

Electronic Supplementary Information (ESI)

for

Simple and rapid monitoring of doxorubicin using streptavidin- modified microparticle-based time-resolved fluorescence immunoassay

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1. Supplementary information of experimental details

1.1 Materials

EZ-Link Sulfo-NHS-LC-Biotin, Imject Mariculture KLH, Pierce® Micro BCA Protein Assay Kit and PageRuler™ Plus prestained protein ladder were purchased from ThermoFisher Scientific (Waltham, MA, USA). Doxorubicin hydrochloride (HPLC grade), Streptavidin (SA), Ovalbumin(OVA), Human serum albumin (HSA), Bovine serum albumin (BSA), 4-morpholineethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (sulfo-NHS), sulfo-NHS-LC-Biotin, ProClin-300, Tween-20, Freund's adjuvant and Freund's incomplete adjuvant were acquired from Sigma-Aldrich (St. Louis, MO, USA). Amicon® Ultra 0.5 mL Centrifugal Filters (10 KD and 50 KD) was purchased from Millipore (Bedford, MA, USA). CmMPs were obtained from JSR Life Sciences (Tokyo, Japan). DELFIA® Eu-Labeling kit and DELFIA® Enhancement Solution were obtained from PerkinElmer (Waltham, MA, USA). Sephadex G-50 was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). BALB/c mice used in this study were obtained from Experimental Animal Center, Southern Medical University (Guangzhou, China). This study had been approved and registered by the laboratory animal welfare and ethics committee of Southern Medical University (Guangzhou, China). The care and use of the animals conform to the Institutional Animal Ethics Committee guidelines. All reagents used were of analytical grade unless otherwise stated. Ultrapure water, obtained by Milli-Q water purification system (Millipore, Bedford, MA, USA), was used throughout the study. 1420 Multi-label Counter (Victor3™) and LS-55 Fluorescence Spectrometer were purchased from PerkinElmer Wallac (Turku, Finland). iMark™ Microplate Absorbance Reader

and ImmunoWash 1575 Microplate Washer were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Other instruments include UV-2550 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan), API 3200 triple quadrupole tandem mass spectrometers (ABI, Inc., Foster City, CA, USA), Prominence LC-20A high-performance liquid chromatograph equipped with LC-20ADXR binary pump, SIL-20ACXR autosampler and CTO-20A Column Oven (Shimadzu, Inc., Chiyoda-ku, Tokyo, Japan), Poroshell 120 EC-C18 column (4.6*50 mm, 2.7 μm).

1.2 Solutions

Buffer solutions used throughout this study were as follows: coating buffer (50 mM Tris-base pH 7.2), gelatin blocking buffer (50 mM Tris-base containing 0.1M saccharose, 0.05% NaN_3 and 0.1% gelatin, pH 7.2), binding buffer (50 mM MES, pH 5.0), blocking buffer (50 mM Tris-HCl, 0.05% NaN_3 and 5% BSA, pH 7.2), conjugating reagent A (10 mg mL^{-1} EDC, in 50 mM MES, pH 5.0), conjugating reagent B (10 mg mL^{-1} sulfo-NHS, in 50 mM MES, pH 5.0), stock solution (25 mM Tris-base, 150 mM NaCl, 0.05% Tween-20, 5% BSA, 0.05% ProClin300 and 1% trehalose, pH 7.2), elution buffer (50 mM Tris-HCl, 0.9 % NaCl and 0.05 % proclin-300, pH 7.8), washing buffer (50 mM Tris-HCl, 0.01% Tween-20, and 150mM NaCl, pH 7.8), labeling buffer (50 mM Na_2CO_3 , pH 9.6), standard buffer (50 mM Tris-base, 150 mM NaCl, 0.1% NaN_3 , 0.01% Tween-20 and 7.5% BSA, pH 7.8), assay buffer (50 mM Tris-HCl buffer, 1.5% polyethylene glycol 6000, 0.3 mM BSA, 0.01% Proclin-300, 150 mM NaCl, 0.02% (w/v) bovine globulin and 0.01% Tween-20, pH 7.8), All solutions were freshly prepared before use.

1.3 LC-MS/MS procedure

LC-MS/MS method was practiced following several former researches with slight adjustment¹⁻⁴ and using DAU as internal standard (IS). Working solutions of DOX (200 µg mL⁻¹) and DAU (200 µg mL⁻¹) were prepared in methanol. The working solutions were further diluted with 50% methanol (methanol: water=1:1, v:v) to obtain working standards (0, 1, 10, 50, 100, 500, 1000 ng mL⁻¹). Each standard sample was spiked with IS with the final concentration of 200 ng mL⁻¹. An extraction procedure previously shown with satisfactory recovery was chosen to extract DOX from serum samples or urine samples⁵. Sample treatment serum and urine involved a single protein precipitation with perchloric acid. Perchloric acid (35%, v/v) was added to the 50 µL of serum or urine samples followed by 62.5 µL of mobile phase. The samples were vortexed for 30 s, followed by centrifugation at 12,000×g for 10 min. 10 µL of the supernatant was used directly for LC-MS/MS.

Mass analysis was performed on API 3200 triple quadrupole tandem mass spectrometers following HPLC separation. The mobile phase used during separation contained phase A (DI water containing 0.1% formic acid) and phase B (methanol containing 0.1% formic acid). Separation was achieved by automated injection of 10 µL samples onto a Poroshell 120 EC-C18 column under isocratic conditions at a flow rate of 0.8 mL min⁻¹ at 40 °C. The column effluent was introduced into the mass spectrometer using an electrospray source positioned orthogonally to the orifice. High purity nitrogen was used as the curtain and collision gas. The result was analyzed by ABI Analyst[®] Software.