Application Note: 415

Experimental Strategies to Determine Distribution of Erlotinib in Pancreatic Tumors by MALDI Using a Linear Ion Trap Mass Spectrometer

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Key Words
- LTQ XL™ Ion Trap
- Biodistribution
- MALDI
- Tissue Imaging

Overview

Purpose: Use imaging mass spectrometry to determine the biodistribution of erlotinib (Tarceva®) in pancreatic tumors from treated and untreated mice.

Methods: SCID mice bearing human pancreatic tumor xenografts were dosed once with 2.5 mg erlotinib in 6% Captisol™ by oral gavage. Tumors were surgically removed from the mice and MALDI matrix was sprayed on the tissue by commercial airbrush and analyzed with a Thermo Scientific LTQ XL linear ion trap with MALDI source.

Results: Single Reaction Monitoring (SRM) experiments were used to monitor the *m/z* 278 fragment characteristic of both erlotinib (*m/z* 394) and its metabolite (*m/z* 380). It appeared to be distributed evenly over the tumor. Significantly less of the *m/z* 278 fragment was found in the untreated specimens as compared with the erlotinib-treated ones.

Introduction

Imaging Mass Spectrometry (IMS) using matrix-assisted laser desorption ionization (MALDI) is being investigated using a linear ion trap with the overall goal of analyzing the distribution of various targeted therapies within patients' tumors. The drug chosen for evaluation is erlotinib (Figure 1) which targets the epidermal growth factor receptor (EGFR), a type I receptor tyrosine kinase (TK) involved in cellular differentiation and proliferation, by binding to the ATP pocket and inhibiting the autophosphorylation of the receptor. Erlotinib has demonstrated clinical activity in non-small cell lung cancer, head and neck cancer, and ovarian cancer in Phase II studies. The sensitivity and MS/MS capabilities of the LTQ XL are exploited for the unambiguous determination of the distribution of this drug within human pancreatic tumors. Custom software was used to acquire data while rastering the tissue and data visualization software used to display the 2- and 3-dimensional images.

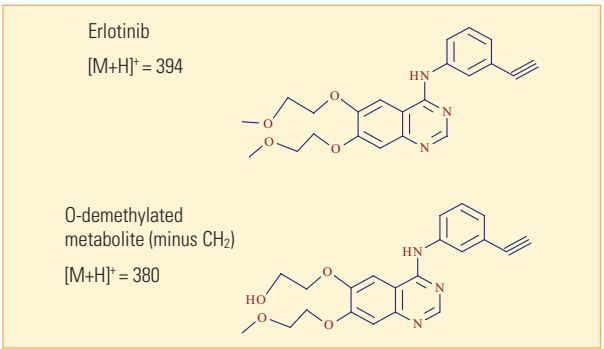

Figure 1: Structure of erlotinib and its principal metabolite

Methods

Solution studies: A series of matrices were tested with erlotinib and erlotinib/Captisol, looking for best ionization of the analyte of interest. 6-aza-2-thiothymine (ATT) and 2,5-dihydroxybenzoic acid (DHB) were selected as the matrices to use with tissue samples. A 70/30 v/v MeOH/0.1% TFA solvent mix was chosen for the matrix solutions, as erlotinib is slightly soluble in both methanol and water and insoluble in acetonitrile. Aqueous solubility of erlotinib hydrochloride is pH dependent, with maximal solubility (0.4 mg/mL) at pH 2 (due to protonation of the secondary amine), which is approximately the pH of MALDI solutions when 0.05-0.1% of trifluoroacetic acid is used in the solvent. Captisol, a polyanionic β-cyclodextrin derivative, is a safe drug carrier with superior water solubility (>900 mg/mL).

Tissue studies: SCID mice bearing patients' pancreas tumors grown as xenografts were dosed once with 2.5 mg erlotinib in 6% Captisol, Captisol alone, or left untreated. Erlotinib and Captisol were delivered by oral gavage. Sixteen hours following the administration of treatment or the vehicle, the pancreas tumors were resected, snap frozen immediately, and then 10 micron-thick tissue sections were prepared with a cryotome and placed on various MALDI surfaces. Surfaces were stainless steel or Indium-Tin-Oxide (ITO) coated glass slides. Nebulized matrix was applied with a commercial airbrush. 2,5-DHB was used at a concentration of 50 mg/mL and ATT at a concentration of 10 mg/mL.

Mass Spectrometry: Custom Tune Plus software (Figure 2) was used to acquire data in a raster mode. The laser spacing was set to 100 µm. Data was acquired with Automatic Gain Control (AGC) On, a standard feature of all LTQ mass spectrometers, to optimally fill the ion trap. Four microscans were averaged per laser spot. Localization of the analyte within the tissue was plotted in two- and three-dimensions with in-house software. The software allows visualization of MS spectra at each tissue pixel.

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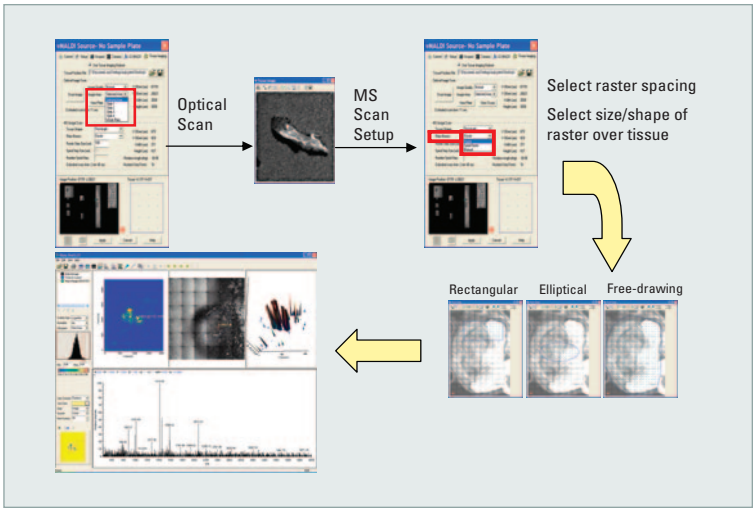

Figure 2: Workflow for tissue imaging data acquisition: acquire optical scan, select raster spacing and tissue area to raster, use data visualization tool to map the analyte.

Results

Solution studies: The MALDI matrices 2,5-DHA, 2,6-DHA, S-DHB, THAP, ATT, and 2,5-DHB were tested in order to find the best matrix for erlotinib ionization. Some of the matrices showed an unexplained fragment at *m/z* 278 upon collision-induced fragmentation (CID) of *m/z* 394 (data not shown), similar to the expected fragmentation of the parent drug (Figure 3). For this reason, these were not selected as matrices for the tissue samples. Erlotinib in both DHB and ATT ionized well and showed no peaks interfering with erlotinib ionization, except for a very small contribution close to *m/z* 278 and 336 in ATT matrix (Figure 3).

Treated pancreatic tumors: Pancreatic tumor sections treated with Erlotinib/Captisol were analyzed with both ATT and DHB matrices. Either CID or SRM experiments were conducted on erlotinib (*m/z* 394) and its O-demethylated metabolite (*m/z* 380), depicted in Figure 1. Due to the complexity of tissue samples, neither analyte was detected when data was acquired in MS mode. The major CID fragment reported from either analyte has been the *m/z* 278 peak when measured from human plasma[1] Other expected fragments would be *m/z* 250, 304, 322, and 336. We found most of these fragments in tissue, from precursor isolation of both *m/z* 380 and 394 (Figure 4). The relative abundance of the fragments varied according to matrix, number of times the particular tissue section was analyzed, and whether the parent drug or metabolite was fragmented. In general, DHB yielded higher relative abundance than ATT and fragmentation of *m/z* 394 yielded higher abundance of fragment signals than the metabolite.

Untreated pancreatic tumors: Figure 5 shows averaged spectra for the untreated pancreas tumors. The *m/z* 278 fragment was not found in the untreated samples. The MS/MS of 380 shows some contribution from fragments close to the *m/z* 322 and 336 peaks of treated samples. These could well be due to small contributions from the ATT matrix itself.

2D distribution of analytes in tissue: Figure 6 shows a comparison of the distribution of *m/z* 278 and 336 fragments (MS/MS 380) in both the erlotinib-treated and untreated pancreas tumors. The data in the four maps were treated similarly, with histogram equalization, and plotted at the same intensity scale. A histogram of the selected 2D distribution displays data as number of pixels vs. MS Intensity. Histogram equalization involves the selection of the MS intensity range that contains the data of interest in order to optimize visualization of the data. One can also display data normalized by the total ion current when Mass Range/TIC is selected to view the data (data now shown). The treated tumor samples show a fairly even distribution within the tissue while the untreated pancreas samples show a low level background that is not evenly distributed. The *m/z* 336 map shows a better coverage in its distribution throughout the tumor than the *m/z* 278 map, while both fragments display similar ion abundance, as depicted by the color scheme of yellow more intense > green > blue (indicating no signal). The higher density coverage displayed by the *m/z* 336 fragment ion might be due to a higher background level (seen in the untreated tumor) for the *m/z* 336 as compared to the *m/z* 278 ion.

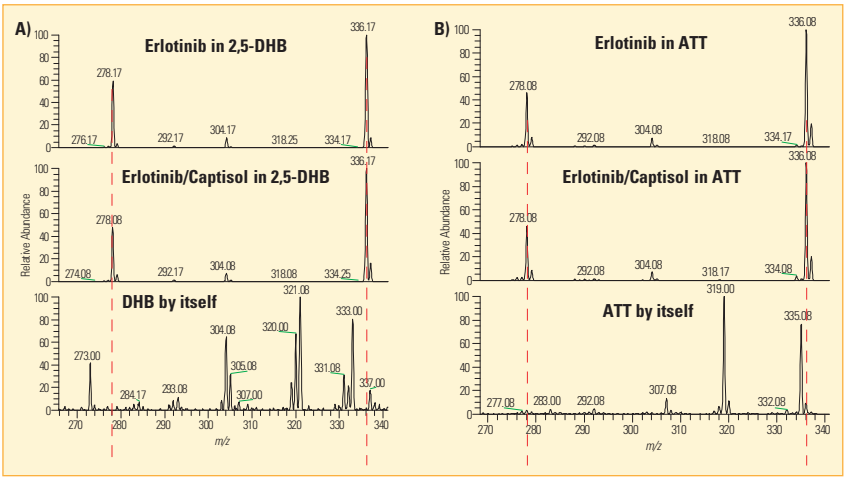

Figure 3: Solution studies showing characteristic peaks (in red) for erlotinib in 2,5-DHB and ATT with none or very little interference from the matrices themselves. A) 2,5- DHB : MS/MS of *m/z* 394 for erlotinib (top), erlotinib/Captisol (middle), and DHB by itself (bottom). B) ATT : MS/MS of *m/z* 394 for erlotinib (top), erlotinib/Captisol (middle), and ATT by itself (bottom).

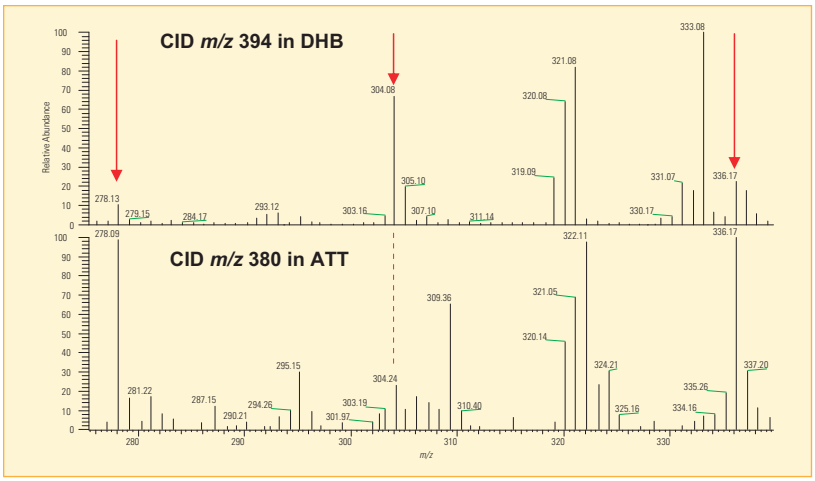

Figure 4: Averaged spectra for erlotinib-treated pancreas tumors, data collected directly off tissue. Characteristic fragments of erlotinib are *m/z* 278, 304, 322, 336. Top trace is fragmentation of parent drug in DHB matrix (*m/z* 394). Bottom trace is fragmentation of metabolite in ATT matrix (*m/z* 380).

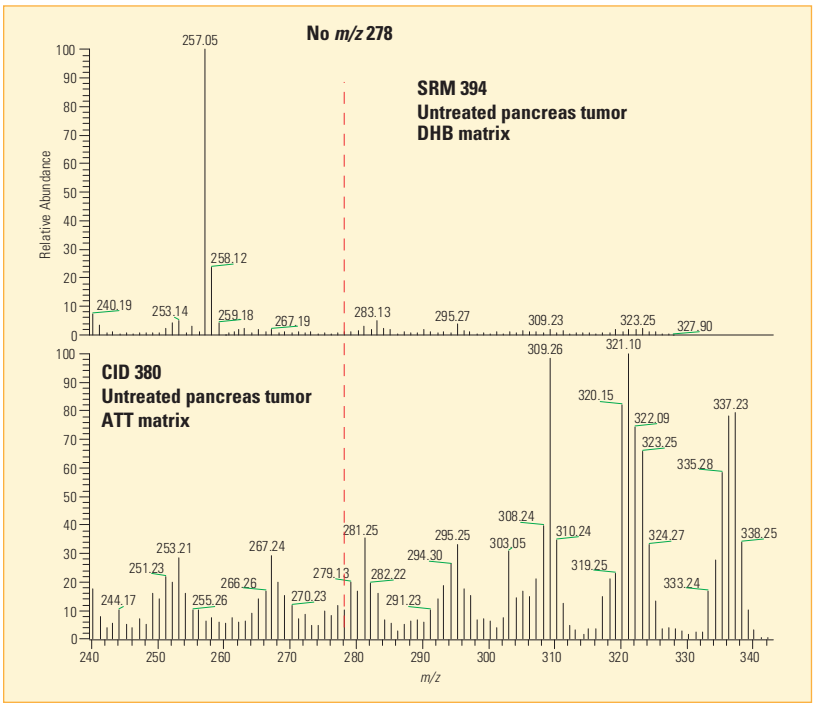

Figure 5: Averaged spectra for untreated pancreas tumors, data collected directly off tissue. The *m/z* 278 fragment characteristic of erlotinib is not present in these tissue sections. Top trace is fragmentation of parent drug in DHB matrix (*m/z* 394). Bottom trace is fragmentation of metabolite in ATT matrix (*m/z* 380).

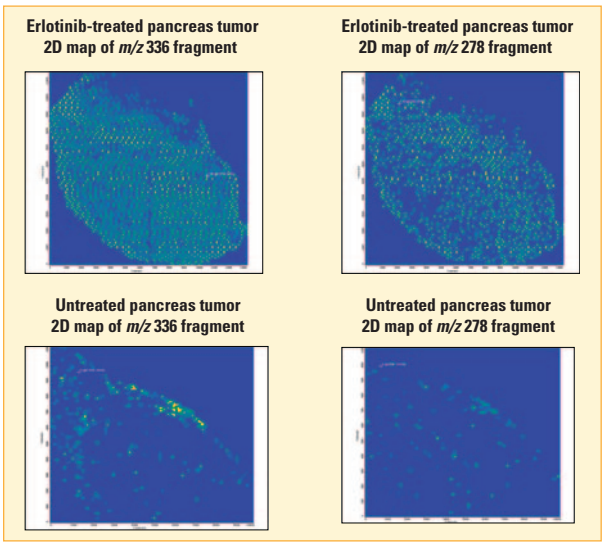

Figure 6: 2-Dimensional maps of erlotinib characteristic fragments (MS/MS 380), ATT matrix in treated (top) vs. untreated (bottom) pancreas tumors.

Conclusions

The LTQ with MALDI source makes for an outstanding platform for the study of drug distribution and its metabolites directly off tissue. The sensitivity of the linear ion trap, in particular, allows for an MS/MS spectrum with less than 10 laser shots at each location.

MALDI matrix, solvent selection, and the pH at which the drug is most soluble are critical parameters in the analysis of tissue by MALDI. The matrix should not have interfering peaks in the *m/z* of interest (both MS and MS/MS). One has to ensure that matrix application is efficient in its extraction of the analyte.

Three of the characteristic erlotinib fragment ions were found in treated pancreas tumors by CID of both parent drug and metabolite in two different matrices (not all spectra shown).

Significantly less of the *m/z* 278 and *m/z* 336 fragment ions were found in the untreated specimens as compared with the erlotinib-treated ones.

MALDI as conducted in this study, without the use of isotopic labels or internal standards, is not considered a quantitative technique. However, data processing for both treated and untreated tissues were kept consistent and normalized to the same intensity scale. A relative comparison and visualization of the different distribution and amounts of measurable drug between the two was therefore possible. The use of tissue replicates would be recommended for future studies.

References

[1] Zhao, M, He, P. Rudek, M. A., Hidalgo, M., Baker, S. D. 2003. Specific method for determination of OSI-774 and its metabolite OSI-420 in human plasma by using liquid chromatography-tandem mass spectrometry. *J Chromatography B*, 793, 413-420.

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