Electronic Supplementary Material (ESI) for Analytical Methods.
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† Electronic Supplementary Information (ESI)

Evaluation of separation performance for eggshell-based reversed-phase HPLC columns by controlling particle size and application in quantitative therapeutic drug monitoring

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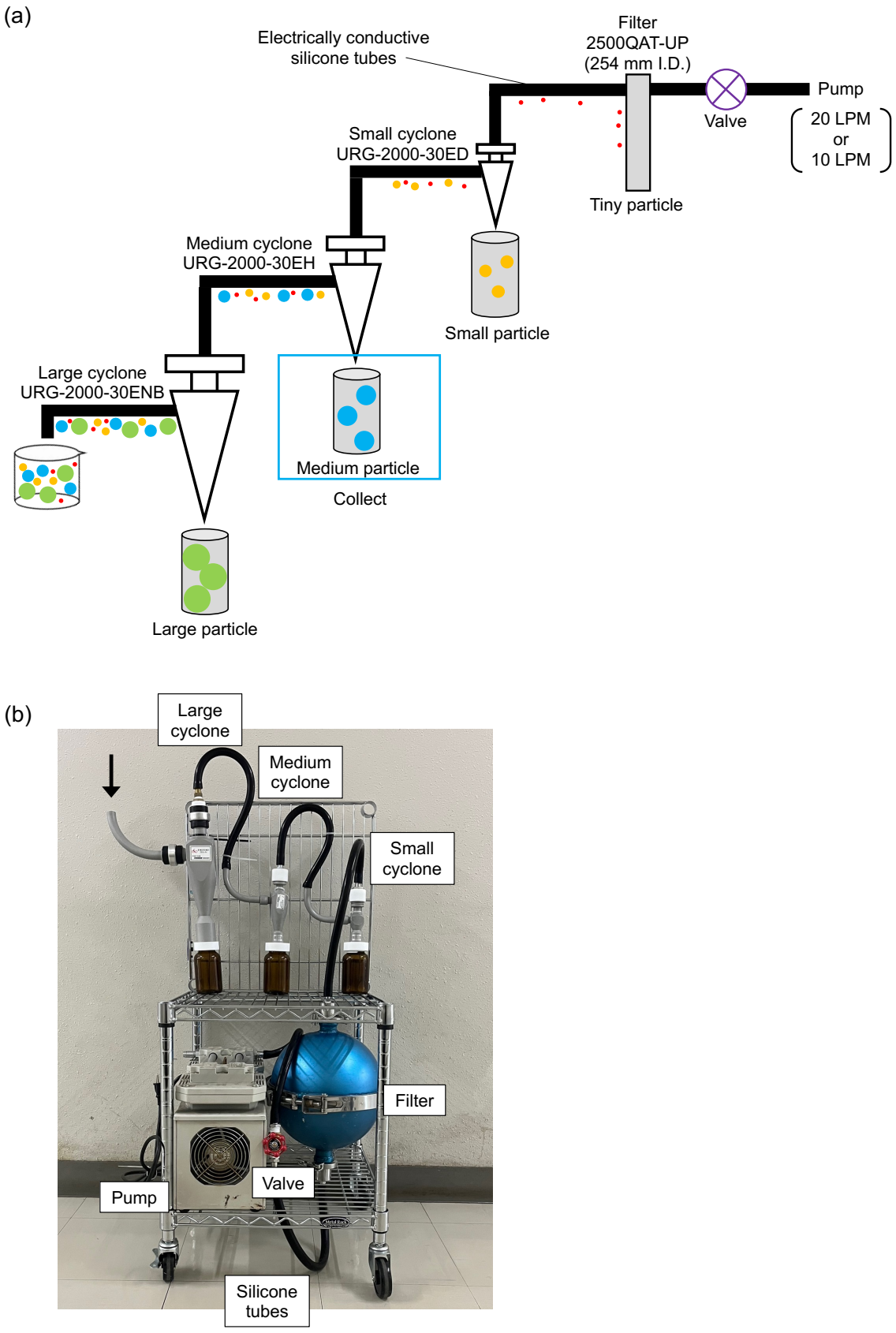

Fig. S1 (a) Shcematic illustration and (b) photograph of classification system with cyclones.

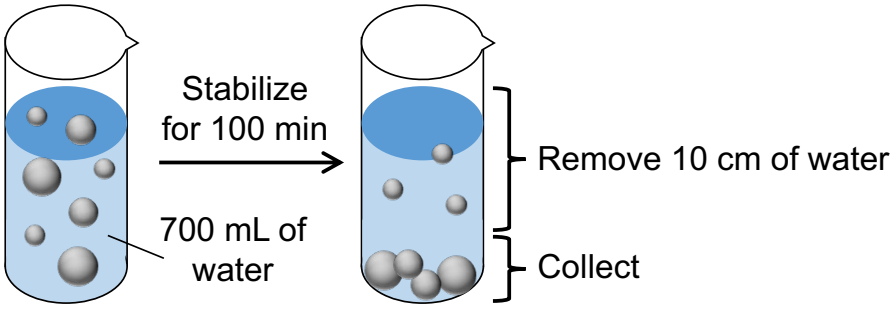

Fig. S2 Removal of tiny particles by sedimentation separation.

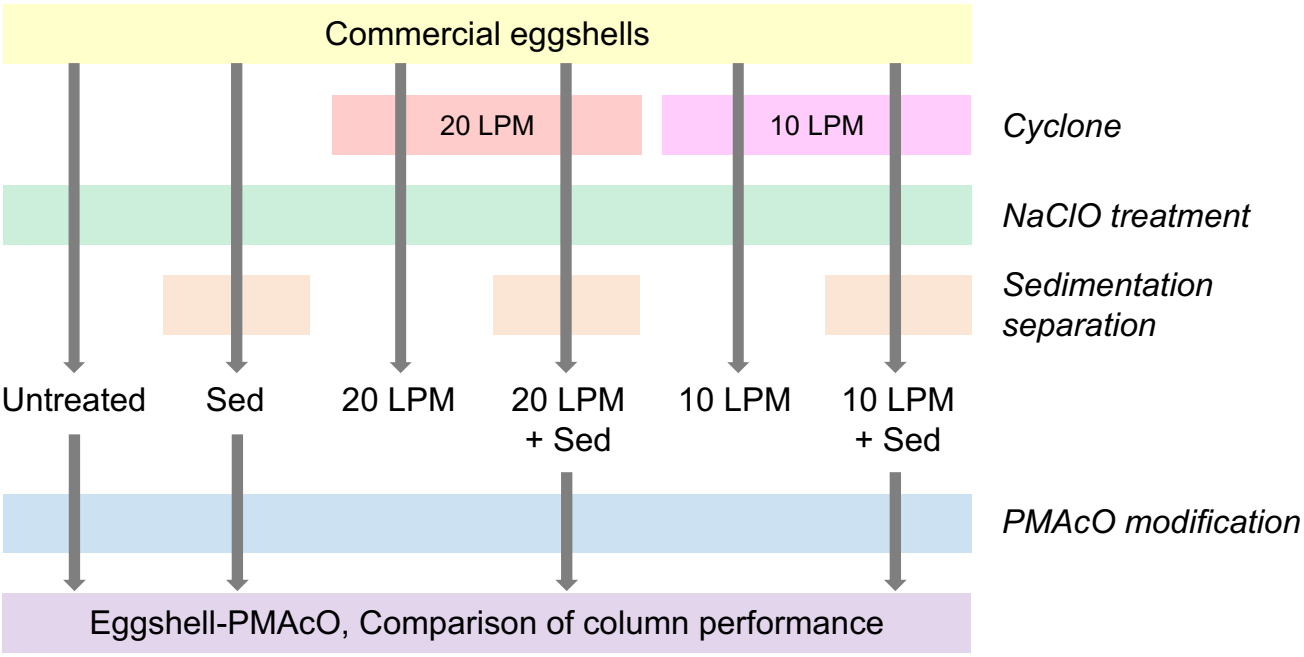

Fig. S3 The treatment performed on each particle.

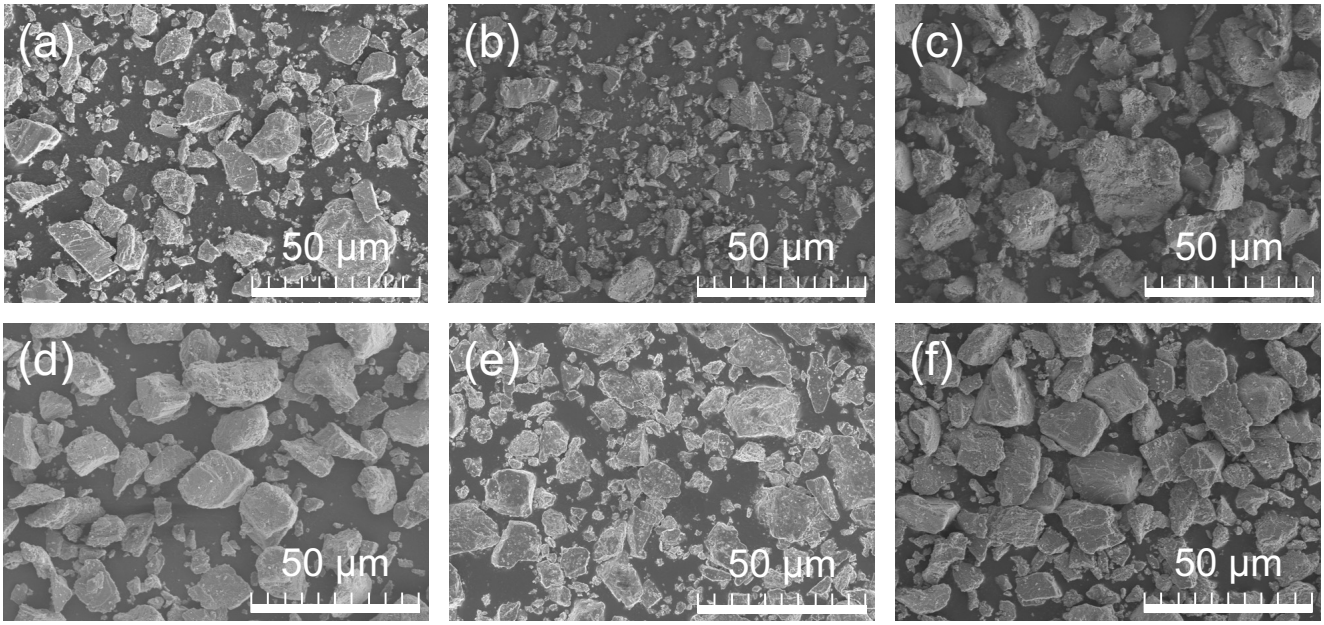

Fig. S4 Higher magnification SEM images of of Fig. 2 in the main text. (a) Untreated, (b) 20 LPM, (c) 10 LPM, (d) Sed, (e) 20 LPM + Sed, and (f) 10 LPM + Sed.

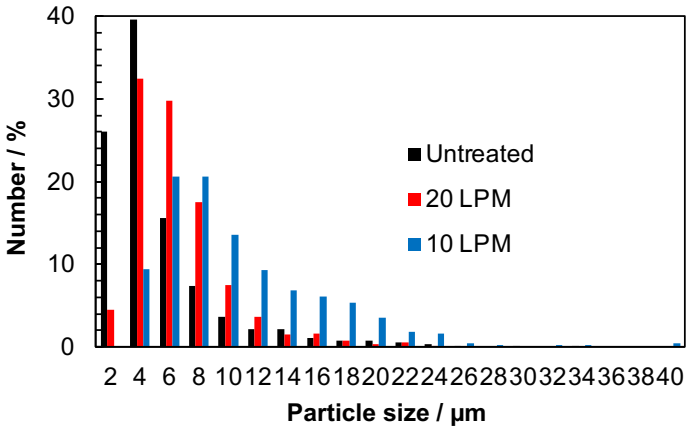

Fig. S5 Particle size distributions of eggshells before and after classification by cyclones.

Table S1 The percentage of particles smaller than 4 μm.

	Before sedimentation separation/ %	After sedimentation separation / %
Untreated	65.6	42.9
20 LPM	37.0	21.0
10 LPM	9.43	2.85

Table S2 Organic contents in the particles.

	Before modification with PMAcO / %	After modification with PMAcO/ %
Untreated	0.69	0.95
Sed	0.66	1.04
20 LPM + Sed	0.70	1.11
10 LPM + Sed	0.70	1.00

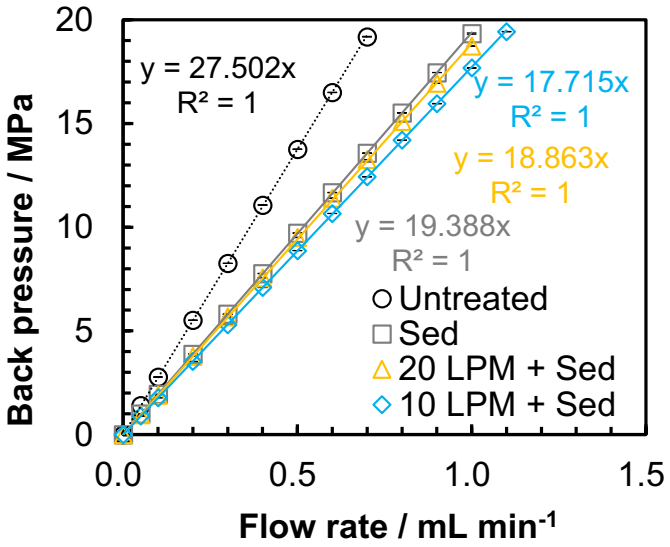

Fig. S6 Back pressure of the columns relative to the flow rate of mobile phase. Analytical conditions: water/methanol (60/40, v/v), $n = 3$.

Table S3 *A, B* and *C* terms of van Deemter equation.

	A	*B*	*C*
Sed	31.0	0.652	14.7
20 LPM + Sed	35.5	2.10	19.6
ODS	0.307	3.28	7.32

Table S4 R_S of alkylbenzenes from the chromatogram shown in Fig. 4a of the main text.

C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
–	3.10	5.26	5.72	5.11	4.39	3.81	3.30	2.94	3.03

Table S5 R_S of steroids from the chromatogram shown in Fig. 4b of the main text.

Prednisolone	Hydrocortisone	Hydrocortisone acetate	Dexamethasone	Testosterone
–	1.61	1.55	3.75	3.01

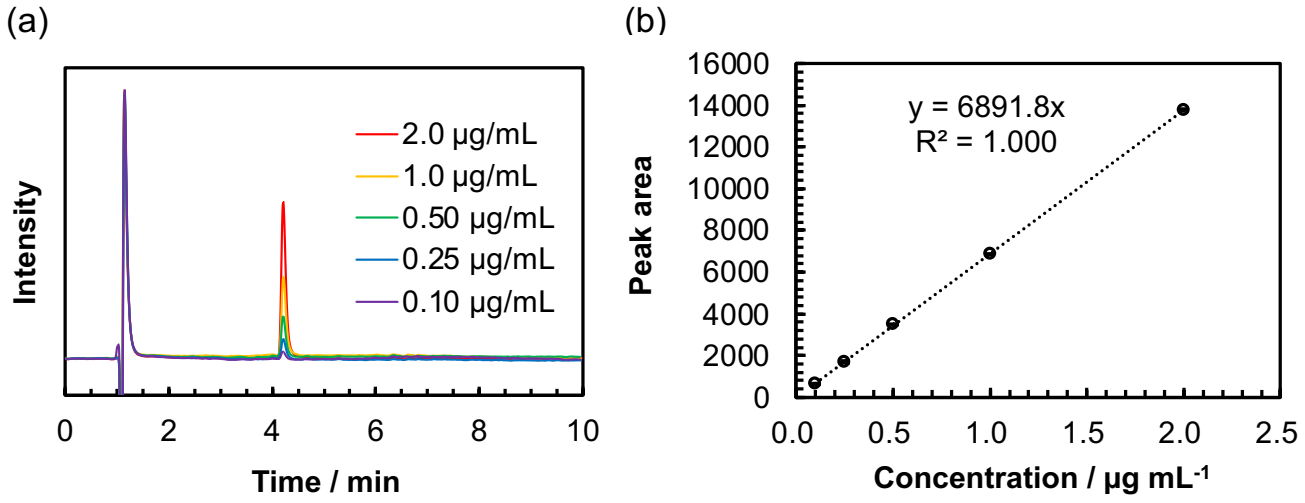

Fig. S7 (a) Chromatograms of voriconazole. Analytical conditions: water/methanol (90/10, v/v) for 1 min, followed by the gradient elution to 30/70 (v/v) for 4 min and finally 30/70 (v/v) for 5 min, 1.0 mL/min, 0.1–2.0 µg/mL voriconazole, 10 µL injection, detected at 254 nm. (b) Calibration curve of voriconazole based on the chromatograms in Fig. S7a, $n = 3$.

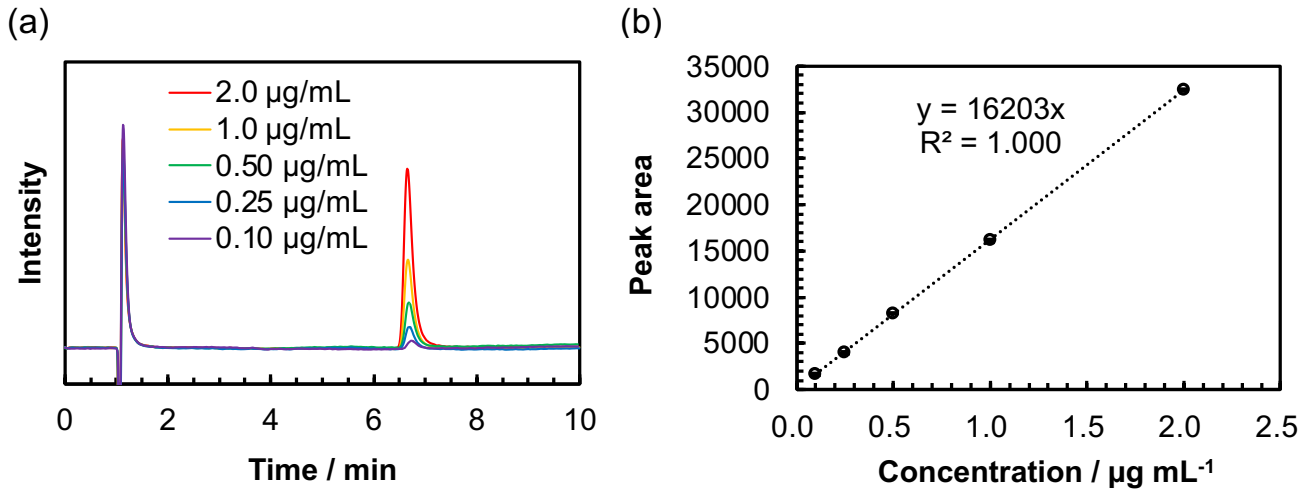

Fig. S8 (a) Chromatograms of imatinib. Analytical conditions: 10 mM $Na_2B_4O_7$ buffer (pH = 10.8)/methanol (90/10, v/v) for 1 min, followed by the gradient elution to 70/30 (v/v) for 3 min and finally 70/30 (v/v) for 6 min, 1.0 mL/min, 0.1–2.0 µg/mL imatinib, 10 µL injection, detected at 285 nm. (b) Calibration curve of imatinib based on the chromatograms in Fig. S8a, $n = 3$.

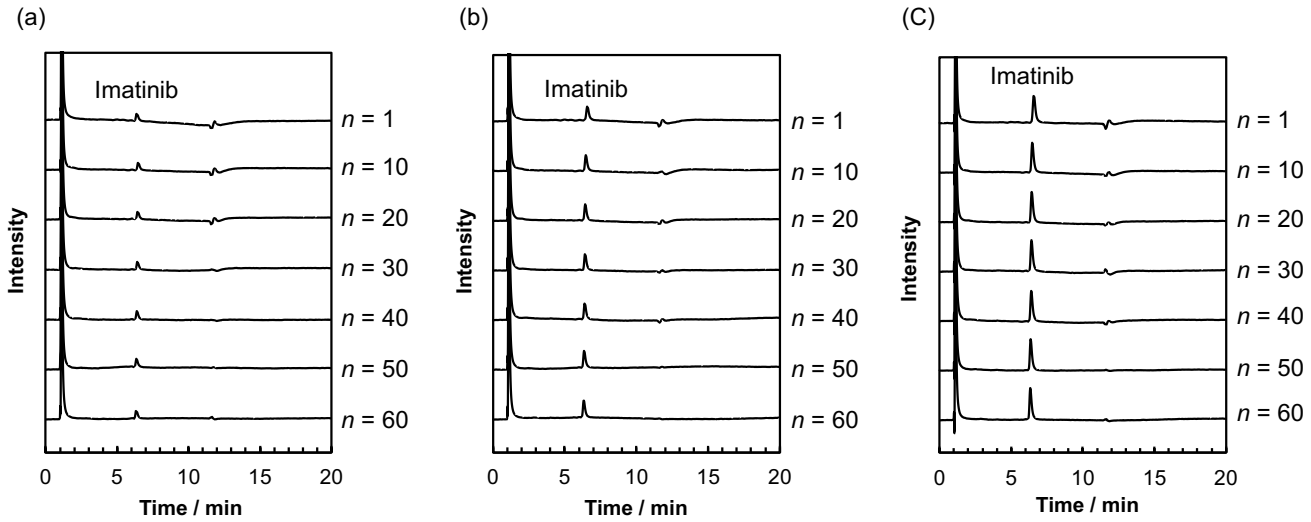

Fig. S9 Chromatograms during routine analysis of three different concentrations of imatinib-spiked whole blood samples (a) 0.75 µg/mL, (b) 1.5 µg/mL, (c) 3.0 µg/mL. Analytical conditions: 10 mM $Na_2B_4O_7$ buffer (pH = 10.8)/methanol (90/10, v/v) for 1 min, followed by the gradient elution to 70/30 (v/v) for 3 min, 70/30 (v/v) for 6 min, and finally 90/10 (v/v) for 10 min, 1.0 mL/min, 0.75–3.0 µg/mL imatinib in whole blood (After pretreatment: 0.15–0.6 µg/mL), 10 µL injection, detected at 285 nm.

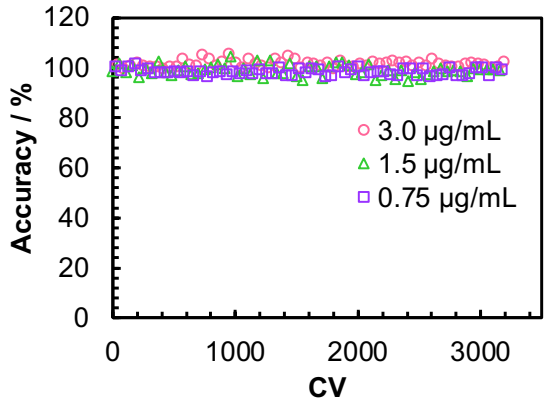

Fig. S10 Change in accuracy during routine analysis of three different concentrations of imatinib-spiked whole blood samples. Analytical conditions: 10 mM $Na_2B_4O_7$ buffer (pH = 10.8)/methanol (90/10, v/v) for 1 min, followed by the gradient elution to 70/30 (v/v) for 3 min, 70/30 (v/v) for 6 min, and finally 90/10 (v/v) for 10 min, 1.0 mL/min, 0.75–3.0 µg/mL imatinib (After pretreatment: 0.15–0.6 µg/mL), 10 µL injection, detected at 285 nm.

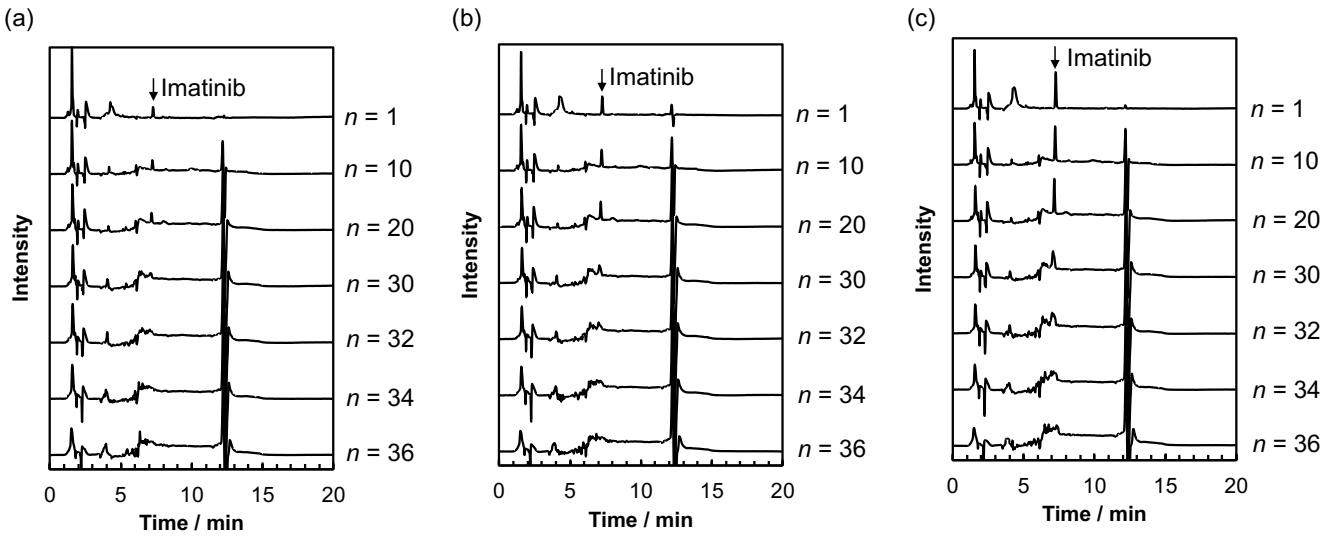

Fig. S11 Chromatograms of three different concentrations of imatinib-spiked whole blood samples (a) 0.75 µg/mL, (b) 1.5 µg/mL, (c) 3.0 µg/mL using a commercial ODS column. Analytical conditions: 10 mM $Na_2B_4O_7$ buffer (pH = 10.8)/methanol (90/10, v/v) for 1 min, followed by the gradient elution to 20/80 (v/v) for 3 min, 20/80 (v/v) for 6 min, and finally 90/10 (v/v) for 10 min, 1.0 mL/min, 0.75–3.0 µg/mL imatinib in whole blood (After pretreatment: 0.15–0.6 µg/mL), 10 µL injection, detected at 285 nm.

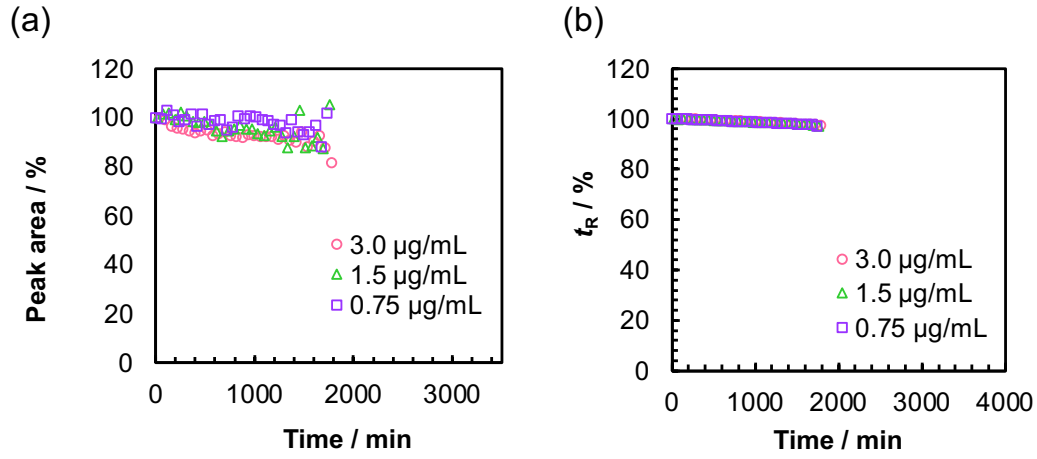

Fig. S12 Changes in (a) peak areas and (b) retention times of three different concentrations of imatinib-spiked whole blood samples using a commercial ODS column. Analytical conditions: 10 mM $Na_2B_4O_7$ buffer (pH = 10.8)/methanol (90/10, v/v) for 1 min, followed by the gradient elution to 20/80 (v/v) for 3 min, 20/80 (v/v) for 6 min, and finally (90/10, v/v) for 10 min, 1.0 mL/min, 0.75–3.0 µg/mL imatinib in whole blood (After pretreatment: 0.15–0.6 µg/mL), 10 µL injection, detected at 285 nm.