学位論文要旨

WHO warned that Substandard and Falsified medical products (SFs) can harm patients and fail to treat the diseases for which they were intended and they lead to loss of confidence in medicines, healthcare providers and health systems, and affect every region of the world. Development of analytical procedure for detecting SFs is the key to grasp the distribution of the SFs and to understand the physical and chemical properties of the SFs to take measure to suppress the public health damage. There are various analytical methods defined for the analysis of a specific medical product, such as published in the pharmacopeias, but the discrimination methods to investigate the authenticity for detecting SFs which are actually distributed on the global market are still limited. Further, there are also few studies on how to apply the analytical technologies to discriminate the SFs with the portable device for in-situ measurements, non-destructive methods for evidence preservation of SFs, easy to use and low cost for easy introduction of those technologies, speedy measurements in order to grasp the actual situation immediately and to suppress damage to public health. This paper is composed of three chapters.

Chapter I delivers how to visualize the physical and chemical properties of falsified medical products with the combination technology of Handheld Raman Spectroscopy and X-ray Computed Tomography. Research to understand how the physical and chemical properties of SFs can be most effectively applied to distinguish the SFs from authentic products has not yet been investigated enough. Here, we investigated the usefulness of two analytical methods, handheld Raman spectroscopy (handheld Raman) and X-ray computed tomography (X-ray CT), for detecting SFs among oral solid anti-hypertensive pharmaceutical products containing candesartan cilexetil as an active pharmaceutical ingredient (API). X-ray CT visualized at least two different types of falsified tablets as shown in Figure 1, one containing many cracks and voids and the other containing aggregates with high electron density, such as from the presence of the heavy elements.

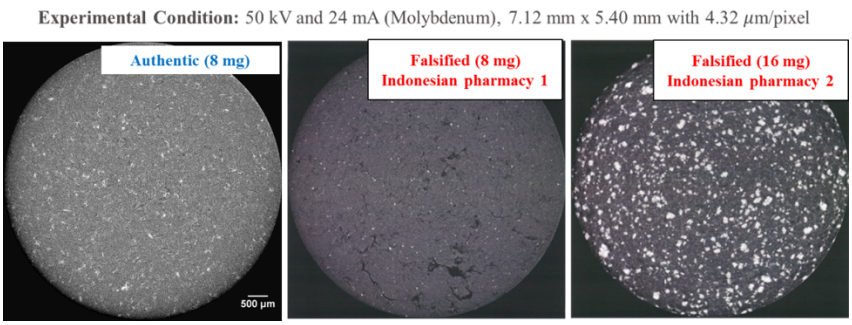

Figure 1. X-ray CT images of the internal structure of tablets.

The Raman spectra of the API, lactose monohydrate (excipient) and the tablets are shown in Figure 2. The area surrounded by the dashed line covers the specific peak of API that does not overlap with the peaks of excipients. Other peaks are due to excipients, mainly from lactose monohydrate. The Raman spectra of each tablets including both API and lactose monohydrate are very similar. These results suggested that the handheld Raman technique can distinguish falsified products that are grossly different from authentic products, but cannot discriminate suspect samples with similar composition including an insufficient quantity of API. Generic products that purported to contain equivalent amounts of API to the authentic products were discriminated from the authentic products by the different physical structure on X-ray CT.

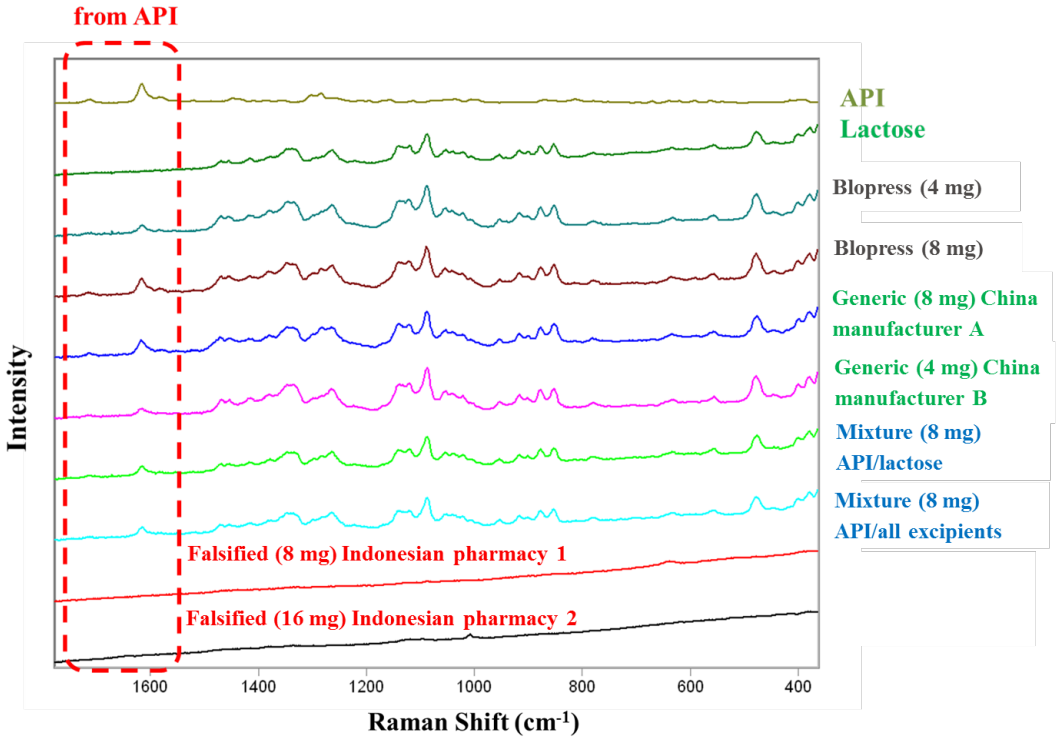

Figure 2. The Raman spectra of the API, lactose monohydrate (excipient) and the tablets.

Approach to investigate both the chemical and physical properties with handheld Raman and X-ray CT, respectively, promise the accurate discrimination of the SFs, even if their visual appearance is similar with authentic products. We present a decision tree (Figure 3) for investigating the authenticity of samples purporting to be authentic commercial tablets. Our results indicate that the combination approach of visual observation, handheld Raman and X-ray CT is a powerful strategy for non-destructive discrimination of suspect samples.

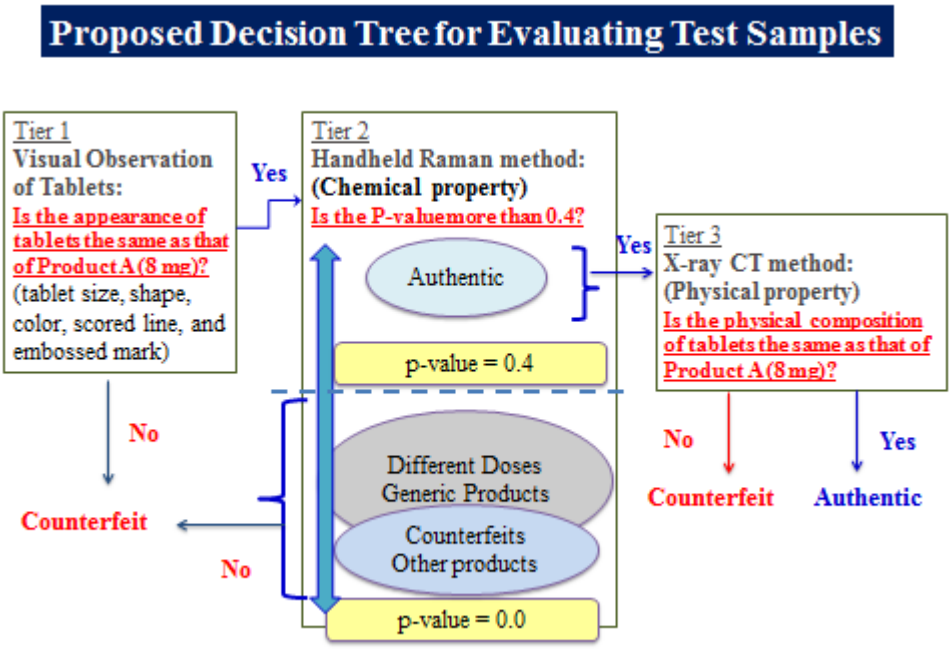

Figure 3. Decision tree of combination approach for non-destructive discrimination between test samples and authentic product (8 mg).

Chapter II shows the result of the survey to identify substandard and falsified tablets of hypertension medical products collected in China, Indonesia and Myanmar using the Japanese pharmacopeial analytical procedures for quality control, together with principal component analysis (PCA) of Raman spectra obtained with a handheld Raman spectrometer. The International Conference on Harmonisation (ICH) Q6A provides guidance to establish a harmonized set of global specifications consisting of analytical procedures and acceptance criteria for new drug substances (DS) and drug products (DP) for human use (1999). Specifications of DS and DP are proposed and justified by the manufacturer, and approved by regulatory authorities in each country. The specifications and acceptance criteria are focused on those chemical, physical and biological properties considered to be important for ensuring the safety and efficacy of DS and DP. Thus, they can be adopted to identify substandard products. Possible issues include 1) out-of-specification content of API, 2) significant dissolution delay, 3) contamination with toxic substances, and 4) lack of sterility. These points can be checked by means of assay, content uniformity testing, measurements of dissolution properties and impurities, and microbial tests. This research focused on methodology for detecting substandard and falsified medicines by PCA of raw data obtained by handheld Raman spectroscopy. It was aimed to clarify the chemical features of substandard medicines by comparing them with authentic medicines, and by extracting the principal components of the Raman spectrum to visualize the relationships among the tablets. We chose the handheld

Raman device as a simple spectroscope suitable for the speedy, easy to use, robust and in-situ observation in anywhere, and we employed PCA as a means to extract critical information despite the limited resolution and sensitivity of the device. We also compared signal preprocessing methods for PCA, and selected the multiplicative scattering correlation (MSC) method as being particularly suitable to extract the desired signals from the strong fluorescence background. The data interval of the hand-held device is around 1.4 to 2.2 cm^{-1}, and the noise level is high, so preprocessing of the spectroscopy spectra is critical for accurate PCA calculation. It was used the Savitzky-Golay method to smooth each segment of the original Raman spectrum in a small window by fitting to a polynomial function. The MSC method was also applied to eliminate baseline shift caused by the multiplicative shift of the baseline tilts and the additive shift of the baseline shifts up and down. MSC can utilize data from many wavelengths to distinguish between light absorption and light scattering, correcting spectra according to a simple linear univariate fit to a standard spectrum by means of least-squares regression using the standard spectrum. The result of some samples showed delayed dissolution and failed to meet the Japanese pharmacopeial specification, while others failed the assay test. These products appeared to be substandard. PCA result showed that all Raman spectra could be explained in terms of two components: the concentration of the active pharmaceutical ingredient (API) and the kind of the excipient. These Raman spectra were conducted preprocessing and subjected to PCA in order to investigate the similarity of chemical components among samples. The calibration result and cross validation result in PCA model were compared and the result suggested that the difference among the samples can be clarified by using the two principal components of PC1 and PC2 and the intensity change of Raman spectrum can be sufficiently expressed by PC1 and PC2. Therefore, the score plot was shown with the score of the PC1 and PC2 on the horizontal axis and the vertical axis, respectively for each tablet. Data set of the Raman spectra, which includes peaks from the API and main excipients, showed the intuitive interpretation score plot in the PCA result. Tablets collected in Myanmar were distributed around authentic Blopress tablets in the score plot, suggesting that similar excipients were used in both cases. On the other hand, the tablets collected in China showed a wide distribution on the score plot, suggesting that different excipients were used by different manufacturers. Notably, one manufacture's tablets were placed very far from the other tablets, and there was a high positive correlation in PC2 and the falsified products collected in Indonesia were located similarly in the plot. In the loading of each PC in the calculated PCA model. The contribution rates were 84% of PC1, 7% of PC2 and 5% of PC3. PC2 was shown as a component extracted the characteristics of the signal derived from API, while PC1 showed the characteristics of the excipients of the lactose and others excipients. The locations of samples

within the PCA score plot varied clearly according to the source country, suggesting that manufacturers in different countries employ different excipients as shown in Figure 4.

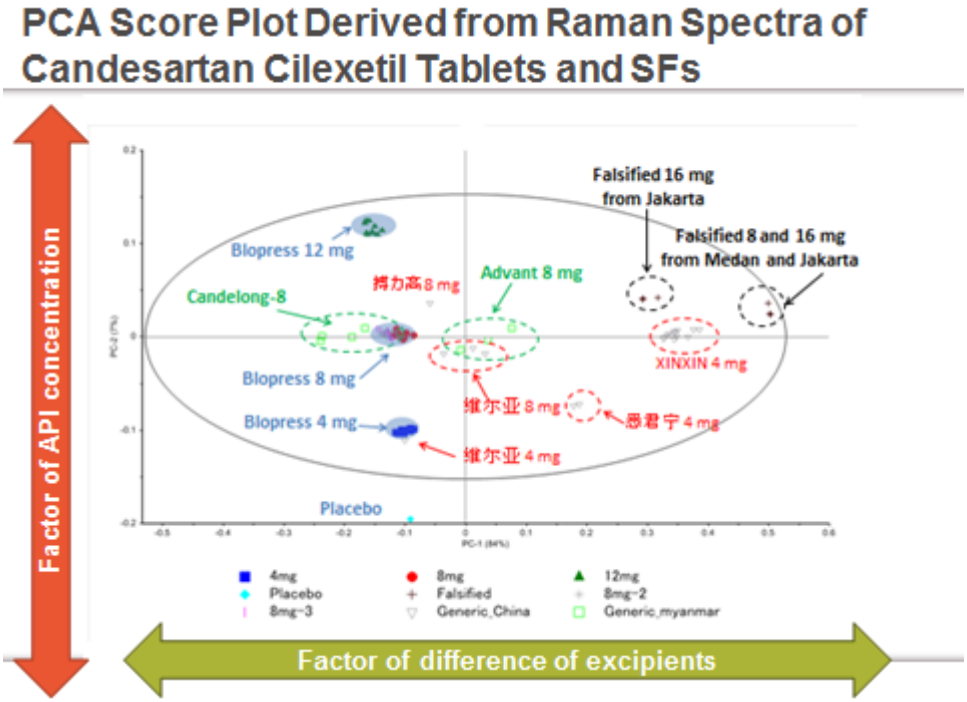

Figure 4. PCA score plot derived from the Raman spectra of candesartan cilexetil tablets, including falsified tablets, collected in China and Myanmar.

The results indicate that the handheld Raman device will be useful for detection of SFs and understand the correlation between the authenticities and other samples. PCA of that Raman data clarify the difference in chemical properties between good quality products and SFs that circulate in the Asian market.

Chapter III is about the development and application of speedy and in-situ 3D fluorescence method for the injectable products. The falsified medicines for the injections have serious risks of the health hazards because the API is administrated directly into the blood, muscle and subcutaneous. It is conceivable that it is important to analyze quickly and to detect counterfeit drugs speedy at an early stage of the distribution. Since the injectable products that are not guaranteed the quality and that may have been prepared in poor environment lead to serious health damage, it is necessary to quickly detect the falsified products and to take measure. The falsified cases have been reported that imitated or stolen glass vial and packaging as same as those of the authentic products were used. Further, the injectable products are often clear liquids and it is difficult to visually identify the contents and to detect the falsified medicines easily. I investigated the analytical method for observing the contents in the glass vial from the outside of the glass vial without removing the

injectable solution from the glass vial. Especially, it was aimed to develop the speedy detection of the presence or absence of the active pharmaceutical ingredient (API). Further, both injections of Avastin and Herceptin targeted as the falsified medicines are molecular targeted therapeutic medicines and are compounds having the high molecular weight and the larger physical three-dimensional structure. Many APIs in the injectable solution include anticancer drugs with a relatively large molecular weight, and medicinal ingredients having large three-dimensional structures such as peptides, antibodies, and antibacterial drugs. These compounds usually have their own fluorescent properties. Therefore, the speedy and in-situ three-dimensional (3D) fluorescence method was developed to detect the API's fluorescence of various injections without removing the contents from the outside of the glass vial or preprocessing of the solution for the measurement. The sample was irradiated with excitation light of various wavelengths, and the emitted fluorescence intensity was measured every 5 nm. By plotting the excitation wavelength on the vertical axis, the fluorescence wavelength on the horizontal axis, and the fluorescence intensity in the height direction, it is possible to draw a three-dimensional contour diagram. A method for measuring such excited fluorescent three-dimensional matrix is called the EEMs (Excitation-Emission Matrices) method. To identify the API by EEMs method has high specificity based on the three-dimensional structure of the API in the injectable solution which was affected by the concentration of the API, buffer solution, excipients, pH, viscosity and temperature in the injection. The result of the EEMs showed the critical specificity of the characteristic fluorescent fingerprints derived from API itself were obtained in any of pharmaceuticals products such as peptides, small molecules and antibodies, and it was confirmed that they have high discriminating ability. And the new approach to measure was possible to measure the fluorescence from API in the parenteral products over the glass vial/ampoule without opening the lid of the glass vial/ampoule. This method is not limited by the size and shape of the glass container. This development of the cell unit was conducted in the joint with Hitachi. It was confirmed this method has phenomenal sensitivity compared with the method connecting an external optical fiber more than 100 times. For the injectable products including API which has fluorescence properties, the fluorescence EEMs measurement is very effective to discriminate the authenticity of the medical products. This research succeeded in measuring the specific fluorescence fingerprints of the API in the injectable products from the outside of the glassware material without affecting the shape and size of the glass with enough intensity of the fluorescence. In recent years, a non-destructive analysis method of the fluorescence by using the fiber probe has been developed. However, in this fiber probe method, the attenuate of the fluorescence intensity is observed due to the physical distance between the spectrometer and the measurement samples. Therefore, it was difficult to detect the EEMs with

the high sensitivity analysis in the conventional fiber probe method. In this study, by removing the conventional cell unit and measuring the samples of the injectable formulation, the detection sensitivity was improved and the detection strength. Even if the low molecules which have week fluorescent, it was confirmed that this speedy and in-situ 3D measurement is able to sufficiently detect to a low concentration of around 10 ppm and it is very powerful tool to discriminate the medical products in the injectable formulation.

The developed analytical methods shown in Chapter I to III are expected to be applied widely as the powerful tools for detecting SFs in the research institutes, authorities and the pharmaceutical industry. This investigation succeeded to detect of SFs for the oral solid pharmaceutical products and the injectable solutions circulating in the Asian market using the pharmacopeial quality tests and non-destructive spectroscopy using the X-ray CT, the portable Raman, PCA, and the fluorescence spectrophotometer. The physical and chemical properties ant those correlation among the samples were clarified in this study. Through the chapter I to III, the discrimination methods of the substandard and falsified medical products for both the solid formulation and injectable formulation were developed using the pharmacopeial quality tests, non-destructive spectroscopy of Raman spectroscopy and X-ray CT, decision tree and PCA model. The application results of the developed methods to the SFs and the medical products showed that these methods have sufficient performance to detect the SFs with speedy and in-situ and to understand the physical and chemical properties and risks of the SFs which are distributing in the actual market. These methods are expected to contribute for detecting the SFs and to help people are able to access the reliable health care.

審査結果の要旨

垣尾智子氏の業績は非破壊分析法を応用して、偽造医薬品の検出法と異同識別法を開発したことである。第一にカンデサルタンシレキセチル錠を用いて携帯ラマン分光とＸ線ＣＴを併用して、真正品と後発品、試作品、偽造品を視覚により判別可能であることを示し、さらに、真正品か偽造品かのデシジョンツリーを作成した。第二に、カンデサルタンシレキセチル錠の携帯ラマン散乱光の主成分が主に有効成分（PC2）と添加剤（PC1）に帰属することを突き止め、サンプルのスペクトルを PC1（水平軸）と PC2（垂直軸）のスコアプロットで表すことにより、有効成分含量と添加剤の異同について直観的に把握することを可能にした。上海、インドネシア、ミャンマーの流通品並びにインターネット購入品について局方試験により把握した品質について、主成分分析のスコアプロットによりさらに詳細に洞察することに成功した。品質試験に合格したミャンマー収集品と先発品 Blopress は似通った添加剤が使用され、中国製品の添加剤は会社ごとに様々であり、特に品質試験不合格の XinXin 製品の添加剤は他の中国製品とは異なっており、むしろ正規品とは異なる添加剤を使用しているインドネシア偽造医薬品に近いことが分かった。さらに、16 ㎎と表記されていた偽造医薬品も 60%程度しか API を含有せず、8 ㎎含有と表示された偽造医薬品とほぼ同量の有効成分しか含有しないこともスコアプロットから明らかになった。第三には注射薬の偽造品検出を可能とする蛍光非破壊分析法の提案である。励起放射マトリックス法と組み合わせてガラス製アンプルの外から含有内容有効成分を検出することに成功し、流通する注射薬の不良品、偽造品の迅速な検出と排斥に有用な方法と考えられた。以上より、本審査委員会は，審査員全員一致で，垣尾智子氏に対して博士（創薬科学）の学位を授与することが適当であると判断した。