博 士 論 文

Development and Application of Discrimination Method of Substandard and Falsified Medical Products using Quality Test and Non-destructive Spectroscopy

品質試験および非破壊分光法を用いた品質不良薬・偽造薬の検出方法の開発と応用

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Preface

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 shows that 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. Chapter II shows the result

of the survey to identify substandard and falsified tablets of hypertension medical products collected in China, Indonesia and Myanmar with pharmacopeial quality control tests and principal component analysis of handheld Raman spectroscopy. The measurements were conducted using the quality control tests such as assay and dissolution tests published in the pharmacopeia and the principal component analysis (PCA) of the Raman spectra obtained by using handheld Raman spectroscopy. Chapter I and Chapter II describe about the analytical technologies for the oral solid pharmaceutical products. In the chapter III, about the development and application of speedy and in-situ 3D fluorescence method for the parenteral products are shown. The fluorescence spectroscopy was applied to the active pharmaceutical ingredient (API) in the parenteral pharmaceutical products. 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.

Chapter I

Classification and Visualization of Physical and Chemical Properties of Falsified Medicines with Handheld Raman Spectroscopy and X-ray Computed Tomography

ABSTRACT

Analytical methods for the detection of Substandard and Falsified medical products (SFs) are important for public health and patient safety. 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, one containing many cracks and voids and the other containing aggregates with high electron density, such as from the presence of the heavy elements. Generic products that purported to contain equivalent amounts of API to the authentic products were discriminated from the authentic products by the handheld Raman and the different physical structure on X-ray CT. 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 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.

1.1   INTRODUCTION

In 2015, 128 countries were impacted by pharmaceutical crime, including sale of substandard and falsified medicines (SFs), and the number of incidents in the Asia Pacific region exceeded one thousand for the first time.[1-9]   The World Health Organization (WHO) and the European Commission have warned about the danger posed to public health by SFs, and pointed out the need for measures to detect and prevent distribution of SFs.[10,11]

1.1.1   Definition of substandard and falsified medical products

The term of falsified medicines means any medicinal product with a false representation of: (a) its identity, including packaging, labelling, name or composition, as regards any of the ingredients including excipients and the strength of those ingredients; (b) its source, including manufacturer, country of manufacture, country of origin or marketing authorisation holder; or (c) its history, including records and documents relating to the distribution channels used.[11] Meanwhile, the term of substandard medicines refers to genuine medicines produced by manufacturers authorized by the relevant National Medicines Regulatory Authority, but which do not meet quality specifications set out for them by the national standards.[10] The United States Food and Drug Administration (FDA) has investigated various analytical methods to discriminate SFs from genuine medical products.[12]

1.1.2 Non-destructive spectroscopic method

Non-destructive analytical technologies that can identify and quantify APIs and excipients in pharmaceutical tablets include Raman spectroscopy[13-17], Near Infrared spectroscopy (NIR)[18-26], X-ray diffraction (XRD)[27-28], NMR spectroscopy[29], terahertz spectroscopy[30], and chemical imaging with combinations of vibrational spectroscopy and multivariate spectral analysis etc.[23,24,28,31-34] In particular, Raman spectroscopy provides sharp, characteristic spectral peaks, and therefore Raman spectroscopy is particularly suitable for identifying APIs and excipients in tablets. Non-destructive spectroscopic methods are summarized in the following figure.

Non-Destructive Spectroscopic Method

Non-Destructive methods without preprocessing of samples
- Raman spectroscopy
- Near Infrared spectroscopy (NIR)
- X-ray diffraction (XRD)
- NMR spectroscopy
- Terahertz spectroscopy
- SEM
- Microscope
- Surface MS
- EDX (Energy Dispersive X-ray spectroscopy)
- X-ray CT

etc.

✓ Chemical ID/Sharp peak from API
✓ On-site inspection at airports and customs
✓ Auto-judgment by the similarity of the spectra
✓ Non-destructive/for use as evidence

Handheld Raman (ThermoFisher)

✓ Physical properties
✓ Visualization inside tablets
✓ Non-destructive/for use as evidence

X-ray CT (Rigaku)

1.1.3 Handheld instrument to detect SFs

In addition, hand held instruments are now widely available for Raman[35] and NIR spectroscopies, and devices covering the spectral region from ultraviolet (UV) to the infrared (IR)[12]. These instruments are suitable for on-site inspection to detect SFs at airports and customs, and to detect substandard medicines at manufacturing sites. Since these instruments are relatively inexpensive and require little or no sample preparation, they are especially suitable for use in low and middle-income countries (LMICs).[36] Further, these methods are non-destructive, so that specimens found in surveys remain available for use as evidence for legal and regulatory purposes.

1.1.4 Profiling of falsified products

Information allowing the manufacture of falsified products is readily available. For examples, the contents of the drug product, including the API identity and quantity, and all excipients, are stated in the drug package insert. Other pertinent information, such as the shape, color, special markings and ID code can also be obtained easily from the drug package insert or website information. Pfizer Inc. has reported the existence of the falsified medicines containing the same effective ingredient as the authentic medicine, and with very similar appearance to the authentic tablets, in the global market.[37]

1.1.5 Physical structure by X-ray CT

To detect such falsified products, which may be indistinguishable from authentic products visually and chemically, X-ray CT can be used to visualize differences in the physical structure of the tablets, such as particle size, uniformity of granules, film coating thickness, and the existence of pores or voids, which may result from differences in the manufacturing process and conditions, or grade of additive. These are important, because defects of physical structure can influence both stability and dissolution properties. Further, X-ray micro CT is a powerful tool to observe the distribution of elements in tablets by utilizing the difference of the electron density. For example, the distribution of magnesium in magnesium stearate, an additive used as a lubricant has been visualized by X-ray CT.[38]

1.1.6 Combination method of handheld Raman and X-ray CT

In this study, we investigated the effectiveness of handheld Raman spectroscopy and X-ray CT to discriminate SFs from authentic medicines of product A (Blopress Tablets), focusing on anti-hypertensive tablets containing candesartan cilexetil as API which is an angiotensin II receptor blocker, because they are widely prescribed worldwide for the treatment of hypertension. Authentic medicines were collected from Japan manufacture. We used generic products collected in China as example of products containing the same API but having differences in the kind of excipients and the manufacturing process. As known falsified tablets, we used falsified tablets from Indonesia, whose outer packaging and press through pack (PTP)

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packaging were very similar with the authentic medicine, but were inconsistent with those of authentic medicines.

1.2   EXPERIMENTAL

The collected and measured samples are summarized in the following.

Sample Collection

Category	Sample Name (Active Ingredient Dose)
Authentic	Blopress Tablets (2 mg), (4 mg), (12 mg), (16 mg), Placebo
	Blopress Tablets (8 mg) as reference
Generic	Generic Product (8 mg) from China, Manufacture A
	Genetic Product (4 mg) from China, Manufacture B
Model formulation of physical mixture	Direct compression of API (8 mg) and Lactose monohydrate
	Direct compression of API (8 mg) and all excipients
Falsified Product	Falsified Product (8 mg) from Indonesia
	Falsified Product (16 mg) from Indonesia

Authentic formulation; API, lactose monohydrate, corn starch, maize starch, carboxymethylcellulose calcium (ECG-505), hydroxypropyl cellulose (HPC-L), polyethylene glycol 6000 (PEG 6000), and magnesium stearate (St-Mg).

Model formulation (Physical mixture of API and excipients);
Compressing the mixture at a pressure of 11 kN without a granulation process. One of them contained the 8 mg API and lactose monohydrate to make the same total weight as that of the authentic tablets, and the other contained 8 mg API and all excipients in the same proportions.

The authentic commercial tablets of Blopress including 0 mg (Placebo), 2 mg, 4 mg, 8 mg, 12 mg and 16 mg of API, together with lactose monohydrate, corn starch, maize starch, carboxymethylcellulose calcium (ECG-505), hydroxypropyl cellulose (HPC-L), polyethylene glycol 6000 (PEG 6000), and magnesium stearate (St-Mg) were used. The weight and size of the 4 mg, 8 mg and 12 mg tablets are equivalent, as the amount of lactose monohydrate is adjusted according to the weight of API to maintain a constant total weight. Tablets distributed

as generic products in China from two different manufactures and two falsified products discovered in Jakarta, Indonesia in 2011 and 2012 were also used in this study. The falsified products were identified based on visual inspection of the packaging (differences from the authentic product included the color of the printed letters and the printing positions on the surface of the aluminum blister). These falsified products had been collected in different pharmacies in Jakarta, and their distribution was reported to Forensics, Brand Protection and Investigations. Two types of model formulations of falsified tablets were also prepared by physically mixing the API and excipients, and directly compressing the mixture at a pressure of 11 kN without a granulation process. One of them contained the 8 mg API and lactose monohydrate to make the same total weight as that of the authentic tablets, and the other contained 8 mg API and all excipients in the same proportions as in the authentic tablets.

1.2.1   Handheld Raman Spectroscopy

All tablets were evaluated with a handheld Raman spectrometer (TruScan®, Thermo Scientific), and chemical equivalence between the authentic product and the other samples was examined based on the similarity of the Raman spectra. The Raman spectrum of the authentic tablets was registered in the instrument as the reference spectrum, and the similarity of the Raman spectra between the authentic tablets and test tablets was automatically calculated and assigned the p-value. The test tablet is judged "pass" if the p-value for similarity is more than

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0.05, and "fail" if less than 0.05. In other words, "fail" means that the Raman spectrum of the

test sample does not match that of the authentic product. The calculation algorithm for p-value

in the instrument has been validated, but has not disclosed and is designed not to be modifiable.

We confirmed the validity and the accuracy of the judgment by extracting and examining the

raw data of the Raman spectra. If the device encounters a completely different Raman spectral

pattern, it stops accumulating data and judges that the p-value is 0. Blow is a diagram of the

flow of the discrimination by using handheld Raman.
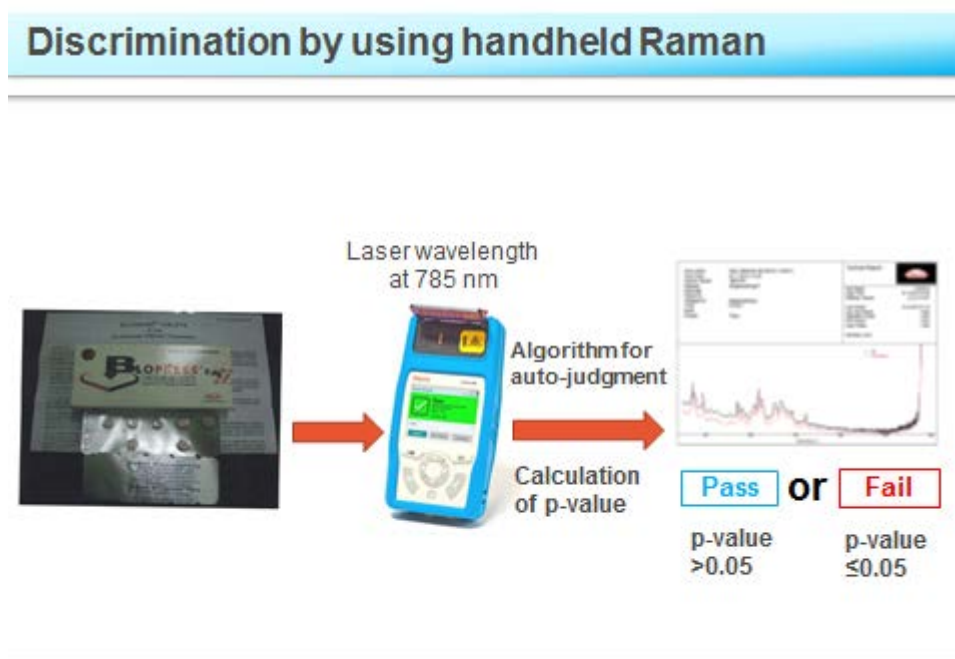
1.2.2 X-ray CT and Image acquisition

The X-ray micro CT consists of a combination of a high-intensity X-ray generator and a

high-resolution X-ray camera. It is capable of observing the three-dimensional internal structure

of tablets at the micron-scale, based on the different X-ray absorptions of the constituent materials. The sample is placed on a rotating stage located between the X-ray generator and the X-ray detector. Multiple 2-dimension X-ray transmission images are recorded at different sample-rotation angles, and are converted into a three-dimensional image by a tomographic reconstruction algorithm. X-ray tomography measurements of tablets were conducted using a high resolution 3D X-ray microscope (nano3DX, Rigaku). The incident X-ray was generated using a rotating-anode generator with a molybdenum target operated at the tube voltage and current of 50 kV and 24 mA, respectively. The temperature was maintained at 22-24ºC during data acquisition. The 2160 lens, which allowed measurements in a 7.12 mm x 5.40 mm field of view with a resolution of 4.32 um/pixel, was used. Each CT reconstruction was conducted using 1800 projection shots with an exposure time of 5 seconds/shot. The analyses were done using the nano3D Calc software (Rigaku) and Image.[39]

1.3   RESULTS

Figure 1.1 shows the appearance of each tablet. The 4 mg authentic product A (T-1) is white scored tablets, and the 8 mg dosage strength authentic product A (T-2) is a reddish white scored tablet. An embossed character was observed on the surface of both authentic tablets. The generic Chinese products, labeled T-3 and T-4 were white scored tablets with no embossed character on the surface. Two model formulation tablets, labeled T-5 and T-6, were prepared by

direct compression without granulation. T-5 contains 8 mg API and lactose monohydrate in an appropriate quantity to make tablet of the same shape and size as the authentic product A. T-6 includes API and all excipients in the same proportions as the authentic product A. T-5 and T-6 were white tablets with no scored line on the surface. The falsified products T-7 and T-8 from Indonesia, where product A is legally marketed were reddish white scored tablets and reddish tablets with no scored line, respectively. Without detailed knowledge of physical appearance of genuine tablets, it would be difficult to distinguish the falsified products simply by visual inspection.

Figure 1.2 shows the Raman spectra of the API and the authentic tablets of Blopress tablets (2 mg), (4 mg), (8 mg), (12 mg), (16 mg) and Placebo. The characteristic peaks from API were confirmed in the region from 1780 cm^{-1} to 1700 cm^{-1}, which are not overlap with the peaks from the excipients. Further, the intensity of the API peak at around 1610 cm^{-1} increased with the increase of the concentration of the API in the tablets. The Raman spectra of tablets T-1 to T-8 were compared with that of the authentic product. Table 1 shows the p-values and the auto-judgment results for each tablet. The authentic product T-2 was evaluated correctly as "pass" (p = 0.5045). Authentic tablets with a different dose of API (4 mg, T-1), the generic tablets T-3 and T-4 from China, and the model formulations T-5 and T-6 including same dose of API as authentic product T-2 were also discriminated as "pass", although the p-values were lower than that of the authentic product T-2. The falsified products T-7 and T-8 were evaluated as "fail" based on the obvious difference of the Raman spectra from that of the authentic

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product. 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.

The Raman spectra obtained with the handheld instrument are shown in Figure 1.3. Samples T-1 to T-6 showed very similar Raman spectra to that of the authentic product. The spectral features are mainly due to the API and lactose monohydrate. Characteristic peaks of the API that do not overlap with peaks due to the excipients were observed in the region from 1780 cm^{-1} to 1700 cm^{-1} as shown in Figure 1.3. The peak intensity from API in this region was reported to increase linearly with increase of API content in the tablets.[27] The Raman spectra of tablets T-1 to T-6 illustrate the difficulty of discriminating authenticity correctly among compositionally similar samples based only on chemical properties. On the other hand, the p-values of the falsified products T-7 and T-8 were 0.0000; their spectra showed no characteristic peaks of API, and the baselines rose from higher to lower wavelengths, suggesting the presence of a fluorescent component. Thus, falsified products that do not contain the API can be evaluated correctly as "fail".

Figure 1.4 shows the average p-values calculated automatically by the handheld Raman instrument with reference to the authentic product A (8 mg), T-2. Good repeatability of the p-value was confirmed using 3 different lots of the authentic product A (8 mg) with ten replicate measurements. The other doses (4 mg and 12 mg) of the authentic products gave average p-values of 0.2127 and 0.2725, respectively, compared with the range of 0.5059 to

0.6768 for three different lots of 8 mg product A. These results suggested an appropriate criterion of the p-value should be set for discriminating the authenticity of the product A (8 mg) from falsified products. Based on the average p-values, a p-value of less than 0.4 might be a suitable criterion for judgment of falsified or substandard products containing less than 50% or more than 150% API compared to the content of the authentic product.

Next, samples T-1 to T-8 were examined by X-ray CT, as shown in Figure 1.5. Uniform granule powders and white spots were seen in images of the authentic products T-1 and T-2. The white spots showed an elongated planar shape and were considered to be Magnesium stearate (St-Mg), a known component of the authentic tablets. Magnesium has a high electron density, and therefore has a higher X-ray absorption than the other components, so that St-Mg is clearly visualized in the CT image. The CT image of generic product T-3 showed much larger granules of non-uniform size and pores with a diameter of over 400 nm. Generic products T-3 and T-4 did not show evidence of the presence of St-Mg. Generic product T-3 showed aggregates with a diameter of 200-600 μm order. The model formulations T-5 and T-6 showed a uniform and smooth appearance, presumably due to the direct compression process without granulation. However, the density unevenness resulting from the lack of enough mixing process is confirmed by the color unevenness as in the black portion of the CT imaging. Also, in the CT imaging of T-5 and T-6, the distribution of the larger particle as seen in T-1 and T-2 are not confirmed.

The images data of falsified product T-7 showed many pores and cracks inside the tablets, suggestive of a poor manufacturing process. The image of falsified product T-8 shows a large amount of an unknown higher specific gravity component dispersed throughout the tablets.

1.4   DISCUSSION

Visual inspection of the appearance of a drug product is a critical test item for detecting the falsified products. However, in the present study, falsified products T-7 and T-8 showed a similar color to the authentic product T-2. Thus, instrumental methods are important to identify SFs.

Although the spectrum resolution of the handheld Raman instrument is only about one-tenth in the comparison with the high resolution bench-top instrument, the handheld device is cheap and easily portable for the field use, such as at the airports, customs, manufacturing sites in the developing countries, owing to its small size. This instrument covers the required region of $2875 cm^{-1}$ to $250 cm^{-1}$ to observe APIs and some excipients in the tablets. In this study, it was found that the detection and discrimination of the falsified products of the product A were achieved by using the handheld Raman instrument. Although the Raman spectra of tablets T-1 to T-6 were quite similar, the behavior of the repeatability in the same lot and the variation of the p-value among the lots suggested that a criterion p-value of not more than 0.4 could be used to discriminate SFs. The p-value was also clearly different among the original product A, the

generic products and the model formulations. This difference may be from the difference of the kind of and/or the grade of the excipients and the difference of the manufacturing process. When the suspect sample is evaluated whether it is product A or not, the trend of the p-value should be considered.

Our results are in agreement with those of a research group in University of Washington (Kovacs et al., 2014), which compared various solutions for detecting SFs in LMICs and evaluated handheld Raman as one of the top solutions, offering multiple advantages, including no-need for sample preparation, high performance, speed, easy of use, low cost, no requirement for electricity supply. Further, handheld Raman technology can be employed without opening the PTP package, because the laser is focused on the tables, and the peaks from the plastic do not overlap the peaks of API. Therefore, this technology is particularly suitable for on-site inspection throughout the supply chain, such as at custom, posts, airports, and also in manufacturing sites.

The X-ray CT approach enables detailed examination of the internal structure of tablets. Many voids were observed inside the falsified product T-7, while falsified product T-8 contained a large amount of excipients with high electron density. Also, the physical mixture, T-5 and T-6 showed X-ray CT images that were clearly distinct from those of authentic product A, reflecting the omission of the granulation process. Further, generic products which included the same amount of API but contained different excipients and granules of the different sizes could be easily distinguished. The results of X-ray CT of T-1 and T-2 suggested that the API is not

identified nor localized in a specific location in the tablets. This is one of the important information about the quality of T-1 and T-2 to discriminate the SFs from authentic samples. Thus, the combination of Raman spectroscopy and X-ray CT can provide detailed information about API content and internal structure and uniformity of the tablet. At present the CT images have to be visually evaluated, but developments in equipment and software should make it possible to score images objectively in the future.

Overall, these results indicate that the combination approach of visual observation, handheld Raman and X-ray CT should be a powerful strategy for non-destructive discrimination of SFs. Figure 1.6 shows a decision tree for using these combination methods to discriminate the authenticity of samples purporting to be product A (8 mg). The first step is the visual observation of the tablet size, shape, color, scored line and embossed mark (Tier 1). Next, handheld Raman should be applied to identify the API and to detect differences of the kind of the excipients, based on the p-value (Tier 2). Generic products and different doses of product A should be discriminated at this point. Then, if necessary, the physical composition of the tablet can be investigated by X-ray CT (Tier 3). This combination approach should enable accurate detection of even falsified products that have very similar properties to the authentic products. Further it should provide detailed information to assist in tracking the source of the falsified products and for monitoring trends in SFs, as well as assessing the efficacy of regulatory procedure.

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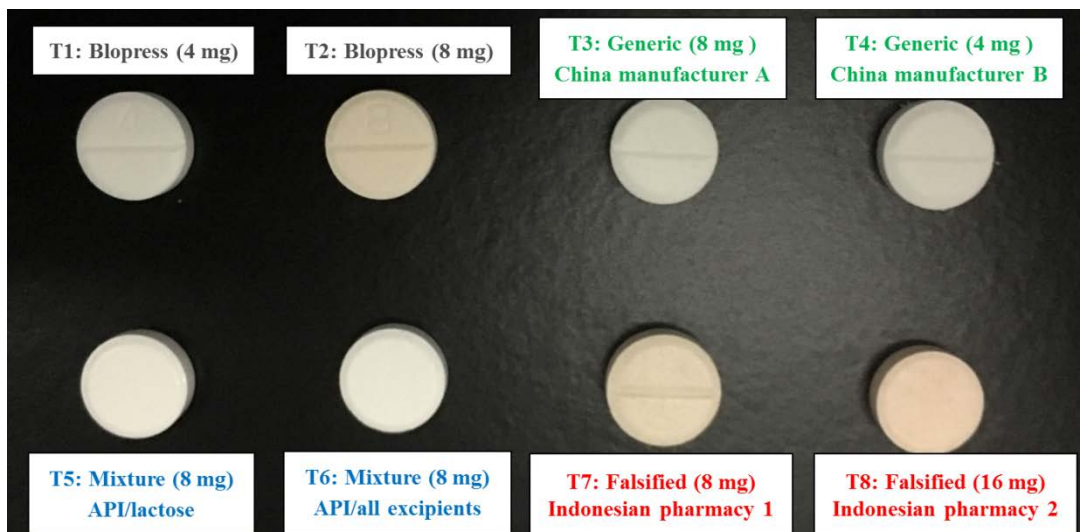
Figure 1.1. **Visual inspection of tablets:** T-1; authentic product A (4 mg), T-2; authentic product A (8 mg), T-3 and T-4; generic products from different Chinese manufacturers, T-5; model formulation including API and lactose monohydrate, obtained by direct compression, T-6; model formulation including API and the same excipients as in the authentic product A, obtained by direct compression, T-7 and T-8; counterfeits found in Indonesia.
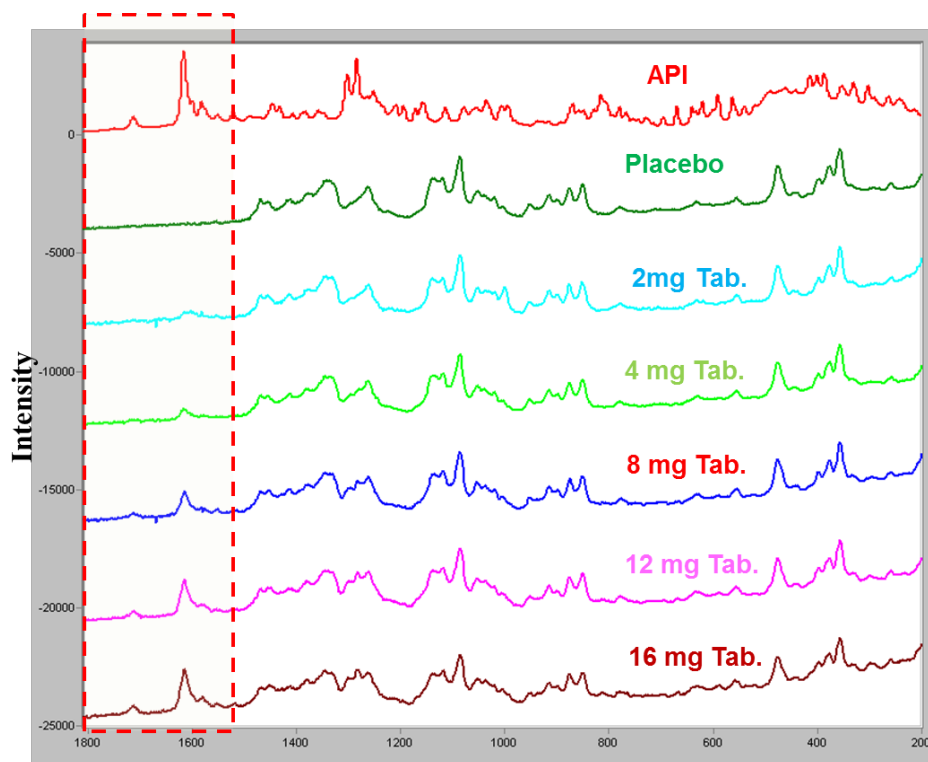
Figure 1.2. Raman spectra of the active pharmaceutical ingredients (API), Blopress tablets (2 mg), (4 mg), (8 mg), (12 mg), (16 mg) and Placebo.

Table 1.1   The results of handheld Raman examination of authenticity, based on the correlation of Raman spectra between test samples and the authentic product A (8 mg). The result of the instrumental judgment based on the p-value is also shown.

No.	Category	Sample Name (Active Ingredient Dose)	p-value	Judgment
T-1	Authentic	Product A (4 mg)	0.2332	pass
T-2	Authentic	Product A (8 mg)	0.5045	pass
T-3	Generic	Generic Product B (8 mg) from China	0.2645	pass
T-4	Generic	Genetic Product C (4 mg) from China	0.1483	pass
T-5	Model formulation	Direct compression of API (8 mg) and Lactose monohydrate	0.1348	pass
T-6	Model formulation	Direct compression of API (8 mg) and all excipients	0.1040	pass
T-7	Falsified Product	Falsified Product of product A (8 mg) from Indonesia	0.0000	fail
T-8	Falsified Product	Falsified Product of product A (16 mg) from Indonesia	0.0000	fail
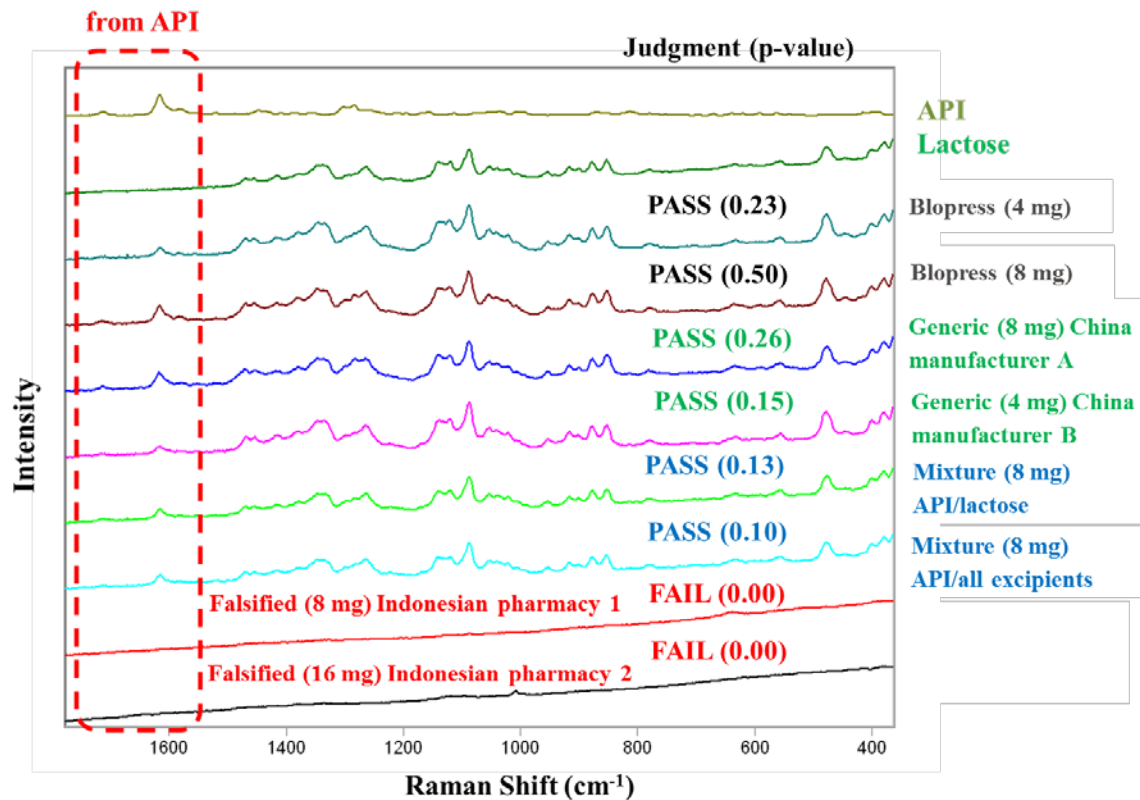
Figure 1.3. The Raman spectra of the API, lactose monohydrate (excipient) and the tablets.

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 all tablets except the falsified tablets, which includes both

API and lactose monohydrate   are similar, but the falsified tablets (T-7 and T-8) show

distinctly different spectra.
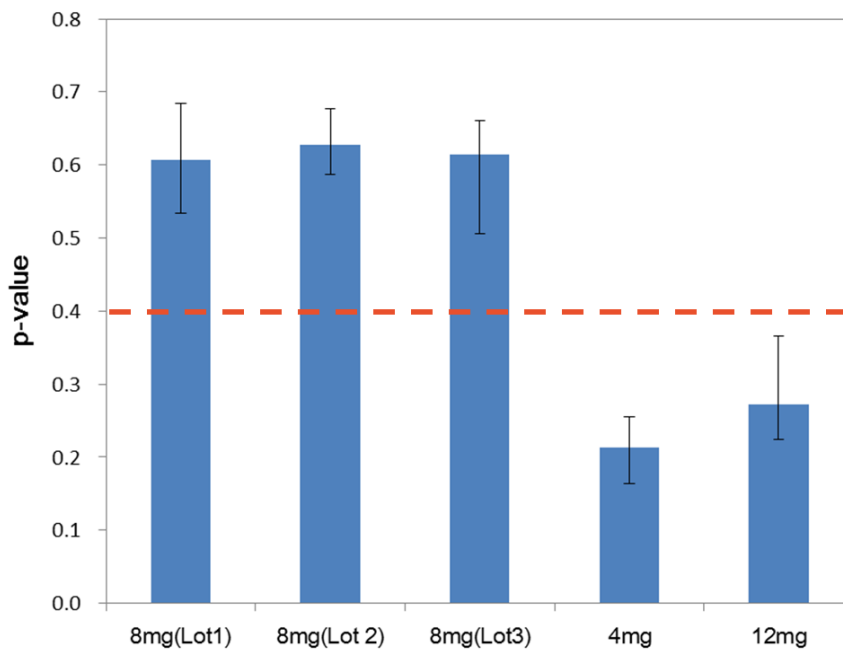
Figure 1.4.   Average p-values of authentic products calculated automatically by the handheld

Raman device for similarity to the authentic product A (8 mg). Repeatability of the p-value was

confirmed by ten replicate measurements. The range of the p-value from the maximum to the

minimum value is shown by a bar.

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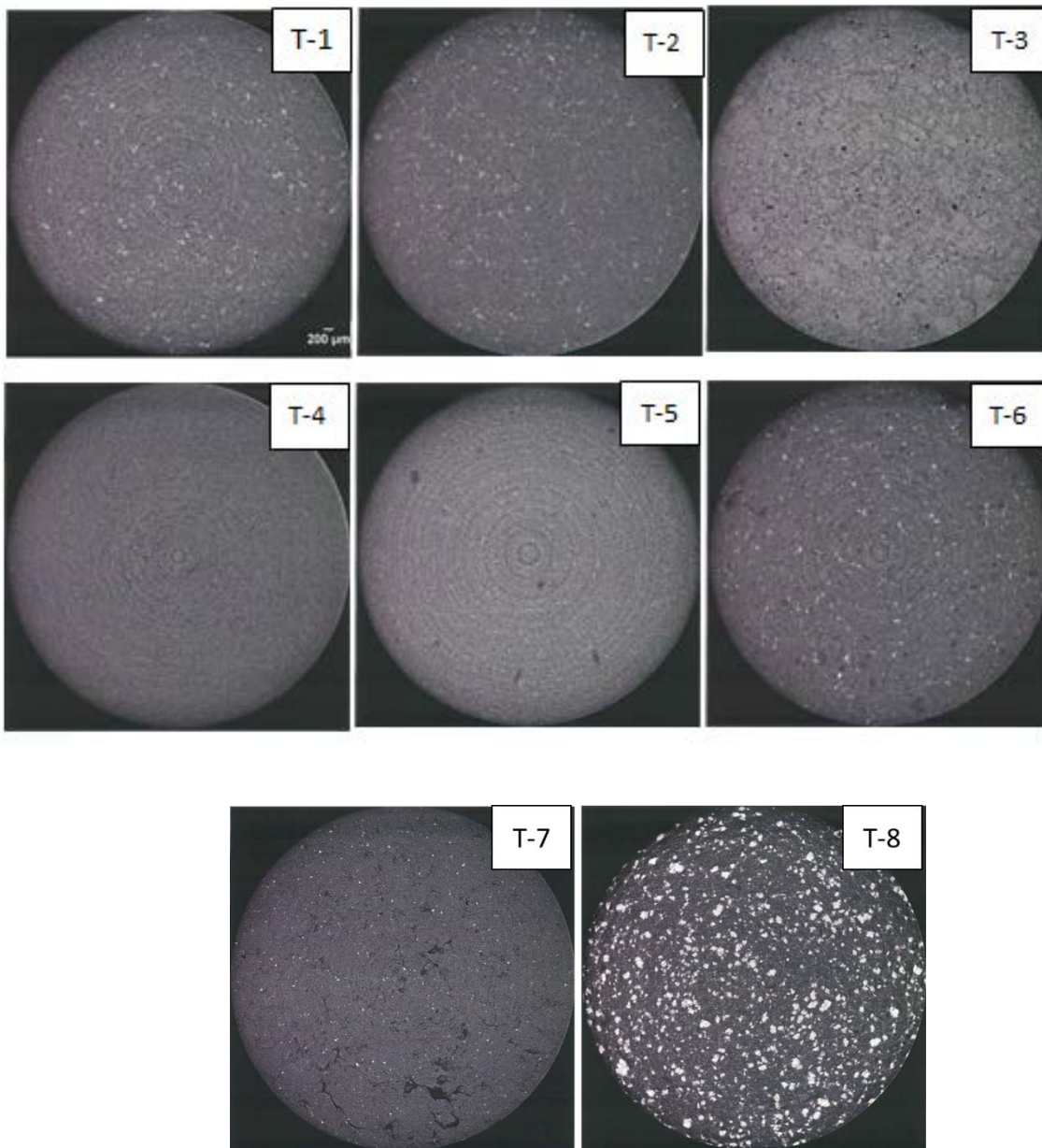
Figure 1.5. X-ray CT images of the internal structure of tablets T-1 to T-8.
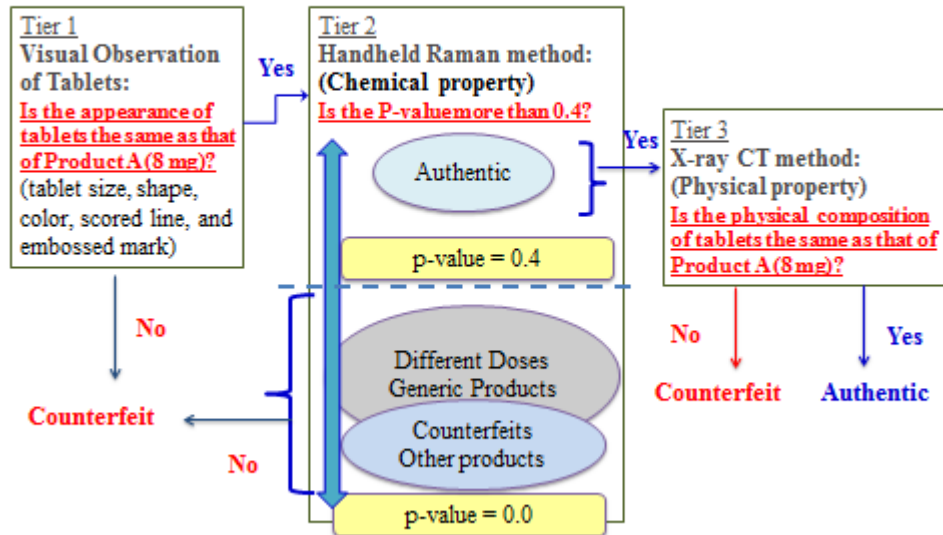
Figure 1.6. Decision tree of combination approach for non-destructive discrimination.

Chapter II

Survey to Identify Substandard and Falsified Tablets in Several Asian

Countries with Pharmacopeial Quality Control Tests and Principal

Component Analysis of Handheld Raman Spectroscopy

ABSTRACT

The World Health Organization (WHO) has warned that substandard and falsified medical products (SFs) can harm patients and fail to treat the diseases for which they were intended, and they affect every region of the world, leading to loss of confidence in medicines, healthcare providers and health systems. Therefore, development of analytical procedures to detect SFs is extremely important. In this study, we investigated the quality of pharmaceutical tablets containing the antihypertensive candesartan cilexetil, collected in China, Indonesia, Japan, 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. Some samples showed delayed dissolution and failed to meet the pharmacopeial specification, while others failed the assay test. These products appeared to be substandard. PCA 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. Examination of the PCA score plot indicated that the excipients of the substandard and falsified tablets showed similar Raman spectra, in contrast to authentic products. The locations of samples within the PCA score plot varied according to the source country, suggesting that manufacturers in different countries employ different excipients. Our results indicate that the handheld Raman device will be useful for detection of SFs in the field. PCA of that Raman data clarify the difference in chemical properties between good quality products and SFs that circulate in the Asian market.

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2.1   INTRODUCTION

2.1.1   SF statement in WHO 2017

In May 2017, definitions of substandard and falsified medical products (SFs) were announced by the World Health Organization (WHO).[1] Substandard medical products (also called "out of specification") are authorized by national regulatory authorities, but fail to meet either national or international quality standards or specifications – or in some cases, both. On the other hand, falsified medical products deliberately or fraudulently misrepresent their identity, composition or source.[1-5]

2.1.2   Survey on falsified medical products

Many surveys of falsified medical products and analytical procedures for investigation of the authenticity of medical products have been reported by various public research institutes.[2-12] In 2015, the Pharmaceutical Security Research Institute (PSI) reported that Asia experienced the highest incidence of drug crime cases among seven regions in the world. In that year, a total of 3,002 cases of drug crime were recorded, among which around 1,000 involved the Asia-Pacific region.[13] Many cases where defective products have been transported across national borders have been reported.[14]

2.1.3 Survey of substandard medical products

Relatively little work has been done on analytical methods for investigating the actual status of substandard medical products, including their distribution, and their physical and chemical properties.[14-15] One reason for this may be concern about the possibility of excessively hindering the development of medicines and access to medicines in developing countries.[16] Also, regular quality control and surveillance of medicines after marketing tend to be more difficult in developing countries for various reasons, including high cost, the need for sophisticated equipment and skilled technicians, and lack of pharmacological knowledge to recognize the need for implementation of countermeasures.[6, 15, 17-19] Further, medicines may be transported across national borders without proper quality checks through various distribution channels.[14,20] These are serious issues to be taken measures, because SFs can cause treatment failure, development of antimicrobial resistance, and serious adverse drug reactions, thereby damaging public confidence in medicines.[2,21-22]

2.1.4 Quality control by analytical procedures and acceptance criteria

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).[23] 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,[24-25] 2) significant dissolution delay,[24] 3) contamination with toxic substances,[26-27] and 4) lack of sterility.[28-29] These points can be checked by means of assay, content uniformity testing, measurements of dissolution properties and impurities, and microbial tests.

2.1.5   Sample Collection

Candesartan cilexetil tablets were collected in China, Indonesia, Japan and Myanmar, and subjected to quality control tests (assay, content uniformity and dissolution tests) according to the Japanese pharmacopeia. The acceptance criteria for these tests in the Japanese pharmacopeia were adopted as thresholds for identification of SFs.

2.1.6   Impact on quality by excipients

Many issues of quality and bioavailability are considered to be due to technical deficiencies in the manufacturing process design and differences in the nature of the excipients[30-32]. Although many studies have shown that excipients influence quality, the excipients are not

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generally stipulated in quality tests. Our previous study found that the types of excipients used in candesartan cilexetil tablets differ depending on the manufacturer, and Raman spectra of the tablets showed different patterns reflecting the chemical nature of the excipients.[33]

2.1.7   Principal component analysis (PCA) of Raman spectra

Here, we focused on methodology for detecting substandard and falsified medicines by principal component analysis (PCA)[12,22,34-42] of raw data obtained by handheld Raman spectroscopy. We 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 MSC method as being particularly suitable to extract the desired signals from the strong fluorescence background. [42] This approach proved highly effective to evaluate the degree of similarity among samples.
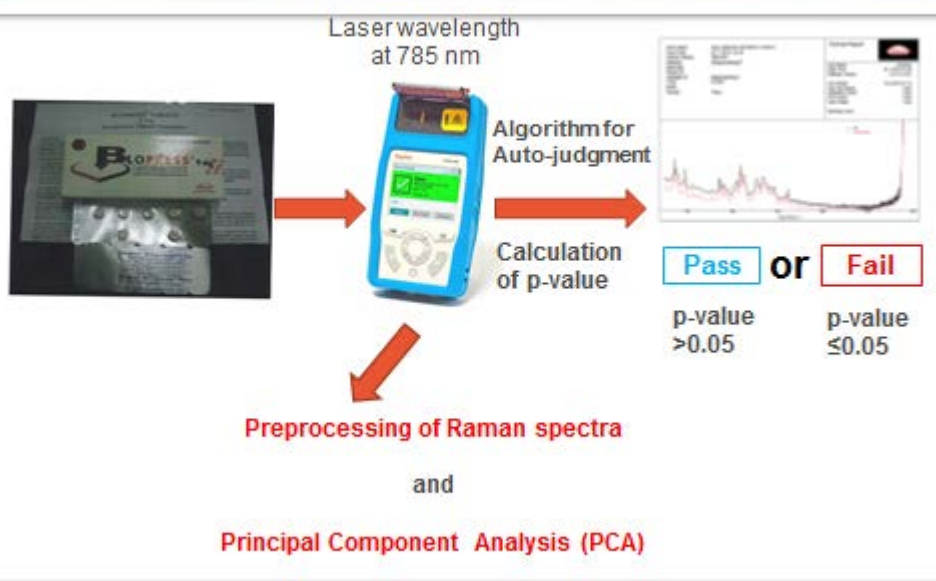
2.2 EXPERIMENTAL

2.2.1 Sample Collection

Authentic candesartan cilexetil tablets were supplied by a Japanese manufacturer as reference samples. Samples of candesartan cilexetil tablets were collected from hospitals and clinic in China, Indonesia and Myanmar, and also purchased via the internet (2009-2015).

2.2.2 Visual Inspection

First, we observed the outer package and package insert, PTP or Al-blister packaging. The product name, dose, component, formulation, packaging unit, manufacturer, manufacturing date, expiration date and manufacturing number were recorded. The cartons were examined visually and microscopically and compared to reference samples. Printing on the edge of the tape seal was carefully observed to check fine details.

2.2.3 Quality Control Test

Content uniformity[43], Assay and Dissolution tests of candesartan cilexetil tablets were conducted according to the Japanese pharmacopeia.

Acetonitrile (for high-performance liquid chromatography), polyoxyethylene 20, and sorbitan monolaurate (for biochemistry) were purchased from Wako Pure Chemical Industries, Ltd., and acetic acid and acenaphthene were purchased from Nacalai Tesque Co., Ltd. Candesartan cilexetil reference tablets were supplied by Takeda Pharmaceutical Co., Ltd.

2.2.4 Content Uniformity and Assay

Candesartan cilexetil in tablets were extracted in a mixture of acetonitrile and water (3:2), and measurements were carried out at a wavelength of 305 nm using a spectrophotometer (U-3210, Hitachi, Tokyo, Japan). Since the number of the collected samples was limited, 2, 3 or 10

tablets were used for each evaluation of content uniformity and the mean of the content was calculated as the result for assay. The acceptance criterion for the assay was set to 95.0-105.0%.

2.2.5 Dissolution

The dissolution test was performed under the conditions described for Apparatus 2 (paddle method) with 50 rpm agitation in 900 mL of a dissolution medium containing 10 w/v% polyoxyethylene (20) sorbitan monolaurate at 37 °C. A sample was taken at the time point of 45 minutes, and examined by high-speed liquid chromatography (HPLC, L-7200 autosampler, D-7000 interface, L-7100 pump, L-7300 column, L-7405 UV detector, Hitachi, Tokyo, Japan). Acenaphthene was added to the test solution as the internal standard. HPLC conditions: 5 μm ODS column (Shim-pack CLC-ODS (M) 4.6 mm × 15 cm, Shimadzu, Kyoto, Japan), flow rate 1.8 mL/min, column temperature 25 °C, injection volume 50 μL, detection wavelength 254 nm. The mobile phase was a mixture of acetonitrile, water and acetic acid (57:43:1). The mean of the dissolution rates of 2, 3 or 6 tablets were evaluated, respectively. The criterion adopted was that the dissolution rate of candesartan cilexetil should be more than 75% at the 45-minute sampling point.[44]

2.2.6 Handheld Raman Spectroscopy

All tablets were evaluated by the handheld Raman spectroscopy (TruScan®, Thermo Fisher Scientific). The chemical equivalent in structural component between the reference authentic products including 8 mg of API and the others were examined based on the similarity of the Raman spectra. The similarity of the Raman spectra between the authentic and the test tablets was calculated automatically as the p-value. The calculation algorithm for p-value in the handheld Raman spectroscopy is validated, not disclosed and designed not to be modified. After the auto-judgment by the handheld Raman device, to investigate trend of the chemical properties of the candesartan cilexetil tablets using PCA, the raw data of the Raman spectra were extracted and investigated. To input the factor of the API in the Raman spectra to the PCA, in addition to measuring the collected tablets, the original authentic tablets including 0 mg (Placebo), 4 mg, 8 mg and 12 mg of the API were also measured with handheld Raman, respectively and applied to PCA. The weight and size of 4 mg, 8 mg and 12 mg tablets are equivalent, and same weights of each excipient except Lactose monohydrate to adjust total weights of them.

2.2.7 Preprocessing of Raman spectra

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. We used the Savitzky-Golay (SG) method[45] to smooth each segment of the original Raman spectrum in a

small window by fitting to a polynomial function[45-46]. The multiplicative scattering correlation (MSC) method[46-49] 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.[46-48] 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 observed spectrum $Y(\omega)$ is considered to depend on wavelength as follows:

$$Y(\omega) = \overline{Y(\omega)} + a\,\omega + b + e(\omega) \qquad\qquad (1.2)$$

where $\overline{Y(\omega)}$ is the standard spectrum and $e(\omega)$ represents the residual. *a* and *b* are adjusted in order to minimize the term $e(\omega)$, to make these discrete deviations as small as possible.[47]

2.2.8 Principal component analysis (PCA)

Unscrambler® software (CAMO Software) was used for PCA. The Raman spectral data set consisting of 85 samples and 476 wavenumbers was calculated and it was decomposed into a linear combination of scores t_n and loadings p_n consisting of several principal components, allowing the spectrum to be understood clear with a limited number of principal components.

That is, the data set X is decomposed into a linear combination of the score and the loading as shown in equation 1.3 and the schematic figure below.

$$X = t_1 p_1 + t_2 p_2 + t_3 p_3 + \cdots t_N p_N \qquad (1.3)$$
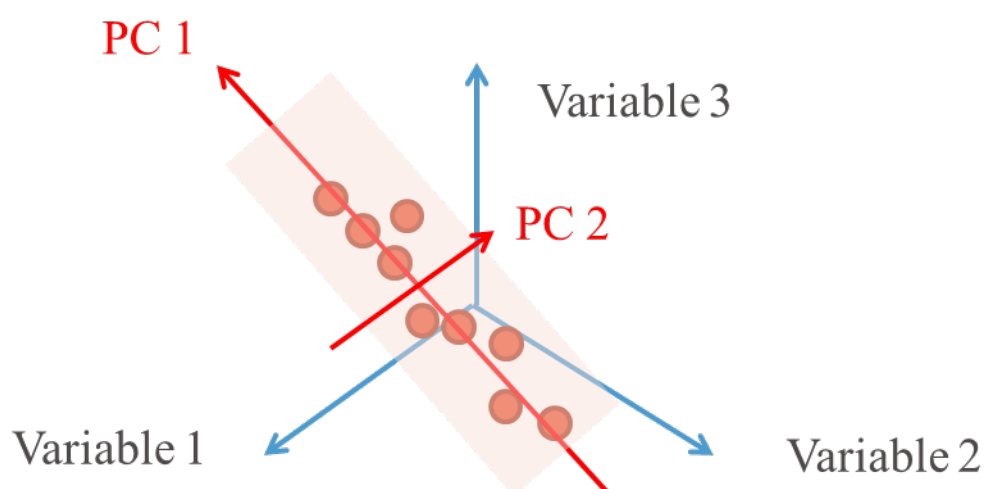
The validity and robustness of the calculated PCA model were confirmed by cross-random validation.

2.3 RESULTS

More than fifteen brands of candesartan cilexetil tablets were available in China, as judged from an internet survey, but only 4 brands were found to be distributed in hospitals and clinics in Shanghai in China. The collected 7 samples of Japanese manufactures (Blopress), 3 kinds of

samples of Chinese manufactures (2 samples of 悉君宁, 4 samples of 维尔亚, 13 samples of XINXIN in distributed in Shanghai in China were used for quality tests and PCA. In Mandalay in Myanmar, three brands, which are Blopress manufactured in Japan, Candelong-8 manufactured in India and Advant manufactured in Pakistan, were found in private hospitals, community pharmacies and wholesalers. No obvious deficiencies in the PTP packaging, package insert, pillows, or tablets were found in visual inspection of all collected samples. Table 2.1 shows the summary of the results of dissolution, assay, contents uniformity and chemical similarity of candesartan cilexetil tablets.

2.3.1   Content Uniformity, Assay and Dissolution Behavior

Figure 2.1 summarizes the results of the dissolution test for the collected samples. Dissolution delay was confirmed in two samples of XINXIN candesartan cilexetil tablets, which failed to meet the criterion dissolution rate of more than 75% at 45 minutes. Other all samples met the criterion and was observed the enough dissolution at 45 minutes. Figure 2.2 summarizes the content uniformity and assay results for the collected samples.The assay values of 12 out of 13 samples of XINXIN candesartan cilexetil tablets exceeded the upper limit of 105.0%. The difference in assay of these samples was large (Max 112.8% to Min 104.4%) and the content un-uniformity was observed from the standard deviation among each sample up to Max 4.8%. (see Tablet 2.1). Two samples of 悉君宁 and one sample of Advant candesartan cilexetil tablets

gave an assay value below the lower acceptance limit of 95.0%. Other samples met the criterion of assay.

2.3.2   Handheld Raman spectroscopy and PCA

Raman spectra obtained with the handheld instrument are shown in Figure 2.3. The spectral features are mainly due to the API and excipients, including lactose monohydrate. The Raman spectra of the falsified products and XINXIN products showed a distinctive upward slope of the baseline toward high wavenumber. The API peak intensity in this region was reported to increase linearly with increase of API concentration in the tablets,[27] and a similar result was also obtained in this study. These relationships of the quantitation between the API peak intensity and the assay of the API in tablet were also confirmed in not only the weight measurement of API in tablets vs the peak intensity of Raman spectra but the relationship between the weight measurements of API vs the peak intensity of X-ray diffraction measurement.[27]

These Raman spectra were conducted preprocessing and subjected to PCA in order to investigate the similarity of chemical components among samples. Figure 2.4 shows the spectra after the preprocessing of Savitzky-Golay method for smoothing and MSC method for baseline correction for the Raman spectra. The calibration result and cross validation result in PCA model were compared as shown in Figure 2.5. 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 as shown in Figure 2.6. Data set of the Raman spectra in the range of 1780 to 1000 cm^{-1}, 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, XINXIN 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. The falsified products including the API were plotted on the PCA score plot suggesting those tablets include around 60% assay of the dose (16 mg) displayed in the package. This result is agreement with the result of the assay measured by high performance liquid chromatography. In addition, both SFs contained almost the same amount of API, despite being labelled on the packages as having 8 mg and 16 mg, respectively, suggesting that these were falsified products with poor quality control.

Figure 2.7 shows 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 in the wave number region of 1200 to 1000 cm^{-1}. PC3 appeared to be mainly due to lactose factor.

2.4   DISCUSSION

Candesartan cilexetil tablets distributed in China, Indonesia and Myanmar were made by various manufacturers and contained different kinds of excipients. Testing identified a number of samples with unacceptable API contents above or below the criterion limits of the Japanese pharmacopeia, and others with excessive dissolution delay. Several products which meet the criteria of the assay, CU and dissolution were plotted in the center on the PCA plot. The failed samples which did not meet the criteria were all located far from the center position on the PCA score plot. This PCA model was very effective to distinguish the products include the different excipients, which appeared in different regions of the score plot and itwas able to explain all spectra clearly with two components of the concentration of the medicinal ingredient and the kinds of the excipients. The PCA result decomposed spectrum reflected the elements of pure Raman spectrum on PC 2 without interference by background of strong florescence substances. In this study, with an appropriate spectral preprocessing and PCA combination, even in market research using a large amount of Raman spectrum of various kinds of tablets including some unknown excipients, the elements of the API and the kinds of the excipients are clearly extracted, and the similarity and correlation are clearly visualized.

A key feature of the present work was the use of the MSC method for Raman signal preprocessing. This method proved to be more effective than other commonly used methods, such as the second derivative and standard normal variate methods, for extracting the desired signals from the strong fluorescence background. It was found that how to extract the chemical information itself from the spectrum of the spectroscopic, not the experimental devices and methods, is a significant powerful and effective solution for detecting SFs. These results suggest that the handheld Raman device we used could be a useful tool to detect SFs in the field, despite its relatively low sensitivity and low resolution.

In conclusion, the combination of pharmacopeial quality control tests and PCA score plots calculated from Raman spectra proved to be very effective methodology for detecting SFs. Application of this approach to candesartan cilexetil tablets collected in several Asian countries uncovered a number of examples of out-of-specification content and inadequate dissolution. The handheld Raman device is expected to be useful in field surveys to detect SFs. PCA of that Raman data clarify the difference in chemical properties between good quality products and SFs that circulate in the Asian market.

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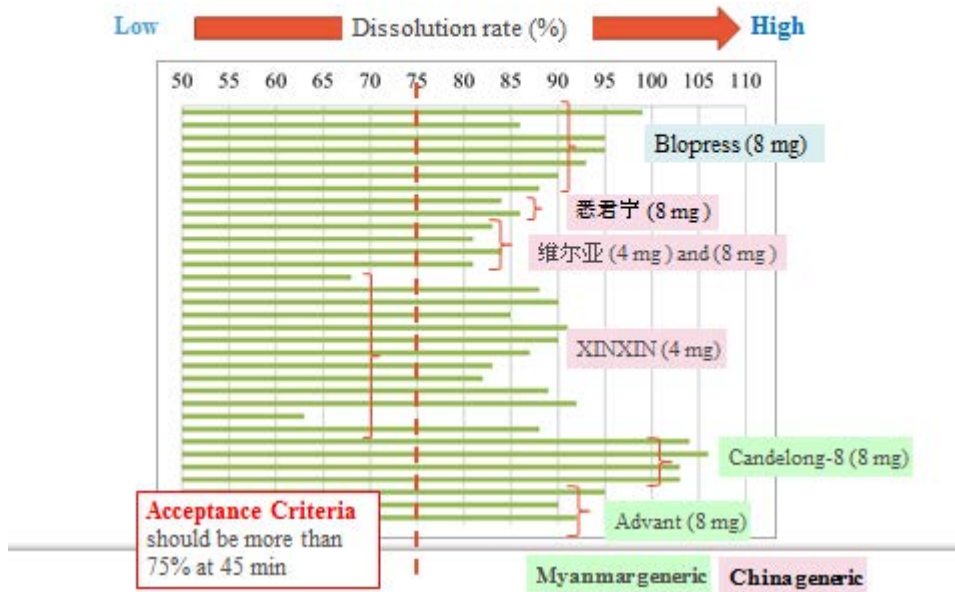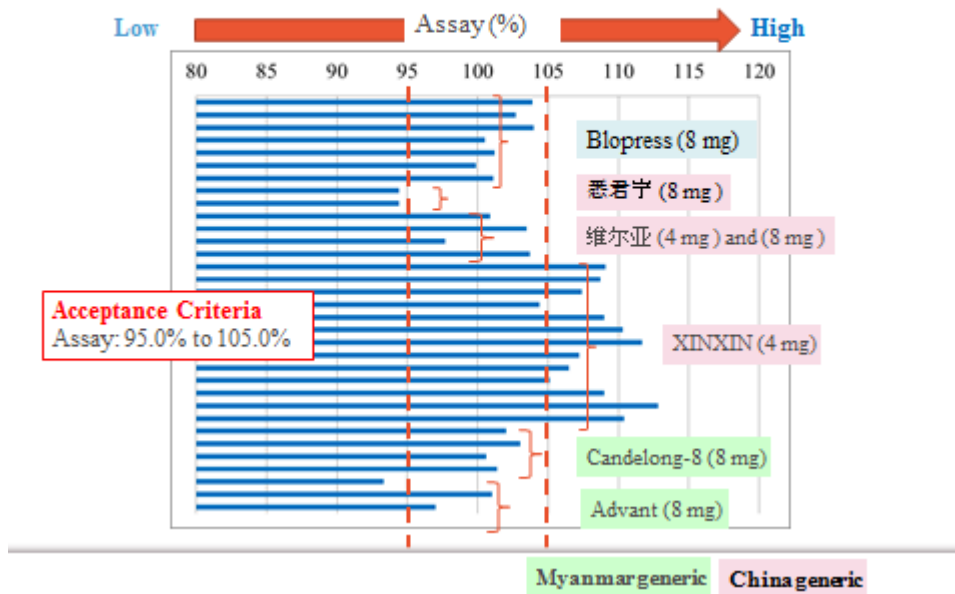
Figure 2.1.   Dissolution result of candesartan cilexetil tablets.

Figure 2.2.   Content uniformity and assay results of candesartan cilexetil tablets.

Table 2.1   Summary of the results of dissolution, assay, content uniformity and chemical

similarity of candesartan cilexextil tablets.

| No.	Product Name	Dose (mg)	Dissolution				Assay				Chemical Similarity by Raman spectra
			Mean of dissolution rate (%)	Standard deviation	Number of tablets	Judgment	Mean of content (%)	Standard deviation	Number of tablets	Judgment	
1	BLOPRESS	8	99	1.9	3	Pass	103.9	0.4	3	Pass	0.7159
2	BLOPRESS	8	86	0.6	3	Pass	102.7	3.3	3	Pass	0.5689
3	BLOPRESS	8	95	0.4	3	Pass	104.0	0.4	3	Pass	0.6546
4	BLOPRESS	8	95	0.4	3	Pass	100.5	0.1	3	Pass	0.6914
5	BLOPRESS	8	93	2.2	2	Pass	101.2	0.6	3	Pass	0.6763
6	BLOPRESS	8	90	1.8	3	Pass	99.9	0.7	3	Pass	0.5910
7	BLOPRESS	8	88	1.3	3	Pass	101.1	1.7	3	Pass	0.6158
8	悉君宁	4	84	0.6	3	Pass	94.4	2.2	3	Fail	0.0000
9	悉君宁	4	86	3.1	3	Pass	94.4	2.1	3	Fail	0.0000
10	维尔业	8	83	1.5	3	Pass	100.9	1.5	3	Pass	0.0000
11	维尔业	8	81	4.1	3	Pass	103.5	4.8	3	Pass	0.0000
12	维尔业	4	84	0.2	3	Pass	97.7	0.6	3	Pass	0.1483
13	维尔业	8	81	4.0	3	Pass	103.7	0.8	3	Pass	0.0000
14	XINXIN	4	68	15.9	3	Fail	109.1	1.6	3	Fail	0.0000
15	XINXIN	4	88	3.8	3	Pass	108.7	1.8	3	Fail	0.0000
16	XINXIN	4	90	4.1	3	Pass	107.4	2.1	3	Fai	0.0000
17	XINXIN	4	85	1.0	3	Pass	104.4	2.5	3	Pass	0.0000
18	XINXIN	4	91	0.2	3	Pass	109.0	2.7	3	Fail	0.0000
19	XINXIN	4	90	4.4	3	Pass	110.3	0.4	3	Fail	0.0000
20	XINXIN	4	87	4.4	3	Pass	111.7	1.2	3	Fail	0.0000
21	XINXIN	4	83	1.2	3	Pass	107.2	4.8	3	Fail	0.0000
22	XINXIN	4	82	1.2	3	Pass	106.5	2.5	3	Fail	0.0000
23	XINXIN	4	89	3.1	3	Pass	105.1	1.8	3	Fail	0.0000
24	XINXIN	4	92	0.1	3	Pass	109.0	1.1	3	Fail	0.0000
25	XINXIN	4	63	13.3	3	Fail	112.8	4.0	3	Fail	0.0000
26	XINXIN	4	88	0.9	3	Pass	110.4	1.8	3	Fail	0.0000
27	Candelong-8	8	104	0.4	6	Pass	102.0	1.2	10	Pass	0.0337
28	Candelong-8	8	106	0.2	6	Pass	103.0	0.3	2	Pass	0.0969
29	Candelong-8	8	103	2.9	6	Pass	100.6	2.2	10	Pass	0.0457
30	Candelong-8	8	103	1.1	6	Pass	101.4	2.1	10	Pass	0.0302
31	Advant	8	95	2.9	6	Pass	93.3	3.8	10	Fail	0.1725
32	Advant	8	90	0.1	6	Pass	101.0	1.9	2	Pass	0.2416
33	Advant	8	92	1.8	6	Pass	97.0	3.0	10	Pass	0.1421
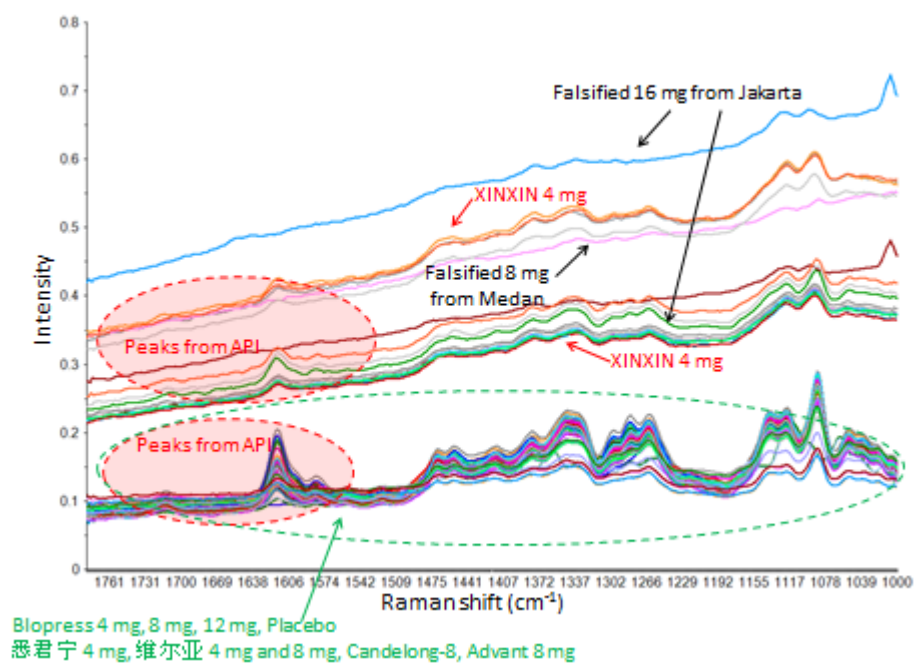
Figure 2.3. Raman spectra of authentic candesartan cilexetil tablets and SFs.

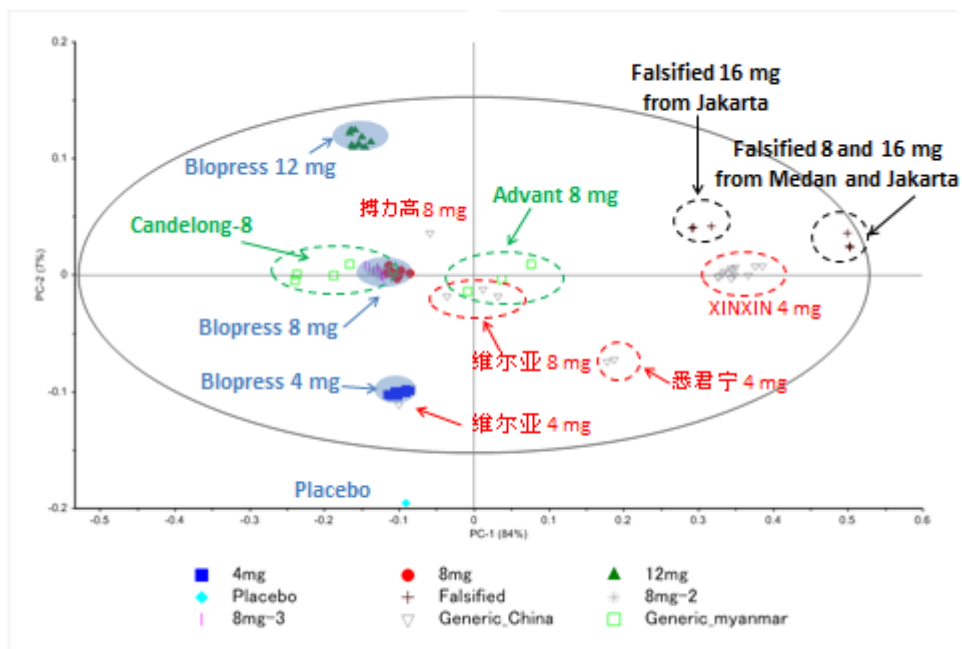(Before MSC preprocessing of spectra)
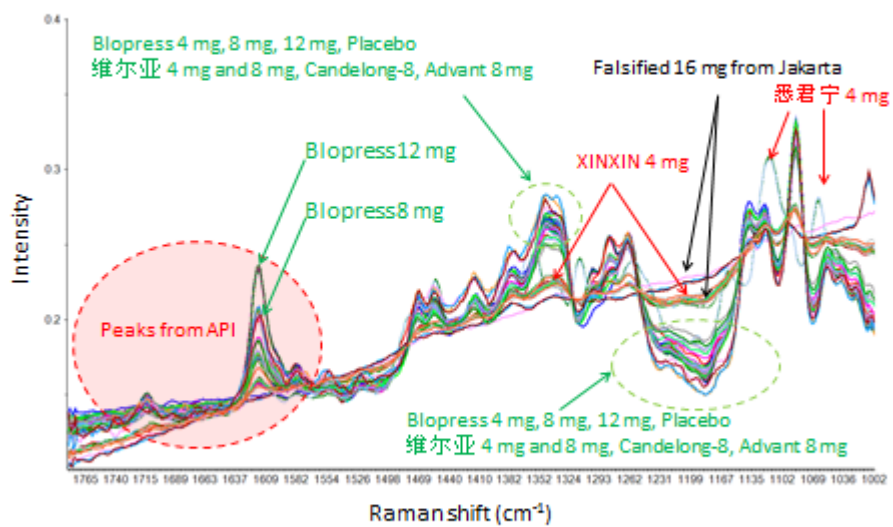
**Figure 2.4.**   Raman spectra of authentic candesartan cilexetil tablets and SFs.

(After MSC preprocessing of spectra)
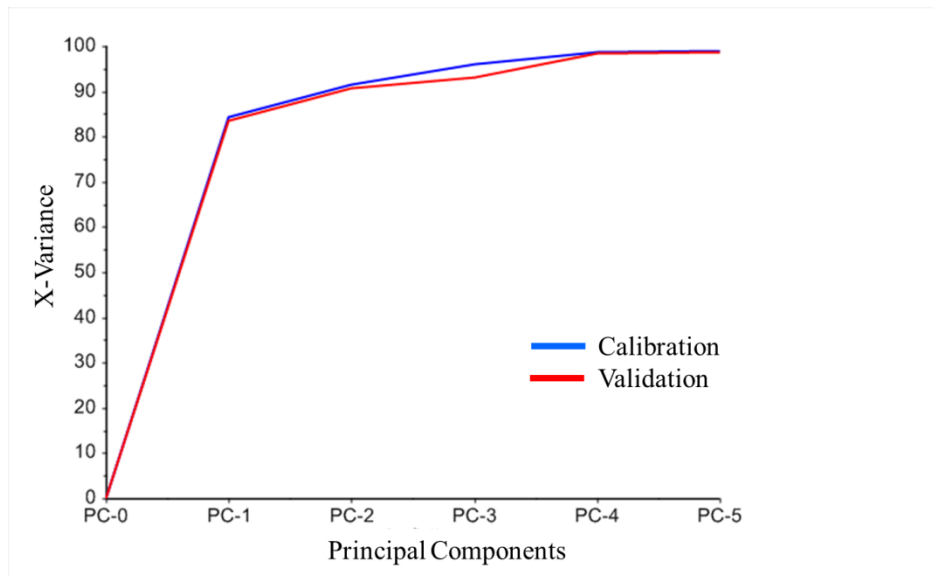
Figure 2.5. Comparison between the calibration and validation result in PCA model.

Figure 2.6. PCA score plot derived from the Raman spectra of candesartan cilexetil tablets,

including falsified tablets, collected in China, Indonesia, Japan and Myanmar.
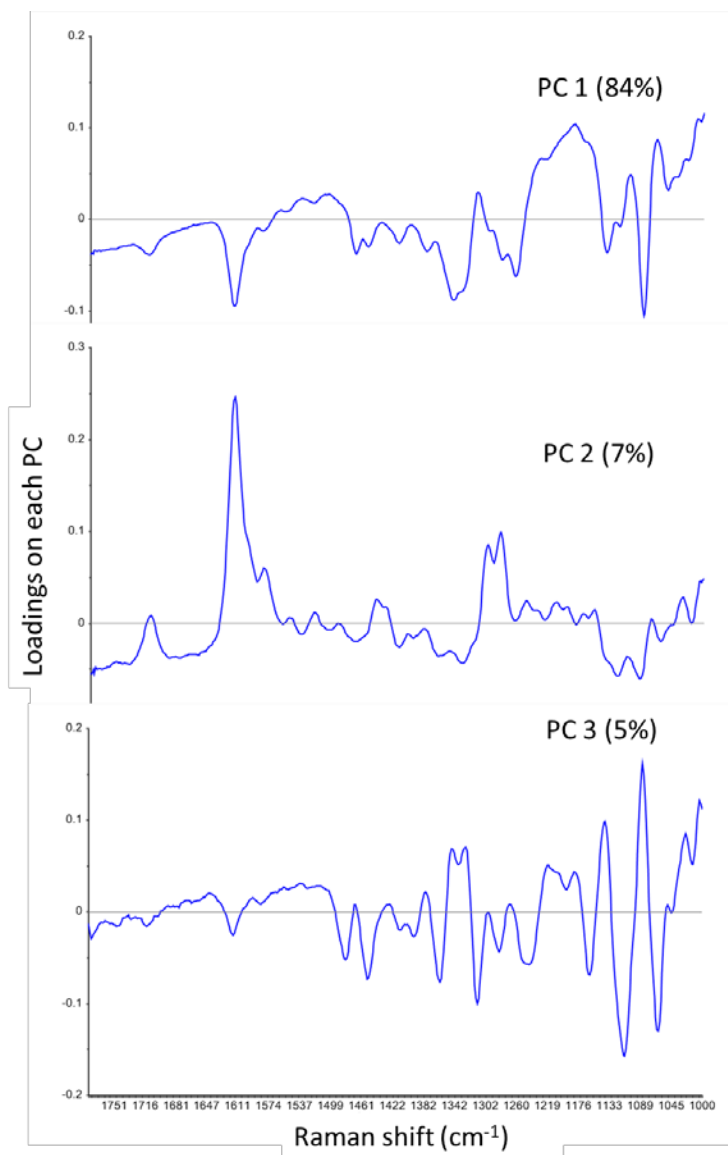
Figure 2.7. Loading on PC1, PC2 and PC3 in the PCA model calculated

by using Raman spectra of candesartan cilexetil tablets.

2.6   CHALLENGES FOR FUTURE SF RESEARCH

In addition, it is necessary to consider the effectiveness of the following means as a way of future investigation method of SF. Not only the results of physicochemical experiments such as quality tests and spectroscopy of each sample but also information on the collected samples (product name, active ingredient name, API dose, dosage form, package, manufacturer, country of manufacture, Product information such as lot number, manufacturing date, expiration date, packing date, packing etc), confirm the validity of the import manufacturer registration number by the manufacturer, check the validity of the import registration number, It is also considered to be one of the useful means to check whether the sample is permitted to sell on the market based on the result of the test. In addition to the results of physicochemical experiments, it is expected to extract the characteristic elements of SF from a lot of information from manufacturers and authorities.

Chapter III

Development and Application of Speedy and in-situ 3D Fluorescence Method to

Detect Substandard and Falsified Parenteral Products

3.1 INTRODUCTION

The anlytical methods for the oral solid form are described in Chpater 1 and 2, but Chpater 3 shows the analytical methods of the falsified medicines for the injectable formulation. The falsified medicines for the injections have serious risks of the health hazards because the API enter directly into the blood. 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 a poor environment lead to serious health damage, it is necessary to quickly detect the falsified products and to take measure.

3.1.1 Distribution of Falsified Avastin in U.S.

The U.S. Food and Drug Administration (FDA) warns medical staff and patients about the distribution of the falsified medical products imitating of Avastin (400 mg / 16 mL).[1] Avastin is an anticancer drug for injection binding to protein of VEGF (Vascular endothelial growth factor) secreted by cancer cells and inhibiting the neovascularization. The falsified products of the Avastin did not include bevacizumab of the active pharmaceutical ingredient (API). The FDA has announced that the falsified products of the Avastin were used for 19 medical practices in the United States and patients were unable to access the necessary health treatment. Those falsified products were labeled with Rosh as manufacture, but the FDA-approved version

of Avastin for use in the United States is marketed by Genentech only. Roche does not manufacture for the United States market.

3.1.2   Distribution of Falsified Herceptin in EU

European Medicines Agency (EMA) alerted that vials of Herceptin injection (trastuzumab) for cancer treatment, which has been stolen from hospital in Italy, were illegally used and flowed into the pharmaceutical supply chain. The medical personnel throughout the European Union (EU) are notified about the characteristic information of counterfeited Herceptin vials. The batch number and expiration date stated in the vials of these falsified products did not match the indication of the batch number and expiration date on the package on the outside. Further, it is pointed out that rubber plugs, crimp caps or lids have also been tampered with.

3.1.3   Evaluation of API in injectable products by Spectrofluorophotometer

In the actual incident which are shown in above,, the imitated or stolen glass vial and packaging as same as those of the authentic products were used. Further, the injections are often clear liquids and it is difficult to visually identify the contents and to detect the falsified medicines easily. We 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.

3.1.4   Excitation-Emission Matrices Method

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 as shown in Figure 3.1. A method for measuring such excited fluorescent three-dimensional matrix is called the EEMs (Excitation-Emission Matrices) method.[4] To identify the API by EEMs method has high specificity based on the three-dimensional structure of the API in solution which was

affected by the concentration of the API, buffer solution, excipients, pH, viscosity and temperature in the injection.[5-8]

3.2   EXPERIMENTAL

3.2.1   Principle and Advantage of Fluorescence Measurement

A molecular electronic transition to excited states is induced by the irradiation of light with a wavelength in the ultraviolet and visible region. The molecular emits the fluorescence when it comes back to the ground state.[3] As some advantages applying fluorescence measurement, the following points are mainly mentioned. 1) Possible to irradiate wide range of light from visible light to ultraviolet light. 2) Compounds emitting fluorescence have specific fluorescence intensities for specific excitation wavelengths. This phenomenon is a characteristic value derived from the three-dimensional structure of each compound. 3) Detection sensitivity is two or three orders better comparing UV method.[3] In this study, spectrofluorometor (F-7000, HITACHI, Japan) was used for the evaluation of API in the injectable products. The model formulations including fluorescence API compound in parenteral products for the small molecule, the antibody, and the peptide injection were measured in the following EEMs method.

3.2.2   Fluorescence Excitation-Emission matrices (EEMs) method[4-5]

The EEMs data were obtained by using the spectrofluorometor (F-7000, HITACHI, Japan) in the following conditions. At the photomal voltage of 600 V, the sample was irradiated with excitation light of the wavelengths of 200 nm to 600 nm (or 400 nm) and the emitted fluorescence intensity was scanned in the region of 200 nm to 700 nm with the scan speed at 60000 nm/min. The slit range of 5 nm was applied for the both the irradiation and the detection. By plotting the excitation wavelength on the vertical axis, the detected fluorescence wavelength on the horizontal axis, and the fluorescence intensity in the height direction, it is possible to draw a three-dimensional contour diagram as shown in Figure 3.1.

3.2.3 Conventional Fluorescence Measurement vs New Approach

In the conventional fluorescence measurements, the test solution to be measured had to be transferred to the cell from glass vial or ampoule to a quartz cell and it is measured in the fluoro-photometer as shown in Figure 3.2. This method is not able to measure each container without destroying the sample as the evidence, and it is difficult to analyze with a plurality of measurement methods quickly. Here, we removed the cell unit and make open space at the position where the direction of the irradiation light and the direction in which the light enters the fluorescence detector are orthogonal as shown in Figure 3.3.

3.3 RESULTS

3.3.1   Identification of API in injectable product by EEMs

Figure 3.4 shows the result of EEMs measured by using spectrofluorometor for 3 kinds of API (peptide, small molecule and antibody) in the injectable products. the comparison between placebo solution without API and the DP with API. 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.

3.3.2   New Approach without Cell Unit

This new approach as shown in Figure 3.3 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.

3.4   DISCUSSION

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.

We 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.

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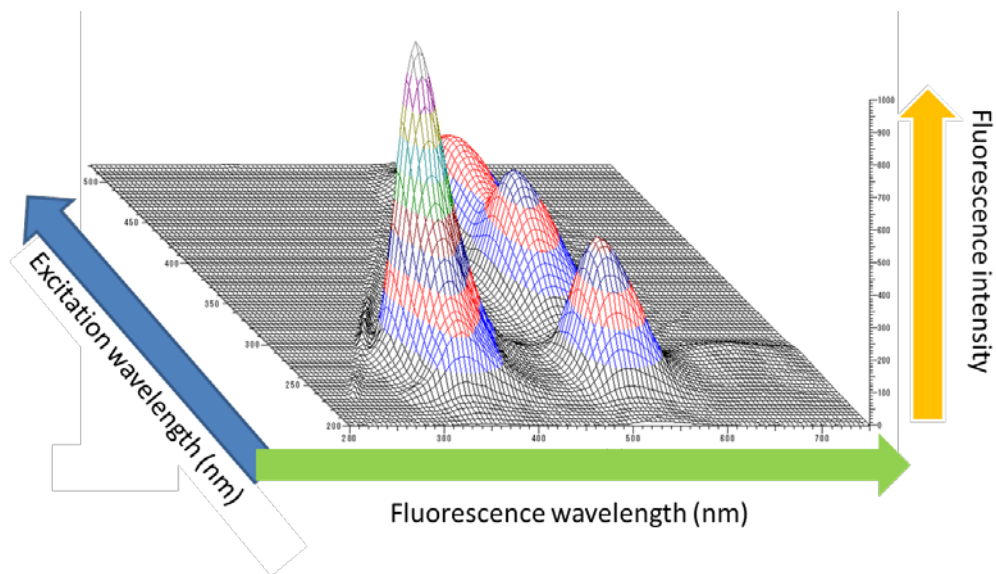
Figure 3.1. EEMs of injectable products including peptide and vitamin.
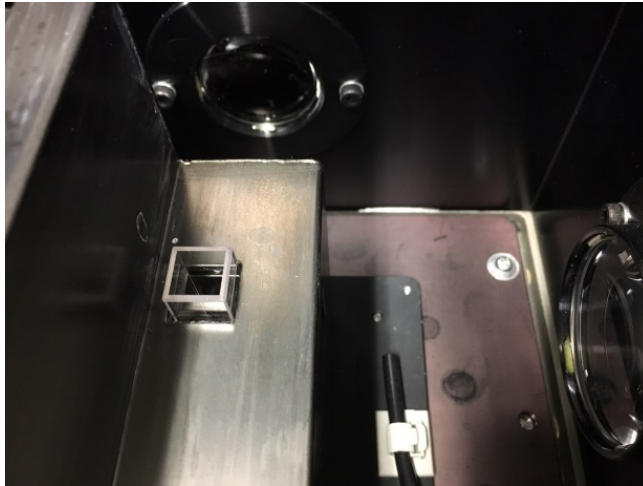
Figure 3.2.   Conventional fluorescence measurement cell unit.
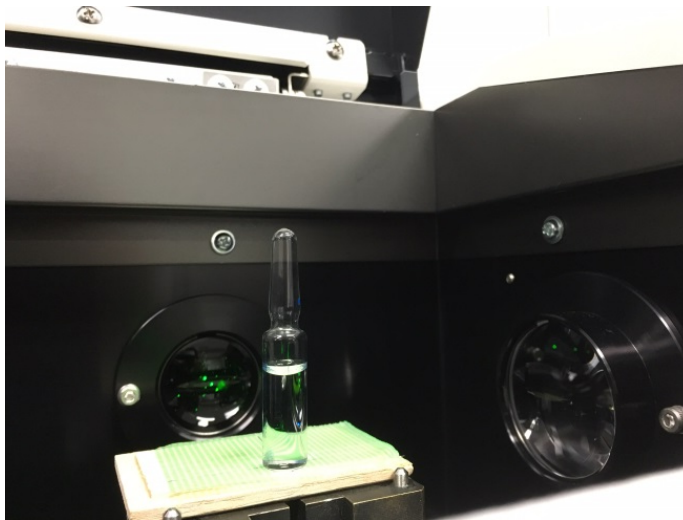
Figure 3.3.   New approach to measure the fluorescence compound in parenteral product

from the outside of the glass ampoule.
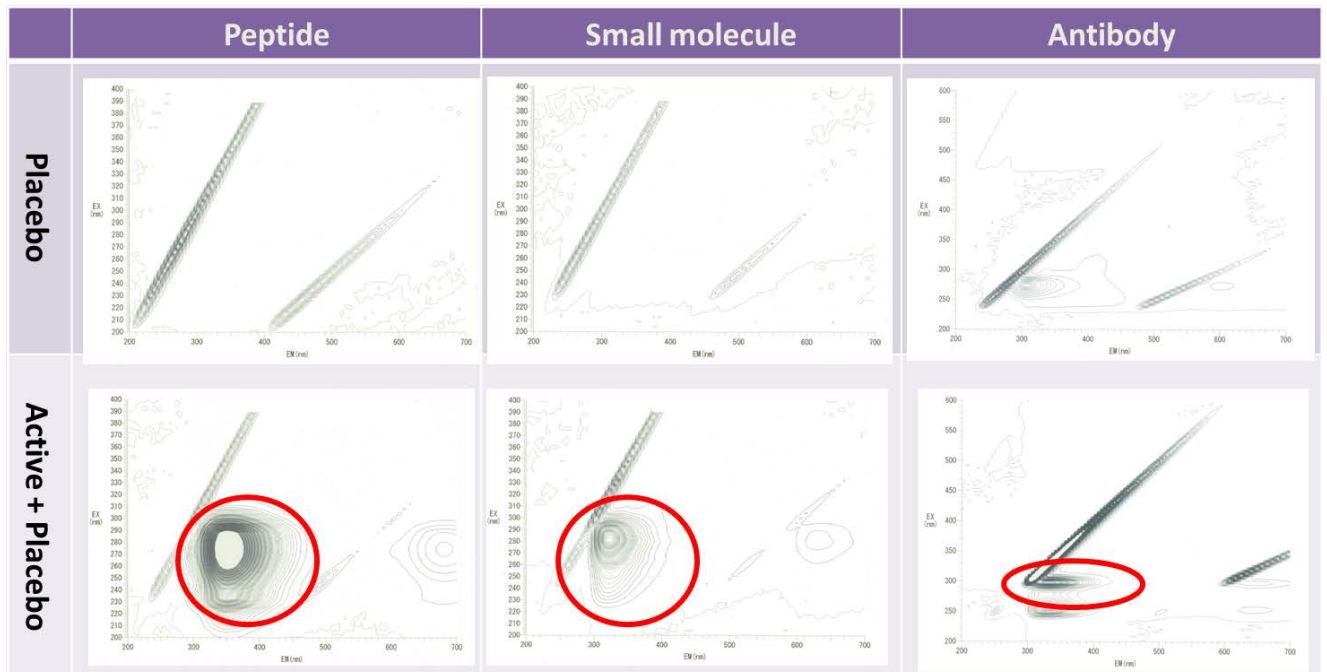
Figure 3.4. EEMs data of peptide, small molecule and antibody in injectable products.

Conclusion and summary

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.

In chapter I, it was showed 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. And the decision tree was presented for the judgment of authenticity of Blopress tablets. In chapter II, the results of the survey on the substandard and falsified products of hypertension medical products collected in Asian marked were shown. This survey was clarified about the existing of some substandard products by using the quality tests, the handheld Raman spectroscopy and PCA. Further, in chapter III, the method development and the application for the parenteral products were conducted for detecting the falsified medical products for injectable formulation with speedy and in-situ 3D fluorescence method. By combination method of the EEMs and the new approach to measure the sample from over the glass ampoule without operation to cut the glass ample and take out the contents, we successfully detected the API itself in injectable solutions with high specificity and sensitivity.

Through the chapter I to III, the discrimination methods of the substandard and falsified medical products for the solid formulation and injectable formulation were developed using the quality test and non-destructive spectroscopy. The application of the developed methods for the actual medical products showed that these methods have sufficient performance to detect speedy and in-situ SFs and to understand the physical and chemical properties and risks of the SFs which are distributing in the actual market. There methods are expected to contribute for the analysis of the medical products for people to access the appropriate health care.

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List of Papers (2014-2017)

Development of Quantitative Analysis for Polymorph of Drug Substances in Pharmaceutical Oral Dosage Forms by XRPD and Raman Spectroscopy

Tomoko Kakio, Takashi Hiroshima, Yukihiro Ikeda

Classification and Visualization of Physical and Chemical Properties of Falsified Medicines with Handheld Raman Spectroscopy and X-ray Computed Tomography

Tomoko Kakio, Naoko Yoshida, Susan Macha, Kazunobu Moriguchi, Takashi Hiroshima, Yukihiro Ikeda, Hirohito Tsuboi, Kazuko Kimura

Survey to Identify Substandard and Falsified Tablets in Several Asian Countries with Pharmacopeial Quality Control Tests and Principal Component Analysis of Handheld Raman Spectroscopy

Tomoko Kakio, Hitomi Nagase, Takashi Takaoka, Naoko Yoshida, Kaoru Sumitomo, Junichi Hirakawa, Susan Macha, Takashi Hiroshima, Yukihiro Ikeda, Hirohito Tsuboi, Kazuko Kimura