Detection of Sulfonamide Residues by Paper Spray Mass Spectrometry

Mr. Surased Suraritdechachai

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2018
Copyright of Chulalongkorn University

การตรวจวัดสารตกค้างกลุ่มซัลโฟนาไมด์ด้วยเปเปอร์สเปรย์แมสสเปกโทรเมตรี

นายสุรเศรษฐ์ สุรฤทธิ์เดชาชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

Thesis Title   Detection of Sulfonamide Residues by Paper Spray Mass Spectrometry

By   Mr. Surased Suraritdechachai

Field of Study   Chemistry

Thesis Advisor   Assistant Professor Dr. THANIT PRANEENARARAT

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.... Dean of the Faculty of Science

(Professor Dr. POLKIT SANGVANICH)

THESIS COMMITTEE

.... Chairman

(Associate Professor Dr. VUDHICHAI PARASUK)

.... Thesis Advisor

(Assistant Professor Dr. THANIT PRANEENARARAT)

.... Examiner

(Assistant Professor Dr. Puttaruksa Varanusupakul)

.... External Examiner

(Assistant Professor Dr. Nakorn Niamnont)

สุรเศรษฐ์ สุรฤทธิ์เดชาชัย : การตรวจวัดสารตกค้างกลุ่มซัลโฟนาไมด์ด้วยเปเปอร์สเปรย์แมสสเปกโทรเมตรี. (Detection of Sulfonamide Residues by Paper Spray Mass Spectrometry) อ.ที่ปรึกษาหลัก : ผศ. ดร.ธนิษฐ์ ปราณีนรารัตน์

ยาปฏิชีวนะกลุ่มซัลโฟนาไมด์เป็นยาสัตว์ที่ใช้กันทั่วไปในปศุสัตว์เพื่อป้องกันหรือรักษาโรคติดเชื้อ การตกค้างของสารเหล่านี้ในอาหารเป็นประเด็นสำคัญเนื่องจากสามารถก่อให้เกิดผลเสียต่อสุขภาพและการพัฒนาของแบคทีเรียดื้อยา ในงานวิจัยนี้ได้พัฒนาวิธีสำหรับตรวจวัดและหาปริมาณซัลฟาเมทาซีนซึ่งเป็นหนึ่งในยาปฏิชีวนะกลุ่มซัลโฟนาไมด์ในตัวอย่างของเหลวจากร่างกายหมูโดยใช้การสกัดที่เรียบง่ายและเปเปอร์สเปรย์แมสสเปกโทรเมตรี วิธีที่ได้มีขั้นตอนการเตรียมตัวอย่างที่น้อยและใช้ระยะเวลาวิเคราะห์สั้น ขณะที่มีความไวและความแม่นยำในการตรวจวัดสูงทั้งในตัวอย่างเลือดหมู (ขีดจำกัดของการตรวจวัด = 10.1 ng/mL, ร้อยละการได้กลับคืน = 95.4-104.1%) ซีรัมหมู (ขีดจำกัดของการตรวจวัด = 4.6 ng/mL, ร้อยละการได้กลับคืน = 103.2-105.4%) และปัสสาวะเทียม (ขีดจำกัดของการตรวจวัด = 3.5 ng/mL, ร้อยละการได้กลับคืน = 99.0-103.4%) จากความสัมพันธ์ระหว่างระดับของซัลฟาเมทาซีนในของเหลวในร่างกายกับเนื้อหมูซึ่งทราบกันอยู่แล้ว วิธีนี้สามารถนำไปใช้ตรวจสอบหายาตกค้างอย่างรวดเร็ว ซึ่งจะเป็นประโยชน์สำหรับการจัดการตัดสินใจในอุตสาหกรรมปศุสัตว์

สาขาวิชา	เคมี	ลายมือชื่อนิสิต ....
ปีการศึกษา	2561	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

6072007023 : MAJOR CHEMISTRY

KEYWORD: sulfonamides, paper spray, mass spectrometry

Surased Suraritdechachai : Detection of Sulfonamide Residues by Paper Spray Mass Spectrometry. Advisor: Asst. Prof. Dr. THANIT PRANEENARARAT

Sulfonamide antibiotics are commonly used veterinary drugs in livestock to prevent or treat infectious diseases. Their residues in food are of concern since they can cause adverse health effects and development of bacterial resistance. In this work, a practical method for detection and quantification of sulfamethazine, one of sulfonamide antibiotics, in pig body fluid samples is developed via the combination of simple extraction and paper spray mass spectrometry. The method required minimal sample preparation and low analysis time while providing high sensitivity and accuracies in pig whole blood (LOD = 10.1 ng/mL, %recovery = 95.4-104.1%), pig serum (LOD = 4.6 ng/mL, %recovery = 103.2-105.4%), and synthetic urine (LOD = 3.5 ng/mL, %recovery = 99.0-103.4%). From previously known correlation between the level of sulfamethazine in pig body fluids and edible tissue, this method could be used for rapid screening of drug residue which can aid the managerial decision in the livestock industry.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Field of Study:	Chemistry	Student's Signature	....
Academic Year:	2018	Advisor's Signature	....

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

LIST OF FIGURES

LIST OF TABLES

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

LIST OF ABBREVIATIONS

CE	Collision energy
CID	Collision-induced dissociation
DESI	Desorption electrospray ionization
ESI	Electrospray ionization
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
FA	Formic acid
GC	Gas chromatography
HPLC-FLD	High-performance liquid chromatography-fluorescence detector
HPLC-UV	High-performance liquid chromatography-ultraviolet detector
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MeCN	Acetonitrile
MeOH	Methanol
MRL	Maximum residue limit
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
PABA	*p*-aminobenzoic acid
PS-MS	Paper spray mass spectrometry
SPE	Solid phase extraction
SRM	Selected reaction monitoring

CHAPTER I

INTRODUCTION

1.1 Veterinary drugs

Veterinary drugs have been widely used in livestock industry for prevention and treatment of animal diseases. There are several classes of veterinary drugs such as antibiotics, anti-inflammatory drugs, antiparasitic drugs, sedatives and so on. Excessive or improper uses of these drugs can cause drug accumulation in animals and lead to unwanted residues in products from these animals such as tissue, egg, and milk. The drug residues in foods can have negative impacts on consumer health either from drug side effects or drug allergies.[1] Therefore, the drug residues have become a great concern in food safety. There are several measures to control the drug usage and maximum residue limit (MRL) of various veterinary drugs have been established.

1.2 Sulfonamide antibiotics

Sulfonamide antibiotics is a class of synthetic drugs based on a parent compound 4-aminobenzenesulfonamide or sulfanilamide. Its derivatives are typically based on the sulfanilamide with different substituents at the nitrogen of the sulfonamide group such as sulfacetamide, sulfamethazole, sulfadiazine (**Figure 1.1**).
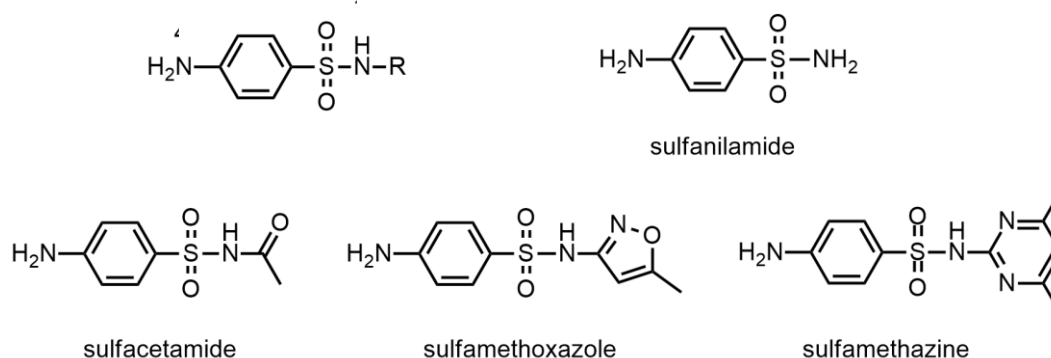
Figure 1.1 General structure of sulfonamide antibiotics and structure of sulfanilamide and some examples of sulfonamide drugs.

Sulfonamide antibiotics inhibit bacterial growth by interfering with the nucleic acid synthesis of these organisms. This is possible due to the structural similarity of these compounds to that of *p*-aminobenzoic acid (PABA). PABA is a substrate for the synthesis of the coenzyme tetrahydrofolic acid, which is crucial for bacterial nucleic acids production. Sulfonamides compete with PABA and thus inhibit the tetrahydrofolic acid synthesis (**Figure 1.2**). Therefore, the growth of bacteria is suppressed.
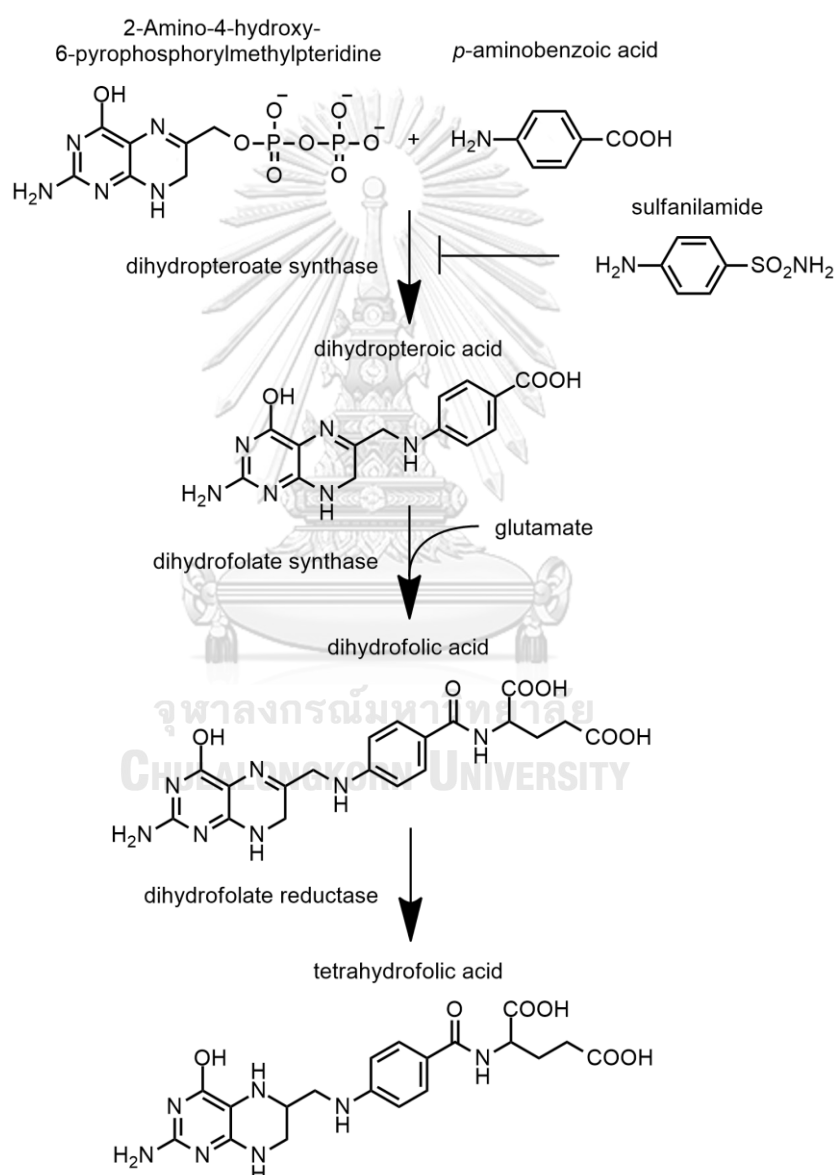
Figure 1.2 Synthesis pathway of tetrahydrofolic acid and inhibition by sulfanilamide

Sulfonamide antibiotics generally have a broad spectrum of activities against bacteria and some microorganisms.[2] As veterinary drugs, sulfonamides are used for prophylactic and therapeutic purposes and can also be used as growth-promoters.[1,3] Residue of sulfonamides in food are of concern because they can cause allergic reaction in consumers and some of them could be carcinogenic.[3-4] Sulfonamides are relatively stable. Their residues can also accumulate in environment such as soil or natural water. Residues in food and environment could induce bacterial resistance which are a great health concern as well. As a consequence, an MRL of sulfonamides has been established by many regulatory organizations around the world. For example, the MRL for EU is set at 100 ng/g in all kinds of tissues.[1,3-4] Thailand, which has a relatively mature swine industry and is a growing global exporter of pork, also set the MRL of 100 ng/g, as imposed by the Thai Food and Drug Administration.[5]

1.3 Conventional detection techniques

Various detection methods for veterinary drug residues have been developed. Most of the conventional techniques were based on chromatographic separation due to the complexity of the samples such as gas chromatography (GC) and liquid chromatography (LC). GC is suitable for volatile and thermally stable compounds. However, veterinary drugs are typically non-volatile and then require derivatization to increase the volatility. For example, the derivatization of sulfonamides with azomethane and trifluoroacetic anhydrides for GC analysis have been reported (**Figure 1.3**).[6-7]
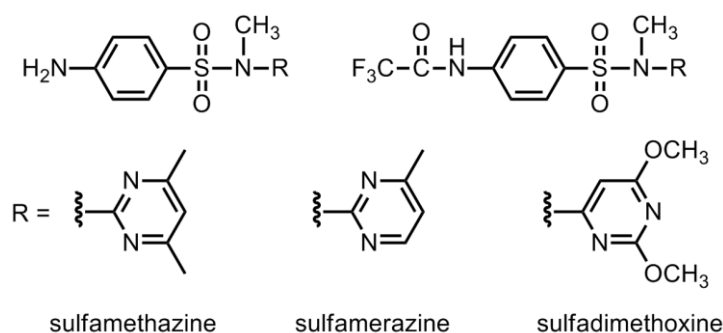
Figure 1.3 Examples of sulfonamide derivatives for GC analysis

LC techniques have gained more interest in detection and quantification of various veterinary drugs. It is more suitable for the analysis of polar non-volatile compounds. Several type of detectors can be used, such as, high-performance liquid chromatography-ultraviolet detector (HPLC-UV)[8-11], high-performance liquid chromatography-fluorescence detector (HPLC-FLD)[12-14], liquid chromatography-mass spectrometry (LC-MS)[15-16], and liquid chromatography-tandem mass spectrometry (LC-MS/MS).[17-20]

1.4 Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) is a technique using multiple steps of mass separation with fragmentation of ions of interest. Typically, an analyte ion, called parent ion, is selected by one mass analyzer and undergo fragmentation into smaller ions called product ions or daughter ions. Another mass separating step analyzed the generated ions and sent then the selected ion to the detector. In some advance mass analyzers, the product ion may undergo the fragmentation step again to provide more structural information. Examples of common mass analyzers that can perform tandem mass spectrometry experiments include triple quadrupole, ion trap, and quadrupole-time-of-flight.

Liquid chromatography-tandem mass spectrometry is one of the widely accepted analytical techniques which can provide both sensitivity and specificity.[3-4] The multiple steps of mass selection can increase the specificity in the analysis, reduce interferences, and thus improve the sensitivity. Specific compounds can be confirmed by LC-MS/MS from its retention time, mass to charge ratio of both parent and product ions.

A triple quadrupole mass spectrometer consists of three quadrupoles (**Figure 1.4**). Two of them act as mass filters that separate the ions with desired *m/z* values. The other one is placed between the two quadrupoles and acts as a chamber for fragmentation called collision-induced dissociation (CID). A selected ion from the first quadrupole is accelerated and collides with unreactive gases such as N_2 and Ar in the collision cell. This resulted in bond cleavage of the parent ion into smaller fragments which are then analyzed by the third quadrupole and sent to the detector.
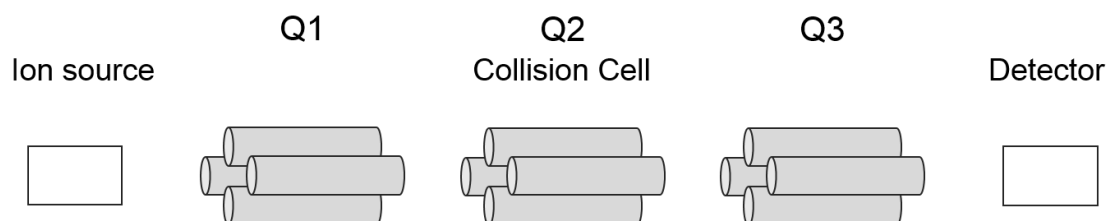
Figure 1.4 Diagram of triple quadrupole mass analyzer

With the triple quadrupole mass analyzer, quantification is usually operated in the selected reaction monitoring (SRM) mode. In this mode, only selected parent ion passed through the collision cell and only a few ranges of selected *m/z* values of the product ions were monitored. This allows rapid detection of ions with higher sensitivity than the full scan mode.

Although LC-MS/MS provides powerful sensitivity and specificity, intensive sample preparation and time-consuming chromatographic separation are required in case of complex matrixes. For example, Shao and co-workers[18] analyzed sulfonamide residues in porcine meat, liver, and kidney using extraction with acetonitrile, fat removal by hexane, reconstitution, and solid-phase extraction (SPE). The eluent was evaporated and reconstituted again for LC-MS/MS analysis. Kung and co-workers[20] developed a method for detection of sulfonamides in fish samples. The method used extraction with acetonitrile, centrifugation, dispersive SPE by C18 sorbent, and another centrifugation step. The supernatant was used for LC-MS/MS analysis.

1.5 Ambient mass spectrometry

Ambient ionization is a group of recently emerged techniques in which ionization of the analytes from sample occur under ambient condition with no or minimal sample preparation. The first ambient technique called desorption electrospray ionization (DESI) has been introduced in 2004.[21] After that, several ambient ionization techniques have been developed. This group of techniques directly ionize the samples in an open-air environment into the mass spectrometer. Thus, the analysis is simplified and the rapid screening of compounds with very low analysis time can be

performed since no chromatography is required. Several applications have been demonstrated such as drug monitoring, compound screening, and chemical imaging.[22]

1.6 Paper spray mass spectrometry (PS-MS)

In 2010, a novel ambient ionization called paper spray ionization has been developed to simplify the analysis.[23-24] In this method, a high voltage is applied to a triangle-shaped paper wetted with a solvent to generate an electrospray droplet (**Figure 1.5**). The charged droplets are generated as a result of the high electric field at the tip of the paper. The ions are then generated by solvent desolvation and passed into the mass spectrometer.[24] When the solvent is depleted, the electrospray process is stopped.
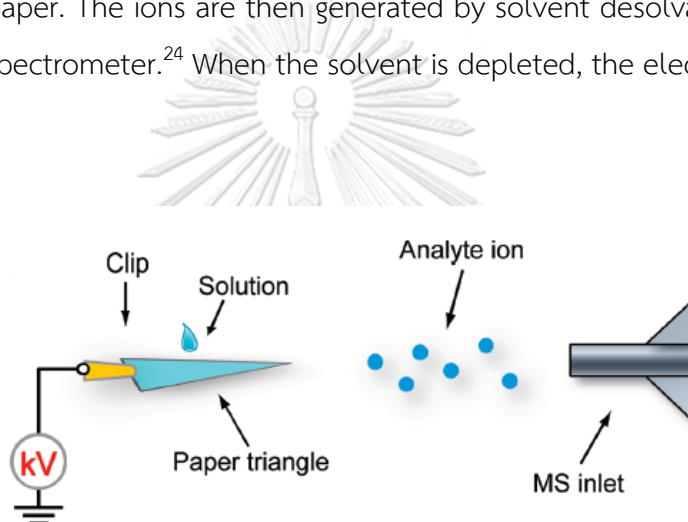
Figure 1.5 Illustration of paper spray mass spectrometry[23]

Many approaches of sample introduction are used for PS-MS including direct analysis of solutions containing the analyte, analysis from wiping the analyte from the sample surfaces, and analysis of dried sample spots containing the analyte on paper. Direct analysis of solution is simple and rapid. It is suitable for liquid samples with low matrix since the obtained results may be complicated when there are many interferences in the mixture.[24] The wiping method allows for collection of the analytes from solid surface. This approach has been used in analysis of agrochemicals from fruit and vegetable samples.[24-25] Analysis of dried sample spots separates the sample deposition and the ionization. This might reduce the interferences in the analysis of complex samples because the analyte could be extracted by the appropriate solvent whereas the interferences still retain on the paper.

Spray solvent affects not only the ionization but also the elution of the analyte. The elution of lipids in blood sample was investigated using silica coated paper as a substrate. The abundance of lipids was higher when using 50:50 MeOH/acetone compared to using pure MeOH.[26] This is due to the increased extraction of lipids with acetone in the solvent. The paper substrate also affects the analysis. The signal of hemoglobin in fresh blood sample was prominent when using paper modified with hydrophobic perfluoroalkyl group but could not be observed when normal hydrophilic chromatography paper were used.[26] This could be explained by the strong interaction between hemoglobin and the paper which led to poor elution of hemoglobin to the tip of paper.

Paper spray ionization has gained much interest because of the ease of sampling method and simple instrumentation. Paper is also a readily available material with low cost. In addition, the use of paper as a medium offers a relatively economical solution, which also creates a logistical advantage due to the ease of transporting large numbers of samples in the form of dry sheets of paper. Consequently, the technique has been used to detect various types of compounds ranging from small molecules to proteins such as dyes[27-29], pesticides[25], therapeutic drugs[23,30], illicit drugs[23], and veterinary drugs[27,31] in various complex matrixes such as blood[23-24,32] and foodstuffs.[27-29,33-34]

Food testing application can undoubtedly benefit from this technique. For example, PS-MS was employed for the analysis of 4-methylimidazole, a side product from caramel production (**Figure 1.6**).[33] This work demonstrated the compatibility of paper spray ionization to three different mass analyzers including triple quadrupole, ion trap, and orbitrap. The sample preparation was only the dilution of a caramel sample with water. The analysis time was 1.5 minute per sample.

Donna *et al*. reported the analytical method for resveratrol, an antioxidant found in red wine (**Figure 1.6**).[34] SPE was utilized to remove interferences in wine samples prior to the paper spray analysis. The limit of detection (LOD) value was 0.5 µg/mL with the analysis time of 2 minutes per sample.
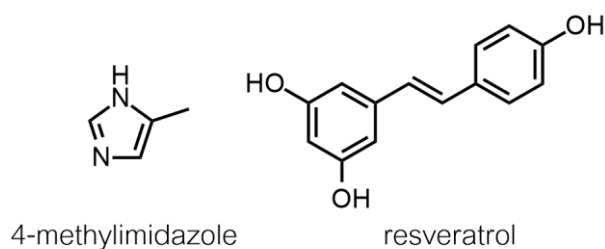
Figure 1.6 Structures of 4-methylimidazole and resveratrol

PS-MS were also applied for analysis of solid samples. For instance, Sudan dyes in powdered chili pepper were analyzed after extraction with 7:3 $CH_3CN/CHCl_3$ followed by centrifugation (**Figure 1.7**).[29] The supernatant was spotted onto the triangle paper for PS-MS analysis. The LOD values were 1 μg/g. The analysis time were 1 minute.
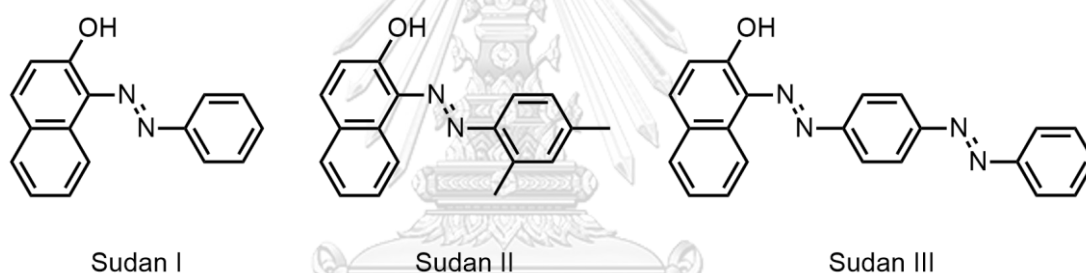
Figure 1.7 Structures of some Sudan azo dyes

Paper spray ionization was used for the detection of four β-agonists in pork and bovine muscle tissue (**Figure 1.8**).[27] The meat homogenate was spotted on the paper and dried before the analysis using 9:1 MeOH:water as the spray solvent. The LOD values were in a range of 1–5 ng/g. The analysis time was 1 minute per sample.
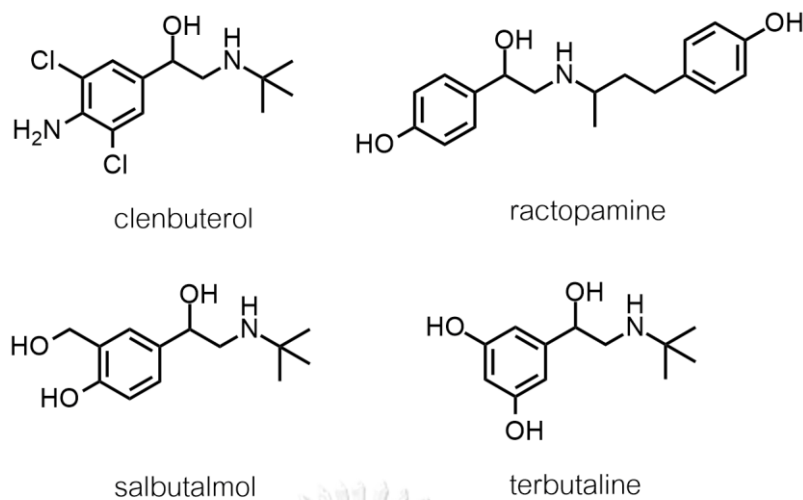
Figure 1.8 Structures of four β-agonists in the work of Zhang *et al.*

Su *et al.* developed a method for the detection of four groups of antibiotics in food samples including quinolones in chicken meat, macrolides in beef, β-lactams in milk, and tetracycline in egg white (**Figure 1.9**).[31] All compounds could be detected at the level equal to or below the established MRLs with the analysis time of 3 minutes.
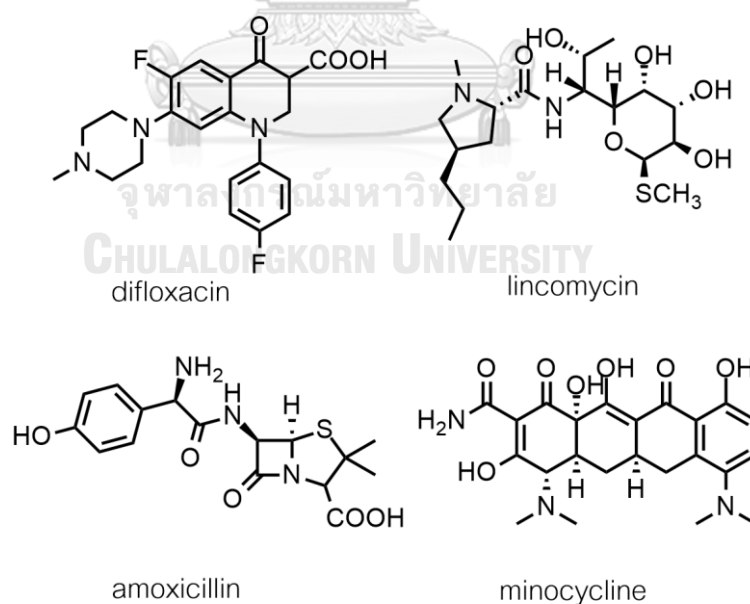
Figure 1.9 Selected antibiotics in group of quinolone, macrolide, β-lactam, and tetracycline in the work of Su *et al.*

Apart from the detection of drug residues in food, the determination of the residues in animal body is also important. There are several reports on the correlation between the amount of drugs in tissue and in body fluids.[35-37] These thus allow for an estimation of the drug content in foods by monitoring the level of the drug in the fluids when the animals are still alive. The obtained data is crucial since it helps determine whether the estimated drug level is under the regulatory limit or not. Thus, the approach prevents unnecessary loss of animals and occurrence of the non-compliant product.

To simplify the analysis of complex biological samples, in this work, a rapid PS-MS method for detection and quantification of sulfonamide residues in pig body fluids including pig whole blood and serum was developed. Sulfamethazine was selected as the analyte of interest since it is prohibited by Thai Food and Drug Administration.[5] Furthermore, synthetic urine was also used to test the applicability for analyzing the drug in real pig urine samples. The method could be useful for the nonlethal detection and monitoring of the drug in livestock industry.

CHAPTER II

EXPERIMENT

2.1 Chemicals

Sulfamethazine
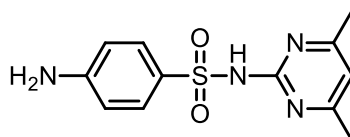
Sulfamethazine-d_4
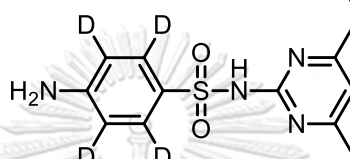
Urea
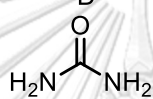
Creatinine
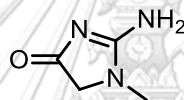
Acetonitrile (MeCN)

Diethyl ether (Et$_2$O)

Ethyl acetate (EtOAc)

Formic acid (FA)

Methanol (MeOH)

NaCl

KH$_2$PO$_4$

Na$_2$HPO$_4$ · 2H$_2$O

2.2 Samples

Pig blood and serum samples were provided by Charoen Pokphand Foods PCL (Thailand).

Synthetic urine was prepared with an adaptation from a previous paper.[38] The synthetic urine consists of 0.33 M urea, 0.12 M NaCl, 0.016 M KH$_2$PO$_4$, 0.004 M Na$_2$HPO$_4$ ·2H$_2$O, and 0.007 M creatinine dissolved in deionized water.

2.3 Triangular paper preparation

Whatman 1Chr chromatography paper was cut into isosceles triangular paper with 6 mm base width and 13 mm height by a commercial laser cutter. The obtained paper pieces were briefly cleansed by being immersed in deionized water with shaking at 240 rpm for 5 min twice and with MeOH for 5 min to remove any impurities followed by drying at room temperature.

2.4 Stock preparation

Stock solution of sulfamethazine was prepared by dissolving sulfamethazine in MeOH at 1 mg/mL. This stock solution was further diluted with MeOH to the desired concentration.

2.5 Sample preparation

Aliquot of 50 µL of sample was transferred to a microcentrifuge tube. 5 µL of 4 µg/mL sulfamethazine-d4 solution was added as an internal standard and mixed by vortexing. 100 µL of EtOAc was then added and mixed by vortexing for 1 minute followed by 0.5 minute of centrifugation using a Mini Centrifuge at 10000 rpm. After the extraction step, 3 µL of the upper EtOAc layer was spotted on the triangle paper. The paper was dried at room temperature for at least 30 minutes.

2.6 Mass spectrometric analysis

Mass spectrometric analysis was performed by using Thermo-scientific TSQ Quantum Ultra EMR mass spectrometer. TSQ Tune software was used for to control the mass spectrometer and the data acquisition while Xcalibur software was used to process the recorded data. The ionization was operated in the positive mode with the capillary temperature of 300 °C. The triangle paper was held by a copper clip at a distance of approximately 0.5 cm from the inlet of the mass spectrometer. A solvent of 20 µL was deposited onto the paper to elute the analyte. A voltage of 5 kV was then applied to the clip by a DC voltage power supply (3B scientific model U33010). The experimental setup is showed in **Figure 2.1**. The analysis time was 1.5 min. Fragmentation by collision-induced dissociation (CID) was done by using Argon gas

(Ultra high purity, Linde) as the collision gas at the pressure of 1.5 mTorr. Quantification was performed in the selected reaction monitoring (SRM) mode with the following parameters: scan width, 1.000; scan time, 0.100 s, Q1 peak width, 0.70; Q3 peak width, 0.50. Area under the curve for 1 min after applying the voltage was used for quantitation.

Ion selection and optimization for SRM parameters was performed by direct infusion and ionization using an electrospray ionization (ESI) source. The experiment setup for direct infusion is showed in **Figure 2.2**. The spray voltage was set at 3000 V. Nitrogen gas (High purity, Linde) was used as the sheath gas with the sheath gas pressure of 3 unit. The parent and product ion of the analytes, along with the optimized collision energy and tube lens voltage is presented in **Table 2.1**.

Figure 2.1 Experiment setup for paper spray mass spectrometry.
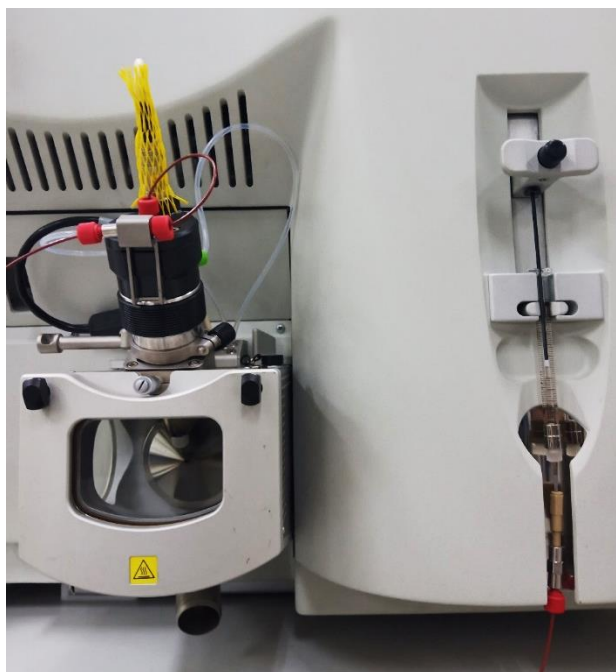
Figure 2.2 Experiment setup for direct infusion.

Table 2.1 Parent and product ions, tube lens voltages, and collision energy for sulfamethazine and sulfamethazine-d_4

Compound	parent ion m/z	product ion m/z	collision energy	tube lens voltage
sulfamethazine	279.0	124.2	21	91
		186.0*	17	91
sulfamethazine-d_4	283.0	124.2	22	92
		186.0*	17	92

*The selected product ions were used for quantification.

2.7 Preparation of calibration curves

Matrix-matched calibration curves for blood, serum, and synthetic urine were prepared as follows. The 195 µL of blank sample matrix was spiked with 5 µL of 20 µg/mL sulfamethazine solution. The obtained spiked sample at 500 ng/mL was serially diluted with the blank sample matrix to prepare the samples with lower concentration of sulfamethazine. The calibration range was 5, 10, 25, 50, 100, 200, 500 ng/mL. The sample preparation was then performed as described in the section 2.2.

The ratio of the signal from sulfamethazine to the signal from the internal standard was plotted against the analyte concentration. The calibration curves in the form of y = mx + c for each matrix were obtained from the linear regression. Limit of detection (LOD) and limit of quantitation (LOQ) values were calculated from 3.3 times and 10 times of the standard deviation of the intercept divided by the slope, respectively.

2.8 Recovery test

For recovery test, sulfamethazine solutions at 4, 8, 16 µg/mL in MeOH were prepared. A portion of 5 µL of each solutions was then added to 195 µL of the blank matrix sample to create samples with sulfamethazine concentration of 100, 200, and 400 µg/mL, respectively. Aliquots of 50 µL of the spiked samples were then used for the sample preparation as described in section 2.2. The %recovery value was calculated by the following equation.

$$\%recovery = \frac{found\ concentration}{spiked\ concentration} \times 100$$

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

CHAPTER III

RESULTS AND DISCUSSION

In this work, the method for sulfamethazine detection in pig fluid samples was developed. The experiments consisted of optimization of parameters of the mass spectrometer, method optimization for sulfamethazine detection in real samples, and evaluation of the developed method.

3.1 Direct infusion experiment

Standard solutions of sulfamethazine and sulfamethazine-d_4 in MeOH were infused to the mass spectrometer and ionized in the positive mode by the ESI source. As shown in **Figure 3.1**, the dominant ion of sulfamethazine was the protonated ion at m/z 279 ($[M+H]^+$). The less abundant metal adducts at 301 ($[M+Na]^+$) and 317 ($[M+K]^+$) m/z might be attributed to the trace metal ions in the solvent. The fragmentation patterns of sulfamethazine by CID were in agreement with those reported in the literatures.[17,20] The product ions at 156 ($[M-RNH_2]^+$), 108 ($[M-RNH_2-SO]^+$), and 92 ($[M-RNH_2-SO_2]^+$) m/z were common to the sulfonamide antibiotics. These ions came from the loss of the aromatic side chain, whereas the product ions at 186 ($[M+H-93]^+$) and 124 ($[M+H-155]^+$) m/z were characteristic ions of sulfamethazine from the loss of sulfonamide part.

The mass spectra of sulfamethazine-d_4 were almost identical to the spectra of sulfamethazine (**Figure 3.2**). The protonated ion at m/z 283 ($[M+H]^+$) was also the base peak of the mass spectrum with two less abundant metal adducts at 304 ($[M+Na]^+$), and 321 m/z ($[M+K]^+$). The fragmentation pattern of sulfamethazine-d_4 was quite similar to that form sulfamethazine. The characteristic ions were still present at 124 and 186 m/z since these ions did not contain any deuterium, while some fragment ions were shifted by 4 m/z unit due to the presence of 4 deuterium atoms.
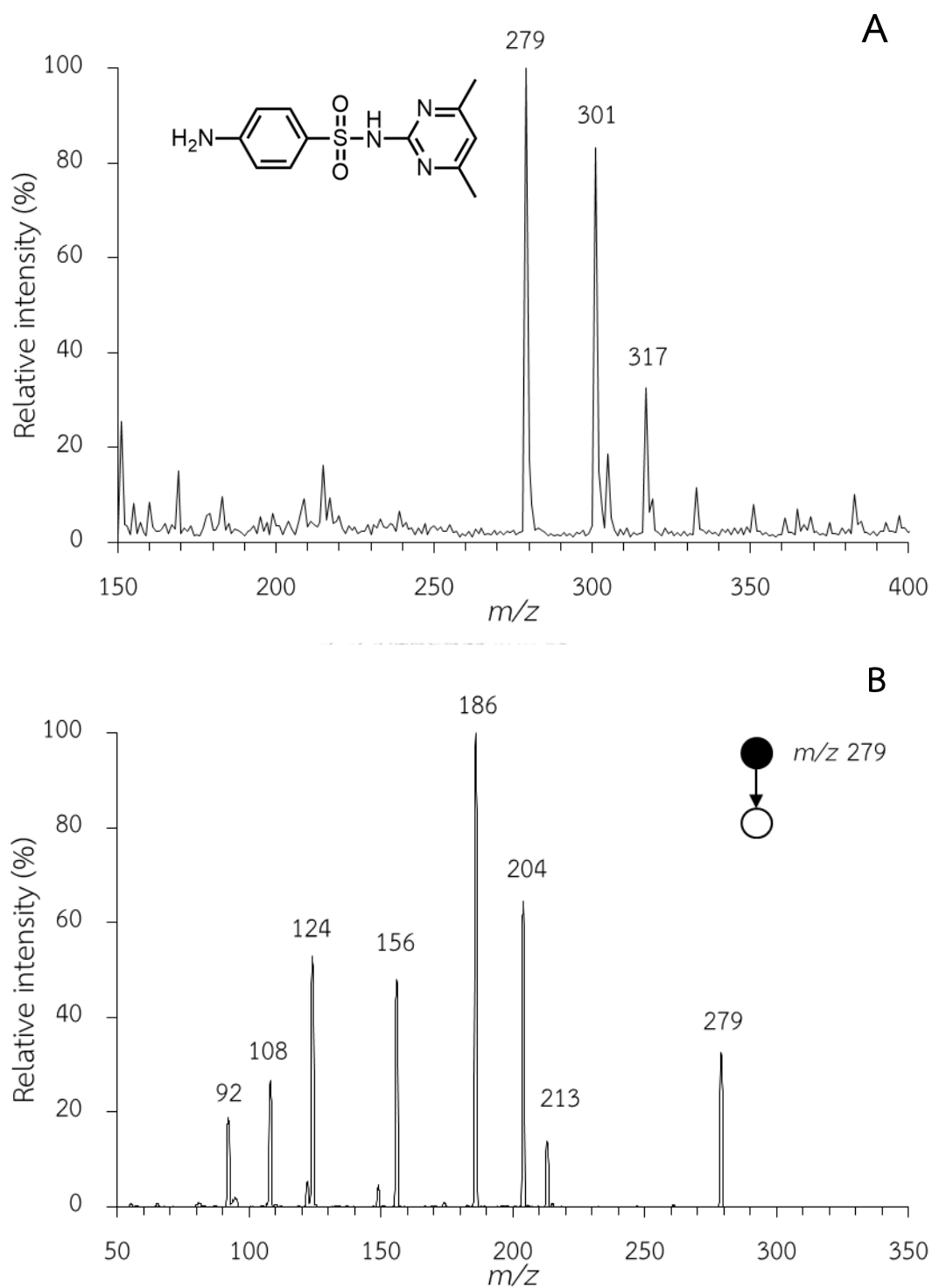
Figure 3.1 (A) Mass spectrum and (B) MS/MS spectrum (CE = 20) of 1 μg/mL sulfamethazine in MeOH by direct infusion.
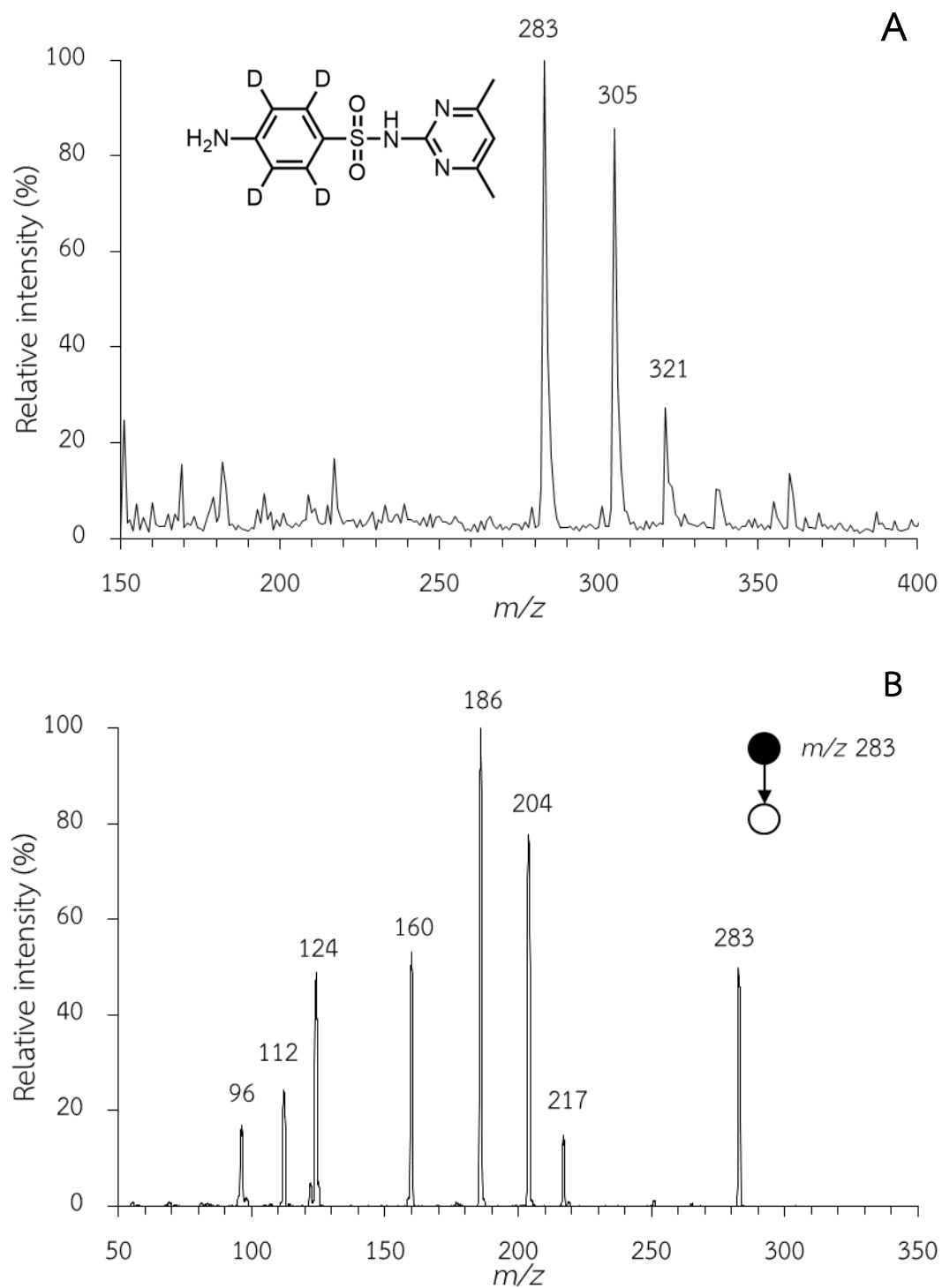
Figure 3.2 (A) Mass spectrum and (B) MS/MS spectrum (CE = 20) of 1 µg/mL sulfamethazine-d_4 in MeOH by direct infusion.

The structures of some fragment ions of sulfamethazine are shown in **Figure 3.3**. For the analysis in SRM mode, the fragment ions of sulfamethazine and its labeled analog were selected based on their intensity from automatic optimization in the software. Collision energy was optimized to improve the sensitivity. Too low value of collision energy resulted in low fragmentation while too high value could lead to uncontrolled fragmentation. The selected product ions and the optimized parameter were shown in **Table 2.1**.
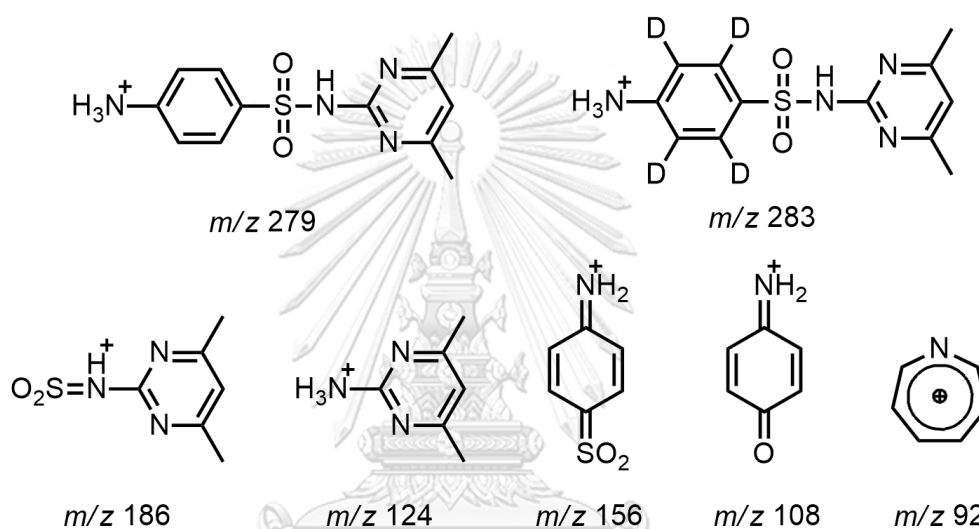
Figure 3.3 Structures of parent ions of sulfamethazine (*m/z* 279), sulfamethazine-*d₄* (*m/z* 283) and some important fragment ions.

3.2 Paper spray mass spectrometry

For typical PS-MS analysis, 20 µL of standard solution of sulfamethazine in MeOH was deposited on the triangular paper and then the voltage was applied. The signal of protonated ion at *m/z* 279 along with the sodium and potassium adducts could be observed in the mass spectrum from PS-MS (**Figure 3.4**).

The MS/MS spectrum of the parent ion at 279 *m/z* in PS-MS gave similar fragmentation pattern to that from direct infusion experiment (**Figure 3.1**). It should be noted that there was a prominent ion at 149 *m/z* in the MS/MS spectrum obtained from paper spray. This product ion was also found in the control paper spray experiment without sulfamethazine (**Figure 3.5**). This ion might come from dibutyl

phthalate, a common plasticizer in paper industry, which has the same molecular mass as sulfamethazine ([M+H]$^+$ at 279 *m/z*).[39] This compound has been previously reported to give signal in paper spray experiment.[24] Extension of paper washing process or washing with other solvents did not substantially remove this signal. However, the fragment ion at *m/z* 149 did not overlap with any fragment ions of sulfamethazine. Therefore, it did not affect with the analysis of sulfamethazine in SRM mode since this mode scanned only narrow ranges of selected product ions.
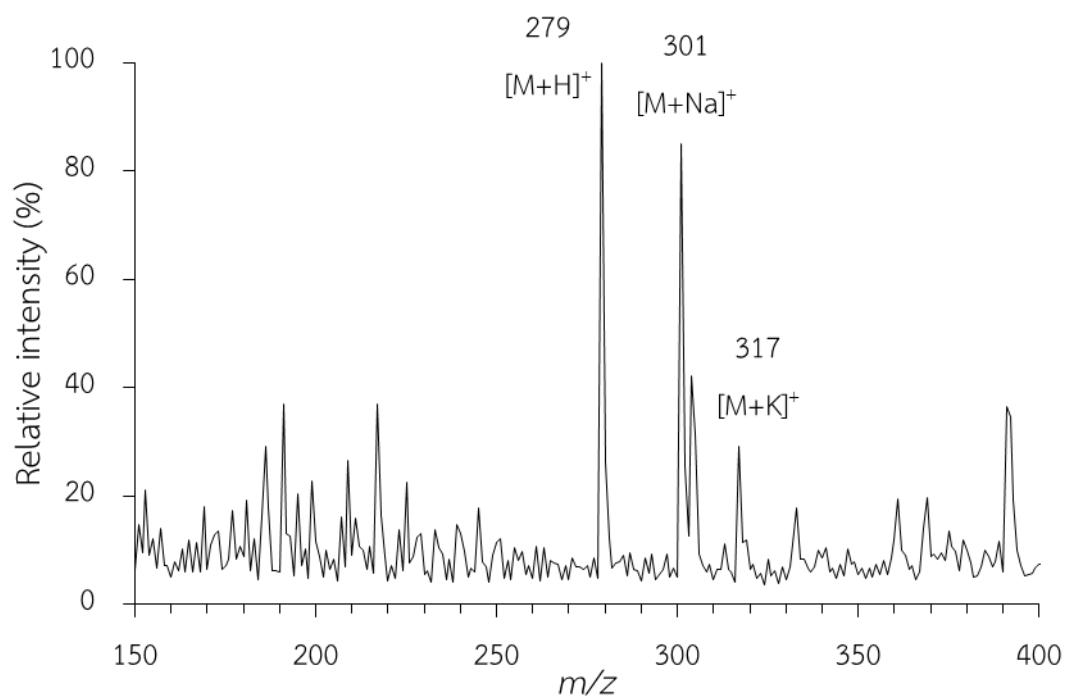
Figure 3.4 Mass spectrum of 500 ng/mL sulfamethazine in MeOH from direct PS-MS.

Figure 3.5 MS/MS spectrum (CE = 20) of (A) 500 ng/mL sulfamethazine in MeOH and (B) blank MeOH from direct PS-MS with structures of dibutyl phthalate and its product ion.

To test the real sample analysis, sulfamethazine was spiked into the matrices. The sample were directly spotted on the paper and air dried. This was then followed by adding fresh solvent for spraying from the paper substrate to enable mass spectrometric analysis. The signal dramatically decreased and provided insufficient sensitivity. This was likely due to high ion suppression effect.

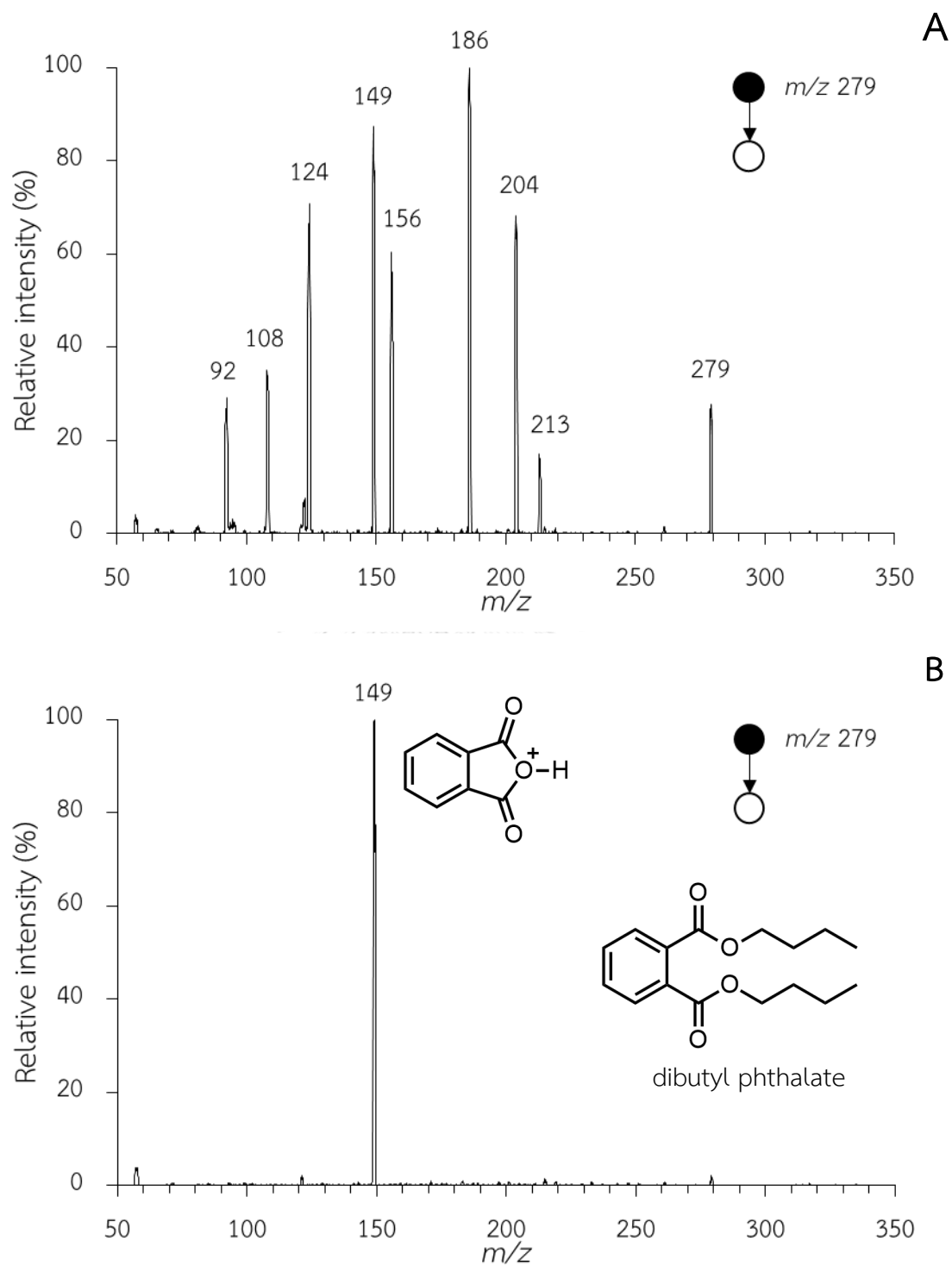This suppression phenomenon is not surprising with complex matrices. Although there have been reports on the PS-MS analysis of drugs from dried blood spot, it was also reported that the performance depended on the structure of the analyte. The compound with less basic groups are not ionized well in the positive mode and likely to be interfered by the matrix.[32] The analyte could also be interfered by various salts in the sample if it easily formed the metal ion adducts.

In the case of high ion suppression, various methods with additional pretreatments have been reported to improve the sensitivity of PS-MS analysis. For instance, the PS-MS analysis of resveratrol in red wine were performed after a clean-up by solid-phase extraction (SPE) with hydrophobic C18 sorbent to remove salts and sugars.[34] Another work form Zhang and Manicke developed a cartridge for SPE and PS-MS analysis of various drugs including sulfamethazine in plasma.[40] The pretreatments based on liquid-liquid extraction were also reported. Liquid-phase microextraction in a microsyringe were employed to analyze malachite green and crystal violet from lake water samples.[41] Also, a study from Yang and co-workers utilized slug-flow microextraction to extract illicit drugs from blood and urine for PS-MS analysis.[42] In this work, the liquid-liquid extraction is of interest since the process is simple while still being amenable to PS-MS without the need for special materials or instrumental setup.

3.3 Optimization of extracting solvent

The extraction process was simplified by using a microcentrifuge tube as a reservoir and a micropipette as a transferring device. Organic solvents could help precipitate proteins and remove various salts that might interfere with the analysis. Excluding chlorinated solvents, hydrocarbons, and substantially water-miscible

solvents, three solvents including diethyl ether (Et$_2$O), ethyl acetate (EtOAc), and acetonitrile (MeCN) were tested for their performance in the extraction process. These organic solvents were added to the sample, mixed by vortexing and separated by centrifugation. The organic layer was then spotted on the triangular paper for PS-MS analysis. The result is shown in **Figure 3.6**. Although MeCN has the highest polarity among these solvents, it gave the lowest signal intensity of sulfamethazine. The other two solvents provided much higher intensity compared to that of MeCN. The interferences were presumably co-extracted with the analyte and exhibited the suppression effect due to the miscibility of MeCN and water. Since EtOAc gave the higher intensity than Et$_2$O, EtOAc was selected as the solvent for extraction.
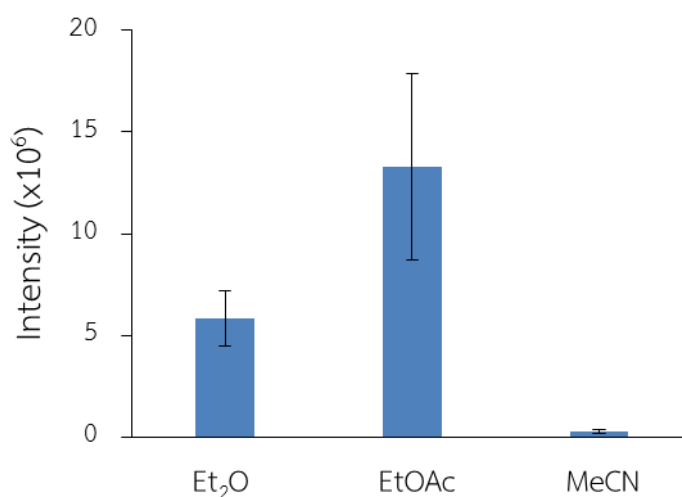
Figure 3.6 Extraction efficiencies of various organic solvents with MeOH as the spraying solvent.

The volume of EtOAc was varied from 25, 50, and 100 µL. The separation between organic solvent and aqueous phase was not clear with 25 µL of EtOAc. Meanwhile, the separations between two phases were obvious when 50 and 100 µL of EtOAc were used (**Figure 3.7**). The result showed that there was no difference in the intensity when using 50 µL and 100 µL of extracting solvent (**Figure 3.8**). For ease of transferring the solvent to the paper, 100 µL of EtOAc was selected for extraction.
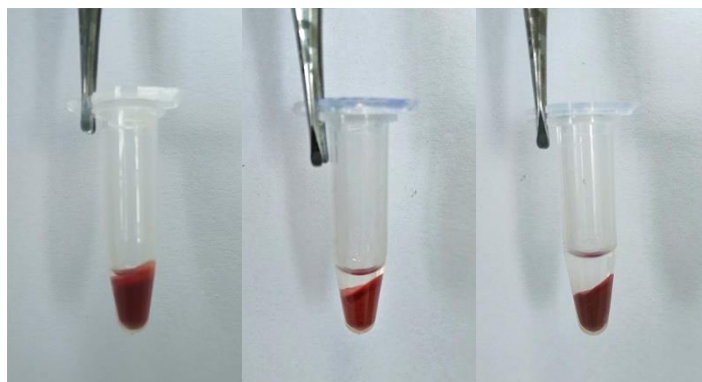
Figure 3.7 50 µL of spiked blood sample after extraction with 25, 50, and 100 µL of EtOAc followed by centrifugation
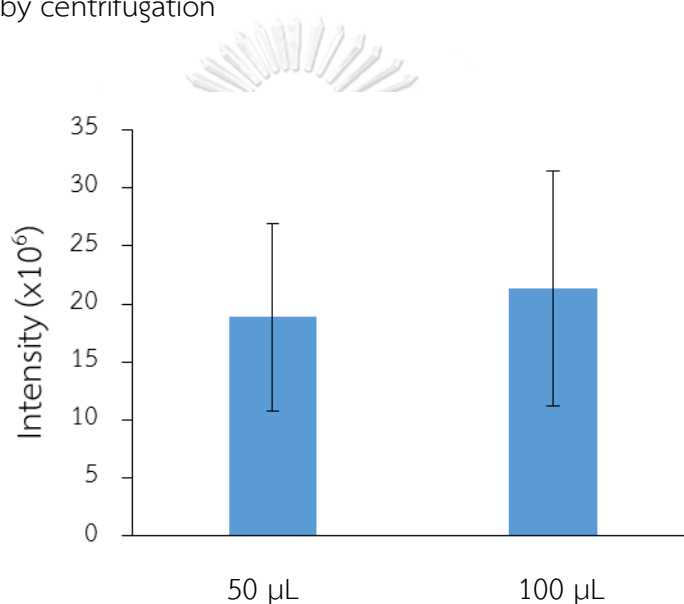
Figure 3.8 Effect of EtOAc volume

Since sulfamethazine contains both basic amino group and acidic sulfonamido group, it can appear in either unionized or ionized form depending on pH of the solution. Therefore, the pH effect of the sample on the extraction was investigated by adding 20 µL of 100-mM phosphate buffer solutions with pH ranging from 5–9 to the whole blood sample to adjust the pH before extraction with EtOAc. The results revealed that the pH seemed to exhibit no obvious effect on the extraction efficiency (**Figure 3.9**). The pH of pig whole blood, pig serum, and synthetic urine were measured to be 7.28, 8.29, and 6.26, respectively. Given that the pH range tested covered the

pH of all sample matrices and that the samples used here have some buffer capacity, it was determined that no buffer was needed for the sample preparation.
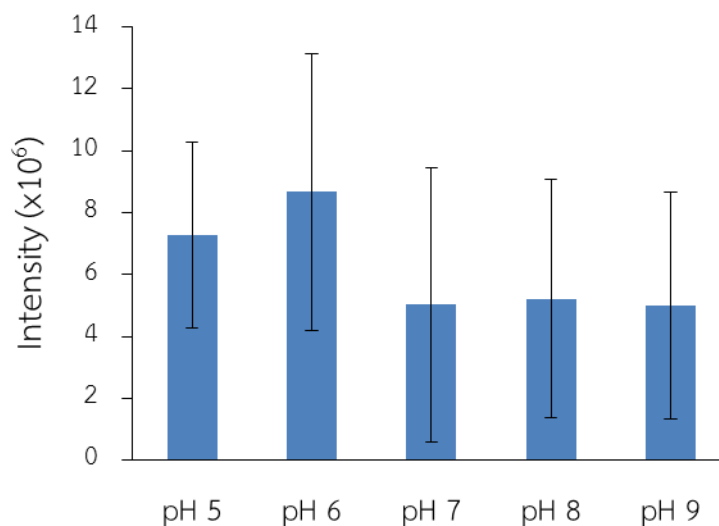
Figure 3.9 pH effect on extraction efficiency with EtOAc as the extracting solvent and MeOH as the spraying solvent.

3.4 Optimization of spraying solvent

Spraying solvent plays an important role in the analysis of dry sample spots. The solvent had to dissolve and elute the analyte to the tip of the triangle paper. There are also several factors contributed to the ionization efficiency such as polarity and volatility. Therefore, the spray solvents were optimized to improve the sensitivity (**Figure 3.10**). The spiked sample was extracted with EtOAc, spotted on the paper and sprayed with 20 µL of various solvents. It was found that 80:20 MeOH:H_2O provided the best result, surpassing other pure solvents including MeOH and MeCN. The higher signal probably come from longer spray duration due to slower solvent depletion on the paper and from improved elution of the analyte from the paper. The addition of formic acid (FA), over the range of 0.01–1% v/v, did not increase the signal intensity (**Figure 3.11**). Therefore, 80:20 MeOH:H_2O was selected as the spray solvent. With the use of PS-MS, the analysis time for each sample was only 2 minutes.
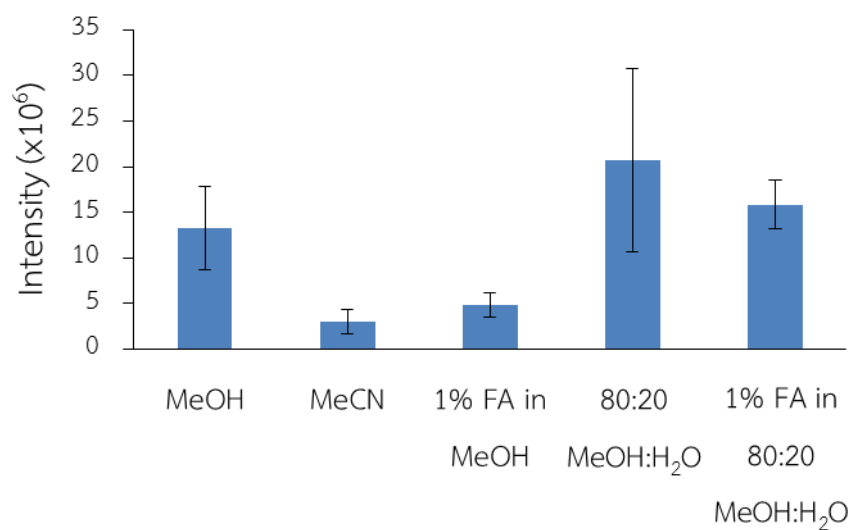
Figure 3.10 Effect of various spray solvents on the signal of sulfamethazine in spiked blood after extraction with EtOAc
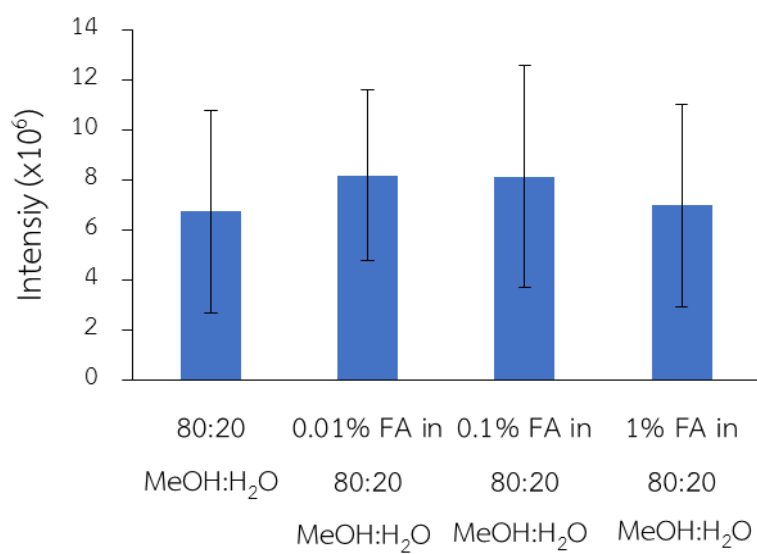
Figure 3.11 Effect of formic acid in the spray solvent

3.5 Calibration curves

The optimized extraction PS-MS method dramatically increased the signal of sulfamethazine in all matrices. This can be clearly seen in **Figure 3.12**. The fragment ions of sulfamethazine could be observed in the MS/MS spectra of the sample with extraction, while no prominent signal appeared in the spectra without the extraction step. Each blank sample matrix was also analyzed and there was no any signal at the same *m/z* of fragment ions of sulfamethazine. In addition, it should be noted that the fragment ion at 149 *m/z* from dibutyl phthalate was also suppressed without the extraction step. These results indicated that the matrix effect was relieved by extraction with EtOAc.

The calibration curves that accounted for pig blood, pig serum, and synthetic urine were created (**Figure 3.13**). With the use of internal standard to compensate signal fluctuation and matrix effect, good linearity was obtained in all matrices. Using linear regression analysis, LOD and LOQ values for each matrix were calculated (**Table 3III.1**). The LOD values were 10.1 ng/mL for pig blood, 4.6 ng/mL for pig serum, and 3.5 ng/mL for synthetic urine. The corresponding LOQ values were 30.7 ng/mL, 14.1 ng/mL, and 10.6 ng/mL, respectively.

Table 3III.1 Calibration curves, linear range, LOD, and LOQ values of sulfamethazine in pig blood, pig serum, and synthetic urine.

matrix	equation	Linear range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Pig blood	y = 0.0024 + 0.0228	10-500	10.1	30.7
Pig serum	y = 0.0025 + 0.0446	5-500	4.6	14.1
Synthetic urine	y = 0.0021 − 0.0046	5-500	3.5	10.6
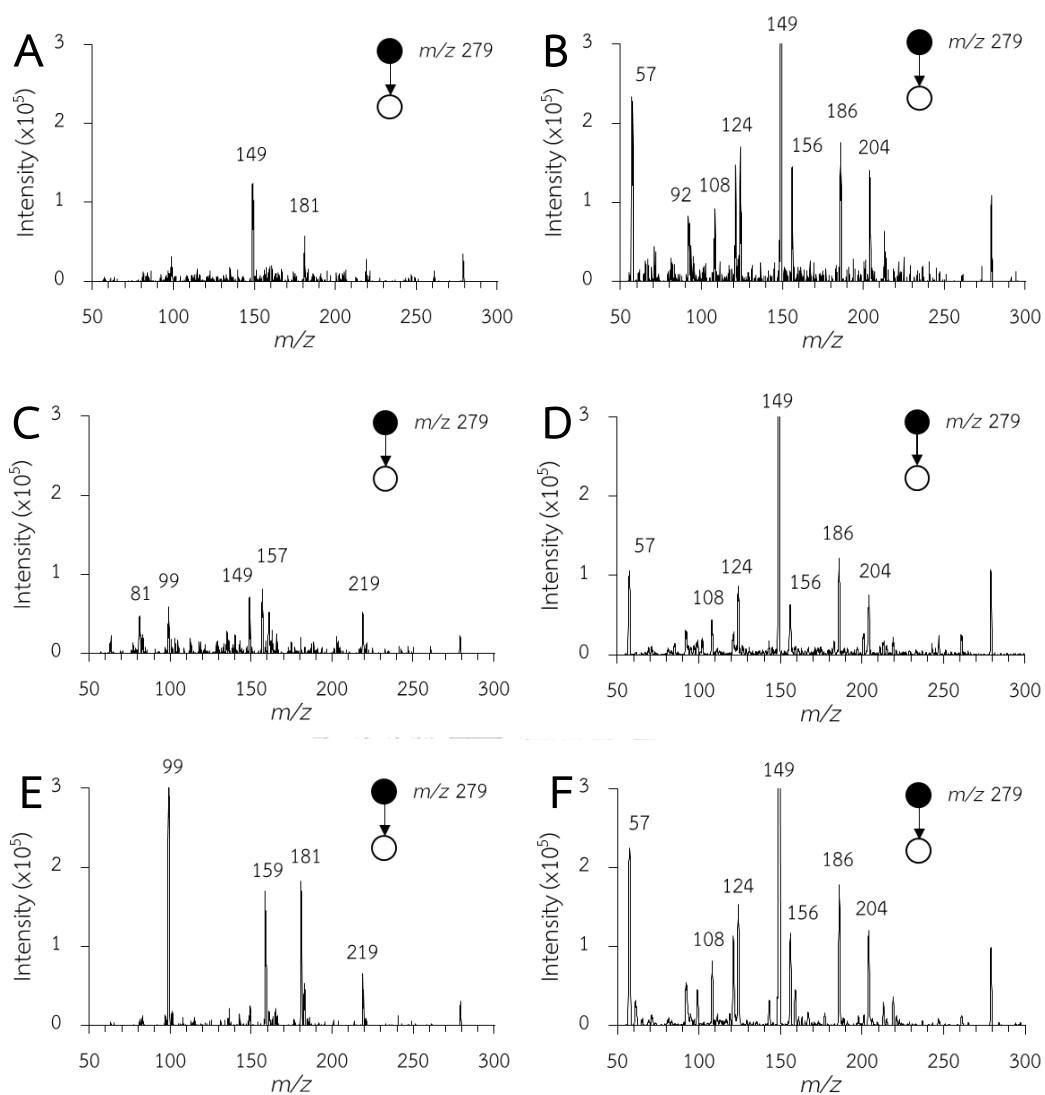
Figure 3.12 Comparison of MS/MS spectra of 500 ng/mL sulfamethazine from PS-MS (A,C,E) before and (B,D,F) after extraction with EtOAc in (A,B) pig blood, (C,D) pig serum, and (E,F) synthetic urine.
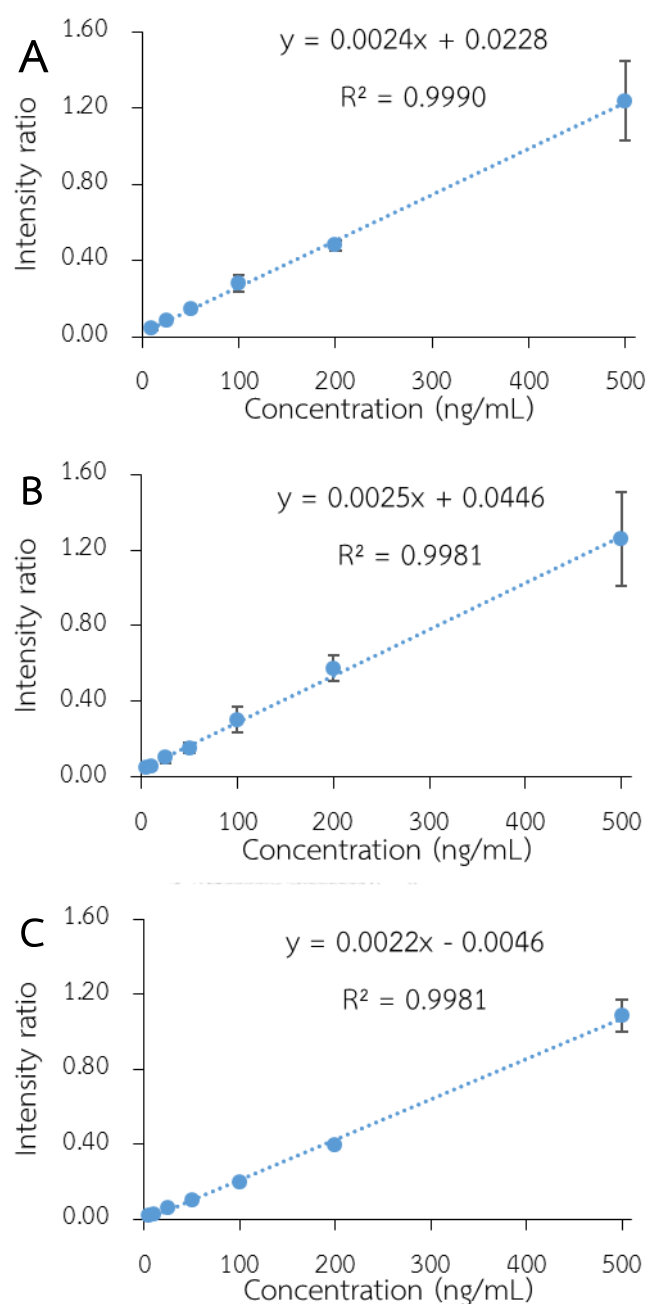
Figure 3.13 Calibration curves of sulfamethazine in (A) pig whole blood, (B) pig serum, and (C) synthetic urine. The intensity ratio was calculated from the analyte signal divided by the signal of the internal standard.

Notably, there have been previous studies showing a correlation between the amount of drug in body fluids and edible tissues. It was found that the sulfamethazine content in kidney, liver, and muscle tissue will exceed the MRL of 100 ng/g when the

compound can be detected in pig serum at the level above 190, 110, and 420 ng/mL, respectively. For urine, the corresponding amount were found to be higher at the level of 630, 370, and 1300 ng/mL.[37] Clearly, this means that the developed method can detect sulfamethazine at the level significantly below the required limits. Therefore, the method can be adopted for practical uses in nonlethal detection of blood or urine sample to provide crucial data before slaughtering.

3.6 Recovery test

After calibration curves with sufficient sensitivity were established, the performance of the method was evaluated by spiking sulfamethazine into blank sample matrices at three concentrations including, 100, 200, and 400 ng/mL. The amount of sulfamethazine in the samples were obtained from the calibration curves and the %recovery were calculated (**Table 3.2**). The results showed that the obtained %recovery was generally good, ranging from 95.4–105.4 %, with acceptable precision in all matrices. The obtained performance demonstrated the viability of the developed method for rapid and efficient analysis of sulfamethazine in samples from pigs.

Table 3.2 %recovery of sulfamethazine spiked at three levels in three matrices.

matrix	Spiked concentration (ng/mL)	Found concentration (ng/mL)	% RSD	% recovery
Pig whole blood	100	104.1 ± 17.0	16.4	104.1
	200	195.0 ± 16.8	8.6	97.5
	400	381.5 ± 42.9	11.2	95.4
Pig serum	100	105.4 ± 13.4	12.7	105.4
	200	207.8 ± 17.4	8.4	103.9
	400	413.0 ± 34.4	8.3	103.2
Synthetic urine	100	103.0 ± 19.2	18.7	103.0
	200	206.7 ± 21.3	10.3	103.4
	400	396.1 ± 88.7	22.4	99.0

The performance of this developed method is comparable with other previous studies that detect sulfamethazine or other sulfonamides in blood samples with the LOD values ranging from 1.4–12.3 ng/mL.[43-45] These related studies required more sophisticated sample preparation including the use of ionic liquids for extraction, multiple steps of pretreatments, and the need for chromatographic separation. The combination of extraction and PS-MS provides a promising alternative for the detection of sulfamethazine in complex biological samples with minimal sample preparation, low solvent, materials, and time consumption.

CHAPTER IV

CONCLUSION

In this work, a rapid method for detection and quantification of sulfamethazine, one of sulfonamide antibiotics, in swine body fluids is developed. The analyte was simply extracted with ethyl acetate to remove interferences and improve the sensitivity. Then PS-MS analysis allows rapid and efficient quantification of the sulfamethazine. The calibration curves showed good linearity in all matrices. The procedure was simple, fast, and practical while still providing suitable performance in terms of sensitivity, accuracy, and precision. This method is suitable for high-throughput analysis due to reduced analysis time, solvent consumption, and waste production. Thus, the developed method provides a promising alternative for the analysis of sulfamethazine in complex biological samples. It should also be applicable to other compounds where nonlethal monitoring of drug levels in pig is desired.

REFERENCES

1. Stolker, A. A. M.; Brinkman, U. A. T., Analytical Strategies for Residue Analysis of Veterinary Drugs and Growth-Promoting Agents in Food-Producing Animals—a Review. *J. Chromatogr. A* **2005,** *1067,* 15-53.

2. Croubels, S.; Daeseleire, E., Veterinary Drug Residues in Foods. In *Chemical Contaminants and Residues in Food*, Schrenk, D., Ed. Woodhead Publishing Limited: Cambridge, 2012; pp 148-182.

3. Balizs, G.; Hewitt, A., Determination of Veterinary Drug Residues by Liquid Chromatography and Tandem Mass Spectrometry. *Anal. Chim. Acta* **2003,** *492,* 105-131.

4. Gentili, A.; Perret, D.; Marchese, S., Liquid Chromatography-Tandem Mass Spectrometry for Performing Confirmatory Analysis of Veterinary Drugs in Animal-Food Products. *TrAC, Trends in Anal. Chem.* **2005,** *24*, 704-733.

5. Suppadit, T.; Pripwai, N.; Chaowittayangkul, N.; Jaturasitha, S., Sulfamethazine Residues in Pork with Different Withdrawal Periods and Pig Tissues in Chiang Mai Market. *J. Agr. Rural Dev. Trop.* **2004,** *80*, 214-223.

6. Suhre, F. B.; Simpson, R. M.; Shafer, J. W., Qualitative/Quantitative Determination of Sulfamethazine in Swine Tissue by Gas Chromatographic/Electron Impact Mass Spectrometry Using a Stable Isotope Labeled Internal Standard. *J. Agric. Food Chem.* **1981,** *29*, 727-729.

7. Reeves, V. B., Confirmation of Multiple Sulfonamide Residues in Bovine Milk by Gas Chromatography–Positive Chemical Ionization Mass Spectrometry. *J. Chromatogr. B: Biomed. Sci. Appl.* **1999,** *723*, 127-137.

8. Zotou, A.; Vasiliadou, C., Selective Determination of Sulfonamide Residues in Honey by SPE-RP-LC with UV Detection. *Chromatographia* **2006,** *64*, 307-311.

9. Tolika, E. P.; Samanidou, V. F.; Papadoyannis, I. N., Development and Validation of an Hplc Method for the Determination of Ten Sulfonamide Residues in Milk According to 2002/657/EC. *J. Sep. Sci.* **2011,** *34*, 1627-1635.

10. Hela, W., Determination of Sulfonamides in Animal Tissues Using Cation Exchange Reversed Phase Sorbent for Sample Cleanup and HPLC–DAD for Detection. *Food Chem.* **2003,** *83,* 601-608.

11. Wen, Y.; Zhang, M.; Zhao, Q.; Feng, Y.-Q., Monitoring of Five Sulfonamide Antibacterial Residues in Milk by in-Tube Solid-Phase Microextraction Coupled to High-Performance Liquid Chromatography. *J. Agric. Food Chem.* **2005,** *53,* 8468-8473.

12. Zotou, A.; Vasiliadou, C., A Fluorescence-LC Method for the Determination of Sulfonamides in Biological Fluids with Pre-Column Derivatization. *Chromatographia* **2009,** *70,* 389-397.

13. Maudens, K. E.; Zhang, G.-F.; Lambert, W. E., Quantitative Analysis of Twelve Sulfonamides in Honey after Acidic Hydrolysis by High-Performance Liquid Chromatography with Post-Column Derivatization and Fluorescence Detection. *J. Chromatogr. A* **2004,** *1047,* 85-92.

14. Borras, S.; Companyo, R.; Guiteras, J., Analysis of Sulfonamides in Animal Feeds by Liquid Chromatography with Fluorescence Detection. *J. Agric. Food Chem.* **2011,** *59,* 5240-5247.

15. Kaufmann, A.; Butcher, P.; Maden, K.; Widmer, M., Ultra-Performance Liquid Chromatography Coupled to Time of Flight Mass Spectrometry (UPLC-TOF): A Novel Tool for Multiresidue Screening of Veterinary Drugs in Urine. *Anal. Chim. Acta* **2007,** *586,* 13-21.

16. Koesukwiwat, U.; Jayanta, S.; Leepipatpiboon, N., Solid-Phase Extraction for Multiresidue Determination of Sulfonamides, Tetracyclines, and Pyrimethamine in Bovine's Milk. *J. Chromatogr. A* **2007,** *1149,* 102-111.

17. Gentili, A.; Perret, D.; Marchese, S.; Sergi, M.; Olmi, C.; Curini, R., Accelerated Solvent Extraction and Confirmatory Analysis of Sulfonamide Residues in Raw Meat and Infant Foods by Liquid Chromatography Electrospray Tandem Mass Spectrometry. *J. Agric. Food Chem.* **2004,** *52,* 4614-4624.

18. Shao, B.; Dong, D.; Wu, Y.; Hu, J.; Meng, J.; Tu, X.; Xu, S., Simultaneous Determination of 17 Sulfonamide Residues in Porcine Meat, Kidney and Liver by Solid-Phase Extraction and Liquid Chromatography–Tandem Mass Spectrometry. *Anal. Chim. Acta* **2005,** *546,* 174-181.

19. Sheridan, R.; Policastro, B.; Thomas, S.; Rice, D., Analysis and Occurrence of 14 Sulfonamide Antibacterials and Chloramphenicol in Honey by Solid-Phase Extraction Followed by LC/MS/MS Analysis. *J. Agric. Food Chem.* **2008,** *56*, 3509-3516.

20. Kung, T.-A.; Tsai, C.-W.; Ku, B. C.; Wang, W.-H., A Generic and Rapid Strategy for Determining Trace Multiresidues of Sulfonamides in Aquatic Products by Using an Improved Quechers Method and Liquid Chromatography–Electrospray Quadrupole Tandem Mass Spectrometry. *Food Chem.* **2015,** *175*, 189-196.

21. Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G., Mass Spectrometry Sampling under Ambient Conditions with Desorption Electrospray Ionization. *Science* **2004,** *306*, 471.

22. Shelley, J. T.; Badal, S. P.; Engelhard, C.; Hayen, H., Ambient Desorption/Ionization Mass Spectrometry: Evolution from Rapid Qualitative Screening to Accurate Quantification Tool. *Anal. Bioanal. Chem* **2018,** *410*, 4061-4076.

23. Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z., Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. *Angew. Chem. Int. Ed. Engl.* **2010,** *49*, 877-880.

24. Liu, J.; Wang, H.; Manicke, N. E.; Lin, J.-M.; Cooks, R. G.; Ouyang, Z., Development, Characterization, and Application of Paper Spray Ionization. *Anal. Chem.* **2010,** *82*, 2463-2471.

25. Evard, H.; Kruve, A.; Lõhmus, R.; Leito, I., Paper Spray Ionization Mass Spectrometry: Study of a Method for Fast-Screening Analysis of Pesticides in Fruits and Vegetables. *J. Food Compos. Anal.* **2015,** *41*, 221-225.

26. Ren, Y.; Wang, H.; Liu, J.; Zhang, Z.; McLuckey, M. N.; Ouyang, Z., Analysis of Biological Samples Using Paper Spray Mass Spectrometry: An Investigation of Impacts by the Substrates, Solvents and Elution Methods. *Chromatographia* **2013,** *76*, 1339-1346.

27. Zhang, Z.; Cooks, R. G.; Ouyang, Z., Paper Spray: A Simple and Efficient Means of Analysis of Different Contaminants in Foodstuffs. *Analyst* **2012,** *137*, 2556-2558.

28. Guo, T.; Zhang, Z.; Yannell, K. E.; Dong, Y.; Cooks, R. G., Paper Spray Ionization Mass Spectrometry for Rapid Quantification of Illegal Beverage Dyes. *Anal. Methods* **2017,** *9*, 6273-6279.

29. Taverna, D.; Di Donna, L.; Mazzotti, F.; Policicchio, B.; Sindona, G., High-Throughput Determination of Sudan Azo-Dyes within Powdered Chili Pepper by Paper Spray Mass Spectrometry. *J. Mass Spectrom.* **2013,** *48*, 544-547.

30. Manicke, N. E.; Yang, Q.; Wang, H.; Oradu, S.; Ouyang, Z.; Cooks, R. G., Assessment of Paper Spray Ionization for Quantitation of Pharmaceuticals in Blood Spots. *Int. J. Mass Spectrom.* **2011,** *300*, 123-129.

31. Su, Y.; Ma, X.; Ouyang, Z., Rapid Screening of Multi-Class Antimicrobial Residues in Food of Animal Origin by Paper Spray Mass Spectrometry. *Int. J. Mass Spectrom.* **2018,** *434*, 233-239.

32. Manicke, N. E.; Abu-Rabie, P.; Spooner, N.; Ouyang, Z.; Cooks, R. G., Quantitative Analysis of Therapeutic Drugs in Dried Blood Spot Samples by Paper Spray Mass Spectrometry: An Avenue to Therapeutic Drug Monitoring. *J. Am. Soc. Mass Spectrom.* **2011,** *22*, 1501-1507.

33. Li, A.; Wei, P.; Hsu, H. C.; Cooks, R. G., Direct Analysis of 4-Methylimidazole in Foods Using Paper Spray Mass Spectrometry. *Analyst* **2013,** *138*, 4624-4630.

34. Di Donna, L.; Taverna, D.; Indelicato, S.; Napoli, A.; Sindona, G.; Mazzotti, F., Rapid Assay of Resveratrol in Red Wine by Paper Spray Tandem Mass Spectrometry and Isotope Dilution. *Food Chem.* **2017,** *229*, 354-357.

35. B Ashworth, R.; L Epstein, R.; H Thomas, M.; T Frobish, L., Sulfamethazine Blood/Tissue Correlation Study in Swine. *Am. J. Vet. Res.* **1986,** *47*, 2596-2603.

36. Buur, J. L.; Baynes, R. E.; Craigmill, A. L.; Riviere, J. E., Development of a Physiologic-Based Pharmacokinetic Model for Estimating Sulfamethazine Concentrations in Swine and Application to Prediction of Violative Residues in Edible Tissues. *Am J Vet Res* **2005,** *66*, 1686-1693.

37. Bevill, R. F., Cause, Detection, and Correction of Sulfonamide Residues in Swine. In *Animal Drugs and Human Health*, Crawford, L. M.; Franco, D. A., Eds. CRC Press: Boca Raton, FL, 1994; p 93.

38. Haglock-Adler, C. J.; Hurley, A.; Strathmann, F. G., Use of Synthetic Urine as a Matrix Substitute for Standard and Quality Control Materials in the Clinical Assessment of Iodine by Inductively Coupled Plasma Mass Spectrometry. *Clin. Biochem.* **2014,** *47*, 80-82.

39. Guo, X.; Bruins, A. P.; Covey, T. R., Characterization of Typical Chemical Background Interferences in Atmospheric Pressure Ionization Liquid Chromatography-Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2006,** *20*, 3145-3150.

40. Zhang, C.; Manicke, N. E., Development of a Paper Spray Mass Spectrometry Cartridge with Integrated Solid Phase Extraction for Bioanalysis. *Anal. Chem.* **2015,** *87*, 6212-6219.

41. Fang, L.; Deng, J.; Yu, Y.; Yang, Y.; Wang, X.; Liu, H.; Luan, T., Coupling Liquid-Phase Microextraction with Paper Spray for Rapid Analysis of Malachite Green, Crystal Violet and Their Metabolites in Complex Samples Using Mass Spectrometry. *Anal. Methods* **2016,** *8*, 6651-6656.

42. Yang, Y.; Wu, J.; Deng, J.; Yuan, K.; Chen, X.; Liu, N.; Wang, X.; Luan, T., Rapid and on-Site Analysis of Amphetamine-Type Illicit Drugs in Whole Blood and Raw Urine by Slug-Flow Microextraction Coupled with Paper Spray Mass Spectrometry. *Anal. Chim. Acta* **2018,** *1032*, 75-82.

43. Yu, W.; Liu, Z.; Cui, S.; Zhang, S.; Yang, X.; Lei, L.; Zhang, H.; Yu, A., In-Syringe Ionic Liquid Dispersive Liquid–Liquid Microextraction for the Determination of Sulfonamides in Blood Using High-Performance Liquid Chromatography. *Anal. Methods* **2014,** *6*, 2545-2552.

44. Liu, Z.; Yu, W.; Zhang, H.; Gu, F.; Jin, X., Salting-out Homogenous Extraction Followed by Ionic Liquid/Ionic Liquid Liquid-Liquid Micro-Extraction for Determination of Sulfonamides in Blood by High Performance Liquid Chromatography. *Talanta* **2016,** *161*, 748-754.

45. Zhang, Y.; Xu, X.; Liu, H.; Zhai, Y.; Sun, Y.; Sun, S.; Zhang, H.; Yu, A.; Wang, Y., Matrix Solid-Phase Dispersion Extraction of Sulfonamides from Blood. *J. Chromatogr. Sci.* **2012,** *50*, 131-136.

VITA

NAME	Surased Suraritdechachai
DATE OF BIRTH	24 Nov 1994
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Department of Chemistry, Faculty of Science, Chulalongkorn University
HOME ADDRESS	214/3 Charoen Krung Road, Si Phraya, Bangrak, Bangkok, 10500
PUBLICATION	Suraritdechachai, S.; Charoenpakdee, C.; Young, I.; Maher, S.; Vilaivan, V.; Praneenararat, T., Rapid Detection of the Antibiotic Sulfamethazine in Pig Body Fluids by Paper Spray Mass Spectrometry. J. Agric. Food Chem. 2019, 67, 3055-3061.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY