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HPLC-MS/MS determination of a peptide conjugate prodrug of doxorubicin, and its active metabolites, leucine-doxorubicin and doxorubicin, in dog and rat plasma

Claude Mazuel [a,*], Jeffrey Grove [a], Geneviève Gerin [a], Kevin P. Keenan [b]

[a] *Laboratoires Merck-Sharp and Dohme-Chibret Route de Marsat–Riom, 63963 Clermont-Ferrand Cedex 9, France*
[b] *WP 45-222 Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract

A HPLC-MS/MS Electrospray (ESI) method was developed and validated to quantify a peptide conjugate prodrug of doxorubicin (Dox-Con) and its active metabolites leucine-doxorubicin (Leu-Dox) and doxorubicin (Dox) in dog and rat plasma. The analytes were extracted from plasma by solid-phase extraction on a Bond Elut® C8 cartridge and eluted with chloroform–methanol (2:1). Eluates were evaporated and reconstituted in acetonitrile–5 μM sodium trifluoroacetate in 0.1% aqueous formic acid (20:80) and injected onto a Waters Oasis® HLB column. Analytes were eluted from the column with a solvent gradient into the mass analyzer. The ions were quantified in the selected reaction-monitoring mode (SRM), using positive ions, on a triple quadrupole mass spectrometer. The lower limits of quantification for Dox-Con, Leu-Dox, and Dox in plasma, were approximately 5, 1 (dog)/6 (rat), and 0.5 ng/ml, respectively. Intra- and inter-assay accuracy (% of nominal concentration) and precision (%CV) for all analytes were within 15 and 16%, respectively.

Keywords: Peptide doxorubicin conjugate; Doxorubicin; HPLC-MS/MS

1. Introduction

A seven-amino acid peptide conjugate (Dox-Con) was selected as a prodrug for the tumor-selective delivery of doxorubicin (Dox), with the objective to lower general systemic exposure to the active drug in the treatment of human prostate cancer [1]. Therefore, there was an interest for a sensitive bioanalytical method to monitor simultaneously the parent compound and two of its active metabolites Leu-Dox and Dox (Fig. 1) in plasma. Bioanalytical methods had been published for the determination of Dox-Con and its active metabolites by HPLC with fluorescence detection and by HPLC-MS/MS [2–4]. In these methods, Dox-Con and its metabolites were extracted from plasma by solid-phase extraction (SPE) on C8 cartridges and then chromatographed on a C8 HPLC column.

* Corresponding author. Tel.: +33-473-67-5072; fax: +33-473-38-5691.
E-mail address: claude_mazuel@merck.com (C. Mazuel).

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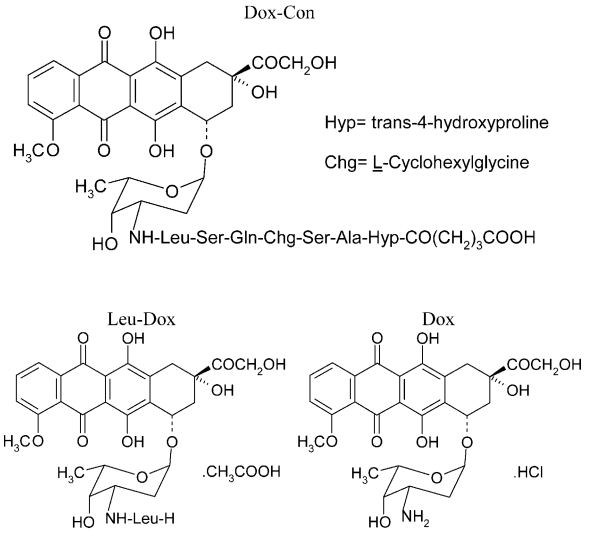

Fig. 1. Chemical structure of doxorubicin conjugate (Dox-Con), leucine-doxorubicin (Leu-Dox), and doxorubicin (Dox).

The MS/MS detection of all analytes was done in the positive ion mode using a sodium adduct for Dox-Con. Fast chromatography with ultra-high flow rates had been reported for the quantitative bioanalyses of drugs, by HPLC-MS/MS, following direct on column injection of plasma samples [5–7]. These analyses were done on small columns containing large particle size packing (30 μm). After an initial separation of proteins and large biomolecules, not retained on the column under the ultra-high flow conditions, the analytes were coeluted, by a step gradient, into the mass spectrometer. These analytical conditions, which give very short analysis time, were initially tested in our laboratory for Dox-Con and its metabolites. However, elution of the analytes at higher flow rates using the step gradient afforded poor quantitative reproducibility. During preliminary experiments, reproducibility was improved by reducing the flow rate and the gradient slope leading to a partial separation of the analytes and better formed peaks. The cross-talk, between coeluting Leu-Dox and Dox, estimated to less than 0.1 area% was considered acceptable. Because of the limited stability of the analytes in plasma, it was chosen to extract the plasma samples rather than keep them for extended periods at ambient temperature on the autosampler. In the method presented, two different internal standards: IS-I for the metabolites and IS-II (Fig. 2) for Dox-Con were used to maximize reproducibility. The adduct formation, which could not be successfully reproduced, was forced by addition of sodium trifluoroacetate to the mobile phase.

2. Experimental

2.1. Reagents and chemicals

Acetonitrile, methanol, and chloroform HPLC grades were purchased from Merck KgaA (Darmstadt, Germany). SPE cartridges Bond Elut® C8 1 cc/100 mg were purchased from Varian (Palo Alto, CA, USA). Water used for preparing reagents and mobile phase was deionized with an Alpha-Q® purifier Millipore (Bedford, USA). Formic acid, sodium trifluoroacetate (NaTFA), Dox, and IS-I were obtained from Sigma Co. (St. Louis, MO, USA). The Dox-Con, Leu-Dox, and IS-II were synthesized at Merck Research Laboratories (West-Point, PA, USA).

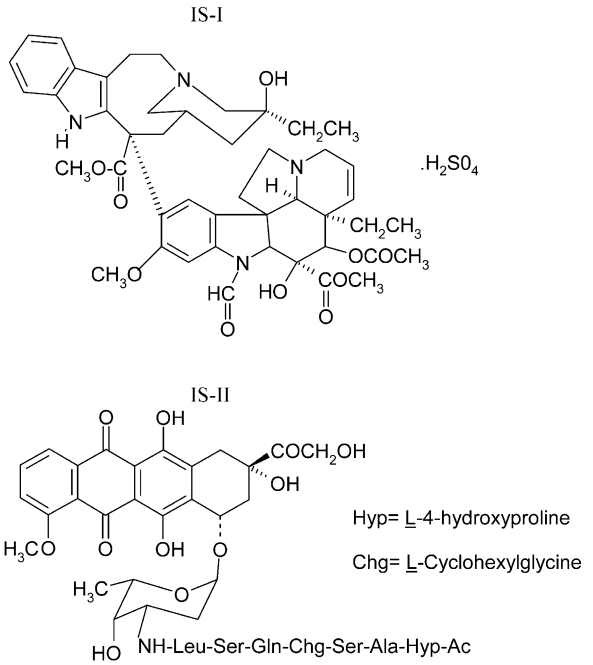

Fig. 2. Chemical structure of the internal standards vincristine (IS-I) and (IS-II).

2.2. Instrumentation

Triple quadrupole mass spectrometer TSQ® 7000 with XCALIBUR® and LCQUAN® software from ThermoFinnigan (San Jose, CA, USA) equipped with an electrospray ion source (API 1) and a divert valve. The chromatographic system consisted of an HPLC pump model 616 and Controller 616 S from Waters (Milleford, MA, USA) and an autosampler Series 200 from Perkin–Elmer (Norwalk, CT, USA). The chromatography column used was Oasis® HLB (30 μm particle size, 1 × 50 mm) from Waters.

2.3. Preparation of standards and quality control (QC) samples

Separate stock solutions of each analyte and each internal standard were prepared in acetonitrile–0.1% aqueous formic acid (20:80). Working standard solutions combining the three analytes were then prepared by dilutions of the stock solutions, with acetonitrile–0.1% aqueous formic acid (20:80), to obtain several levels over the concentration ranges of 20–31 000, 4–6700, and 2–3200 ng/ml for Dox-Con, Leu-Dox, and Dox, respectively. These solutions were stable up to 34 days when stored refrigerated. Separate working internal standard solutions were prepared by dilutions of each stock solution, with acetonitrile–0.1% aqueous formic acid (20:80), to obtain concentrations of approximately 1200 and 3200 ng/ml for IS-I and IS-II, respectively. Calibration standards were prepared by spiking 200 μl of drug-free plasma with 50 μl of the working standard solutions to obtain several levels equivalent to concentrations in plasma ranging approximately from 5 to 7600, 1/6 (dog/rat) to 1700, and 0.5 to 800 ng/ml, for Dox-Con, Leu-Dox, and Dox, respectively. These calibration ranges were selected, based in part on previously published work and made as wide as possible to avoid unnecessary repeat assays caused by samples falling outside the limits. In order to cover well these wide ranges, up to 12 levels of standards were prepared. QC samples, that simulate future study samples, were prepared in a similar fashion to obtain levels equivalent to plasma concentrations of approximately 40, 2500, and 5000 ng/ml for Dox-Con, 8, 480, and 960 ng/ml for Leu-Dox, and 5, 290, and 580 ng/ml for Dox. Fifty μl of each working internal standard solution was added to each plasma standard and QC sample.

2.4. Plasma study samples

Four male and four female Beagle dogs were each given a single intravenous injection of Dox-Con at the dose of 10 mg/kg. Blood samples were collected in heparinized tubes and cooled on ice at 0.25, 0.5, 1, 2, 4, 8, 24, 48, and 72 h post-dose. Shortly after blood collection, plasma was separated by centrifugation at approximately 4 °C and stored at −70 °C until analysis. Before extraction, 50 μl of each working internal standard solution and 50 μl of acetonitrile–0.1% aqueous formic acid (20:80), were added to 200 μl of plasma sample.

2.5. Extraction procedure

Calibration standards, QC, and study samples were diluted 50% with water and centrifuged for 5 min at 12 000 rpm at 4 °C. An approximately 500 μl aliquot of supernatant was transferred to a C8 SPE cartridge preconditioned with 1.0 ml of methanol and 1.0 ml of water. The cartridge was washed successively with 0.8 ml of water and with 0.8 ml of 10% methanol. Analytes were then eluted with two 0.8 ml portions of chloroform–methanol (2:1). The eluate was evaporated to dryness under nitrogen at approximately 50 °C and the dried extract was reconstituted with 200 μl of acetonitrile–5 μM NaTFA in aqueous 0.1% formic acid (20:80). The three analytes were stable in this solvent for approximately 48 h under ambient laboratory conditions.

2.6. Mass spectrometric conditions

Electrospray-ionization was performed in the positive ion mode. The heated capillary was set at 245 °C, the spray voltage at 4.5 kV. Nitrogen was used as the sheath and auxiliary gas set at 90 lb/in^2 and 25 arbitrary units, respectively. The argon collision gas pressure was set to 2.5 mTorr.

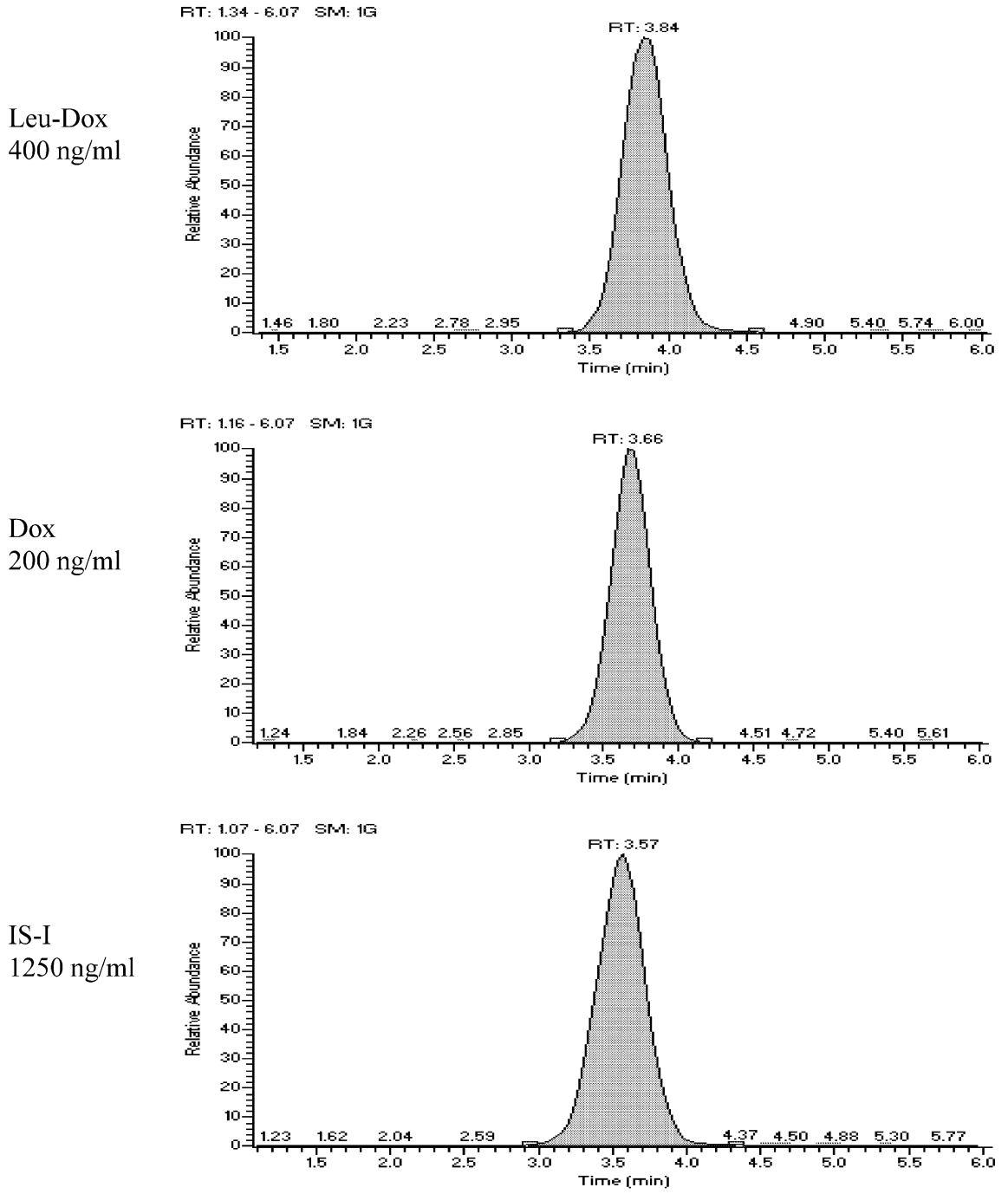

Fig. 3. Typical reconstructed ion chromatograms of Dox-Con metabolites and their internal standard in dog plasma extracts.

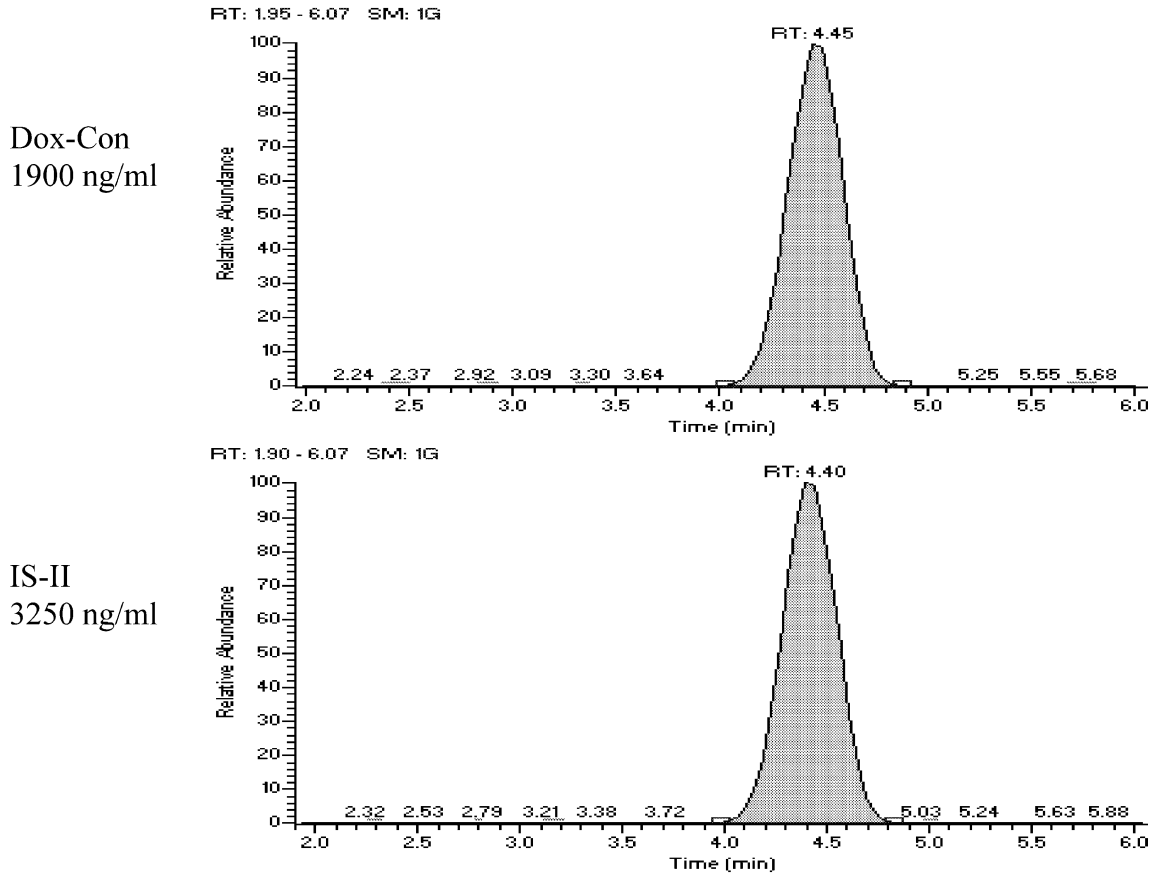

Fig. 4. Typical reconstructed ion chromatograms of Dox-Con and its internal standard in dog plasma extracts.

Analytes and internal standards were detected, in the positive ion mode, using the selected reaction monitoring of the following transitions: m/z 1419→1023 for Dox-Con, m/z 657→243 for Leu-Dox, m/z 544→397 for Dox, m/z 413→353 for IS-I, and m/z 1347→951 for IS-II.

2.7. Chromatographic conditions

Fifty μl of reconstituted solutions were chromatographed at ambient temperature on the Oasis® HLB column. Solvent A was acetonitrile and Solvent B was 5 μM sodium trifluoroacetate in 0.1% aqueous formic acid. The analytes were chromatographed with a 0.8 ml/min flow rate and the following linear gradient: 0 min, 5% A; 0.5 min, 5% A; 5.5 min, 82% A. The gradient was followed by a 2.0 ml/min rinse for 1.0 min with 100% methanol, and 30 s with 95% acetonitrile, and then by column reequilibration to the initial conditions. The flow was diverted into the mass spectrometer only during the elution of the analytes/internal standards and to waste during the rest of the chromatographic cycle. Typical reconstructed ion chromatograms of plasma standards containing all analytes and internal standards are shown in Figs. 3 and 4.

2.8. Calculations

Concentrations of Dox-Con and its metabolites were calculated with the LCQUAN® quantitation

Table 1
Accuracy and precision of calibration standards in dog plasma

Dox-Con nominal concentration	4.73	9.45	23.6	47.3	94.5	189	378	945	1890	3030	6050	7550
Average found (ng/ml)	4.70	9.67	23.3	46.4	95.6	192	370	949	1910	3020	6080	7510
Accuracy (%)	99.4	102	98.7	98.1	101	102	97.9	100	101	99.7	100	99.5
Precision (%CV)	2.81	6.38	4.59	2.17	3.15	4.63	2.37	4.27	2.85	4.56	3.58	1.73
Number of replicates	5	5	5	5	5	5	5	5	5	5	5	5
Leu-Dox nominal concentration	1.06	2.11	5.28	10.6	21.1	42.3	84.3	211	423	675	1350	1690
Average found (ng/ml)	1.03	2.19	5.55	10.0	20.8	42.4	86.0	214	426	638	1340	1720
Accuracy (%)	97.2	104	105	94.3	98.6	100	102	101	101	94.5	99.3	102
Precision (%CV)	5.27	7.85	7.62	9.48	3.06	2.17	4.04	4.50	2.35	9.14	3.14	2.41
Number of replicates	4	5	5	5	5	5	5	5	5	5	5	5
Dox nominal concentration	0.500	1.00	2.50	5.00	10.0	20.0	40.0	99.8	200	320	638	798
Average found (ng/ml)	0.498	1.01	2.62	4.60	9.61	20.1	41.4	103	205	310	637	799
Accuracy (%)	99.6	101	105	92.0	96.1	101	104	103	103	96.9	99.8	100
Precision (%CV)	6.12	12.6	6.35	4.75	4.51	4.22	4.82	3.16	3.67	6.84	2.29	0.737
Number of replicates	5	5	5	5	5	5	5	5	5	5	5	5

Table 2
Accuracy and precision of calibration standards in rat plasma

Dox-Con nominal concentration	4.75	9.50	23.8	47.5	95.0	190	380	950	1900	3050	6080	7600
Average found (ng/ml)	4.67	9.74	24.6	47.8	90.7	189	387	947	1840	3090	6140	7560
Accuracy (%)	98.3	103	103	101	95.5	99.5	102	99.7	96.8	101	101	99.5
Precision (%CV)	1.80	7.82	9.91	5.17	3.23	10.3	7.08	4.33	6.12	4.53	3.77	2.70
Number of replicates	3	3	3	3	3	3	3	3	3	3	3	3
Leu-Dox nominal concentration	5.60	11.2	22.4	44.8	89.3	223	445	713	1430	1780		
Average found (ng/ml)	5.62	11.3	21.7	44.1	93.5	211	451	741	1440	1740		
Accuracy (%)	100	101	96.9	98.4	105	94.6	101	104	101	97.8		
Precision (%CV)	5.44	10.4	9.18	8.96	2.20	4.55	6.21	7.26	3.67	1.45		
Number of replicates	3	3	3	3	3	3	3	3	3	3		
Dox nominal concentration	0.500	1.00	2.50	5.00	10.0	20.0	40.0	100	200	320	640	800
Average found (ng/ml)	0.512	0.979	2.48	4.88	9.82	19.3	42.0	101	200	337	647	777
Accuracy (%)	102	97.9	99.2	97.6	98.2	96.5	105	101	100	105	101	97.1
Precision (%CV)	5.25	9.14	7.34	6.62	2.54	5.77	0.476	1.74	3.50	5.78	3.31	0.876
Number of replicates	2	3	3	3	3	3	3	3	3	3	3	3

software by plotting the area ratios of analyte to internal standard, obtained on the chromatograms, against standard concentrations.

3. Results and discussion

3.1. Selectivity

The selectivity of the method was demonstrated by the absence of substantial endogenous interference on the chromatograms of plasma extracts verified on three separate sources of plasma from untreated dogs and rats.

3.2. Extraction recovery from plasma

Absolute recoveries of each analyte, determined on plasma spiked at three concentrations equivalent to the QC samples and processed as in Section 2.4, was not less than 70 and 49% from dog and rat plasma, respectively.

Table 3
Intra- and inter-assay accuracy and precision for the dog plasma QC samples

	Intra-assay			Inter-assay		
Dox-Con QC nominal concentration	40.5	2530	5050	40.5	2530	5050
Average found (ng/ml)	38.4	2310	4590	40.7	2390	4700
Accuracy (%)	94.8	91.3	90.9	100	94.5	93.1
Precision (CV%)	2.65	3.27	1.81	5.57	3.40	2.65
Number of replicates	10	10	10	10	10	10
Leu-Dox QC nominal concentration	7.65	478	955	7.65	478	955
Average found (ng/ml)	8.49	495	983	8.62	506	984
Accuracy (%)	111	104	103	113	106	103
Precision (CV%)	10.2	11.5	11.7	9.27	10.8	9.92
Number of replicates	10	10	10	10	10	10
Dox QC nominal concentration	4.65	290	583	4.65	290	583
Average found (ng/ml)	4.21	282	583	4.92	313	606
Accuracy (%)	90.5	97.2	100	106	108	104
Precision (CV%)	14.6	7.01	10.5	12.1	8.54	9.61
Number of replicates	10	10	10	10	10	10

Table 4
Intra- and inter-assay accuracy and precision for the rat plasma QC samples

	Intra-assay			Inter-assay		
Dox-Con QC nominal concentration	40.8	2550	5100	40.8	2550	5100
Average found (ng/ml)	41.0	2410	4690	39.7	2440	4750
Accuracy (%)	100	94.5	92.0	97.3	95.7	93.1
Precision (CV%)	8.83	6.69	1.52	10.4	6.89	1.36
Number of replicates	6	6	6	6	6	6
Leu-Dox QC nominal concentration	7.53	470	940	7.53	470	940
Average found (ng/ml)	8.15	484	987	8.02	458	924
Accuracy (%)	108	103	105	107	97.4	98.3
Precision (CV%)	14.6	2.37	9.55	15.8	4.73	10.4
Number of replicates	6	6	6	6	6	6
Dox QC nominal concentration	4.73	295	593	4.73	295	593
Average found (ng/ml)	4.90	304	628	4.84	295	588
Accuracy (%)	104	103	106	102	100	99.2
Precision (CV%)	6.22	5.52	5.59	5.55	3.73	4.90
Number of replicates	6	6	6	6	6	6

3.3. Accuracy and precision of calibration standards

Accuracy and precision of calibration standards in dog and rat plasma were determined for each analyte on five replicate analyses of the complete set of standards. Because of the wide concentration range, the calibration curves for all three analytes were fitted with quadratic models (zero ignored) using a $1/x^2$ weighting factor. This was found to best accommodate the reponse profile within the precision required, through the entire range. Concentrations of analytes were calculated from each curve. The accuracy was obtained by comparing the average calculated concentrations to their nominal values (% of nominal) and the precision by the percent coefficient of variation (%CV). Results for dog plasma are given in Table 1. The accuracy and precision for Dox-Con ranged from 97.9 to 102% and 1.73 to 6.38%, respectively.

Table 5
Stability of the combined analytes in dog plasma after three freeze–thaw cycles between −70 °C and melting ice temperature

Nominal concentration (ng/ml)			Percentage of initial concentration		
Dox-Con	Leu-Dox	Dox	Dox-Con	Leu-Dox	Dox
51.3	11.5	5.41	107	89.6	117
2420	540	255	102	86.6	88.3
4840	1080	510	106	90.4	98.4

The accuracy and precision for Leu-Dox ranged from 94.3 to 105% and 2.17 to 9.48%, respectively. The accuracy and precision for Dox ranged from 92.0 to 105% and 0.737 to 12.6%, respectively. Results for rat plasma are given in Table 2. The accuracy and precision for Dox-Con ranged from 95.5 to 103% and 1.80 to 10.3%, respectively. The accuracy and precision for Leu-Dox ranged from 94.6 to 105% and 1.45 to 10.4%, respectively. The accuracy and precision for Dox ranged from 96.5 to 105% and 0.476 to 9.14%, respectively.

3.4. Lower limits of quantitation

The lower limits of quantification were the lowest concentrations of the calibration standards, as determined in Section 3.3, that had an accuracy within ±20% of nominal and a precision within ±20% CV. They were approximately 4.7, 1.1, and 0.5 ng/ml in dog plasma, and approximately 4.8, 5.6, and 0.5 ng/ml in rat plasma for Dox-Con, Leu-Dox, and Dox, respectively.

3.5. Intra- and inter-assay accuracy and precision of QC samples

The intra- and inter-assay accuracy and precision for each analyte were determined on replicate analyses of each QC sample. The accuracy was obtained by comparing the average calculated concentrations to their nominal values (% of nominal) and the precision by the %CV. Results for dog plasma are given in Table 3. For Dox-Con, the intra-assay accuracy and precision ranged from 90.9 to 94.8% and 1.81 to 3.27%, respectively. The inter-assay accuracy and precision ranged from 93.1 to 100% and 2.65 to 5.57%, respectively. For Leu-Dox, the intra-assay accuracy and precision ranged from 103 to 111% and 10.2 to 11.7%, respectively. The inter-assay accuracy and precision ranged from 103 to 113% and 9.27 to 10.8%, respectively. For Dox, the intra-assay accuracy and precision ranged from 90.5 to 100% and 7.01 to 14.6%, respectively. The inter-assay accuracy and precision ranged from 104 to 108% and 8.54 to 12.1%, respectively. Results for rat plasma are given in Table 4. For Dox-Con, the intra-assay accuracy and precision ranged from 92.0 to 100% and 1.52 to 8.83%, respectively. The inter-assay accuracy and precision ranged from 93.1 to 97.3% and 1.36 to 10.4%, respectively. For Leu-Dox, the intra-assay accuracy and precision ranged from 103 to 108% and 2.37 to 14.6%, respectively. The inter-assay accuracy and precision ranged from 97.4 to 107% and 4.73 to 15.8%, respectively. For Dox, the intra-assay accuracy and precision ran-

Table 6
Stability of the combined analytes in dog plasma after 13 months at approximately −70 °C

Nominal concentration (ng/ml)			Percentage of nominal concentration		
Dox-Con	Leu-Dox	Dox	Dox-Con	Leu-Dox	Dox
51.3	11.5	5.41	114	89.6	91.7
2420	540	255	115	80.6	91.8
4840	1080	510	111	75.9	89.2

Table 7
Mean concentrations of Dox-Con and its metabolites in dog plasma after a 10-mg/kg single intravenous injection of Dox-Con

Bleed time (h)	Mean concentration (ng/ml) ±S.E.M.[a]		
	Dox-Con	Leu-Dox	Dox
0.25	9630±685	1700±100	14.0±0.598
0.5	2590±186	842±19.9	14.5±0.607
1	462±38.5	365±23.1	16.5±1.16
2	68.6±4.20	127±7.63	16.8±1.02
4	14.2±0.719	28.8±1.81	17.3±1.05
8	<LLQ[b]	3.46±0.356	18.8±1.16
24	<LLQ	<LLQ	8.29±0.292
48	<LLQ	<LLQ	5.47±0.248
72	<LLQ	<LLQ	3.81±0.180

[a] S.E.M., standard error of the mean.
[b] LLQ, lower limit of quantitation.

ged from 103 to 106% and 5.52 to 6.22%, respectively. The inter-assay accuracy and precision ranged from 99.2 to 102% and 3.73 to 5.55%, respectively.

3.6. Freeze–thaw stability

A preliminary experiment had shown that analyte stability was limited in plasma at ambient temperature. Therefore, plasma samples were kept in an ice bath and freeze–thaw stability was studied between −70 °C and melting ice temperature. Under these conditions, the analytes were considered stable in dog plasma after three cycles; results are shown in Table 5.

3.7. Freezer stability

Long-term stability at −70 °C was evaluated on dog plasma samples spiked with the three combined analytes. Possible conversion to the metabolites was monitored, concomitantly, on two sets of dog plasma samples: One set spiked with Dox-Con only and one set spiked Leu-Dox only. Based on the assay results, presented in Table 6, the combined analytes were considered stable for at least 13 months at this temperature. No substantial conversion from Dox-Con to Leu-Dox or from Leu-Dox to Dox occurred during this period.

3.8. Analysis of study samples

Dox-Con and its metabolites were quantitated in the dog plasma samples collected after administration of a single 10-mg/kg intravenous injection of Dox-Con. Samples, with concentrations exceeding the upper limit of the calibration ranges, were assayed after dilution with blank plasma obtained from non-treated animals. Results presented in Table 7 show the applicability of the method to sample from an in vivo study.

4. Conclusion

A sensitive HPLC-MS/MS method was developed and validated to monitor, in a single analysis, concentrations of Dox-Con and its two active metabolites Leu-Dox and Dox in dog and rat plasma. This analysis was performed on Oasis® HLB columns after SPE extraction of the analytes from plasma. The lower limits of quantification were approximately 4.7, 1.1, and 0.5 ng/ml in dog plasma, and approximately 4.8, 5.6, and 0.5 ng/ml in rat plasma for Dox-Con, Leu-Dox, and Dox, respectively. This method was used successfully for the analysis of plasma samples collected from dogs treated with a 10-mg/kg single intravenous injection of Dox-Con.

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References

[1] V.M. Garsky, P.K. Lumma, D.-M. Feng, J. Wai, H.G. Ramjit, M.K. Sardana, A. Oliff, R.E. Jones, D. Defeo-Jones, R.M. Freidinger, J. Med. Chem. 44 (2001) 4216–4224.

[2] D. Defeo-Jones, V.M. Garsky, B.K. Wong, D.-M. Feng, T. Bolyar, K. Haskell, D.M. Kieffer, K. Leander, E. McAvoy, P. Lumma, J. Wai, E.T. Senderak, S.L. Motzel, K. Keenan, M. Van Zwieten, J.H. Lin, R. Freidinger, J. Huff, A. Oliff, R.E. Jones, Nat. Med. 6 (2000) 1248–1252.

[3] B.K. Wong, D. Defeo-Jones, R.E. Jones, V.M. Garsky, D.-M. Feng, A. Oliff, M. Chiba, J.D. Ellis, J.H. Lin, Drug Metab. Dispos. 29 (2001) 313–318.

[4] M.S. Schwartz, B.K. Matuszewski, J. Chromatogr. 780 (2002) 171–182.

[5] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, Rapid Commun. Mass Spectrom. 11 (1997) 1953–1958.

[6] M. Jemal, Yuan-Qing, D.B. Whigan, Rapid Commun. Mass Spectrom. 12 (1998) 1389–1399.

[7] J. Ayrton, R.A. Clare, G.J. Dear, D.N. Mallett, R.S. Plumb, Rapid Commun. Mass Spectrom. 13 (1999) 1657–1662.