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Technical note

Influence of Cremophor EL on the quantification of paclitaxel in plasma using high-performance liquid chromatography with solid-phase extraction as sample pretreatment

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Abstract

For the quantitative determination of paclitaxel in human plasma reversed-phase high-performance liquid chromatographic (HPLC) methods with solid-phase extraction (SPE) as sample pretreatment procedure are frequently used. Recovery problems arose during the quantification of paclitaxel in plasma samples of patients. The major problems were a large batch-to-batch difference in performance of the SPE columns and the effects of the pharmaceutical vehicle Cremophor EL on the performance of the SPE. Cremophor EL concentrations exceeding 1.0% (v/v) had a great impact on the absolute recovery of paclitaxel from human plasma with the SPE procedure. The recoveries decreased approximately 10 to 40% depending on the quality of the batch SPE columns. The problems are avoided by using 2′-methylpaclitaxel as the internal standard. This study points out the importance of including the effects of a pharmaceutical vehicle, like Cremophor EL, in the validation programme of a bioanalytical assay and the use of an internal standard in HPLC paclitaxel assays preceded by SPE as sample pretreatment procedure. © 1998 Elsevier Science B.V.

Keywords: Cremophor EL; Paclitaxel

1. Introduction

The taxoids constitute a new class of antineoplastic agents which act by promoting and stabilizing microtubuli. This group of antitumour agents is highly active against a wide variety of solid tumours including ovarian, breast, and non-small cell lung cancer [1]. A major problem associated with the use of taxoids is their poor solubility in water and in most accepted pharmaceutical solvents. The current commercially available formulation of paclitaxel (Taxol, Bristol Myers Squibb, Syracuse, NY, USA; Paxene, Norton Baker, Miami, FL, USA) is a sterile 6 mg/ml solution, in a solvent consisting of polyoxyethyleneglycerol triricinoleate 35 (Cremophor EL) and dehydrated alcohol, USP (1:1, v/v). A high-performance liquid chromatography (HPLC) procedure with solid phase extraction (SPE) as sample pretreatment procedure has been developed

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and validated for the bioanalysis of paclitaxel and metabolites in human plasma [2–4]. The power of this assay as compared to the earlier described methodologies is the lower limit of quantitation (0.010 μg/ml) and the detection of metabolic products. We used this HPLC assay for the analysis and quantitation of paclitaxel and metabolites in human plasma in several pharmacokinetic studies [3,5–8]. So far 5000 plasma samples have been analyzed with 12 different batches of SPE cyano bond elut columns. In one batch of SPE columns the recovery was <80%, which was below our defined criterium of acceptance, and which provided paclitaxel concentrations in clinical samples lower than expected. However, analysis of paclitaxel spiked plasma samples supplied by another laboratory (''cross-validation'' samples) were within the required accuracy and precision limits of 15%. Adaptation of a washing step [with methanol–0.01 *M* ammonium acetate pH 5.0 (2:8, v/v)] from 2 to 1 ml improved the recovery to above the desired 80%. Then, the effects of Cremophor EL on the performance of the SPE columns were investigated. We reported earlier the impracticability of quantitating paclitaxel with HPLC preceded by SPE, in human urine containing 5% of a mixture of Cremophor EL–dehydrated alcohol [9]. This mixture was added to prevent crystallization of paclitaxel during storage of the samples. Plasma samples from patients contain variable amounts of Cremophor EL at each time point, with peak concentrations of this vehicle up to 1% [10]. This substance is not routinely added to the plasma standard samples (and it was also not added to the cross-validation samples). The aims of this study were thus to investigate the performance of several batches cyano bond elut SPE columns and the influence of Cremophor EL on the SPE.

2. Experimental

Paclitaxel was assayed in plasma using the HPLC assay with SPE as sample pretreatment procedure as previously described (sample processing, HPLC instrumentation and conditions) [2,3]. For recovery experiments samples were spiked with Cremophor EL to obtain 0, 0.2 and 1% (v/v) concentrations and with three different paclitaxel concentrations 50, 500, and 5000 ng/ml. The extraction efficiencies of the analytes were determined by comparing the peak area of paclitaxel in human plasma to the paclitaxel peak area of standard concentrations prepared in acetonitrile–methanol–distilled water (4:5:1, v/v/v). Three different batches (130585, 130247 and 132814) of cyano bond elut columns (100 mg; 1 ml; Varian, Harbor City, CA, USA) were used to test the effect of Cremophor EL on the performance of the assay. The concentration of the internal standard 2′-methylpaclitaxel (Bristol Myers Squibb, Syracuse, NY, USA) in plasma samples is 500 ng/ml.

3. Results and discussion

In 1992 The Netherlands Cancer Institute participated in a large randomized multicentre European–Canadian trial which investigated the safety and antitumour efficacy of paclitaxel in ''high'' (175 mg/m^2) versus ''low'' (135 mg/m^2) dose and long (24 h) versus short (3 h) infusion duration in platinum pretreated ovarian cancer patients [11]. This was an unique opportunity to investigate the pharmacokinetic behaviour of this compound. Several HPLC methods including various sample pretreatment procedures had been reported for the analysis of paclitaxel in biological matrices at that time. These methods were, however, relatively insensitive and could not detect metabolic products in human plasma [12–15]. A more sophisticated and sensitive HPLC method with a SPE procedure as sample pretreatment was then developed [2,3]. The assay in human plasma was fully validated at our laboratory. For the acceptance of each run we predefined that the following criteria had to be met: (1) <15% relative standard deviation of standard or quality control (QC) replicate response values; (2) <15% deviation of predicted concentration from nominal for at least four of the six QC's; >0.9950 correlation coefficient for the regression analysis of the standards; (3) recovery >80%.

Recovery problems, however, arose in December 1992–January 1993 (Fig. 1). Although the extraction coefficient was 92% during the validation procedure it dropped to approximately 60% when another batch of SPE columns was used. Paclitaxel concentrations in clinical samples processed with these columns

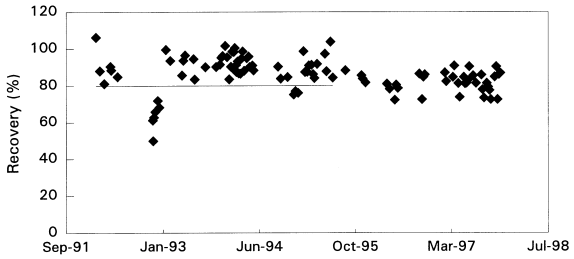

Fig. 1. Historical quality control data (recovery as determined in each analytical run) for the analysis of paclitaxel (December 1991 to December 1997). The horizontal line represents a 80% recovery of paclitaxel. From September 1995 the internal standard is added.

were lower than expected. The amount of paclitaxel was then quantitated in each washing step. A significant amount of paclitaxel could be detected in the second washing step with the methanol–0.01 *M* ammonium acetate pH 5.0 (2:8, v/v) mixture. A reduction in this washing step from 2 to 1 ml normalized the recovery to an acceptable level, i.e, >80%. Blinded spiked plasma samples from another laboratory (''cross validation'' samples) were analyzed and did meet the criteria as previously mentioned even with the use of the batch of the low performance SPE columns and the initially applied 2 ml second washing step. The hyperlipemic state of the clinical plasma samples and impracticability of the quantification of paclitaxel in human urine containing a 5% mixture of Cremophor EL–dehydrated alcohol with SPE [9] alerted us to the possibility that the vehicle Cremophor EL might also influence the quantification of paclitaxel here in human plasma. The calibration and QC samples used for the validation of this assay did not contain Cremophor EL in contrast to plasma material obtained from paclitaxel treated patients. The cross validation samples also did not contain Cremophor EL.

The recovery data for the quantification of paclitaxel in human plasma (nominal concentrations 50, 500 and 5000 ng/ml) as a function of the Cremophor EL concentration, using 1 or 2 ml washing steps and for three batches SPE columns is tabulated in Table 1. A large batch-to-batch variability was observed,

Table 1
Influential factors on the quantification of paclitaxel

Cremophor EL conc. (%)	Nom. conc. (ng/ml)	Wash step (ml)	Solid-phase extraction columns								
			Batch 132814			Batch 130585			Batch 130247		
			Pacl. area (%)	Ratio P:M (%)	Rec. (%)	Pacl. area (%)	Ratio P:M (%)	Rec. (%)	Pacl. area (%)	Ratio P:M (%)	Rec. (%)
0	50	1	100	100	100	100	100	62	100	100	79
	500	1	100	100	92	100	100	73	100	100	94
	5000	1	100	100	92	100	100	68	100	100	94
	50	2	79	81	79	69	76	43	119	118	94
	500	2	97	94	90	71	85	52	101	111	94
	5000	2	91	91	84	77	96	53	92	96	86
0.2	50	1	88	88	88	81	86	50	99	106	78
	500	1	102	102	94	87	105	64	102	102	95
	5000	1	101	101	93	60	101	41	96	102	90
	50	2	82	85	82	55	71	34	119	123	94
	500	2	97	98	89	73	97	54	103	102	97
	5000	2	95	95	88	77	91	52	89	98	84
1.0	50	1	75	81	75	69	111	43	66	79	52
	500	1	95	100	88	62	99	45	87	97	81
	5000	1	89	91	82	63	99	43	83	95	78
	50	2	66	71	67	28	50	17	75	92	59
	500	2	82	91	76	43	74	31	76	102	71
	5000	2	81	85	75	58	91	40	72	89	68

P, Pacl.=paclitaxel; M=2′-methylpaclitaxel.

which was most pronounced at the lowest concentration. Modification of washing steps from 2 to 1 ml led to an improvement in recovery in cases where the recoveries were below the level of acceptance. The influence of the solvent Cremophor EL on the absolute recovery was most pronounced with plasma Cremophor EL concentrations of 1.0% (Fig. 2). A reduction in absolute recovery between 4 and 28% was observed for the paclitaxel concentration of 500 ng/ml (Fig. 2). This effect occurred for batch 130585 also with lower Cremophor EL concentrations (0.2%). The improvement in recovery when the washing step was reduced from 2 to 1 ml methanol–0.01 *M* ammonium acetate pH 5.0 (2:8, v/v) indicated that a substantial amount of paclitaxel is lost during the SPE. This might be due to a loss of interaction between paclitaxel and the SPE column sorbent in the presence of Cremophor EL.

Recently, with the development of a reversed-phase HPLC method for Cremophor EL, peak plasma levels up to 1.2% were measured in paclitaxel (Taxol) treated patients (175 mg/m^2) [10,16]. These concentrations may thus have a profound impact for the HPLC determination of paclitaxel with a SPE procedure.

At the time of the development of our bioanalytical assay no internal standard was available and validation requirements were met. Therefore, there was no urgent need to implement an internal standard [3]. Recently, 2′-methylpaclitaxel was purified and could be used as an internal standard. Since that time we add 2′-methylpaclitaxel 500 ng/ml as internal standard to human plasma samples. From Table 1 it becomes clear that with the internal standard and a 1 instead of 2 ml washing step in the SPE acceptable relative paclitaxel recoveries (>70–80%) are obtained even in situations when a batch of SPE columns is used with lower absolute paclitaxel recoveries.

We conclude that: (1) there is a large variability in the performance of the SPE columns from batch to batch; (2) that Cremophor EL has a negative effect on the absolute recovery of paclitaxel and (3) that a substantial amount of paclitaxel is lost with a 2 ml wash step during the SPE when the column performance was below 80%. The use of an internal standard can avoid the recovery problems (Table 1). We therefore advise that besides the 15% accuracy and precision limits the assay performance should also meet the 80% recovery criterium for the SPE columns when no internal standard is available; the use of an internal standard, e.g., 2′-methylpaclitaxel is however strongly to be preferred.

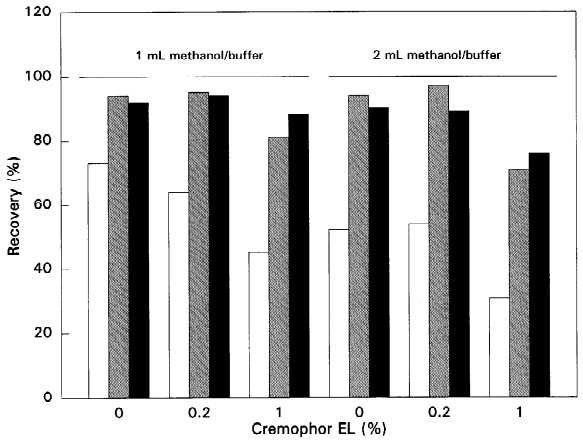

Fig. 2. The inluences of Cremophor EL and a 1 and 2 ml SPE wash step on the recovery efficiency of different SPE cyano bond elut columns. On the *x*-axis the Cremophor EL concentration and on the *y*-axis the recovery efficiency. White bars represent the SPE batch 130585, shaded bars the SPE batch 130247 and black bars the SPE batch 132814.

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