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Determination of GDC-0449, a small-molecule inhibitor of the Hedgehog signaling pathway, in human plasma by solid phase extraction-liquid chromatographic-tandem mass spectrometry

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ABSTRACT

To support clinical development, a solid phase extraction (SPE) liquid chromatographic-tandem mass spectrometry (LC-MS/MS) method for the determination of GDC-0449 concentrations in human plasma has been developed and validated. Samples (200 μl) were extracted using an Oasis MCX 10 mg 96-well SPE plate and the resulting extracts were analyzed using reverse-phase chromatography coupled with a turbo-ionspray interface. The method was validated over calibration curve range 5–5000 ng/mL. Quadratic regression and $1/x^2$ weighing were used. Within-run relative standard deviation (%RSD) was within 10.1% and accuracy ranged from 88.6% to 109.0% of nominal. Between-run %RSD was within 8.6% and accuracy ranged from 92.4% to 105.3% of nominal. Extraction recovery of GDC-0449 was between 88.3% and 91.2% as assessed using quality control sample concentrations. GDC-0449 was stable in plasma for 315 days when stored at −70 °C and stable in reconstituted sample extracts for 117 h when stored at room temperature. Quantitative matrix effect/ion suppression experiment was performed and no significant matrix ion suppression was observed. This assay allows for the determination of GDC-0449 plasma concentrations over a sufficient time period to determine pharmacokinetic parameters at relevant clinical doses.

1. Introduction

GDC-0449 (Fig. 1) is a small-molecule inhibitor of the Hedgehog signaling pathway which is currently in clinical development. Hedgehog Pathway Inhibitors have shown anticancer activity in several preclinical models, including xenograft models of basal cell carcinoma (BCC) [1], medulloblastoma [2], and colon and pancreatic adenocarcinoma [3]. Favorable preclinical pharmacokinetic properties in mouse, rat, dog and monkey, such as low *in vivo* clearance, good oral availability, and metabolic stability in addition to toxicology data supported GDC-0449 advancement to clinical development [4]. Recently, GDC-0449 has demonstrated anticancer activity in a Phase I trial in patients with advanced BCC, for whom prognosis is poor, without significant adverse event [5]. GDC-0449 is currently in Phase II clinical trials for metastatic or locally advanced BCC, metastatic colorectal carcinoma, and late-stage ovarian carcinoma.

A SPE LC-MS/MS method for the determination of GDC-0449 concentrations in human plasma was developed and validated using Oasis 96-well MCX SPE plates. The method is better and cleaner than the protein precipitation methods used for preclinical assessment [4] and for the first time allowed determination of the pharmacokinetic behavior of the molecule in a phase I trial in healthy volunteers (SHH4433g: A Phase I, Open-Label, Pharmacokinetic Study of Systemic Hedgehog Antagonist GDC-0449 in Healthy Female Subjects of Non-Childbearing-Potential), the results of which are included in this report. The method is also in use for the determination of GDC-0449 concentrations in human plasma for other Phase I and Phase II clinical trials and will be continuously used for upcoming clinical trials.

2. Experimental

2.1. Materials

Test compound GDC-0449 and internal standard GDC-0449-D5 were synthesized at Genentech with a purity of 99.1% and 100%,

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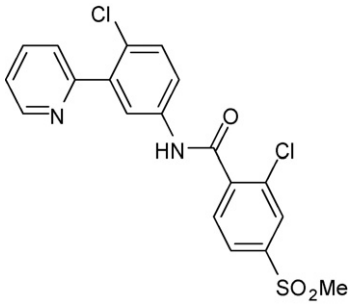

Fig. 1. Structure of GDC-0449 (pK_a = 3.5).

respectively. Acetonitrile, methanol and acetone (HPLC grade) were obtained from EMD (Gibbstown, NJ, USA). N,N-dimethylformamide (HPLC grade) was obtained from Burdick and Jackson (Morristown, NJ, USA). Formic acid and ammonium hydroxide (ACS grade) was also obtained from EMD. Trifluoroacetic acid (HPLC grade) was from J.T. Becker (Phillipsburg, NJ, USA). Deionized water (type 1, typical 18.2 MΩ cm) or equivalent was generated in Tandem Labs (Salt Lake City, UT, USA). All reagents were used as received. Oasis MCX (10 mg) 96-well SPE plates were obtained from Waters Inc. (Milford, MA, USA). Human plasma (K_2EDTA) was obtained from Bioreclamation (Hicksville, NY, USA).

2.2. Instrumentation

The LC-MS/MS system consisted of a Shimadzu SCL-10A system controller, two LC-10AD Shimadzu pumps (Columbia, MD, USA) and an API-3000 mass spectrometer with a turbo-ionspray interface (400 °C) from Applied Biosystems PE Sciex (Foster City, CA, USA) equipped with HSID upgrade (Ionics MSV Ltd., Cheshire, UK). Data was collected and processed using Analyst software (version 1.3.2) from Applied Biosystems. The SPE procedure was performed using a Tomtec Quadra 96® (Hamden, CT, USA) and a CEREX® Channel SPE System 96™ Positive Pressure SPE Manifold from SPEWare Corp. (San Pedro, CA, USA). Samples were introduced to the LC/MS/MS system using a Leap CTC PAL Autosampler (Chapel Hill, NC, USA) and an electronically actuated six-port high pressure switching valve from Valco Instruments Co. (Houston, TX, USA).

2.3. LC-MS/MS conditions

The mobile phases were water containing 0.1% formic acid (Mobile phase A), acetonitrile (Mobile phase B), and 90:10 (v/v) acetone:water (Mobile phase C). Extracted samples were analyzed using a reverse-phase liquid chromatograph and isocratic conditions with 60% mobile phase B at a flow rate of 0.30 mL/min on a Thermo Fisher Scientific (Waltham, MA, USA) Betasil C_{18} 2.1 mm × 100 mm column. A Cera column oven from SPEWare Corporation (Baldwin Park, CA, USA) was used to maintain the column temperature at 30 °C. To protect the analytical column, an UNIGUARD guard cartridge (Thermo Fisher Scientific, Waltham, MA, USA) was also used. The column was back flushed for 1 min after elution of each sample with mobile phase C at a flow rate of 0.80 mL/min (back pressure ~200 bar).

A neat solution of GDC-0449 and the internal standard (IS) GDC-0449-D5 was separately infused into the mass spectrometer using a Harvard Apparatus syringe pump (Holliston, MA, USA) to optimize the mass spectrometer parameters. GDC-0449 and GDC-0449-D5 were ionized using a turbo-ionspray source operating in the positive ionization mode. For quantitation, the mass spectrometer was operated in the selected reaction monitoring mode (SRM). The following SRM transitions were monitored: m/z 421.1 to m/z 139.2 for GDC-0449 and m/z 426.1 to m/z 139.1 for GDC-0449-D5. The dwell time was 100 ms for each transition. The ionspray voltage was 5500, the declustering potential was 50 V and the collision energy was 60 V. Analyst software (Applied Biosystems) was used to optimize the MS parameters and for data acquisition.

2.4. Preparation of standards and quality control samples

All standard solutions were prepared using N,N-dimethylformamide (DMF) solvent. The initial stock solution containing GDC-0449 was diluted using DMF to give working standards, which were spiked into plasma to prepare calibration standards ranging from 5 to 5000 ng/mL. The working IS solution was prepared in DMF at a concentration of 5000 ng/mL and spiked into the samples, resulting in final concentration of 1000 ng/mL. The resulting plasma standards were used to quantitate clinical human samples containing GDC-0449 over the concentration range of 5–5000 ng/mL.

High, middle, low, lower limit of quantitation (LLOQ) and dilution quality control (QC) samples containing GDC-0449 at concentrations of 4000, 2000, 15, 5 and 20,000 ng/mL, respectively, were prepared by diluting the stock solutions with control human plasma. Following preparation, aliquots of quality control samples were transferred to cryogenic vials capped and stored at −60 to −80 °C. These quality control samples were used for the validation and the analysis of clinical human plasma samples.

2.5. Extraction of human plasma

A 200-μL aliquot of plasma for each calibration curve standard, QC, control blank, blank and samples was pipetted into a clean 96-well plate. A 50-μL aliquot of 5000 ng/mL working IS solution was added into each well of the plate except for the wells containing the plasma blanks. The wells containing the plasma blanks received a 50-μL aliquot of DMF. Followed the addition of 100 μL of water containing 5% formic acid to all wells of the plate the plate was vortexed briefly and centrifuged at 3000 rpm for about 5 min.

Each well in an Oasis MCX 96-well SPE plate was conditioned using 500 μL of methanol and 500 μL of 5% formic acid in water. The buffered sample was then loaded to the plate and washed sequentially with 500 μL of 5% formic acid in water followed by 750 μL of methanol and eluted slowly with 400 μL of freshly prepared 5% (v/v) ammonium hydroxide in methanol into a 96-well collection plate. Each eluted sample was evaporated to dryness using a turbovap set at 5 °C and reconstituted with 200 μL of 0.1% formic acid in acetonitrile:water (1:1, v/v). After reconstitution, the 96-well collection plate was capped, vortexed, centrifuged at 3000 rpm for about 1 min, and transferred to an autosampler tray for injection (5–20 μL) onto the LC-MS/MS system.

2.6. Stability

Quality control samples (n = 6 at low and high QC concentrations) were subjected to five freeze-thaw cycles consisting of storage at room temperature, vortexing, and then refreezing at −70 °C for at least 12 h. After five freeze-thaw cycles the samples were analyzed using freshly prepared calibration standards. To evaluate bench-top stability, quality control samples (n = 6 at low and high QC concentrations) were left on the bench-top at room temperature for 6 h prior to extraction. To determine the stability of extracted samples after sitting in the autosampler tray at room temperature, calibration standards (n = 2 at each concentration) and QC samples (n = 6 at each concentration) were extracted and reinjected after storage in the autosampler tray at room temperature for 117 h. Long-term stability was evaluated using quality control samples (n = 6 at low and high QC concentration levels) after

Table 1
Within-run and between-run accuracy and precision of GDC-0449 quality control samples.

	LLOQ (5 ng/mL)	Low QC (15 ng/mL)	Middle QC (2000 ng/mL)	High QC (4000 ng/mL)	Dilution QC (20,000 ng/mL)
Within-run mean (n = 6)	4.68	16.0	1980	4360	19,900
Accuracy[a] (%)	93.6	106.7	99.0	109.0	99.5
RSD[b] (%)	2.5	1.7	0.7	2.5	2.4
Within-run mean (n = 6)	4.75	15.6	2010	3980	
Accuracy[a] (%)	95.0	104.0	100.5	99.5	
RSD[b] (%)	5.3	1.9	1.2	1.3	
Within-run mean (n = 6)	4.43	15.7	1990	3650	
Accuracy[a] (%)	88.6	104.7	99.5	91.3	
RSD[b] (%)	7.4	2.6	0.9	10.1	
Between-run mean (n = 18)	4.62	15.8	2000	4000	
Accuracy[a] (%)	92.4	105.3	100.0	100.0	
RSD[b] (%)	2.9	1.0	0.8	8.6	

[a] Expressed as [(mean observed concentration)/(nominal concentration)] × 100.
[b] Relative standard deviation.

frozen storage at a temperature between −60 and −80 °C for 315 days prior to extraction.

2.7. Pharmacokinetics of GDC-0449 in healthy volunteers

Pharmacokinetics of GDC-0449 was studied in an open-label, single-dose phase I study in three healthy females of non-childbearing-potential following Institutional Review Board approval of the protocol. Upon obtaining informed consent, meeting institutional and federal requirements and meeting study entry criteria, GDC-0449 was administered orally as a single 150 mg dose with 240 mL room temperature tap water after a minimum of an 8-h fast. After drug administration, twenty-two serial blood samples were obtained and plasma harvested during an 8-week sampling period for the determination of GDC-0449 plasma concentration over time.

Pharmacokinetic calculations were performed using WinNonlin® (Pharsight Corporation, version 5.2). All AUC values were calculated using the linear trapezoidal method when the concentrations were rising and the logarithmic trapezoidal method when the concentrations were declining (Linear up/Log Down rule in WinNonlin®). Below the limit of quantitation (BLQ) values at predose were considered as zero for PK analysis. Actual blood collection time was used to calculate PK parameters. PK parameters were reported as their means and standard deviations (SD).

3. Results and discussion

3.1. LC-MS/MS

Using a turbo-ionspray source GDC-0449 and internal standard GDC-0449-D5 yielded protonated molecules at m/z 421 and 426, respectively. Full scan product ion mass spectra of GDC-0449 and GDC-0449-D5 are shown in Fig. 2. For quantitative analysis, the predominant SRM transitions m/z 421 to m/z 139.1 for GDC-0449 and m/z 426 to m/z 139.1 for IS GDC-0449-D5 were monitored. Under the isocratic chromatographic condition described earlier, the retention time was 1.7 min for both GDC-0449 and GDC-0449-D5. The total HPLC run time was 4 min.

3.2. Accuracy and precision

Within-run and between-run accuracy and precision were obtained from experiments performed on 3 separate days with 2 calibration curves and 6 replicates of quality control samples at each concentration. Within-run and between-run accuracy and precision from quality controls are summarized in Table 1. The within-run relative standard deviation (%RSD) from QC samples ranged from 0.7% to 10.1%, while the accuracy ranged from 88.6% to 109.0% of nominal at all concentrations. The between-run %RSD from QC samples varied from 0.8% to 8.6%, while the accuracy was 92.4–105.3% of nominal at all concentrations including the LLOQ quality control 5 ng/mL.

3.3. Sensitivity

Six replicates of LLOQ QC samples at concentrations of 5 ng/mL were extracted and analyzed in three validation runs to determine the accuracy and precision (%RSD) at the LLOQ level. The accuracy and precision at the LLOQ of 5 ng/mL were within 88.6% of nominal and within 7.4%, respectively (Table 1). The LLOQ at 5 ng/mL had an average signal to noise ratio of 48 which was sufficient for determining GDC-0449 concentrations in human plasma samples in these clinical studies. Representative chromatograms of GDC-0449 at LLOQ and IS GDC-0449-D5 at final concentration of 1000 ng/mL are shown in Fig. 3.

3.4. Selectivity and matrix effect

Selectivity was tested using six lots of blank plasma. Interference peaks in all six lots of blank plasma at the retention time of the analyte were ≤20% of the mean response for the analyte at the LLOQ. The interference peaks in all six lots of blank plasma at the retention time of the IS were ≤5% of the mean response for the IS for all tested samples. There was no significant interference found at the retention time of the analyte and IS. Representative chromatograms of extracted blank plasma from GDC-0449 and IS GDC-0449-D5 channels are shown in Fig. 4.

The matrix effect was investigated using LLOQ QCs prepared in six lots of blank plasma to ensure that precision, selectivity and sensitivity were not compromised. The LLOQ QCs for validation met acceptance criteria: 5 out of 6 LLOQ QCs should be within 20% of their target concentrations and the %CV must be ≤20% (Table 2).

The matrix factor, defined as a ratio of the analyte peak response in the presence of matrix ions to the analyte peak response in the absence of matrix ions [6] was quantitatively measured. Six lots of blank human plasma were extracted following the validated extraction procedure. After extraction and evaporation to dryness, 200 μL of solution containing 50 ng/mL GDC-0449 was spiked into six tubes containing dried six lots of blank plasma extract. The LC/MS responses of the resulting solutions were compared to six GDC-0449 neat solutions at 50 ng/mL concentration. Measured matrix factor (99.7%) indicated that there was no significant matrix suppression observed (Table 3).

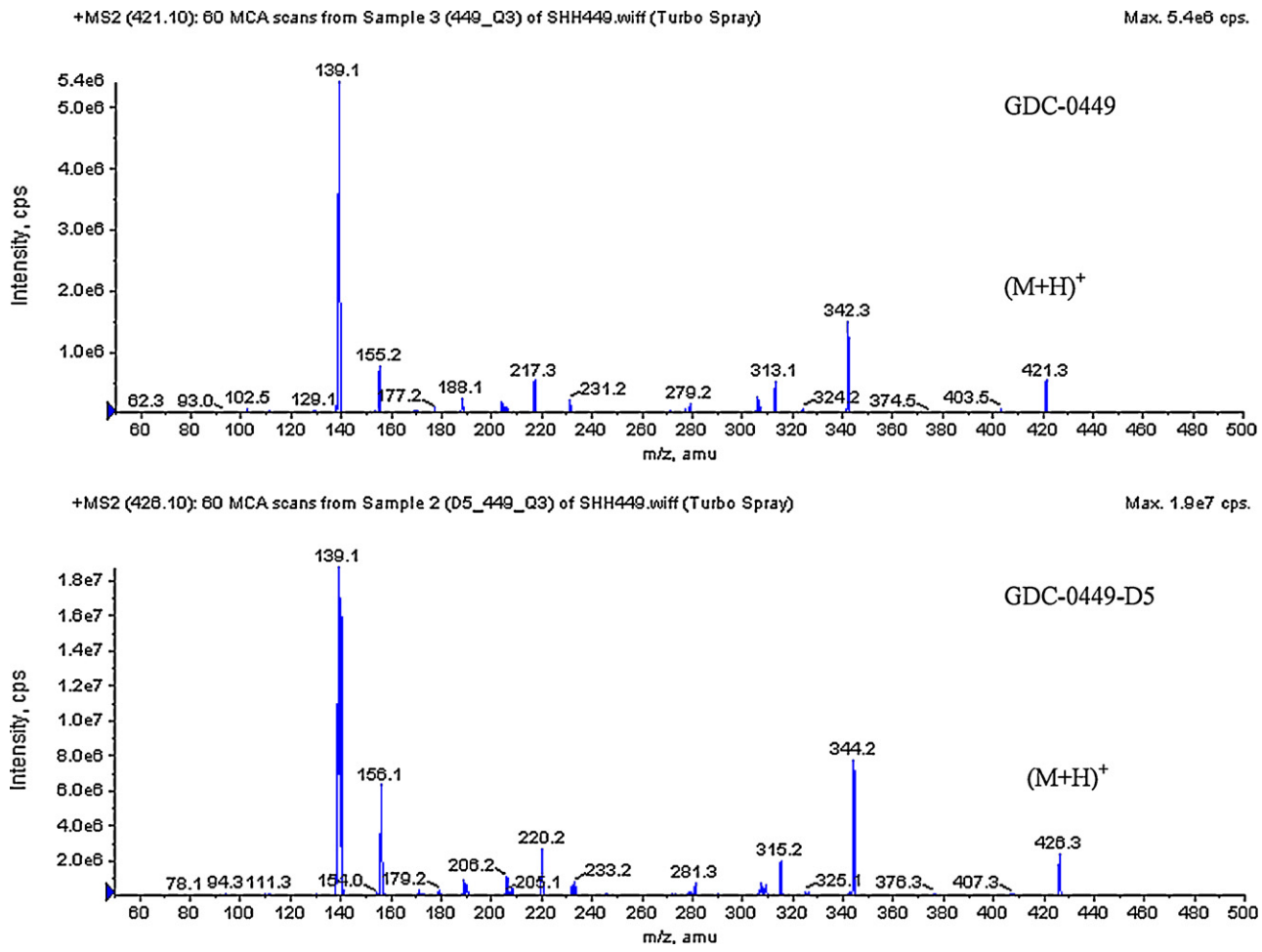

Fig. 2. Product ion mass spectra of GDC-0449 and GDC-0449-D5.

Table 2
Selectivity and matrix effect at the lower limit of quantitation for GDC-0449.

Nominal conc. (ng/mL)	LLOQ 1	LLOQ 2	LLOQ 3	LLOQ 4	LLOQ 5	LLOQ 6	Mean	Accuracy[a] (%)	RSD[b] (%)
5	4.75	4.83	4.97	4.84	4.55	4.72	4.78	95.6	2.9

[a] Expressed as [(mean observed concentration)/(nominal concentration)] × 100.
[b] Relative standard deviation.

3.5. Integrity of dilution

To demonstrate the ability to dilute samples with acceptable accuracy and precision, a dilution QC containing GDC-0449 was prepared and diluted ten fold. Dilution was performed using QC samples at a concentration of 20,000 ng/mL. The accuracy of dilution QCs at 20,000 ng/mL was within 99.5% of nominal and the precision within 2.4% (Table 1).

3.6. Stability

Freeze-thaw, bench-top, reinjection and long-term stability assessments were carried out and the results are summarized in Table 4. GDC-0449 was determined to be stable in human plasma after five freeze-thaw cycles and stable after storage on the bench-top at room temperature for 6 h prior to extraction. GDC-0449 was also stable after extracted GDC-0449 QC samples were stored in the autosampler tray at room temperature for 117 h. Long-term stability was evaluated after GDC-0449 was stored in the freezer at −60 to −80 °C for 315 days and analyzed against freshly prepared calibration curves. Stability assessments demonstrated that GDC-

Table 3
Matrix effect–measured matrix factor for GDC-0449.

	Area ratio[a] Plasma extract	Area ratio[a] Neat solution
Lot 1	0.548	0.543
Lot 2	0.549	0.548
Lot 3	0.548	0.551
Lot 4	0.551	0.556
Lot 5	0.551	0.552
Lot 6	0.549	0.558
Mean	0.549	0.551
RSD[b] (%)	0.2%	1.0%
Matrix factor[c]	99.7%	

[a] Expressed as [(area of GDC-0449)/(area of GDC-0449-d5)].
[b] Relative standard deviation.
[c] Expressed as [(mean area ratio of plasma extract)/(mean area ratio of neat solution)] × 100.

Table 4
Stability assessments for GDC-0449.

Nominal conc. (ng/mL)	Bench-top stability at room temperature for 6 h			Storage at −60 to −80 °C for 315 days		
	Determined mean (ng/mL, $n=6$)	Mean %accuracy ($n=6$)	RSD (%) ($n=6$)	Determined mean (ng/mL, $n=6$)	Mean %accuracy ($n=6$)	RSD (%) ($n=6$)
15	15.8	105.3	1.1	15.8	105.3	3.6
4000	3890	97.3	1.9	3830	95.8	14.4

Nominal conc. (ng/mL)	Stability after 5 freeze-thaw cycles			Storage in the autosampler at room temperature for 117 h		
	Determined mean (ng/mL, $n=6$)	Mean %accuracy ($n=6$)	RSD (%) ($n=6$)	Determined mean (ng/mL, $n=6$)	Mean %accuracy ($n=6$)	RSD (%) ($n=6$)
15	15.6	104.0	3.0	16.0	106.7	0.8
2000				2010	100.5	0.6
4000	4230	105.8	5.9	4350	108.8	0.9

0449 was stable in human plasma for 315 days under these storage conditions.

3.7. Extraction recovery

Stationary phase of Oasis MCX is equipped with a mixed-mode cation-exchange and reversed-phase sorbent. At buffered acidic condition (5% formic acid in water) the positively charged amine and amide groups of GDC-0449 interact with negatively charged MCX stationary phase through ionic interactions. In addition to the ionic interactions, mid- to low-polarity parts of the GDC-0449 molecule interact with MCX reversed-phase sorbent through hydrophobic interaction. GDC-0449 molecules are retained to the stationary phase through these interactions, allowing wash off all impurities that are not retained at acidic condition. GDC-0449 was eluted with the basic solvent (5% ammonium hydroxide in methanol) that neutralizes ionic interaction between the analyte and the stationary phase.

Extraction recovery was evaluated for GDC-0449 at low, medium and high QC concentrations: 15, 2000 and 4000 ng/mL. Extraction recovery was determined by comparing the absolute peak areas of the QC samples spiked in human plasma before extraction to control human plasma extracted in the same manner then spiked post-extraction with a known amount of the GDC-0449. Extraction recoveries for GDC-0449 at low, medium and high QC levels were 88.3%, 90.0% and 91.2%, respectively.

3.8. Pharmacokinetic analysis

Validation was performed at calibration curve range 5–5000 ng/mL. The lower limit of quantitation of 5 mg/mL was sufficient to detect the plasma concentrations at the lowest dose given in the study. The upper limit of quantitation of 5000 ng/mL allowed minimum sample dilution and maximum sample throughput. Two standard curves were processed for each batch run. GDC-0449 concentrations were calculated from the equation $y = ax^2 + bx + c$, by weighted $(1/x^2)$ quadratic regression of the calibration line constructed from peak area ratios of GDC-0449 to internal standard versus nominal GDC-0449 concentration.

Fig. 5 shows the GDC-0449 concentration versus time profiles for each of the three healthy postmenopausal women enrolled in the phase I study that were administered a single 150 mg oral dose. GDC-0449 plasma concentrations were within the calibration curve for all subjects and ranged from 2532 to 38 ng/mL, with

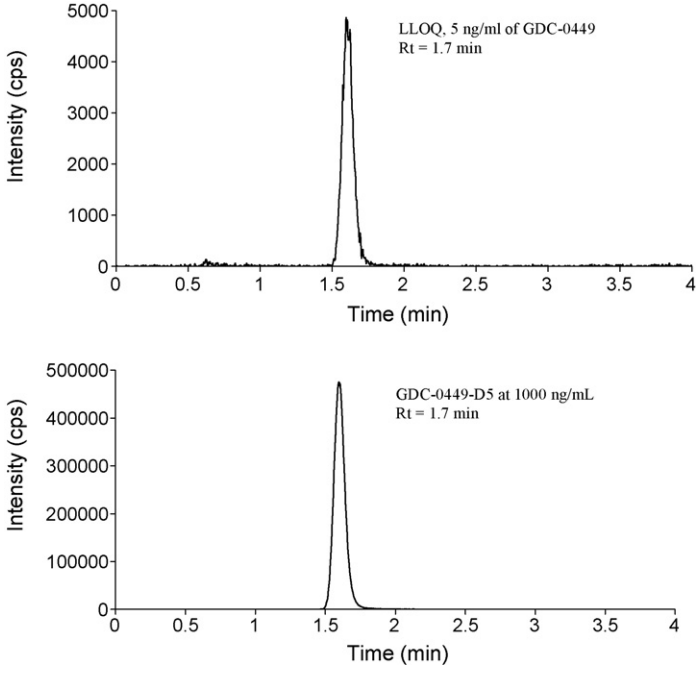

Fig. 3. Ion chromatograms for a calibration standard at the LLOQ, 5 ng/ml of GDC-0449 and internal standard GDC-0449-D5 at final concentration of 1000 ng/mL.

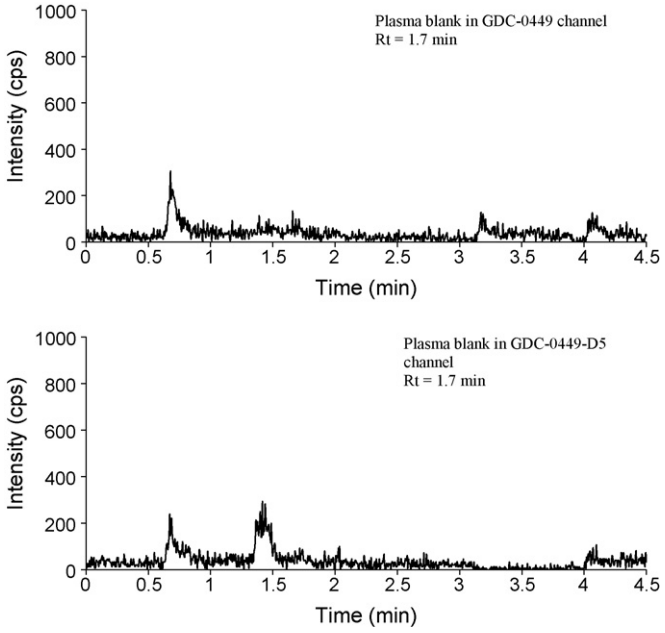

Fig. 4. Ion chromatograms of extracted blank plasma for the GDC-0449 and internal standard GDC-0449-D5 channels.

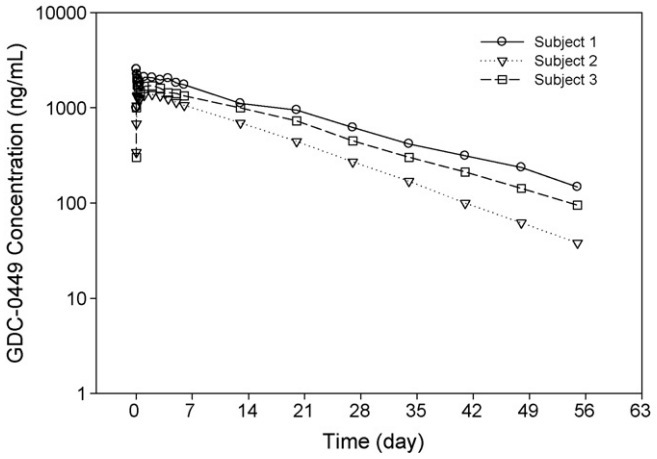

Fig. 5. Individual concentration versus time profiles for 3 healthy volunteers following a single 150 mg oral dose of GDC-0449 in a Phase I clinical trial.

C_{max} ranging from 2532 to 1380 ng/mL and C_{last} ranging from 147 to 38.0 ng/mL. Over a 56-day blood sampling period, the area under the concentration–time curve and terminal elimination rate constant was adequately determined despite the long $t_{1/2}$, with only 4.5% of the AUC extrapolated from T_{last} to infinity.

Mean (SD) pharmacokinetic data for these three healthy volunteers were: C_{max} = 1984 (577) ng/mL, AUC_{0-t} = 1910 (577) μM × h, AUC_{0-inf} = 2000 (644) μM × h, $t_{1/2}$ = 286 (42.6) h, CL/F = 0.192 (0.0652) L/h, and Vz/F = 76.4 (14.2) L.

4. Conclusions

An automated 96-well SPE LC-MS/MS method for the determination of GDC-0449 in human plasma was developed and validated. The validated method met the regulatory requirements for accuracy, precision, selectivity and stability, and was applied successfully to the analysis of human plasma samples generated in a phase I clinical study. The validated method described herein is currently in use to evaluate GDC-0449 steady state PK in ongoing phase I and phase II clinical [5].

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References

[1] M. Athar, C. Li, X. Tang, S. Chi, X. Zhang, A.L. Kim, S.K. Tyring, L. Kopelovich, J. Hebert, E.H. Epstein Jr., D.R. Bickers, J. Xie, Cancer Res. 64 (2004) 7545.
[2] J.T. Romer, H. Kimura, S. Magdaleno, K. Sasai, C. Fuller, H. Baines, M. Connelly, C.F. Stewart, S. Gould, L.L. Rubin, T. Curran, Cancer Cell 6 (2004) 229.
[3] R.L. Yauch, S.E. Gould, S.J. Scales, T. Tang, H. Tian, C.P. Ahn, D. Marshall, L. Fu, T. Januario, D. Kallop, M. Nannini-Pepe, K. Kotkow, J.C. Marsters, L.L. Rubin, F.J. de Sauvage, Nature 455 (2008) 406.
[4] H. Wong, J.Z. Chen, B. Chou, J.S. Halladay, J.R. Kenny, H. La, J.C. Marsters Jr., E. Plise, P.J. Rudewicz, K. Robarge, Y. Shin, S. Wong, C. Zhang, S.C. Khojasteh, Xenobiotica Sep. (2009) 1.
[5] D.D. Von Hoff, P.M. LoRusso, C.M. Rudin, J.C. Reddy, R.L. Yauch, R. Tibes, G.J. Weiss, M.J. Borad, C.L. Hann, J.R. Brahmer, H.M. Mackey, B.L. Lum, W.C. Darbonne, J.C. Marsters Jr., F.J. de Sauvage, J.A. Low, N. Engl. J. Med. 361 (2009) 1164.
[6] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, AAPS J. 9 (1) (2007) E30.