Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 809 (2004) 51–58
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simple determination of capecitabine and its metabolites by liquid chromatography with ultraviolet detection in a single injection

L. Zufía*, A. Aldaz, J. Giráldez

Pharmacy Department, University Hospital of Navarra, c/Pío XII s/n, 31008 Pamplona, Spain

Received 26 January 2004; received in revised form 19 May 2004; accepted 7 June 2004

Available online 2 July 2004

Abstract

Capecitabine (N^4-pentoxycarbonyl-5′-deoxy-5-fluorocytidine, Xeloda®), a prodrug of 5-fluorouracil (5-FU), is an oral tumor-selective fluoropyrimidine carbamate approved in the treatment of colorectal and breast cancer. It has a preferential activation to 5-FU by thymidine phosphorilase (TP) in target tumor tissues through a series of three metabolic steps minimizing the exposure of normal tissues to 5-FU. It offers the potential of less gastrointestinal toxicity and advantages in terms of convenience and quality of life for the patient, in addition to cost-effectiveness as compared with intravenous 5-FU chemotherapy. We developed a high performance liquid chromatography assay for the determination of plasma capecitabine and its nucleoside metabolite concentrations and 5-FU catabolite dihydro-5-fluorouracil in a single step extraction and a single HPLC injection. The retention times of dihydro-5-fluorouracil, 5-FU, 5′-deoxy-5-fluorouridine (5′-DFUR) and capecitabine were 3.6, 4.4, 11.4 and 20.4 min, respectively and the internal standard retention times were 8.7 and 12.2 min for 5-bromouracil (5-BU) and tegafur, respectively. The limit of detection was 0.01 μg/ml for capecitabine and its nucleoside metabolites and the limit of quantification was 0.025 μg/ml. Extraction efficiency was >80% with a single solvent mixture extraction step for all analytes of interest. The assay had good precision, the within-day and between-day standard deviation of the mean (R.S.D.) being <10% in the linear range 0.025–10 μg/ml. The authors conclude that the method described here is ideally suited for the therapeutic monitoring of capecitabine and its metabolites.

Keywords: Capecitabine; 5-Fluorouracil; Dihydro-5-fluorouracil

1. Introduction

In the last few years, several new oral fluoropyrimidines have been developed and studied in colorectal cancer and other tumors (tegafur, doxifluridine, UFT, 5-ethynyl-uracil, S-1, BOF-A2 and capecitabine) in response to the need for a more convenient agent with an improved safety profile and equivalent/superior efficacy compared with intravenous 5-FU [1–7]. The oral fluorinated pyrimidines are compounds that may emulate the effects of continuous infusion of 5-FU. Thus, there are some important reasons to investigate oral fluoropyrimidines in the treatment of colorectal and breast cancer [1] to allow more convenient administration, [2] to exploit a tumor-specific prodrug activating enzyme to reduce toxic effects and increase activity, [3] to achieve pharmaco-economic advantages and, possibly, to improve the quality of life of patients.

Capecitabine (N^4-pentoxycarbonyl-5′-deoxy-5-fluorocytidine, Xeloda®), a new fluoropyrimidine carbamate, was developed as an orally-administered prodrug of 5-FU and tumor-selective cytotoxic agent [8]. Capecitabine is converted to 5-FU via a sequential triple enzymatic pathway that exploits the higher concentrations of the enzyme thymidine phosphorylase (TP) present in tumor tissue (Fig. 1). Following rapid and almost complete absorption of the intact molecule from the gastrointestinal tract [9], capecitabine becomes effective after selective conversion of its major metabolites 5′-deoxy-5-fluorocytidine (5′-DFCR) and 5′-deoxy-5-fluorouridine (5′-DFUR or doxifluridine) to 5-FU within human cancer cells. It is converted to 5′-deoxy-5-fluorocytidine (5′-DFCR) by the hepatic enzyme, carboxylesterase. 5′-DFCR is then converted to 5′-DFUR by the enzyme cytidine deaminase, which is present in both the liver and tumor tissue. The third and

* Corresponding author. Tel.: +34 948 296692; fax: +34 948 175278.
E-mail address: lzufia@unav.es (L. Zufía).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.06.004

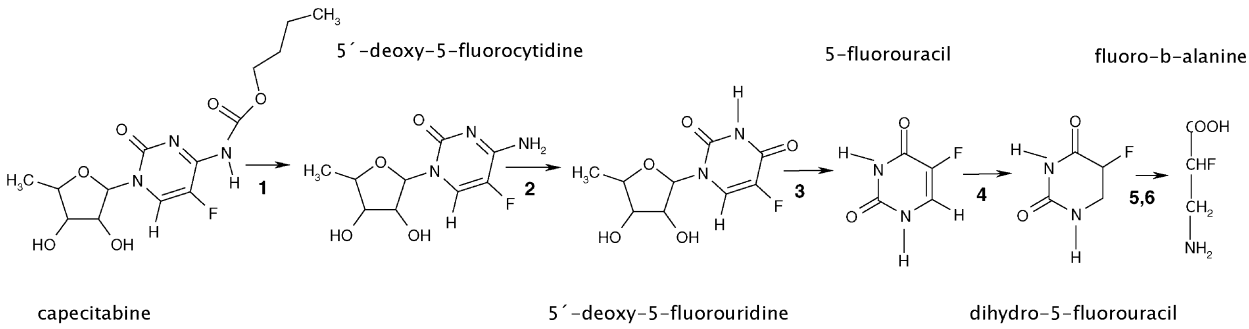

Fig. 1. Metabolism of capecitabine. The enzymes are as follows: (1) carboxylesterase; (2) cytidine deaminase; (3) thymidine phosphorylase; (4) dihydropyrimidine dehydrogenase; (5) dihydropyrimidinase; (6) β-alanine synthase.

final activation step is the conversion of 5′-DFUR to 5-FU by TP, an important enzyme which is up-regulated in solid tumors, is associated with tumor angiogenesis and has shown anti-apoptotic properties [10]. 5-FU is enzymatically cleared from plasma, and the initial, rate-limiting step is catalyzed by dihydropyrimidine dehydrogenase (DPD) to produce dihydro-5-fluorouracil (FUH_2); two subsequent steps result in the formation of fluoroureidopropionic acid (FUPA) and α-fluoro-β-alanine (FBAL), respectively, with release of CO_2 and NH_3 [11]. It has been shown that high expression of TP is associated with resistance to conventional 5-FU treatment in various gastrointestinal tract tumors, particularly colon cancer [12]. Consequently, capecitabine potentially offers a means of overcoming this type of resistance. Furthermore, data from human tumor xenografts have shown that the ratio of TP to DPD, the rate-limiting catabolising enzyme for fluoropyrimidines, can be used to predict response to capecitabine [13].

Capecitabine is rapidly and almost completly absorbed and converted to its metabolites following the oral administration of single doses in cancer patients [9,14–16]. The peak plasma concentration for the drug and its metabolites 5′-DFCR, 5′-DFUR and 5-FU occurs shortly (median t_{max} 2.0 h) after oral administration and its elimination half life was approximately 1 h [17]. The mean elimination half-life of FBAL, the final catabolite of 5-FU, is longer, ranging from 2.6 to 11.5 h.

Pharmacokinetic studies require sensitive, specific and accurate detection methods. The high-performance liquid chromatography (HPLC) with ultraviolet detection determination of capecitabine and its metabolites 5′-DFCR and 5′-DFUR, and the sensitive gas chromatography–mass spectrometry (GC–MS) determination method for 5-FU and its catabolites are the most widely used instrumental techniques [15]. This method used two different liquid chromatography columns and separate conditions for the analysis of capecitabine and 5′-DFCR/5′-DFUR, respectively. Liquid chromatography tandem mass spectrometry (LC–MS–MS) was developed by Roche Laboratories [18] but MS–MS is a relatively expensive technology not often available in a research laboratory setting. An LC–MS method has recently been published that permits the simultaneous measurement of plasma capecitabine, 5′-DFCR, and 5′-DFUR with direct sample injection, but this method involves on-line plasma sample extraction and it is not possible to quantify concurrently the cytotoxic 5-FU [19]. The published HPLC methods for 5-FU determination are relatively sensitive but also time-consuming and sometimes require long and involved extraction procedures [20–29]. A simple and sensitive HPLC method for determination of 5-FU in plasma was developed in our laboratory [30] and another simple and rapid HPLC method (also optimized and validated in our laboratory) for simultaneous quantification of both 5-FU and its fluoropyrimidine prodrug Tegafur in human plasma and tissue has recently been published [31].

This report describes optimization and validation of a simple HPLC assay method with ultraviolet detection which permits the simultaneous quantification of capecitabine and its metabolites 5′-DFUR, 5-FU and the 5-FU catabolite dihydro-5-fluorouracil. Sample handling and chromatographic run times were minimized to provide fast quantitative results while maintaining the sensitivity, specificity, accuracy and precision required for pharmacokinetic evaluation and therapeutic monitoring of capecitabine in the clinical setting. Cancer chemotherapy is often associated with high toxic risks and adaptive dosing and therapeutic drug monitoring based on pharmacokinetic parameters is often an important way to reduce toxicity and even enhance efficacy [32].

2. Experimental

2.1. Reagents

Capecitabine (98% minimum purity) and dihydro-5-fluorouracil (FUH_2) (98%) reference standards were kindly supplied by Nippon Roche Research Center (Kanagawa, Japan). 5′deoxy-5-fluorouridine (5′DFUR) (98%), 5-fluorouracil (5-FU) (99%) and 5-bromouracil (5-BU) (98%) were from Sigma–Aldrich Chemical S.A. (Madrid, Spain) and tegafur reference standard (98% minimum purity) was generously provided by Almirall Prodesfarma (Barcelona, Spain). 99.9% purity HPLC grade solvents (methanol and acetoni-

trile) were obtained from Panreac (Barcelona, Spain), HPLC grade ethyl acetate and analytical-reagent-grade formic acid were purchased from Panreac (Barcelona, Spain).

Stock standard solutions of capecitabine, 5′DFUR and the internal standard Tegafur were prepared by dissolving appropriate amounts of compounds in a known volume of methanol and stored at 4 °C while stock standard solutions of 5-FU, FUH_2 and the internal standard 5-bromouracil (5-BU) were prepared in Milli Q water and stored at 4 °C. Working standard solutions were prepared by appropriate dilutions of the stock standard solutions in methanol or Milli Q water.

2.2. Sample preparation

Blood samples were collected in heparinized tubes and immediately placed on ice and transported to the pharmacokinetic laboratory where they were centrifuged at 5000 × *g* for 5 min at room temperature. The resulting plasma samples were stored at −30 °C until analysis. Each sample was allowed to thaw at room temperature and was then homogenized by vortex-mixing.

2.2.1. Liquid–liquid extraction

A volume of 25 μl of a solution of 5-BU (30 μg/ml) as internal standard to determine 5-FU and FUH_2, 25 μl of a solution of tegafur (10 μg/ml) as internal standard to determine 5′DFUR and capecitabine, and 25 μl of orthophosphoric acid (5%) were added to a glass tube with 500 μl of human plasma. The tube was vortex-mixed for 10 s and 5 ml of acetonitrile/ethyl acetate (1/4, v/v) were added as extraction solvent. The sample was vortexed vigorously for 5 s, shaken for 5 min on a shaker IKA KS 130 basic and then centrifuged at 5000 × *g* for 10 min at room temperature to separate aqueous and organic layers. The organic layers were placed in glass tubes and evaporated to dryness in a vortex evaporator. The residues were reconstituted in 100 μl of mobile phase and after vortex-mixing for 20 s, these samples were centrifuged at 10,000 × *g* for 3 min at room temperature. The clear supernatant was transferred to a microvial and the autosampler programmed to inject 40 μl into the HPLC system.

Spiked plasma samples used as calibration standards were prepared by addition of 25 μl of the corresponding standard solution of capecitabine, 5′DFUR, 5-FU or FUH_2 at each concentration level, 25 μl of orthophosphoric acid (5%) and 25 μl of internal standards 5-BU and Tegafur solution to 475 μl of drug-free human plasma from healthy volunteer donors. Quality control samples were prepared in human plasma at the concentrations of 0.1, 1 and 10 μg/ml, as described above for the calibration standards. Calibration standards and quality control samples were analyzed in the same way as patient plasma samples.

2.3. High-performance liquid chromatography

The chromatographic system used in the study was a Hewlett-Packard 1100 series with a Model G1311A quaternary pump, Model G1313A autosampler, Model G1315A diode array detector, Model G1316A column compartment and Model G1322A degasser. Data were acquired and processed with HP Chem Station chromatography manager software from Hewlett-Packard (Santa Clara, CA, USA).

Table 1
Gradient elution method

Time (min)	A (%)	B (%)	C (%)	Flow (ml/min)
0	10	0	90	1.4
20	10	70	20	1.4
30	10	0	90	1.4

Separation of compounds was achieved using an Atlantis™ dC_{18} (5 μm, 4.6 mm i.d. × 150 mm) analytical column protected by an Atlantis™ dC_{18} precolumn (5 μm, 4.6 × 20 mm). The method used gradient elution with a total run time of 30 min, 1% formic acid was the solvent A in the gradient elution. Methanol and HPLC grade water were used as the solvent B and C, respectively. The last 10 min are given in order to allow the column to reach its initial conditions for the subsequent analysis (Table 1).

The column was maintained at 30 °C and the eluent was monitored at a wavelength of 266 nm for 5′DFUR and 5-FU, 205 nm for FUH_2 and 310 nm for capecitabine.

2.4. Quantification of capecitabine and metabolites in plasma

A standard curve was prepared for each compound by injecting various concentrations of capecitabine, 5′DFUR, 5-FU and FUH_2 solutions in plasma from healthy donors. The peak area ratios of capecitabine, 5′DFUR, 5-FU and FUH_2 to internal standards (5-BU or Tegafur) were used to construct the calibration curves, which were obtained by weighted $1/x$ least-squares linear regression analysis of known drug concentrations versus peak areas.

The concentrations of the plasma and quality control samples were calculated by using the regressed equation of the straight line $y = ax + b$.

The limit of detection in plasma was calculated as three times the standard deviation of the lowest concentration included in the calibration graph (0.01 μg/ml).

The limit of quantification (LOQ) was defined as the lowest concentration of the drug that could be assayed with a good level of precision and accuracy. The limit of quantification was calculated as the minimum concentration at which the analyte can be reliably quantified (0.025 μg/ml) with relative standard deviation of the mean (R.S.D.) and relative mean error (RME) both <20%. The R.S.D. and RME are indicators of precision and accuracy, respectively. The signal-to-noise ratio for these concentrations was approximately 10:1.

2.5. Determination of recovery, precision and accuracy

The absolute recovery of capecitabine, 5′DFUR, 5-FU and FUH_2 from plasma was evaluated in three analytical runs,

by peak areas obtained by direct injection of five replicates of the standard solutions containing capecitabine, 5′DFUR, 5-FU, FUH_2 at three concentration levels and its internal standards in mobile phase to those obtained in plasma samples prepared from blank plasma spiked with the compounds at the same levels and subjected to the complete extraction procedure. By comparing the areas of pure standards with those of extracted plasma samples containing the same amount of standards, we determined the recovery coefficient.

Five replicates with three different concentrations ranging from 0.025 to 10 μg/mL of capecitabine, 5′DFUR, 5-FU and FUH_2 in human plasma were processed as described above to determine the within-day and between-day reproducibility.

The precision of the method at each concentration was calculated as the relative standard deviation of the mean (R.S.D.) using the following equation: R.S.D. = (S.D./mean) × 100.

The accuracy of the procedure was determined as the relative mean error (RME) using the following equation: RME = [(mean-spiked concentration of the analyte)/spiked concentration of the analyte] × 100.

2.6. Application to clinical pharmacokinetic studies

To demonstrate that this HPLC method is applicable to pharmacokinetic studies, it will be utilised to determine capecitabine and metabolite concentrations in plasma from rectal cancer patients receiving capecitabine orally under the specific protocol used and developed in the Oncology Department in the Universitary Hospital of Navarra (Spain).

3. Results and discussion

3.1. HPLC assay development

The absorption spectra of Capecitabine metabolites 5-FU and 5′-DFUR and the internal standard 5-BU exhibited two absorbance maxima at approximately 205 and 266 nm. Tegafur presented maximum absorption at about 270 nm; so the wavelength of 266 nm (the second of the absorbance maxima for capecitabine metabolites), was selected for the simultaneous determination of 5-FU, 5′-DFUR and the internal standards 5-BU and tegafur. This wavelength does not permit us to visualize the dihydrogenated catabolite of 5-FU, FUH_2, which have an absorbance maximum at 205 nm, so we selected this wavelength for determination of this analyte. The wavelength 266 nm permits us to visualize capecitabine which presented maximum UV absorption at 310 nm, the wavelength selected to accomplish capecitabine quantification.

Among the analytes of interest, except for capecitabine, the other compounds are quite hydrophilic in nature. Since these compounds elute rapidly from reversed-phase columns with mobile phases containing a low percentage of organic content, they present a challenge to chromatographic method development. With commonly used reversed-phase columns, it is not easy to separate 5-FU from structurally related compounds such as other pyrimidines. Furthermore, more published methods use two different liquid chromatography columns and separate conditions for the analysis of capecitabine and 5′-DFCR/5′-DFUR, and 5-FU (15).

For the present study, we had tested several reversed-phase columns for the separation and simultaneous determination of all the analytes. The best results were obtained with the Atlantis™ dC_{18} (5 μm, 4.6 mm i.d. × 150 mm) analytical column which provide enhanced retention of polar compounds as compared to an embedded polar group containing stationary phase and excellent peak shape. In addition, the difunctional bonding chemistry of the Atlantis™ packing material provides excellent stability under acidic mobile phase condition and extended column lifetime at low pH.

Rather than 5-BU, (the internal standard for 5-FU and its catabolite FUH_2), we choosed tegafur as the internal standard for capecitabine and its metabolite 5′DFUR because it is chemically more similar to them and more suitable for chromatography. The tegafur peak appears in an area of the chromatogram which is almost free from interfering peaks and nearer to 5′DFUR and capecitabine than 5-BU.

Different liquid–liquid extraction procedures for capecitabine and metabolites and the internal standards were tested in our laboratory. Some of the compounds such as capecitabine, 5-FU or tegafur differ considerably in their lipophilic properties and so finding a suitable solvent was the greatest challenge in developing a single-step extraction method with good recovery for all of then. Several solvents, solutions and volumes were assayed. We tried solvents and mixtures such us the recently published for tegafur and 5-FU [31]. We found that the mixture of ethyl acetate, isopropanol and dichloromethane (53:10:37) gave recoveries for both 5-FU and tegafur of greater than 80% but recoveries were not so good for capecitabine or 5′DFUR. Some solvents showed interferences of endogenous compounds with some of the compounds of interest. Others had an insufficient extraction yield to allow proper pharmacokinetic application.

Sample acidification is necessary to ensure that weak organic acids such as our analytes exist in the un-ionised form thereby guaranteeing extraction with an organic solvent [30]. Of the different solvents examined, the mixture acetonitrile/ethyl acetate, condensed in just one phase two common operations in sample treatment: protein precipitation and solvent extraction with the best recovery for all the compounds. So, acetonitrile/ethyl acetate solvent extraction after a sample acidification was finally the analytical procedure selected for simultaneous plasma extraction of capecitabine and metabolites. This analytical method was put into practice in a similar way to that used in previously published procedures.

3.2. Assay validation

The analytical methodology was validated in terms of selectivity, recovery, linearity, limit of quantification, precision and accuracy.

3.2.1. Selectivity

A useful analytical method should permit resolution and detection of the analytes of interest and the internal standard from other interfering metabolites and co-eluting endogenous compounds.

Possible interferences from endogenous constituents of human plasma were evaluated by analyzing plasma samples obtained from different donors. No interfering peaks were observed and no significant peaks were found at the retention times of analytes or the internal standards. Fig. 2 shows the chromatographic separation of human plasma extracts without capecitabine (A), of a plasma extract spiked at the quantification limit (0.025 μg/ml) (B) and from a human plasma extract after the oral administration of 825 mg/m^2 of capecitabine (C). The approximate retention times for dihydro-5-fluorouracil, 5-FU, 5′-deoxy-5-fluorouridine (5′-DFUR) and capecitabine were 3.7, 4.4, 11.4 and 20.4 min, respectively, and the internal standard retention times were 8.7 and 12.1 min for 5-bromouracil (5-BU) and tegafur respectively, and all eluted as sharp symmetrical peaks. A gradient elution method that uses 1% formic acid and methanol as solvents has been optimized resulting in good liquid chromatographic separation of all compounds of interest and in order to obtain the best peak shape and separation factor for the analytes in an overall chromatogram run time of 30 min.

3.2.2. Recovery

Recovery was determined according to the ratio of the areas of extracted samples to those in corresponding standard solutions. The overall mean recoveries of capecitabine, 5′DFUR, 5-FU and FUH_2 were (mean ± S.D.) 90.34 ± 9.48, 90.47 ± 8.95, 95.24 ± 3.96 and 91.49 ± 3.31 (mean ± S.D.), respectively. As shown in Table 2, recoveries for all compounds were not only high, but also similar for every concentration studied. The recovery of the internal standards 5-BU and tegafur, respectively, measured at their defined concentrations (1.5 μg/ml) and (0.5 μg/ml) were (mean ± S.D.) 81.25 ± 5.93% ($n = 10$) and 93.4 ± 8.39% ($n = 10$).

Table 2
Recovery of capecitabine, 5′DFUR, 5-FU and FUH_2 from human plasma

Compounds	Recovery (%)		
	10 μg/ml	1 μg/ml	0.1 μg/ml
Capecitabine	101.12 ± 4.08	86.6 ± 7.83	83.3 ± 5.41
5′DFUR	100.55 ± 6.45	83.47 ± 5.88	87.4 ± 5.88
5-FU	97.97 ± 5.89	97.05 ± 5.65	90.7 ± 7.33
FUH2	88.28 ± 6.5	94.9 ± 6.7	91.3 ± 8.63

3.3. Linearity and the lower limit of quantification

The range of reliable response was established on the basis of five triplicate standards in plasma covering the concentration range of 0.025–10 μg/ml for all studied compounds. The calibration lines

$$y = (0.69034 \pm 0.0084)x + (0.0008 \pm 0.00012) \text{ for capecitabine}$$

$$y = (0.7504 \pm 0.081)x + (-0.001 \pm 0.00067) \text{ for 5′DFUR}$$

$$y = (2.2654 \pm 0.047)x + (-0.000583 \pm 0.00097) \text{ for 5-FU}$$

$$y = (1.7485 \pm 0.041)x + (-0.00235 \pm 0.0034) \text{ for } FUH_2$$

represent the mean of the three graphs corresponding to the triplicate standards. The correlation coefficient (r) for each calibration graph was greater than 0.999 and the R.S.D.s of the response factors (RFs) for each concentration assayed were below 10% (≤20% for the LLQ). In both equations, y is the area ratio of the peak area of capecitabine or 5′DFUR to that of tegafur (I.S), or the peak area of 5-FU or FUH_2 to that of 5-BU (I.S), and x is the unknown concentration of capecitabine, 5′DFUR, 5-FU or FUH_2.

The limit of detection was 0.010 μg/mL since the peak area for this concentration was distinguishable from the responses given by the capecitabine, 5′DFUR, 5-FU and FUH_2 free control plasma.

The lower limit of quantification was 0.025 μg/mL, the signal-to-noise for this concentration was approximately 10:1 and analyte response with this concentration gave an R.S.D. ≤ 20% and an accuracy of 80–120.

3.3.1. Precision and accuracy

Table 3 shows the within-day and between-day precision (R.S.D.) and accuracy (RME) for this assay. As can be seen in these tables, precision and accuracy of this method are acceptable and hardly depend on the concentration assayed on the day of the assay.

3.4. Application of the analytical method in clinical pharmacokinetic studies

We developed our method to measure capecitabine and metabolites in plasma concentrations of patients receiving this drug orally under the specific protocol used and developed in the University Hospital of Navarra (Spain). This assay was useful to determine plasma concentrations of capecitabine and metabolites in a patient after the administration of a standard treatment as a previous phase of the clinical trial. We have confirmed the applicability of our method which provides fast quantitative results with specificity, accuracy, and precision. An accurate and precise quantification

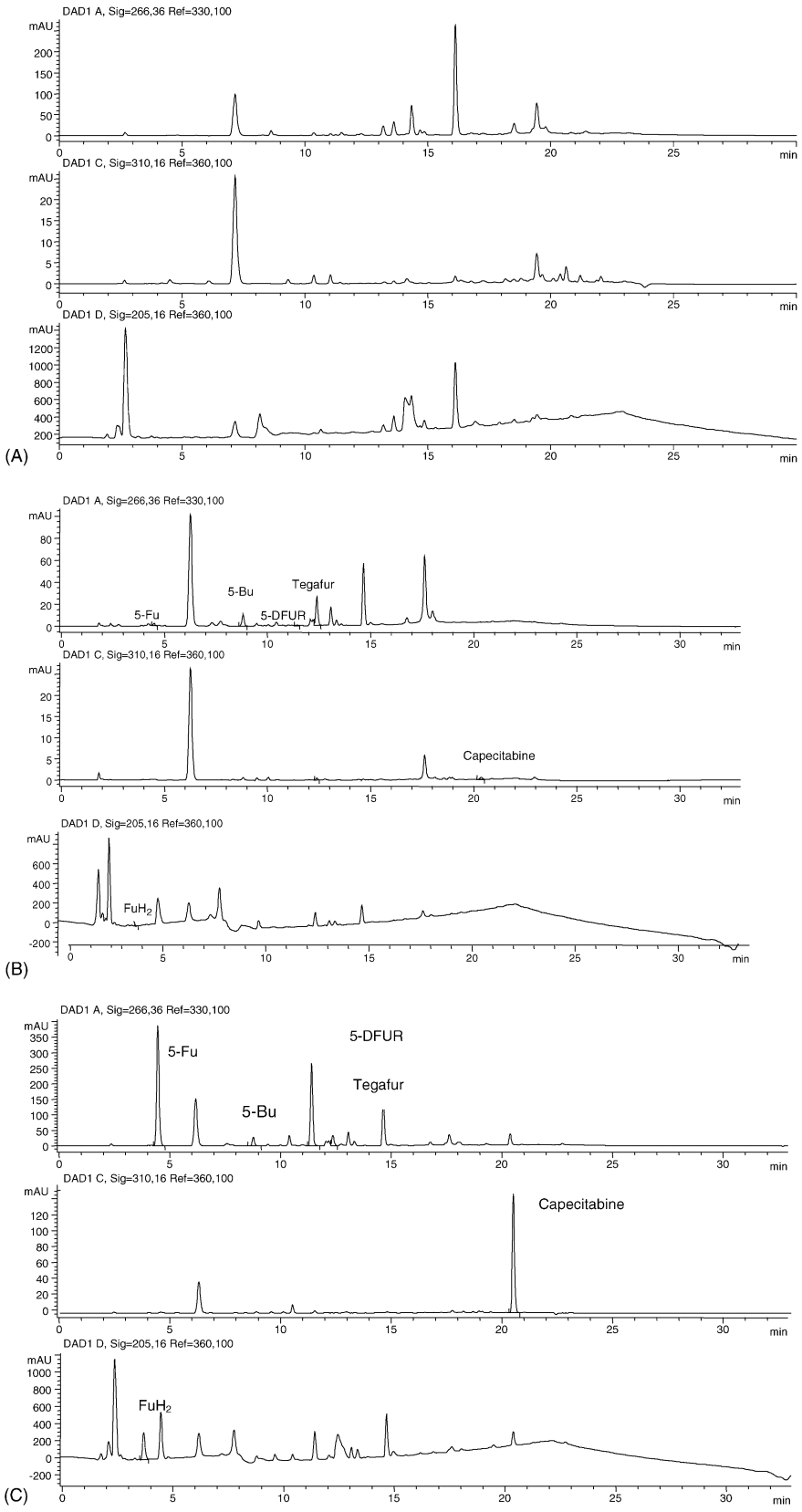

Fig. 2. Chomatographic separation of a human plasma extract without capecitabine (A), from a human plasma extract at the LLQ (B) and from a human plasma extract 2 h after the oral administration of 825 mg/m^2 of capecitabine (C). The experimental conditions are described in Section 2.3. Concentrations of capecitabine, 5′-DFUR, 5-FU and FUH_2 were 7.07, 6.23, 5.97 and 5.58 μg/ml, respectively, while the internal standars Tegafur and 5-BU concentrations were 0.5 and 1.5 μg/ml, respectively.

Table 3
Within-day and between-day accuracy and precision of the HPLC determination of capecitabine, 5′DFUR, 5-FU and FUH_2 in human plasma

Compounds	10 μg/ml		1 μg/ml		0.1 μg/ml		LLQ (0.025 μg/ml)	
	R.S.D. (%)	RME (%)	R.S.D. (%)	R.S.D. (%)	RME (%)	RME (%)	R.S.D. (%)	RME (%)
Within-day								
Capecitabine	2.53	2.4	8.5	5.8	2.9	−7.3	7.1	5.5
5′DFUR	5.3	7.2	7.1	4.4	−5.8	−2.5	5.9	1.3
5-FU	4.7	−7.2	6.6	2.3	6.7	4.4	4.7	−7.2
FUH_2	2.5	10.4	6.8	8.6	3.6	1.8	13	10.4
Between-day								
Capecitabine	4.4	5.6	3.4	3.8	6.4	5.35	7.5	−8.3
5′DFUR	7.2	7.4	5.8	6.2	8.1	4.2	7.1	−5.6
5-FU	2.6	3.9	6.7	6.3	8.4	8.8	6.8	13
FUH_2	3.5	7.7	9.8	5.5	−6.2	11	11	6.4

of drugs and a fully validated analytical method to quantify the drugs is critical in therapeutic drug monitoring. Pharmacokinetic parameters, pharmacokinetic variability, dose adjustment and all potential variables required for a successful implementation of therapeutic drug monitoring represent a challenge for further studies.

4. Conclusions

We report an assay for the simultaneous determination of capecitabine and metabolites and the 5-FU catabolite FUH_2 in a one-step extraction and a single HPLC injection. The assay is accurate, sensitive, and precise.

Good recovery is achieved in a single solvent mixture extraction step and HPLC quantification requires only a single injection. Because determination of all compounds is simultaneous, the assay is quick and simple and has a sufficiently rapid turnaround time to enable the laboratory to routinely provide useful pharmacokinetic data in time to adjust patient regimens. Apart from therapeutic drug monitoring, the assay should facilitate pharmacokinetic research in clinical laboratory settings. Clinical pharmacokinetics of capecitabine will be studied in detail in further investigations.

Acknowledgements

The authors wish to thank the partial financial support of RTIC Cancer 10/03 FIS for promoting research in our hospital and making this study possible.

References

[1] A.A. Adjei, Br. J. Clin. Pharmacol. 48 (1999) 277.
[2] C.A. Bunnell, E.P. Winer, Oncology 10 (Suppl. 7) (1998) 43.
[3] R. Diasio, Drugs 58 (Suppl. 3) (1999) 126.
[4] M. Hoff, M. Royce, D. Medgyesy, D. Brito, R. Pazdur, Semin. Oncol. 26 (1999) 646.
[5] J.S. MacDonald, Am. J. Clin. Oncol. 22 (1999) 480.
[6] N.J. Meropol, Eur. J. Cancer 34 (1998) 1513.
[7] Y.M. Rustum, S. Cao, Semin. Oncol. 26 (1999) 620.
[8] C.E. Myers, R. Diasio, H.M. Eliot, B.A. Chabner, Cancer Treat. Rev. 3 (1975) 183.
[9] I.R. Judson, P.J. Beale, J.M. Trigo, T. Crompton, D. Jones, E. Bush, B. Reigner, W. Aheme, Invest. New Drugs 17 (1999) 56.
[10] M. Caudry, C. Bonnel, A. Floquet, C. Marsault, P. Quetin, J. Pujol, O. Maton, J.P. Dujols, Y. Caudry, P. Skawinski, Am. J. Clin. Oncol. 18 (1995) 125.
[11] J.L. Grem, Invest. New Drugs 18 (2000) 313.
[12] C.G. Leichman, T.R. Fleming, F.M. Muggia, C.M. Tangen, B. Ardalan, J.H. Doroshow, F.J. Meyers, R.F. Holcombe, G.R. Weiss, A. Mangalik, J. Clin. Oncol. 13 (1995) 1311.
[13] P. Rougier, B. Paillot, A. LaPlanche, F. Morvan, J.F. Seitz, C. Rekacewicz, P. Laplace, J. jacob, S. Grandjouan, J.M. Tigand, M.C. Fabri, M. Luboinski, M. Ducreux, Eur. J. Cancer 33 (1997) 1793.
[14] J. Cassidy, C. Twelves, D. Cameron, W. Steward, K. O'Byme, D. Jodrell, L. Banken, T. Goggin, D. Jones, B. Roos, E. Bush, E. Weidekamm, B. Reigner, Cancer Chemother. Pharmacol. 44 (1999) 460.
[15] B. Reigner, J. Verweij, L. Dirix, J. Cassidy, C. Twelves, D. Allman, E. Weidekamm, B. Roos, L. Banken, M. Utoh, B. Osterwalder, Clin. Cancer Res. 4 (1998) 948.
[16] C. Twelves, R. Glynne-Jones, J. Cassidy, J. Schuller, T. Goggin, B. Roos, L. Banken, M. Utoh, E. Weidekamm, B. Reigner, Clin. Cancer Res. 5 (1999) 1702.
[17] B. Reigner, K. Blesch, E. Weidekamm, Clin. Pharmacokinet. 40 (2001) 104.
[18] B. Reigner, S. Clive, J. Cassidy, D. Jodrell, R. Schulz, T. Goggin, L. Banken, B. Roos, M. Utoh, T. Mulligan, E. Weidekamm, Cancer Chemother. Pharmacol. 43 (1999) 315.
[19] Y. Xu, J.L. Grem, J. Chromatogr. B 783 (2003) 285.
[20] P. Compagnon, L. Thiberville, N. Moore, C. Thuillez, C. Lacroix, J. Chromatogr. B 677 (1996) 383.
[21] E. Gamelin, M. Boisdron-Celle, A. Turcant, F. Larra, P. Allain, J. Robert, J. Chromatogr. B 695 (1997) 416.
[22] W.E. Wung, S.B. Howell, Clin. Chem. 26 (1980) 1708.
[23] A.R. Buckpitt, M.R. Boyd, Anal. Biochem. 106 (1980) 437.
[24] F.P. LaCreta, W.M. Williams, J. Chromatogr. 414 (1987) 201.
[25] W. Wattanatorn, H.L. McLeod, J. Cassidy, K.E. Kendle, J. Chromatogr. B 692 (1997) 237.
[26] M.J. Del Nozal, J.L. Bernal, A. Pampliega, P. Marinero, M. Pozuelo, J. Chromatogr. B 656 (1994) 405.

[27] M.J. Moore, P. Bunting, S. Yuan, J.J. Thiessen, Ther. Drug Monit. 15 (1993) 399.
[28] N. Christophidis, G. Mihaly, F. Vajda, W. Louis, Clin. Chem. 25 (1979) 86.
[29] L.J. Schaaf, D.G. Ferry, C.T. Hung, D.G. Perrier, I.R. Edwards, J. Chromatogr. 342 (1985) 313.
[30] J. Escoriaza, A. Aldaz, E. Calvo, J. Giráldez, J. Chromatogr. B 736 (1999) 102.
[31] L. Zufía, A. Aldaz, C. Castellanos, J. Giráldez, Ther. Drug Monit. 25 (2003) 228.
[32] K. Kobayashi, D.I. Jodrell, M.J. Ratain, Cancer Surv. 17 (1993) 579.