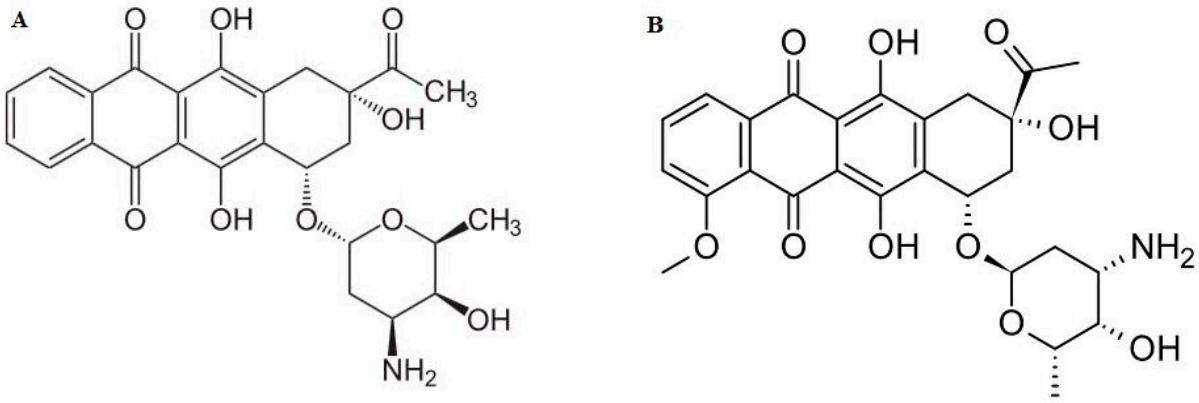

Figure S1: Chemical structure of IDA (A) and DAU (I.S.)

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Table S1. Comparison of LC methods for the quantification of IDA in biological samples reported in the literature

Biological matrix	Determined substances	Extraction procedure	Absolute recovery (%)	Chromatographic method				Range of the linearity	LOD (ng/mL)	LOQ (ng/mL)	Ref.
				Stationary phase	Mobile phase	Detection conditions	Total analysis time (min)				
Human plasma 2 mL	IDA and idarubicinol	SPE C18 *Eluting solvent*: 3 NH,PO_4:ACN: H_2O:$(C_2H_5)N$ (20:40:39.95:0.05, *v*/*v*/*v*/*v*)	No data	C18 (250 x 4.6 mm, 5 µm)	*Isocratic elution:* 3 M H_3PO_4:ACN: H_2O: TEA (20:40:39.95:0.05, *v*/*v*/*v*/*v*)	FL λ_{ex} = 250 nm λ_{em} = 570 nm	No data	0.1-50 ng/mL	0.1 ng/mL	No data	[6]
Human plasma 1 mL and urine 50-100 µL	IDA and idarubicinol	Deproteinization with MeOH for plasma; dilution with water for urine before SPE with Isolute C18 *Eluting solvent*: 0.25 M HCl in MeOH	No data	Waters µBondapak phenyl column (300 x 3.9 mm, no data)	*Isocratic elution:* 0.4 M Ammonium formate (pH 4.0) : ACN (7:3, *v*/*v*)	FL λ_{ex} = 254 nm λ_{em} = 550 nm	No data	1-100 ng/mL for plasma No data for urine	No data	No data	[7]
Human plasma	IDA and idarubicinol	SPE with C18 *Eluting solvent*: No data	84	Supelcosil LC-CN (250 x 4.6 mm, 5 µm)	*Isocratic elution:* 10 mM KH_2PO_4 : ACN (pH 2.5) no data	FL λ_{ex} = 470 nm λ_{em} = 580 nm	No data	1.05-350 ng/mL	0.1 ng/mL	0.3 ng/mL	[9]

1-2 mL; Cerebrospinal fluid 1-2 mL										
Human urine 5 mL	IDA DAU Doxorubicin Epirubicin	SPE with Bond Elut C18 *Eluting solvent*: DCHM:2-propanol (1:1, *v*/*v*) pH 7	90.7	BDS C8 (150 x 4.6 mm, 5 μm)	*Gradient elution:* Component A: 0.1% HCOOH in H_2O:ACN:MeOH (70:25:5, *v*/*v*/*v*), Component B: 0.1% HCOOH in H_2O:ACN (70:30, *v*/*v*)	LC/MS/MS *m*/*z* = 498 *m*/*z* = 291	25	100-2000 ng/mL	10 ng/mL	30 ng/mL	[14]
Human plasma 0.5 mL	IDA DAU Doxorubicin Epirubicin and their 13-dihydrometabolites	LLE with chloroform: 1-heptanol (9:1, *v*/*v*) at pH 8.4 and re-extraction into 0.1 M H_3PO_4	93-109	Supelcosil LC-CN (250 x 4.6 mm, 5 μm)	*Isocratic elution:* 50 mM NaH_2PO_4: ACN (65:35, *v*/*v*) adjusted to pH 4.0 with H_3PO_4	FL λ_{ex} = 230, 254 and 480 nm λ_{em} = 560 nm	15	0.4-10000 ng/mL	0.4 ng/mL	0.4 ng/mL	[15]
Human plasma 400 μL and saliva 200 μL	IDA DAU Doxorubicin Epirubicin	Deproteinization with ethanol, followed by LLE extraction with DCHM at pH 8.5 (phosphate buffer)	81.7-87.4 for plasma, 76.0-77.4 for saliva	Purospher Star RP-18 (150 x 4.6 mm, 5 μm)	*Gradient elution:* Component A: 0.1% formic acid in water	FL λ_{ex} = 480 nm λ_{em} = 555 nm	14	1-1000 ng/mL for both fluids	0.3 ng/mL for both fluids	1 ng/mL for both fluids	[16]

	and their 13-dihydrometabolites				Component B: 0.1% formic acid in ACN						
Human serum 0.5 mL	IDA DAU Doxorubicin Epirubicin and three their 13-dihydro-metabolites	SPE with Bond Elut C18 *Eluting solvent*: Chloroform: 2-propanol (4:1 *v*/*v*)	85-105	Symmetry C18 (150 x 1 mm, 3.5 μm)	*Isocratic elution:* 5 mM ammonium formate buffer (pH 3.0) :ACN (70:30, *v*/*v*)	ESI-MS m/z = 291 m/z = 333	30	5-2000 ng/mL	1 ng/mL	5 ng/mL	[17]
Human plasma 0.5-2 mL Urine no data	IDA Idarubicinol 4-Demotoxy-daunomycinone	SPE with Bondelut C18 *Eluting solvent*: H_2O:MeOH (3:1, *v*/*v*) and 0.03 M H_3PO_4 in MeOH	91.5	Cyanopropyl column (250 x 4.6 mm, 5 μm)	*Gradient elution:* Component A: 10 mM KH_2PO_4: ACN (78:22, *v*/*v*) Component B: [10 mM KH_2PO_4 + 0.006 M H_3PO_4]: ACN (30:70, *v*/*v*)	FL λ_{ex} = 470 nm λ_{em} = 580 nm	20	0.14-35.3 ng	0.2 ng/mL	No data	[18]
Rat plasma 100 μL	IDA and idarubicinol	Deproteinization with ACN	95.6	LiChrosphe r 100 RP-18 (250 x 4 mm, 5 μm)	*Isocratic elution:* H_2O:ACN:THF: H_3PO_4:TEA (312:165:20:1:2, *v*/*v*/*v*/*v*/*v*) adjusted to pH 2.2 with 5 M HCl	FL λ_{ex} = 485 nm λ_{em} = 542 nm	15	1-500 ng/mL	0.25 ng/mL	No data	[19]

Rabbit plasma 0.5 mL Heart, liver and kidney tissues: 100-500 mg	IDA and idarubicinol	LLE chloroform: 2-propanol (9:1, *v*/*v*) at pH 8.2 (0.5 M Na_2HPO_4)	92.2	Ultracarb 5 ODS (150 x 4.6 mm, 5μm)	*Isocratic elution:* H_2O:ACN:THF: H_3PO_4:TEA (68.4:28:3:0.4:0.2, *v*/*v*/*v*/*v*/*v*) adjusted to pH 2.2 with 5 M HCl	FL λ_{ex} = 485 nm λ_{em} = 560 nm	No data	2-500 ng/mL	No data	2 ng/mL	[20]
Human plasma 0.5 mL Urine 1 mL	**IDA**	**pH modification with 0.1 HCl before SPE with Supel Select HLB** ***Eluting solvent:*** **MeOH**	**99.4 for plasma 95.2 for urine**	**Discovery HS C18 (150 × 4.6 mm, 5 μm)**	***Isocratioc elution*** **ACN:0.1% formic acid in water: (33:67, *v*/*v*) for plasma (32:68, *v*/*v*) for urine**	**FL λ_{ex} = 487 nm λ_{em} = 547 nm**	**8 for plasma 10 for urine**	**0.1-50 ng/mL for plasma 0.25-200 ng/mL for urine**	**0.05 ng/mL for plasma 0.125 ng/mL for urine**	**0.1 ng/mL for plasma 0.25 ng/mL for urine**	**Method in this study**

ACN – acetonitrile; DAU – daunorubicin; IDA – idarubicin; LLE – Liquid-liquid extraction; MeOH – methanol; SPE – solid phase extraction; TAE – trimethylamine; THF – tetrahydrofuran

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Table S2. Results of a stablity study for IDA in human plasma and urine under various experimental conditions

Storage conditions	QC	Conc. added (ng/mL)	Conc. found * (ng/mL)	Precision RSD (%)	Accuracy (%)
			Urine		
Long-term stability	LQC	50	47.3 ± 4.8	10.1	94.6
(-80°C, 2 months)	MQC	100	102.3 ± 8.4	8.2	102.4
stability	HQC	150	148.7 ± 10.3	6.9	99.1
Three freeze-thaw	LQC	50	46.7 ± 5.4	11.6	93.4
cycles stability	MQC	100	101.6 ± 8.2	8.1	101.6
	HQC	150	152.1 ± 10.5	6.9	101.4
Ambient storage	LQC	50	51.3 ± 4.1	8.0	102.6
(25°C, 12 h)	MQC	100	98.9 ± 6.6	6.7	98.9
	HQC	150	148.9 ± 8.4	5.6	99.3
Post-preparative	LQC	50	48.3 ± 4.6	9.5	100.6
storage (4°C, 24 h)	MQC	100	103.5 ± 7.2	6.9	103.5
	HQC	150	151.7 ± 7.9	5.2	101.1
Auto sample stability	LQC	50	50.3 ± 3.9	7.7	99.6
(6 ± 2°C, 24 h)	MQC	100	97.9 ± 6.8	6.9	97.9
	HQC	150	149.5 ± 8.2	5.5	99.7
			Plasma		
Long-term stability	LQC	0.5	0.49 ± 0.05	10.2	98.0
(-80°C, 2 months)	MQC	5	4.44 ± 0.33	7.4	88.8
stability	HQC	15	15.82 ± 1.01	6.4	105.5
Three freeze-thaw	LQC	0.5	0.53 ± 0.04	7.5	106.0
cycles stability	MQC	5	5.15 ± 0.26	5.0	103.0
	HQC	15	14.71 ± 0.78	5.3	98.1
Ambient storage	LQC	0.5	0.51 ± 0.03	5.9	102.0
(25°C, 12 h)	MQC	5	4.89 ± 0.25	5.1	97.8
	HQC	15	14.22 ± 0.68	4.8	94.8
Post-preparative	LQC	0.5	0.47 ± 0.04	8.5	94.0
storage (4°C, 24 h)	MQC	5	5.34 ± 0.37	6.9	106.8
	HQC	15	15.66 ± 0.77	4.9	104.0
Auto sample stability	LQC	0.5	0.52 ± 0.03	5.8	104.0
(6 ± 2°C, 24 h)	MQC	5	5.07 ± 0.13	2.6	101.4
	HQC	15	14.37 ± 0.57	4.0	95.8

* mean ± SD value from three samples