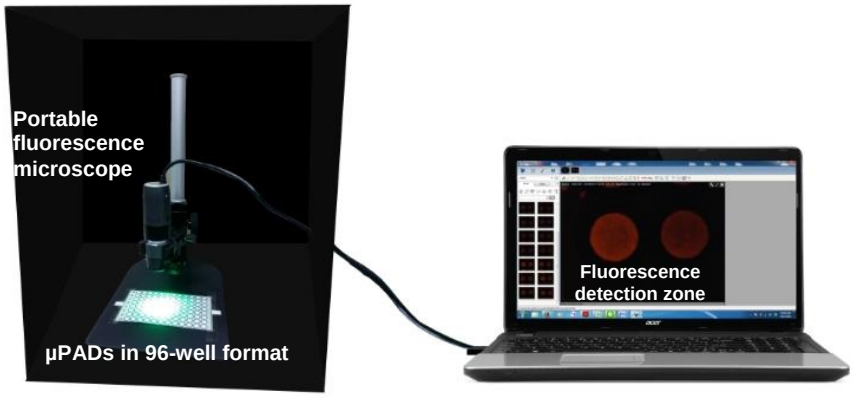
Paper-based fluorescence assays of CYP450 activities using portable microscope

Mananya Laosuksakul[1], Jiraporn Leanpolchareanchai[2], Leena Suntornsuk[1] and Nantana Nuchtavorn[1*]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Rd., Rajathevee, Bangkok 10400, Thailand*
[2]*Department of Pharmacy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Rd., Rajathevee, Bangkok 10400, Thailand*

*e-mail: nantana.nuc@mahidol.ac.th

Cytochrome P450 (CYP450) enzymes involve in drug metabolism, particularly with regard to drug interaction. Thus, CYP450 screening is essential in drug discovery in order to avoid the drug-enzyme interaction. Paper-based microfluidic devices (µPADs) in 96-well format was developed for the enzyme assay because of its low-cost, portability and low reagent consumption. Method development was performed using microplate reader (λ_{ex} 570 nm, λ_{em} 600 nm) by varying types and concentrations of buffer, and incubation time. The optimal reaction using resorufin benzyl ether as a fluorescence substrate was in 50 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM dithiothreitol, 1.3 mM NADPH and 0.02% poloxamer188, and incubation at 37°C for 25 and 30 min in the conventional plastic plate and µPADs, respectively. The fluorescence intensity obtained from µPADs (15,349.2±614.6) was superior to the plastic plate (4,434.41±105.6) due to the high surface to volume ratio of the paper. Subsequently, the fluorescence intensity of the reaction zone was measured using the microscope and ImageJ. This method showed good linearity ($r^2 \geq 0.995$ over 2.5-15 µM) and precision (RSDs < 5.2%). Limits of detection and quantitation were 0.23 µM and 0.71 µM, respectively. The validated method would be applied for screening of the potential drug candidates.



Keywords: Cytochrome P450, Portable microscope, Fluorescence substrate