Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 840 (2006) 44–49
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simultaneous determination of gemcitabine di- and triphosphate in human blood mononuclear and cancer cells by RP-HPLC and UV detection

R. Losa*, M.I. Sierra, M.O. Gión, E. Esteban, J.M. Buesa

Laboratory of Medical Oncology, Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Hospital Central de Asturias, C/Julián Clavería s/n, 33006 Oviedo, Asturias, Spain

Received 1 December 2005; accepted 21 April 2006
Available online 24 May 2006

Abstract

A reverse-phase HPLC method based on ion-pair formation with UV detection was set up for the simultaneous determination of gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP) in human cells. The separation was achieved on a Tracer Excel ODSA column (100 mm × 4.6 mm i.d., 3 μm particle size) at room temperature. Nine nucleotides were separated by isocratic elution in 26 min. Accuracy, linearity, sensitivity and precision studies for dFdCDP, dFdCTP, adenosine diphosphate (ADP) and triphosphate (ATP) validated this method. This assay was used to provide data from gemcitabine treated patients and in vitro grown human cancer cells.

Keywords: dFdCTP; dFdCDP; Reverse-phase HPLC; Peripheral blood mononuclear cells; Gemcitabine

1. Introduction

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine; dFdC) is a prodrug with significant activity against solid tumours [1,2]. Structurally, it is a deoxycytidine analog with a modification at the 2′ position of the sugar ring. It is metabolized by cytidine deaminase to 2′,2′-difluoro-2′-deoxyuridine (dFdU), which represents the main catabolic pathway [3]. Gemcitabine penetrates the cells with the concourse of nucleoside transporters [4,5], and thereafter it is phosphorylated to its mononucleotide by deoxycytidine kinase, and subsequently by nucleotide kinases to its active metabolites gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP) [6]. Gemcitabine triphosphate is mainly incorporated into DNA leading to masked chain termination, while gemcitabine diphosphate inhibits ribonucleotide reductase (RR) activity, what leads to a reduction in the concentration of deoxynucleotides required for DNA synthesis and reparation [7].

The cytotoxic activity of dFdC has been correlated both with the intracellular concentration of dFdCTP and with its incorporation into DNA [8] and, for this reason intracellular kinetics of dFdCTP in peripheral blood mononuclear cells (PBMC) has been studied in the framework of clinical trials. Recently, some authors have found a relation between ribonucleotide reductase expression and the efficacy of a combination schedule of cisplatinum and gemcitabine [9]. Since, as aforementioned, dFdCDP is a RR activity inhibitor, simultaneous determination of the intracellular levels of gemcitabine diphosphate and triphosphate in PBMC from gemcitabine treated patients may offer additional information on this drug activity. Some ion-exchange chromatographic methods for dFdCTP determination have been published, but only one of them was fully validated [10]. However, only a few studies have quantified both dFdCTP and dFdCDP by means of anion-exchange HPLC in gradient mode with UV detection [11,12]. None of them provided details of any validation procedure.

Although ion-exchange and ion-pair chromatography are the most important methods for the determination of nucleotides, ion-exchange columns are often poorly reproducible lot to lot and stable since they have to be operated with low pH mobile phases to elute ionic analytes [13]. Ion-pair chromatography on reversed-phase columns offers higher efficiency than fixed-site ion exchanger and – by exploiting additional secondary equilibria for optimizing the separation – also greater versatility [13].

* Corresponding author. Tel.: +34 985 10 61 00x36400.
E-mail address: raquel.losa@hca.es (R. Losa).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.04.036

In this work, the use of an ion-pair reverse-phase HPLC as an alternative method to aforementioned is reported. The separation of these compounds was performed in isocratic mode at room temperature using UV detection.

The developed method was employed to monitor intracellular levels of both dFdCDP and dFdCTP in PBMC from patients treated with gemcitabine at a dose of 1800 mg per square meter of body surface area. The drug was delivered intravenously at a fixed dose rate of 10 mg m^{-2} min^{-1}. This method was also used in two ovarian cancer cell lines (A2780 and SKOV-3) exposed to 25 μM gemcitabine.

2. Experimental conditions

2.1. Chemicals and reagents

dFdCTP was kindly supplied by Eli Lilly (Indianapolis, IN, USA) and dFdCDP was primarily identified as the main impurity of that dFdCTP standard accounting for a 3% on peak area basis. Nucleotide triphosphates, tri-*n*-octylamine and the ionic pair reagent, tetrabutylammonium chloride (TBACl), were obtained from Sigma–Aldrich (St. Louis, MO, USA). Tetrahydrouridine (THU) was purchased from Calbiochem (La Jolla, CA, USA). Inorganic reagents (analytical grade) and methanol (HPLC grade) were obtained from Merck (Hohenbrunn, Germany); 1,1,2-trichlorotrifluorethane Chromasolv for HPLC was obtained from Riedel-deHaën (Seelze, Germany). Phosphate buffer saline (PBS) Ca^{2+} and Mg^{2+} free (1× Dulbecco's A) pH 7.3 was purchased from Oxoid Limited (Hampshire, England). Lymphoprep, a sodium diatrizoate/polysucrose solution for the in vitro isolation of human mononuclear cells was obtained from Axis-Shield (Oslo, Norway).

Purified water (Milli-Q system, Millipore, Milford, MA, USA) was used. Drug free human PBMCs were obtained from healthy volunteers.

2.2. Gemcitabine diphosphate identification

dFdCDP was synthesized from dFdC nucleoside according to the method of Krishna et al. [14] to confirm that it was the main impurity detected in the dFdCTP standard. Reaction products were injected in an HPLC system using SAX and RP-HPLC columns. With both methods, a small peak at the retention time of the putative dFdCDP was noted with an UV spectrum and retention time similar to that of the impurity of the dFdCTP standard.

Further identification of dFdCDP was accomplished when the impurity of a vial of standard dFdCTP was enriched. Direct injection, at a flow rate of 0.2 mL min^{-1}, of the HCl acidified water solution (600 μg mL^{-1}) on a mass spectrometer (Hewlett-Packard MS 1100) operated in the positive electrospray ionization mode, showed the characteristic molecular mass information corresponding to $[dFdCDP-H]^+$ at *m/z* 423.5 and $[dFdCDP-Na]^+$ at *m/z* 446. This result was obtained using nitrogen as nebulizer gas at a flow rate of 10 L min^{-1}, the vaporizer temperature was set at 300 °C and the capillary voltage at 4000 V.

2.3. Chromatographic equipment

Chromatographic analyses were performed on the following configuration: a system controller model SCL-10A^{VP}, two LC-10AD^{VP} pumps, a Sil-10AD^{VP} autosampler and a Photodiode Array detector model SPD-M10 A^{VP} (all from Shimadzu, Kyoto, Japan). Data were recorded on a personal computer equipped with a Class VP Chromatographic Data System version 5.1 (Shimadzu, Kyoto, Japan).

2.4. Chromatographic conditions

The chromatographic separation was accomplished on a Tracer Excel ODSA C_{18} analytical column (100 mm × 4.6 mm, 3 μm particle size) with a Tracer guard column (10 mm × 2 mm) (Teknokroma S. Coop. C. Ltda., Barcelona, Spain). The separation of target compounds was performed in isocratic mode but a clean-up step at the end of each injection was necessary in order to elute the compounds more strongly retained in the stationary phase. Two solutions were used: KH_2PO_4 10 mM/TBACl 10 mM pH 7 with 0.25% methanol (A) and KH_2PO_4 250 mM/TBACl 10 mM pH 7:methanol (85:15, v:v) (B) mixed in a 64:36 (v:v) proportion. After each injection, a step with 100% B over 15 min was performed and the column re-equilibrated during 10 min at initial conditions. Prior to use, all eluents were filtered trough a membrane filter, pore size 0.22 μm and degassed. Elution was performed at room temperature, the flow-rate was 1.2 mL min^{-1}, and the detection wavelength was set at 271 nm. All compounds were identified by their retention times and spectral comparison with pure single standards in the 250–400 nm range.

2.5. Standard solutions

Stock solutions were prepared by dissolving the dFdC-derived standard, in water to concentrations of 0.360 mg mL^{-1}. ATP and ADP aqueous solutions were prepared at 1 mg mL^{-1}. These stock solutions were further diluted to obtain standards of suitable working concentrations: 0.18, 0.50, 1, 2, and 3 μg mL^{-1} for dFdCDP and 0.2, 0.9, 1.8, 2.5, and 3 μg mL^{-1} dFdCTP. Due to the scarcity of dFdCDP standard, and as according to Sparidans et al. [10] the molar extinction coefficients of the diphosphate and triphosphate forms are presumably equal, the dFdCDP concentration values were obtained using the dFdCTP calibration curves. Five different concentrations were prepared for ADP and ATP: 0.3, 2.5, 7, 11, and 15 μg mL^{-1} and 2, 10, 20, 30, and 42 μg mL^{-1}, respectively. Calibration curves of ADP, ATP and dFdCDP were obtained by simultaneous injection of these three nucleotides whereas dFdCTP was injected alone to avoid interfering with dFdCDP results.

2.6. Peripheral blood mononuclear cells isolation and sample preparation

Patient blood samples (10 mL) were collected into heparin-containing tubes to which 50 μL of THU (inhibitor of plasma cytidine deaminase) were added immediately before use. This

solution, prepared by dissolving 10 mg vials in 1 mL of purified water, was stable for at least 4 weeks when stored refrigerated [15]. Blood samples, kept at 4 °C, were diluted (1:1) with PBS and 10 mL of mixture was layered over 5 mL Lymphoprep contained in 15 mL conical tubes. PBMC were isolated according to manufacturer specifications. Briefly, tubes were centrifuged at 600 × *g* and 4 °C for 30 min, and the interface containing PBMC was carefully collected, and washed three times with 10 mL of ice-cold PBS. Cells were resuspended in PBS reaching a final volume of 240 μL and counted in a Neubauer chamber. Samples were stored at −80 °C until analysis.

Before analysis, the samples were treated with 80 μL 40% trichloroacetic acid (TCA). The mixture was vortexed and placed in an ice bath for 20 min allowing protein and nucleic acids precipitation. After centrifugation at 16,000 × *g* during 10 min at 4 °C, the supernatant was collected and 640 μL of freshly prepared trioctylamine:freon (1,1,2-trichlorotrifluorethane) mixture (1:4) were added. After centrifugation (2 min, 16,000 × *g*, 4 °C), the organic layer was carefully removed. This extraction procedure was repeated three times, allowing the concomitant cleaning and neutralisation of the sample. Finally, 200 μL of the aqueous layer was injected in the HPLC system. The concentration data obtained in μg mL^{-1} were recalculated according to the total sample volume, the cell number, and the molecular weights of the analytes studied, so the final nucleotide concentrations were expressed as pmol 10^{-6} cells.

2.7. Validation procedure

The selectivity of this assay was evaluated by analyzing six different samples from ovarian cancer cells and from PBMC before any treatment with dFdC.

The linearity of the method was proven with standards at five known concentrations [16]. The calibration curves were obtained using the unweighted linear least squares regression procedure. The linearity of these curves was checked using two different statistical tests (linearity and proportionality tests).

The precision was determined by the study of the instrumental repeatability, method repeatability and inter-day reproducibility. The instrumental repeatability was calculated as the relative standard deviation (R.S.D.) of the areas obtained from 10 injections of 4 million PBMC in 240 μl PBS spiked with aqueous solutions of standards to reach final concentrations of 0.5 and 1.4 μg mL^{-1} for dFdCDP, and 0.5 and 2 μg mL^{-1} for dFdCTP. The method repeatability was evaluated by analysis of eight PBMC samples from a single healthy donor spiked with 0.5 and 2 μg mL^{-1} dFdCDP and dFdCTP, processed independently. For ADP and ATP, the R.S.D. was calculated from the peak areas of the endogenous nucleotides in both cases. Different analysts studied the inter-day precision of the method in five different days.

Recovery studies were performed in order to assess the accuracy of the method. It was calculated by analysis of blank PBMC spiked with known amounts of dFdCDP (0.5, 1.5, 2.5 μg mL^{-1}), dFdCTP (0.5, 1.5, 2.5 μg mL^{-1}), ADP (2.5 and 5 μg mL^{-1}) and ATP (8 and 16 μg mL^{-1}). Five replicates of each level were injected in the chromatographic system, and recovery values calculated from the difference between the amount experimentally determined in the spiked samples and that initially present in the blank samples, respect to the added concentrations.

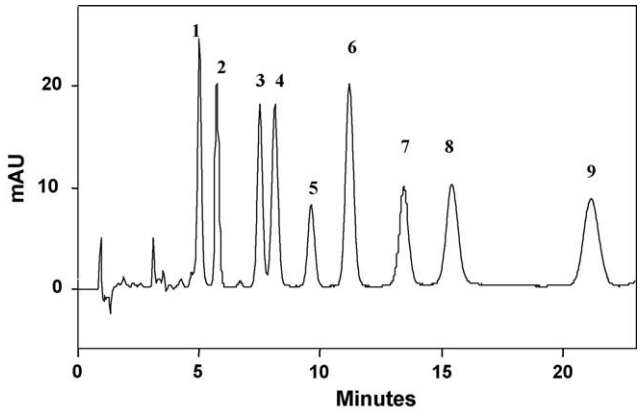

Fig. 1. Chromatogram corresponding to a mixture of nucleotide standards. Concentrations were 2 μg mL^{-1} except in the case of dFdCDP 0.5 μg mL^{-1} and dFdCTP 1 μg mL^{-1}. Chromatographic conditions were: column Tracer Excel C_{18} (100 mm × 4.6 mm, 3 μm particle size); the mobile phase was a mix of A/B (64:36, v:v) being phase A: KH_2PO_4 10 mM/TBACl 10 mM pH 7 with 0.25% methanol and phase B: KH_2PO_4 250 mM/TBACl 10 mM pH 7: Methanol (85:15). Flow rate 1.2 mL min^{-1}. Peaks: 1: CTP; 2: dCTP; 3: UTP; 4: GTP; 5: dFdCDP; 6: ADP; 7: TTP; 8: dFdCTP; 9: ATP.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during usage [16]. In this study, the influence of little changes in: pH values (±0.2), mobile phase counter-ion concentrations (±2 mM), ionic strength (±0.02 M) and methanol percentage (±2%), on retention times and peak area of the analytes was evaluated.

3. Results and discussion

Quantification of nucleotides in cell extracts requires a protein precipitation step that can be achieved with either trichloroacetic or perchloric acids. Our selection of trichloroacetic acid was based on the negative effect of perchloric extraction on the retention behaviour of nucleotides in ion-pair chromatography [18,19]. An important aspect on sample preparation is the following extraction step; freon:trioctylamine (4:1) yields clean extracts and neutralizes the samples. Furthermore, the repetition of this extraction three times renders cleaner extracts that allow extending the HPLC column life-time. Under our conditions 300 injections could be performed in a column without losing resolution. There is a shift on retention times along column ageing, leading to differences of several minutes on retention times obtained with new columns than with heavily used ones. This fact can be clearly seen when comparing Figs. 1 and 2, shorter retention times correspond to aged columns but the analytes resolution is never lost.

In order to maintain the stability of nucleotide triphosphates, rigorous temperature control at 4 °C during sample preparation was required and the intact PBMC were kept at −80 °C, until analysis.

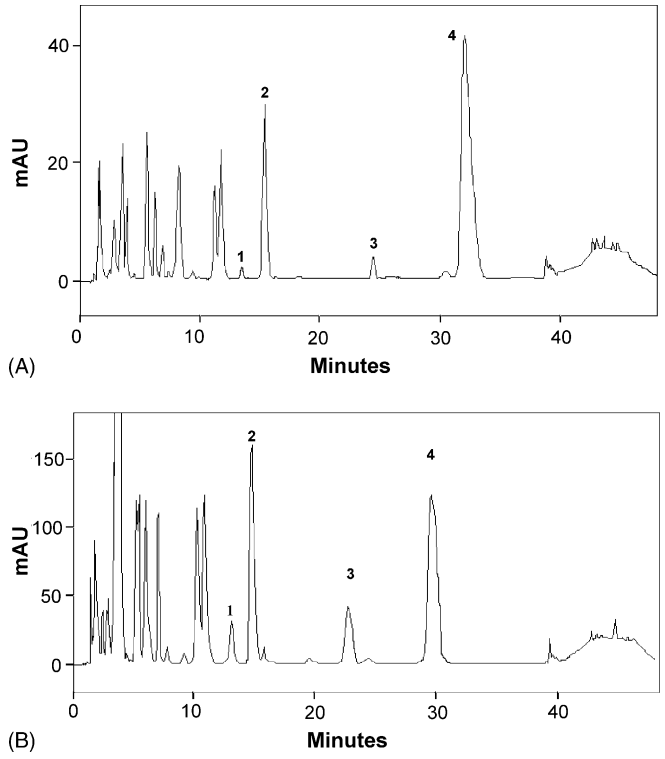

Fig. 2. (A) PBMC sample from a patient treated with 1800 mg m^{-2} gemcitabine intravenously infused at a fixed dose rate of 10 mg m^{-2} min^{-1}. The chromatogram corresponds to a sample taken at the end of gemcitabine infusion, i.e. 3 h from the beginning of treatment. Analyte concentrations in pmol 10^{-6} cells were: 1: dFdCDP 56.85, 2: ADP 1683, 3: dFdCTP 199.18, and 4: ATP 3316. (B) Sample of the A2780 cell line exposed for 24 h to 25 μM gemcitabine. Samples were processed as described in Section 2. Analyte concentrations in pmol 10^{-6} cells were: 1: dFdCDP 961.9, 2: ADP 2656, 3: dFdCTP 1430, and 4: ATP 6230. Chromatographic conditions are those stated in Fig. 1.

To optimize the HPLC assay conditions, the effect of the methanol percentage, ionic strength and pH were studied. The organic percentage in the phase B proved to be a useful tool for the optimization of resolution of the following nucleotide pairs dFdCDP/ADP and dFdCTP/ATP. Different pH values (from 6 to 7.7) did not affect the separation and therefore a value of 7.0 was chosen. In this way, the retention time of the analytes was short enough and the stability of nucleotides was well preserved [17]. The ionic strength had a strong effect on the retention time of the analytes. An increase of phosphate salt concentration in the mobile phase reduces the retention time of all nucleotides.

We have used this method to determine the cellular contents of dFdCDP, dFdCTP, ADP and ATP. These two last nucleotides were included in the validation procedure as controls of sample manipulation and even though a good separation of other endogenous nucleotides was reached avoiding interferences with our targets, they were not validated.

Nine nucleotides: CTP, dCTP, UTP, GTP, dFdCDP, ADP, TTP, dFdCTP and ATP were separated in 25 min (Fig. 1) with resulting retention times of 5, 5.8, 7.5, 8.2, 9.6, 11.2, 13.4, 15.4, and 21.2 min, respectively. Fig. 2 shows two chromatograms corresponding to a PBMC sample from a patient treated with intravenous gemcitabine (a) and to an ovarian cancer cell line (A2780) exposed to 25 μM dFdC over 24 h (b).

Selectivity is the ability of a bioanalytical method to measure unequivocally an analyte and to differentiate it from other components which may be present in the matrix. The analysis of six different samples from ovarian cancer cells and from PBMC blanks did not show peaks that could interfere with the quantification of our target compounds, proving the selectivity of the method. The current use of this HPLC method at our laboratory in the analysis of samples from patients treated with gemcitabine combined with other anticancer drugs (doxorubicin, docetaxel, vinorelbine, dacarbazine and liposomal doxorubicin) did not show any interference with our target metabolites, proving the required method's selectivity.

Linearity, sensitivity and precision of this method were studied, but due to the high inter-patient variability detected in gemcitabine metabolites by other authors, we had to perform a previous screening to know which concentration range should be studied. Patients treated with different gemcitabine doses, from 800 to 1800 mg per square meter of body surface area, were analyzed, and a wider concentration range than found was chosen to asses the linearity of our method. Data obtained from triplicate determinations of five concentrations in the range found in patients: dFdCDP (0.18, 0.5, 1, 2, and 3 μg mL^{-1}), dFdCTP (0.2, 0.9, 1.8, 2.5, and 3 μg mL^{-1}), ADP (0.3, 2.5, 7, 11, and 15 μg mL^{-1} and ATP (2, 10, 20, 30, and 42 μg mL^{-1}) were fitted by linear least-square analysis with respective r values of 0.9993, 0.998, 0.999, and 0.998. For the linearity test we used the regression variance analysis, which yielded an F value lower than the tabulated at the 0.05 significance level. In the proportionality test, the Student t-test values calculated for the intercept were always lower than the tabulated ones for $\alpha = 0.05$, and those obtained for the slope were always higher than those tabulated for the same level of significance. Linearity was thus demonstrated.

The detection limit, defined as the lowest concentration of analyte that can be clearly detected above the baseline signal, was estimated as three times the signal to noise ratio [20], and 10 times this ratio was the quantitation limit. In our case the limits obtained for each analyte are showed in Table 1.

The precision of this method was investigated using human PBMC spiked with dFdCDP and dFdCTP. The instrumental repeatability was calculated as the relative standard deviation of the areas obtained from 10 injections of spiked PMBC (Section 2). The values obtained were lower than 3.3 for dFdCDP and 3.6 for dFdCTP. For endogenous ADP and ATP the R.S.D. values were both inferior to 4%.

Table 1
Values of detection and determination limits obtained

Nucleotide	Detection limit ($k = 3$) (μg mL^{-1})	Determination limit ($k = 10$) (μg mL^{-1})
dFdCDP	0.04	0.13
dFdCTP	0.07	0.23
ADP	0.09	0.30
ATP	0.55	1.83

Calculation outlined in Section 3.

Table 2
Recovery study conducted in PBMC spiked with known dFdCDP, dFdCTP, ADP and ATP amounts performed five times

Analyte	Concentration added ($\mu g\,mL^{-1}$)	Concentration found ($\mu g\,mL^{-1}$)	Recovery (%)	R.S.D. (%)
dFdCDP	0.50	0.50	100.8	7.5
	1.50	1.44	96.2	5.9
	2.50	2.46	98.5	3.4
dFdCTP	0.50	0.52	105.3	3.9
	1.50	1.48	98.5	7.2
	2.50	2.23	89.3	4.1
ADP	2.5	2.58	103.5	3.2
	5	4.99	99.9	4.5
ATP	8	7.79	97.4	3.8
	16	15.76	98.5	6.4

The R.S.D. of the areas obtained to calculate the method repeatability were lower than 9% for dFdCDP and 6% for dFdCTP. For ADP and ATP, the R.S.D. was calculated from the peak areas of the endogenous nucleotides, and it was always inferior to 5%. All these analyses were performed under identical conditions in a single day. Different analysts studied the inter-day reproducibility of the method in five different days, and R.S.D. values obtained were in all cases lower than 10%. All values of precision met the demands for a bionalytical assay: ≤20% R.S.D. for amounts close to the quantification limit, and ≤15% for higher levels [21,22].

Recovery experiments were performed to assess the accuracy of the method as described in Section 2. The average recoveries obtained, ranging from 89 to 105%, proved the accuracy of the proposed method (Table 2). Recovery studies were also performed in the cell lines A2780 and SKOV-3, with two dFdCDP and dFdCTP levels tested, and values are shown in Table 3.

During the robustness studies several parameters were followed. Small changes in all of them, except the ion-pair concentration affected the retention time of the analytes, with variations lower than 7% (R.S.D.). But under all conditions tested the target analytes were perfectly resolved. These changes in the retention time did not affect the analytes peak area.

As an example of the applicability of this method, Fig. 3 shows the concentration of dFdCDP and dFdCTP versus time in PBMC from a patient treated with an intravenous infusion of $10\,mg\,m^{-2}\,min^{-1}$ gemcitabine during 180 min. This method was also applied to measure the levels of those analytes in two ovarian cancer cell lines with a different sensitivity to gemcitabine. The results after a 24 h incubation period with 25 μM gemcitabine are shown in Table 4.

Table 3
Recovery study performed in two ovarian cancer cell lines (SKOV-3 and A2780) spiked with two known amounts of dFdCDP and dFdCTP ($n = 5$)

Cell line	Nucleotide	Concentration added ($\mu g\,mL^{-1}$)	Recovery ± R.S.D. (%)
SKOV-3	dFdCDP	0.60	102.3 ± 6.1
		1.50	108.2 ± 3.3
	dFdCTP	0.6	98.0 ± 6.6
		2.0	105.5 ± 7.3
A2780	dFdCDP	0.60	99.4 ± 5.8
		1.50	103.8 ± 3.1
	dFdCTP	0.6	96.6 ± 4.4
		2.0	103.0 ± 2.7

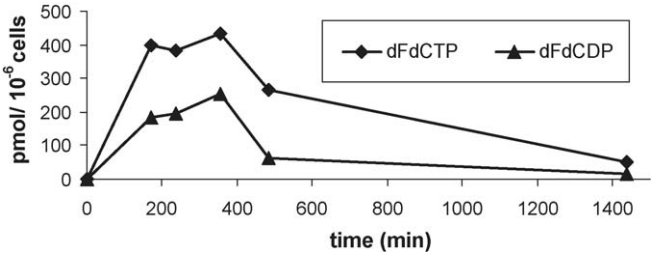

Fig. 3. Concentration vs. time plot of dFdCDP and dFdCTP levels in PBMC from a patient treated with $1800\,mg\,m^{-2}$ gemcitabine intravenously infused at a fixed dose rate of $10\,mg\,m^{-2}\,min^{-1}$.

Table 4
Intracellular levels of nucleotides (pmol 10^{-6} cells and in $\mu g\,mL^{-1}$ 10^{-6} cells) in two ovarian cancer cell lines (SKOV-3 and A2780) after 24 h exposure to 25 μM gemcitabine

Cell line	dFdCDP	dFdCTP	ADP	ATP
SKOV-3	829 ± 40	417 ± 50	1882 ± 151	1799 ± 165
	1.16 ± 0.06	0.71 ± 0.08	2.51 ± 0.2	3.09 ± 0.28
A2780	985 ± 34	1516 ± 123	2669 ± 53	6353 ± 195
	1.38 ± 0.05	2.59 ± 0.21	3.56 ± 0.07	10.93 ± 0.33

4. Conclusion

An ion-pair HPLC method was developed for the simultaneous determination of dFdCDP and dFdCTP, two intracellular metabolites of gemcitabine. The described procedure provides an alternative for anion exchange chromatography, and combines the required sensitivity, accuracy and precision criteria defined for analytical methods. Furthermore, the chromatographic method demonstrated its usefulness for drug monitoring of gemcitabine treated patients and in vitro studies performed with human cancer cell lines.

Acknowledgements

Grant support: Eli Lilly and Company (Spanish Affiliate); Program FEDER-FSE of the European Community; FIS 01/0319 from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (Madrid, España); and Obra Social Cajastur, Asturias, Spain. This study was performed within frame of Program 9 of the Redes Temáticas de Investigación Cooperativa de Centros de Cáncer (C03/10), Instituto de Salud Carlos III, Spain. This work was realized in the Laboratory of Medical Oncology pertaining to the Instituto de Oncología del Principado de Asturias (IUOPA).

References

[1] C.J.A. Van Moorsel, G.J. Peters, H.M. Pinedo, The Oncologist 2 (1997) 127.
[2] A. Thomas, W.P. Steward, Ann. Oncol. 9 (1998) 12653.
[3] J.R. Mackey, R.S. Mani, M. Selner, D. Mowles, J.D. Young, J.A. Belt, C.R. Crawford, C.E. Cass, Cancer Res. 58 (1998) 434.
[4] K.A. Granham, J. Leithoff, I.R. Coe, D. Mowles, J.R. Mackey, J.D. Young, C.E. Cass, Nucleosides Nucleotides Nucleic Acids 19 (2000) 415.
[5] V. Heinemann, L.W. Hertel, G.B. Grindey, W. Plunkett, Cancer Res. 48 (1988) 4024.
[6] W. Plunkett, P. Huang, V. Gandhi, Anticancer Drugs 6 (1995) 713.
[7] V. Heinemann, Y. Xu, S. Chubb, A. Sen, L.W. Hertel, G.B. Grindey, Cancer Res. 52 (1992) 533.
[8] P. Huang, S. Chubb, L.W. Hertel, G.B. Grindey, W. Plunkett, Cancer Res. 51 (1991) 6110.
[9] R. Rosell, K.D. Danenberg, V. Alberola, G. Bepler, J.J. Sanchez, C. Camps, M. Provencio, D. Isla, M. Taron, P. Diaz, A. Artal, Clin. Cancer Res. 15 (2004) 1318.
[10] R.W. Sparindans, M. Crul, J.H.M. Schellens, J.H. Beijnen, J. Chromatogr. B 780 (2002) 423.
[11] W. Plunkett, P. Huang, C.E. Searcy, V. Gandhi, Semin. Oncol. 23 (1996) 3.
[12] V. Heinemann, Y. Xu, S. Chubb, A. Sen, L.W. Hertel, G.B. Grindey, W. Plunkett, Cancer Res. 52 (1992) 533.
[13] M. Cichna, M. Raab, H. Daxecker, A. Griesmacher, M.M. Müller, P. Markl, J. Chromatogr. B 787 (2003) 381.
[14] P. Krishna, Q. Fu, W. Lam, J. Liou, G. Dutschman, Y. Cheng, J. Biol. Chem. 277 (2002) 5453.
[15] K.B. Freeman, S. Anliker, M. Hamilton, D. Osborne, P.H. Dhair, R. Nelson, S.R.B. Allerheiligen, J. Chromatogr. B 665 (1995) 171.
[16] M. Candela, A. Ruiz, F.J. Feo, J. Chromatogr. A 890 (2000) 273.
[17] F. Carlucci, A. Tabucchi, E. Consolmagno, P. Tagliaferri, B. Porcelli, E. Marinillo, R. Leoncini, R. Pagani, Biomed. Pharmacother. 46 (1992) 109.
[18] J.E. van Doorn, P.F.J. Goormachtig, A. de Haan, J. Chromatogr. 496 (1989) 441.
[19] L.A. Decosterd, E. Cottin, X. Chen, F. Lejeune, R.O. Mirimanoff, J. Biollaz, P.A. Coucke, Anal. Biochem. 270 (1999) 59.
[20] M. Laufer, S. Ramalingam, M.P. Schoenberg, M.E. Haisfield-Wolf, E.G. Zuhowski, I.N. Trueheart, M.A. Eisenberger, O. Nativ, M.J. Egorin, J. Clin. Oncol. 21 (2003) 697.
[21] H. Rosing, W.Y. Man, E. Doyle, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 329.
[22] V.P. Shah, K. Midha, S. Dighe, Eur. J. Drug Metab. Pharmacokin. 16 (1991) 249.