CherryPicker II system:
A powerful tool for HTS-ADME automated assay

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Introduction

High-throughput screening *in vitro* absorption, distribution, metabolism and excretion (HTS-ADME) has become an essential part of lead optimization in drug discovery. HTS-ADME involves various assessment such as metabolic stability, membrane permeability, and plasma protein binding. The assay portfolio continues to expand in clinically relevant areas such as CYP inductions and drug-transporter interactions. In this poster, we introduce a custom solution for efficiency of HTS-ADME using combined standalone instruments, including the newly developed mosquito® X1 Cherry Picker II (CP2).

Problem

- Time-consuming labor of hit-picking from compound libraries
- Expensive reagent costs
- Complicated manual operation
- Low accuracy and precision of sub-microliter dispensing

Solution

- Automated dispensing of compounds from 384-plate based library
- Cost saving by assay miniaturization
- Standardized to automatic operation
- High accuracy and precision of nanoliter dispensing

Keywords: HTS-ADME, automation, nano-dispensing, miniaturization, expensive reagent, cost-saving

2. Technology

Compact automated cherry picking

The mosquito® X1 Cherry Picker II workcell enables high-speed, automated hit-picking from microplates. It comprises a mosquito® X1 low volume liquid handler with 2 or 5 deck positions, a PF400 plate handling robot, 2 or 4 plate stacks (up to 40 plate capacity) and a barcode reader for autonomous operation.

mosquito® X1 is a single channel liquid handler based on positive displacement pipetting that utilizes low-cost, disposable tips.

Key features include:

- accurate low volume liquid handling (25 nL – 1.2 µL)
- ability to access any well in any plate, including sealed source plates
- rapid cycle times: only 6 seconds to aspirate, dispense and tip change
- outstanding accuracy – less than 2% error
- unrivalled precision – CVs less than 3.0%

3. Cherry picking software seamlessly accessed by KNIME

Cherry Picker II (CP2) software creates an optimal pipetting protocol by reading the pipetting list (CSV file format) created by the workflow of the KNIME Analytics Platform* developed by JT. Barcoded plates can be stacked in any order in the input stacks. As a plate is loaded on the mosquito® X1 deck, the barcode is read and the correct transfer for that plate is carried out. Subsequent plates are loaded and processed in the same fashion. The Cherry Picker II software automatically optimizes all protocols for efficient tip usage and optimal dispense heights, and, if required, could also track the source plate volumes for subsequent reporting.

*Free open-source software

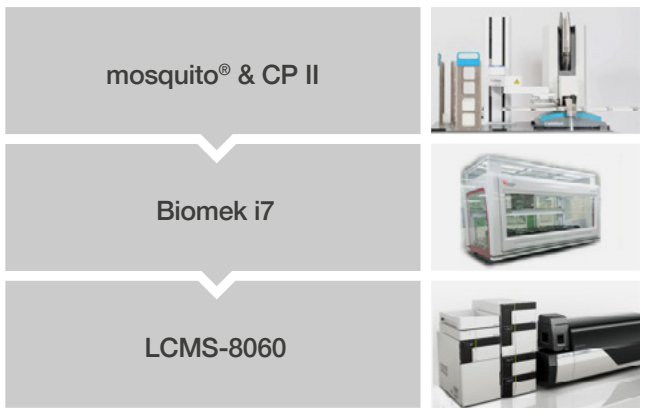
Fig. 1 Cherry Picker software followed by KNIME

4. HTS-ADME automated assay system

In order to operate various ADME screenings efficiently, JT constructed an HTS-ADME automated assay system using the CP2, Biomek i7 and LCMS-8060 (Fig. 2). The assay plates are prepared by the automated dispensing of compounds from a 384-plate compound library using CP2. The assay plates are then set on the Biomek i7 platform and the assays are performed automatically to prepare samples for measurement. The analytical instruments (LCMS-8060) are unified across assessments to use mutual optimization results and methods. Here, CP2 is specialized for compound dispensing and dilution, allowing it to operate in parallel with the Biomek i7, maximizing the capacity of each instrument. The nano-dispensing (25-1200 nL) of CP2 enabled us to miniaturize the volume of assays and save the running cost significantly by reducing reagents (as described in section 6). In addition, by preparing a complex mix of more than 10 compounds, which was not possible with conventional methods, the number of samples were reduced to less than 1/10, resulting in a greatly improvement of the throughput.

Fig. 2 HTS-ADME automated assay

HTS-ADME automation assay	
mosquito® & Cherry Picker II	
Biomek i7	
LCMS-8060	
Biomimetic Chromatography	Solubility
Liver Microsome Stability	SF-LogD
Protein Binding	Permeability
CYP inhibition	Hepatocyte Stability
CYP indcution	*in vitro* Kpuu



5. Accuracy and precision

The accuracy and precision of dispensing is important to increase the reliability of data. In this study, we evaluated the accuracy and precision of dry dispenses and serial dilutions of Ponceau S in DMSO solution. 96-well microplates (flat bottom) were used for the dry dispenses, and the results showed that the accuracy and precision of aliquots were 100±2% and <3% respectively at 50 nL or more. (A). For the serial dilutions, the accuracy was 100 ± 7% and the precision was 3%CV (B).

Table 1 Accuracy and precision for (A) DMSO dry dispensing and (B) serial dilution

A

Vol (nL)	25	50	100	500
Accuracy (%)	100.3	101.3	100.5	99.8
CV (%)	6.7	2.6	2.8	1.8

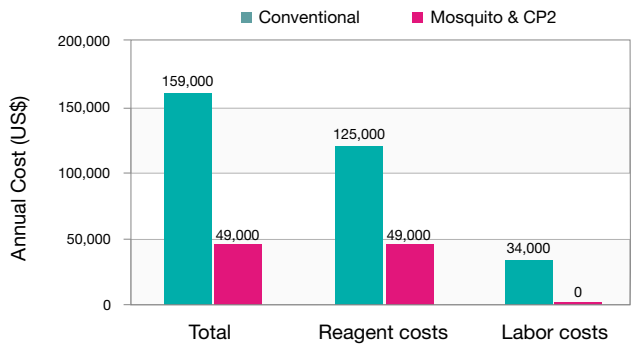
B

	1µM	3µM	10µM
Accuracy (%)	95.3	93.2	94.3
CV (%)	2.5	2.8	2.0

6. Cost savings

Human liver microsomes, an expensive reagent, are used for metabolic stability evaluation. To reduce costs, we developed a miniaturized metabolic stability assay using CP2. In our system, the reduction rates of the amounts of 10mM compounds and microsomes compared to a conventional method were 40% and 12%, respectively. In addition, the labor required for dispensing and dilution of the compounds was significantly reduced thanks to CP2. In total, US$ 110,000 was saved in an annual cost.

Fig. 3 Cost savings



Conclusions

The CP2 system was introduced for HTS-ADME.

- By combining CP2 with Biomek i7 and LCMS-8060, an automated assay system for various ADME screenings was constructed.
- The efficiency of HTS-ADME was improved by standardizing the complicated manual operation.
- High accuracy and precision of nanoliter dispensing were obtained from the CP2.
- The running cost was reduced significantly.