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Simultaneous online SPE–HPLC–MS/MS analysis of docetaxel, temsirolimus and sirolimus in whole blood and human plasma

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ABSTRACT

Docetaxel and temsirolimus are some of the most used drugs in a wide range of solid tumors. In preclinical studies, mTOR inhibitors such as temsirolimus have demonstrated synergistic cytotoxic effects with taxanes providing the rationale for combination studies. These anticancer agents exhibit a narrow therapeutic concentration range and due to their high inter- and intra-individual pharmacokinetic variability, therapeutic dose monitoring by highly sensitive methods as LC–MS/MS are important for clinical research. Therefore, the aim of this study was to develop and validate a sensitive, fast and convenient method for the simultaneous identification and quantification of docetaxel, temsirolimus and its main metabolite, sirolimus, using paclitaxel, another anticancer drug, as the internal standard. These analytes were quantified by an integrated online solid phase extraction–high performance liquid chromatography–tandem mass spectrometry (SPE–HPLC–MS/MS) system. Separation was performed on a Zorbax eclipse XDB-C8 (150 mm × 4.6 mm, 5 μm) column. The mass spectrometer tandem quadruple detector was equipped with jet stream electrospray ionization, monitored in multiple reactions monitoring (MRM) and operated in positive mode. A combination of protein precipitation with methanol/zinc sulphate (70:30) (v/v) and online SPE using a Zorbax eclipse plus C8 (12.5 mm × 4.6 mm, 5 μm) cartridge was used to extract the compounds. This method allows the use of the same reagents, sample treatment and analytical technique independently of whether the samples are whole blood or plasma. The method has been successfully validated and applied to real samples. It is a suitable method for dose adjustment and for evaluating potential drug interactions during combined treatments.

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1. Introduction

Docetaxel (Taxotere®), temsirolimus (Torisel®) and its active metabolite sirolimus [1,2] are important agents with broad spectra of antitumoral activity. Nowadays, all of them are commonly used in a variety of solid tumors in chemotherapy [3–7], either alone or in combination. Docetaxel is an inhibitor of microtubule depolymerization to free tubulin, which leads to cell death [7]. This antitumoral compound belongs to the taxoide family. Temsirolimus is an analog of the macrolide sirolimus [2] as it is shown in Fig. 1, and both macrolides have shown a mammalian target to rapamycin (mTor) inhibition that results in antiangiogenic effect [8]. According to some in vitro studies rapamycin and other inhibitors of mTor have a symbiotic effect with taxanes.

These cytotoxic agents have narrow therapeutic windows and their quantification in blood or plasma samples is important for establishing pharmacokinetic parameters allowing the dose adjustment and assessing inter-individual and intra-individual metabolism variability [9,10].

Therapeutic drug monitoring (TDM) is widely used to optimize the treatment of patients with cancer and there is a lack of information about the security of these agents in combined chemotherapy. For the practical use of the analytical method to monitor these drugs, it should be a fast and easy to perform method carried out with minimal sample preparation.

Docetaxel is highly protein-bound in the plasma [11] generally measured in plasma samples as bound or unbound drug. Some studies add paclitaxel, another taxane structurally related to docetaxel, as an internal standard [9,12,13], its structure is included in Fig. 2.

The usual clean-up method in the analysis of docetaxel is solid phase extraction (SPE) with columns consist of silica packaging coated with ethyl or cyanopropyl groups [9,11,12,14–17] and

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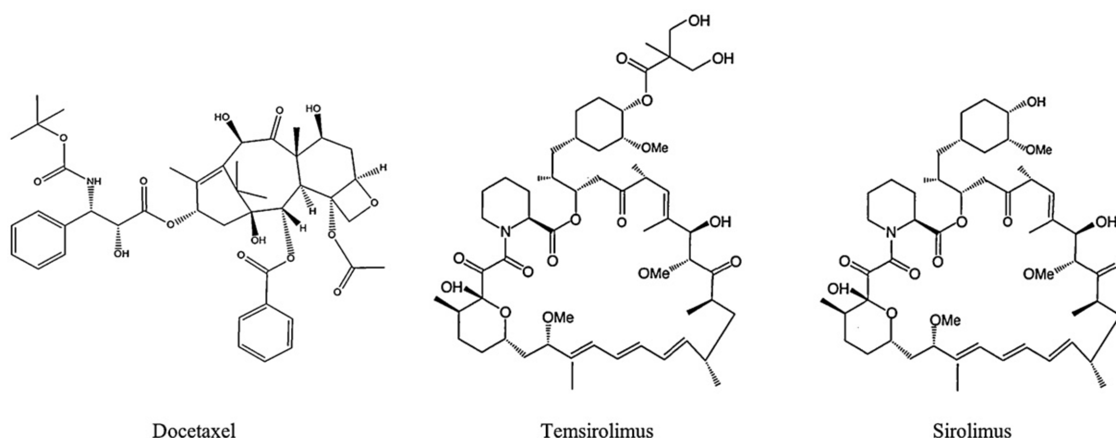

Fig. 1. Structures of the drugs tested in the study.

liquid–liquid extraction (LLE) [4,18] procedures. Both strategies need several steps that may not be appropriate for multi-sample analysis in human pharmacokinetics studies or routine drug monitoring programs [19].

Sirolimus distribution is approximately 95% inside the erythrocytes, so this macrolide and its related compound temsirolimus should be measured in whole blood samples [10,20], although Raymond et al. [21] quantified sirolimus in both whole blood and plasma. A temsirolimus derivative as d7-CCI-779 [21] or a sirolimus derivative as nor-rapamycin [20], are often used as internal standards. Most of the assays use zinc sulphate as protein precipitation agent that allows the erythrocytes to lyse and produce a very clean supernatant [10,20,22–24]. After protein precipitation, the common sample treatments are also SPE [10,23] and LLE [20,21,24,25].

Recent researches have described online-SPE methods to automate simultaneous anticancer extractions obtaining more accurate and precise quantization with faster sample preparation and lower human errors [26–28].

The concentrations of these antitumorals in plasma and in whole blood samples are in the μg/L range (ppb), thus to quantify them accurately, highly sensitive methods are required. Several reports have described some assays based on liquid chromatography (LC) in combination with tandem mass spectrometry (MS/MS) instrumentation [7,9–11,18,20,23,29–31] [. As Koal et al. described in their study [26], MRM detection mode in MS/MS system, allows simultaneous quantitative determination of antitumoral drugs with high specificity, low detection limits and very little time of analysis. These methods are much more sensitive than HPLC–UV methods although they have been widely used [4,12,14,17,24,25]. The aim of the current study was to develop a sensitive, fast and convenient method for the simultaneous identification and quantification of docetaxel, temsirolimus and its main metabolite, sirolimus, using paclitaxel as internal standard. The experiment was performed by an integrated online solid phase extraction–high performance liquid chromatography–tandem mass spectrometry (SPE–HPLC–MS/MS) system. The method has been successfully validated and applied to real samples and it is suitable for monitoring these anticancer agents during pharmacokinetics studies.

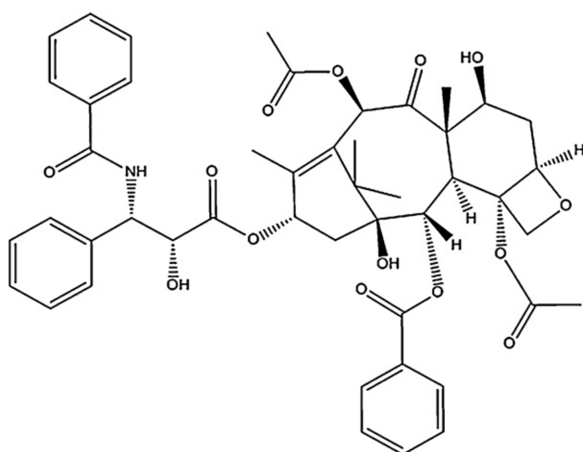

Fig. 2. Structure of paclitaxel.

2. Materials and methods

2.1. Automated online-SPE–LC–MS/MS instrumentation and operating conditions

The chromatographic system combines an Agilent 1260 Infinity quaternary pump and an Agilent 1290 Infinity LC (Agilent Tecnologies, Palo Alto, CA). It was composed of a binary pump with integrated degasser, high performance autosampler with thermostat, temperature-controlled column compartment and 2 position/10 ports switching valve (Santa Clara, CA, EE.UU.).

The analyte enrichment on the Online-SPE was achieved through a Zorbax Eclipse plus C8 (12.5 mm × 4.6 mm, 5 μm) cartridge. Compounds separation was performed by a Zorbax Eclipse XDB-C8 Column (150 mm × 4.6 mm, 5 μm). Both columns were maintained at 65 °C. Injection volume was 5 μL, the auto sampler temperature was 4 °C .The positions of the switching valve were: 0.0 min – position 1; 1 min – position 2; 3.50 min – position 1. Fig. 3 displays the connections among the ports in both positions. Samples were washed for 1 min with a mobile phase of A: water + 0.1% formic acid – B: methanol + 0.1% formic acid (95:5) (v/v). The flow of the loading pump (binary pump) was 2.0 mL/min. After 1 min the switching valve position changes and the analytes were backflushed from the online-SPE to the analytical column at 1 mL/min. 3.5 min after injection, extraction column was washed with 100% mobile phase B for 2.5 min at 2 mL/min. Flow rates and gradients for the analytical pump (quaternary pump) are shown in Table 1.

Column eluates were analyzed with an Agilent 6460 triple quadrupole mass spectrometer, with jet stream electrospray ionization (ESI). Nitrogen (purity 99.9999%) was the collision gas. The detector was operated in multiple reaction monitoring (MRM) in the positive mode. The conditions of the source parameters were: nebulizer 35 psi, drying gas flow 10 L/min, drying gas temperature 350 °C, sheath gas temperature 400 °C, sheath gas flow 12 L/min and the capillary voltage 4000 V.

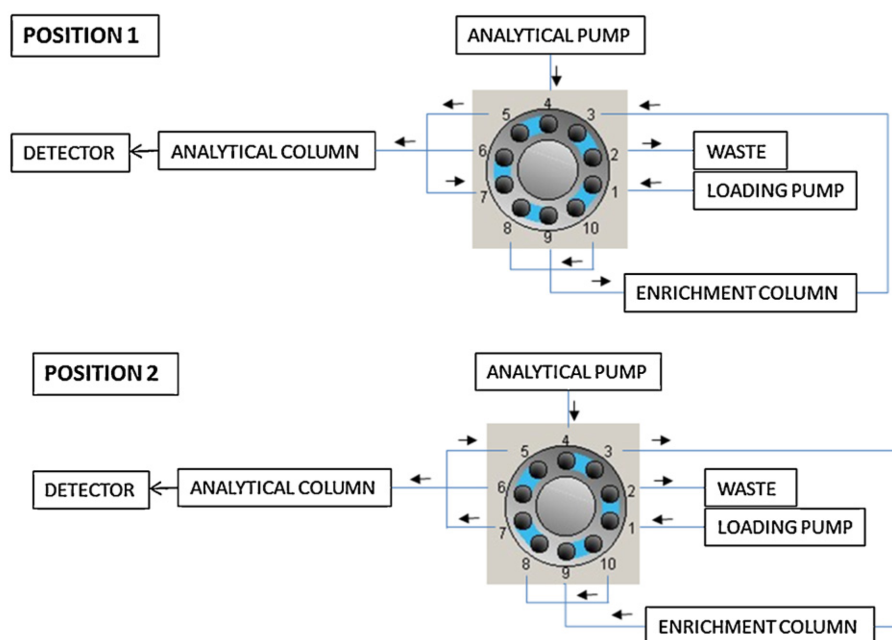

Fig. 3. Two positions–ten ports valve diagram.

Table 1
Gradient and flow in the analytical pump.

Quaternary pump (analytical pump)		
Time	Flow	% Solvent B
0.00	1.000	68.0
1.00	1.000	68.0
3.00	1.000	95.0
3.50	1.000	95.0
3.51	0.500	95.0
5.00	0.500	95.0
6.50	0.500	95.0
6.60	1.000	95.0
10.00	1.000	32.0

Table 2
Optimized method data.

Compound	Precursor ion	Product ion	Dwell time	Fragmentor voltage (V)	Collision energy (V)
Paclitaxel	876.3	531	300	160	31
Docetaxel	830.3	599.1	300	160	25
Temsirolimus	1052.5	461	300	300	75
Sirolimus	936.4	409.1	300	210	70

The first quadrupole was set to select their sodium adducts as the precursor ion of the three analytes and the internal standard: for paclitaxel (I.S. m/z 876.3), for docetaxel (m/z 830.3), for temsirolimus (m/z 1052.5) and for sirolimus (m/z 936.4). The product ion of each analyte was selected by the third quadrupole, for paclitaxel (m/z 531), for docetaxel (m/z 599.1), for temsirolimus (m/z 461), for sirolimus (m/z 409.1). All these ions and conditions for MRM detection are included in Tables 2 and 3. Peak area ratios obtained from MRM mode of the mass transitions were used for quantitation.

Table 3
Optimized method time segments.

Segment	Start time	Valve	Delta EMV (+)
1	0	To waste	0
2	3.6	To MS	400
3	5.4	To MS	400

The retention time of paclitaxel, docetaxel, temsirolimus and sirolimus were 4.5 min, 4.7 min, 6.0 min and 6.2 min, respectively.

2.2. Software and data analysis

System control and data analysis were acquired and processed with Agilent Mass Hunter QQQ Qualitative and Quantitative software programs (version B.04.00).

2.3. Chemicals

Docetaxel analytical reference standard was supplied by Sanofi-Aventis (Barcelona, Spain). Paclitaxel was supplied from Sigma–Aldrich (Saint Quentin Fallavier, France). Temsirolimus and sirolimus analytical reference standard were purchased from Pfizer (Madrid, Spain). Zinc sulphate was obtained from Fluka (Bunch, Switzerland) and formic acid (minimum 95%) from Sigma (St. Louis, MO, USA). Methanol HPLC grade quality was obtained from Riedel-de Haën (Sleeze, Germany) and water was purified with a Milli-Q plus system from Millipore (Bedford, MA, USA).

2.4. Plasma and whole blood samples

Plasma and whole blood samples were obtained from patients with advanced solid tumors belonged to a clinical trial conducted by Centro Integral Oncológico Clara Campal (Fundación Hospitales de Madrid). All patients signed informed-consent forms approved by the ethics committee of these hospitals, Madrid (Spain).

Blood and plasma samples from healthy volunteers for method development and validation were obtained from personnel directly related to this research. For plasma samples, blood samples were collected in lithium heparinized tubes. After centrifugation, the plasma samples were transferred to polypropylene tubes and stored at −80 °C. Whole blood samples were collected in EDTA tubes and stored at −80 °C.

2.5. Standard solutions

Individual 100 mg/L stock standard solutions of paclitaxel, docetaxel, temsirolimus and sirolimus, were prepared in methanol and stored at −20 °C. Intermediate 2.5 mg/L solutions were prepared from the stock solutions by dilution with methanol. Working standards solutions were prepared by appropriate dilutions of the intermediate standard solutions in methanol and both stored at +4 °C. They were stable for several months. Calibration samples were prepared by spiking blank blood or plasma with the analytes.

2.6. Sample treatment procedure

Samples were thawed at room temperature 1 h before the analysis and then homogenized by vortex mixing for 1 min

Quality control plasma samples: 10 μL of a solution containing 2.5 mg/L of internal standard (paclitaxel) in methanol and 10 μL of a solution 2.5 mg/L of docetaxel in methanol were added to 450 μL of drug-free human plasma in an eppendorf. The sample was vortexed vigorously for 1 min and then 1350 μL of methanol:0.2 M zinc sulphate (70:30) (v/v) was added for precipitation of proteins. After vortexing again for 1 min, the samples were centrifuged for 5 min at 9300 × *g* at 4 °C. The clear supernatant was transferred to a vial.

Patient plasma samples: 10 μL of a solution of 2.5 mg/L of internal standard (paclitaxel) in methanol and 10 μL of methanol (to keep the same dilution) were added to 450 μL of patient plasma in an eppendorf. The sample was vortexed vigorously for 1 min and then 1350 μL of methanol:0.2 M zinc sulphate (70:30) (v/v) was added to precipitate the proteins. After vortexing for 1 min, the samples were centrifuged at 9300 × *g*, at 4 °C for 5 min. The clear supernatant was transferred to a vial.

Quality control blood samples: 10 μL of a solution of 2.5 mg/L of internal standard (paclitaxel) in methanol, 10 μL of a solution of 2.5 mg/L of temsirolimus in methanol and 10 μL of a solution of 2.5 mg/L of sirolimus in methanol, were added to 450 μL of drug-free human blood sample in an eppendorf. The sample was vortexed vigorously for 1 min and then 1350 μL of methanol:0.2 M zinc sulphate (70:30) (v/v) was added to precipitate the proteins. After vortexing again for 1 min, the samples were centrifuged for 5 min at 9300 × g, at 4 °C. The clear supernatant was transferred to a vial.

Patient whole blood samples: 10 μL of a solution of 2.5 mg/L of internal standard (paclitaxel) in methanol and 20 μL of methanol (to keep the same dilution) were added to 450 μL of patient blood sample in an eppendorf. The sample was vortexed vigorously for 1 min and then 1350 μL of methanol:0.2 M zinc sulphate (70:30) (v/v) was added to precipitate the proteins. After vortexed again for 1 min, the samples were centrifuged at 9300 × g at 4 °C for 5 min. The clear supernatant was transferred to a vial.

2.7. Validation study

The method was validated for the analysis of docetaxel in plasma and for the analysis of sirolimus and temsirolimus in whole blood in terms of linearity, intra-day, inter-day and instrumental precision, accuracy, limit of quantification, limit of detection and selectivity.

Linearity was tested by the triplicate analysis of plasma samples containing docetaxel over a concentration range (10–200 μg/L, $n = 5$) and the internal standard (paclitaxel) in a final concentration of 50 μg/L. Calibration samples were prepared by spiking blank blood or plasma with the analytes. This was also done for whole blood samples but containing temsirolimus and sirolimus over the same concentration range and using the same internal standard. An ordinary least-squared regression was selected for calibration model. The calibration curves were constructed using nonweighted linear regression.

Instrumental precision was tested to check the constancy of instrumental response to a given analyte in the mid-range of the calibration curve. It was evaluated by multiple injections, $n = 10$, of a homogeneous quality control plasma and quality control whole blood. *Intra-day and inter-day precision* were studied by the analysis of plasma and whole blood samples containing docetaxel and temsirolimus, sirolimus respectively, at 50 μg/L, as the internal standard, in individual preparations ($n = 6$) on each day.

Accuracy study was also performed in triplicate over the same concentration range (10–200 μg/L, $n = 5$). Since calibration samples were prepared with spiked blood or plasma, accuracy was tested with the same samples, by comparing the theoretical area in the linearity curve and the experimental value. As there are no other previous methods based on the proposed sample treatment for docexatel in plasma samples, another accuracy study was performed by comparison of our results with the results obtained by the method of Gardner [30] for some real patient plasma samples ($n = 6$).

The limit of quantification (LOQ) was assessed as the lowest concentration of the drug that could be assayed with a good level of precision. LOQ was calculated following EURACHEM method [32] by injecting six replicates at four levels of concentration in the lower range (0.08–10 μg/L). LOQ was established by representing R.S.D. of the six replicates versus concentration and interpolating the concentration corresponding to 10%. The detection limit (LOD) was calculated by means of the relation LOD: (3/10) × LOQ and checked experimentally.

To demonstrate the *selectivity* of the internal standard and the analytes, patient's samples of both matrix samples were analyzed with and without paclitaxel (IS) to demonstrate that endogenous constituents of the human samples do not interfere with the analyte. The selectivity of the rest of the analytes was demonstrated by measuring two samples of the same patient, one pre-infusion (drug-free), and the other post-infusion. Similar procedure was performed to demonstrate the selectivity of the method with drug-free plasma and blood from healthy volunteers.

3. Results and discussion

3.1. Method development process

The goal of this investigation was to develop a robust, sensitive and easy method to apply a unique protocol for the analysis of these three analytes in blood and plasma samples, to validate and to apply this method to the samples from patients receiving combined antitumor therapy.

For protein precipitation, zinc sulphate was the agent that allows the erythrocytes to lyse and it allows a very clean supernatant and method of Taylor [10] was performed for both fluids. The clear superpernatant, free of proteins, was transferred to an HPLC vial for the analysis.

For SPE, online option offered many advantages as automatization, lower variability, saving operator time, higher stability of extracted samples in the autosampler at +4 °C, etc. Extraction column and analytical column were the same as the method of Zhang [22]. Chromatographic conditions (for extraction and separation) were modified for improving the separation of the analytes, so aqueous content in mobile phase was incremented at time zero for decreasing its elutrophic power, especially important for obtaining a good resolution between docetaxel and paclitaxel. As docetaxel, paclitaxel, temsirolimus and sirolimus have constant log *D* from pH 0 to 10, variation of pH does not influence the retention in reverse mode LC. Formic acid in the mobile phase was added to enhance

Table 4
Validation results for docetaxel in plasma.

Plasma samples		Docetaxel
Linearity ($n=15$)	Slope	0.02898 ± 0.00063
	Intercept	-0.036 ± 0.066
	R	0.999
Range	(μg/L)	10–200
Instrumental precision	($n=10$) RSD (%)	1.5
Sample precision	Inter-assay ($n=12$) RSD (%)	2.1
	Intra-assay ($n=6$) RSD (%)	1.5
Accuracy	%	99.7
	RSD	4.1
LOQ	(μg/L)	0.14
LOD	(μg/L)	0.043

the positive MS detection. After checking different gradients in both pumps (extraction and analytical), the best results were obtained with the conditions described in Table 1.

During validation assays linearity for docetaxel standard was processed both in a solvent (methanol) and added to the matrix (plasma) with the aim of checking not only the linearity range but also recovery and/or potential suppression effects. Linearity range was tested from 10 to 100 ppb. The internal standard (paclitaxel) was also included.

Response factor for docetaxel in methanol was 0.021 (RSD 6.5%) while in plasma was 0.015 (RSD 7.1%). This lower response when working in plasma could be associated to either a decrease in recovery due to sample treatment or some suppression effect. However, in both cases, the problem was solved by performing the calibration with the standard spiked into the matrix. Data for calibrations in blood or plasma are included in Section 2.7. Real samples were measured against this calibration curve and therefore those effects were compensated. Therefore, calibration standards were prepared in human drug-free blood or plasma samples by spiking whole blood with 0, 10, 25, 50, 100 and 200 μg/L of temsirolimus and sirolimus, and with the same concentrations of docetaxel for plasma samples. Quality control samples were prepared as 50 μg/L calibration standards.

3.2. Validation

A complete validation of the analytical method was performed for the analysis of docetaxel in plasma samples and for the analysis of temsirolimus and sirolimus in whole blood samples. A summary of the validation parameters is shown in Tables 4 and 5, and will be briefly discussed.

Table 5
Validation results for temsirolimus and sirolimus in whole blood.

Blood samples		Temsirolimus	Sirolimus
Linearity ($n=15$)	Slope	0.00968 ± 0.00066	0.00893 ± 0.00055
	Intercept	0.019 ± 0.071	-0.039 ± 0.059
	R	0.993	0.994
Range	(μg/L)	10–200	10–200
Instrumental precision	($n=10$) RSD (%)	3.1	3.2
Sample precision	Inter-assay ($n=12$) RSD (%)	7.2	7.0
	Intra-assay ($n=6$) RSD (%)	4.3	4.8
Accuracy	%	102.5	99.7
	RSD	9.0	9.8
LOQ	(μg/L)	0.69	0.76
LOD	(μg/L)	0.21	0.23

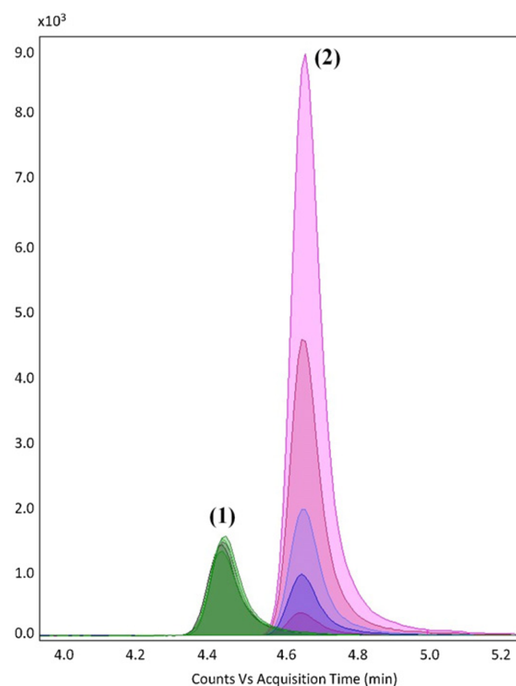

Fig. 4. Linearity of docetaxel in plasma over the concentration range 10–200 μg/L. (1) Paclitaxel; (2) docetaxel. For LC–MS/MS conditions see the text.

The validation parameters obtained for plasma are included in Table 4, the linearity study was performed with calibration samples and fit the linear model ($r > 0.99$) for docetaxel and no bias was found, because the confidence limits of the intercept include the zero value. Study of precision for docetaxel was based on the quality control plasma samples. Instrumental precision gave RSD of 1.5% ($n=10$)RSD of 1.5% (n = 10). Intra-assay precision offered 1.5% day one ffered 1.5% day one ($n=6$) and Inter-assay offered 2.1% ($n=12$)ffered 2.1% (n = 12) on two different days.

Recoveries ranged from 93.0 to 105.0% and 7.0% taking into account their RSDs, they did not statistically differ from 100 ($p \leq 0.05$). As final evaluation of the analysis method, docetaxel was determined by the proposed method in six different patient plasma samples (concentration range: 50–200 μg/L) and compared with the results obtained by a previously published method, for determination of total docetaxel, based on protein precipitation with ACN [30]. As the addition of methanol/zinc sulphate to the

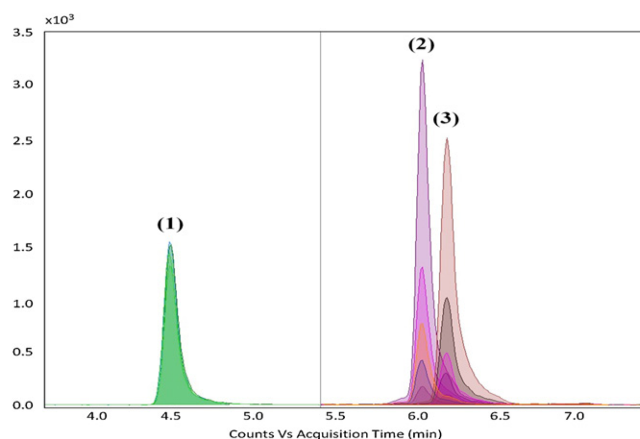

Fig. 5. Linearity of temsirolimus and sirolimus in whole blood over the concentration range 10–200 μg/L. (1) Paclitaxel; (2) temsirolimus; (3) sirolimus. For LC–MS/MS conditions see the text.

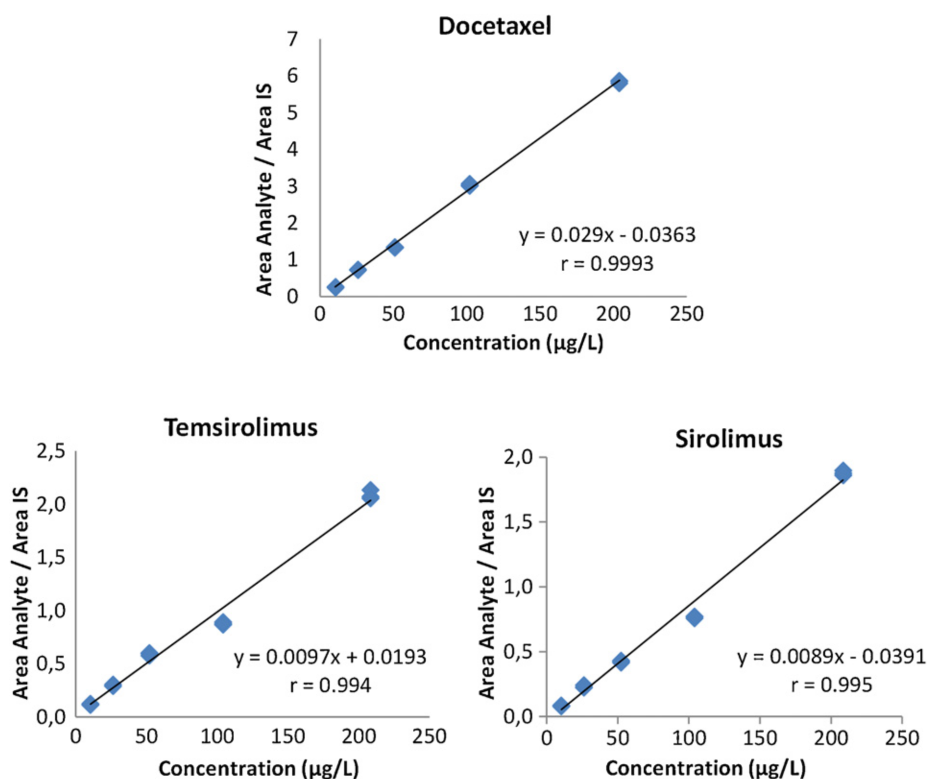

Fig. 6. Calibration curves obtained in the linearity study for docetaxel, temsirolimus and sirolimus.

plasma is a new strategy, an accuracy study is recommended. The obtained results with the method of Gardner [30] ranged from 53.3 to 168.0 μg/L and with this method 56.5–154.9 μg/L. The paired Student t-test was performed and the results are in good agreement, for ($p < 0.05$) t_{cal}: 0.818 and t_{tabl}: 2.447.

The limit of quantification considering 10% RSD with 6 consecutives injections was 144 ng/L, good enough compared to others previously published with SPE–LC–MS methods [11] and much better than others using off-line SPE even with LC–MS.

The study of selectivity by the comparison of $n = 10$ different blank plasma samples demonstrate no interference peaks with internal standard and also with docetaxel.

Regarding the validation parameters obtained for blood seen in Table 5, the linearity study was performed with calibration samples and fit the linear model ($r > 0.99$) for temsirolimus and sirolimus and no bias was found, because the confidence limits of the intercept include the zero value.

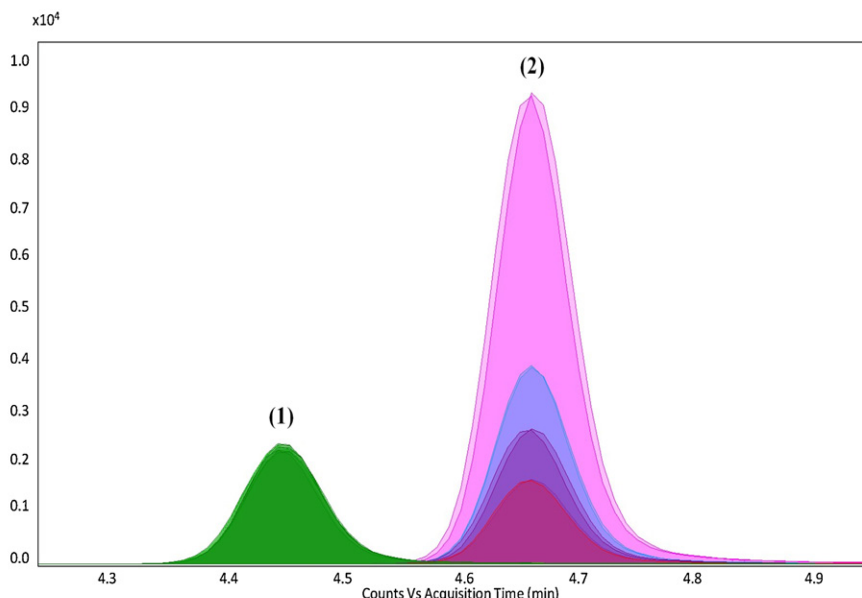

Fig. 7. Online SPE–LC–MS/MS chromatographic profile of patient plasma in treatment with temsirolimus and docetaxel during docetaxel cycle. (1) Paclitaxel; (2) docetaxel.

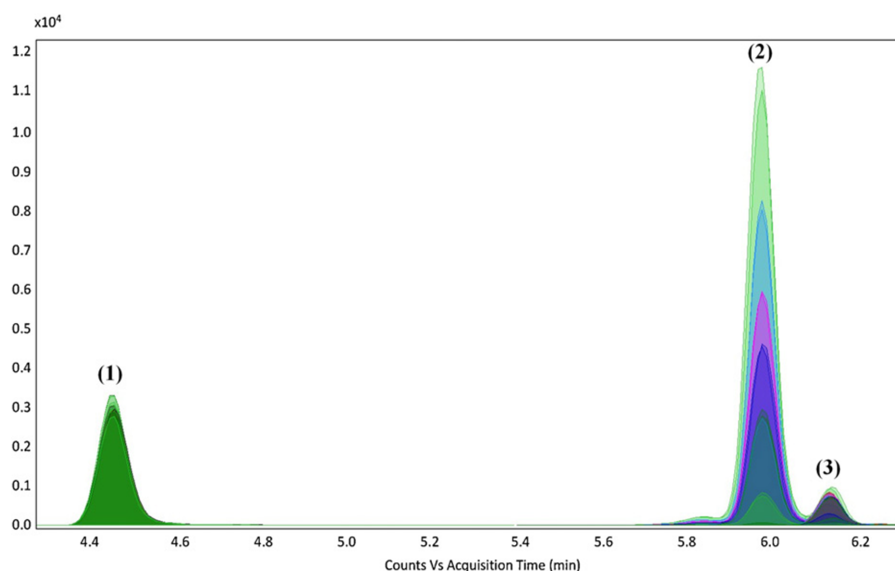

Fig. 8. Online SPE–LC–MS/MS chromatographic profile of patient whole blood in treatment with temsirolimus and docetaxel during temsirolimus cycle. (1) Paclitaxel; (2) temsirolimus; (3) sirolimus.

A precision study was based also on the quality control blood samples. Instrumental precision for temsirolimus gave RSD of 3.1% and for RSD of 3.1% and for sirolimus gave RSD of 3.2 (n = 10).RSD of 3.2 (n = 10). Intra-assay precision offered 4.3% and 4.8% for temsirolimus and sirolimus, respectively (n = 6) and the inter-assay offered 7.2% and 7.0%, respectively (n = 12) on two different days.

Recoveries ranged from 85.1 to 114.6% for temsirolimus and 85.7 to 114.3% for sirolimus and taking into account their RSDs, they did not statistically differ from 100 ($p \leq 0.05$).

The limit of quantification considering 10% RSD with 6 consecutive injections were 695 ng/L for temsirolimus and 756 ng/L for sirolimus, concordant value with a previously study [20].

The study of selectivity by comparison of the n = 10 different blank blood samples demonstrates no interferences either with the internal standard, temsirolimus or sirolimus.

The quantitation of patient samples were performed with quality control samples containing 50 μg/L of docetaxel in plasma samples and with quality control samples containing 50 μg/L of temsirolimus and 50 μg/L of sirolimus in whole blood samples during the pharmacokinetic studies.

Fig. 4 shows the plasma chromatograms in the linearity study of docetaxel and Fig. 5, the whole blood chromatographic profiles in the linearity study of temsirolimus and sirolimus. Fig. 6 shows the calibration curves obtained in the linearity study for docetaxel, temsirolimus and sirolimus.

Table 6
Comparison of docetaxel concentrations in plasma samples for two different patients (A and B) under the same dose: docetaxel 100 mg during 60 min. Patient A concentrations correspond to the chromatogram showed in Fig. 7.

Time from the beginning of the infusion (h)	Docetaxel concentration (μg/L)	
	Patient A	Patient B
−0:30	ND	ND
02:00	120.43	176.17
03:00	50.19	68.15
04:00	34.73	39.52
07:00	27.77	22.92

ND: not detected value.

Table 7
Comparison of temsirolimus concentration in two different patients (C and D) under the same dose: temsirolimus 15 mg during 30 min. Patient C concentrations correspond to the chromatogram showed in Fig. 8.

Time from the beginning of the infusion (h)	Temsirolimus concentration (μg/L)		Sirolimus concentration (μg/L)	
	Patient C	Patient D	Patient C	Patient D
−00:05	ND	ND	ND	ND
00:45	317.60	156.75	31.27	37.03
01:30	160.22	88.79	29.02	33.26
02:30	138.59	64.49	31.51	30.56
04:30	84.79	43.36	23.44	27.90

ND: not detected value.

3.3. Applicability to treated samples

The method was used to quantify the concentrations of docetaxel in plasma and temsirolimus and sirolimus in two whole blood samples of patients who received combined therapy with docetaxel and temsirolimus by infusion. Samples were obtained at different times before, during and post infusion to assess the pharmacokinetics parameters. Sirolimus was also analyzed because it is the main active metabolite of temsirolimus metabolism.

The samples were quantified by correction with the internal standard. Fig. 7 shows the plasma concentration–time profile of a patient receiving a combination therapy of docetaxel and temsirolimus in the docetaxel cycle after receiving 100 mg of docexatel. Fig. 8 shows the whole blood concentration-time profile of a patient with the same treatment, but in the temsirolimus cycle after receiving 15 mg of temsirolimus, both by intravenous infusion.

The obtained concentrations for two patients, before and four different times after the infusion of 100 mg of docetaxel during 60 min, are included in Table 6 for plasma samples and Table 7 for temsirolimus and sirolimus in whole samples for two patients after the infusion of 15 mg of temsirolimus for 30 min.

4. Conclusions

The assay reported in this investigation provides a general strategy for the LC–MS analysis of three anti-tumors as docetaxel, temsirolimus and sirolimus in blood and plasma samples during a combined therapy assay.

Some methods have been published for docetaxel, temsirolimus and sirolimus, but were different for each group of compounds and the preparation time was time consuming. The described method allows the use of the same reagents, sample treatment and analytical technique independently of whether starting with whole blood or plasma samples, for the quantification of the studied drugs. The described method will be faster, simpler and less expensive to implement, and to maintain a routine clinical laboratory; moreover, it is easier to automate. Harmonized methodologies in clinical laboratory could help in saving time and resources.

Combined chemotherapy involves lower doses of each drug that results in very low concentration of them in plasma and blood samples [9]. Therefore one of the biggest challenges was to determine the lowest concentration in both matrix samples for these drugs.

The method has been successfully validated and applied to real samples and it is a suitable method for dose adjustment and for evaluating potential drugs interactions during combined treatments.

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References

[1] P. Cai, R. Tsao, M.E. Ruppen, Drug Metab. Dispos. 35 (2007) 1554.
[2] F. Chan, E.E. Samlowski, W.E. Samlowski, Clin. Med. Insights: Ther. 1 (2009) 167.
[3] E.A. Eisenhauer, J.B. Vermorken, Drugs 55 (1998) 5.
[4] W.J. Loos, J. Verweij, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. B: Biomed. Sci. Appl. 693 (1997) 437.
[5] S.N. Sehgal, K. Molnar-Kimber, T.D. Ocain, B.M. Weichman, Med. Res. Rev. 14 (1994) 1.
[6] M.E. Trudeau, Can. J. Oncol. 6 (1996) 443.
[7] L. Zufía López, A. Aldaz Pastor, J.M. Aramendia Beitia, J. Arrobas Velilla, J. Giraldez Deiró, Ther. Drug Monit. 28 (2006) 199.
[8] D. Del Bufalo, L. Ciuffreda, D. Trisciuoglio, M. Desideri, F. Cognetti, G. Zupi, M. Milella, Cancer Res. 66 (2006) 5549.
[9] R.A. Parise, R.K. Ramanathan, W.C. Zamboni, M.J. Egorin, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 783 (2003) 231.
[10] P.J. Taylor, A.G. Johnson, J. Chromatogr. B: Biomed. Sci. Appl. 718 (1998) 251.
[11] D.L. Gustafson, M.E. Long, J.A. Zirrolli, M.W. Duncan, S.N. Holden, A.S. Pierson, S.G. Eckhardt, Cancer Chemother. Pharmacol. 52 (2003) 159.
[12] J.C. Vergniol, R. Bruno, G. Montay, A. Frydman, J. Chromatogr. 582 (1992) 273.
[13] R. Wang, X.Y. Wu, H. Xie, H. Zhang, J. Ma, et al., J. Bioanal. Biomed. 2 (2010) 023.
[14] H. Rosing, V. Lustig, F.P. Koopman, W.W. ten Bokkel Huinink, J.H. Beijnen, J. Chromatogr. B: Biomed. Sci. Appl. 696 (1997) 89.
[15] P.F. Brunsvig, A. Andersen, S. Aamdal, V. Kristensen, H. Olsen, BMC Cancer 7 (2007) 197.
[16] M.B. Garg, S.P. Ackland, J. Chromatogr. B: Biomed. Sci. Appl. 748 (2000) 383.
[17] A. Andersen, D.J. Warren, P.F. Brunsvig, S. Aamdal, G.B. Kristensen, H. Olsen, BMC Clin. Pharmacol. 6 (2006) 2.
[18] S.D. Baker, M. Zhao, C.K. Lee, J. Verweij, Y. Zabelina, J.R. Brahmer, A.C. Wolff, A. Sparreboom, M.A. Carducci, Clin. Cancer Res. 10 (2004) 1976.
[19] J. Ciccolini, J. Catalin, M.F. Blachon, A. Durand, J. Chromatogr. B: Biomed. Sci. Appl. 759 (2001) 299.
[20] D.W. Holt, T. Lee, K. Jones, A. Johnston, Clin. Chem. 46 (2000) 1179.
[21] E. Raymond, J. Alexandre, S. Faivre, K. Vera, E. Materman, J. Boni, C. Leister, J. Korth-Bradley, A. Hanauske, J.P. Armand, J. Clin. Oncol. 22 (2004) 2336.
[22] Y.L. Zhang, J. Bendrick-Peart, T. Strom, M. Haschke, U. Christians, Ther. Drug Monit. 27 (2005) 770.
[23] F. Streit, U. Christians, H.M. Schiebel, K.L. Napoli, L. Ernst, A. Linck, B.D. Kahan, K.F. Sewing, Clin. Chem. 42 (1996) 1417.
[24] D.C. French, M. Saltzgueber, D.R. Hicks, A.L. Cowper, D.W. Holt, Clin. Chem. 47 (2001) 1316.
[25] G. Khoschsorur, Clin. Chem. 51 (2005) 1721.
[26] T. Koal, M. Deters, B. Casetta, V. Kaever, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 805 (2004) 215.
[27] M. Karapirli, M. Kizilgun, O. Yesilyurt, H. Gul, Z.I. Kunak, E.O. Akgul, E. Macit, T. Cayci, Y. Gulcan Kurt, I. Aydin, H. Yaren, M. Seyrek, E. Cakir, H. Yaman, Sci. World J. 2012 (2012) 571201.
[28] N. Ansermot, M. Fathi, J.L. Veuthey, J. Desmeules, S. Rudaz, D. Hochstrasser, Clin. Biochem. 41 (2008) 728.
[29] P.J. Larger, M. Breda, D. Fraier, H. Hughes, C.A. James, J. Pharm. Biomed. Anal. 39 (2005) 206.
[30] E.R. Gardner, W. Dahut, W.D. Figg, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 862 (2008) 213.
[31] H. Yamaguchi, A. Fujikawa, H. Ito, N. Tanaka, A. Furugen, K. Miyamori, N. Takahashi, J. Ogura, M. Kobayashi, T. Yamada, N. Mano, K. Iseki, J. Chromatogr. B 893–894 (2012) 157.
[32] EURACHEM Working Group, the Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics. Available from: http://www.eurachem.org/guides/pdf/valid.pdf