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An API LC/MS/MS quantitation method for ansamitocin P-3 (AP3) and its preclinical pharmacokinetics

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Abstract

Ansamitocin P-3 (AP3) is a potent maytansinoid antitumor agent isolated from microorganisms and mosses. In this study, a highly sensitive and specific electrospray ionization (ESI) liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for quantitation of AP3 was developed and validated. AP3 was extracted from rat plasma along with the internal standard, depsipeptide FK228 (NSC-630176, FR) with ethyl acetate. Components in the extract were separated on a 50 mm × 2.1 mm Betabasic C 85 μm stainless steel column by isocratic elution with 70% acetonitrile/0.9% formic acid. The liquid flow was passed through a pre-source splitter and 5% of the eluent was introduced into the API source. The components were analyzed in the multiple-reaction-monitoring (MRM) mode as the precursor/product ion pair of *m/z* 635.2/547.2 for AP3 and of *m/z* 541.5/424.0 for the internal standard FR. Linear calibration curves were obtained in the range 1–500 ng/mL using 0.2 mL rat plasma. The within-day coefficients of variation (CVs) were 12.9, 6.7, and 5.5% and the between-day CVs were 10.4, 6.5, and 6.4% (all $n = 5$) at 1, 10, and 200 ng/mL, respectively. A formulation based on normal saline and PEG300 was then developed and Sprague–Dawley male rats were given this formulated drug by i.v. bolus. Plasma drug concentrations were measured by this method and the pharmacokinetics were analyzed by standard techniques. Plasma concentration–time profiles were found to follow a triexponential decline and the terminal phase was nearly flat, suggesting that the drug distributed in deep tissue compartments or organs and then equilibrates slowly with the blood stream.

Keywords: Ansamitocin P-3; Maytansinoids; Anticancer agents; Pharmacokinetics; Metabolism; Liquid chromatography–tandem mass spectrometry

1. Introduction

AP3 (Fig. 1, inset) is a potent maytansinoid antitumor agent isolated from microorganisms and mosses [1–6]. Its structure elucidation showed that AP3 possesses a macrolactam skeleton. In vitro studies [7] demonstrated that AP3 showed antitumor activity toward human leukemia P-388, some antimicrobial activity against Hamigera avellanea IFO 7721 at a concentration of 100 (g/mL, and potent cytotoxicity against human solid tumor cell lines A-549 and HT-29. AP3 was found to block the assembly of purified leishmanial tubulin and inhibited the growth of *Leishmania donovani promastigotes* at micromolar concentrations. When a derivative of AP3 was conjugated to the monoclonal antibody C422 [8], the immunoconjugate showed high cytotoxicity toward cultured colon cancer cells in an antigen-specific manner and remarkable antitumor efficacy in vivo such as eradication of large colon tumor xenografts at doses that showed very little host toxicity. Several immunoconjugates of AP3 are currently in clinical trials. The objective of this study was to develop a validated, sensitive, and specific LC/MS/MS analytical method, and to evaluate the pharmacokinetic properties of AP3 in rats using this methodology.

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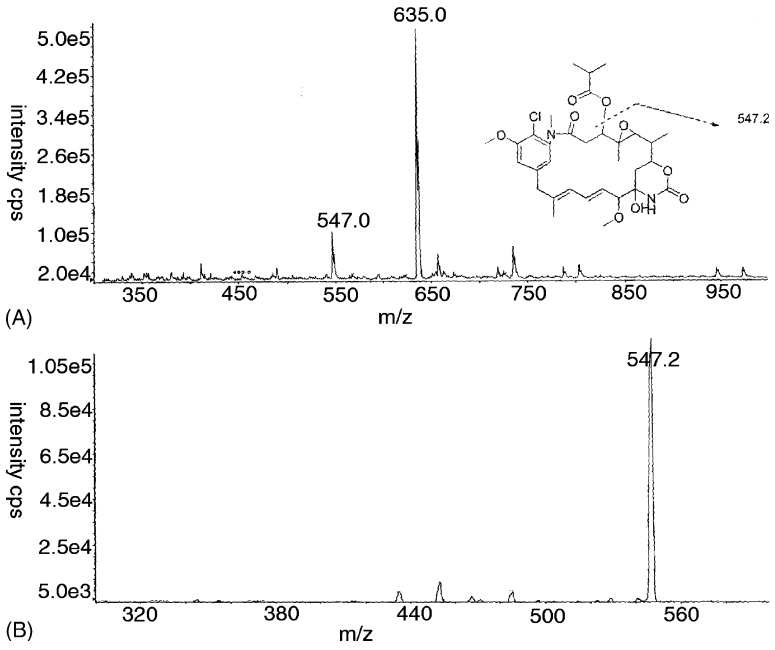

Fig. 1. LC/electrospray mass spectrum (A) of AP3 and collision-induced dissociation mass spectrum (B) of AP3. The parent ion is at *m*/*z* 635.0. Inset: the proposed fragmentation.

2. Experimental

2.1. Materials

AP3 was provided as a white powder by Takeda Chemical Industries, Osaka, Japan, and used without further purification. The internal standard, depsipeptide FK228 (NSC-630176, FR, Fig. 2), was obtained from the National Cancer Institute (NCI) and used without purification.

The HPLC grade water (>18 mΩ) was obtained from an E-pure water purification system (Barnstead, Dubuque, IA). Acetonitrile (HPLC grade) and ethyl acetate (reagent grade) were purchased from Fisher Scientific (Pittsburgh, PA) and formic acid (reagent grade) from Sigma (St. Louis, MO). Ketamine HCl injection (USP) was purchased from the Ohio State University of Pharmacy (product of Ben Venue Labs, Inc., Bedford, OH). Drug-free heparinized rat plasma was purchased from Harlan Bioproducts for Science, Inc. (Indianapolis, IN). All chemicals and reagents were used as received.

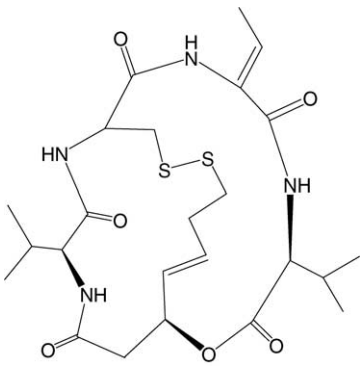

Fig. 2. Structure of depsipeptide FR.

2.2. Instrumentation

The LC/MS system used consisted of a Perkin-Elmer Sciex API 300 triple–quadruple mass spectrometer (Thornhill, Ontario, Canada) coupled to a Shimadzu HPLC system (Shimadzu, Columbia, MD). The HPLC system was equipped with an SCL-10A system controller, an LC-10AD pump and an SIL-10A autosampler (Shimadzu, Columbia, MD).

2.3. HPLC chromatographic and mass spectrometric conditions

AP3 and internal standard FR were separated on a 50 mm × 2.1 mm Betabasic C 85 μm stainless steel column (Thermo Hypersil-Keystone, Bellefonte, PA), which was coupled to a 2 μm precolumn filter (Thermo Hypersil-Keystone, Bellefonte, PA). The components were eluted with a mobile phase consisting of 70% (v/v) aqueous acetonitrile containing 0.9% (v/v) formic acid at a flow rate of 0.4 mL/min. The run time was 5 min. The LC eluate was introduced into the API source at 20 μL/min after a 95:5 (LC/MS) split.

The mass spectrometer was operated using electrospray ionization (ESI) with an ionspray voltage of +4620 V. The positive ion multiple-reaction-monitoring (MRM) mode analysis was performed using nitrogen as the collision gas. The curtain gas (nitrogen) flow and the ionspray flow were set at 0.6 and 0.9 L/min, respectively. The pressure in the collision cell was set at 0.29 Pa. The orifice voltage and

ring voltages were set to +35 and +400 V, respectively. A dwell time of 600 ms and a pause time of 5 ms between scans were used to monitor the following precursor/product ion pairs: *m/z* 635.2/547.2 for AP3 and *m/z* 541.5/424.0 for FR. The reason for selection of FR as the internal standard was based on its similar molecular weight and lipophilicity and well-characterized analytical behaviors [9,10]. The mass spectrometer was tuned to its optimum sensitivity and mass accuracy by infusion of a standard calibration solution of polypropylene glycol (PPG) on a daily basis. This tuning was further adjusted by injection of a fresh standard solution of AP3 at 5 ng/mL in the HPLC mobile phase as described above. This tuning was found essentially similar to that by the internal standard FR. Data acquisition was performed using the PE Sciex software Sample Control 1.2 and the data were analyzed by PE Sciex software MacQuan 1.4.

2.4. Sample preparation

AP3 and FR in plasma were extracted with ethyl acetate. To each polypropylene centrifuge tube (Falcon, Fisher, Philadelphia, PA) was added 0.1 mL of rat sample plasma and 0.1 mL of blank rat plasma containing 10 μL of FR solution (2 μg/mL in acetonitrile). The content was allowed to stand at room temperature for 1 min. The solution was then extracted with 2.0 mL of ethyl acetate and the mixture was vortexed for 1 min and centrifuged at 3000 × *g* for 15 min. The organic layer was transferred to a clean polypropylene tube and evaporated to dryness under a stream of nitrogen. The residues were dissolved in 200 μL of 70% acetonitrile/0.9% formic acid and a 50-μL aliquot was introduced into the LC/MS system for analysis.

2.5. Assay validation

Plasma samples for the standard curves were prepared by spiking 0.2 mL of rat plasma each with various amounts of AP3 and a constant amount of FR. The linearity was evaluated in the concentration range of 1–500 ng/mL of rat plasma. The within-day precision values were determined in five replicates at each concentration of 1, 2, 5, 10, 20, 50, 100, 200, 500 ng/mL of AP3 in rat plasma, and these replicates were processed independently. The between-day precision was determined across nine concentrations at five different days, and the mean concentrations and coefficients of variation were calculated. The accuracy of the assay was determined by comparing the nominal concentrations with the corresponding calculated concentrations via linear regression. The specificity of the assay was established by simultaneously monitoring one major product ion from the molecular ion of AP3 in blank plasma; no trace of interfering substance was found at the same retention time as AP3. The recovery of AP3 was estimated by comparing the peak area of the extract AP3 to that of the unextracted AP3 at four concentrations of 1, 5, 50, and 500 ng/mL.

2.6. In vitro plasma protein binding studies

Ultrafiltration in an Amicon device (MW cut-off 30,000) was used to assess the plasma protein binding of AP3 in rat plasma. AP3 was added to rat plasma as an acetonitrile solution at 0, 10, 100, and 1000 ng AP3/mL of plasma with a final acetonitrile concentration of 0.5%. The mixture was allowed to stand at room temperature for 45 min, then transferred to the Amicon filter tube, and the tube was centrifuged at 25 °C, 2000 × *g* for 3 h. Concentrations of AP3 before centrifugation and of the free AP3 in the filtrate were analyzed using the previously validated HPLC/MS/MS method.

2.7. Distribution of AP3 in rat blood

AP3, 5 μg in acetonitrile (1 μg/μL), was incubated with 1 mL of fresh Sprague–Dawley male rat heparinized blood at 37 °C for 1 h and with 0.6 mL of rat plasma. Then the mixtures were centrifuged at 15,000 × *g* for 3 min. The concentration of AP3 in the plasma of the rat blood and the rat plasma was analyzed by the previously validated HPLC/MS/MS method.

2.8. Preliminary pharmacokinetic study of AP3 in Sprague–Dawley male rats

Six Sprague–Dawley male rats weighing about 300 g were used in the pharmacokinetics studies. The right jugular vein of each rat was cannulated under ketamine anesthesia (100 mg/kg), and the rat was allowed to recover for about 12 h prior to drug administration. AP3, dissolved in 50% PEG300 and 50% normal saline, was given as an i.v. bolus at a dose of 1.5 mg/kg through the jugular vein cannula followed by flushing the cannula with 0.25 mL of normal saline. Approximately 0.2 mL each of heparinized blood was withdrawn according to a typical schedule of 0 (predose), 2, 5, 10, 20, 30, 45, 60, 120, 180, 300, 480, 720, and 1440 min after dosing, and the loss of fluid was replaced by flushing the cannula with an equal volume of normal saline. The blood samples were centrifuged at 15,000 × *g* for 1 min and the supernatants of each were collected and kept frozen at −80 °C until analysis.

2.9. Data analysis

Plasma concentration–time profiles were analyzed by the WinNonlin computer software, version 4.0 (Pharsight Corporation, Mountain View, CA), using an appropriate model and model-independent methods.

3. Results

3.1. LC/MS/MS assay

Under ESI conditions, AP3 gave MH^+ at *m/z* 635.0 as the base ion (Fig. 1A). This ion was selected for collision-induced dissociation (CID) experiments, which generated one major product ion at *m/z* 547.2, representing the cleavage of the ester bond (Fig. 1B, inset).

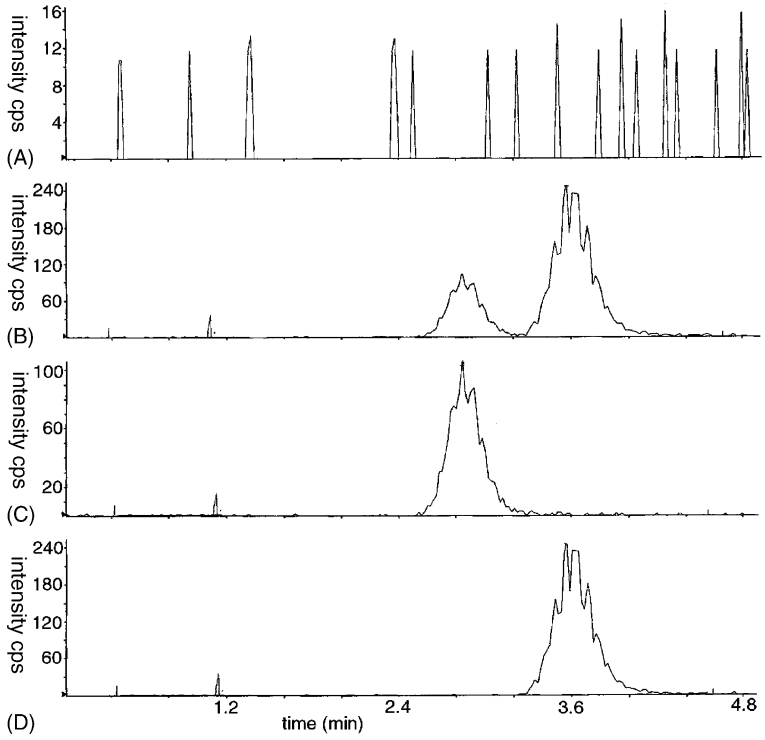

Fig. 3. (A) The total ionic chromatogram (TIC) of AP3 and FR in blank rat plasma; absence of a peak at the corresponding retention time indicated no significant chemical interference. (B) The total ionic chromatogram of rat plasma spiked with AP3 (10 ng/mL) and FR (10 ng/mL). (C) The extracted ion chromatogram of AP3 in rat plasma spiked with 10 ng/mL AP3. (D) The extracted ion chromatogram of FR in rat plasma spiked with 10 ng/mL.

The precursor/product ion pair at *m/z* 635.0/547.2 (Fig. 1) was selected in the MRM mode for quantitation of AP3. As previously reported [9], the precursor/product ion pair at *m/z* 541.5/424.0 of the internal standard FR was selected for the assay.

3.2. Assay validations

The assay was validated in rat plasma. The MRM chromatograms of blank rat plasma and rat plasma spiked with AP3 are shown in Fig. 3. As shown, AP3 and FR were baseline

Table 1
Assay validation characteristics of AP3 in rat plasma by ESI LC/MS/MS

Concentration (ng/mL plasma)		Within-day calculated				Average ± S.D.	CV (%)	Accuracy (%)
1	1.1	1.0	0.9	0.8	0.9	0.9 ± 0.1	12.9	93.6
2	2.1	1.9	2.2	2.1	1.7	2.0 ± 0.2	10.0	100.1
5	4.4	4.0	4.1	5	4.5	4.4 ± 0.4	9.2	88.2
10	8.3	8.7	9.6	8.6	9.6	9.0 ± 0.6	6.7	89.5
20	19.8	16.9	18.4	19.4	20.8	19.1 ± 1.5	7.8	95.3
50	48.4	42.3	52.4	46.9	47.3	47.5 ± 3.6	7.6	94.9
100	97.8	103	107	100	95.1	101 ±4.6	4.6	100.6
200	173	195	184	189	172	183 ± 10.0	5.5	91.3
500	511	504	509	504	516	509 ± 5.1	1.0	101.8
		Between-day calculated				Average ± S.D.	CV (%)	
1	1.0	1.1	0.8	0.9	0.9	0.9 ± 0.1	10.4	
2	1.8	2.1	2.0	1.9	2.1	2.0 ± 0.1	6.1	
5	5.9	5.3	5.2	4.0	4.6	5.0 ± 0.7	14.5	
10	8.8	9.5	10.3	8.8	9.5	9.4 ± 0.6	6.5	
20	17.0	19.6	20.5	17.1	17.9	18.4 ± 1.6	8.5	
50	44.8	51.8	49.6	44.8	52.7	48.7 ± 3.8	7.7	
100	93.1	92.0	104.0	93.1	102.0	96.8 ± 5.7	5.9	
200	185.0	208	200.0	185.1	212.0	198 ± 12.6	6.4	
500	506.0	499.8	498.0	506.0	492.0	500 ± 5.9	1.2	

separated (Fig. 3B), and the absence of signals at the retention times of AP3 and FR in the blank established the specificity of the assay (Fig. 3A). The limit of quantitation (LOQ) was set at 1 ng/mL in rat plasma, on the basis of a signal-to-noise level above 3:1 (Fig. 3C).

The assay was linear from 1 to 500 ng/mL, using 0.2 mL rat plasma, with regression coefficient (r^2) >0.9995. The within-day precision, expressed as %CV is given in Table 1. As shown, the values ranged from 1.0 to 12.9%. The between-day precision values ranged from 1.2 to 10.4%. The accuracy values of the assay varied from 89.3 to 101.7%. The validation results for quality control samples at low (5 ng/mL), medium (50 ng/mL), and high (500 ng/mL) were also incorporated into Table 1. The use of the internal standard was necessary, since the within-day CV values for these QC samples increased to 24.8, 30.4, and 4.0% with corresponding accuracy values of 144.8, 118.1, and 99.7%, and the between-day CV values changed to 29.6, 12.4, and 2.4%, respectively, when the internal standard was omitted. Additionally, the r^2 of the calibration curve mostly decreased to <0.9995. The mean recovery values of AP3 for the entire procedure were found to be 48.9, 45.4, 48.8, and 48.4% at 500, 50, 5, and 1 ng/mL in rat plasma (all n = 6), and since these were rather constant, they were considered to be adequate.

3.3. *In vitro plasma protein binding, distribution of AP3 in plasma and red blood cells, and pharmacokinetics of AP3 in Sprague–Dawley rats*

Using the LC/MS/MS method, the protein binding of AP3 in rat plasma was determined and it was found that the protein binding was nearly constant across a concentration range from 10 to 1000 ng/mL. AP3 was found to bind to rat plasma proteins to the extent of 82.3, 81.4, and 82.5% at 10, 100, and 1000 ng/mL, respectively. The distribution of AP3 in the plasma and red blood cells was found to be nearly equal.

The pharmacokinetics of AP3 after i.v. bolus administration in Sprague–Dawley male rats were studied. A typical plasma concentration–time profile of AP3 in rats following i.v. dosing at 1.5 mg/kg is presented in Fig. 4. As shown, plasma levels of AP3 reached nearly 300 ng/mL (0.47 μM) in 2 min and declined rapidly by about one order of magnitude within 1 h. The decline of the drug concentration then became slower and the drug was still detectable after 24 h at low ng/mL levels. The plasma concentration–time data were fitted to a three-compartment model and the relevant pharmacokinetic parameters were computed and are shown in Table 2. The mean half-life of the terminal phase was found to be 220 h and the area under the concentration–time curve (AUC) was calculated to be 6.73 μM h. The total clearance values from individual animals were rather variable with a mean value of 233 mL/h. These data were also analyzed with the noncompartment method and the relevant pharmacokinetic parameters are summarized in Table 2. The mean resident time was calculated to be 593 min, the 24 h AUC to be 5.05 μM h, and the volume of distribution at steady state was 28,313 L/kg.

4. Discussion

Due to the lack of a strong chromophore in AP3, an HPLC-UV assay is not expected to have adequate sensitivity for the analysis of AP3 in biological samples. Electrospray LC/MS/MS methods have recently been widely used for drug analysis in biological media, especially in the pharmaceutical industry [9–18]. These methods are generally highly sensitive and specific, and they simplify the sample cleanup. Frequently the elution time can be kept short, such as in the present case, making the sample analysis very efficient. Initially, we employed an isocratic program to completely resolve the AP3 and the internal standard FR peaks in 5 min as shown in Fig. 3 in case of interference by the matrix. No appreciable ion suppression was found in the MRM transitions of 635.2 → 547.4 and 541.5 → 424 for AP3 and FR, respectively, when the ethyl acetate extraction residue of rat plasma was spiked with the analytes. Later, we were able to shorten the retention time to about 3 min with some degree of peak overlap between AP3 and the internal standard FR without compromising the specificity of the detection and quality of the results.

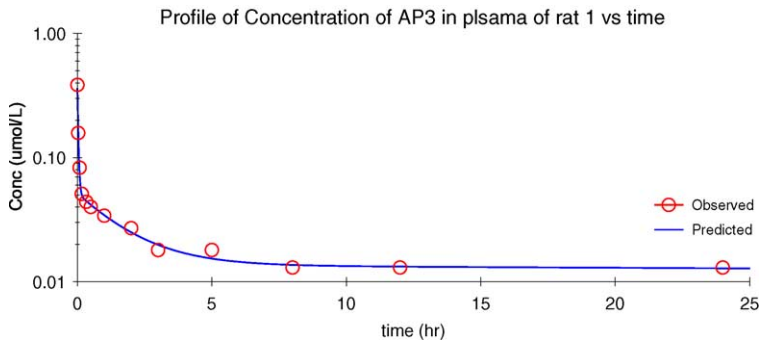

Fig. 4. A representative plasma concentration–time profile of AP3 in the rat following its i.v. bolus at 1.5 mg/kg (◯) measured concentration and (—) line fitted to a three-compartment model.

Table 2
Relevant pharmacokinetic parameters of AP3 in Sprague–Dawley rats following i.v. dosing at 1.5 mg/kg using compartment method except as indicated

Parameter	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average ± S.D. (range)
C_{2min} (μM)[a]	0.385	0.362	0.418	0.510	0.408	0.291	0.396 ± 0.072
A (μM)	0.731	7.54	14.9	8.55	5.79	5.13	8.20 ± 3.49
B (μM)	0.606	0.427	0.577	1.07	0.537	0.177	0.567 ± 0.292
C (μM)	0.204	0.156	0.164	0.259	0.261	0.231	0.213 ± 0.046
α (h^{-1})	19.3	27.6	40.8	20.4	18.2	20.4	24.4 ± 8.7
$t_{1/2\alpha}$ (min)	2.18	1.50	1.02	2.04	2.28	2.03	1.9 (1.02–2.28)
β (h^{-1})	0.70	0.98	1.12	1.26	2.31	0.85	1.20 ± 0.58
$t_{1/2\beta}$ (h)	0.99	0.71	0.62	0.55	0.30	0.82	0.67 (0.30–0.99)
γ (h^{-1})	0.00525	0.00145	0.00104	Very small	0.0328	0.00534	0.00918 ± 0.0134
$t_{1/2\gamma}$ (h)	132.1	487.7	339.7	Very large	21.1	129.7	220.3 (21.1–487.7)
$AUC_{0-24\,h}$ (μM h)[b]	0.43	0.46	0.41	0.68	0.48	0.57	0.51 ± 0.10
AUC_{μ} (μM h)[c]	3.09	10.9	14.3	3.90[b]	0.965	4.45	6.73 ± 5.61
CL (mL/h/kg)	214.1	65.5	49.5	189.1[b]	668	151.3	222.9 ± 227.7
Vdss (L/kg)	44.3	44.1	47.9	9.4	23.2	29.8	36.5 ± 10.1
MRT (h)[d]	9.3	10.0	9.6	10.8	8.8	10.8	9.9 ± 0.8
Dose (μg)	430.5	454.5	448.5	468	409.5	427.5	439.3 ± 21.2

[a] From actual data.
[b] Calculated from actual data to 24 h, using linear trapezoidal rule.
[c] Measured value plus extrapolation from WinNonlin.
[d] Calculated from non-compartment method.

There are three common ways of extracting drugs from plasma: organic solvent protein precipitation [19], organic solvent extraction [20] and solid-phase extraction (SPE) [21], the first method being the simplest. However, the recovery of AP3 from rat plasma using acetonitrile protein precipitation was only 20%, possibly due to co-precipitation of the drug with the proteins. The recovery of AP3 from rat plasma after extraction with ethyl acetate was about 50%. The condition was adequate with the use of an internal standard FR, of which the recovery from plasma using ethyl acetate extraction was previously found to be 86% at 100 ng/mL [9]. Associated with the validation, we determined drug levels of freshly prepared plasma samples and compared with the same following storage for at least 1 month at −80 °C, no appreciate changes were found, suggesting that AP3 was stable for at least one freeze–thaw cycle. Using this method, a linear dynamic range from 1 to 500 ng/mL was achieved for AP3, using 0.2 mL of rat plasma.

Using the present method, the pharmacokinetics of AP3 was investigated at a low i.v. dose of 1.5 mg/kg. Plasma AP3 levels were detectable for at least 24 h. The drug showed a long terminal phase, suggesting the distribution of the drug into a deep tissue compartment. The facts that reasonable plasma levels were achievable in circulation and that the agent has a long terminal half-life make the pharmacokinetic properties favorable. However, we do not know enough about the toxicology of the agent; therefore, it is not certain at this time what dose range could be used without high toxicity. The rats in this study all survived at least 24 h. Further pharmacokinetic characterization with respect to different doses, including oral dosing, is needed. Preliminary metabolism studies of AP3 and maytansine have been initiated and will be reported elsewhere.

5. Conclusion

A highly sensitive and specific LC/MS/MS method for the quantitation of AP3 in rat plasma has been developed. The method has been validated with a routine sensitivity limit of 1 ng/mL using 0.2 mL rat plasma. The assay has been successfully applied to a preliminary investigation of the pharmacokinetics of AP3 in the rat following i.v. administration at 1.5 mg/kg. Plasma drug levels were detected at low ng/mL at 24 h.

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References

[1] E. Higashide, M. Asai, K. Ootsu, S. Tanida, Y. Kozai, T. Hasegawa, T. Kishi, Y. Sugino, M. Yoneda, Nature 270 (1977) 721–722.
[2] S. Tanida, T. Hasegawa, K. Hatano, E. Higashide, M. Yoneda, J. Antibiot. 33 (1980) 192–198.
[3] K. Nakahama, M. Izawa, M. Asai, M. Kida, T. Kishi, J. Antibiot. 34 (1981) 1581–1586.
[4] K. Suwanborirux, C.-J. Chang, R.W. Spjut, J.M. Cassady, Experientia 46 (1990) 117–120.
[5] M. Izawa, Y. Wada, F. Kasahara, M. Asai, T. Kishi, J. Antibiot. 34 (1981) 1591–1595.
[6] J.M. Cassady, K.K. Chan, H.G. Floss, E. Leistner, Chem. Pharm. Bull. 52 (2004) 1–26.
[7] K. Hatano, E. Higashide, M. Yoneda, Agric. Biol. Chem. 48 (1984) 1889–1890.

[8] C. Liu, B.M. Tadayoni, L.A. Bourret, K.M. Mattocks, S.M. Derr, W.C. Widdison, N.L. Kedersha, P.D. Ariniello, V.S. Goldmacher, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 8618–8623.
[9] Z. Li, K.K. Chan, J. Pharm. Biomed. Anal. 22 (2000) 33–44.
[10] K.K. Chan, R. Bakhtiar, C. Jiang, Invest. New Drugs 15 (1997) 195–206.
[11] T.R. Covey, E.D. Lee, J.D. Henoine, Anal. Chem. 58 (1986) 2453–2460.
[12] W. Feng, K.K. Chan, J.M. Covey, J. Pharm. Biomed. Anal. 28 (2002) 601–602.
[13] R. Pacifici, S. Pichini, I. Altieri, A. Caronna, A. Passa, P. Zuccaro, J. Chromatogr. B 664 (1995) 329–334.
[14] A. Xu, K. Linderholm, L. Peng, J. Hulse, J. Pharm. Biomed. Anal. 14 (1996) 1675–1680.
[15] J. Zagrobelny, C. Chavez, M.L. Constanzer, B.K. Matuszewski, J. Pharm. Biomed. Anal. 15 (1997) 1427–1433.
[16] Y. Wu, J. Zhao, J. Henion, W.A. Korfmacher, A.P. Lapiguera, C. Lin, J. Mass Spectrom. 32 (1997) 379–387.
[17] R.E. Calaf, M. Carrascal, E. Gelphi, J. Abian, Rapid Commun. Mass Spectrom. 11 (1997) 75–80.
[18] P.K. Bennett, Y. Li, R. Edom, J. Henion, J. Mass Spectrom. 32 (1997) 739–749.
[19] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882–889.
[20] S. Ptacek, J. Macek, J. Klima, J. Chromatogr. B 789 (2003) 405–410.
[21] M. Carrascal, K. Schneider, R.E. Calaf, S. Leeuwen, D. Canosa, E. Gelphi, J. Abian, J. Pharm. Biomed. Anal. 17 (1998) 1129–1138.