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Pharmacokinetic Study of Azoxystrobin and Isopyrazam in Rat by LC-MS/MS and Evaluation of Its Toxicity

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Abstract

A combination of azoxystrobin and isopyrazam is a commonly used pesticide, but its widespread exposures has potential adverse effects. A physiologically based pharmacokinetic model was developed to quantitatively assess the azoxystrobin/isopyrazam in plasma by a high-performance liquid chromatography–tandem mass spectrometric (LC–MS/MS) method. The plasma concentration was determined following administration of low and high oral doses in rats. Phenacetin was used as an internal standard and a dual liquid extraction with ethyl acetate as plasma samples pretreated technic. Optimal chromatographic separation was achieved based on an Inertsil ODS-2 analytical column (5 μm, 4.6 × 150 mm) and isocratic elution with a mobile phase consisting of methanol: water (80:20, v/v) containing 0.1% (v/v) formic acid. All requirements of selectivity, linearity, precision, accuracy and stability met generally acceptable limit in bioanalysis. The study could be particularly useful in detecting pesticide residue and estimating toxicological risk of pesticide exposure to human.

Keywords

Azoxystrobin; Isopyrazam; HPLC-MS/MS; Pesticide exposure; Pharmacokinetic study

Introduction

Pesticides are accessible poisonous substances with potentially adverse effects on human health and environment. In the last few decades, due to the production of pesticides has increased significantly, the scientific community concern about their toxicity and other hazard may cause to man and environment [1]. Some of the authoritative organizations whose provide scientific advice on safety issues, including the European Food Safety Authority (EFSA), the World Health Organization (WHO), and the Food and Agriculture Organization of the United Nations (FAO), have been emphasis the significance of monitoring pesticides of exposure to evaluate risks to public health [2]. It is reported that pesticides have potential effect on the normal hormonal function by interfering their synthesis, transport, secretion or metabolism in the body [3]. In addition, exposure to pesticides is also associated with adverse health effects such as cancers, neurodegenerative disorders and reproductive disorders [4]. Therefore, great efforts should be addressed to study the effects on humans caused by their widespread use.

Cucumber (*Cucumis sativus L.)* is a popular vegetable and is cultivated commercially worldwide as a seasonal crop [5]. However, the powdery mildew caused by the fungus has become the largest threat for cucumber, which will lead to reduction of fruit quality, shrinkage of marketable yields and severe economic losses [6]. Using fungicides is one of the commonest management methods for decreasing the incidence of powdery mildew [7]. Currently, a combination of azoxystrobin (AZT, 17.8%) and isopyrazam (IZM, 11.2%) is commonly used to treat powdery mildew in cucumber [8].

AZT, a commonly used broad-spectrum fungicide, has significant effect in increasing production of crops [9]. Whereas, the widespread application of AZT would cause severe threats to ecosystem [10], terrestrial animals, amphibians, birds [11], aquatic organisms [12], freshwater and sea environment [13]. IZM, a new generation broad-spectrum representative of succinate-dehydrogenase inhibitors (SDHIs), is an inhibitor of the fungal mitochondrial respiration chain affecting mitochondrial electron transport [14]. It is reported that some pathogenic organisms of plant resistant to IZM, increasing the rate of gene mutation [15]. Consequently, additional information should be given by a novel approach to track human exposure to pesticides. Currently, cultivation of cucumber

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involves unregulated applications of pesticides, leading to potential health hazards to consumers. Food safety and public health arouse severe concern, and pesticide residues have become a vital problem in the food industry. These fungicides not only have reproductive toxicity but also lead to poisoning symptoms includes suppression of the immune system, nausea, vomiting and other gastrointestinal traumas [16].

China has become the world's largest producer and consumer of chemical pesticides [17], so that overuse of pesticides has become the effective method to increase product yields. It is reported that 357 kilograms per hectare pesticide was used China's arable land in 2013, which is approximately equivalent to the total amount of pesticide used in both the United States and India, and far exceeds the internationally recognized standard to limit use [18]. Evidently, the highly intensive and unscientific application of pesticide poses a potential risk to the ecological environment, increasing the exposure hazard to human [19].

The determination of chemicals or their metabolites in human specimens is commonly used for assessing human exposure to pesticides, but it was limited by high costs and ethical consideration in sampling [20]. Therefore, the establishment of vitro models is substitutable method for evaluating chemicals' potential side effects and mechanism of action [21]. Hence, this study was mainly purposed to establish plasmatic concentration curves of both AZT and IZM after oral administration with subsequent pharmacokinetic analysis on a rodent animal model. This assay could be helpful for evaluating inherent risk of pesticide residue and drug accumulation for human, and would provide relevant information to clinical therapeutics and to further toxicity studies of fungicides.

Methods

Materials and reagents

A standard of AZT (purity 98%) was purchased from Aladdin (Shanghai, China), IZM (purity 99.6%) was purchased from Sigma (St. Louis, MO), and phenacetin (internal standard, purity 99%) was purchased from Dalian Meilun biological technology co., LTD (Dalian, China). Methanol, acetonitrile, ethyl acetate and formic acid were all of chromatographically pure grade and obtained from Merck (Darmstadt, Germany). Other chemicals and solvents (analytical grade) used were acquired from Kermel (Chengdu, China). The Lvfei ™ (AZT 17.8% + IZM 11.2%) was supplied by Syngenta (Basel, Switzerland).

Liquid chromatographic and mass spectrometric conditions

High Performance Liquid Chromatography was an Agilent 1100 Series (Agilent, USA), consisted of a binary gradient pump, an online degasser, a thermostatic column compartment and an autosampler. Chromatographic separation was achieved on an Inertsil ODS-2 (5 μm, 4.6 × 150 mm) column. The mobile phase consisted of methanol/0.1% formic acid in water (80: 20, V/V) at a flow rate of 0.8 mL·min^{-1} (split ration 1:3). The analysis run time was 10 minutes and injection volume was 5 μL. flowed on an Inertsil ODS-2 column (5 μm, 4.6 ×150 mm).

Mass spectrometric detection was performed on a triple quadrupole tandem mass spectrometer (AB Sciex-API3000, USA) equipped with an electrospray ionization source. The main source parameters were optimized as follows: nebulizer gas, 8 L·min^{-1}; curtain gas, 8 L·min^{-1}; collision activated dissociation (CAD) gas, 4 L·min^{-1}; source temperature, 450 °C and turbo ion spray, 4500 V. Multiple reaction monitoring (MRM) with a dwell time of 100 ms for each transition. Electrospray ionization (ESI) was performed in positive ion mode, m/z 404.3→372.1 for AZT, m/z 360.3→244.1 for IZM, m/z 180.1→110.3 for phenacetin, respectively. Data acquisition and processing were powered by Analyst 1.4.1 software.

Preparation of standards and quality control samples

The primary stock solutions of AZT, IZM and phenacetin were prepared at 1 mg·mL^{-1} in acetonitrile, respectively. The working solutions of each analyte were prepared by diluting the stock solution with methanol. Phenacetin was used as an internal standard (IS) by dissolving appropriate amount in methanol to achieve a concentration of 400 ng·mL^{-1}. Calibration curves were generated using nine calibration standards. Blank plasma samples were prepared by adding working solutions to drug-free plasma to final concentrations of 0.8, 1.6, 3.2, 16, 40, 80, 200, 400 and 800 ng·mL^{-1} for AZT; 0.5, 1, 2, 10, 25, 50, 125, 250 and 500 ng·mL^{-1} for IZM.

The quality control (QC) samples involved high quality control (QC-H), medium quality control (QC-M) and low quality control (QC-L), were similarly prepared at concentration of combined working solutions with blank rat plasma. All the stock solutions, standards and QC samples were stored at -20 °C.

Sample preparation

A dual liquid–liquid extraction method was used to extract analytes from the plasma samples. An aliquot of 100 μL plasma, 20 μL of IS solution (400 ng·mL^{-1}) was added in a 2.0 mL polypropylene tube and mixed for 2 minutes on a Vortex mixer (IKA, Germany). 1 mL ethyl acetate was then added to the samples, vortexed for 5 minutes and followed by centrifugation at 12000 g for 10 minutes. The 0.8 mL organic layer was separated and concentrated to dryness under nitrogen gas. The residue was re-dissolved in 100 μL of mobile phase and vortexed for 2 minutes, centrifuged for 5 minutes. Finally, 20 μL of supernatant was injected into the LC-MS/MS system.

Method Validation

Selectivity and specificity: To investigate whether endogenous matrix constituents would interfere with the assay, the specificity and selectivity of the method were evaluated by analyzing blank plasma, blank plasma extracted by ethyl acetate containing IS and blank plasma spiked with analytes, a plasma sample collected at 45 minutes after oral administration of the pesticide to rats, respectively. All the analytes were determined in positive ion mode due to the superior sensitivity under this condition.

Linearity and LLOQ: Calibration curves were constructed used nine concentrations spiked with plasma samples and were treated in accordance with the "bio-sample pre-treatment". The calibration curves of analytes in bio-samples were obtained by plotting the peak-area ratio of analytes/IS versus the theoretical concentration. The LLOQ, defined as the lowest concentration on the calibration curve, was measured in six replicates on one validation day and had to meet the requirement that signal-noise ratio(S/N) was at least 10:1.

Accuracy and precision: The accuracy and precision of inter-/intra-day measurements of the method were evaluated by analyzing the QC samples at three concentration levels (QC-L, QC-M and QC-H) in six replicates during a single day and on three consecutive days. Samples were quantified used calibration curves constructed during the same batch. The precisions were expressed as relative standard deviation (RSD), which should be within ±15%.

Extraction recovery and matrix effect: The matrix effect and extraction recovery experiments were performed in six replicates at three different QC concentrations. The extraction recovery was investigated by comparing the chromatographic peak areas response of extracted analytes in pre-extract spiked samples (Set A) with those of post-isolation and fortified quality control samples (representing 100% recovery, Set B). The matrix effect was determined by comparing the response of analytes spiked into the extracted blank sample (Set C) with analytes in the reconstitution solution at the same concentration dissolved (Set D), respectively.

Stability: Stability experiments were performed to demonstrate whether all the compounds were stable under different storage and typical process conditions. In this assay, freeze-thaw (-20 °C, three

cycles), long term (-20 °C, 14 days) stability, room temperature stability (25 °C, 6 h) and autosampler (25 °C, 12 h) samples were determined at six replicates at each QC concentration.

Pharmacokinetic study

Ten healthy male rats (220-250 g) were purchased from the Experimental Animal Center of West China, Sichuan University. The rats were allowed one week acclimation period in the animal quarters under air conditioning (25 ± 1) °C and an automatically controlled photoperiod of 12 h light daily. Before the experiments, animals were fasted for 12 h with access to water and were randomly divided into two groups (each 5 rats): the low dosage group and the high dosage group. The Lvfei™ was dissolved in water with 0.5% CMC-Na to achieve a concentration of AZT and IZM were 6.241 mg·mL^{-1}, 3.927 mg·mL^{-1} for the low dosage group, 17.98 mg·mL^{-1}, 11.31 mg·mL^{-1} for the high dosage group, respectively. Each group was received an oral administration of 10 mL·Kg^{-1}. Venous blood samples (300 µL) were collected at the following times after administration: 0.000, 0.167, 0.333, 0.500, 1, 2, 4, 6, 8, 12, 24, and 48 h. Blood samples were centrifuged at 8000 g for 10 minutes, and then 100 µL of plasma supernatant was collected. Plasma samples were stored at -20 °C until analysis and were processed as per the extraction procedure described earlier. The study protocol was approved by the Animal Ethics Committee of Sichuan University (Chengdu, Sichuan, China). The pharmacokinetic parameters of analytes were calculated using Phenix Winnolin 6.3 (friendly provided by XPiscoric).

Results

LC–MS/MS method development and optimization

In order to obtain good sensitivity and chromatographic resolution in a short run time, a reliable LC-MS/MS method was developed and validated to simultaneously detect both analytes in a single biological sample. For the optimization of chromatographic conditions, an Agilent SB-C8 column (5 µm, 4.6 ×250 mm) and an Inertsil ODS-2 (5 µm, 4.6 ×150 mm) were evaluated, the results showed that Inertsil ODS-2 column (5 µm, 4.6 ×150 mm) performed better peak shape and more appropriate retention time. Better chromatographic separation was observed using methanol than acetonitrile. It was confirmed that the addition of formic acid and 10 mM ammonium acetate had indistinctive improvement in chromatographic separation compared to the mobile phase only added formic acid. The retention time of AZT, IZM and IS were 2.75, 7.60 and 3.14 minutes, respectively.

Development of sample pretreatment method

Because of the extremely high sensitivity of LC-MS/MS for each of the analytes, it was essential to establish a method for extracting the analytes from relatively large complex endogenous biological matrices. In this study, the optimization of pretreatment was investigated by comparing protein precipitation with liquid-liquid extraction. It is showed that protein precipitation performed significant endogenous impurities interference; low response of analytes and unstable results of determination, while liquid-liquid extraction generated a narrow and symmetric chromatographic peak with an optimal resolution, the extraction recovery is better and more stable. Taking into the lipid solubility of analytes and environmental friendly extractive solvents, ethyl acetate was used as an extractant instead of dichloromethane and chloroform.

Method validation

All the analytes in the text produced a prominent, protonated molecular ion $[M+H]^+$ in positive-ion mode The MRM transitions and optimized, collision-induced dissociation conditions are described in Table 1, the most intensive precursor→fragment transitions are shown in Figure 1. Typical MRM chromatograms for blank blood plasma, blank plasma spiked with M-QC levels of analytes and plasma collected from 45 minutes after oral administration of Lvfei™ are shown in Figure 2. No significant interference was observed in drug-free plasma sample at the retention times of the target drugs and IS.

Analyte	Precursor (m/z)	Product (m/z)	DP (V)	CE (eV)	CXP (V)
AZT	404.3	372.1	57.01	22.86	9.33
IZM	360.3	244.1	61.80	33.66	15.64
IS	180.1	110.3	54.98	30.89	19.12

Table 1: MRM transitions and MS fragmentation parameters for the analytes and internal standard (IS)

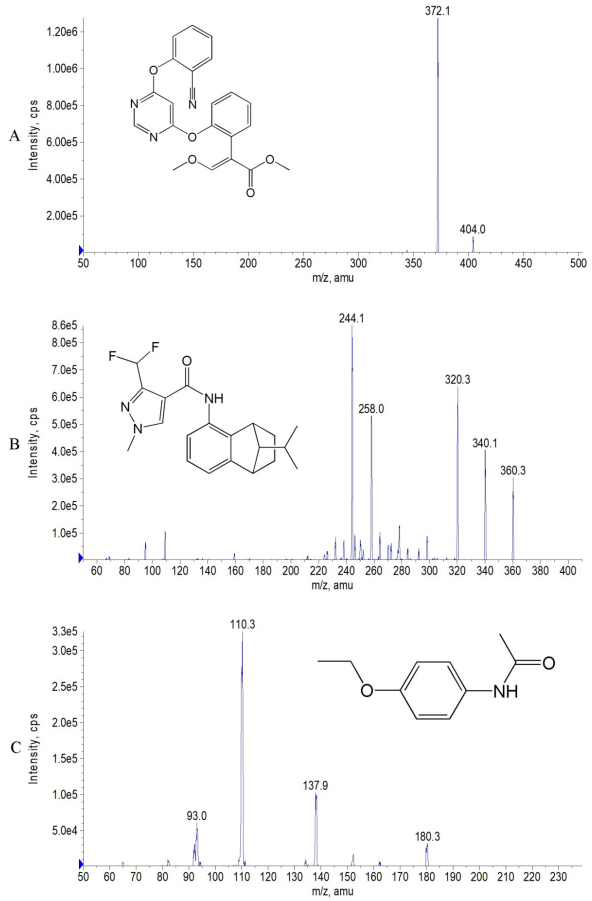

Figure 1: MS/MS spectra and chemical structures of AZT (A), IZM (B) and IS(C)

The nine-point calibration curves for each analyte were linear over the ranges 0.8-800 ng·mL^{-1} for AZT, 0.5-500 ng·mL^{-1} for IZM with correlation coefficients (R^2) greater than 0.999 [Table 2]. The best-fit line of the calibration curves was obtained by using a weighting factor of 1/x for AZT and IZM. LLOQ at 0.8 ng·mL^{-1} for AZT and 0.5 ng·mL^{-1} for IZM.

The precision and accuracy of intra- and inter-day were within the acceptable limit for both analytes [Table 3]. The intra-day accuracies (RE%) ranged between 0.09% and 7.37% with precisions (RSD%) of 3.92% - 8.06% at the low, middle, and high concentrations. The inter-day data were also accurate and reproducible with accuracies between 0.60% and 8.79%, precisions between 3.86% and 9.67%.

Results summarized in Table 4 showed that extraction recoveries and matrix effect for AZT and IZM. The extraction recoveries ranged between 80.79% to 94.34% and the average matrix factor values ranged from 96.96% to 110.5%. No significant endogenous interference was observed near the retention times between the different lots assays. Due to the significant differences in polarity of

Analyte	Regression equation	R2	Concentration range(ng·mL^{-1})	LLOQ (ng·mL^{-1})
AZT	y = 0.0201x + 0.1101	0.9995	0.8-800	0.8
IZM	y = 0.0083x + 0.0019	0.9999	0.5-500	0.5

Table 2: Regression parameters of standard curves and LLOQ for determination of two fungicides in plasma during method validation

Analyte	Level	Intra-day(n=6)			Inter-day(n=6)		
	(ng·mL^{-1})	Mean (ng·mL^{-1})	RSD (%)	RE (%)	Mean (ng·mL^{-1})	RSD (%)	RE (%)
AZT	1.60	1.61	7.66	0.09	1.60	6.75	-0.60
	40.0	43.29	5.94	7.8	43.69	6.43	8.79
	400	374.3	3.92	-6.80	375.4	3.86	-6.52
IZM	1.00	1.02	4.06	1.41	1.02	9.67	1.46
	25.0	26.75	8.06	6.67	26.73	7.32	6.59
	250	269.3	6.61	7.37	256.1	8.61	2.13

Table 3: Intra- and inter-day accuracy and precision of analytes

Analyte	Level (ng·mL^{-1})	Set A (Mean±CV, %)	Set B (Mean±CV, %)	Recovery[a] (%)	Set C (Mean±CV, %)	Set D (Mean±CV, %)	Matrix effect[b] (%)
AZT	1.60	140804±9.70	161906±2.71	86.97	45172±5.08	43807±3.30	103.1
	40.0	1098860±3.34	1311180±9.29	83.81	1095560±5.50	1123140±6.09	97.54
	400	9563680±4.09	10258600±10.5	93.23	9636440±3.45	9398780±4.43	102.5
IZM	1.00	15670±3.93	19396±4.38	80.79	6230±0.40	6425±1.33	96.96
	25.0	149274±9.24	168402±1.60	88.64	171706±4.11	175750±6.95	97.70
	250	1378740±5.74	1461500±10.0	94.34	1604020±12.12	1452240±4.45	110.5

Table 4: Extraction recovery and matrix effect of the analytes and IS (n=6)

a. Recovery%= Set A/ Set B× 100%

Matrix effect%= Set C/ Set D × 100%

Analyte	Level (ng·mL^{-1})		Bench-top (25 °C,6 h)		Autosampler (25 °C, 12 h)		Freeze-thaw (-20°C ,three cycles)		Long-term (-20°C ,14 days)	
			RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
AZT	1.6		7.17	-5.7	1.98	-6.76	10.00	2.93	1.29	3.31
	40		5.91	-0.73	8.57	0.23	9.21	-5.90	2.81	7.70
	400		4.80	7.99	4.23	2.65	5.19	-11.11	5.63	2.11
IZM	1		7.78	-6.31	7.08	1.48	11.19	2.55	6.51	2.27
	25		13.40	-7.48	6.71	-10.56	6.56	-11.48	3.88	-12.80
	250		13.06	0.06	4.83	3.46	5.48	-11.89	11.23	-8.86

Table 5: Stability data of analytes in rat plasma (n=6)

three substrates, some components such as AZT was unable to be extracted completely, only arrived at 80.79%, approximately. These values were from combined contributions from recovery loss and matrix suppression.

Stability had been tested for bench-top (25 °C, 6 h), autosampler (25 °C, 24 h), freeze-thaw (-20 °C, three cycles), short-term (-20 °C, 7 days) and long-term (-20 °C, 14 days) stabilities. The results summary of stability [Table 5] indicated that each analyte in bio-samples was of good stability.

Application of the method in pharmacokinetic study

The developed method was successfully applied to simultaneously determine concentrations of the target drugs in rat plasma after concomitant oral administration of pesticide to 10 healthy rats (the half rats with low dose and half with high dose). The pharmacokinetic parameters were calculated using Phenix Winnolin 6.3 and summarized in Table 6. The mean plasma concentration–time profiles of AZT and IZM were shown in Figure 3. Comparing two different doses for single analyte, significant influences for pharmacokinetic parameters were observed. High dose group has higher C_{max}, longer T_{max}, and greater AUC_{0-t} for both analytes. The low and high doses for AZT were detected 1.0 h after dosing with C_{max} values of 247.8 and 450.2 ng·mL^{-1}, respectively. The peak of AZT in high dose continued for a period time after arrived C_{max}. The C_{max} values of IZM in two doses were detected 26.69 and 53.42 ng·mL^{-1}, respectively. In addition, AZT and IZM showed similar pharmacokinetic characteristics in high dose groups, presenting the double peaks. It is suggested that AZT and IZM at high concentration might be reabsorbed in vivo. None of earlier studies refered to pharmacokinetic characteristics of AZT or IZM.

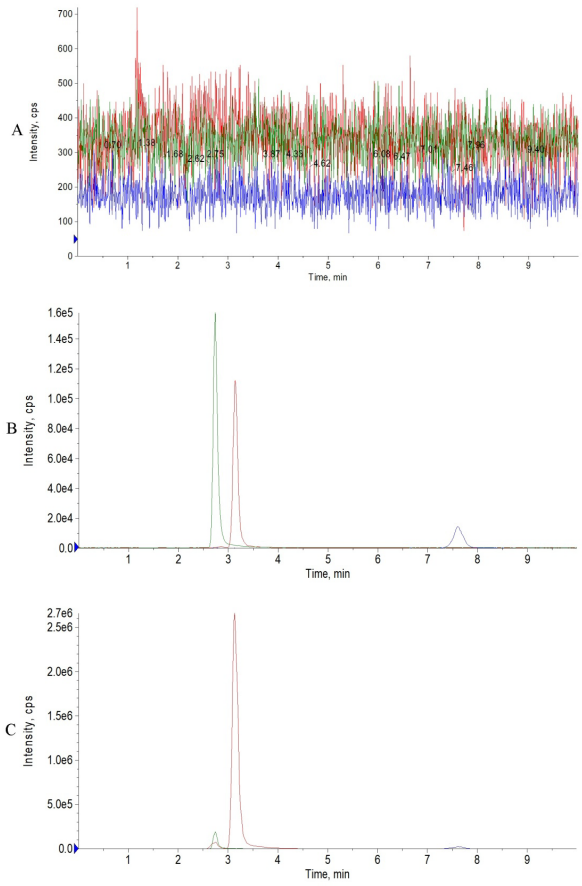

Figure 2: Representative MRM chromatograms of AZT, IZM and phenacetin (IS) in rat plasma: (A) blank plasma; (B) blank plasma spiked with two analytes (QC-M level) and IS; (C) plasma at 1 h after oral administration of the suspensions of two fungicides

Parameter (unit)	AZT		IZM	
	Low dose	High dose	Low dose	High dose
C_{max} (ng·mL^{-1})	247.84*	450.2	26.69	53.42
T_{max} (h)	2.00	12	0.25*	12
$T_{1/2}$ (h)	5.21*	2.97	12.87*	5.28
AUC_{0-t} (ng·h·mL^{-1})	3273.80**	14289.06	378.11**	1304.97
$AUC_{0-\infty}$ (ng·h·mL^{-1})	3288.00**	14294.72	398.27**	1315.02
MRT_{0-t} (h)	10.86**	18.11	15.38	16.66
$MRT_{0-\infty}$ (h)	11.06**	18.12	17.97	16.96
Cl/F (L·h^{-1}·Kg^{-1})	18.98	12.57	98.60	86.01
Vz/F (L·Kg^{-1})	142.60	53.89	1830.57	654.64

Table 6: Pharmacokinetic parameters of AZT and IZM at different levels

*P<0.05, significantly different from high dose group; **P<0.01, highly significantly difference

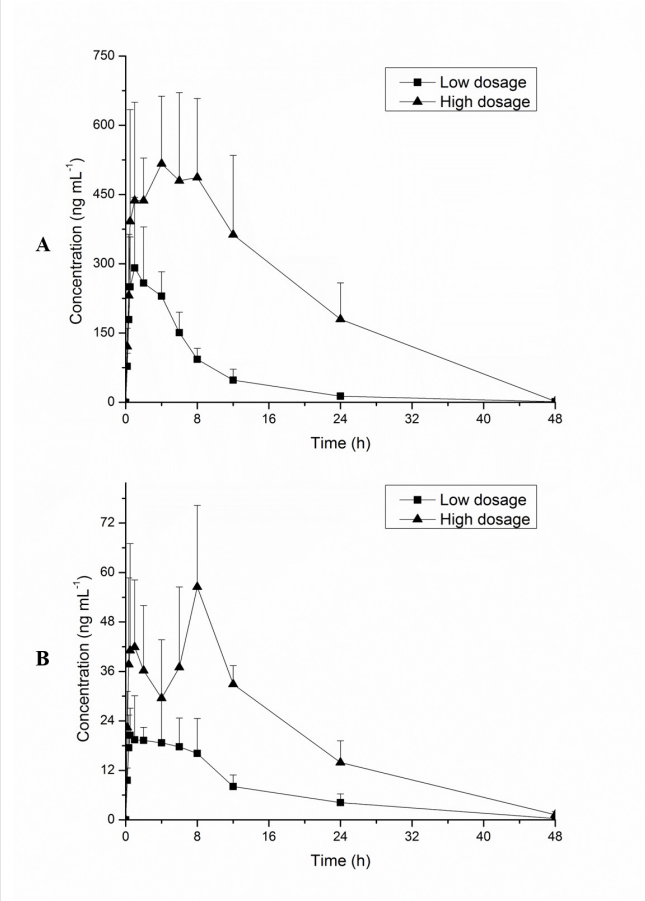

Figure 3: Plasma concentration-time curves of AZT (A) and IZM (B) in rats after oral administration of low and high dosage. Data represent mean ± SD (n=5)

Therefore, it is necessary to explore the metabolic pattern of single analyte to investigate combined medication whether have potential drug–drug interactions among them on metabolism for further study.

Statistical analysis

AZT and IZM activities were evaluated by comparing their pharmacokinetic behaviors in low dose and high dose groups, the statistically significant differences of pharmacokinetic results between two treatment groups were calculated by Student's t-test in SPSS 24.0. Differences that reached a *p* value less than 0.05 were considered statistically significant. Results showed that there were significant differences (P<0.05) in the all parameters except T_{max} and Cl for AZT, while the differences in kinetic parameters of IZM were relatively small, only T_{max}, $T_{1/2}$ and AUC showed significant differences.

Discussion and Conclusion

The present work is the first time to report a HPLC-MS/MS method for simultaneous determination of the AZT and IZM in biological matrix, and it has been fully validated reliable, precise and sensitive. Besides, the method required a small volume of plasma and the sample preparation technique was efficiency and inexpensive. The low LLOQs and wide range of linearity confirm our method selective and credibly acceptable. Results reveal that the method is available for pharmacokinetic study with satisfying precision and accuracy.

General population is exposed to pesticides not only primarily through diet, but also through inhalation of polluted air. Castorina et al. have ever reported that residues of the non-persistent pesticides and their metabolites were measured in human maternal, umbilical cord sera, and urine [22]. Apparently, the potentially highly exposed

through dermal and inhalation pathways are unavoidable, especially for farmers that handle or apply pesticides as well as other professional pesticide-handlers. Even more so, it can't exclude the possibility of some accidental/incidental sources [23]. So, biomonitoring techniques for determining internal doses of chemicals have become quantitative tools for evaluating human exposure [24].

Although the trace of pesticide residua in most of crops [25], considering response of determination, the experimental dose of toxicological analysis is determined to the lowest dose value reported to elicit an adverse response (i.e. the LOAEL value) or the highest possible value reported to elicit no adverse effect at all (i.e. the NOAEL value). Due to lack of reference of literature and analytical limitations of detection, the two optimize oral administrations are conducted in current study by incorporating these considerations.

The pharmacokinetic of specific environmental contaminants and consumer chemicals has been extensively analyzed in the past. However, they mainly focus on carbamate pesticides or organochlorine pesticides, there are few literatures reported that relevant pharmacokinetic of SDHIs (IZM) and Methoxy acrylate pesticide (AZT). The quantitation of pesticides in blood, although technically challenging, represents the most relevant biomarker of exposure [26]. Taking ethical and cost-related reasons to consideration, it has been increasingly selected that use of animals for assessing mode of action, metabolism and toxicity of pesticides.

The physiologically based pharmacokinetic (PBPK) models are ideally suited for assessing dosimetry and biological responses following exposures. The present study was part of a larger research project, the PK model of AZT and IZM were conducted, which assumes the pharmacokinetic response in rats and humans is independent of gender. With the development of PBPK/PD model, a future aim will be to establish a pharmacodynamics (PD) model of AZT and IZM, combining with PK data from this study, a comprehensive PBPK/PD model will better fit mechanism of action in human body. In a word, this PK model is capable of quantifying dosimetry in plasma and dynamic response in rats and can be used to estimate toxicological risk for human as a biological base.

Due to a lack of knowledge regarding internal uptake of AZT and IZM into the human, the relative importance concerning their metabolism in biological matrix (e.g. blood) need to be clarified. In addition, this in vivo experiment was to elucidate potential mechanisms of action as well as to determine the potency of the pesticides. Indiscriminate use of pesticide is an important factor that leads to health and environmental problems. This paper elucidated indirectly influences of pesticide on the human by monitoring the pharmacokinetic behaviors in rats. What's more, the different dosages used in the study to investigate the tolerability and toxicity of two fungicides. Most of rats had symptoms of loose stools and breathe with noise, which were observed 0.33 h and 6 h after oral administration, respectively. Upon evaluation of the toxicological data, the results showed that great cautions should be taken to control the use of fungicide, a strong residual administration of fungicides also should be run before crops came into market to reduce adverse reactions for human and avoid drug accumulation. In addition, both AZT and IZM showed double peaks in high dose groups. This phenomenon suggested that there may be two high-risk periods after toxication of AZT or IZM, which should arouse great cautions in clinical treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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