SUPPLEMENTARY INFORMATION

Minimally-invasive method for the point-of-care quantification of lymphatic vessel function

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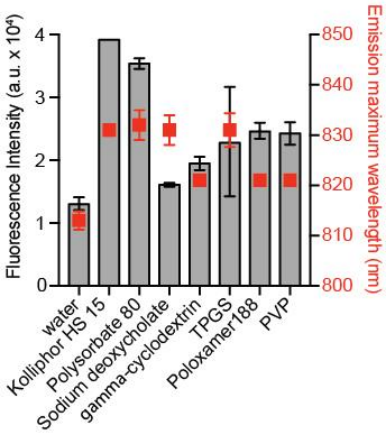
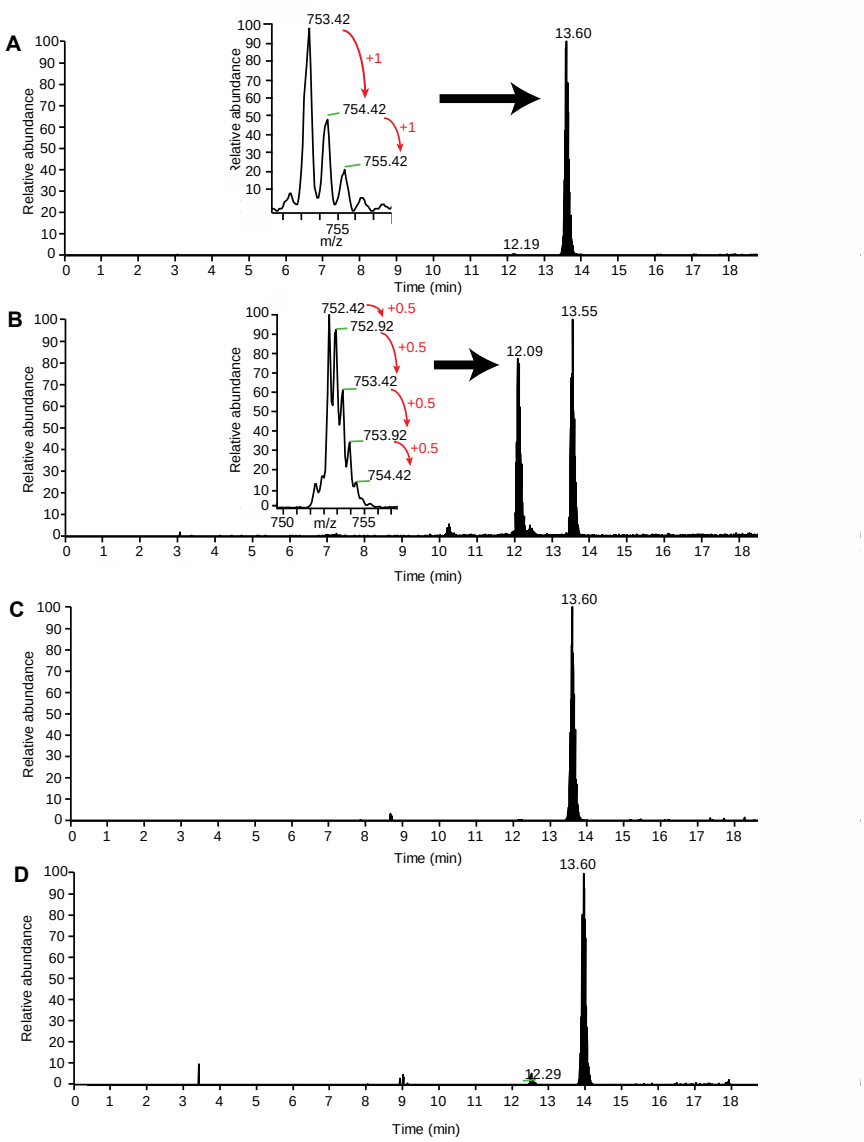


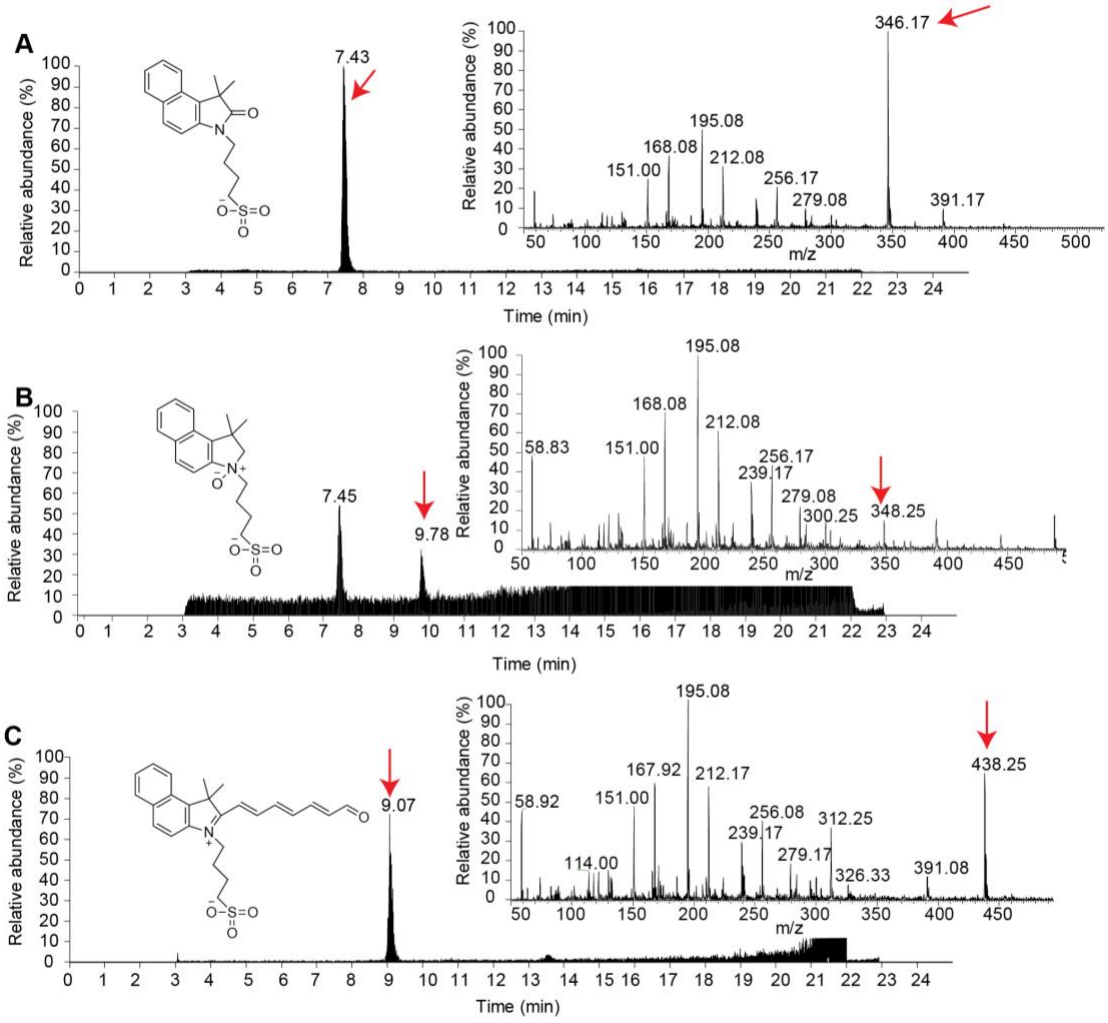
Supplemental Figure 1. Fluorescence intensities and emission maxima of ICG in the presence of various injectable excipient (Concentrations of ICG and excipients are mentioned in the Methods section). Data shown as mean ± SD. In some cases the S.D. are smaller than the bars and symbol and are not visible.

LC-MS analysis

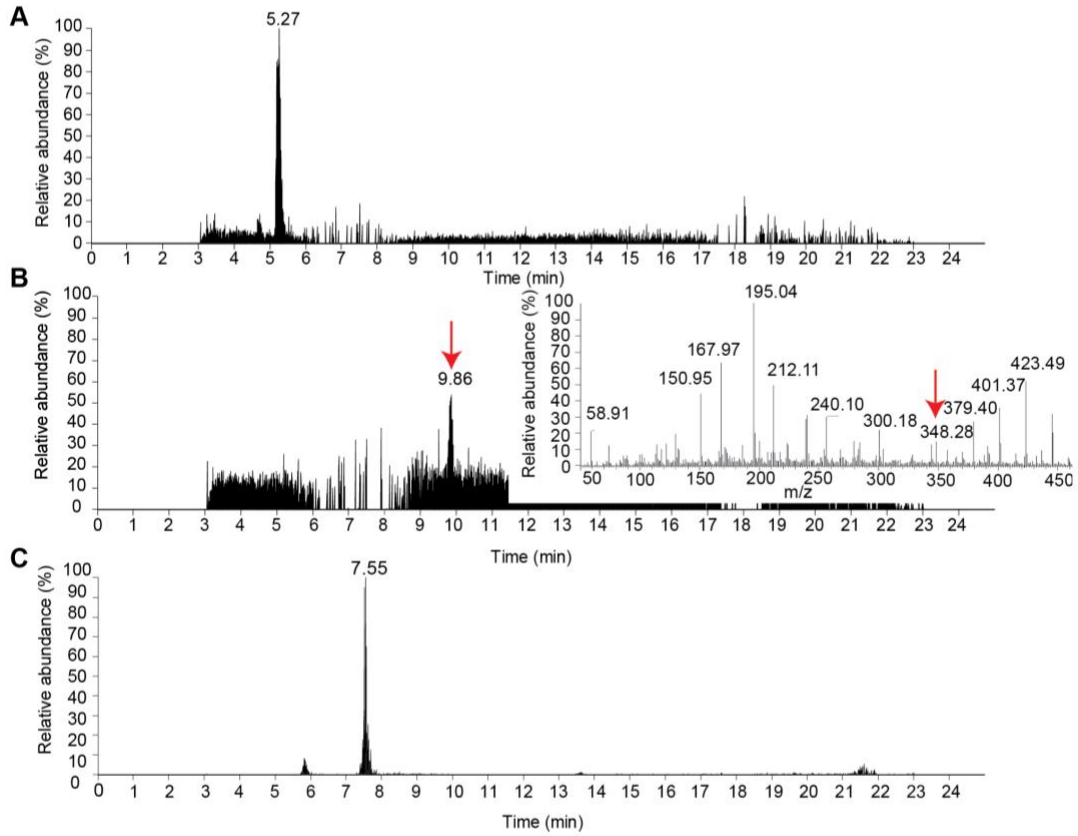
Qualitative LC-MS analysis of a fresh solution of ICG in water revealed the presence of the main ICG peak that eluted at 13.6 min (m/z = 753.42, **Supplemental Figure 2A**). After 24 h of incubation (r.t., protected from light), a second peak at around 12.09 min appeared (m/z = 752.42) with an isotopic distribution pattern characteristic for a dimer molecule (m/z + $n \times 0.5$) (**Supplemental Figure 2B**). Moreover, the peaks from degradation products resulting from *via* the saturation and cleavage of the polymethine chain of ICG were detected (m/z = 346, 348 and 438, **Supplemental Figures 3A-C)**. In ICG-Kolliphor HS15 solutions, the peak emerging from the plausible dimer was barely visible even in samples after 106 days after preparation (**Supplemental Figure 2D)** Only one leucoform at m/z = 348, presumably emerging from N-oxidation of the indole ring was detected in this solution (**Supplemental Figure 4**).



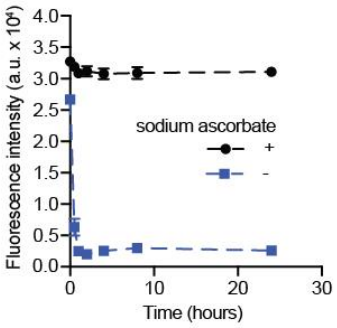
Supplemental Figure 2. A) LC-MS chromatogram of a fresh ICG-water solution at m/z = 753 and LC-MS spectrum of the main peak (R_t =13.6 min) in the chromatogram (inset). B) LC-MS chromatogram of a 1-day old ICG-water solution and LC-MS chromatogram at m/z = 753 of the degradation peak (R_t =12.09 min) in the chromatogram (inset). C) LC-MS chromatogram of a fresh ICG-Kolliphor HS15 solution at m/z = 753. D) LC-MS chromatogram of a 106-day old ICG-Kolliphor HS15 solution at m/z = 753.



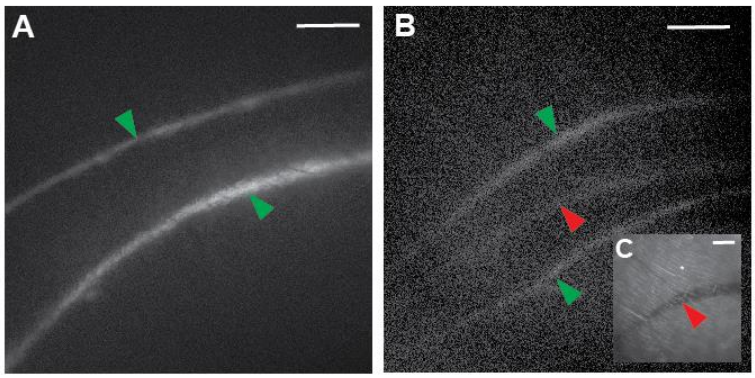
Supplemental Figure 3. LC-MS analysis of degradation products of ICG in water (1 day-old). A) LC-MS chromatogram at m/z = 346 and and LC-MS spectrum of the main peak (R_t =7.43 min) in the chromatogram (inset). B) LC-MS chromatogram at m/z = 348 and LC-MS spectrum of the main peak (R_t =9.78 min) in the chromatogram (inset). C) LC-MS chromatogram at m/z = 438 and LC-MS spectrum of the main peak (R_t =9.07 min) in the chromatogram (inset).



Supplemental Figure 4. LC-MS analysis of degradation products of ICG in Kolliphor HS15 (106 day-old). A) LC-MS chromatogram at m/z = 346. B) LC-MS chromatogram at m/z = 348 and and LC-MS spectrum of the main peak (R_t =9.86 min) in the chromatogram (insert). C) LC-MS chromatogram at m/z = 438.



Supplemental Figure 5. Fluorescence intensity of ICG (0.05 mg/ml) in 150 mg/ml solution of PVP that was pre-incubated for 24-h with 0.5% sodium ascorbate (w/w, against PVP mass).



Supplemental Figure 6. Imaging of lymphatic vessels with ICG (0.0075 mg/ml) in Kolliphor HS15 (10 mg/ml) and in water in the hind limb of a mouse. Representative fluorescent stereomicroscope video frames after intradermal injection of 5 µl of ICG into the hind limb of a mouse. Scale bars: 500 µm. Green and red arrows indicate lymphatic and blood vessels, respectively. A) The region in the hind limb after injection of ICG-Kolliphor HS15 and after applying external compression. B) The region in the hind limb after injection of ICG in water and after applying external compression. Images A and B are shown at different display levels. C) Autofluorescence image (FITC channel) demonstrating the location of the popliteal vein (red arrow).

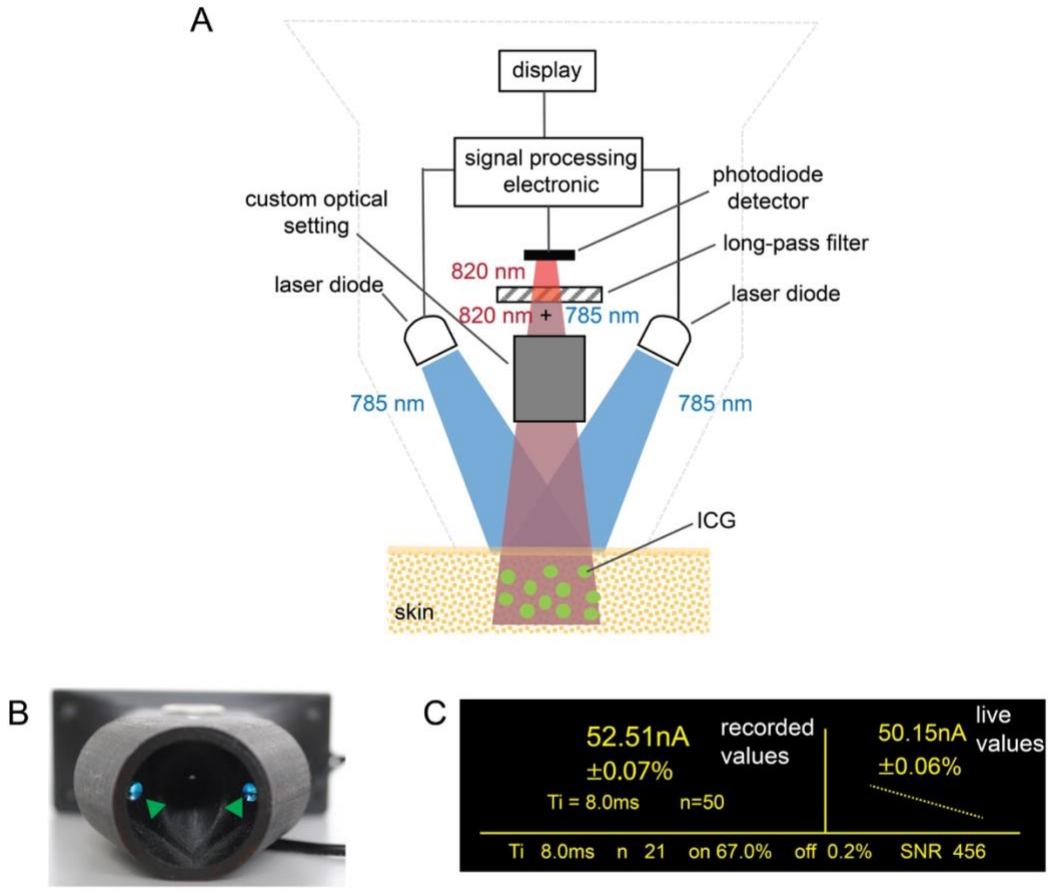
Supplemental text

Device electronics and control algorithm

The user triggers the acquisition of a new measurement by pressing a button on the side of the device. To obtain final measurement values, the device executes several integration cycles of duration Ti. In the first integration cycle ("on" cycle), the excitation laser diodes (Panasonic LNCT22PK01WW) are switched on to illuminate the skin area containing the ICG. A long-pass filter (Semrock BLP01-808R-25, cut to 10 mm diameter) blocks the reflected excitation light, but not the fluorescence light emitted by the ICG. The detector photodiode (Osram BPW34S) converts the resulting fluorescence light to an electrical current, which the signal processing electronic integrates to a voltage **(Supplemental Figure 7A**). At the end of the integration cycle, the control algorithm samples the voltage with an analog-digital convertor (ADC) and uses the result to estimate the average voltage on the photodiode. The estimated average voltage is proportional to the average power of the fluorescence light received by the photodiode during the integration interval. Next, the same integration procedure is repeated ("off" cycle), but with the laser diode switched off, to estimate the background signal from the external environment. To avoid saturation of the ADC, the integration time Ti is automatically adjusted in the range of 0.5 – 64 ms according to the fluorescence light intensity reaching the detector.

The device repeats the described "on" and "off" integration cycles n number of times each, computes the final measurement by averaging over the estimated currents and shows it on the display **(Supplemental Figure 7C**). The number of cycles n is chosen such that the time to obtain the final measurement is always 2 seconds, keeping the total integration and averaging time is constant. The periodically, in 200 ms intervals updated live readings on the right side of the display are obtained by averaging over fewer integration cycles, which guarantees responsive readings during positioning of the device.

The displayed on and off values (in percent of total dynamic range) serve as quality control and indicate the use of the ADC's dynamic range during the on and off integration cycle, respectively. The on value must always be below 100% (to avoid saturation) and the off value below 1% (indicates absence of external interferences). The signal-to-noise ratio (SNR) represents the ratio between the signals during on and off cycles.



Supplemental Figure 7. A) Scheme representing the optical set-up of the portable custom device for NIR fluorescence measurements. The laser diodes excite the ICG in the skin. An optic consisting of lenses and a pinhole focuses the fluorescence light on the photodiode. A long-pass filter blocks the reflected excitation light at a wavelength of 785 nm, but not the fluorescence light emitted by the ICG with wavelengths longer than 810 nm. The signal processing electronic controls the device and estimates the photocurrent induced by the fluorescence light. B) View of the lower part of the device (measuring head). The inner diameter of the measuring head is 3 cm. Green triangles indicate the laser diodes, which are located behind lenses. C) Display of the device. Left upper panel shows the acquired average value, its relative standard deviation (RSD) over n integration cycles and the integration period Ti. Right upper panel indicates live values together with RSD. Lower panel shows live Ti and the number of averaged measurements n to obtain the live value. For measurement validation purposes, the current on and off values and the SNR are shown, too.

Supplemental Table 1. *In vitro* intra-day variability of the fluorescence signal of ICG in Kolliphor HS15 solution.

ICG concentration (mg/ml)	Day 1 (*n* = 5)			Day 2 (*n* = 5)			Day 3 (*n* = 5)		
	Average signal	S.D:	%COV	Average signal	SD	%COV	Average signal	SD	%COV
0.0075	29.2	1.6	5.30	30.5	1.7	5.47	27.6	1.0	3.64
0.005	20.5	0.8	3.90	23.0	1.0	4.42	19.9	0.7	3.39
0.0025	10.3	0.5	5.00	12.3	0.5	3.90	10.0	0.4	3.58
0.001	4.5	0.1	1.95	4.4	0.2	3.48	4.0	0.1	3.31
0.00075	3.4	0.3	9.20	3.1	0.0	1.12	3.1	0.1	3.25
0.0005	2.0	0.1	4.87	2.1	0.1	2.81	2.1	0.1	3.64

Supplemental Table 2. Inter-day variability of the fluorescence signal of ICG in Kolliphor HS15 solution.

Day 1, 2, 3 (*n* = 15)			
ICG concentration (mg/ml)	Average signal	S.D.	%COV
0.0075	29.1	1.8	6.24
0.005	21.1	1.6	7.60
0.0025	10.9	1.1	10.55
0.001	4.3	0.2	5.68
0.00075	3.2	0.2	7.53
0.0005	2.1	0.1	3.72

Supplemental Table 3. Average fluorescence values obtained from all 8-11 measurements, together with the number of readings per injection site (*n*), standard deviations (SD) and relative standard deviation (RSD%) in pig 1

Injection	Time (h)	*n*	Average signal	SD (nA)	RSD (%)
	0	8	319	15.2	4.8
	0.5	8	328	3.6	1.1
	1	8	310	10.3	3.3
	1.5	8	297	7.9	2.7
1	2	8	296	2.2	0.7
	2.5	8	283	8.4	3.0
	3	8	260	5.3	2.0
	3.5	8	238	4.5	1.9
	4	8	218	6.4	2.9
Injection	**Time (h)**	***n***	**Average signal**	**SD (nA)**	**RSD (%)**
	0	8	357	11.2	3.1
	0.5	8	346	9.5	2.7
	1	8	249	9.7	3.9
	1.5	8	233	22.2	9.5
2	2	8	189	10.3	5.4
	2.5	8	170	7.1	4.2
	3	8	147	6.8	4.6
	3.5	8	136	2.2	1.6
	4	8	111	4.1	3.7
Injection	**Time (h)**	***n***	**Average signal**	**SD (nA)**	**RSD (%)**
	0	9	345	12.5	3.6
	0.5	9	348	28.7	8.2
	1	9	351	14.4	4.1
	1.5	9	333	11.2	3.4
3	2	9	329	8.7	2.6
	2.5	10	319	13.0	4.1
	3	9	315	22.8	7.2
	3.5	10	312	21.2	6.8
	4	9	302	3.9	1.3
Injection	**Time (h)**	***n***	**Average signal**	**SD (nA)**	**RSD (%)**
	0	8	340	16.1	4.7
	0.5	8	385	10.7	2.8
	1	10	321	9.0	2.8
	1.5	8	268	17.7	6.6
4	2	8	261	7.8	3.0
	2.5	8	229	13.7	6.0
	3	10	219	4.9	2.2
	3.5	11	193	4.2	2.2
	4	8	171	9.2	5.3

Cont'd Supplemental Table 3. Average fluorescence values obtained from all 8-11 measurements, together with the number of readings per injection site (*n*), standard deviations (SD) and relative standard deviation (RSD%) in pig 1

Injection	Time (h)	*n*	Average signal	SD (nA)	RSD (%)
	0	8	344	4.7	1.4
	0.5	8	420	11.5	2.7
	1	8	417	12.3	3.0
	1.5	9	407	34.9	8.6
5	2	9	433	16.1	3.7
	2.5	8	383	52.3	13.7
	3	8	422	3.7	0.9
	3.5	9	411	18.4	4.5
	4	8	379	9.2	2.4
Injection	**Time (h)**	***n***	**Average signal**	**SD (nA)**	**RSD (%)**
	0	9	407	9.4	2.3
	0.5	9	447	14.8	3.3
	1	9	388	14.8	3.8
	1.5	9	346	10.1	2.9
6	2	9	329	14.8	4.5
	2.5	9	334	5.8	1.7
	3	9	304	4.7	1.6
	3.5	10	266	9.8	3.7
	4	9	260	16.8	6.5