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Sensitive and specific quantification of the anticancer agent ZD1839 (Gefitinib) in plasma by on-column focusing capillary liquid chromatography–tandem mass spectrometry

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Abstract

The development of an on-column focusing gradient capillary LC method coupled to tandem mass spectrometry (quadrupole-linear ion trap) for the quantitative determination of the anticancer agent ZD1839 (Gefitinib, Iressa) in blood plasma is described. Plasma samples (0.2 ml) were extracted with methyl *tert*-butyl ether. The analytes of interest, ZD1839 and the internal standard [$^2H_8$]ZD1839 (ZD1839-d8) were eluted on a 50 mm × 1 mm, 5 μm particle size, capillary ODS Hypersil column using an aqueous ammonium acetate gradient at 40 μl/min. Mass spectrometric detection was performed by a Q-Trap tandem mass spectrometer with electrospray positive ionisation, and monitored in the multiple reaction monitoring transitions 447 > 128 and 455 > 136, respectively. The limit of quantification of ZD18395 was 0.1 ng/ml. The method proved to be robust, allowing quantification of ZD1839 with sufficient precision, accuracy and sensitivity.
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Keywords: Iressa; On-column focusing; Capillary liquid chromatography; Tandem mass spectrometry; ZD-1839; Plasma

1. Introduction

ZD 1839 (Gefitinib, Iressa) has been developed to block growth stimulatory signals in cancer cells. These signals are mediated in part by enzymes called tyrosine kinases (TK). Iressa blocks several of these TKs, including the one associated with Epidermal Growth Factor Receptor (EGFR). Inhibition of EGFR has been achieved by various methods, including inhibition of the EGFR-TK by small molecule inhibitors [1–8]. ZD1839, 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline, is an orally active, selective EGFR-TK inhibitor (EGFR-TKI). This low molecular substance (Fig. 1) shows in vitro and in vivo activity against a wide variety of human solid tumours [7–10]. Iressa can be given orally over prolonged periods of time and its favourable tolerability has been seen both in volunteers and cancer patients [11–13]. In order to follow its fate in cancer patients, a sensitive assay for ZD1839 has been developed by Jones et al. [14]. The assay is based on liquid–liquid extraction of human plasma and subsequent processing over a liquid chromatographic (LC)–tandem mass spectrometric system. The extracts are chromatographed on an Inertsil ODS3 (15 mm × 4.6 mm i.d.) column eluted with acetonitrile/ammonium acetate. Here we present a capillary LC–tandem MS system for the analysis of Iressa in plasma and demonstrate its advantages like superior sensitivity, robustness and a minimal solvent use over the system published previously [14].

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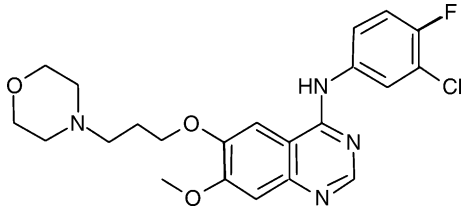

Fig. 1. Chemical structure of ZD1839 (Iressa).

2. Experimental

2.1. Reagents and materials

All solvents were HPLC grade and were used without further purification. ZD1839 was a kind gift of AstraZeneca Pharmaceuticals (Macclesfield, UK) with a purity of 99%. Deuterated [$^2H_8$]ZD1839 (ZD1839-d8) was supplied by the Drug Metabolism and Pharmacokinetics Department, AstraZeneca (Macclesfield, UK). Acetonitrile, methyl *tert*-butyl ether (MTBE), methanol, ammonium acetate and sodium hydroxide analytical-reagent grade were supplied by Across Organics (Geel, Belgium).

2.2. Stock solution preparation

Stock solutions of ZD1839 and the internal standard (IS), ZD1839-d8, were prepared by dissolving appropriate amounts, typically 100 mg of solid compound ZD1839 to 100 ml of methanol and 30 mg of ZD1839-d8 to 100 ml of methanol to give respectively, 1 mg/ml and 0.3 mg/ml of ZD1839 and ZD1839-d8. Next, four working solutions (1 μg/ml, 100 ng/ml, 10 ng/ml and 1 ng/ml) were prepared by serial dilution of the above described stock solutions. The ZD1839 working solutions were used to prepare the calibration standards in plasma (0.1, 0.4, 2, 6, 20, 50, 80 and 100 ng/ml) and a 75 ng/ml solution in methanol of deuterated ZD1839 was used as IS.

2.3. Sample preparation

0.2 ml of blanco plasma from a standard or clinical sample was dispensed into a Sarstedt tube and 100 μl of a 75 ng/ml solution of the IS was added. 0.5 ml of an aqueous sodium hydroxide solution (1 M) and 5 ml of MTBE were added and the tube content was mixed thoroughly by vortex mixing for 2 min. Each tube was centrifuged for 1 min to separate the organic and aqueous layers. Subsequently, the organic layer was transferred to a fresh disposable glass tube and evaporated to dryness at room temperature by vacuum centrifugation. The dry residues were reconstituted in 1 ml of a aqueous ammonium acetate (1% w/v)/CH_3CN solution (65/35), vortex mixed, transferred to autosampler vials (Alltech Associates, Lokeren, Belgium) and 75 μl was injected onto the reversed phase capillary liquid chromatography (CapLC) system. Fig. 2 shows the schematic overview of the extraction procedure.

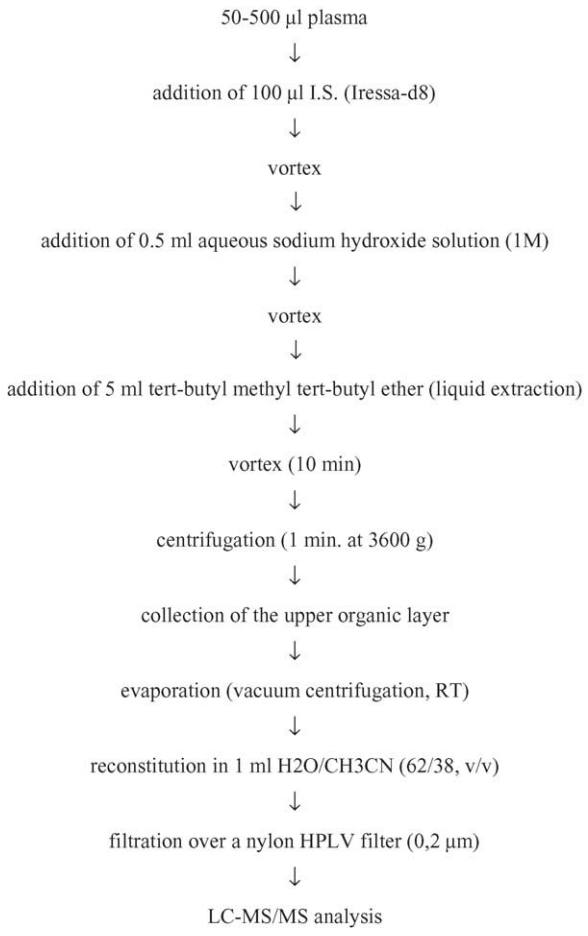

Fig. 2. Flow chart sample pretreatment procedure.

2.4. Chromatography and mass spectrometry

Chromatography was performed on a 50 mm × 1 mm, 5 um particle size, capillary ODS Hypersil column (Thermo Hypersil-Keystone, Thermo Electron Corporation, San Jose, CA, USA) and subjected to gradient elution. The capillary LC system consisted of a Waters CapLC system, with a liquid chromatography pump using an aqueous ammonium acetate (1% w/v)/CH_3CN gradient at 40 μl/min. The autosampler used was a Model SPH (Spark, Holland). The system operated at ambient temperature, and the injection volume was 75 μl. Mass spectrometric detection was performed by a Q-Trap tandem mass spectrometer with electrospray positive ionisation (ES^+) (MDS-Sciex, Concord, ON, Canada). The linear ion trap (LIT) was operated in the quadrupole mode (Q3) and multiple-reaction monitoring was performed for quantitative work. The needle voltage was set at 5500 V. Nitrogen was used as curtain (value of 10) and collision gas (Argon, set to high). Nebulizer and heater gas were set to a value of 20 and 30, respectively. The declustering potential was at 40 V and the collision energy was set at 40 eV. In order to establish the appropriate MRM conditions for ZD1839 and its deuterated analogue, solutions of the standards (0.1 mg/ml in HPLC mobile phase) were infused into the mass spectrometer

and the cone voltage (CV) optimised to maximize the intensity of the protonated molecular species $[M + H]^+$. Collision-induced dissociation (CID) of each protonated molecule was performed. ZD1839 and ZD1839-d8 were monitored in the MRM transitions 447 > 128 and 455 > 136, respectively. Multiple reaction monitoring scans were employed with a dwell time of 100 and 2 ms pause. The scans were acquired for 10 min.

2.5. Calibration and quality control

Calibration and quality control was executed as described earlier by us for another signal transduction inhibitor, i.e. STI-571 [15]. The guidance for industry bioanalytical method validation was followed [16].

3. Results and discussion

Capillary LC resulted in minimized solvent usage as well as high sensitivity detection. Data of CapLC–MS/MS showed that liquid–liquid extraction of ZD1839 gave clean extracts without endogenous interference, both in plasma and cellular extracts (Fig. 3). The chromatographic conditions proved to be robust and linear calibration curves were achieved in the range from 0.1 to 100 ng/ml. The analytes of interest, ZD1839 and ZD1839-d8, were eluted at a flow rate of 40 μl/min on the Thermo Hypersil-Keystone ODS Hypersil column (50 mm × 1 mm i.d., 5 μm particle size) using an aqueous ammonium acetate (1% w/v)/CH_3CN gradient. The optimal gradient was found to be a change from 95% H_2O (1% NH_4Ac)/5% CH_3CN to 20% H_2O/80% CH_3CN in 2 min after 1 min at 95% of mobile phase A (H_2O, 1% NH_4Ac). By using this gradient, the compounds of interest are, after a large volume injection (75 μl), trapped as a small front on the entrance of the column and elute as sharp symmetric peaks of 10 s. Since the flow is 40 μl/min, the compounds elute in a volume of 6.7 μl. Because of the concentration sensitivity-like behaviour of the ES-source, this means a 10-fold gain in sensitivity. The mobile phase returned to its initial conditions after 5 min at 80% CH_3CN to clean the column. ZD1839 and its deuterated analogue were detected by electrospray tandem mass spectrometry (Q-Trap) in the positive mode and monitored in the multiple reaction mode transitions 447-128 and 455-136, respectively. The strong gradient (5% CH_3CN to 80% CH_3CN in 2 min) allowed on-column focussing of large volume injections (75 μl), which is almost twice the column volume (40 μl). The resulting peaks exhibit an excellent peak shape and resolution and elute after 5.1–5.3 min.

Data of linearity, recovery, limit of quantitation, specificity, precision/accuracy, stability and quality control in routine analyses demonstrate that the present assay can be applied in routine therapeutic drug monitoring and in vitro and in vivo cellular experiments as well. Linear responses in plasma were obtained ($r^2 > 0.99$) over the range investigated (0.1–100 ng/ml). A LOQ of 0.1 ng/ml was achieved.

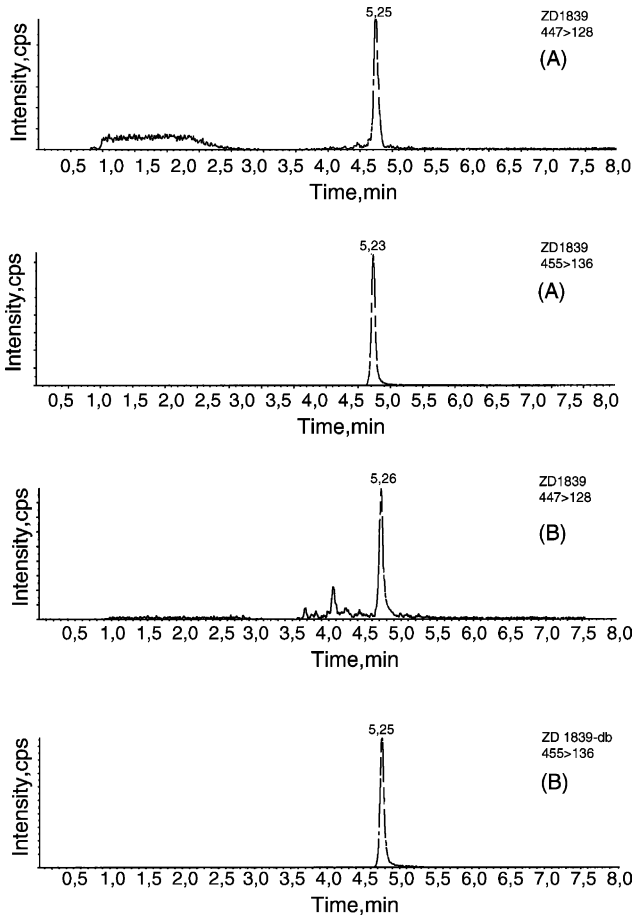

Fig. 3. MRM chromatogram of ZD1839 and ZD1839-d8 obtained with a 75 μl injection of spiked whole blood (0.1 ng/ml) after extraction of the isolated plasma (A) and with a 75 μl injection of a spiked whole blood (0.1 ng/ml) after extraction of the isolated red blood cells (B).

The precision of the assay was assessed by performing replicate ($n = 5$) extractions of plasma samples containing low, medium and high concentration of the analyte for each concentration range (i.e. 0.5, 5 and 60 ng/ml). Within-run and between-run precisions were less than 10% and average accuracies were between 90 and 110%.

After analyses of 600 patient samples in a phase I clinical trial (Iressa + Cetuximab), the column pressure, the peak area of the internal standard and the retention times remained stable. It was not necessary to use a pre-column. It can be stated that the developed method is robust. In none of the real samples, interfering peaks of endogenous and exogenous compounds were remarked.

The Food and Drug Administration (FDA) has approved Iressa tablets as single agent treatment for patients with advanced non-small cell lung cancer (NSCLC), the most common form of lung cancer in the US.

Iressa is of interest in the treatment of patients whose cancer has continued to progress despite treatment with platinum-based and docetaxel chemotherapy, two drugs that are currently the standard care in this disease. A significant safety concern associated with Iressa emerged recently with

Japanese reports, which described the occurrence of, serious and sometimes fatal interstitial lung disease in patients treated with Iressa. This toxicity may be well related with aberrant in vivo drug behaviour and this is one of the current points of interest for the application of the present assay, together with its use in cellular experiments, both in vivo and in vitro. Especially for the latter, the assay based on CapLC offers great advantages for monitoring ZD1839 in different cell cultures, possibly even at the single cell level.

4. Conclusion

A method has been developed for the analysis of ZD1839 concentrations in human plasma and cellular extracts as well. The assay has been shown to have adequate capacities for both clinical and experimental settings of ZD1839 monitoring. The assay has been applied already to a clinical trial with the cell signalling inhibitor.

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