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Validated assays for the determination of gemcitabine in human plasma and urine using high-performance liquid chromatography with ultraviolet detection

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Abstract

Procedures are described for the determination of gemcitabine, a new anti-tumor agent, and its uridine metabolite in human plasma and in human urine. The sample preparation for the plasma assay involves precipitation of plasma proteins with isopropanol and ethyl acetate. Following this, the solids are discarded and the supernatant is evaporated to dryness. For the urine assay, the sample is diluted with methanol and evaporated to dryness. For both procedures, the residue is reconstituted in mobile phase prior to injection into a normal-phase (amino column) liquid chromatographic system followed by UV detection at 272 nm. The limits of quantitation for both compounds are 50 ng/ml in plasma and 20 μg/ml in urine. The procedures were used to provide pharmacokinetic data for both compounds in man following the intravenous administration of a 1000 mg/m^2 dose of gemcitabine.

1. Introduction

Gemcitabine, 2′-deoxy-2′,2′-difluorocytidine (I, Fig. 1), [1] is a pyrimidine antimetabolite with broad spectrum activity against murine leukemias, murine solid tumors, and human tumor xenografts [2–4]. Clinical investigations are underway in non-small cell lung cancer, pancreatic carcinoma, and other solid tumors.

In mice, rats, and dogs, the primary circulating metabolite of gemcitabine is the uridine derivative, 2′-difluoro-2′,2′-deoxyuridine or 2-dFdU (II, Fig. 1), formed by the action of cytidine deaminase [5]. In an early clinical trial, 2-dFdU was also the primary metabolite observed in human plasma and urine [6]. Intracellularly,

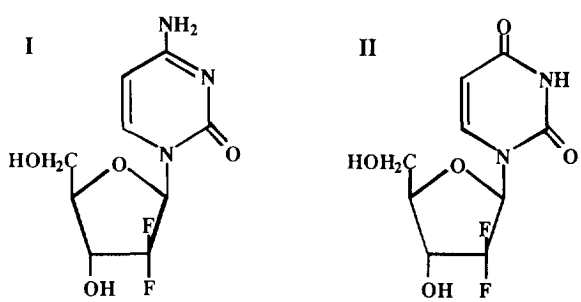

Fig. 1. Structures of gemcitabine (I) and the metabolite, 2-dFdU (II).

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gemcitabine is metabolized by deoxycytidine kinase and other nucleotide kinases to the active triphosphate metabolite [7].

In order to fully characterize the pharmacokinetics and metabolism of gemcitabine in humans, sensitive assays for the determination of gemcitabine and 2-dFdU in human plasma and urine are required. However, nucleosides present unique challenges to isolate from biological fluids. Typical bioanalytical sample preparation techniques such as extraction into an organic solvent are not effective because of the extremely hydrophilic nature of the compounds. Early investigations of the plasma concentrations of gemcitabine and 2-dFdU in humans were accomplished by directly injecting 20 μl of plasma into a gradient reversed-phase HPLC system. A limit of detection of 265 ng/ml was achieved [6,8]. But this method was reported to be unable to measure urinary concentrations because of significant interferences [9]. Analysis of the urinary excretion in one study was only able to be accomplished with radiolabeled drug [6]. Two other techniques have also been explored for the analysis of these compounds. An ELISA assay with a limit of detection of 75 ng/ml [6] has been described but sufficient quantities of the antibody are no longer available. Another study used 19F-NMR for the analysis of both compounds in plasma and urine [10]. The estimated limit of detection at 2.6 μg/ml was not comparable to the reversed-phase HPLC method for plasma, but the technique was an improvement for quantitation of the compounds in urine.

We have developed HPLC assays for the analysis of gemcitabine and 2-dFdU in human plasma and in human urine. The plasma assay incorporates a relatively simple sample preparation step and has a limit of quantitation of 50 ng/ml. For both assays, the analysis is accomplished using an isocratic normal-phase separation with UV detection (272 nm). This paper describes the validation of these assays and their application to the analysis of gemcitabine and 2-dFdU in samples from a Phase I clinical study. These data were used to determine the pharmacokinetics in patients with advanced cancer. As part of this study, certain patients received radiolabeled drug and the urine assay was also used to examine the excretion profile.

2. Experimental

2.1. Reagents and materials

Gemcitabine hydrochloride, ^{14}C-radiolabeled gemcitabine, and 2-dFdU were provided by Lilly Research Laboratories (Eli Lilly and Company, Indianapolis, IN, USA). The internal standard, 2′-deoxycytidine, was obtained from Sigma (St. Louis, MO, USA). Tetrahydrouridine (THU) was purchased from Calbiochem (San Diego, CA, USA). Cyclohexane (HPLC-grade), triethylamine and 1,2-dichloroethane (HPLC-grade) were obtained from EM Science (Gibbstown, NJ, USA). HPLC-grade methanol and ethyl acetate were purchased from Burdick and Jackson (Division of Baxter Healthcare, Muskegon, MI, USA). Isopropanol (USP grade) and glacial acetic acid (AR grade) were obtained from Mallinckrodt (Paris, KY, USA). Purified water (Milli-Q System, Millipore, Milford, MA, USA) was used for all aqueous solutions. All other chemicals were of analytical reagent grade. Drug-free human plasma (heparinized) and urine were obtained from healthy volunteers.

2.2. Chromatographic equipment and conditions

The HPLC system consisted of a Beckman (San Ramon, CA, USA) Model 114M isocratic pump, a Perkin-Elmer (Norwalk, CT, USA) ISS-100 autosampler, and an Applied Biosystems (Foster City, CA, USA) Model 783 variable-wavelength detector set at 272 nm and 0.01 AUFS. The analytical column was either an Adsorbosphere or Econosphere NH_2 (Alltech Associates, Deerfield, IL, USA), 5 μm particle size, 250 × 4.6 mm I.D. cartridge column. Guard columns were also obtained from Alltech Associates: Adsorbosphere NH_2, 5 μm particle size, and 10 × 4.6 mm I.D. The column temperature was ambient. A Hewlett-Packard Model

1000 computer with in-house developed software was used for on-line data acquisition and subsequent calculations.

The mobile phase for the plasma assay was prepared by mixing 630 ml of cyclohexane, 150 ml of 1,2-dichloroethane, 220 ml of methanol, 1 ml of purified water, 0.5 ml of glacial acetic acid, and 1 ml of triethylamine. The mobile phase flow-rate was 1.5 ml/min. The peaks of interest eluted within 20 min. An additional 20 min was allowed between injections for the elution of more strongly retained endogenous interferences. An injection volume of 50 μl was used.

For the urine assay, the mobile phase consisted of 600 ml of cyclohexane, 150 ml of 1,2-dichloroethane, 250 ml of methanol, 1 ml of purified water, and 0.5 ml of glacial acetic acid. The flow-rate was increased to 1.7 ml/min for the urine method and a chromatographic run time of 20 min was required per sample. An injection volume of 10 μl was used for the urine assay.

2.3. Collection and storage of subject samples

Gemcitabine is metabolized by plasma cytidine deaminase ex vivo, however this can be inhibited by tetrahydrouridine or THU [11,12]. Tetrahydrouridine, provided as 10 mg vials, was dissolved in 1 ml of purified water. This solution was stable for at least 4 weeks when stored refrigerated. Subject plasma samples were each collected in a heparinized 10-ml vacutainer to which 100 μl of the THU solution described above had been added. Normally human urine should be protein-free and there should be no need to inhibit enzymatic activity. However the presence of protein in the urine may occur in some patients and therefore 1 ml of the THU solution was added to each patient's urine collection for the first 24 h. Based on the half-life of gemcitabine estimated from preliminary studies [6], THU was not added to the other urine collections since parent gemcitabine was not expected to be excreted after the first 24 h. Plasma and urine samples were stored at −70°C until analysis.

2.4. Preparation of THU-treated plasma and urine

The THU solution described above was mixed with control plasma or urine in a 1:100 (v/v) ratio. THU-treated plasma or urine was stored at −20°C until needed to prepare standards and control samples.

2.5. Preparation of plasma standards and controls

A standard stock solution containing gemcitabine and 2-dFdU was prepared in methanol at a concentration of approximately 50 μg/ml (free base) of each compound. A stock solution in methanol of the internal standard, 2′-deoxycytidine, was prepared at a concentration of approximately 100 μg/ml. The stock solutions were stable for at least three weeks when stored refrigerated. A working solution of the internal standard was prepared fresh daily by diluting 4 ml of the 100 μg/ml solution to 50 ml with purified water.

Plasma calibration standards were prepared at concentrations of approximately 50, 150, 300, 600, 1200, 1800, and 2400 ng/ml by diluting appropriate aliquots of the 50 μg/ml solution with THU-treated plasma. The standards were stored at −70°C until needed. Duplicate standards at each concentration were assayed on each day.

Plasma control samples were prepared from a separate stock solution at concentrations of approximately 100, 1000, and 2000 ng/ml. These samples were stored with the subject samples at −70°C. Each concentration was assayed in duplicate on each day that samples were analyzed.

2.6. Preparation of urine standards and controls

A standard stock solution containing gemcitabine and 2-dFdU was prepared in methanol at a concentration of approximately 2 mg/ml (free base) of each compound. A working solution in methanol of the internal standard, 2′-deoxycytidine, was prepared at a concentration of approximately 30 μg/ml. The stock solutions

were stable for at least three weeks when stored refrigerated.

Urine calibration standards were prepared at concentrations of approximately 20, 50, 75, 100, 200, 300, and 400 μg/ml by diluting appropriate aliquots of the 2 mg/ml solution with THU-treated urine. The standards were stored at −70°C until needed. Duplicate standards at each concentration were assayed on each day.

Urine control samples were prepared from a separate standard solution at concentrations of approximately 40, 160, and 320 μg/ml. These samples were stored with the subject samples at −70°C and each concentration was assayed in duplicate with each set of study samples.

2.7. Plasma sample preparation procedure

Individual 0.2-ml aliquots of plasma standards, controls, and subject samples were pipetted into 12-ml disposable glass tubes with PTFE-lined screw caps. For sample concentrations above 2400 ng/ml, sample volumes from 0.01 to 0.1 ml were used and diluted to 0.2 ml with THU-treated plasma. A 50-μl aliquot of the working plasma internal standard solution was added to each tube. The samples were briefly vortex-mixed. Then 1 ml of isopropanol was added and they were vortex-mixed again. The samples were allowed to sit for 5 min. A 2.5-ml aliquot of ethyl acetate was added and vortex-mixed. The samples were centrifuged at approximately 2500 *g* for 10 min at 5°C to pellet the precipitate. The supernatant was transferred to a fresh tube and evaporated to dryness at 40°C under a stream of nitrogen. The samples were reconstituted with 250 μl of mobile phase. A slight white insoluble residue remained visible in the tubes. The samples were filtered through Ultra-free MC units (Millipore, Bedford, MA, USA) and transferred to glass autosampler vials.

2.8. Urine sample preparation procedure

Individual 0.2-ml aliquots of urine standards, controls, and subject samples were pipetted into 12-ml disposable glass tubes with PTFE-lined screw caps. For sample concentrations above 400 μg/ml, sample volumes from 0.01 to 0.1 ml were used and diluted to 0.2 ml with THU-treated urine. A 1-ml aliquot of the methanol internal standard solution for urine samples was added to each tube. The samples were briefly vortex-mixed and then evaporated to dryness at 40°C under a stream of nitrogen. The samples were reconstituted with 1-ml of mobile phase and transferred to glass autosampler vials.

2.9. Data handling and calculations

A calibration curve for each analyte was fit with a linear least-squares analysis of the standards as concentration vs. peak-height ratio (analyte peak height/internal standard peak height) The control and subject sample concentrations were calculated from comparison of their peak-height ratios to the calibration curve after correction for dilution factors.

2.10. Validation procedure

The precision and accuracy of the plasma method with the Adsorbosphere column was determined by analyzing five pools of plasma spiked with known concentrations of the compounds (approximately 50, 500, 1000, 2000, and 2800 ng/ml). Five replicates of each pool were analyzed on three different days by the same analyst. The linearity of the standard curves was assessed on each of these days. The characterization of the method with the Econosphere column involved the analysis of five replicates of each of four concentrations (approximately 50, 100, 1000, and 2000 ng/ml) on a single day. The appropriateness of the dilution method for samples was evaluated by analyzing a spiked solution of plasma containing approximately 2800 ng/ml of each compound at sample volumes of 0.01, 0.02, 0.05, 0.1, and 0.2 ml. The recovery of the sample preparation procedure was calculated by comparing the peak heights of extracted plasma control samples at three concentrations (approximately 100, 1000, and 2000 ng/ml) to equivalent concentration absolute standards prepared by directly diluting the stock standard solution with

mobile phase. Five replicates of the controls and absolutes were assayed at each concentration.

The precision and accuracy of the urine method was determined by analyzing five pools of urine spiked with known concentrations of gemcitabine and 2-dFdU (20, 60, 120, 240, and 480 μg/ml). Five replicates of each pool were analyzed on three different days by the same analyst. The linearity of the standard curves was assessed on each of these days. The appropriateness of the dilution method for samples was evaluated by analyzing a spiked solution of urine containing approximately 480 ng/ml of each compound at sample volumes of 0.01, 0.02, 0.05, 0.1, and 0.2 ml. The recovery of the sample preparation procedure was calculated by comparing the peak heights of extracted standard samples to equivalent concentration absolute standards. The absolute standards were prepared by evaporating appropriate aliquots of the stock standard solution to dryness and reconstituting with mobile phase. Four replicates of the standards and absolutes were assayed at each concentration.

2.11. Determination of stability

The stability of gemcitabine and 2-dFdU in plasma and urine was determined by preparing samples at known concentrations of the compounds in THU-treated plasma or urine. The samples were stored at room temperature, 4°C, −20°C, and −70°C. Aliquots of each pool were assayed at various time points during storage. Freeze–thaw stability of the compounds in each matrix was evaluated through three cycles. The stability of processed samples was determined by comparing a set of standards stored at room temperature.

2.12. Method application–Phase I clinical trial

Twelve patients with advanced cancer were treated with 1000 mg/m^2 gemcitabine infused over a 30-min period. The patients received a gemcitabine infusion once per week for 3 weeks followed by a rest week (no treatment). In five of the patients a portion of the dose consisted of 98.6 μCi of radiolabeled gemcitabine. Blood samples were obtained periodically for 1 week following the first dose. Urine was also collected for 1 week after the initial gemcitabine infusion. In addition to quantitation of all of the samples by the HPLC-UV methods, the samples from the patients receiving radiolabeled drug were analyzed by liquid scintillation counting. Triplicate aliquots were added to Scintisol scintillation cocktail (United Technologies Packard) and a Beckman liquid scintillation counter Model LS, 5000 TD (San Ramon, CA, USA) was used.

3. Results and discussion

3.1. Chromatographic conditions

The hydrophilic nature of the nucleosides presented a challenge for the development of a sensitive and selective assay for gemcitabine and 2-dFdU. The resolution of the analytes from equally polar endogenous materials is difficult. For sample preparation, we explored liquid–liquid and solid-phase extractions but were unable to obtain sufficient recovery or selectivity. The protein precipitation step used for the plasma method affords protection of the analytical column but the chromatographic separation imparts the selectivity to the method.

A number of different options were explored in the development of the separation. The highly polar nature of the analytes causes them to elute quite rapidly from reversed-phase columns even with very low organic content mobile phases. We were unable to achieve sufficient resolution from endogenous interferences under reversed-phase conditions. Gemcitabine and 2-dFdU were more strongly retained on a normal-phase amino column with a mobile phase consisting of methanol, cyclohexane and dichloroethane. However varying levels of free silanols from lot-to-lot and even column-to-column appeared to cause reproducibility problems as evidenced by shifting retention times and varying peak shapes. The variability was controlled by adding small quantities of triethylamine and acetic acid to the mobile phase. A small quantity of water was also added

to achieve a constant level of hydration of the chromatographic system.

The method was initially developed in the UK with a Rosil amino column. In the course of addressing the reproducibility problems, a number of different amino columns were compared for suitability in the assay using the mobile phase described. Zorbax, Waters, and Hypersil columns were unable to provide an adequate separation under those conditions. Alltech Adsorbosphere and Econosphere columns produced equivalent separations with sufficient retention (and thereby resolution) of the analytes and were used for the analysis of the samples from the Phase I study. Representative chromatograms using an Adsorbosphere column are shown in Fig. 2 for plasma standards and Fig. 3 for urine standards. Recently we have examined an Alltech Econosil amino column that has irregularly-shaped particles and greater surface area. This column appears to provide even greater retention of the analytes and better separation from endogenous interferences.

Other drugs which may be concomitantly administered with gemcitabine were tested for potential interference in the assay. Of the compounds listed in Table 1 only nitrofurantoin coeluted with 2-dFdU and could potentially interfere with the analysis. The presence of nitrofurantoin can be distinguished however at

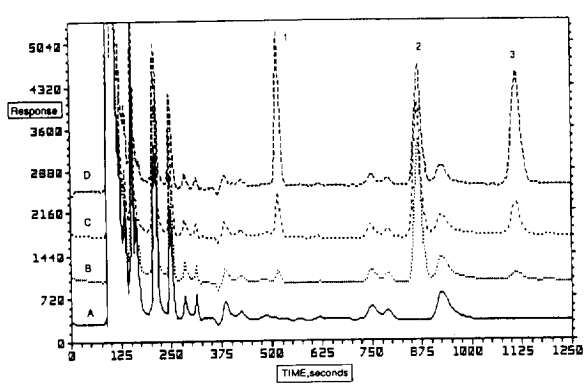

Fig. 2. Chromatogram of human blank plasma (A) and standards in human plasma at concentrations of 150 ng/ml (B), 600 ng/ml (C), and 2400 ng/ml (D). Peaks: 1 = 2-dFdU; 2 = internal standard; 3 = gemcitabine.

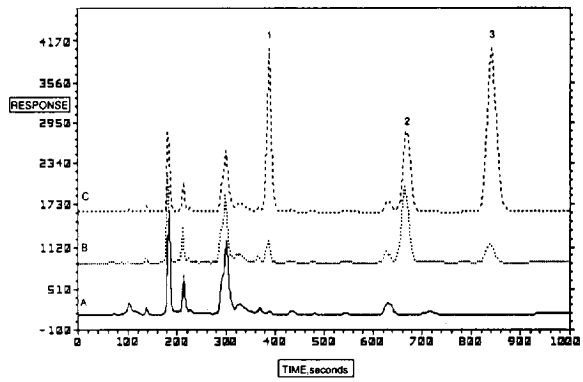

Fig. 3. Chromatograms of human blank urine (A) and standards in human urine at concentrations of 50 μg/ml (B) and 400 μg/ml (C). Peaks: 1 = 2-dFdU; 2 = internal standard; 3 = gemcitabine.

367 nm where it has an additional absorption band, but 2-dFdU does not absorb.

3.2. Recovery

The recovery of gemcitabine and 2-dFdU from plasma averaged 96.9 and 103%, respectively. The recoveries from urine averaged 68.2 and 67.0%, respectively, which was somewhat low but appeared to be consistent throughout the concentration range tested.

3.3. Precision and accuracy

The precision and accuracy for each procedure was determined by spiking gemcitabine and 2-

Table 1
Drugs tested for interference in the assay[a]

Acetaminophen	Hydrocodone
Atenolol	Ibuprofen
Atropine	Indomethacin
Dexamethasone	Loperamide
Digoxin	Nitrofurantoin
Diphenhydramine	Phenytoin
Dipyridamole	Prochlorperazine
Fluoxetine	Theophylline
Furosemide	Warfarin

[a] These drugs were tested for interference in the assay as stated. Only nitrofurantoin presented a potential interference with the determination of 2-dFdU.

Table 2
Summary of assay precision and accuracy data for gemcitabine in human plasma

Day		Theoretical concentration (ng/ml)				
		50.68	506.8	984.6	1969	2838
1	Mean ($n = 5$)	52.25	482.5	919.8	1915	2863
	%R.S.D.	19.68	1.83	2.48	1.93	1.39
	% of Theory	103.1	95.2	93.4	97.3	100.9
2	Mean ($n = 5$)	50.90	509.2	922.5	1929	2857
	%R.S.D.	19.00	3.62	2.59	0.78	1.31
	% of Theory	100.4	100.5	93.7	98.0	100.7
3	Mean ($n = 5$)	56.75[a]	508.6	924.5	1917	2789
	%R.S.D.	12.38	3.30	4.37	2.32	0.77
	% of Theory	112.0	100.4	93.9	97.3	98.3
Overall	Mean ($n = 15$)	53.05[b]	500.1	922.2	1920	2836
	%R.S.D.	17.47	4.09	3.27	1.79	1.81
	% of Theory	104.7	98.7	93.7	97.5	99.9

[a] $n = 4$.
[b] $n = 14$.

dFdU into drug-free, THU-treated plasma and urine at five concentrations. Five replicates from each pool were assayed on each of three days so that both within-day and between-day precision and accuracy could be determined. The results for gemcitabine and 2-dFdU in human plasma are shown in Tables 2 and 3, respectively. The relative standard deviations and the relative

Table 3
Summary of assay precision and accuracy data for 2-dFdU in human plasma

Day		Theoretical concentration (ng/ml)				
		50.15	501.5	1011	2022	2808
1	Mean ($n = 5$)	55.68	499.7	932.9	1941	2791[a]
	%R.S.D.	5.87	1.25	1.22	5.03	0.66
	% of Theory	111.0	99.6	92.3	96.0	99.4
2	Mean ($n = 5$)	60.04	515.6	919.5	1964	2781
	%R.S.D.	7.77	2.94	6.44	0.70	3.19
	% of Theory	119.7	102.8	91.0	97.2	99.0
3	Mean ($n = 5$)	54.56[a]	505.2	976.4	1990	2828
	%R.S.D.	7.24	1.58	3.91	1.64	1.03
	% of Theory	108.8	100.7	96.6	98.4	100.7
Overall	Mean ($n = 15$)	56.92[b]	506.8	942.9	1965	2800[b]
	%R.S.D.	8.06	2.45	5.03	3.05	2.04
	% of Theory	113.5	101.1	93.3	97.2	99.7

[a] $n = 4$.
[b] $n = 14$.

Table 4
Summary of assay precision and accuracy data for gemcitabine in human urine

Day		Theoretical concentration (μg/ml)				
		20.00	60.00	120.0	240.0	480.0
1	Mean ($n = 5$)	21.49	62.43	120.5	236.7	484.3
	%R.S.D.	0.78	0.80	0.44	0.52	0.32
	% of Theory	107.4	104.1	100.4	98.6	100.9
2	Mean ($n = 5$)	21.03	61.71	120.1	236.9	483.3
	%R.S.D.	1.06	0.63	0.55	0.74	0.74
	% of Theory	105.2	102.9	100.1	98.7	100.7
3	Mean ($n = 5$)	21.20[a]	61.82	120.6	236.4	483.0
	%R.S.D.	0.85	0.22	0.27	0.48	0.39
	% of Theory	106.0	103.0	100.5	98.5	100.6
Overall	Mean ($n = 15$)	21.24[b]	61.99	120.4	236.7	483.5
	%R.S.D.	1.38	0.83	0.46	0.53	0.52
	% of Theory	106.2	103.3	100.3	98.6	100.7

[a] $n = 4$.
[b] $n = 14$.

errors for the plasma method were less than 10% at all concentrations except at the lower limit of quantitation where they were less than 20%. The precision and accuracy data for the urine assay are shown in Tables 4 and 5 for gemcitabine and 2-dFdU, respectively. For the urine procedure, the statistics were within less than 10% at all concentrations examined.

Table 5
Summary of assay precision and accuracy data for 2-dFdU in human urine

Day		Theoretical concentration (μg/ml)				
		20.00	60.00	120.0	240.0	480.0
1	Mean ($n = 5$)	20.76	62.68	122.7	240.4	490.0
	%R.S.D.	3.03	2.75	2.38	2.99	3.19
	% of Theory	103.8	104.5	102.3	100.2	102.1
2	Mean ($n = 5$)	20.76	62.81	124.8	243.0	485.6
	%R.S.D.	2.77	2.73	0.72	3.27	3.80
	% of Theory	103.8	104.7	104.0	101.3	101.2
3	Mean ($n = 5$)	20.94[a]	61.26	119.9	238.5	485.8
	%R.S.D.	1.38	2.74	3.41	0.29	0.34
	% of Theory	104.7	102.1	99.9	99.4	101.2
Overall	Mean ($n = 15$)	20.81[b]	62.25	122.5	240.6	487.1
	%R.S.D.	2.57	2.81	2.94	2.57	2.87
	% of Theory	104.1	103.8	102.1	100.3	101.5

[a] $n = 4$.
[b] $n = 14$.

3.4. Linearity

The linearity of the response for the plasma assay was established over the concentration range 50–2400 ng/ml for each gemcitabine and 2-dFdU. Typical correlation coefficients were greater than 0.999. The urine method was linear over the concentration range 20–400 μg/ml with correlation coefficients greater than 0.998.

3.5. Limits of quantitation

The lower limit of quantitation for the plasma assay was 50 ng/ml. Based on the wide range of plasma levels of gemcitabine and 2-dFdU seen in patient samples, it was often necessary to dilute high concentration samples with THU-treated plasma to achieve concentrations within the standard curve range. A one-day characterization was performed to assess the within-day precision and accuracy of these dilutions. It was verified that up to 20-fold dilutions could be performed with patient samples.

The urine method had a lower limit of quantitation of 20 μg/ml. Dilution of high concentration urine samples was examined using either water or THU-treated urine. Better results were obtained using urine as the diluent for up to 20-fold dilutions.

3.6. Stability

Gemcitabine and 2-dFdU were found to be exceedingly stable compounds under all the conditions examined. The compounds were stable in THU-treated human plasma for at least 8 months at −20°C and for at least 21 months at −70°C. Under refrigerated and room temperature conditions, the compounds were stable in plasma for at least 2 months. In human urine stored at either −20°C or −70°C, gemcitabine and 2-dFdU were determined to be stable for at least one year. The stability in urine under refrigerated conditions was at least 1 month and at room temperature was at least 1 week. The compounds in either plasma or urine were unaffected by three freeze–thaw cycles. Gemcitabine and 2-dFdU were stable in the injection solvent of the plasma method for at least 4 days at room temperature. Processed urine samples were stable for at least 40 h.

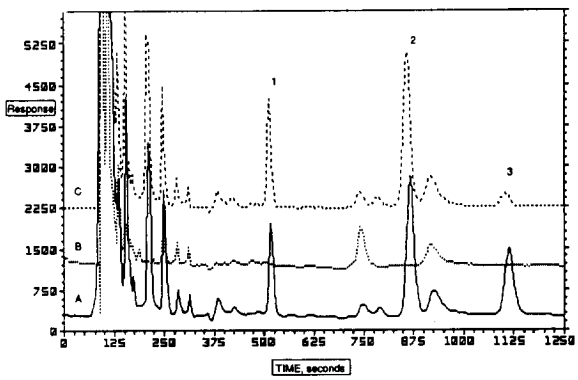

Fig. 4. Chromatograms from a patient administered a 30-min infusion of 1000 mg/m^2 of gemcitabine. Chromatograms: (A) human plasma standard (1200 ng/ml); (B) patient sample prior to infusion; (C) patient sample at 60 min post-infusion. Peaks: 1 = 2-dFdU; 2 = internal standard; 3 = gemcitabine.

3.7. Application of the method in pharmacokinetic studies

The validated procedures were used to provide pharmacokinetic data in man following a 30-min infusion of 1000 mg/m^2 of gemcitabine. Analysis of plasma and urine samples collected prior to

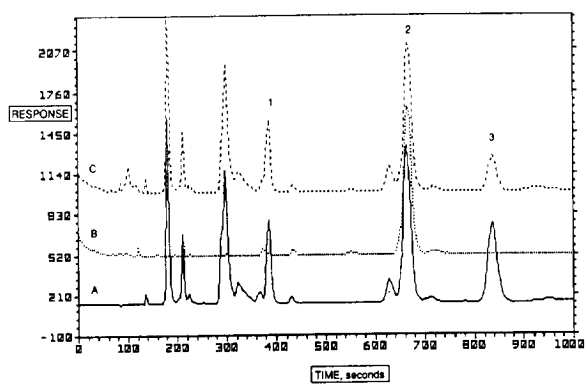

Fig. 5. Chromatograms from a patient administered a 30-min infusion of 1000 mg/m^2 of gemcitabine. Chromatograms: (A) human urine standard (100 μg/ml); (B) patient sample prior to infusion; (C) patient sample from the 0–2 h post-infusion urine collection. Peaks: 1 = 2-dFdU; 2 = internal standard; 3 = gemcitabine.

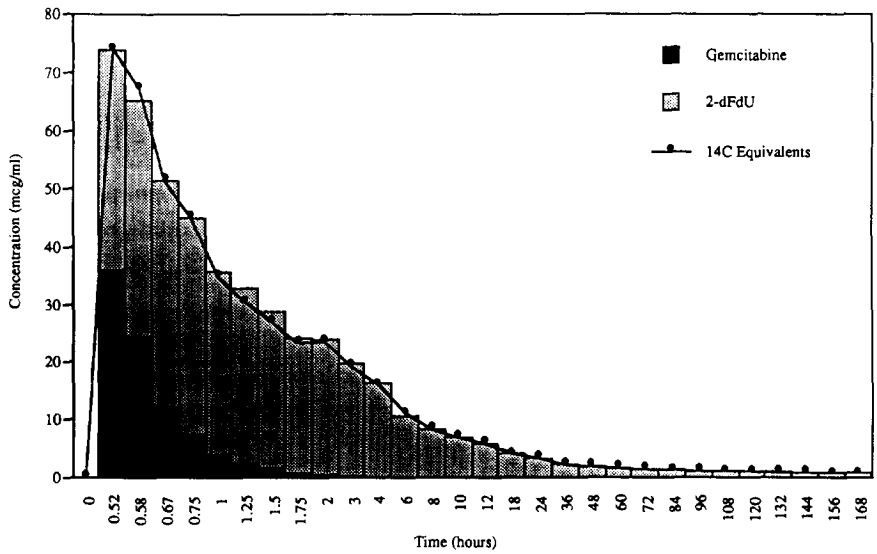

Fig. 6. Profile of the concentrations in human plasma of gemcitabine and 2-dFdU determined by the HPLC method as compared to the concentration of the radiolabel determined by scintillation counting. The patient was administered a 30-min infusion of 1000 mg/m^2 of gemcitabine with a portion consisting of 98.6 μCi of radiolabeled gemcitabine.

drug administration demonstrated that endogenous components did not interfere with the analyses. Representative patient chromatograms from plasma and urine samples are shown in Figs. 4 and 5, respectively.

For the subjects receiving a portion of the dose as radiolabeled gemcitabine, the HPLC method was used to isolate fractions of the urine for scintillation counting. Urinary excretion accounted for over 99% of the recovered dose with

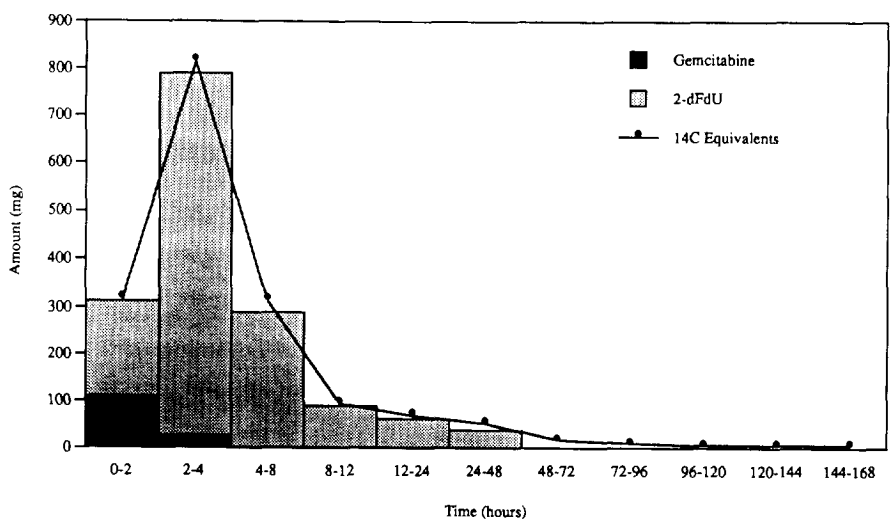

Fig. 7. Profile of the concentrations in human urine of gemcitabine and 2-dFdU determined by the HPLC method as compared to the concentration of the radiolabel determined by scintillation counting. The patient was administered a 30-min infusion of 1000 mg/m^2 of gemcitabine with a portion consisting of 98.6 μCi of radiolabeled gemcitabine.

less than 10% excreted as unchanged drug. The total radioactivity in the plasma and urine was equal to the sum of the concentrations of gemcitabine and 2-dFdU as measured by the HPLC-UV methods (Figs. 6 and 7, respectively). This metabolic information indicates that the HPLC-UV methods presented here can be used to accurately account for the disposition of gemcitabine.

4. Conclusions

Gemcitabine and 2-dFdU may be accurately determined in plasma and urine by the described procedures. The method was used to analyze the samples from a Phase I pharmacokinetic study. Comparison with the radiolabel analyses indicates that the described HPLC-UV methods account for the disposition of gemcitabine in humans. The plasma procedure has since been transferred to contract laboratories for the analysis of samples from Phase II/III studies, demonstrating the ruggedness of the methodology.

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