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A full validated hydrophilic interaction liquid chromatography–tandem mass spectrometric method for the quantification of oxaliplatin in human plasma ultrafiltrates

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ABSTRACT

Oxaliplatin is a platinum agent that is used for treatment of colorectal cancer. A sensitive and selective hydrophilic interaction liquid chromatography–tandem mass spectrometric method for the quantification of oxaliplatin was developed. Human plasma ultrafiltrates were precipitated by acetonitrile containing carboplatin as an internal standard and further diluted with acetonitrile. Chromatographic separation of oxaliplatin and the internal standard was achieved with a column modified with phosphorylcholine and an isocratic mobile phase (acetonitrile/water/acetic acid = 90:10:0.1, v/v/v) at the flow rate of 0.2 mL/min. The lower limit of quantification for oxaliplatin was 25 ng/mL. The linearity range of the method was from 25 to 5000 ng/mL. The intra-day precision and inter-day precision (RSD) ranged from 0.8 to 6.1%, and the accuracy (RE) was within ±4.5%. The extraction recoveries from human plasma ultrafiltrates were 83.6–91.6%, and ion suppression caused by matrix components was 86.7–88.5% at three different levels, respectively. This method was applied to a clinical pharmacokinetic study of oxaliplatin in a cancer patient. The maximum concentration of colorectal cancer patient administered oxaliplatin was 1650 ng/mL.

1. Introduction

Oxaliplatin (1R,2R-diaminocyclohexane-oxalatoplatinum(II)) is a third-generation of platinum agent that is used for treatment of colorectal cancer. Oxaliplatin was developed to reduce the nephrotoxicity of cisplatin as the first-generation platinum agents and myelosuppression of carboplatin as the second-generation platinum agents. Platinum agents are activated intracellularly to form reactive platinum complexes that bind to nucleophilic groups producing both inter- and intra-strand crosslinks in DNA, thereby inhibiting DNA replication [1]. The majority of previously untreated metastatic colorectal cancer patients are treated with oxaliplatin and infusional 5-fluorouracil/leucovorin or capecitabine (FOLFOX or XELOX) [2]. It has been reported that the AUC of platinum agents correlates with the degree of adverse events such as thrombopenia [3,4]. Besides, oxaliplatin was one of major organic cation transporter (OCT) 2 substrates. OCT2 is located on the basolateral membrane of the proximal tubular epithelium in the kidney and related to uptake of cationic drugs in the kidney. Burger et al. indicated that high OCT2 expression caused to platinum toxicity [5]. Therefore, therapeutic drug monitoring of platinum agents could be an effective tool to reduce adverse effects. To establish therapeutic drug monitoring of platinum agents, it is necessary to determine the plasma target concentration and to clarify the cause of variation in pharmacokinetics.

Methods using atomic absorption spectrometry [6] and inductively coupled plasma-mass spectrometry [7] have been developed for the determination of oxaliplatin. However, the total quantity of the element platinum is measured by these methods, and the intact form cannot be distinguished from inactive platinum intermediates. For pharmacokinetic investigation, it is necessary to measure intact oxaliplatin. Ficarra et al. reported the HPLC-UV method for measuring intact oxaliplatin [8]. However, the inherent

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lack of favorable UV absorption properties of these agents has led to poor sensitivity. They reported that the limit of quantification of oxaliplatin was 70 μg/mL [8]. To obtain more sensitivity with UV detection, post-column derivatization techniques have been needed [9,10].

The LC/MS/MS system has been widely used for the determination of drugs because of its high sensitivity and selectivity. Luo et al. reported the LC/ESI-MS method for the determination of the intact oxaliplatin and its biotransformation products in rat plasma [11]. Recently, Zhang et al. reported the LC/MS/MS method for the determination of the intact oxaliplatin in human plasma [12]. The sensitivity of their method is comparable with that of atomic absorption spectrometry. However, they used a long ODS column (250 mm) to avoid the matrix effect by endogenous impurities. Hydrophilic interaction chromatography (HILIC) using bare silica or polar bonded phase and low aqueous/high organic mobile phase has been shown to be a suitable method for the quantification of polar compounds in biological samples. HILIC is superior separation of polar compounds in biological samples with reversed retention to the traditional reversed-phase liquid chromatography [13–16]. Recently, Hemström et al. and Falta et al. reported the ICP-MS method combined with HILIC for the determination of total platinum concentrations in human plasma ultrafiltrates [17,18]. However, the method was not validated, so we considered that their method did not have higher confidence. Their method could determine total platinum concentrations, but that could not distinguish intact oxaliplatin from platinum intermediates which do not have biological activity. Moreover, the method was not applied to the actual patients, who were administered oxaliplatin.

In this paper, we describe a HILIC/MS/MS method for the determination of oxaliplatin in human plasma ultrafiltrates and results of application of the method to a pharmacokinetic study in a cancer patient. The sample preparation of our established method employs protein precipitation and dilution with acetonitrile without evaporation step, meaning that not only it needs simple technique but also the exposure of antineoplastic drugs could be avoidable.

2. Materials and methods

2.1. Materials

Oxaliplatin, carboplatin, and acetic acid were purchased from Wako (Osaka, Japan). Purity of oxaliplatin standard was 97.0+%. All other solvents and regents were of HPLC grade. Human plasma with sodium heparin as the anticoagulant was obtained from Terumo (Tokyo, Japan).

2.2. Sample preparation

2.2.1. Preparation of stock and working solution

A stock solution of oxaliplatin was prepared in methanol/water (50:50, v/v) at a concentration of 100 μg/mL and was stored at −80 °C. As an internal standard solution, 10 μg/mL carboplatin in acetonitrile was stored in the dark at −80 °C.

2.2.2. Preparation of calibration standards and validation samples

We obtained human plasma ultrafiltrates using Centrifree® Ultrafiltration Devices purchased from Millipore (Tokyo, Japan). Calibration standards of oxaliplatin in human plasma ultrafiltrates were prepared by diluting a stock solution at a concentration of 5000 ng/mL and serially diluting a plasma working solution at concentrations of 25, 50, 100, 200, 500, 1000 and 2000 ng/mL. Quality Control (QC) samples of oxaliplatin in human plasma ultrafiltrates were prepared by diluting human plasma ultrafiltrates working solutions at concentrations of 25, 50, 500 and 5000 ng/mL. All solutions were stored at −80 °C.

2.3. Sample preparation

Human plasma ultrafiltrates were precipitated by acetonitrile containing carboplatin as the internal standard. To 40-μL sample volumes, 60 μL internal standard in acetonitrile containing 10,000 ng/mL of internal standard was added. The samples were then centrifuged for 10 min at 10,000 × *g*. The supernatant was diluted twenty-fold with acetonitrile. About 10 μL of solution was injected into the HPLC column.

2.4. Chromatographic and mass spectrometric conditions

The HPLC system consisted of a fully equipped Prominence20A (Shimadzu, Kyoto, Japan).

For the analytical column, we used PC HILIC (50 mm × 2.0 mm i.d., 3 μm, Shiseido, Tokyo, Japan). The column temperature was maintained at 40 °C. Mobile phase A consisted of acetonitrile/water/acetic acid (90:10:0.1, v/v/v), and mobile phase B consisted of acetonitrile/water (50:50, v/v). The initial mobile phase composition was 100% mobile phase A, pumped at a flow rate of 0.2 mL/min for 5.0 min. From 5.0 to 5.5 min, mobile phase B was increased linearly from 0 to 100%. For removal of insoluble salts and matrix components, this setting was held for 4.5 min. From 10.0 to 10.5 min, mobile phase B was decreased to 0% and kept at that rate until 15.0 min, after which the next sample was injected. From 0 to 5 min, the flow was introduced into a mass spectrometer using a switching valve. The overall run time was 15.0 min.

Mass spectrometry was carried out in an API3200 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Positive ionization electrospray mass spectrometry was performed. For the determination oxaliplatin, the ionspray voltage was set at 5000 V. The turbospray gas (N_2) probe was heated at 500 °C. Nitrogen was used as curtain gas, gas 1 and gas 2, and their flows were set to 50, 60 and 80 units, respectively. Unit mass resolution was set in both mass resolving quadrupole Q1 and Q3. The declustering potential (DP) and collision energy (CE) for oxaliplatin were 32 V and 19 V, and these for internal standard were 46 V and 27 V, respectively. The transitions of m/z 398 → 306 and m/z 372 → 294 on selected reaction monitoring (SRM) were used for monitoring oxaliplatin and carboplatin, respectively. The dwell time was 500 ms. Data were collected and processed using Analyst 1.4.2 data collection and integration software (Applied Biosystems).

2.5. Method validation

2.5.1. Linearity and lower limit of quantification (LLOQ)

For validation, oxaliplatin standards (eight non-zero standards of the analyte, 25, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL for oxaliplatin) were prepared in control human plasma ultrafiltrates and analyzed. Linear regression of ratio of the areas of the analyte and internal standard peaks versus the concentration were weighted by $1/x$ (reciprocal of the concentration). LLOQ was defined as the concentration with a signal-to-noise ratio of at least 10 and acceptable precision and accuracy data (RSD and RE less than 20%).

2.5.2. Precision and accuracy

Intra-day ($n = 6$) and inter-day ($n = 6$) precision and accuracy were investigated at four different levels, 25, 50, 500 and 5000 ng/mL, for oxaliplatin. The precision (RSD) was determined on the basis of the coefficient of variation (RSD (%)), and the accuracy was calculated as ((found concentration − theoretical concentration)/theoretical concentration) × 100 (RE (%)).