Application Note: 413

The Distribution of Metabolites of Di-(2-ethylhexyl) Phthalate on a Whole Rat by Imaging MS Using a MALDI Ion Trap

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Key Words

- LTQ™ MS^n
- DEHP
- Metabolite Distribution
- Quantitative Whole-Body Autoradiography (QWBA)
- Tissue Imaging

Overview

Purpose: Demonstrate mass spectral imaging in whole rat tissue and compare it to results from quantitative whole-body autoradiography (QWBA) technology.

Methods: Parallel dosing of test animals with radioactive and non-radioactive DEHP. Perform QWBA on the hot animal and MS Imaging analysis on the cold animal and compare results.

Results: The [phthalic acid-H_2O+H]$^+$ fragment ion was most abundant upon fragmentation of DEHP in the ion trap in both solution (*in vitro* experiment) and in tissue. Monitoring and mapping the MEHP metabolite showed more specific distribution within the tissue, as compared to the parent drug.

Introduction

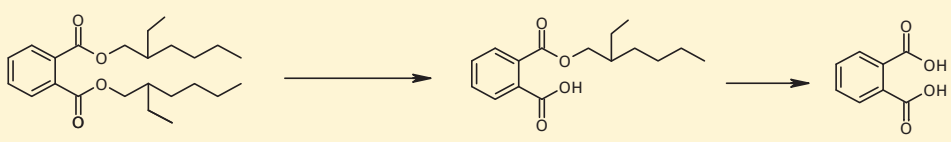
Di-(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer (softener for polyvinyl chloride or PVC), of most concern when used in medical devices. Exposure to high levels of DEHP causes a wide range of effects in rodents[1] including cancer, liver damage, birth defects and damage to the male reproductive system. DEHP has also been correlated to spina bifida occurrence in mice. DEHP in rat, mouse[2] and human is rapidly metabolized to its hydrolytic monoester, mono-(2-ethylhexyl) phthalate (MEHP), which is further metabolized to several Phase I oxidative metabolites (see below). Several of these metabolites have longer elimination half lives than DEHP, and it is likely that some of these have a greater toxicological effect than the parent compound. Quantitative whole body autoradiography (QWBA) is a rapid way to visualize the distribution of the total radioactivity introduced into the animal, using animals dosed with radio-labeled test compounds. High activity in rodents has been reported in brown fat, liver, gall bladder, intestines, kidney, testicles and urinary bladder. However, QWBA can not distinguish between different forms of the radioactive chemical species (metabolites and test compound) so mass spectral imaging was investigated as an important tool to visualize the distribution of parent compound and metabolites.

Methods

DEHP in solution. Neat DEHP was diluted 1:4 in methanol. Further dilution was done directly in 100 mg/mL 2,5-DHB (50/50 v/v acetonitrile/0.1% TFA), for spotting on to a MALDI stainless steel plate.

Tissue studies. Three male S-D rats were orally dosed daily at 300 mg/kg for 14 days and sacked on day 17. One rat was dosed with ^{14}C-labeled DEHP, another with non-labeled DEHP and a third control rat was similarly dosed with corn oil vehicle to compare any differences in the levels of the three metabolites. The rats were frozen in a dry ice/hexane bath and stored at -80°C before cryotome sectioning. Twenty µm thick tissue samples were deposited on acetate film tape and divided into posterior and anterior sections. The tissue sections were fitted atop two standard, microtiter-size, stainless steel MALDI sample plates. 2,5-Dihidroxybenzoic acid (40 mg/mL in 70/30 v/v MeOH/0.1% TFA) was sprayed (Aztec commercial air-brush) on top of the tissue on both treated and control samples (earlier work with a MALDI TOF was done using saturated α-cyanohydroxycinnamic acid in 60/40 v/v methanol/water[3]). The MS signal was optimized for DEHP on tissue in the positive ionization mode on the LTQ using a MALDI source. QWBA was performed on the hot animal on the Beta-Imager 2000 (BioSpace, France), and sections of the cold-dosed and control rats were sent for MS imaging analysis.

Mass Spectrometry: An LTQ linear ion trap with MALDI source was used with modified acquisition software (LTQ Tune Plus™) for tissue imaging applications. The laser raster pattern was set to 400 µm. Single Reaction Monitoring (SRM) experiments, *m/z* 391 → *m/z* 167, *m/z* 391 → *m/z* 279, to monitor the parent compound DEHP, and *m/z* 279 → *m/z* 167 to monitor MEHP, were used to determine the distribution of the main analytes. ImageQuest 1.0 software was used to visualize the two-dimensional distribution of compounds in the rat.



Di-(2-ethylhexyl) Phthalate
MW 390

Mono-(2-ethylhexyl) Phthalate
MW 278

Phthalic Acid
MW 166

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Results

Figure 1 shows the collision-induced dissociation (CID) spectrum for DEHP in solution in the MALDI LTQ (MS/MS of 391). The concentration in Figure 1 is 2.5 µg/mL or ~6 µM. Main fragment ions are *m/z* 149 (phthalic acid – H_2O+H), 167 (phthalic acid+H) and 279 (the protonated hydrolytic monoester, MEHP).

Results for the quantitative whole-body autoradiography (Figure 2) show that DEHP and its metabolites concentrate in the cecum, intestines, stomach, and liver. The dots outside the tissue are 14C calibration spots. A back of the envelope radioactivity calculation gives the following amounts for each organ: liver=5.56 nCi/g; stomach=10.6 nCi/g; intestine=8.38 nCi/g; cecum=20.7 nCi/g. This translates to an estimated dioctyl phthalate concentration in mg/kg of approximately: liver=18.2; stomach=34.7; intestine=27.4 ; cecum=67.8.

An enlarged area of the dosed rat posterior is shown in Figure 3. The distribution of the parent compound (DEHP), mapped by the strongest fragment ion in the spectrum, the [phthalic acid–H_2O+H]$^+$ at *m/z* 149, is shown in comparison with a photograph of the tissue with the different organs labeled. The first attempt to obtain imaging data followed the SRM transition *m/z* 391 → *m/z* 279, which yielded a distribution with weak intensity for this fragment ion. The tissue was then sprayed with a mixture of 50/50 v/v ethanol/methanol solvent with the intention of better solubilizing DEHP and recrystallizing matrix and analyte. The 2D image in Figure 3 was acquired after solvent spraying. The cecum, intestines, seminal vesicles, and possibly surrounding fat show the highest concentrations of unmetabolized DEHP. The liver did not show obvious concentration of DEHP. The highest concentrations appear in red, followed by yellow and green. Blue is no compound detected. The anterior portion of the rat showed non-localized, low level DEHP (data not shown). Figure 4 shows 2-dimensional images of the dosed vs. the control tissue for the same region of rat. A third imaging experiment over the same tissue yielded the 2D image shown in Figure 5, bottom, in which a decrease in concentration of DEHP can be observed.

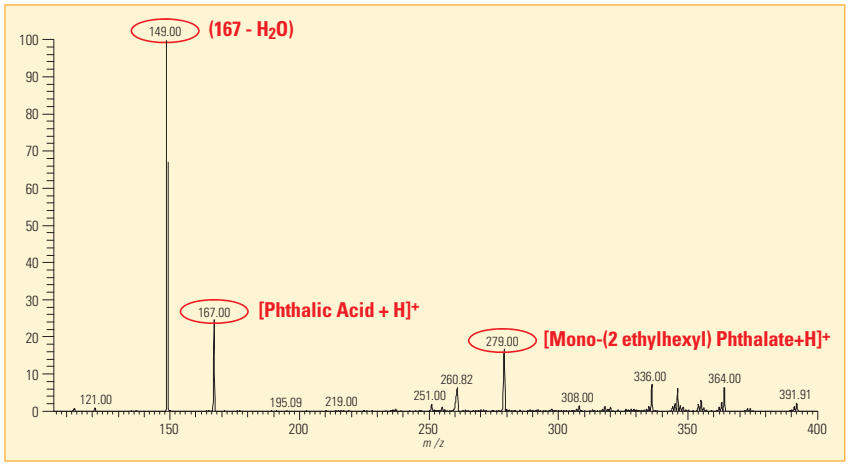

Figure 1: MS/MS spectrum of DEHP (2.5 µg/mL) in solution: precursor [M+H]$^+$ = 391. Matrix is 100 mg/mL of 2,5-DHB (50/50 v/v acetonitrile/0.1% TFA).

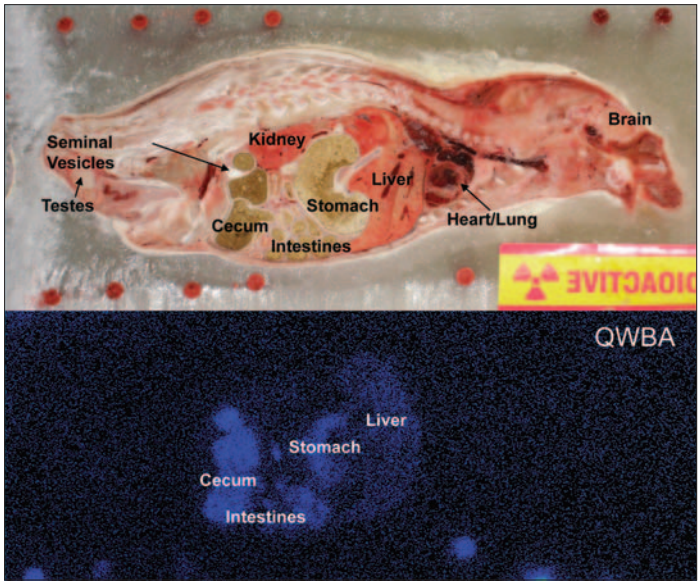

Figure 2: Comparison of optical image (top) with quantitative whole body autoradiography results (bottom). Dots in the figures are for calibration purposes.

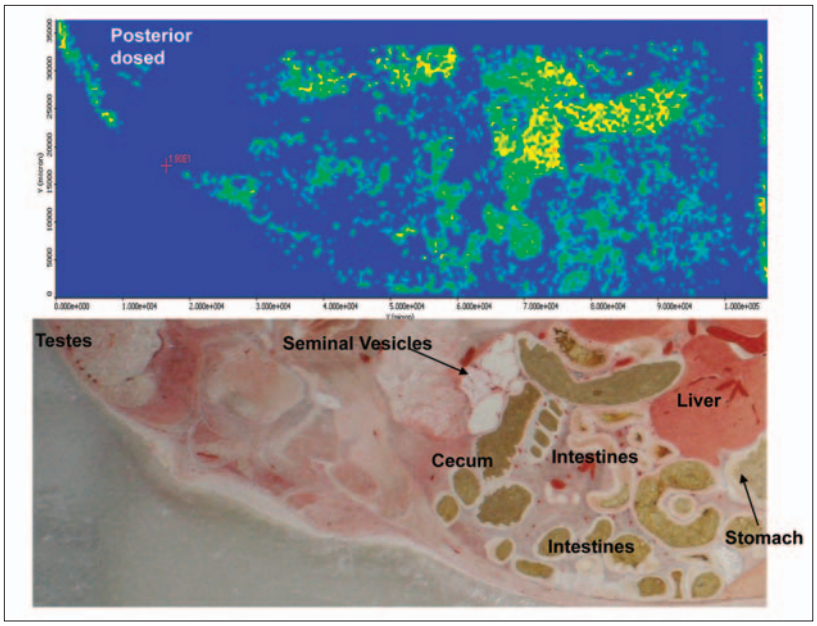

Figure 3: MALDI MS/MS 2D-Image of *m/z* 149 (DEHP SRM transition 391 → 149) compared to an optical image of the similar area, obtained from a different tissue section off the same specimen. About 1/4 of the actual rat tissue is shown in these images.

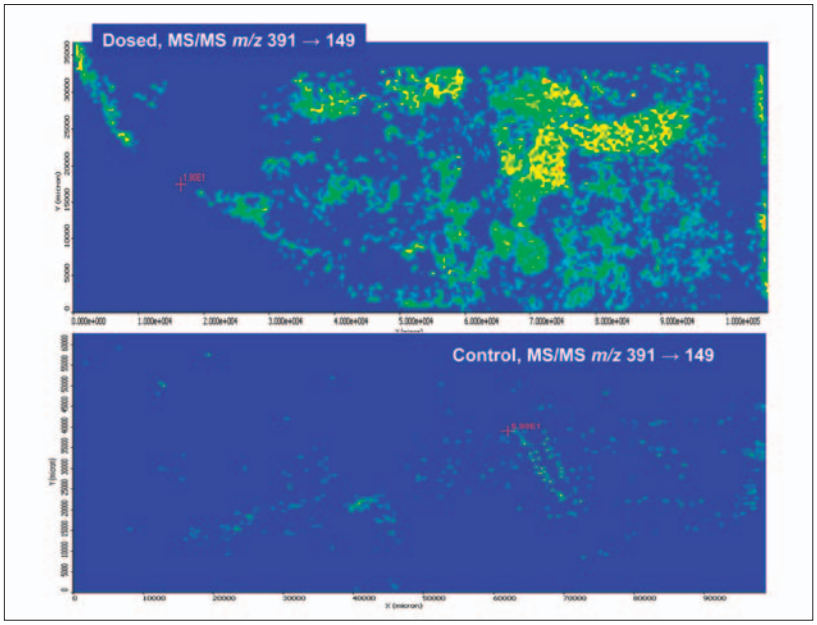

Figure 4: MALDI MS/MS 2D-Images for DEHP-dosed rat (top) and Control (bottom). The SRM transition 391 → 149 was monitored, mapping the *m/z* 149 ion. Images shown reflect 1/4 of the whole rat.

DEHP is known to rapidly clear as the diester in urine and feces, but in chronic studies it has been detected in heart, testes and fat fifteen months after the final dose. Our results seem to indicate that there is some remaining, un-metabolized DEHP in the cecum, intestines and seminal vesicles.

The 2D image showing results for the SRM experiment with the MEHP metabolite (SRM *m/z* 279 → *m/z* 167) appears in Figure 6. This metabolite appears concentrated in fewer regions, mainly the testes, seminal vesicles and part of the intestines. Tissue from the anterior portion of the rat showed less localized and less abundant results (data not shown) for the same experiment.

Conclusions

Mass spectral imaging was demonstrated as a complementary technique to quantitative whole body autoradiography.

The sensitivity of the LTQ with MALDI source for tissue imaging application and utility for displaying distribution of particular compounds and metabolites was demonstrated.

The unmetabolized DEHP was not completely eliminated out of the rat in the timeframe of the experiment, and this compound was found mostly concentrated in the intestinal region.

Monitoring a specific metabolite (SRM of MEHP) showed more specificity in the distribution within the rat as compared to the parent, unmetabolized compound.

Parallel experiments, where tissues for the dosed, control, and quantitative whole body autoradiography came from different rats, made comparisons difficult due to variability among animals.

References

[1] Silva, M.J. et al. *Toxicology* (**2006**) *219* 22-32.

[2] Lingren, A. et al. *Toxicology* (**1982**) *23* 149-158.

[3] Snow, T., Fasano, W.J., Manning, L.A. Imaging MS, Sanibel Island, FL, January 19-22, 2007.

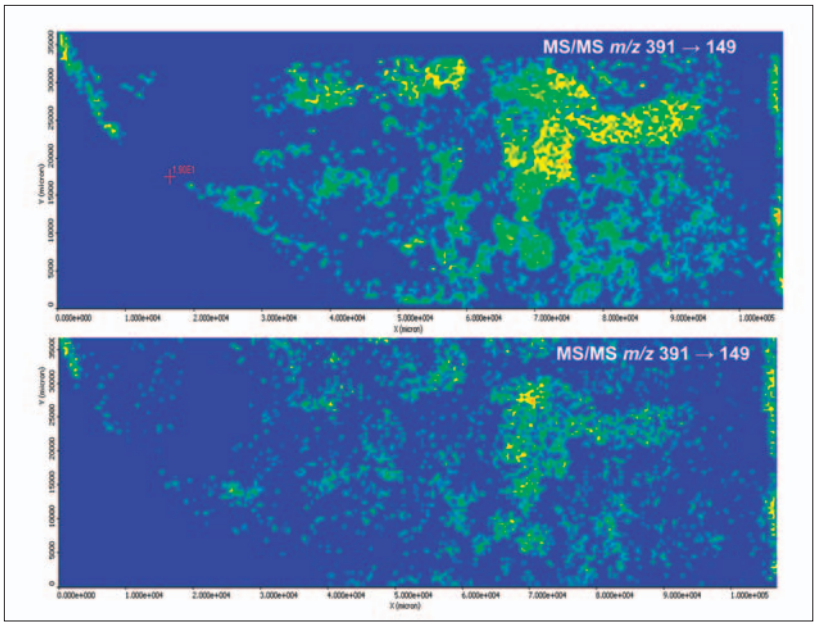

Figure 5: MALDI MS/MS 2D-Images of *m/z* 149 (DEHP SRM transition 391 → 149) second data acquisition over tissue (top) vs. third pass (bottom) showing decreased intensity of the precursor. About 1/4 of the actual rat tissue is shown in these images.

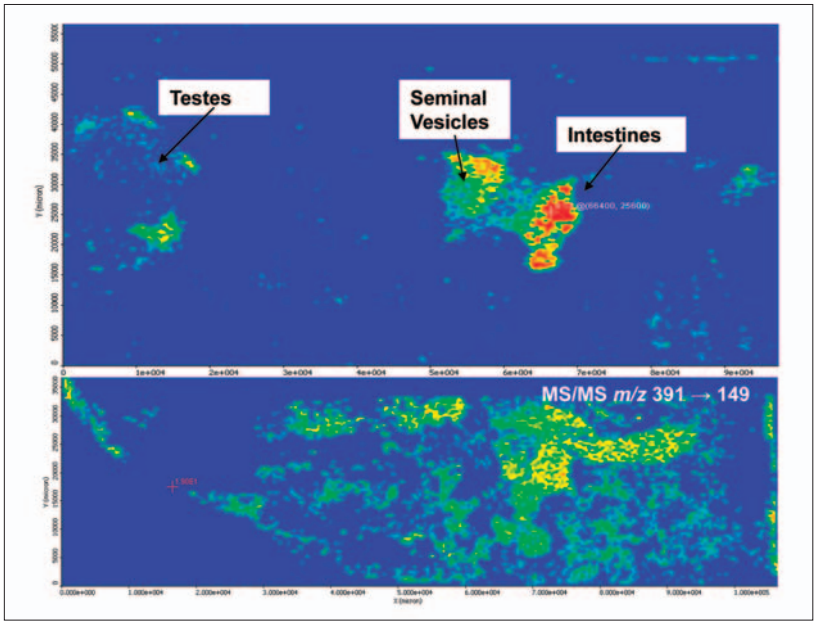

Figure 6: Posterior rat MS/MS image for the metabolite MEHP SRM 279 → 167. Fragment ion at *m/z* 104 mapped. Top image shows distribution of MEHP in the whole rat posterior; bottom image is the bottom half of this same tissue.

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