard deviation (%)
Acetaminophen 1	98.1	0.954
Acetaminophen 2	99.1	0.655
Acetaminophen 3	98.9	0.723

3.2 The verification of method validation using NIR spectral methods

In order to verify that it has specificity, all raw materials constituting the product including acetaminophen, the main ingredient, are measured at 10000 to 4000 cm^{-1} in the case of using the NIR spectrum method. The peak wavelength of the main component and other excipient do not show overlap. It means that there is a specificity in the analysis method using the NIR spectrum (Fig. 3). The fact that the peak wavelengths do not overlap means that there is no interference between the main raw material and the excipient, which means that quantification using the NIR spectrum is possible. The spectrum of the sample without acetaminophen did not appear at this wavelength. In order to know the linearity and range, the experiment was conducted by NIR spectral analysis. Linear regression analysis was performed within the range of concentrations that could be predicted by the calibration curve of the NIR measurement method, and the standard value and the predicted value by the NIR measurement method were expressed as a

linear relationship of two sets of values. the linear relationship was expressed as a correlation coefficient of the regression line.

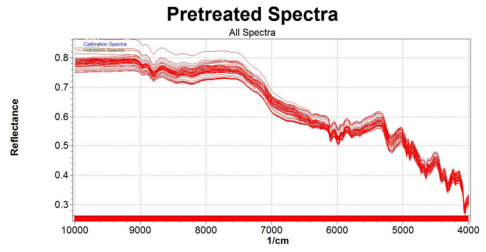

Fig. 3. NIR original spectra specificity test

As a result, the following spectra curve was well obtained in the range of 80 to 120% by calculating a statistically reliable range. In the case of the mixing process, it can be seen that the sample for calibration of the predicted value with respect to the reference analysis value shows a regression linear correlation coefficient(r) of 0.998 and the sample for validation is 0.993. It also confirmed that there was linearity inferred from these results(Fig. 4).

As for the acceptance criteria for the accuracy and precision in NIR, the recovery rate and relative standard deviation was 100.43±1.6% and RSD was 0.6%. So, it could be confirmed that the acetaminophen analyzed by NIR had the accuracy and precision as well.

Fig. 4. NIR original spectra linearity test

4. Conclusion

In this study, we established calibration models for AAP, a drug effective for analgesic medication using NIR spectrum analysis method, which has recently been spotlighted as a new validation analysis method in order to derive accurate analysis results in a short time instead of the HPLC method which has many problems. Quantitative analysis and analytical method validation by near infrared spectroscopy was applied to confirm the accuracy, precision, specificity and range of the experimental method compare the results with the HPLC analysis method. Both the HPLC analysis method and the NIR spectrum analysis method used in the above experiment satisfy the parameters of method validation (specificity, accuracy, linearity, etc). In conclusion, it was confirmed that a new analysis method, NIR analysis could be applied in the case of acetaminophen used as an anti-inflammatory analgesic instead of the existing analysis method, HPLC method which had disadvantages such as difficulty in finding solvent composition and long analysis time. Based on these results, it is considered that the analysis time can be relatively shortened and the product shipment speed can be improved if the NIR spectrum analysis method is used for active pharmaceutical ingredients in pharmaceutical field.

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Seong-Wan Cho [Regular member]

- Feb. 1997 : Chungang Univ., Pharmacy, MS
- Feb. 2001 : Chungang Univ., Pharmacy, PhD
- Sep. 2002 : Univ. of Utah, Post Doc.
- Mar. 2005 ~ current : Konyang Univ., Dept. of Pharmaceutical Engineering, Professor

〈Research Interests〉
Drug formulation development and validation, Screening and evaluation of natural products