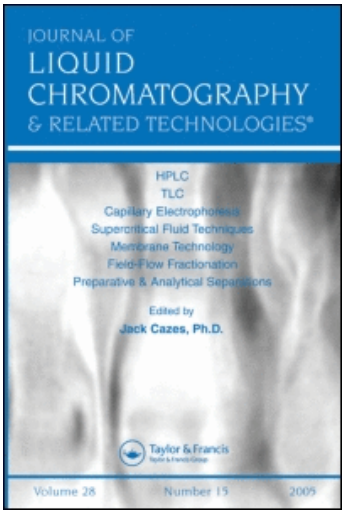

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

PERFORMANCE OF THE ANALYTICAL ASSAYS OF PACLITAXEL, DOCETAXEL, AND CYCLOSPORIN A IN A ROUTINE HOSPITAL LABORATORY SETTING

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Online publication date: 31 October 2001

To cite this Article Malingré, M. M. , Rosing, H. , Koopman, F. J. , Schellens, J. H. M. and Beijnen, J. H.(2001) 'PERFORMANCE OF THE ANALYTICAL ASSAYS OF PACLITAXEL, DOCETAXEL, AND CYCLOSPORIN A IN A ROUTINE HOSPITAL LABORATORY SETTING', Journal of Liquid Chromatography & Related Technologies, 24: 17, 2697 — 2717
To link to this Article: DOI: 10.1081/JLC-100106096
URL: http://dx.doi.org/10.1081/JLC-100106096

J. LIQ. CHROM. & REL. TECHNOL., 24(17), 2697–2717 (2001)

PERFORMANCE OF THE ANALYTICAL ASSAYS OF PACLITAXEL, DOCETAXEL, AND CYCLOSPORIN A IN A ROUTINE HOSPITAL LABORATORY SETTING

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ABSTRACT

The taxanes paclitaxel and docetaxel are important anticancer agents. To optimize therapy of these drugs, many studies have been performed by us with pharmacokinetic monitoring of the compounds. The numerous determinations of paclitaxel and docetaxel in our laboratory enabled us to monitor performance of the bioanalytical assays over a prolonged period of time. In addition, we analyzed the performance of the bioanalytical assay of cyclo-

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sporin A, a compound co-administered to enhance absorption of orally administered paclitaxel and docetaxel. Here, we report our experience with these assays over the past four years.

Paclitaxel and docetaxel were analyzed by validated high-performance liquid chromatography (HPLC) assays developed at our Institute. Cyclosporin A was analyzed with use of a specific fluorescence polarization immunoassay (s-FPIA) developed and validated by Abbott Laboratories. For acceptance of an analytical run, we used the criteria for calibration and quality control samples issued by the conference on Analytical Methods Validation (1990). Quality control samples have been used to monitor performance of the assays.

In the past four years, all three analytical assays showed excellent performance. In this period, we performed 84 analytical runs of paclitaxel, 19 runs of docetaxel, and 131 runs of cyclosporin A. Accuracies of the paclitaxel, docetaxel, and cyclosporin A assays were 92-102%, 103-112%, and 103-105%, respectively. Precisions of the paclitaxel and docetaxel assays were less than 10% for all concentrations. For the cyclosporin A assay, the coefficients of variation were always less than 12%. It can be concluded that the validated analytical assays of paclitaxel, docetaxel, and cyclosporin A showed very good performance in a routine hospital laboratory setting for a prolonged period of time.

INTRODUCTION

Over the last 10 years the taxanes paclitaxel and docetaxel have obtained a prominent place in anticancer chemotherapy and are widely used in the treatment of breast, ovarian, and lung cancer.(1,2) The drugs are routinely administered intravenously, either as single agent or in combination therapy, at different dosages and time schedules. To optimize therapy of paclitaxel and docetaxel, we have performed many studies, which were pharmacokinetically supported by bioanalysis of the compounds.(3-13) At our Institute high-performance liquid chromatographic (HPLC) assays, with solid-phase extraction (SPE) as sample pretreatment, were developed and validated for determination of paclitaxel(3,14,15) and docetaxel(16) and their major metabolites.

Further optimization of cancer treatment by paclitaxel and docetaxel is continued. We are currently exploring the oral route of administration of paclitaxel, and docetaxel and have recently demonstrated profound enhancement of systemic exposure of the drugs by co-administration of the P-glycoprotein inhibitor cyclosporin A.(17,19) Based on the first promising results, we have

continued with further development of oral treatment of paclitaxel and docetaxel.(20-22)

In order to pharmacokinetically support the clinical studies of paclitaxel and docetaxel we performed numerous determinations of these analytes in the last couple of years. In addition, many bioanalytical measurements of cyclosporin A were performed. The latter has been analyzed with use of a specific fluorescence polarization immuno-assay (s-FPIA) developed and validated by Abbott Laboratories.(23-26) This assay is generally used in therapeutic drug monitoring of cyclosporin A in transplant patients.(24-26) Robustness of an analytical method is a critical evaluation parameter,(27,28) which can be obtained by long-term experience. Here, we present an overview of the performance of the bioanalytical assays of paclitaxel, docetaxel, and cyclosporin A in a routine hospital laboratory setting over the past four years.

EXPERIMENTAL

Paclitaxel Analysis

Development and validation of the HPLC bioanalytical assay of paclitaxel has been described previously.(3,14,15) The sample pretreatment involves a solid phase extraction (SPE) using 0.5 mL plasma, buffered with 0.5 mL of 0.2 M ammonium acetate pH 5.0, onto 1-mL Cyano Bond Elut columns. 2'-Methylpaclitaxel is used as internal standard. The eluent is evaporated under nitrogen and low heat, and reconstituted with the mobile phase, acetonitrile-methanol-water (AMW) (4:1:5, v/v/v) containing 0.01 M ammonium acetate pH 5.0. The samples are chromatographed on a reversed-phase octyl column. Detection of the analytes is performed by UV absorbance measurement at 227 nm.

Each paclitaxel run involved analysis of calibration samples, quality control samples, and study samples, which were processed as a batch. Calibration curves consisted of at least five concentrations measured in duplicate at concentrations between 10-10,000 ng/mL. Quality control samples were measured in duplicate at three concentrations in the low, medium, and high calibration range. Paclitaxel stock solutions were made by dissolving 10 mg of paclitaxel reference material in 2.0 mL methanol. Paclitaxel calibration and quality controls samples were prepared by making the appropriate dilutions in blank, human plasma. The internal standard stock solution consisted of 1 mg/mL 2'-methylpaclitaxel in methanol. The working solution of the internal standard was 10 μg/mL.

Stock solutions, calibration, and quality control samples were stored at -20°C. Every six months fresh stock solutions were made. Study samples were obtained in EDTA or heparinized tubes and immediately centrifuged. Plasma was separated and directly stored at -20°C until analysis. If the available study

sample volume was less than the validated sample volume, the study sample was supplemented with blank human plasma to the validated volume. When the concentration of a study sample was above the highest calibration standard, the sample was diluted with blank human plasma and re-analyzed.

For acceptance of a paclitaxel analytical run, the guidelines issued by a joint conference of the FDA, AAPS, AOAC, HPB, and FIP on Analytical Methods Validation (1990) were used.(27,28) For the calibration samples, the mean percentage deviation of the nominal value and the relative standard deviation of the responses must be less than 15%. For the lower limit of quantitation (LLQ) of the assay, a deviation of 20% is acceptable for both parameters. When calibration samples fall out of these ranges they are excluded from the calibration curve. A minimum of five calibration concentrations should meet the above criteria to accept the run. The correlation coefficient of the calibration curve must be higher than 0.995. For the quality control samples, at least four of the six samples must be within the 20% of their respective nominal values; two of the six samples (not both at the same concentration) may be outside the 20% respective nominal value. The relative standard deviation of the responses must be less than 15%.

Performance of the paclitaxel analytical assay in time has been monitored by use of the quality control samples. The accuracies of the assay were calculated for each quality control concentration by dividing the mean measured concentration by the nominal concentration and multiplication by 100. The assay precisions were obtained by one-way analysis of variance (ANOVA) for each quality control concentration, using the run day as the classification variable. The following formula was used to calculate the precisions:

$$\text{Between - run precision} = \frac{\sqrt{\left(MS_{BG} - MS_{WG}\right)/n}}{GM} \times 100\%$$

where, MS_{BG} is the mean square of the between runs, MS_{WG} the mean square of the within runs, GM the grand mean of the measured quality control concentration, and n the number of determinations per run.

Docetaxel Analysis

Development and validation of the docetaxel HPLC bioanalytical assay has been described previously.(16) A volume of 1.0 mL of plasma is extracted with Cyano end-capped solid phase columns using an ASPEC XL system. 2'-Methylpaclitaxel is used as internal standard. The eluent is evaporated under nitrogen and low heat, and reconstituted in acetonitrile-methanol-water (AMW)

(4:1:5, v/v/v). The samples are chromatographed on an APEX-octyl column with acetonitril-0.02 M ammonium acetate buffer pH 5.0 mixture (36.8:63.2, w/w) as the mobile phase. UV detection is performed at 227 nm.

Each docetaxel run involved analysis of calibration samples, quality control samples, and study samples, which were processed as a batch. Calibration curves consisted of at least five concentration levels measured in duplicate at concentrations of 10-10,000 ng/mL. Quality control samples were measured in duplicate at three concentrations in the low, medium, and high calibration range. Docetaxel stock solutions were prepared by dissolving 1 mg of reference material in 2.0 mL methanol. Docetaxel calibration and quality controls samples were prepared by making the appropriate dilutions in blank, human plasma. The 2'-methylpaclitaxel stock and working solutions were made as described for paclitaxel. Stock solutions, calibration and quality control samples were stored at –20°C. Every six months fresh stock solutions were prepared. Study samples were obtained in EDTA or heparinized tubes and immediately centrifuged. Plasma was separated and stored at -20°C until analysis. Study samples were supplemented with human blank plasma if the sample volume available was less than the validated sample volume. When exceeding the calibration range, samples were diluted with blank human plasma and re-analyzed.

Acceptance of a docetaxel analytical run was based on the same guidelines as used in paclitaxel analysis.(27,28) Performance of the docetaxel analytical assay in time has been evaluated by use of the quality control concentrations as described above for paclitaxel.

Cyclosporin A Analysis

Development and validation of the specific FPIA bioanalytical assay of cyclosporin A (Abbott Laboratories, Amstelveen, The Netherlands) has been described elsewhere.(23-26) The analysis requires 150 µL whole blood to which solubilization reagent (aqueous surfactant with 0.1% sodium azide) and precipitation reagent (zinc sulfate in methanol and ethylene glycol) are added. The samples are mixed, centrifuged, and the supernatant is then analyzed in the TDxFLx analyzer (Abbott Laboratories) and, thereafter, automatically quantified. The reagents provided in the analyzer kit included cyclosporin A antibody (<25% mouse monoclonal in a buffer containing stabilizer with sodium azide) and a <0.01% cyclosporin A monoclonal whole blood fluorescein tracer solution in buffer containing surfactant and a protein stabilizer with sodium azide.

Each analytical run (with a maximum of 20 samples) involved analysis of study samples and cyclosporin A quality control samples at concentrations of 150 ng/mL (Low), 400 ng/mL (Medium), and/or 800 ng/mL (High), which were processed as a batch. Prior to start of cyclosporin A analysis, a calibration curve

was run (0-1500 ng/mL, six concentrations) and stored as long as the quality control samples were within the accepted ranges of their nominal values. Accepted ranges for the quality controls are ± 20% (Low) and ± 15% (Medium and High), which were used for acceptance of the cyclosporin A run.(23) Study samples were obtained in EDTA or heparinized tubes and stored at 4°C until analysis. When study samples were above the highest calibration standard (print output HI), the supernatant was diluted with dilution buffer (phosphate buffer with sodium azide) and re-analyzed.

Performance of the cyclosporin A assay has been monitored by use of the quality control samples. As performed for paclitaxel and docetaxel analysis, accuracies of the assay have been determined for each quality control concentration by dividing the mean measured concentration by the nominal concentration and multiplication by 100. Precisions could not be calculated using one-way analysis of variance (ANOVA) because of the single measurements of the quality control concentrations. We calculated the coefficients of variation in the quality control concentrations by dividing the standard deviations by the mean measured concentrations, multiplied by 100.

RESULTS AND DISCUSSION

Development of the Paclitaxel Analytical Assay

In 1992 The Netherlands Cancer Institute participated in a large randomized multicentre European-Canadian trial, which investigated the safety and antitumor efficacy of paclitaxel in 'high'(175 mg/m^2) versus 'low'(135 mg/m^2) dose and long (24 hours) versus short (3 hours) infusion in platinum pretreated ovarian cancer patients.(29) This was a unique opportunity to investigate the pharmacokinetic behavior of paclitaxel in the two different dose levels and two different infusion schedules. At that time, several HPLC methods, including various sample pretreatment procedures, had been reported for the analysis of paclitaxel in human plasma. However, these methods were relatively insensitive with lower limit of quantitations of 50-100 nM.(30-33) Furthermore, these assays could not detect paclitaxel metabolites. We developed and validated a more sensitive HPLC method for quantification of paclitaxel in human plasma with a SPE procedure as sample pretreatment.(3,14)

The lower limit of quantitation of this assay was 12 nM. During the implementation of this assay, however, recovery problems of paclitaxel arose. The major problems were

1) a large batch-to-batch difference in performance of the SPE columns,

2) loss of paclitaxel during the second wash step with methanol-0.01 M ammonium acetate pH 5 (20:80, v/v), and

3) a reduction in paclitaxel recovery due to the pharmaceutical vehicle Cremophor EL (Taxol® contains 6 mg/mL paclitaxel in ethanol/Cremophor EL 1:1 v/v).(34)

To avoid recovery problems we modified the assay with addition of 2'-Methylpaclitaxel as internal standard. The modified assay was subsequently revalidated for quantification of paclitaxel.(15) In addition, the assay was validated for the three major human paclitaxel metabolites 6α-hydroxypaclitaxel, 3'p-hydroxypaclitaxel, and 6α,3'p-dihydroxy-paclitaxel.(15)

Methods for the quantification of these compounds in human plasma were not described previously, which was most likely caused by the lack of reference compounds. We were able to isolate and purify the metabolites in sufficient amounts from human feces.(35) The molar absorptivities, the extraction recoveries, and the slopes of the calibration curves of 6α-hydroxypaclitaxel and 3'p-hydroxypaclitaxel were in the same range of that of paclitaxel. Therefore, these metabolites can be determined by use of the paclitaxel calibration curve.(15) The quantification of the 6α,3'p-dihydroxypaclitaxel metabolite using the paclitaxel calibration curve needs a correction factor of 1.14, as its extraction recovery is slightly lower than for paclitaxel.(15)

Performance of the Paclitaxel Analytical Assay

Since the development of the assay for quantification of both paclitaxel and the three major metabolites, no modifications of the assay were necessary. We evaluated the analytical runs of paclitaxel from January 1997 up to January 2001. In this four year period, more than 5200 study samples have been analyzed in 84 analytical runs. Six different reversed-phase octyl HPLC columns were used and four different batches of SPE columns.

Performance of the paclitaxel quality control samples is presented in Table 1. The mean measured values of the quality control samples very closely resemble their nominal values and accuracies of the paclitaxel assay yield values of 92-102%. For validation of an analytical assay, acceptance ranges of accuracy of 85-115% are applied.(27,28) Considering the limited amount of variables during validation of an assay, the obtained accuracies in a four year period of 92-102% can be considered as very good. Precisions of the assay were less than 10% for all quality control concentrations. For validation of an assay, values of less than 15% must be obtained.(27,28) Precisions of less than 10% during a four year period can, therefore, be considered as acceptable. It should be noted, that precisions are calculated on quality controls spiked with preparations of different stock solutions, which we have considered as one batch.

Figures 1a-d give the individual values of the most frequently assayed quality control samples in the past four years. For all quality control concentrations,

Table 1. Performance of the Paclitaxel Quality Control Samples (January 1997–January 2001)

Nominal Value (ng/mL)	Measured Value (ng/mL)	Accuracy (%)	Precision (%)	Number of Runs
50	46 ± 4	92	8	6
100	98 ± 9	98	8	59
500	491 ± 39	98	7	82
750	742 ± 39	99	5	35
5000	5005 ± 359	100	6	59
7500	7634 ± 347	102	2	19

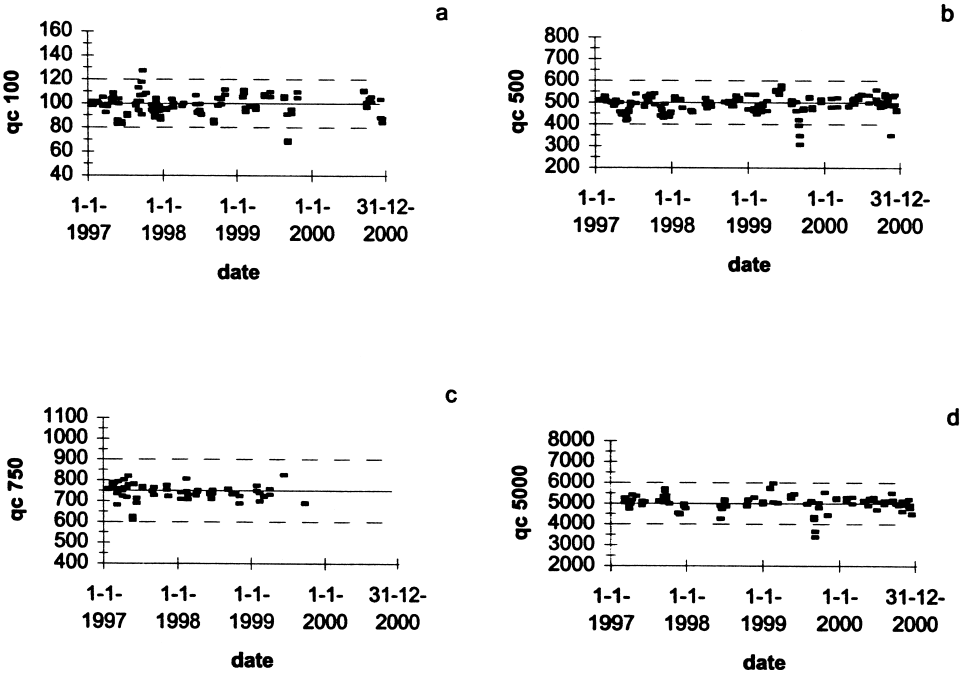

Figure 1. Individual values of the paclitaxel quality control concentrations of 100, 500, 750, and 5000 ng/mL. The dotted horizontal lines represent the 20% acceptance ranges.

scattered patterns around their nominal values are observed and it can, therefore, be concluded that there has not been an obvious trend in the paclitaxel analytical assay. However, trend detection is difficult because new quality controls have been made in time. The charts show that the quality control samples perfectly apply to the 20% ranges of the acceptance criteria.(27,28) However, in one run (September 1999), all quality control samples fell out of the 20% ranges. This has led to preparation of new quality controls, which then were within the 20% acceptance criteria.

In the past four years, a total of 4 different batches of SPE columns were used. Extraction recoveries of paclitaxel were calculated in each run by comparing the area of paclitaxel in human plasma with the area of paclitaxel dissolved in AMW (Figure 2). Previously, we noticed a large SPE batch-to-batch variability

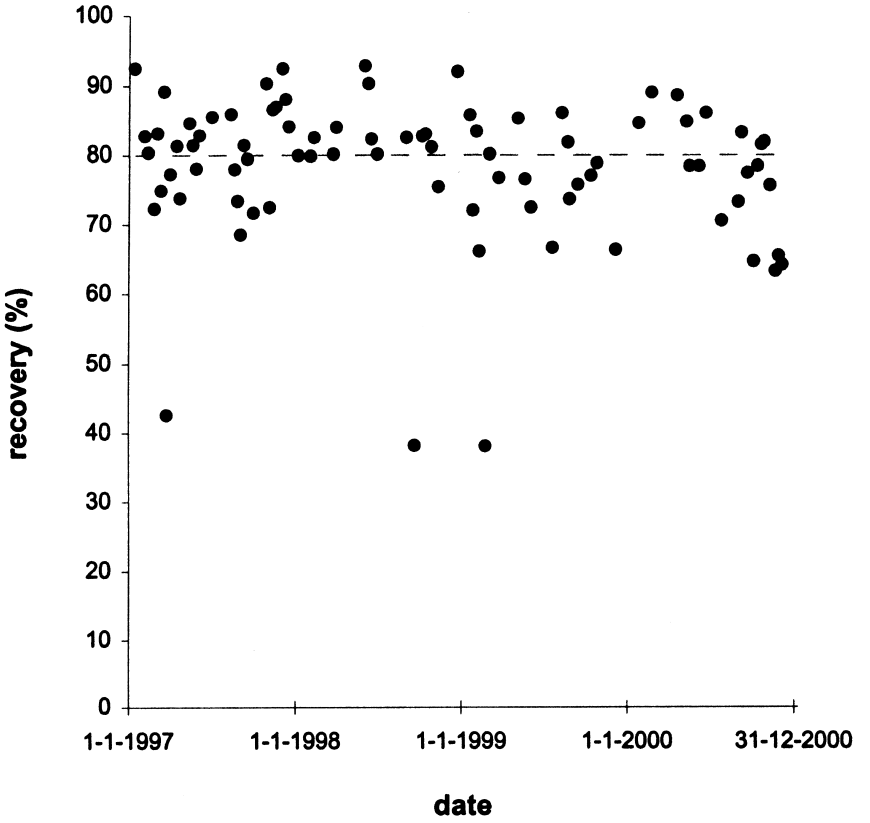

Figure 2. Recovery data of paclitaxel in human plasma. The dotted horizontal line represents an 80% recovery.

in the recovery data of paclitaxel.(34) This has led to application of an internal standard in the assay. For the 4 used batches, we found mean recovery percentages of 79 ± 11% (66 runs), 81 ± 6% (6 runs), 78 ± 2% (2 runs), and 73 ± 8% (9 runs). We did not observe substantial differences between the 4 batches. For three runs, recovery values of paclitaxel were only 40%. These low recoveries were retrieved from the same SPE batch, which, however, also produced recoveries of 70-80%. One possible reason for this difference in recovery in one SPE batch is storage of open packaging, causing loss of active sides of the sorbent under the influence of moisture and air. We, therefore, recommend careful storage of the SPE columns in closed packaging.

Importantly, in these three runs, calibration and quality control samples perfectly applied to the acceptance criteria with mean percentage deviations from their nominal values of less than 5%. These results show the appropriate application of an internal standard resulting in reliable data.

For preparation of the paclitaxel calibration and quality control samples, we made fresh stock solutions of paclitaxel and 2'-methylpaclitaxel every 6 months. During the past four years, we re-evaluated the stability of the stock solutions and the stability of paclitaxel in human plasma when stored at -20°C. Diluted stock solutions were injected and the areas were compared. A percentage of less than 5% deviation was considered acceptable. The paclitaxel stock solution was found to be stable for at least 8 months and the 2'-methylpaclitaxel for at least 13 months. Stability of paclitaxel in human plasma was evaluated at concentrations of 50 and 5000 ng/mL. Three replicates were analyzed at 0, 6, and 14 months. Paclitaxel was found to be stable in human plasma for at least 14 months. Stability of the stock solutions and paclitaxel in human plasma has not been tested for longer periods.

In conclusion, the validated assay of paclitaxel and its three major metabolites in human plasma showed excellent performance over the past four years. Accuracies of 92-102% and precisions of less than 10% were achieved. Recovery data of paclitaxel underline the need of use of an internal standard in the assay. New stability data of the stock solutions of paclitaxel and 2'-methylpaclitaxel and paclitaxel in human plasma allow us to prepare these solutions on a less regular basis.

Development of the Docetaxel Analytical Assay

In order to pharmacokinetically support clinical studies of docetaxel, we recently developed and validated an HPLC analytical assay for determination of docetaxel in human plasma.(16) Furthermore, the assay was also capable of detection of four hydroxylated docetaxel metabolites M1, M2, M3, and M4 for which a limited validation was performed.(16) Quantification of docetaxel

metabolites in plasma had not been described earlier. Only the analysis of parent drug in human plasma had been reported.(36) We were able to isolate and purify the metabolites in sufficient amounts from human feces.(37) The HPLC system used, however, did not separate the metabolites M1 and M2; total concentrations of the products were determined.

The molar absorbtivities, the extraction recoveries, and the slopes of the calibration curves of the metabolites were all in the same range as that of docetaxel. Therefore, the metabolites can be quantified by the use of the docetaxel calibration curve, when these compounds are not available as references.(16) We have used this assay in clinical studies of docetaxel in which we were able to detect docetaxel metabolites in plasma.(13,16)

Performance of the Docetaxel Analytical Assay

We have evaluated the analytical runs of docetaxel from November 1998 up to January 2001. The last docetaxel run before November 1998 was of March 1996. In this more than 2-year period, we have analyzed almost 1500 study samples in 19 runs. One HPLC column was used and 5 different batches of SPE columns.

Performance of the docetaxel quality control samples is presented in Table 2. The mean measured values of the quality control samples closely resemble their nominal values and accuracies of the docetaxel assay yield values of 103-112%. Precisions of the assay were less than 10% for all quality control concentrations. As described above for paclitaxel analysis, these accuracies and precisions can be considered as very good. Figures 3a-d give the individual values of the docetaxel quality controls in the past two years. From the charts it can be seen that in three runs (April 1999) the quality control of 500 ng/mL reveals values exceeding the 20% acceptance ranges. This has led to the preparation of new quality control samples, which perfectly applied to the 20% acceptance criteria. For the other two quality control concentrations, measured values all fall within the 20% acceptance ranges.

Table 2. Performance of the Docetaxel Quality Control Samples (November 1998–January 2001)

Nominal Value (ng/mL)	Measured Value (ng/mL)	Accuracy (%)	Precision (%)	Number of Runs
100	103 ± 10	103	9	19
500	560 ± 50	112	8	19
750	770 ± 53	103	6	19

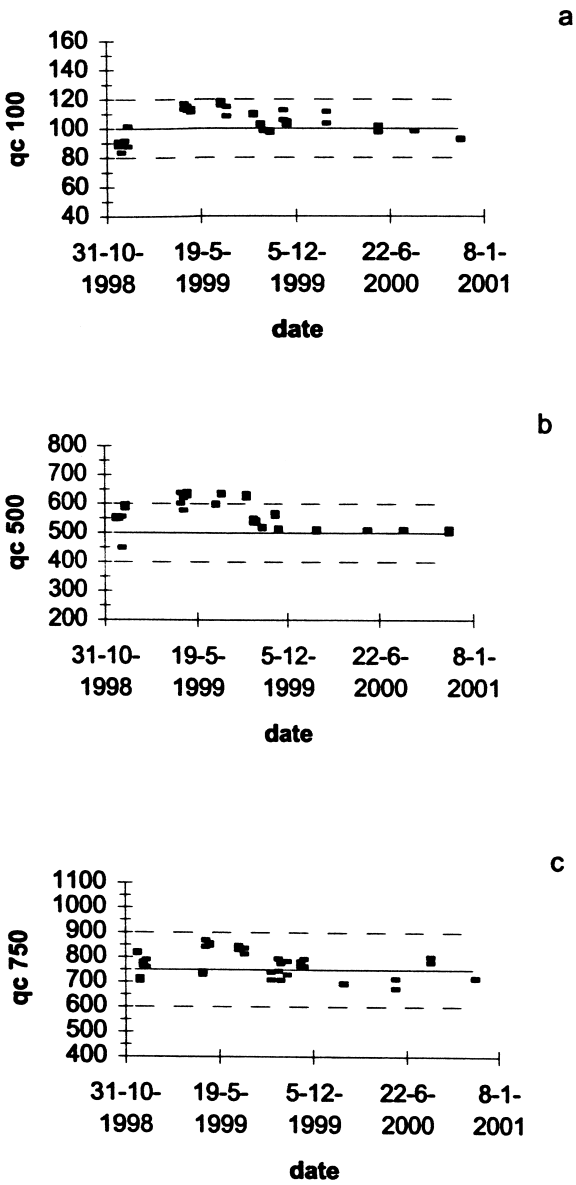

Figure 3. Individual values of the docetaxel quality control concentrations of 100, 500, and 750 ng/mL. The dotted horizontal lines represent the 20% acceptance ranges.

In the past two years, a total of 5 different batches of SPE columns were used. Recoveries of docetaxel were calculated in each run by comparing the area of docetaxel in human plasma with the area of docetaxel dissolved in AMW (Figure 4). During validation of the assay, we determined recoveries of docetaxel from several batches of cyano SPE columns from two suppliers. The best results were obtained with end-capped columns from IST (Sopachem BV, Nieuwegein, The Netherlands).(16) Only these columns have been used in further docetaxel analysis. Mean recovery percentages of the 5 batches were 85 ± 4% (3 runs), 76 ± 9% (4 runs), 70 ± 5% (2 runs), 64 ± 15% (6 runs), and 91 ± 9% (4 runs). The relative low recovery of 64% of one batch is merely caused by the low recovery of 40% obtained in one run (June 1999). In this run, it was observed that the nee-

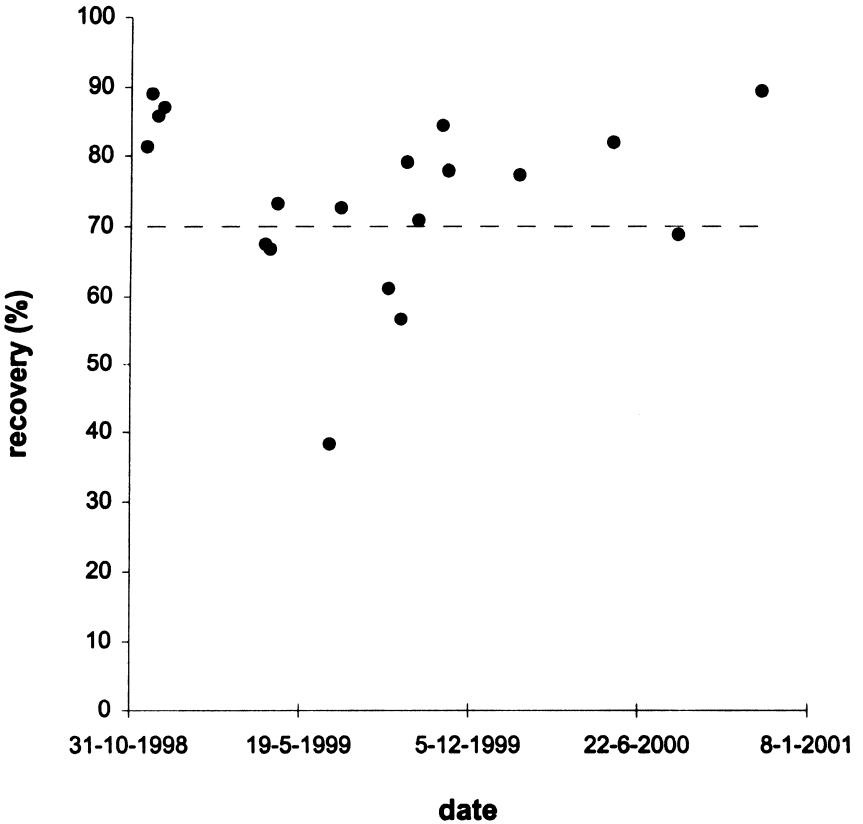

Figure 4. Recovery data of docetaxel in human plasma. The dotted horizontal line represents a 70% recovery.

dle of the SPE equipment was slightly bent, resulting in reduced transfer of plasma to the SPE columns. Most likely, this has caused the low recovery. Calibration and quality control samples in this run perfectly applied to the acceptance criteria, which shows the usefulness of an internal standard.

For preparation of the docetaxel calibration and quality control samples, we made fresh stock solutions of docetaxel and 2'-methylpaclitaxel every 6 months. We re-evaluated stability of the docetaxel stock solution, which was found to be stable for at least 18 months. Stability of the 2'-methylpaclitaxel stock solution was already performed for paclitaxel analysis and determined stable for at least 13 months. Longer periods have not been tested.

In conclusion, the validated assay of docetaxel and the four hydroxylated metabolites showed very good performance over the past two years. Accuracies of 103-112% and precisions of less than 10% were achieved. Variability in the recovery data of docetaxel stresses the use of an internal standard in this assay.

Development of the Cyclosporin A Analytical Assay

The specific FPIA we used for quantification of cyclosporin A in whole blood has been validated and developed by Abbott Laboratories in order to monitor cyclosporin A therapy in transplant patients.(23-26) The assay is a modification of the non-specific FPIA for determination of cyclosporin A in whole blood with less cross-reactivity of the cyclosporin A metabolites.(23-26) The assay uses a competitive immunoassay methodology in which tracer-labeled antigen and patient antigen compete for binding sites on the antibody molecules. The precise relationship between polarization and concentration of the unlabeled drug is established by measuring the polarization values of calibrators with known concentrations of the drug.

Performance of the Cyclosporin A Analytical Assay

The use of cyclosporin A as an enhancer of the absorption of orally administered paclitaxel and docetaxel gave us a large amount of cyclosporin A blood samples to study performance of the assay. From April 1997 up to January 2001 more than 2000 study samples were analyzed in 131 runs. Different from the paclitaxel and docetaxel HPLC assays, cyclosporin A FPIA analytical runs do not involve analysis of calibration samples in each run. Prior to start of analysis, a calibration curve is made, which is stored as long as the quality controls fall within their accepted ranges. Each cyclosporin A run involves analysis of study samples and single measurements of 1-3 of the quality control samples.

Performance of the cyclosporin A quality controls is presented in Table 3. Accuracies of the quality control samples yield values of 103-105%. The coefficient of variation in the quality control samples was 11% for the 150 ng/mL quality control and less than 10% for the 400 and 800 ng/mL quality controls. Applying the 15% values for precision during validation of an analytical assay, these coefficients of variation fall within the predefined range.

Individual values of the cyclosporin A quality control samples are given in Figures 5a-c. From these charts it can be seen that, in general, the cyclosporin A quality controls meet the requirements of acceptance. Furthermore, the charts show scattered patterns, indicating no obvious trend in the cyclosporin A assay. However, it can be seen, that in the period of February-March 1999 cyclosporin A quality control samples exceeded the 15% and 20% acceptance ranges. It can also be seen that, in this period, other quality control samples at the same concentrations yielded values close to the target values and met the requirements of acceptance. In this period, we used different lot numbers of the cyclosporin A reagent pack.

Between the different lot numbers, we observed large differences between the values of the quality controls with acceptable values for one of the lot numbers, however, with unacceptable values for the other. A similar observation was made in May 1999. In the same week, two analytical runs of cyclosporin A were performed with two different reagent lot numbers. In one run, the three quality controls exceeded the acceptable ranges; while in the other run quality controls perfectly met the requirements of acceptance. The cyclosporin A assay guide(23) recommends recalibration of the assay when a new reagent lot is used. Our observations strongly underline this recommendation.

When cyclosporin A study sample concentrations were above the highest calibration standard (1500 ng/mL), the supernatant was diluted with dilution buffer and re-analyzed, as performed, for many other compounds analyzed by the TDxFLx system. However, the cyclosporin A assay guide(23) recommends reanalysis of these study samples after dilution with the Cyclosporine Monoclonal

Table 3. Performance of the Cyclosporin A Quality Control Samples (April 1997–January 2001)

Nominal Value (ng/mL)	Measured Value (ng/mL)	Accuracy (%)	Precision (%)	Number of Runs
150	158 ± 18	105	11	94
400	420 ± 39	105	9	94
800	821 ± 53	103	6	86

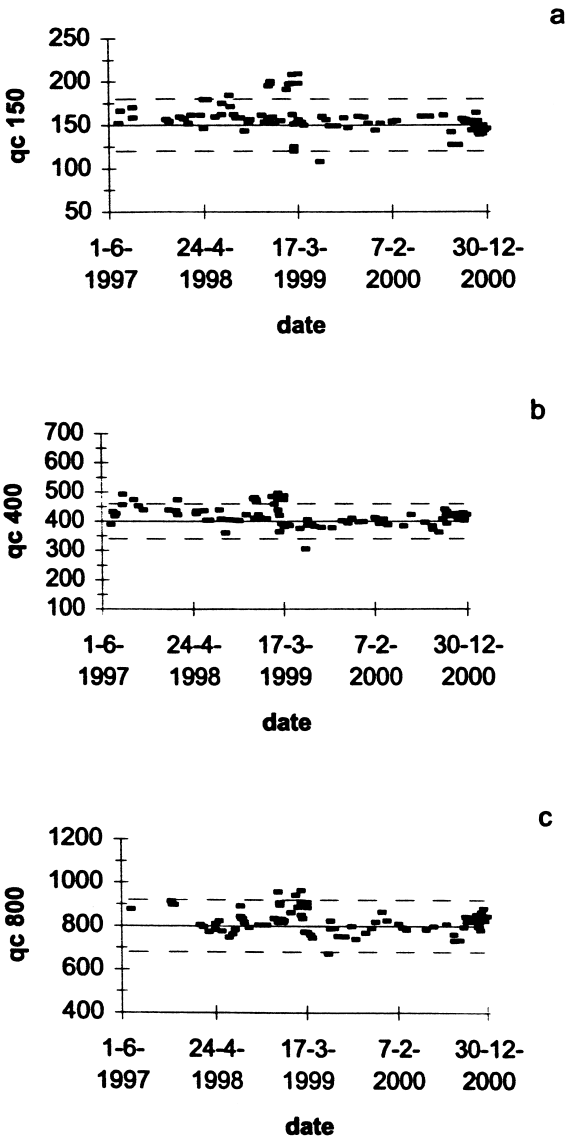

Figure 5. Individual values of the cyclosporin A quality control concentrations of 150, 400, and 800 ng/mL. The dotted horizontal lines represent the 20% (150 ng/mL) and 15% (400 and 800 ng/mL) acceptance ranges.

Table 4. Cyclosporine A Data of the 800 ng/mL Quality Control Diluted with Whole Blood [23], Diluted with Dilution Buffer, and Undiluted

Dilution Protocol	Dilution	Mean Measured Value (ng/mL)	CV (%)	n	DEV (%)
Whole blood	5	917	2.2	2	2.2
Dilution buffer	5	735	3.8	2	–18.1
Undiluted	—	897	—	1	

CV: coefficient of variation.
DEV: deviation from undiluted sample.

Whole Blood Calibrator A (0.0 ng/mL cyclosporin A) prior to performing the solubilization step. We analyzed the 800 ng/mL quality control diluted according to the cyclosporin A assay guide with control whole blood(23) and diluted with dilution buffer. In this run, the undiluted 800 ng/mL quality control was also analyzed. The results are given in Table 4. The data clearly show that only dilutions in control whole blood result in an accurate determination of the concentration. Because dilution of study samples is performed when concentrations are above the 1500 ng/mL, we have applied both dilution protocols to study samples with high expected values. At concentrations above the 1500 ng/mL, differences between the two dilution protocols were very small (less than 3%) and can be considered as negligible.

In conclusion, the specific FPIA for determination of cyclosporin A in whole blood showed very good performance in a routine laboratory setting. Accuracies of the assay yielded values of 103-105% and the coefficients of variation were less than 12%.

CONCLUSIONS

In the past four years, the analytical assays of paclitaxel, docetaxel, and cyclosporin A showed excellent performance. In this period, we performed 84 analytical runs of paclitaxel, 19 runs of docetaxel, and 131 runs of cyclosporin A. Accuracies of the paclitaxel, docetaxel, and cyclosporin A assays were 92-102%, 103-112%, and 103-105%, respectively. Precisions of the paclitaxel and docetaxel assays were less than 10% for all concentrations. For the cyclosporin A assay, the coefficients of variation were less than 12%. It can be concluded that the validated analytical assays of paclitaxel, docetaxel, and cyclosporin A showed very good performance over a prolonged period of time in a routine hospital laboratory setting and can, thus, be considered as robust assays.

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Received April 14, 2001 Manuscript 5536
Accepted May 17, 2001