Journal of Pharmaceutical and Biomedical Analysis 115 (2015) 300–306

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

An ultrasensitive LC–MS/MS method with liquid phase extraction to determine paclitaxel in both cell culture medium and lysate promising quantification of drug nanocarriers release *in vitro*

Tarek Baati[a,**], Thérèse Schembri[a,c], Claude Villard[a,c], Florian Correard[a,b], Diane Braguer[a,b,*], Marie-Anne Estève[a,b]

[a] *Aix Marseille Université, INSERM, CRO2 UMR_S911, Faculté de pharmacie, 27 boulevard Jean Moulin, Marseille 13385, France*
[b] *Assistance Publique - Hôpitaux de Marseille, Hôpital Timone, 254 rue Saint Pierre, Marseille 13385, France*
[c] *Plateforme Protéomique d'Innovation Technologique Timone (PIT2), Faculté de pharmacie, 27 boulevard Jean Moulin, Marseille 13385, France*

ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form 20 July 2015
Accepted 26 July 2015
Available online 29 July 2015

Keywords:
Paclitaxel
Docetaxel
LC–MS/MS
Liquid–liquid extraction
A549 human cancer cells

ABSTRACT

The quantification of paclitaxel, a chemotherapy drug used to treat different types of cancers, has been performed from complete cell culture medium and cell lysate samples using a simple liquid–liquid extraction procedure in conjunction with liquid chromatography tandem mass spectrometry (LC–MS/MS). A simple sample preparation using methanol and acetic acid as a weaker acid was applied to avoid paclitaxel destruction and to achieve recovery exceeding 80 % from both matrices spiked with paclitaxel and docetaxel used as internal standard. This rapid, simple, selective and sensitive method enabled the quantification of paclitaxel within the linear range of 1–250 nM in culture medium and 5–250 nM in cell lysate. The lower limit of quantification was achieved in cell culture medium and cell lysates at 0.2 and 1 pmol, respectively. This method was successfully applied to human non-small cell lung carcinoma cells (A549 cells) in order to quantify the amount of paclitaxel in both cell culture medium and lysate after incubation with 5, 50 and 100 nM of paclitaxel. This ultra-sensitive method promises the quantification of ultra-low concentrations of paclitaxel released from any nanocarriers, allowing the determination of the kinetic profile of drug release, which is an essential parameter to validate the use of nanocarriers for drug delivery in cancer therapy.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Paclitaxel (denoted PT*x*) is a major chemotherapy drug used for treatment of different types of cancers including ovarian, breast, lung and pancreatic cancer [1–3]. Numerous *in vitro* or preclinical experiments suggest that PT*x* and other microtubule-targeting drugs can exert anti-vascular or anti-angiogenic effects and some of these effects are observed at ultra-low concentrations [4–9]. Moreover, poor aqueous solubility and serious side effects associated with commercial preparation of PT*x* (Taxol®) triggered the development of alternative PT*x* formulations without cremophor. Different nanocarrier systems including nanoparticles, liposomes, micelles, bioconjugates and dendrimers have been employed to improve PT*x* solubility and eliminate undesired side effects [10–15]. It was reported that PT*x*, delivered in polyanhydrides, was released very slowly, and only 15% of 0.042 mg/mL of PT*x* was released in 77 days [16]. Since PT*x* release kinetic is time and medium composition depending, a highly sensitive quantification of drug at short time is required to validate the use of such nanocarriers for drug delivery in cancer therapy. Indeed, due to its high hydrophobicity, most of *in vitro* studies of PT*x* release from nanocarriers were performed in simulated physiological medium supplemented with surfactant Tween 80 [11–18]. Numerous analytical methods using liquid phase extraction and solid phase extraction combined to the LC–MS/MS technique were developed for ultrasensitive quantifi-

Abbreviations: LC–MS/MS, liquid chromatography tandem mass spectrometry; PT*x*, paclitaxel; UPLC, ultra-performance liquid chromatography; MS, mass spectrometry; DT*x*, docetaxel; SDS, sodium dodecyl sulfate; A549 cells, human non-small cell lung carcinoma cells; ACN, acetonitrile; QC, quality control sample; LOD, limit of detection; LOQ, limit of quantification; RS, relative standard deviation; PBS, phosphate buffered saline; EDTA, ethylenediamine tetraacetate; MRM, multiple reactions monitoring; ESI, electrospray ionization.

* Corresponding author at: Aix Marseille Université, INSERM, CRO2 UMR_S911, Faculté de pharmacie, 27 boulevard Jean Moulin, Marseille 13385, France.
** Corresponding author.
E-mail addresses: tarek.baati@univ-amu.fr (T. Baati), diane.braguer@univ-amu.fr (D. Braguer).

http://dx.doi.org/10.1016/j.jpba.2015.07.041
0731-7085/© 2015 Elsevier B.V. All rights reserved.

cation of PT*x* in biological samples such as cells, plasma, urine, feces or tissues [19–30]. Nevertheless, most of the published works use high-performance liquid chromatography or spectrophotometric UV–vis absorbance [11–15,17] and radio labeled [16] detection to determine the *in vitro* profile of PT*x* release from nanocarriers. This makes us thinking about to what extend LC–MS/MS reported methods were suitable to quantify PT*x* in biological medium such as complete cell culture medium supplemented with 10% of fetal bovine serum. It is worth noting that these methods recorded a high sensisitivity of PT*x* quantification ranging from 0.1 ng/mL to 0.2 pg/mL [19–21,24,25]. However, none of these methods is actually used to quantify PT*x* release from nanocarriers in complete cell culture medium, in order to determine the drug release profile before exposing to cells. This can be attributed to numerous constraints such as the requirement of large amount of biological sample (minimum 0.5 mL) [28–30], high cost of sample purification procedures [20], more complex and time consuming solid-phase extraction since it is commonly specific to clean-up tissue samples [24–26,30]. Moreover, the use of highly sensitive analytical method such as LC–MS/MS is required to perfectly determine the kinetic profile of PT*x* release from nanocarriers in cell culture medium prior the cytototoxicity studies. Since the liquid phase extraction is very easy to applicate on sample treatment and it allows great specificity with less sample loss and damage, we developed and validated in this work a simple and rapid enabling analytical method with liquid-phase extraction combined to ultra-performance liquid chromatography (UPLC) coupled to mass spectrometry (MS) for ultra-sensitive and selective quantification of PT*x* in complete cell culture medium and cell lysate. This method promises ultra-sensitive quantification of drug release from nanocarriers in any cell culture medium. Finally, this method was applied to quantify PT*x* in cancer cell lysates following exposure to low nanomolar but pharmacologically relevant drug concentrations. This task is challenging because the drug is expected to be present in biological matrices at ultra-low-to-medium pmol levels.

2. Materials and methods

2.1. Reagents and chemicals

PT*x* was obtained from Chemieliva (China). Docetaxel (denoted DT*x*), sodium dodecyl sulfate (SDS), and acetic acid were obtained from Sigma–Aldrich (France). UPLC–MS-grade methanol, acetonitrile (ACN), and water were obtained from Fisher Scientific (France). Human non-small cell lung carcinoma cells (A549 cells) were obtained from American Type Culture Collection.

2.2. Standard solutions and method validation

Standard solutions of PT*x* (PT*x* = 1.0 μM) have been initially prepared in methanol and diluted to the appropriate concentrations, which were used to study the recoveries and the accuracy of the method in cell lysates samples and medium. Stock solution of 1.0 μM of DT*x* (used as internal standard) was prepared in methanol. All the previous solutions were stored at 4 °C. The linearity was tested by the use of PT*x* and DT*x* standards prepared in methanol. For the biological matrix, the linearity was tested by the spiked standards in both cell lysates and complete cell culture medium samples. Calibration standards were prepared in biological matrix by spiking of the appropriate quantity of PT*x* into cell lysates or cell culture medium samples to final concentrations of 1, 2.5, 5, 10, 50, 100 and 250 nM. DT*x* was spiked to a final concentration of 100 nM. In order to validate the method, quality control samples (QC) were prepared in methanol to final concentrations of 1, 5, 10, 50 nM of PT*x* and 100 nM of DT*x*. Weighted least squares linear regression analysis was used for construction of calibration curves from peak area ratios between PT*x* and DT*x* and for samples quantification.

The limit of detection (LOD) and the limit of quantification (LOQ) were measured according to the Food and Drug Administration guidance [31]. The LOD can be defined as the lowest PT*x* concentration that the present assay can reliably differentiate from the background noise (signal noise ≥3). The LOQ was determined by spiking a control cell lysate or culture medium sample with PT*x* at the concentration of the lowest calibrator with a 20% precision and 80–120% accuracy. For method validation, accuracies were obtained by comparing concentrations calculated from standard curves to the nominal concentrations at the fourth quality control levels. Precision of the method was determined by repeated analysis of spiked cell lysates and medium culture samples. The relative standard deviation (RSD, %) of the replicate measurement was calculated to determine the intra- and inter-days variabilities. For intra-day validation, each QC sample was evaluated five times in a day ($n = 5$). For inter-day validation each QC sample was measured on 5 days in a week once per day. The criterion for acceptability of the data was accuracy within ±15% RSD as previously reported for analytical methods [31].

The stability of PT*x* in both cell culture medium and lysate matrices (matrices spiked with PT*x* at 5, 10 and 50 nM) was determined by analyzing the extracted samples that were stored in different conditions including: (1) room temperature and light irradiation for 24 h, (2) post-preparative stability: the extracted samples were stored at 4 °C for 48 h and one week, (3) three cycles of freeze and thaw (freezing at −20 °C for 24 h and thawing at room temperature), (4) long-term storing at −20 °C for one week and one month. For stability assessment, all the samples were analyzed in triplicate. Under manifold storage and processing conditions, the stability of PT*x* in both matrices was evaluated by analyzing three replicate for QC samples at 5, 10 and 50 nM.

2.3. Cell samples preparation

A549 cells were grown in 25 cm^2 flasks in cell culture medium (RPMI 1640) supplemented with 10% fetal bovine serum and 1% (v/v) antibiotics (penicillin–streptomycin) and incubated at 37 °C with 5% CO_2 in a humidified incubators described previously [5]. Cells were used for method validation or PT*x* uptake studies when 90% confluence was reached. To harvest cells for analysis, the cell culture medium was removed and the cell monolayer was washed rapidly with 3 mL of phosphate buffered saline (PBS) and then incubated in 0.5 mL of 0.05% trypsin containing 1 mM ethylenediamine tetraacetate (EDTA). After 5 min of incubation at 37 °C, the dissociated cells were collected into a tube, counted using a Beckman Coulter Z2Counter then seeded at the density of 18,750 cells/cm^2 in a 12-well plate (3.5 cm^2) 24 h before treatment. In order to determine the drug uptake, cells were exposed during 24 h to 2.5; 25 and 75 pmol of PT*x* (5, 50 and 150 nM). An equivalent cell number, used as control, was seeded as previously in three wells and exposed to classical RPMI medium without PT*x*.

2.4. Control and treated samples

24 h after PT*x* treatment, the culture medium (500 μL) was collected from each well and then centrifuged at 14,500 rpm for 10 min. The cell monolayer was rapidly washed with 0.3 mL of PBS and then incubated in 0.3 mL of 0.05% trypsin containing 1 mM of EDTA. After 5 min of incubation at 37 °C, the dissociated cells were collected into a tube and then centrifuged at 14,500 rpm for 10 min. Both the control supernatant and cell pellet were used to validate

Download English Version:

https://daneshyari.com/en/article/7629508

Download Persian Version:

https://daneshyari.com/article/7629508

Daneshyari.com