Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 824 (2005) 181–188
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

A liquid chromatographic–tandem mass spectrometric method for the determination of two selective thymidylate synthase inhibitors, BGC945 and BGC638, in mouse plasma

Nadya Wood [a,*], David D. Gibbs [b], Ann L. Jackman [b], Alan Henley [a], Paul Workman [a], Florence Raynaud [a]

[a] *Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK*
[b] *Section of Medicine, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK*

Received 9 December 2004; accepted 16 July 2005
Available online 2 August 2005

Abstract

A LC–tandem mass spectrometry method to quantify the quinazoline-based thymidylate synthase inhibitors BGC945 and BGC638 in mouse plasma was developed. BGC945 and BGC638 were extracted from mouse plasma using protein precipitation with acetonitrile. Chromatography was performed on a Fluophase RP 5 μm, 100 mm × 2.0 mm i.d. column using a gradient of ammonium acetate and acetonitrile as a mobile phase with a flow rate of 0.2 mL min^{-1}. The injection volume for each sample was 20 μL with a total run time of 7.5 min. This method was validated in the range 25–4000 nM ($r^2 = 0.99$). The analytical assay performance showed that the method was accurate (mean intra- and inter-day assay R.E. were below 12% and 11%, respectively), reproducible (mean intra- and inter-day R.S.D. were less than 13% and 5% for all quality control levels, respectively) and sensitive (lower limit of quantification was 25 nM) in the range studied. This validated method has been used to define the first pharmacokinetic report of BGC945 and BGC638 in mice.

Keywords: BGC945; BGC638; Validation; Liquid chromatography–tandem mass spectrometry

1. Introduction

Thymidylate synthase (TS) is a crucial enzyme involved in DNA synthesis as it is the only source of thymidine nucleotides [1–2]. TS has been recognised as an attractive target for antiproliferative chemotherapy and folate-based TS inhibitors are now used in the clinic as anticancer agents (e.g. raltitrexed) [3]. The first generation of antifolates are transported into cells by the reduced folate carrier (RFC), a transmembrane transporter of folates and antifolates which is ubiquitously expressed in most tumours and normal tissues [4–5]. Therefore, the use of antifolates in the clinic is limited due to TS inhibition in normal proliferating tissues resulting in unwanted toxic side effects. Folates and antifolates can also be transported into cells by the folate receptor (FR). The α-isoform of the folate receptor (α-FR) is a glycosylphosphatidylinositol (GPI)-anchored membrane associated cell surface glycoprotein that internalises substrates by receptor-mediated endocytosis [6–7]. α-FR is partially expressed in normal tissues (largely the luminal surface of the kidney proximal tubules and choroids plexus) but is overexpressed in several epithelial cancers (ovarian and endometrial carcinoma) [8]. A series of highly water-soluble cyclopenta[*g*]quinazoline-based antifolate TS inhibitors have been developed that have a high affinity for the α-FR [9,10]. Two compounds BGC945 and BGC638 were shown to be selective and highly cytotoxic to α-FR overexpressing tumour cell lines in vitro (IC_{50} = 3 nM) [10–13]. BGC945 has been identified as a lead compound because of its α-FR targeting and is therefore predicted to give low toxicity in vivo [12].

* Corresponding author. Tel.: +44 20 87224383; fax: +44 20 87224205.
E-mail address: nadya.wood@icr.ac.uk (N. Wood).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.07.025

This paper describes the development and validation of a liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay for the analysis of two novel compounds, BGC945 and BGC638, together with the results of the first pharmacokinetic study.

2. Experimental

2.1. Chemicals and reagents

The compounds BGC945, BGC638 and the internal standard BGC899 (Fig. 1), were synthesised at The Institute of Cancer Research (Sutton, Surrey, UK). The purity of all three substances was >99%. Dimethyl sulfoxide (DMSO) and ammonium acetate were obtained from Fisher Chemicals (Loughborough, Leicestershire, UK). HPLC grade methanol and acetonitrile were obtained from British Drug House (Poole, Dorset, UK). Phosphate buffered saline (PBS) and sodium bicarbonate were obtained from Sigma (Poole, Dorset, UK). The drug-free (blank) mouse plasma was obtained from Harlem Sera (Loughborough, Leicestershire, UK). Double distilled water was used throughout.

2.2. Instrumentation

The liquid chromatography system consisted of an Alliance Waters 2795 separations module (Waters Ltd., Hertford, UK). The mass spectrometer was a Micromass Quattro Ultima mass detector (Micromass UK Ltd., Manchester, UK). An IBM P260 computer with Micromass MassLynx software was used to control the Waters 2795 liquid chromatograph and the Quattro Ultima mass spectrometer.

2.3. Chromatography

Chromatographic separation was performed on a Thermo Hypersil-Keystone fluophase RP 5 μm, 100 mm × 2.0 mm

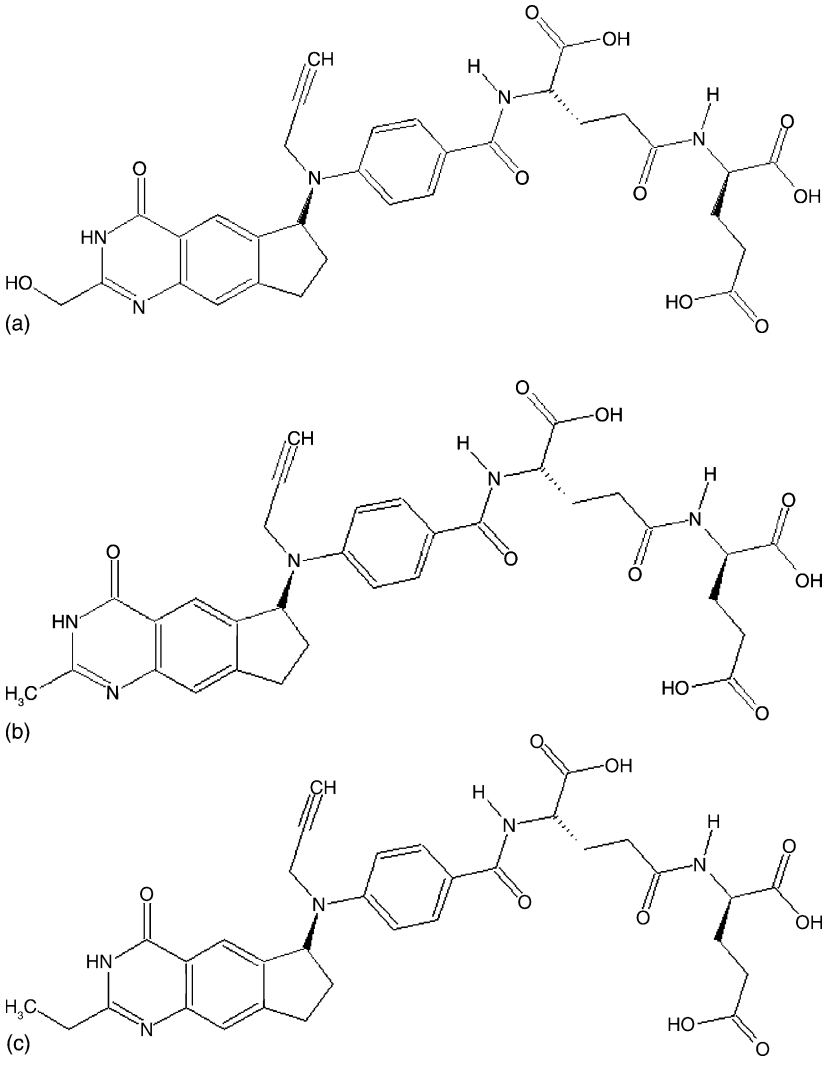

Fig. 1. Chemical structures of (a) BGC945, (b) BGC638 and the internal standard, (c) BGC899.

i.d. column (Thermo Hypersil-Keystone, Cheshire, UK) with a guard column (4.0 mm × 3.0 mm) packed with reversed-phase C_{18} material (Phenomenex, Macclesfield, Cheshire, UK). The mobile phase consisted of acetonitrile and ammonium acetate (10 mM adjusted to pH 8 with ammonium hydroxide), with a linear gradient of 100% organic until 0.5 min, then a linear decease to 20% over 3 min, and then a linear increase back to 100% organic for 3 min. Chromatography was performed at ambient temperature at a constant flow-rate of 0.2 mL min^{-1}. The injection volume for each sample was 20 μL with a total run time of 7.5 min.

2.4. Mass spectrometry

Using the mass spectrometer with electrospray ionisation in positive mode (ESI^+) the capillary voltage (3.5 kV), cone voltage (45 V), source temperature (150 °C), desolvation temperature (350 °C), cone gas flow (45 L h^{-1}) and desolvation gas flow (694 L h^{-1}) were optimised for the detection of the precursor and product ions of BGC945, BGC638 and BGC899. The analytes were infused at 20 μL min^{-1} by an infusion pump into the mass spectrometer.

2.5. Data acquisition and quantification

Transitions from the specific precursor ion to the product ion were monitored by multiple reaction monitoring (MRM). Data acquisition and quantification were performed using Micromass QuanLynx software. Peaks detected were integrated automatically and a calibration curve was constructed using the peak area ratios of BGC945 or BGC638 to the internal standard BGC899 versus corresponding concentrations. Unknown sample concentrations of BGC945 and BGC638 were determined by extrapolation from the calibration curve with least squares regression weighting ($1/y$).

2.6. Stock solutions

Stock solutions of BGC945, BGC638 and internal standard BGC899 were prepared in sodium bicarbonate (0.15 M buffered to pH 8.3 using sodium hydroxide) at a concentration of 1 mM. The working solutions were prepared by serial dilution of the stock solution with sodium bicarbonate solution to give concentrations of 40,000, 20,000, 10,000, 5000, 2500, 1000, 500, and 250 nM for BGC945. Concentrations of 50,000, 25,000, 10,000, 5000, 2000, 1000, and 500 nM were prepared for BGC638. Quality control (QC) samples were prepared at high, medium and low concentrations of 25,000, 4000, and 400 nM, respectively, for BGC945; and 15,000, 7500, and 1500 nM, respectively, for BGC638. All solutions were stored at −20 °C.

2.7. Preparation of calibration and QC samples

A calibration curve for BGC945, with concentrations of 4000, 2000, 1000, 500, 250, 100, 50, and 25 nM, were prepared from 10 μL of the appropriate working solution and 100 μL of blank mouse plasma. For BGC638, concentrations of 5000, 2500, 1000, 500, 200, 100, and 50 nM, were prepared. QC samples were prepared at the lower limit of quantification (LLOQ), 25 nM and at high, medium and low concentrations of 2500, 400, and 40 nM in blank mouse plasma for BGC945 and 1500, 750, and 150 nM for BGC638. Samples were spiked with 10 μL of BGC899 (1500 nM) as the internal standard.

2.8. Sample extraction

Plasma samples were vortex mixed and incubated at 4 °C for 1 h, as this improved the reproducibility of the assay by ensuring all samples were treated the same. Samples were then extracted by protein precipitation with 300 μL of acetonitrile and centrifuged at 2000 × g for 10 min. The supernatant was removed and evaporated to dryness for 90 min, on heat setting 2, in a speed vacuum sample concentrator (Jouan, France), and reconstituted into 200 μL of ammonium acetate (10 mM buffered to pH 8 with ammonium hydroxide).

2.9. Assay performance

The assay performance was investigated to insure that the analytical methods for the quantification of BGC945 and BGC638 in mouse plasma were accurate, reproducible, and sensitive in the range studied. Replicate ($n = 6$) sets of calibration standards, LLOQ and QC samples were analysed in three batches to determine intra- and inter-day validations.

2.10. Linearity of calibration curves

The method was validated according to guidelines set by the US Food and Drug Administration (FDA) for bioanalytical method validation [14]. Briefly, the LLOQ should be ±20% of nominal concentration, all other QCs should be ±15% of nominal concentration and at least 4 out of 6 calibration standards should be within specification. In addition the calibration curve should have a correlation coefficient of determination (r^2) greater than 0.98.

2.11. Accuracy

The mean intra- and inter-batch accuracy of the method is expressed in terms of relative error (R.E.).

2.12. Imprecision

The imprecision between and within batches is expressed as the relative standard deviation (R.S.D.). The mean intra- and inter-batch R.S.D. were calculated from replicate $n = 6$ samples at the LLOQ and QC low, medium and high concentrations for batches 1–3.

2.13. Sensitivity

The precision and accuracy were assessed for the acceptance of the LLOQ from replicate $n = 6$ analysis of the LLOQ (25 nM) for batches 1–3.

2.14. Stability

The stability of BGC945 and BGC638 in mouse plasma was assessed by comparison of stored samples to freshly prepared samples. Bench-top stability was determined after 4 h storage at room temperature. Replicate ($n = 6$) sets of QC samples were analysed for stock solution stability after 1 month storage at −20 °C, freeze–thaw stability after three cycles of freezing at −20 °C and thawing at room temperature for 2 h. The stability of extracted samples at 4 °C in the Alliance Waters 2795 autosampler after 24 h was determined.

2.15. Pharmacokinetic studies

Pharmacokinetic studies for BGC638 were carried out in Balb/C mice. BGC945 pharmacokinetic studies were carried out in KB tumour bearing ICR nude mice. Mice were acclimatised to the laboratory conditions and restricted to a folic acid free diet 24 days prior to the experiment in order to reduce plasma folate levels to those found in humans [15]. The animals weighed ca. 20 g at the time of treatment. BGC945 and BGC638 were administered to KB tumour bearing and Balb/C mice, respectively, as a single agent intravenously (i.v.) at 3 μM in a vehicle of sodium bicarbonate in distilled water (50 mM) with three mice per time point. Blood samples were collected at 5, 15, and 30 min and 1, 2, 4, 6, 8, 16, 24, 48, and 72 h post administration by cardiac puncture into heparinised syringes. The blood was transferred into Eppendorf tubes and centrifuged at 10,000 × *g* for 2 min to obtain the supernatant plasma for sample analysis. Samples were stored at −80 °C until analysis. All experiments were conducted in line with the United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) guidelines for animal welfare [16].

2.15.1. Sample analysis

Samples were thawed on ice. Aliquots of plasma samples (100 μL) were spiked with 1500 nM BGC899 (10 μL) as an internal standard. The unknown samples were extracted in parallel with the known standards.

2.15.2. Pharmacokinetic analysis

The pharmacokinetic parameters of all compounds after i.v. dosing were evaluated up to the lowest detectable time-point by non-compartmental analysis using WinNonlin Version 3.0 (Pharsight Corporation, CA, USA). Values presented include AUC_{0-t}, the area under the plasma concentration-time curve from the time of dosing (0 h) to the last measurable concentration at time = t (h). C_{max}, represents the maximum plasma drug concentration obtained after administration of the compounds. The terminal half-life, $t_{1/2\_\lambda\_z} = \ln 2/\lambda z$ (where, λz is the first order rate constant estimated by the linear regression of log concentration versus time). Clearance, Cl = dose/AUC_{INF} (AUC_{INF} is the AUC from the time of dosing extrapolated to infinity), is the total body clearance for intravenous administration. Volume of distribution at steady state, Vss = MRT·Cl (where, MRT is the mean residence time, this is the average amount of time a particle remains in a compartment), this relates volume directly to the amount of drug in the body.

3. Results

3.1. Method development

In the development of this assay other mobile phase combinations and columns were evaluated to determine optimal analytical conditions. Other mobile phase compositions included: 0.1% formic acid and methanol; 0.1% formic acid and acetonitrile; ammonium acetate (10 mM adjusted to pH 8 with ammonium hydroxide) and acetonitrile. The mobile phases were run isocratically 50:50 (v/v) or using a linear gradient (described in Section 2.3). Mobile phases that contained a low percentage of organic modifiers on the following 5 μm, 50 mm × 2.1 mm i.d. columns: C18 Symmetry (Waters Ltd., Hertford, UK), C6 Luna, Phenyl-Hexyl, Synergi Polar RP, C18 Luna (Phenomenex, Cheshire, UK), ABZ, ABZ^+, and C18 Discovery (Supelco, Sigma–Aldrich, Dorset, UK), resulted in a poor peak shape. With a high percentage of organic modifiers carry-over occurred (data not shown). The optimal analytical conditions were obtained on a Thermo Hypersil-Keystone fluophase RP 5 μm, 100 mm × 2.0 mm i.d. column. The mobile phase consisted of acetonitrile and ammonium acetate (10 mM in water adjusted to pH 8) using a linear gradient as this eliminated peak tailing and carry-over. Chromatography was performed at ambient temperature with a constant flow-rate of 0.2 mL min^{-1}. The injection volume for each sample was 20 μL with a total run time of 7.5 min. The purge solvent was the starting mobile phase, ammonium acetate (10 mM in water adjusted to pH 8), and the needle/seal wash was methanol. BGC945, BGC638 and the internal standard BGC899 were detected using LC–MS/MS with electrospray ionisation in positive mode. The transitions from the specific precursor ion to product ion fragment were monitored by MRM at *m/z* 648.12–434.21 for BGC945, 632.23–434.21 for BGC638 and *m/z* 646.52–448.21 for the internal standard BGC899. Under these LC–MS/MS conditions BGC945, BGC638 and internal standard BGC899 eluted at ca. 4.7 min. No cross-talk was observed between the compounds and the co-elution of BGC945 or BGC638 with the internal standard BGC899 avoided lack of accuracy due to ion suppression. Fig. 2 shows the chromatograms of blank plasma, BGC945 LLOQ (25 nM), and BGC945 QC high (2500 nM) showing no interfering endogenous substances that could affect assay specificity and sensitivity.

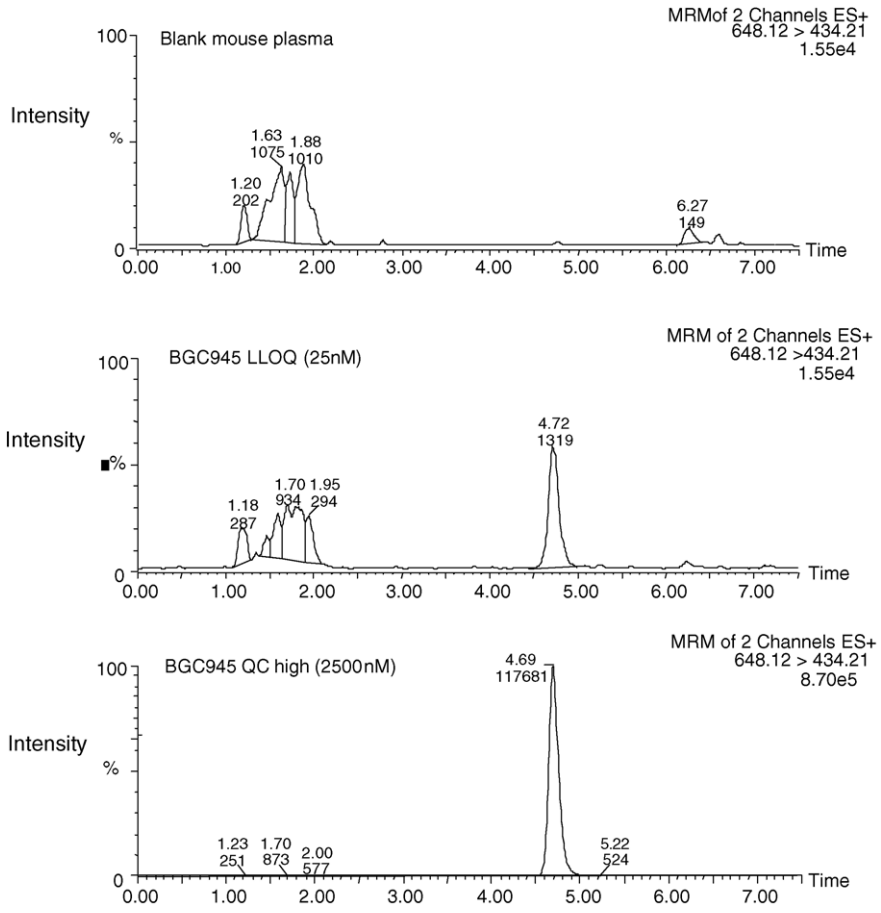

Fig. 2. Representative chromatographic profiles of extracted blank plasma, extracted BGC945 LLOQ (25 nM), and extracted BGC945 QC high (2500 nM) plasma samples.

3.2. Assay performance

The calibration curve for BGC945 was linear in the range 25–4000 nM with a mean correlation coefficient determination of 0.99 ($n = 3$) (data not shown). The concentrations of the validation samples were determined by automatic extrapolation from the calibration curves. BGC638 calibration curve was linear in the range 15–4000 nM and the mean inter-batch imprecision R.S.D. of BGC638 at LLOQ, QC low, medium and high concentrations were 8.2%, 2.5%, 4.1% and 5%, respectively. The assay performance of BGC638 is described in a Ph.D. thesis [17].

3.3. Accuracy

The mean intra- and inter-batch accuracies are expressed as %R.E. at each QC level. The mean intra-batch R.E. remained below 12% (Table 1). The mean inter-batch R.E. of observed concentrations at each QC level were 2.88% at 25 nM (LLOQ); 5.45% at 40 nM (QC low); 10.34% at 400 nM (QC medium) and 4.41% at 2500 nM (QC high) (Table 1).

3.4. Intra-batch imprecision

The mean intra-batch imprecision R.S.D. for replicate $n = 6$ analysis of QC samples in batches 1–3 was calculated. R.S.D. for each level ranged from 5.8% to 12.9 % at 25 nM (LLOQ); 6.6–12.5% at 40 nM (QC low); 5.4–10.4% at 400 nM (QC medium) and 2.4–7.4% at 2500 nM (QC high) (Table 1).

3.5. Inter-batch imprecision

The mean inter-batch precision R.S.D. for replicate ($n = 18$) analysis of QC samples at each level in batches 1–3 were 4.09% at 25 nM (LLOQ); 1.78% at 40 nM (QC low); −1.98% at 400 nM (QC medium) and 4.41% at 2500 nM (QC high) (Table 2).

3.6. Sensitivity

At the LLOQ (25 nM) the mean intra-batch R.S.D. was less than 13%. The mean inter-batch R.S.D. was 4.09%, with a R.E. of 2.88% ($n = 15$) (Tables 1 and 2).

Table 1
Intra-batch quality control samples

QC	LLOQ 25 nM	QC low 40 nM	QC medium 400 nM	QC high 2500 nM
(a) Results for batch 1 ($n = 6$)				
1	23.80	30.30[a]	341.80	2579.60
2	29.30	37.50	342.40	2838.10
3	40.40[a]	44.10	340.60	2718.40
4	29.30	43.70	375.60	2849.90
5	21.70	37.90	337.20	2786.50
6	26.20	39.80	379.20	2747.70
Mean	26.06	40.60	352.80	2753.37
S.D.	3.36	5.06	19.17	99.04
R.E. (%)	4.2	1.5	−11.8	10.1
R.S.D. (%)	12.9	12.5	5.4	3.6
(b) Results for batch 2 ($n = 6$)				
1	47.20[a]	38.40	384.90	2807.50
2	21.90	37.00	444.60	2826.20
3	25.40	37.70	445.90	2693.80
4	29.10	37.50	419.00	2732.60
5	23.60	45.70	450.30	2720.60
6	22.70	35.00	458.10	2859.80
Mean	24.54	38.55	433.80	2773.42
S.D.	2.86	3.69	27.34	66.64
R.E. (%)	−1.8	−3.6	8.5	10.9
R.S.D. (%)	11.7	9.6	6.3	2.4
(c) Results for batch 3 ($n = 6$)				
1	27.20	45.50	418.40	2159.00
2	29.00	43.90	354.60	2191.70
3	25.40	40.20	421.50	2486.80
4	25.80	44.50	341.30	2159.80
5	25.40	45.10	437.00	2281.60
6	41.40[a]	38.70	364.80	2544.00
Mean	26.56	42.98	389.60	2303.82
S.D.	1.55	2.83	40.66	170.85
R.E. (%)	6.2	7.5	−2.6	−7.8
R.S.D. (%)	5.8	6.6	10.4	7.4

[a] Data that is outside the acceptable range for bioanalytical method validation that has been excluded from assay performance calculations.

3.7. Stability

BGC945 and BGC638 were stable as stock solutions after 4 h storage at room temperature. BGC638 was stable for at least 3 months after storage at −20 °C and over four freeze–thaw cycles [17]. BGC945 was stable for a least 1 month after storage at −20 °C and in mouse plasma after three freeze–thaw cycles at −20 °C and the extracted samples were stable in the autosampler after 24 h at 4 °C (Table 3).

Table 2
Precision and accuracy summary of inter-batch quality control samples

	LLOQ 25 nM	QC low 40 nM	QC medium 400 nM	QC high 2500 nM
Mean	25.72	40.71	392.07	2610.20
S.D.	1.05	2.22	40.56	265.53
R.E. (%)	2.88	5.45	10.34	10.17
R.S.D. (%)	4.09	1.78	−1.98	4.41
n	15	17	18	18

Deviation from $n = 18$ due to excluded data in Table 1.

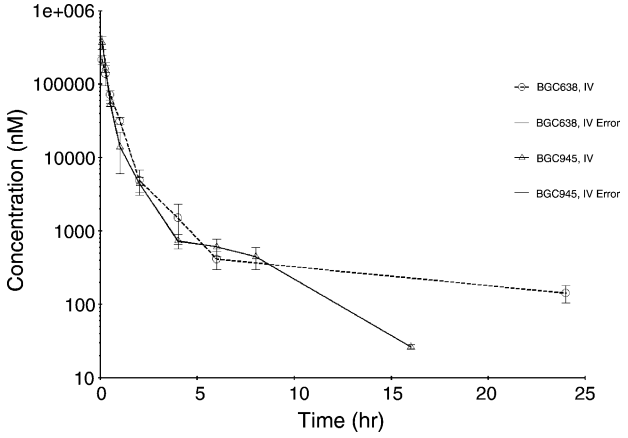

Fig. 3. Plasma concentration of BGC945 and BGC638 vs. time curve, administered to KB tumour bearing and Balb/C mice, respectively, i.v. as a single agent at 3 μM. Data are mean ($n = 3$).

3.8. Pharmacokinetic analysis of BGC945 and BGC638

The plasma concentration–time curve for BGC945 and BGC638 are shown in Fig. 3. Both compounds showed biphasic log-linear concentration decay curves. Following 3 μM i.v. administered to KB tumour bearing ICR nude mice, BGC945 was detectable for up to 16 h (Fig. 3), reached a maximum plasma concentration of 376 μM and the $AUC_{0–16\,h}$ was 147 h μM. The terminal $t_{1/2}$ was 2 h, with a volume of distribution of 0.58 L kg^{-1} and a clearance of 1.05 L h^{-1} kg^{-1} (Table 4). BGC638 was administered at 3 μM i.v. in Balb/C mice; it was detectable for up to 24 h (Fig. 3), the C_{max} was 256 μM and the $AUC_{0–24\,h}$ was 149 h μM. The clearance was 1.05 L h^{-1} kg^{-1} with a terminal $t_{1/2}$ of 4 h and a volume of distribution of 1.87 L kg^{-1} (Table 4). There were no adverse effects following administration to the mice.

4. Discussion

Potent cyclopenta[*g*]quinazoline-based inhibitors of TS with high affinity for the α-FR include BGC945, BGC638 and BGC899. BGC945 and BGC638 were the lead compounds in this series and were therefore chosen for further pharmacokinetic studies. The assay developed was based on a LC–MS/MS method for the quantification of BGC945 and BGC638 in mouse plasma. The assay is accurate, reproducible, and sensitive, fulfilling validation requirements from the FDA. BGC945 and BGC638 were stable after stability procedures to evaluate sample handling during analysis were assessed. The LLOQ was 25 and 15 nM for BGC945 and BGC638, respectively, and the calibration curve was linear in the range 25–4000 nM for BGC945 and 15–4000 nM for BCG638, making this a useful assay for the determination of BGC945 and BGC638 in mouse plasma at low concentrations. The quantitative determination of BGC945 and BGC638 required a specific method due to the presence of a glutamic acid moiety and the basic quinazoline ring.

Table 3
Stability results for BGC945 in mouse plasma ($n = 6$ per concentration)

Treatment	Nominal concentration (nM)	Mean concentration (nM)	R.E. (%)	R.S.D. (%)	Stability (%)
(a) Storage stability (−20 °C)					
0 months	40	41.55	3.9	6.7	
1 months	40	41.25	3.1	9.5	99.3
0 months	400	405.98	1.5	3.7	
1 months	400	395.82	−1.0	2.7	97.5
0 months	2500	2446.10	−2.2	4.0	
1 months	2500	2363.84	−5.4	6.4	96.6
(b) Freeze–thaw stability					
0 cycle	40	41.97	4.9	5.0	
1 cycle	40	44.02	10.1	5.5	
2 cycle	40	39.97	−0.1	9.5	
3 cycle	40	41.08	2.7	5.1	97.9
0 cycle	400	405.65	1.4	4.6	
1 cycle	400	423.52	5.9	5.0	
2 cycle	400	398.68	−0.3	3.0	
3 cycle	400	385.42	−3.6	4.0	97.0
0 cycle	2500	2536.98	1.5	3.7	
1 cycle	2500	2511.88	0.5	5.0	
2 cycle	2500	2571.58	2.9	4.7	
3 cycle	2500	2473.48	−1.1	3.8	97.5
(c) Autosampler stability (24 h)					
0 h	40	41.97	4.9	5.0	
24 h	40	42.42	6.1	7.6	101.1
0 h	400	405.65	1.4	4.6	
24 h	400	411.88	3.0	3.3	101.5
0 h	2500	2600.4	4.0	6.1	
24 h	2500	2545.4	1.8	4.1	97.9

Table 4
Pharmacokinetic parameters for BGC945 administered to KB tumour bearing mice and BGC638 administered to Balb/C mice i.v. as a single agent at 3 μM

Compound	Matrix	Route	AUCLast (h μM)	C_{max} (μM)	$t_{1/2}$ (h)	Cl (L h^{-1} kg^{-1})	Vss (L kg^{-1})
BGC945	Plasma	i.v.	147	376	2	1.05	0.58
BGC638	Plasma	i.v.	149	256	4	1.05	1.87

Data were derived from one experiment with three mice per time point. Parameters were derived using non-compartmental pharmacokinetics using WinNonlin.

This was resolved by the use of ammonium acetate at pH 8 as the mobile phase and a polar base deactivated stationary phase. These optimal conditions prevented peak tailing and carryover.

This method has allowed us to evaluate the pharmacokinetic behaviour of BGC945 and BGC638 in mice. BGC945, administered to KB tumour bearing ICR nude mice, reached the limit of quantification after 16 h and BGC638, administered to Balb/C mice, remained above the LOQ even after 24 h. Further studies will evaluate if the compounds are metabolised and whether there is accumulation in α-FR expressing tissues (i.e. tumours). Both BGC945 and BGC638 had a clearance of 1.05 L h^{-1} kg^{-1}, which is moderate compared to liver blood flow (5.4 L h^{-1} kg^{-1}). The assay that we developed is more sensitive than that described for BGC9331 (ZD9331) (a water-soluble, nonpolyglutamatable, quinazoline-based antifolate inhibitor of TS [18,19].

In conclusion, this is the first pharmacokinetic report on these compounds and the developed assay is shown to be specific, accurate, reproducible, and sensitive by fulfilling validation requirements from the FDA. Further studies will evaluate if this assay is suitable to measure BGC945 and BGC638 in different tissues samples and evaluate if there is accumulation in α-FR expressing tissues.

Acknowledgements

This work is funded by Cancer Research UK and BTG.

References

[1] G.J. Peters, C.L. van der Wilt, Biochem. Soc. Trans. 23 (1995) 884.
[2] P.V. Danenberg, H. Malli, S. Swenson, Semin. Oncol. 26 (1999) 621.
[3] A.L. Jackman, A.H. Calvert, Ann. Oncol. 6 (1995) 871.
[4] G. Jansen, in: A.L. Jackman (Ed.), Antifolate Drugs in Cancer Chemotherapy, Humana Press Inc., Totowa, NJ, 1999, p. 293.

[5] J.R. Whetstine, R.M. Flatley, L.H. Matherly, Biochem. J. 367 (2002) 629.
[6] D.S. Theti, A.L. Jackman, Clin. Cancer Res. 10 (2004) 1080.
[7] S. Sabharanjak, S. Mayor, Adv. Drug Deliv. Rev. 56 (2004) 1099.
[8] H. Elnakat, M. Ratnam, Adv. Drug Deliv. Rev. 56 (2004) 1067.
[9] V. Bavetsias, J.H. Marriott, C. Melin, R. Kimbell, Z.S. Matusiak, F.T. Boyle, A.L. Jackman, J. Med. Chem. 43 (2000) 1910.
[10] D.S. Theti, V. Bavetsias, L.A. Skelton, J. Titley, D. Gibbs, G. Jansen, A.L. Jackman, Cancer Res. 63 (2003) 3612.
[11] A.L. Jackman, D. Gibbs, D. Theti, M. Green, F. Raynaud, M. Bavetsias, Br. J. Cancer 88 (2003) S21.
[12] M.D. Forster, F. Raynaud, N. Wood, D. Theti, F. Mitchell, M. Valenti, A. De Haven Brandon, D. Gibbs, L. McKinney, A.L. Jackman, Proc. Am. Assoc. Cancer Res. 45 (2004) 1066.
[13] A.L. Jackman, D.D. Theti, D.S. Gibbs, Adv. Drug Deliv. Rev. 56 (2004) 1111.
[14] Guidance for industry: Bioanalytical method validation. US Department of Health and Human Services, Food and Drug Administration, Centre for Veterinary Medicine, May 2001.
[15] J.C. Schmitz, G.B. Grindey, R.M. Schultz, D.G. Priest, Biochem. Pharmacol. 48 (1994) 319.
[16] P. Workman, P. Twentyman, F. Balkwill, A. Balmain, D. Chaplin, J. Double, J. Embleton, D. Newell, R. Ramond, J. Stables, T. Stephens, Br. J. Cancer 77 (1998) 1.
[17] D.D. Gibbs, The In Vivo Development of Folate Receptor-Targeted Tymidylate Synthase Inhibitors, University of London, 2003.
[18] A.L. Jackman, R. Kimbell, G.W. Aherne, L. Brunton, G. Jansen, T.C. Stephens, M.N. Smith, J.M. Wardleworth, F.T. Boyle, Clin. Cancer Res. 3 (1997) 911.
[19] M.I. Walton, F. Mitchell, G.W. Aherne, C.J. Medlow, F.T. Boyle, A.L. Jackman, Br. J. Cancer 78 (1998) 1457.