Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

The material-enabled oxygen control in thiol-ene microfluidic channels and its feasibility for subcellular drug metabolism assays under hypoxia in vitro

Iiro Kiiski[a], Päivi Järvinen[a], Elisa Ollikainen[a], Ville Jokinen[b], and Tiina Sikanen[a]*

[a]Faculty of Pharmacy, Drug Research Program, Division of Pharmaceutical Chemistry and Technology, University of Helsinki, P.O. Box 56 (Viikinkaari 5E), Helsinki, FI-00014, Finland. E-mail: tiina.sikanen@helsinki.fi

[b]Department of Materials Science and Engineering, School of Chemical Engineering
Aalto University, Espoo, FI-02150, Finland

Liquid chromatography-mass spectrometry method development and validation parameters

The parameters of the liquid chromatography-mass spectrometry (LC-MS) method used for the analysis of 3'-amino-3'-deoxythymidine (AMT) and zidovudine glucuronide (ATZ-G) in Figure 5 are given in Table S1. The LC-MS method was validated in relevant parts for quantitative detection of AMT and AZT-G. The critical method validation parameters are presented in Figure S1 (selectivity; AMT), Figure S2 (selectivity; AZT-G), Table S2 (range, linearity, accuracy, and precision; AMT), and Table S3 (range, linearity, accuracy, and precision; AZT-G).

Table S1. LC-MS method parameters used for analysis of 3'-amino-3'-deoxythymidine (AMT, m/z 241.24) and zidovudine glucuronide (ATZ-G, m/z 442.15). SRM=selected reaction monitoring.

MS parameters	**AMT, m/z 241.24**	**AZT-G, m/z 442.15**
Instrument	Waters Xevo TQ-S triple quadrupole MS	
Ionization mode	ES+ (positive mode electrospray ionization)	ES- (negative mode electrospray ionization)
Capillary voltage	3 kV	2 kV
Cone voltage	50 V	54 V
Source Offset	50 V	
Source temperature	150 °C	
Desolvation temperature	500 °C	
Cone gas	150 L/h	
Desolvation gas	1000 L/h	
Nebuliser gas	7 bar	
SRM pairs (collision energy)	241.24 -> 116.18 (20 V) 241.24 -> 98.78 (20 V)	442.15 -> 124.34 (24 V) 442.15 -> 112.14 (22 V)
LC parameters		
Instrument	Waters Acquity UPLC	
Column	Waters Acquity UPLC BEH C-18, 100 mm x 2.1 mm (i.d.), 1.7 µm (particle size)	
Precolumn	Waters Vanguard BEH C-18, 5mm x 2.1 mm (i.d.), 1.7 µm (particle size)	
Column temperature	40 °C	
Flow rate	300 µL/min	
Injection volume	1 µL	
Eluent A	0.1% formic acid (aqueous)	
Eluent B	acetonitrile	
LC gradient		
Time	A%	B%
0.00	100%	0%
2.00	100%	0%
7.00	35%	65%
8.00	0%	100%
9.0	0%	100%
9.10	100%	0%
10.0	100%	0%

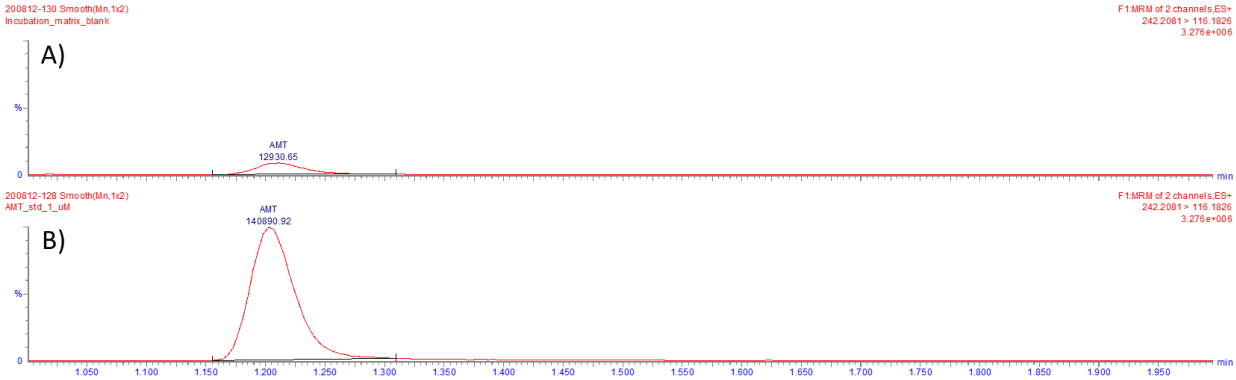
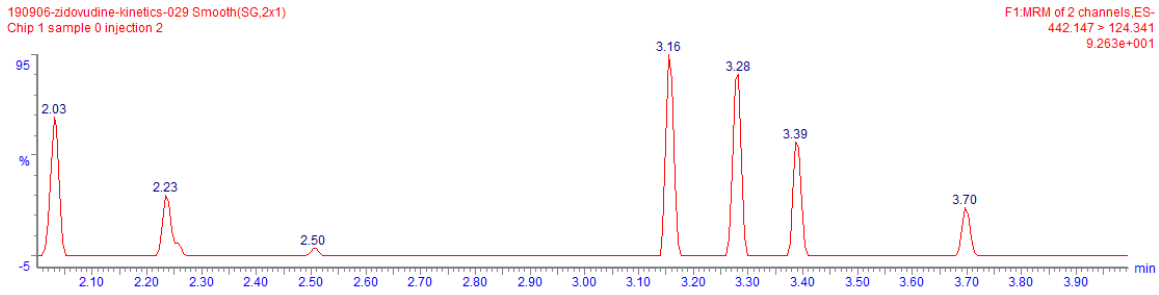


Figure S1. Selectivity of the LC-MS method for AMT. Representative ion chromatograms (SRM) of A) blank enzyme incubation matrix and B) blank enzyme incubation matrix spiked with 1 μM AMT. The blank incubation matrix comprised of 0.1 M Tris 5 mM $MgCl_2$, 3 mM zidovudine (substrate), and 1 mM UDPGA and 1 mM NADPH (cosubstrates*).* The minor background peak at 1.2 min (AMT retention time) in (A) results from photocatalytic, nonenzymatic degradation of zidovudine (substrate) to AMT, as reported by Kurmi et al. (*RSC Adv.* **2017**, *7*, 18803) and was subtracted prior to quantitation of the enzymatically produced AMT.

Table S2. LC-MS method validation parameters for AMT. The linearity was determined by spiking the blank enzyme incubation matrix (0.1 M Tris 5 mM $MgCl_2$, 1 mM zidovudine, 1 mM UDPGA, 1mM NADPH) with AMT standard. The accuracy and precision were determined from n=6 repeated injections of blank incubation matrix spiked with 1.00 μM AMT.

Parameter	**Result**
Range	0.050–10.0 μM
Linearity	y=130668x-4263 (R^2=0.9999)
Accuracy (n=6 injections, 1.00 μM)	107.0%
Precision (n=6 injections, 1.00 μM)	1.2%

A)



B)

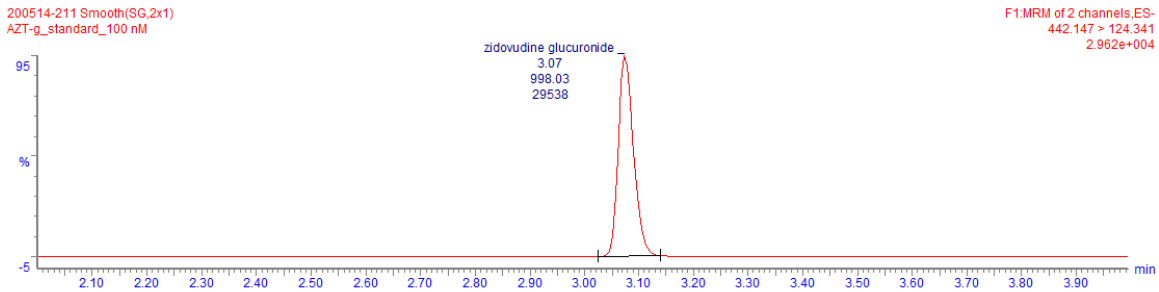


Figure S2. Selectivity of the LC-MS method for AZT-G. Ion chromatograms (SRM) of A) blank enzyme incubation matrix (0.1 M Tris 5 mM $MgCl_2$, 1 mM zidovudine, 1 mM UDPGA) and B) enzyme incubation matrix spiked with 0.10 µM zidovudine-glucuronide.

Table S3. LC-MS method validation parameters for AZT-G. The linearity was determined by spiking the blank enzyme incubation matrix (0.1 M Tris 5 mM $MgCl_2$, 1 mM zidovudine, 1 mM UDPGA) with AZT-G standard. The accuracy and precision were determined from n=6 repeated injections of blank incubation matrix spiked with 0.10 µM AZT-G.

Parameter	**Result**
Range	0.001–5.00 µM
Linearity	y=8.8x+200 (R^2=0.9994)
Accuracy (n=6 injections, 0.100 µM)	108.0%
Precision (n=6 injections, 0.100 µM)	4.4%

Table 4. Best-fit values for the rate constant k (min^{-1}) of oxygen-depletion in rectangular OSTE channels in static conditions for different off-stoichiometric and stoichiometric thiol-ene compositions, including the impact of heat treatment (HT, 110 °C, overnight) as well as surface biotinylation on k. PETMP = pentaerythritol tetrakis(3-mercaptopropionate), TMPTMP = trimethylolpropane tris(3-mercaptopropionate), PI = photoinitiator (0.1%, m/v, TPO-L). The allyl component in all compositions was triallyl-1,3,5-triazine-2,4,6(*1H,3H,5H*)-trione (TATATO). nd = not determined

	Bulk composition			
Thiol component (postcure treatment)	**+50 mol-% thiol**	**+25 mol-% thiol**	**stoichiometric**	**+25 mol-% allyl, w/ PI**
PETMP	0.049 min^{-1}	0.038 min^{-1} a)	0.034 min^{-1}	0.0072 min^{-1}
PETMP (heat treated)	0.0034 min^{-1}	nd	nd	nd
PETMP (biotinylated)	0.039 min^{-1}	nd	nd	nd
TMPTMP	0.014 min^{-1}	nd	0.018 min^{-1}	0.013 min^{-1}
TMPTMP (heat treated)	0.0016 min^{-1}	nd	nd	nd

[a)] For +25 mol-% thiol-rich composition of PETMP, the rate constant was also determined in the presence of the PI and was 0.040 min^{-1}.