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# Liquid extraction surface analysis mass spectrometry (LESA-MS) as a novel profiling tool for drug distribution and metabolism analysis: the terfenadine example

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Liquid extraction surface analysis mass spectrometry (LESA-MS) is a novel surface profiling technique that combines micro-liquid extraction from a solid surface with nano-electrospray mass spectrometry. One potential application is the examination of the distribution of drugs and their metabolites by analyzing *ex vivo* tissue sections, an area where quantitative whole body autoradiography (QWBA) is traditionally employed. However, QWBA relies on the use of radiolabeled drugs and is limited to total radioactivity measured whereas LESA-MS can provide drug- and metabolite-specific distribution information. Here, we evaluate LESA-MS, examining the distribution and biotransformation of unlabeled terfenadine in mice and compare our findings to QWBA, whole tissue LC/MS/MS and MALDI-MSI. The spatial resolution of LESA-MS can be optimized to ca. 1 mm on tissues such as brain, liver and kidney, also enabling drug profiling within a single organ. LESA-MS can readily identify the biotransformation of terfenadine to its major, active metabolite fexofenadine. Relative quantification can confirm the rapid absorption of terfenadine after oral dosage, its extensive first pass metabolism and the distribution of both compounds into systemic tissues such as muscle, spleen and kidney. The elimination appears to be consistent with biliary excretion and only trace levels of fexofenadine could be confirmed in brain. We found LESA-MS to be more informative in terms of drug distribution than a comparable MALDI-MS imaging study, likely due to its favorable overall sensitivity due to the larger surface area sampled. LESA-MS appears to be a useful new profiling tool for examining the distribution of drugs and their metabolites in tissue sections. Copyright © 2011 John Wiley & Sons, Ltd.

Quantitative Whole Body Autoradiography (QWBA) is currently the most commonly utilized technique to examine drug distribution in animals.<sup>[1–3]</sup> Although QWBA is a very sensitive analysis method, which also provides valuable absolute quantitative data, there are two major limitations: autoradiography requires a radiolabeled compound whose synthesis might be expensive and its detection cannot distinguish parent compound from other compound-related material. Thus, autoradiography is unable to differentiate between parent compound and radiolabeled metabolites and does not detect unlabeled metabolites. This can lead to challenges in the interpretation of QWBA data and an inability to study metabolites that could be potentially pharmacologically active or toxic. The characterization of metabolites of new chemical entities remains an important topic in drug development.<sup>[4]</sup>

Any technology that utilizes mass spectrometry (MS) as detector for analytes from whole body animal sections could potentially overcome above limitations of autoradiography. Drug distribution and biotransformation could be characterized

by MS in one step and at reduced cost since no radiolabeled drug would be necessary. These approaches have been coined Mass Spectrometry Imaging (MSI),<sup>[5–11]</sup> and include, but are not limited to, techniques such as Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI MS),<sup>[12–16]</sup> Desorption Electrospray Ionization Mass Spectrometry (DESI-MS),<sup>[17]</sup> Secondary Ion Mass Spectrometry Imaging (SIMS-Imaging),<sup>[18]</sup> Electrospray-assisted Laser Desorption/Ionization MS,<sup>[19]</sup> Laser Ablation Electrospray Ionization (LAESI),<sup>[20]</sup> or live single cell mass spectrometry (nanomanipulation nESI-MS).<sup>[21–23]</sup> MSI approaches have unique capabilities and limitations compared to QWBA.<sup>[24]</sup>

Another more recent development in the context of MSI is Liquid Extraction Surface Analysis Mass Spectrometry (LESA-MS)<sup>[25]</sup> which obtains information from the whole body animal section via liquid extraction of analytes from the solid surface followed by nano-electrospray ionization. The LESA-MS approach has potential advantages over other MSI approaches in that no additional sample preparation is required (which compared to e.g. MALDI could also eliminate potential low molecular weight MALDI matrix mass interferences), the ionization process is soft, potentially revealing fragile drugs or metabolites and it allows analysis of negatively or positively charged analytes by simple exchange of the modifier used in the extraction solvent.

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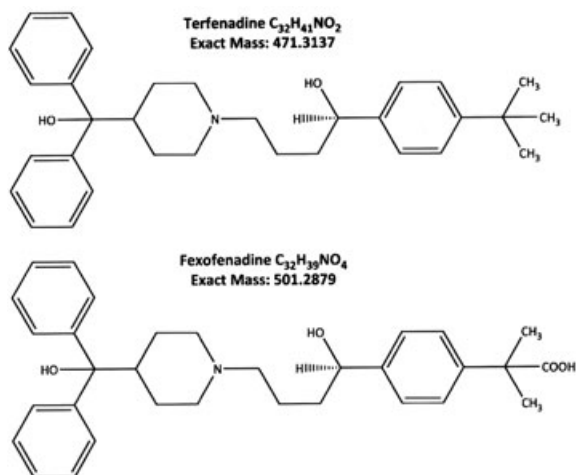
However, LESA is a fairly new analysis tool available for ambient surface analysis and its extraction process on thin tissue sections is not well characterized or understood. Its sensitivity might be compound-dependent and affected by ion suppression of co-extracted matrix molecules. Differences in extraction and ion suppression from different tissues might also limit the ability for relative quantification across a whole body thin section.

Here we report the application of LESA-MS to examine the distribution and metabolism of a small molecule drug in whole body thin tissue sections of mice. We chose terfenadine as the example drug since its pharmacokinetic profile is well characterized<sup>[26,27]</sup> and comparable studies using <sup>14</sup>C-terfenadine<sup>[28]</sup> as well as MALDI imaging MS<sup>[29]</sup> are available for direct comparison and evaluation of LESA-MS.

## EXPERIMENTAL

### Materials

Terfenadine and fexofenadine (Fig. 1) were purchased from Sigma Aldrich (St. Louis, MO, USA), water and methanol purchased as B+J High Purity Solvent from Honeywell (Morristown, NJ, USA), formic acid as 99+ %-grade from Thermo Fisher Scientific (Rockford, IL, USA). Male CD-1 mice (Charles River, Wilmington, MA, USA) with an average weight of 36 g at 9–11 weeks old were dosed with 50 mg/kg terfenadine in 0.5% methylcellulose via oral gavage (control sample was not dosed). At 1 and 4 h post-dose, animals were sacrificed by CO<sub>2</sub> inhalation, immediately frozen in a dry ice/hexane bath using a standard QWBA protocol and the carcasses were embedded in water and frozen until further processing. Whole body sagittal sections (20 µm thick) were collected onto 810 tape (3M, St. Paul, MN, USA) using a CM 3600 cryomicrotome at –20°C (Leica, Frankfurt, Germany). The sections were freeze-dried within the microtome chamber for 48 h followed by desiccation for at least 1 h.<sup>[30,31]</sup> Sections were adhered to 3" × 4" inch, 1.2 mm thick glass slides (Brain Research Laboratories, Newton, MA, USA) and stored at room temperature until further LESA-MS analysis.



**Figure 1.** Chemical structures of terfenadine and fexofenadine investigated by LESA-MS in this study.

### <sup>3</sup>H-Terfenadine QWBA analysis

A CD-1 mouse was dosed P.O. at 50 mg/kg dose of <sup>3</sup>H-terfenadine at 66 µCi/kg. One hour post-dose the mouse was anesthetized using CO<sub>2</sub> and frozen rapidly in a dry ice/hexane bath after whole blood was sampled. The mouse was embedded in 2% carboxymethylcellulose and 40 µm thick sagittal cryo-sections were taken at various levels then dehydrated as described above and exposed to a phosphor imaging plate for 2 weeks and imaged with a Typhoon 9200 phosphor imager (GE Healthcare, Picataway, NJ, USA) at 25 µm resolution. Tissue levels of radioactive material were quantified using co-embedded blood standards of <sup>3</sup>H-glucose using the vendor's image analysis software.

### Whole organ/tissue LC/MS/MS analysis

A CD-1 mouse was dosed 50 mg/kg terfenadine P.O. in 0.5% methylcellulose. One hour post-dose, the mouse was sacrificed via CO<sub>2</sub> inhalation and blood was withdrawn. In addition brain, kidney, liver, muscle and spleen were excised, blotted, and weighed. Respective tissue was homogenized and frozen until liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using positive ion mode atmospheric pressure chemical ionization (APCI) on an API 5000 mass spectrometer (AB Sciex, Toronto, Canada) using single reaction monitoring (SRM) transition 472.4/436.3 for terfenadine (setting of declustering potential (DP) 50 and collision energy (CE) 30) and 502.2/466.4 for fexofenadine (settings of DP 80 and CE 35). The LC run was executed using a Transcend LX2 Multiplexed UPLC system (Thermo Fisher Scientific, Waltham, MA, USA) with an Ascentis Express C18 column (50 mm × 3.0 mm × 2.7 µm) and the following gradient program: 95% solvent A for 15 s, ramping to 5% A at 90 s, washing at 5% A for 25 s and reconditioning to 95% A within 50 s (mobile phase A = water with 0.1% formic acid; mobile phase B = acetonitrile (ACN) with 0.1% formic acid). Flow rate was 750 µL/min.

### LESA-MS analysis

Fixed tissue sections were placed onto a universal adapter plate, an optical image was obtained and the picture was calibrated for robotic sample location and sequence list generation using LESA Points Software (Advion Inc., Ithaca, NY, USA). The sample and adapter were then placed into a LESA-enabled TriVersa NanoMate robot (Advion Inc., Ithaca, NY, USA) without further sample processing and the selected areas were analyzed.

The LESA extraction process has been described previously<sup>[25]</sup> in short: a robotic arm picks up a conductive pipette tip, moves to an extraction solvent reservoir and aspirates the volume required. The tip is then moved in close proximity to the surface location of interest (ca. 50–150 µm above the surface) and the robot dispenses a defined volume of the solvent. The static extraction process starts after formation of a liquid junction. Delay time on target and/or repetitions of the dispense/aspirate step are possible variables to increase extraction efficiency. After the extraction, the solvent is aspirated from the surface and the robotic system moves the tip to make contact with an electrospray ionization (ESI) chip (Advion Inc., Ithaca, NY, USA) to generate a nano-electrospray

directed against the inlet of the mass spectrometer. A new tip and nozzle are used for every location to eliminate cross-contamination.

Two different LESA settings were used in this study to reflect either a lower spatial resolution with improved sensitivity for drug distribution profiling across the whole body section (Set 1) or an improved spatial resolution setting for drug profiling within a single organ (Set 2). Set 1 parameters: 1.7  $\mu\text{L}$  solvent, dispensing 1.2  $\mu\text{L}$  at maximum speed and wait for 1 s, aspirate 1.3  $\mu\text{L}$  and wait for 1 s, repeat dispensation/aspiration cycle two more times. Set 2 parameters: 1.1  $\mu\text{L}$  solvent, dispensing 0.7  $\mu\text{L}$  at maximal speed and wait for 1 s, aspirate 0.9  $\mu\text{L}$  and wait for 1 s, repeat dispense/aspirate cycle two more times. Nano-electrospray was initiated with 1.40 kV and a pressure of 0.35 psi in positive ion mode. The extraction/spray solvent used in this study was 80:20 methanol/water 0.1 vol% formic acid.

### LESA-MS analysis and data processing

MS data were collected on an Exactive Orbitrap mass spectrometer (Thermo Fisher, Bremen, Germany) in the full-scan mode from  $m/z$  100 to 2000 for 1 min using the following settings: HCD gas on, 100 000 nominal mass resolution, positive ion polarity, 1 micro scan, lock masses:  $m/z$  355.06994 and  $m/z$  429.08873, AGC target 5 exp5, 50 ms maximum injection time, 200°C capillary temperature, 70 V capillary voltage, 185 V tube lens voltage and 32 V skimmer lens voltage. Data were averaged for 50 scans and mass accuracy calculated against the theoretical isotopic mass for terfenadine ( $\text{C}_{32}\text{H}_{41}\text{NO}_2$ ;  $[\text{M} + \text{H}]^+ = m/z$  472.3210) and fexofenadine ( $\text{C}_{32}\text{H}_{39}\text{NO}_4$ ;  $[\text{M} + \text{H}]^+ = m/z$  502.2652). Mass accuracy of the MS instrument was <5 ppm within 24 h after calibration using internal lock mass correction.

For experiments using the QTrap 5500 mass spectrometer (AB/Sciex, Toronto, ON, Canada) a 2 min MS method for positive ion mode was set up including Q3 survey scan, MS/MS scans of terfenadine at  $m/z$  472.3 and fexofenadine at  $m/z$  502.3 as well as SRM transitions for both compounds with the following settings: Q3 survey scan:  $m/z$  200–600 at 1000 Da/s (step size 0.1 Da), DP 140, EP 12, CXP 16, CUR 10. MS/MS of  $m/z$  472.3 and  $m/z$  502.3 with  $m/z$  50–550 at 1000 Da/s (step size 0.1 Da), DP 180, EP 10, CXP 14, CUR 10, CAD 8, CE 42 and 46, respectively. Terfenadine SRM scan  $m/z$  472.3/436.4 for

150 ms dwell time and CE 36, DP 140, EP 12, CXP 16, CUR 10, CAD 4. Fexofenadine SRM scan  $m/z$  502.3/171.1 for 150 ms dwell time and CE 50, DP 180, EP 10, CXP 14, CUR 10, CAD 8. Data was read out as SRM signal intensity (counts per second, cps) averaged over the 2 min run time (40 scans).

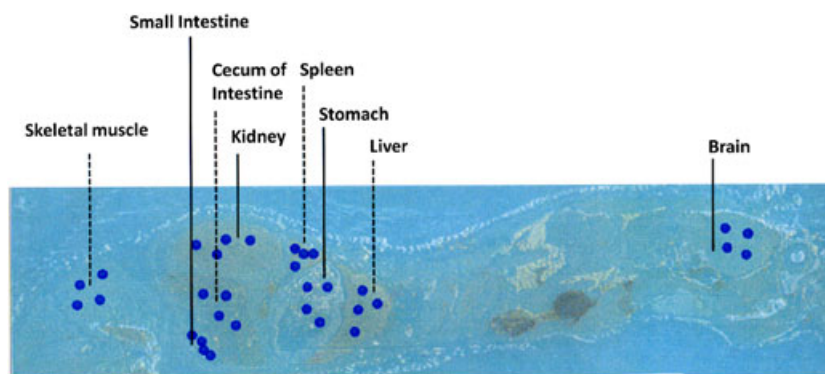
Organs/tissues were sampled in four different locations at up to three sections of different sectioning depth for a total of up to 12 locations per tissue of interest (Fig. 2). SRM data were averaged and the standard deviation (SD) determined. Data were summarized in bar graphs of the average and SD of multiple spot analysis or in a topographic style for individual tissues/organs using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

### Assay grading system

In order to compare the relative tissue distribution of terfenadine and fexofenadine a simple intensity grading system was applied with 0 representing 0–1000 cps, + representing 1000 to 10 000 cps, ++ representing 10 000 to 100 000 cps, +++ representing 100 000 to 1 000 000 cps and ++++ representing the strongest intensity signals with more than 1 000 000 cps (see Table 1).

## RESULTS AND DISCUSSION

Terfenadine and its metabolite fexofenadine can be measured using a LESA-MS workflow resulting in both relative quantification data of compound distribution in organs/tissues as well as single organ drug profiling. Two different sets of LESA parameters were used for the experiments. Parameter Set 1 was a general purpose setting for drug distribution analysis with a ca. 1.5 mm diameter area sampled on target (3.5 mm<sup>2</sup>) using 1.7  $\mu\text{L}$  of extraction solvent. The area sampled on target depends on the extraction solvent used, its contact angle on the surface as well as the volume dispensed from the extraction tip. A solvent with higher organic composition shows slightly better extraction efficiency than a lower organic solvent composition (methanol, iso-propanol, tetrahydrofuran and acetonitrile mixtures with water tested on brain, liver and kidney, data not shown); however, its dispersion across the surface is more pronounced. Dispersion is also more pronounced when using iso-propanol, acetonitrile



**Figure 2.** Example of sample selection with four sampling spots per organ and per cryo-section (for a total of up to 12 locations per organ for final signal intensity analysis).

or tetrahydrofuran as the organic phase. The extraction solvent used in this study was 80:20 methanol/water 0.1 vol% formic acid, which is a good compromise of extraction efficiency and spatial resolution obtainable on the tissue section.

Parameter Set 1 gave an optimal ratio of volume of solvent to surface area sampled and shows an improved sensitivity compared to the second set of parameters used, which was optimized for maximum spatial resolution on the target tissue. Parameter Set 2 resulted in a sampled target area of ca. 1.0 mm diameter (1.6 mm<sup>2</sup> area) using 1.1  $\mu$ L extraction volume (Fig. 3) and was used for profiling the drug distribution within a single organ (see discussion below and Fig. 7).

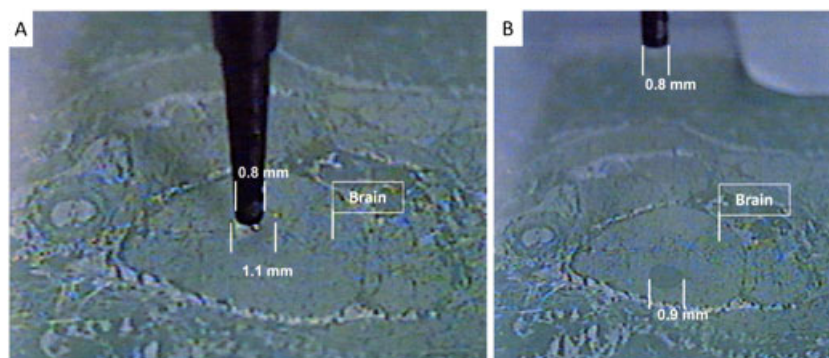
Another interesting aspect of LESA is the liquid extraction of a solid surface and the question of achievable extraction using a static extraction process on a thin tissue section. Figure 4 shows the sequential extraction and analysis ten times of a single spot on the kidney 1 h post P.O. dose of 50 mg/kg terfenadine and the SRM signal intensities of both the parent drug terfenadine and its metabolite fexofenadine. Fexofenadine shows maximum signal intensity at the second to third extraction from the surface whereas signal is reduced thereafter. However, even after the tenth extraction from the same location the signal for terfenadine and fexofenadine is still at ca. 50% of the original signal intensity. An initial increase in signal intensity might be explained by a favorable hydration of the tissue section after its first contact with extraction solvent or might be caused by initial extraction of otherwise signal suppressing compounds such as lipids. The fact that even after 10 replicates both analytes can still be extracted in significant amounts suggests a limited absolute amount extractable at each LESA cycle, which might be caused by an insufficient equilibration time (similar results obtained from liver – data not shown). It is also possible that a slightly different area/volume of the tissue is extracted with every subsequent step. The overall sensitivity of the LESA-MS method used in this study is clearly limited. On the other hand, this does allow for re-analysis of pre-sampled areas for follow-up analysis using different mass spectrometry settings or different MS instruments altogether.

Although sampling and analyzing an entire mouse whole body section at maximum spatial resolution is technically possible, it is generally not necessary for determining the distribution of a drug and its metabolites. It would also consume an unreasonable amount of analysis and processing time. In order to compensate for potential tissue heterogeneity four different locations on up to three different section depths should be analyzed for each tissue/organ of interest as demonstrated (Fig. 5).

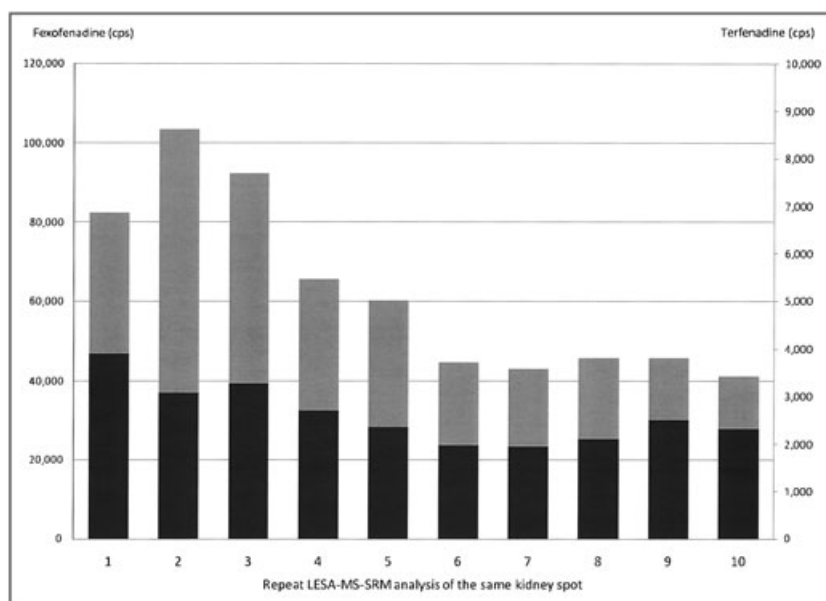
As expected, following 50 mg/kg oral administration of terfenadine, the parent drug shows very strong signals in the gastrointestinal tract including the stomach and intestine. Data indicate that the compound is absorbed rapidly and high levels of the metabolite fexofenadine were observed in the liver, as well as low levels of unchanged parent drug, indicating high first pass metabolism. Levels of terfenadine and fexofenadine in the lower gastrointestinal tract are consistent with biliary excretion of this metabolite. These findings are in agreement with earlier disposition reports using radiolabeled terfenadine<sup>[28]</sup> and MALDI-MSI<sup>[29]</sup> both describing biliary excretion of drug related material and significant elimination via the feces in multiple species including mouse and rat.<sup>[28]</sup>

Terfenadine and its metabolite were also detected in other systemic tissue such as blood, spleen, skeletal muscle and kidney, suggesting that both compounds are systemically distributed via blood after reaching the liver. These findings are supported by earlier reports examining the disposition of <sup>14</sup>C-terfenadine which showed quantifiable levels of terfenadine and higher levels of an unidentified radiolabeled metabolite in major systemic tissues including liver, lung, spleen, kidney, heart, and blood following oral dosing in rats.<sup>[28]</sup> Disposition studies with <sup>14</sup>C-labeled terfenadine in rat, monkey and dog<sup>[28]</sup> conclude that about 10% of the dose is eliminated in the urine and low concentrations of radio-labeled material were detected in the kidney of rat after oral administration of <sup>14</sup>C-terfenadine, supporting LESA-MS findings of terfenadine and fexofenadine in the kidney.

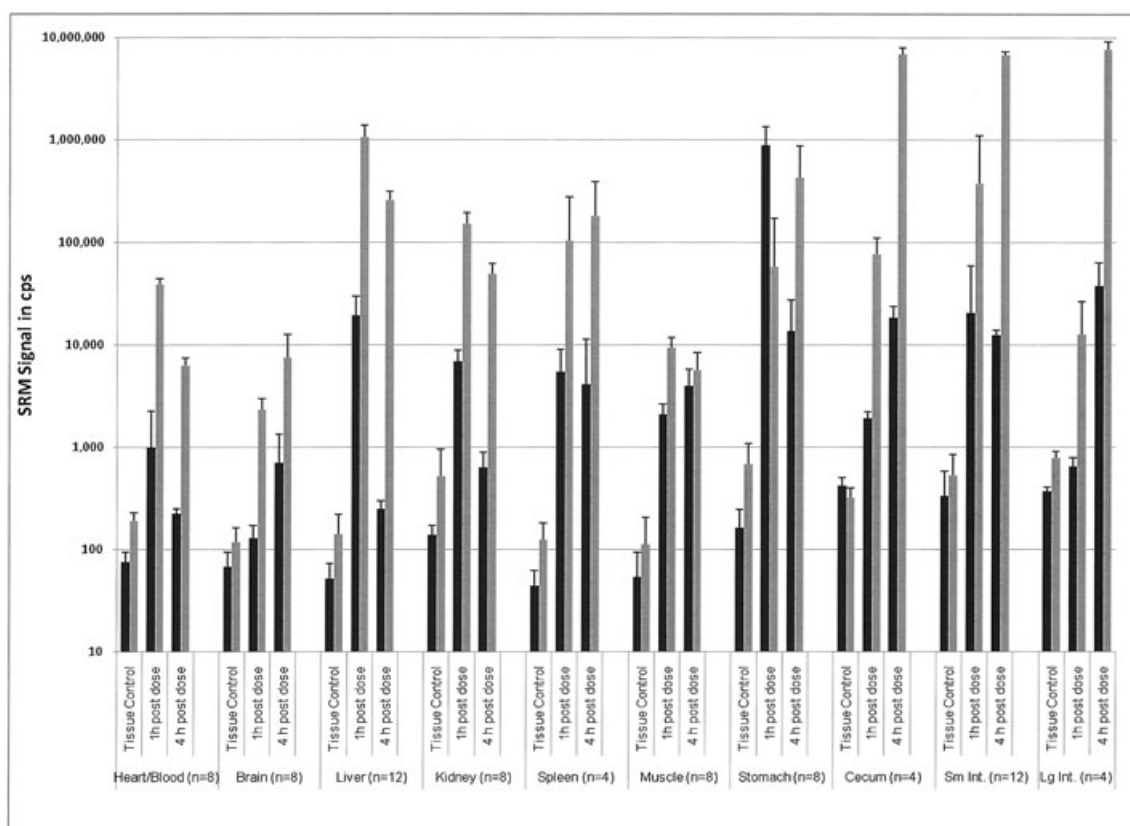
Another finding in our LESA-SRM drug distribution study is that fexofenadine was present in brain 1 h post-dose and both terfenadine and fexofenadine were present in brain



**Figure 3.** Estimation of LESA spatial resolution on whole body cryo-sections of mouse using 80:20 methanol/water 0.1 vol% formic acid as extraction solvent and with parameters optimized for maximum spatial resolution. An area of about 1 mm diameter is sampled (pipette tip has an outside diameter of 760  $\mu$ m and is used as reference). (A) Example of a liquid junction formed on target (size of the liquid junction ca. 1.1 mm at maximum diameter of the drop). (B) Example of surface hydration right after sampling (area hydrated on tissue ca. 0.9 mm at maximum spot diameter).



**Figure 4.** Repeated LESA analysis of the same location on kidney tissue 1 h post P.O. dose of 50 mg/kg terfenadine in mouse. Grey bars demonstrating signal intensity of the SRM transition for fexofenadine, black bars representing the signal intensity of the SRM transition of terfenadine in cps, respectively. After an initial increase in signal intensity for fexofenadine, both analyte signals drop to about 50% of initial signal after 10 extractions demonstrating a rather limited absolute amount of material extracted during each LESA extraction cycle.



**Figure 5.** LESA-MS-SRM analysis of terfenadine (black columns) and fexofenadine (grey columns) at different tissues/organs of mouse whole body cryo-sections and at different times after P.O. dose of 50 mg/kg terfenadine. Average cps signal of 4 to 12 locations from up to three different section depths of each organ and the respective SD is shown. Tissue control samples show an average baseline signal of ca. 100 cps.

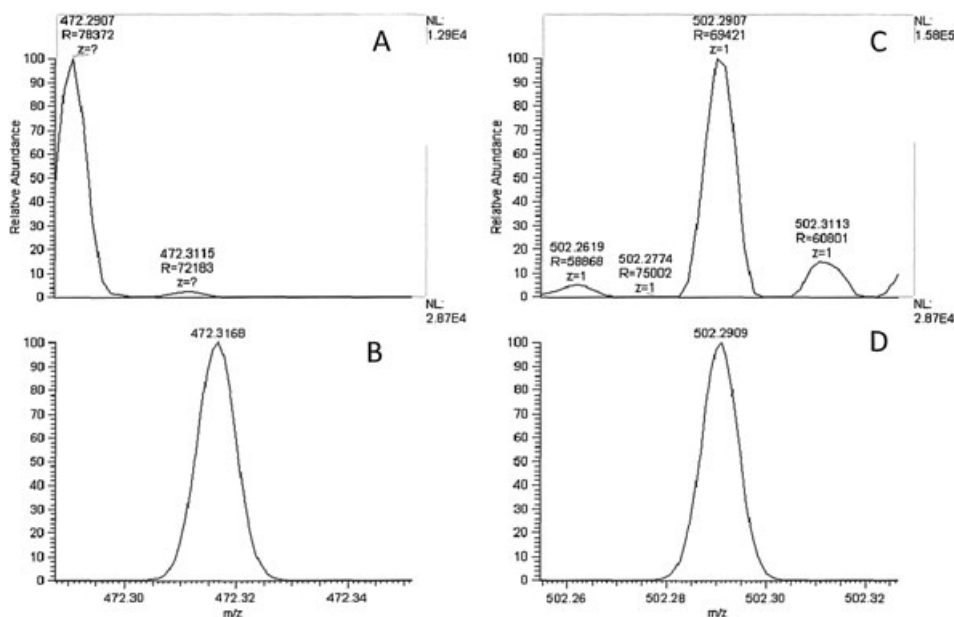
tissue 4 h after a 50 mg/kg P.O. dose of terfenadine (Fig. 5), albeit at low levels compared to other tissues. A LESA-MS analysis using accurate mass and high mass resolution did confirm the presence of the metabolite fexofenadine in brain 1 h post-dose (0.3 ppm deviation from theoretical value), with terfenadine not being detected (Fig. 6). Also, at 4 h post-dose fexofenadine can be detected in the brain via LESA-exact mass measurement, but again terfenadine cannot be confirmed (data not shown). This finding suggests that LESA-SRM might be slightly more sensitive than LESA-accurate mass MS on the respective MS instruments used. Also interesting is the fact that an alternative approach using conventional LC/MS/MS of brain homogenate did show measurable levels of terfenadine and fexofenadine present in brain 1 h post-dose (Table 1). We find that 0.5 ng/g fexofenadine in brain, as determined by LC/MS, can be detected by LESA in brain tissue as well, whereas 5.2 ng/g terfenadine cannot. In blood, 0.5 ng/g terfenadine can be confirmed by LESA-MS as well as 0.5 ng/g fexofenadine in kidney tissue, thus suggesting an overall LESA sensitivity in the low pmol/g range; however, dependent on the compound and tissue investigated.

Previous MALDI-MSI data did not indicate terfenadine or fexofenadine signals in the brain or central nervous system in mice at the same dose and time.<sup>[29]</sup> Also, reports in the literature indicate both compounds show little central nervous system toxicity, suggesting only a minor, if any, distribution into the brain.<sup>[32]</sup> However, it was shown in rat that some radio-labeled material can penetrate the blood-brain barrier (BBB) at elevated doses between 10 and 100 mg/kg terfenadine<sup>[28]</sup> and others also found fexofenadine in rat brain after terfenadine dosage.<sup>[33]</sup> In this context it is noteworthy that in both our LESA as well as LC/MS/MS study animals were sacrificed by CO<sub>2</sub>

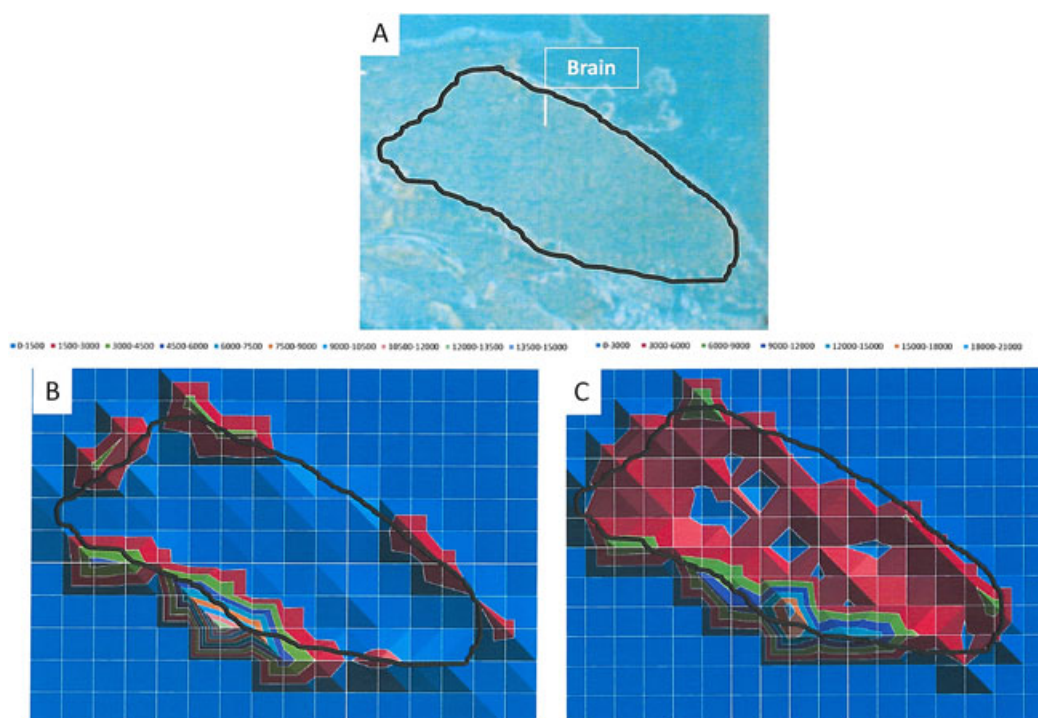
gas inhalation, which is known to alter the BBB<sup>[24]</sup> therefore, it is possible that the detection of at least a portion of these analytes may be due to the sample preparation.

We also analyzed the brain 4 h post-dose with LESA-SRM in a single organ profiling mode using a higher spatial resolution setting (Fig. 7). The single organ profiling setting causes a loss in sensitivity of the LESA method compared to the whole body distribution analysis performed at ca. 1.5 mm spatial resolution since a smaller area is sampled on the tissue of interest (see discussion above). In the single organ profiling mode higher terfenadine levels were in fact only detectable in outermost areas of the brain and border areas to other tissues suggesting co-extraction of the surrounding tissue whereas fexofenadine was clearly distributed within the brain. This could also exemplify that a whole tissue approach such as LC/MS might not reflect the correct concentration level and distribution within the tissue since the whole organ is homogenized prior to analysis, whereas a strength of profiling or imaging approaches would be to distinguish e.g. penetration into the tissue from possible short-term effects due to sample preparation that only effect part of the tissue.

In summary, we can clearly confirm fexofenadine presence in brain after terfenadine administration, which is in agreement with current literature reports.<sup>[26–29,32,33]</sup> However, no definitive answer can be given as to the presence of terfenadine in brain, somewhat demonstrating the current spatial and sensitivity limitation of LESA-MS for the two compounds analyzed. The findings with regard to brain levels of both compounds however do not influence the overall interpretation of the drug metabolism and distribution since both signal intensities measured are marginal compared to other tissues.



**Figure 6.** LESA-MS analysis utilizing high resolution and accurate mass of an Exactive Benchtop Orbitrap mass spectrometer. (A) Average of 50 scans, zoom into the monoisotopic region of terfenadine; (B) Simulation of the theoretical isotopic mass of terfenadine; (C) Average of 50 scans, zoom into the monoisotopic region of fexofenadine; (D) Simulation of the theoretical isotopic mass of fexofenadine. Fexofenadine can be confirmed with 0.3 ppm mass deviation, terfenadine cannot be detected with the nearest signal to the theoretical value being off by 11.2 ppm, well outside the 5 ppm mass deviation limit.



**Figure 7.** LESA-MS-SRM single organ profiling of brain 4 h post P.O. dose of 50 mg/kg terfenadine with parameter settings optimized for 1 mm spatial resolution. Fexofenadine is distributed throughout the brain with low signal intensity, whereas terfenadine is only detected in the border regions of brain and adjacent tissues. (A) Optical image of the mouse whole body cryo-section with outline of the sampled area and annotation of the brain tissue. (B) LESA-MS-SRM analysis of terfenadine and (C) LESA-MS-SRM analysis of fexofenadine. SRM intensity data is shown as topographic map with colors representing the signal intensity in cps, please note that this graphical output does interpolate between data points (color changes between cross sections), the spatial resolution on target is 1 mm as indicated by the white cross-sections.

Overall, the distribution and metabolism data from this study is in good agreement with previously conducted disposition studies using  $^{14}\text{C}$ -labeled terfenadine,<sup>[28]</sup> MALDI-MSI,<sup>[29]</sup> or our own LC/MS/MS investigation. A comparison of all four approaches to drug distribution is summarized in Table 1. Comparing the different approaches, LESA-SRM detects the two compounds in all organs that previously were indicated by autoradiography, TLC-Rad, MALDI-MSI or LC/MS/MS with the exception of terfenadine in brain 1 h post-dose, see discussion above.

Also, relative abundances are in good agreement among the four approaches keeping dosage differences and species differences in mind. Although nano-ESI has been shown to be less prone to matrix effects,<sup>[34]</sup> these findings are surprising and would indicate that LESA is significantly less influenced by the biological matrix extracted from the different surfaces than expected. MALDI-MSI data was collected following a similar in-life experiment compared to this study<sup>[29]</sup>; however, it showed levels of terfenadine and fexofenadine only in the liver and GI tract contents. Therefore, LESA-MS seems to be more informative than MALDI-MSI<sup>[29]</sup> in this example, an effect likely caused by its favorable sensitivity due to the larger spot size analyzed on target. The previously conducted  $^{14}\text{C}$ -terfenadine disposition study in rat used only 6–10 mg/kg and can therefore be considered more sensitive than the LESA-MS analysis approach. This is somewhat expected given the currently limited LESA extraction from tissue and an area of potential

improvement in the future. However, LESA-MS provided valuable molecule-specific information not obtainable through autoradiography studies alone.

## CONCLUSIONS

We have reported here on LESA-MS, a complementary analytical tool to QWBA and MALDI-MSI, in the study of drug distribution and biotransformation. LESA-MS is a recent approach to tissue profiling offering advantages over QWBA such as the elimination of the need for a radiolabeled compound and the ability to distinguish between parent drug and metabolites during the analysis. As such, LESA-MS falls into the category of Imaging Mass Spectrometry (MSI) techniques such as MALDI. LESA-MS combines a micro-liquid extraction from a solid surface followed by nano-ESI coupled with MS analysis of the generated ions to gain information from tissue sections. It can be combined with any type of mass spectrometer to gain information by e.g. accurate mass or SRM analysis.

Although LESA-MS does not provide absolute quantification data, our findings with regard to the distribution and metabolism of terfenadine employing profiling LESA-MS analysis are in agreement with previous studies using the classical approach to disposition using radiolabeled material and autoradiography<sup>[28]</sup> as well as an alternative mass spectrometry imaging approach using MALDI-MS<sup>[29]</sup> or our

**Table 1.** Summary of terfenadine/fexofenadine detection and relative abundance in various tissues comparing different analytical assays typically used in drug distribution and metabolism studies. \*Autoradiography does not distinguish between parent drug and radiolabeled metabolites. A signal intensity grading system is applied to the LESA data as described in the Experimental section; non-quantitative MALDI imaging and QWBA data was graded from no signal intensity to strong signal intensity for easier comparison

Organ/Tissue	LESA-MS (QQQ) 50 mg/kg P.O. mouse 1 h post-dose		LC-MS/MS (QQQ) 50 mg/kg P.O. mouse 1 h post-dose (nmol/g)		<sup>3</sup> H-Terfenadine study* 50 mg/kg P.O. mouse 1 h post-dose ( <sup>3</sup> H-eq-ng/g)		MALDI-MSI 50 mg/kg P.O. mouse 1 h post-dose (signal intensity)		<sup>14</sup> C-Terfenadine study* P.O. rat <sup>[28]</sup>	
	Terfenadine	Fexofenadine	Terfenadine	Fexofenadine	Terfenadine	Fexofenadine	Terfenadine	Fexofenadine	Terfenadine	Fexofenadine
Blood/Heart	+	++	0.6	0.5	<LOQ	<LOQ	none	none	QWBA-6 mg/kg 1 h post-dose	TLC-Rad-10 mg/kg 2 h post-dose (14 C-eq-ng/g)
Brain	0	+	5.2	0.5	<LOQ	<LOQ	none	none	none	340/1500
Liver	++	+++	1174.0	49.0	113	113	none	medium	weak	19200
Kidney	+	+++	17.5	6.0	59	59	none	none	none	4500
Spleen	+	+++	13.3	0.4	<LOQ	<LOQ	none	none	none	3800
Skeletal muscle	+	+	44.4	0.8	<LOQ	<LOQ	none	none	none	none
Stomach	+++	++			9363	9363	medium	none	strong	strong
Small intestine	++	+++			1011	1011	strong	strong	strong	strong
Large intestine	0	++			1744	1744	none	none	none	none

own whole tissue LC/MS/MS analysis. LESA-MS relative abundances in different tissues are also in surprisingly good agreement with LC/MS quantification data suggesting that LESA-nESI might suffer less from matrix effects than expected, a finding that needs further investigation and also needs to be confirmed over a wider range of analytes.

Terfenadine is extensively absorbed in the intestine and undergoes significant first pass metabolism to fexofenadine in liver. Both compounds are distributed to systemic tissues and the results appear consistent with elimination through the bile and feces. However, based on the levels observed in the kidney, a small portion is also likely eliminated via renal filtration. Although minor levels of fexofenadine can be observed in brain, these are marginal compared to other organ concentrations, might have been induced by CO<sub>2</sub> inhalation of the animals and favored by the relatively high dosage used in this study, thus suggesting a limited transfer of this metabolite across the blood-brain barrier.

The spatial resolution of LESA-MS is currently limited to about 1 mm on the surface. This parameter is dependent on the contact angle of the extraction solvent on the surface of interest, the extraction solvent composition and the volume of extraction solvent used. Given its limited spatial resolution compared to other MSI techniques we propose to describe LESA-MS as a profiling tool. Although it is technically possible to profile a whole body mouse section with a 1 mm resolution, a more rapid approach is to sample a limited number of spots per tissue of interest and across different section depths. Typically, a total of 4–12 locations should be sampled to address possible heterogeneity in drug distribution in the tissue.

The major limitation to the overall sensitivity of LESA-MS is currently the limited absolute amount extracted during each LESA cycle, since even a tenth extraction of the same location results in about 50% of the original signal. The extraction profile within the tissue should be further investigated to better understand the underlying cause for this effect. In spite of this limitation, our results show that the LESA-MS analysis approach provides significantly more information about the drug distribution than a comparable MALDI-MSI analysis<sup>[29]</sup> or whole body autoradiography study in rat.<sup>[28]</sup> Both approaches might have been limited due to their respective dynamic detection range.

LESA-MS was also previously shown to allow drug distribution information for propranolol and sulforaphane<sup>[25]</sup> and we conclude that LESA-MS has the potential to be a very useful tool for examining the distribution and metabolism of drugs in the discovery stage of drug development. It does not require a radiolabeled compound and its comparable sensitivity, ease of use, analysis speed and molecule specificity makes it attractive for applications across drug discovery.

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