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Using miniature MS system with automatic blood sampler for preclinical pharmacokinetics study

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Aim: Preclinical pharmacokinetic studies are an essential part of modern drug development. In this work, we explored a new solution with onsite analysis using a miniature MS system, which can significantly improve the efficiency of the preclinical study. **Materials & methods:** A miniature mass spectrometer was used with an automatic blood sampler for onsite quantitation of drug compounds in whole blood samples. Slug-flow microextraction was used to replace the in-lab sample preparation. **Results & conclusion:** Animal studies were carried out using two drug compounds, using the auto sampler to take blood samples at preprogrammed time points. The miniature MS system was used to obtain drug concentrations, which were subsequently used to calculate the pharmacokinetic parameters.

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Preclinical pharmacokinetics (PK) studies are an essential part of modern drug discovery. By performing preclinical PK studies, candidate compounds with improper PK properties are excluded from later, costlier clinical studies [1]. Many new approaches have been explored to improve the throughput of preclinical PK studies, including the recent use of dried blood spots [2], which provides faster sample collection with low sample volumes and easier sample storage. New strategies, such as cassette dosing [3] and snapshot PK [4], were also suggested and have attracted many follow-up studies. In cassette dosing, multiple compounds are given to one animal, whereas in conventional methods, we use only one compound. For cassette dosing, full PK data are collected while for snapshot PK, only partial PK data are collected for a quick decision. These strategies can help to save animal resources as well as time, at an expense of the quality and comprehensiveness of the PK data.

Currently, in the animal labs, sample collection can be highly automated. Automatic blood samplers can now be used for collection of samples through a catheter connected to an animal's artery, while the animal moves freely inside the cage. It is believed that the PK study results could be more reliable without the stress induced by manual handling of the animals [5,6]. It has been demonstrated that the catheterization could create an initial stress to the rats, which could be quickly recovered after the surgery. The subsequent blood draws by the autosampler would not induce corticosterone release in blood [7]. The most common practice for blood sample analysis is to use LC–MS or LC–MS/MS [8]. This analytical method can produce high-quality data; however, when used for screening a large number of drug candidates, high workload is demanded for sample preparation. In a modern analytical lab, the entire analysis process can also be automated. Although both the sample collection at the animal lab and the chemical analysis in the analytical lab can be highly automated, the sample transfer between the labs, however, involves additional logistics that slows down the entire process of the animal studies.

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Figure 1. Chemical structures of the drug compounds used in the study.

The recent development of miniature MS analytical systems has an important implication on the future means of conducting PK studies. The chemical analysis might be performed in real time on the site of sample collection. In this study, a miniature MS system was used for quantitative analysis of blood samples taken by a Culex autosampler (Culex ABS, BASi Inc., IN, USA) for a demonstration in PK study. Two essential components in the technical development for miniaturization of MS systems are the use of ambient ionization to simplify the sample preparation process and the development of the miniature mass spectrometers that can be readily used on site. Since the invention of desorption electrospray ionization [9] and direct analysis in real time [10], many new ambient ionization methods have been developed [11–13]. The most important feature of ambient ionization is that it does not require sample preparation, so direct analysis of raw samples, such as whole blood, urine or tissues, can be performed. Ambient ionization methods have been quickly used in applications such as illicit drug detection [14] and therapeutic drug monitoring [15]. Among many of the ambient ionization methods, the paper spray or paper capillary spray ionization has been proven to be very effective for quantitative analysis of drugs in biofluids, including whole blood, serum and raw urine [16–19]. Coated blade spray method combines the sample collection using solid-phase microextraction and ambient ionization, and was demonstrated for quantitative analysis of urine and plasma samples [20]. Slug-flow microextraction (SFME) was also developed for direct analysis of biofluid samples in liquid forms [21]. This is a single-step in-capillary microextraction method. Two adjacent plugs were formed by sequentially injecting microliters of organic solvent and biofluids. Liquid–liquid extraction would be expected but of low efficiency due to the small area of contact; however, the extraction speed could be significantly increased with the in-plug flows induced by the movements of the two plugs. This is a simple but very efficient technique to extract target analytes directly from whole blood. In this study, this method was adopted for direct analysis of the blood samples taken by the Culex autosampler.

The analyte analysis was performed using a home-built miniature mass spectrometer, Mini 12 [22], which could perform MS/MS analysis. This system was a 25-kg system with pumping system all included. It has MS/MS capability, which is required for direct analysis of complex samples. Contrary to the conventional way of performing analysis by transferring the sample from an animal lab to an analytical lab, the entire PK study can be performed onsite from sample collection (Culex autosampler) to sample preparation (SFME) and then MS analysis (Mini 12). In this study, we demonstrated this concept by measuring the PK of two drugs in rat whole blood samples: sitagliptin (STG, Januvia, Merck Research Laboratories, NJ, USA), an oral antihyperglycemic of the dipeptidyl peptidase-4 inhibitor class used for treating type II diabetes, and imatinib (IMB, Gleevec, Sigma-Aldrich, MI, USA), a protein-tyrosine kinase inhibitor used for treatment of leukemia. Figure 1 shows the chemical structures of these two drug compounds.

Materials & methods

Equipment & chemicals

STG phosphate and STG-d4 were provided by Merck Research Laboratories, IMB-d8 was purchased from Alsachim (Strasbourg, France), and IMB mesylate and all other chemicals were purchased from Sigma-Aldrich.

Figure 2. Workflow of the pharmacokinetics study, Mini 12 and Culex ABS are coupled using offline slug-flow microextraction.

Borosilicate glass capillaries with 1.5 mm outer diameter and 0.86 mm internal diameter were purchased from Sutter Instrument (CA, USA); some of them were used for SFME directly and some were fabricated to have the tips pulled using a micropipette puller (model P-1000, Sutter Instrument) for performing nanoESI.

The miniaturized mass spectrometer Mini 12 was previously reported [22]. Mini 12 was designed to operate with ambient ionization sources and is suitable for point-of-care applications. Blood samples were taken by Culex ABS autosampler, which was manufactured by BASi, Inc. Culex ABS was capable of sampling rodent blood automatically at programmed time points.

Animal studies

Sprague Dawley male rats were purchased from Envigo (Huntington, UK). A catheter was implanted into each rat's artery. The rats fasted for about 15 h before oral gavage with STG or IMB. The dosing solutions were made by dissolving the drugs in deionized water with concentrations of 5 mg/ml for both STG and IMB. The dosage was 20 mg/kg for both STG and IMB. The STG was dosed to one rat and the IMB was dosed to four rats.

The Culex autosampler was programmed for sampling at a series of time points within a 24-h period. The time points chosen for STG were 0.5, 1, 2, 3, 6, 12, 24 h after dosing, and 0.5, 1, 2, 3, 4, 6, 8, 16, 24 h after dosing for IMB. The 0-h concentrations were set as 0 ng/ml. At each time point, 100 µl of whole blood was sampled from each rat and stored in vials coated with heparin at 4°C. PK parameters were calculated using Winnonlin Enterprise v5.3 (Pharsight Corp., CA, USA).

Results & discussion

The workflow used in the study is summarized in Figure 2. Culex ABS was used to collect whole blood samples each of 100 µl automatically from the rat. The internal standards (IS) solution was added into the blood samples. For analysis, 10 µl of the sample, mixed with IS, was transferred to a borosilicate glass capillary for SFME. After SFME, the extraction solvent was transferred to a nanoESI capillary with a pulled tip for quantitative analysis using Mini 12. The blood samples were analyzed shortly after they were collected, the sample preparation and MS analysis took about 5 min, so the requirements of sample transfer and storage was minimized.

MS/MS methods using Mini 12

Mini 12 mass spectrometer featured a discontinuous atmospheric pressure interface (DAPI), which enabled the coupling of ambient ionization sources with a miniature ion trap mass spectrometer of small pumping capacity [23]. The mass analyzer used was a rectilinear ion trap [24]. MS/MS functions were developed and optimized using standard solutions. STG, STG-d4, IMB and IMB-d8 were diluted from high concentration stock solutions to 500 ng/ml ones, using methanol as the solvent. These solutions were sprayed in using nanoESI directly to Mini 12 at a spray voltage of 1800 V. A DAPI open time of 15 ms and a scan rate of 5000 Da/s were used for all analysis.

Figure 3. MS/MS spectra of (A) STG, (B) STG-d4, (C) IMB and (D) IMB-d8 by nanoESI-Mini 12. IMB: Imatinib; STG: Sitagliptin.

To perform MS/MS on Mini 12, a stored-waveform inverse Fourier transform (SWIFT) was used to isolate the precursor ions first, and then an AC voltage was applied to excite the ions for collision-induced dissociation (CID). The trapping RF frequency was 762 kHz and the AC frequencies for CID of STG (m/z : 408), STG-d4 (m/z : 412), IMB (m/z : 494) and IMB-d8 (m/z : 502) were 98.6, 97.3, 80.5 and 78.6 kHz, respectively. The MS/MS spectra are shown in Figure 3. The major product ion peaks observed were at m/z : 235, m/z : 239, m/z : 394 and m/z : 394 for STG, STG-d4, IMB and IMB-d8, respectively. They were selected for subsequent quantitative analysis.

For quantitative analysis using IS, typically the product ion intensities of the analyte and IS were recorded with two adjacent MS/MS scans. In the case of STG using STG-d4 as the IS, however, their product ions are of different m/z values at 235 and 239. A single-scan method was then adopted. A SWIFT with a notch window of 5 kHz centered at 165 kHz was used to isolate both STG m/z : 408 and STG-d4 m/z : 412; the RF amplitude was then lowered and the CID AC signal was then applied twice at two frequencies 98.6 and 97.3 kHz to fragment STG and STG-d4; a spectrum was recorded and the ratio of the intensities of the peak m/z : 235 to m/z : 239 (A/IS ratio, analyte to IS ratio) was obtained using a single scan.

For IMB and IMB-d8, the product ions were the same at m/z : 394. Two scans were used to obtain the A/IS ratio. A SWIFT with a notch window of 5 kHz centered at 165 kHz was used to isolate the precursor ions; the RF amplitude was lowered and the CID AC signal voltage was then applied, either at 80.5 kHz for IMB or 78.6 kHz for IMB-d8, to fragment them. The A/IS ratio was then calculated using the fragment ion intensities obtained from the two adjacent MS/MS scans.

Figure 4. Principle of offline slug-flow microextraction nanoESI with Mini 12.

Quantitative analysis using SFME

SFME was used in this study to extract drug molecules directly from rat whole blood samples. The working principle of offline SFME is shown in Figure 4. Whole blood of 10 μ l was first loaded to the borosilicate glass tube, and then 10 μ l extraction solvent was loaded to the tube, in contact with the blood sample plug. The selection of the extraction solvent was crucial [21]. The solvent needed to be immiscible with blood, had reasonable solubility for the target analytes and could also be used for nanoESI. Ethyl acetate was used in the current work because it met these requirements and both STG and IMB could be extracted from whole blood samples at good efficiencies. A pipette gun was used to make the push-and-pull movements of the two plugs, which induced the turbulence inside the sample and solvent plugs and facilitated efficient extractions [21]. After SFME, the extraction solvent was transferred to a nanoESI capillary for MS/MS analysis using Mini 12.

In our previous research, it was found that the equilibrium of the extraction could be reached during the SFME after a certain number of movement cycles, which could be as low as 5 cycles for extracting drug molecules from blood samples [21]. In this work, 10 cycles were used for SFME of each blood sample to ensure that the equilibrium was achieved. For analysis using Mini 12, the distance between the tip of nanoESI tube and the DAPI inlet was optimized at 1 cm.

Matrix-matched calibrations were made for both STG and IMB. Calibration standards were made by diluting concentrated stock solutions with methanol to target concentrations. The diluted STG or IMB solutions were then added to rat whole blood samples together, and their corresponding IS solutions were also added. The volume ratio of drug solution:IS:rat whole blood was 1:1:98. For STG calibration standards, the final concentrations of STG were 50, 200, 500, 1000 and 2000 ng/ml, with the IS STG-d4 at a concentration of 200 ng/ml in all standards. For IMB calibration standards, the final concentrations of IMB were 50, 200, 500, 1000, 2000 and 5000 ng/ml, and the concentration of IMB-d8 was 500 ng/ml in all standard samples. Each aliquot of 10 μ l blood calibration sample prepared was processed with SFME using 10 μ l ethyl acetate. Three SFME–MS analyses were repeated for each concentration, and the average of 5 MS/MS scans were taken to calculate the A/IS ratio for each standard. The calibration curves are shown in Figure 5.

A good linearity was observed for each of the calibration ranges selected, viz. 50–2000 and 50–5000 ng/ml for STG and IMB. The LOQs were 50 ng/ml for both STG and IMB. The linear range might be further expanded toward the higher concentration ranges, but is considered unnecessary for the PK studies in this work.

In order to validate the method for its accuracy and precision, quality control samples were made separately and analyzed using exactly the same method. These samples were made in the same way as the calibration standards were made, except that their concentrations were 50, 500 and 2000 ng/ml for STG and 50, 1000 and 5000 ng/ml for IMB, covering the low, medium and high concentration levels within the linear range. Both intraday and interday assays were performed. The intraday assays were carried out immediately after the calibration standards were measured, while the interday assays were performed 24 h after intraday assays, using the same samples. The

Figure 5. Calibration curves of (A) STG and (B) IMB. IMB: Imatinib; STG: Sitagliptin.

Table 1. Results of quality control experiments.

Compound	Target concentration (ng/ml)	Concentration found (mean, ng/ml)	Precision (% RSD)	Accuracy (%)
STG	Intraday			
	50	41.8	13.6	83.6
	500	542.5	4.7	108.5
	2000	1978.0	6.5	98.9
	Interday			
	50	39.8	11.4	79.6
IMB	500	497.9	9.9	99.6
	2000	1943.0	12.4	97.2
	Intraday			
	50	58.6	12.2	117.3
	1000	1147.9	1.3	114.8
	5000	4959.3	10.1	99.2
	Interday			
	50	61.7	3.4	123.4
	1000	1129.7	4.0	113.0
	5000	4622.0	5.1	92.4

n = 3.
IMB: Imatinib; STG: Sitagliptin.

results are listed in Table 1. Satisfactory precision and accuracy were obtained with RSD <15% for all samples analyzed intraday or interday and accuracy <15% except for LOQ concentrations for which accuracy <20% was obtained, considered acceptable [25]. These test results indicate that the current method is reliable and could be used for PK studies.

PK analysis with Culex ABS autosampler

As described above, whole blood samples were taken from the rats automatically using Culex autosampler at programmed time points. For each time, 100 μ l whole blood was taken and analyzed without performing typical in-lab sample processing or chromatographic separation. IS solution, 1 μ l containing STG-d4 or IMB-d8, was spiked into the 100 μ l blood samples to make a final concentration of 200 or 500 ng/ml, respectively. Aliquots of 10 μ l were then taken for analysis using SFME and Mini 12. The concentrations were calculated using the

Figure 6. Whole blood concentration profile of sitagliptin.

Figure 7. Whole blood concentration profile of imatinib.

A/IS ratio obtained and the calibration curves established. As a pilot experiment, one rat was dosed with STG to establish the workflow. Figure 6 shows the calculated whole blood concentrations of STG, plotted as a function of time after dosing. The concentration of STG in rat whole blood increased very rapidly after dosing, reaching the maximum concentration of 1790 ng/ml in about 2 h. After that, the concentration began to drop rapidly, reaching an undetectable level at 24 h after dosing. Other PK parameters were calculated using noncompartmental analysis, including the area under the curve from time 0 to infinity ($AUC_{0-\infty}$) as 3999 $\text{ng}\cdot\text{ml}^{-1}\cdot\text{h}$, half-life time ($t_{1/2}$) as 2.58 h and apparent clearance (CL/F) as 4894 $\text{ml}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$.

Figure 7 shows the PK data collected from four rats for IMB. Similar trends were observed for all the rats, with the concentration increasing rapidly immediately after dosing, reaching the maximum in 3–4 h and then dropping rapidly. At 24 h after dosing, there were still significant amounts of IMB presenting in blood for all

Table 2. Pharmacokinetics parameters calculated for imatinib in rats.

	Unit	Rat 01	Rat 02	Rat 03	Rat 04
AUC _{0-inf}	ng•ml ⁻¹ •h	10,418	31,754	20,129	12,931
C _{max}	ng•ml ⁻¹	992.6	3006	1476	2055
T _{max}	h	4	3	3	4
t _{1/2}	h	6.01	3.24	7.37	5.26
CL/F	ml•h ⁻¹ •kg ⁻¹	2066	728	1103	1650

rats. Interindividual differences among rats were observed. The PK parameters were calculated for each rat using noncompartmental analysis and summarized in Table 2.

Conclusion

In summary, we have demonstrated a method for preclinical PK studies using a miniature ion trap MS system with an automatic blood sampler. We were able to extract target compounds from rat whole blood samples using SFME and perform quantitative analysis of the liquid samples without using traditional in-lab sample preparation and chromatographic separation. With limited number of rats used in an animal study, we demonstrated the feasibility of onsite analysis using miniature MS systems for preclinical PK studies.

Future perspective

An integration of a blood autosampler with a miniature mass spectrometer would be a logical next step to create an automated on-rack analytical system for preclinical study. This would certainly provide a unique and useful tool for the biologists to obtain the data readily, without sending the samples to the analytical labs. With the rapid development of ambient ionization and miniature MS systems for direct chemical analysis, the technologies for analysis of biofluid samples will mature and become applicable for biomedical use, including the clinical studies and disease diagnosis.

Summary points

Background

- The benefits of automatic blood sampler for preclinical pharmacokinetics studies.
- The current status of ambient ionization and miniaturization of mass spectrometer.

Results & discussion

- MS/MS method established for quantitation using a miniature MS system Mini 12.
- Quantitative analysis of whole blood samples using slug-flow microextraction (SFME).
- Combination of sampling by autosampler, fast processing by SFME and direct analysis using Mini 12.

Conclusion

- Preclinical pharmacokinetics study was carried out using an automatic blood sampler coupled with Mini MS, with a fast sample processing by SFME.

Conflict of interest

Z Ouyang is the founder of the PURSPEC Technologies, Inc.

Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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