

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/264431517>

Mass Spectrometry Imaging of Cassette-Dosed Drugs for Higher Throughput Pharmacokinetic and Biodistribution Analysis

ARTICLE *in* ANALYTICAL CHEMISTRY · AUGUST 2014

Impact Factor: 5.64 · DOI: 10.1021/ac502217r · Source: PubMed

CITATIONS

8

READS

78

11 AUTHORS, INCLUDING:



Diego Cobice

The University of Edinburgh

15 PUBLICATIONS 54 CITATIONS

SEE PROFILE



Malcolm R Clench

Sheffield Hallam University

110 PUBLICATIONS 1,955 CITATIONS

SEE PROFILE



Per E Andrén

Uppsala University

148 PUBLICATIONS 3,507 CITATIONS

SEE PROFILE



Peter J Webborn

AstraZeneca

24 PUBLICATIONS 677 CITATIONS

SEE PROFILE

Mass Spectrometry Imaging of Cassette-Dosed Drugs for Higher Throughput Pharmacokinetic and Biodistribution Analysis

John G. Swales,^{*,†,‡,⊥} James W. Tucker,[†] Nicole Strittmatter,^{||} Anna Nilsson,[§] Diego Cobice,^{||} Malcolm R. Clench,[⊥] C. Logan Mackay,^{||} Per E. Andren,[§] Zoltán Takáts,^{||} Peter J. H. Webborn,[†] and Richard J. A. Goodwin[†]

[†]Drug Safety and Metabolism, Innovative Medicines, AstraZeneca R&D, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

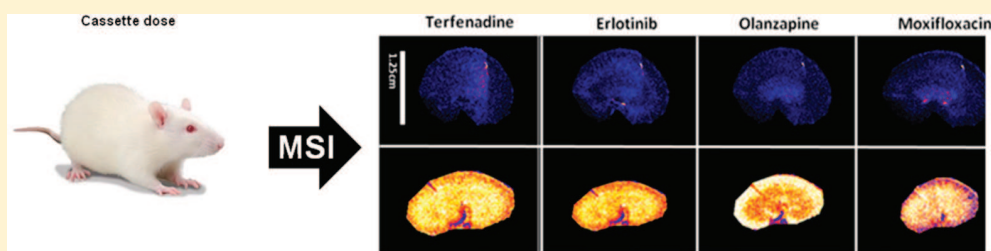
[‡]SIRCAMS, School of Chemistry, University of Edinburgh, Edinburgh EH8 9YL, U.K.

[§]Biomolecular Imaging and Proteomics, National Center for Mass Spectrometry Imaging, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala 751 05, Sweden

^{||}Department of Surgery and Cancer, Sir Alexander Fleming Building, Imperial College, London SW7 2AZ, U.K.

[⊥]Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB, U.K.

S Supporting Information



ABSTRACT: Cassette dosing of compounds for preclinical drug plasma pharmacokinetic analysis has been shown to be a powerful strategy within the pharmaceutical industry for increasing throughput while decreasing the number of animals used. Presented here for the first time is data on the application of a cassette dosing strategy for label-free tissue distribution studies. The aim of the study was to image the spatial distribution of eight nonproprietary drugs (haloperidol, bufuralol, midazolam, clozapine, terfenadine, erlotinib, olanzapine, and moxifloxacin) in multiple tissues after oral and intravenous cassette dosing (four compounds per dose route). An array of mass spectrometry imaging technologies, including matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI), liquid extraction surface analysis tandem mass spectrometry (LESA-MS/MS), and desorption electrospray ionization mass spectrometry (DESI-MS) was used. Tissue analysis following intravenous and oral administration of discretely and cassette-dosed compounds demonstrated similar relative abundances across a range of tissues indicating that a cassette dosing approach was applicable. MALDI MSI was unsuccessful in detecting all of the target compounds; therefore, DESI MSI, a complementary mass spectrometry imaging technique, was used to detect additional target compounds. In addition, by adapting technology used for tissue profiling (LESA-MS/MS) low spatial resolution mass spectrometry imaging (~ 1 mm) was possible for all targets across all tissues. This study exemplifies the power of multiplatform MSI analysis within a pharmaceutical research and development (R&D) environment. Furthermore, we have illustrated that the cassette dosing approach can be readily applied to provide combined, label-free pharmacokinetic and drug distribution data at an early stage of the drug discovery/development process while minimizing animal usage.

The development of mass spectrometric analytical technologies has enabled pharmaceutical research and development to employ an increasingly wide array of high-throughput screening modalities. Compound optimization with early, parallel elucidation of the physiochemical and pharmacokinetic properties enables a broader choice of compound leads from which candidate drugs can be selected and has also been shown to reduce compound attrition in development.¹ The simultaneous dosing of drugs during *in vivo* research is typically termed cassette dosing (though also referred to as *N*-in-one dosing). Cassette dosing was first employed by pharmaceutical companies toward the end of the 1990s, when

simultaneous measurements of five compounds were made during the development of potential therapeutics for the treatment of benign prostatic hyperplasia.² Cassette dosing and analysis has been shown to be applicable for both orally³ and intravenously⁴ administered drugs. Factors that need to be considered when devising a cassette dosing experiment include mass spectrometry instrumentation and liquid chromatography (LC) requirements (positive or negative mode ionization),

Received: June 17, 2014

Accepted: August 1, 2014



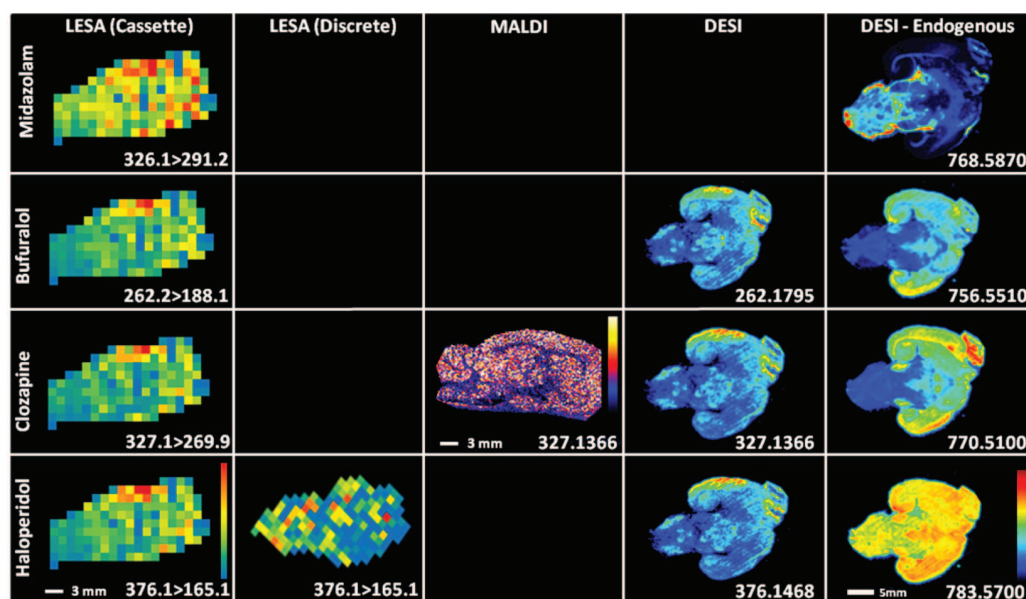


Figure 1. Images of intravenously dosed compounds detected using LESA-MS/MS, MALDI-MS, and DESI-MS after cassette dosing or discrete dosing (haloperidol), highlighting the value of using orthogonal analytical techniques to cover a wider chemical space than that covered by MALDI-MS alone.

whether drugs or their metabolites are likely to have common structures or fragmentation patterns, whether compounds require specific formulation (solubility at specific pH), and careful attention should be given to the dose levels selected. While such factors can be mitigated for by the selection of appropriate combinations of compounds to be analyzed, there remains a risk of drug–drug interactions during *in vivo* administration. This topic is extensively discussed in several reviews on cassette dosing.^{5–7} Interactions mainly arise around competition at clearance pathways. Other reported interactions including heteroactivation of clearance pathways, which is the activation of a metabolic enzyme by a second drug acting through an allosteric mechanism (acting on a site other than the active site). There is also the chance of pharmacological and toxicological effects on organ blood flows and clearances.⁷ However, during early drug discovery, the advantage of parallel preliminary experiments means that the risk of erroneous results from drug–drug interactions is outweighed by the saving in reduced time and cost, as noncassette experiments will always be performed on compounds that are to be progressed later in the discovery pipeline.

Traditionally discovery phase pharmacokinetic analysis has been performed using sensitive triple quadrupole mass spectrometers, usually in combination with rapid chromatography systems and operated with automated or semiautomated optimization software.⁸ This generic approach enables rapid quantification of chemically diverse compounds from biofluids or tissue homogenates. While these methods are ideally suited for deriving pharmacokinetic parameters from homogeneous biofluids, they are less suitable to analysis of heterogeneous tissue samples. Furthermore, any tissue information from homogenates can be misleading due to factors such as residual blood concentration or dilution. Compound biodistribution information is usually collected at a later stage in a compound's life cycle using techniques like quantitative whole body autoradiography (qWBA).⁹ Such analysis retains distribution information lost during homogenization but has major limitations such as the need for a radiolabeled compound,

rendering the technique impractical for discovery use. Label-based techniques are also limited in their inability to distinguish a parent compound from any metabolites that still contain the label, hence producing an ambiguous representation of a compound's distribution.

Mass spectrometry imaging (MSI),^{10,11} regarded as collection of multiple mass spectra by rastering across a tissue section in two dimensions, and mass spectrometry profiling (MSP),^{12–15} generally regarded as collection of mass spectra at discrete points on a tissue section, have recently emerged as technologies that have the capability to obtain label-free compound biodistribution, relative abundance, and quantitation data by analyzing tissue sections directly.

Such methods enable the simultaneous analysis of drugs, metabolites, endogenous small molecules, lipids, and peptides directly from tissue sections. Several comprehensive reviews describe methodologies^{16–18} utilizing a range of sample ionization methods. Matrix-assisted laser desorption/ionization (MALDI) is arguably the most effective for pharmaceutical research and is most commonly employed for MSI. However, alternative ionization methods such as desorption electrospray ionization (DESI) are amenable for use in MSI.²⁵ Techniques like liquid extraction surface analysis (LESA) can be used to increase the sensitivity of tissue analysis when target compounds cannot be detected by other MSI ionization methods, but their use to date has been restricted to low spatial tissue profiling at only a few sampling positions across a tissue section.¹³ While the origins of LESA and DESI can both be traced back to electrospray ionization, they exhibit different but complementary strengths and weaknesses. LESA can have high sensitivity when used in selected reaction monitoring mode on a triple quadrupole MS but lacks the high spatial resolution that DESI is capable of providing. DESI, in contrast, can supply structure-rich mass spectrometry (MS) images (Figure 1, DESI endogenous masses, showing multiplex analysis within the cassette-dosed sections) at high spatial resolution ($\sim 50 \mu\text{m}$)²⁵ but has less apparent sensitivity due to the decrease in sampling area.

The utility of MSI is determined by the ability to detect the target compound at the level present in the sample and is determined by a number of factors such as the ionization efficiency and localized suppression caused by salts and endogenous compounds within the tissues. There is also a risk that the mass of the target compound is masked in the mass spectrum by endogenous compounds or MALDI matrix adduct signal. Therefore, “up-front” MSI ionization assessments are routinely performed to detect target compound in tissue samples before undertaking extensive studies. The risk of failing to detect a target in a sample can be further mitigated by using the orthogonal MSI techniques previously alluded to, such as LESA and DESI, which are becoming increasingly popular as imaging tools. These ionization techniques are essentially based on generic nanoelectrospray ionization and can be used to cover a much wider chemical space than MALDI which is limited by MALDI matrix selection. These ambient ionization techniques are often combined with triple quadrupole, ion trap, or time-of-flight mass spectrometers offering increased sensitivity at the cost of lower spatial resolution.

In a pharmacokinetic study multiple biofluid samples can be taken at required time points post dose; however, samples for MSI require the termination of the subject animal and the dissection and sectioning of a target organ. This means that a greater number of animals are required to generate corresponding time course MSI data. Effective cassette-dosed MSI experiments would be advantageous with a reduction in both the analytical turnaround time but also the animal handling and husbandry costs. Any significant reduction in the number of animals is advantageous, above and beyond cost savings and analysis time, as it is in keeping with industry-wide aims to reduce, reuse, and refine experimental animal numbers, the so-called “3-Rs”.

The research presented here outlines a novel strategy of mass spectrometry tissue imaging and profiling in routine intravenous and orally cassette-dosed pharmacokinetic studies. MSI analysis was not adversely affected by cassette dosing, and the tissue distribution data corresponds with the plasma pharmacokinetic (PK) analysis and highlights the advantage of gaining important pharmacokinetic and distribution data from the same animals at an early phase of drug discovery. Demonstrated for the first time is enhanced tissue profiling technology (LESA) for low spatial resolution imaging. Furthermore, such analysis is combined with near cellular resolution MSI ($\sim 20\ \mu\text{m}$) and demonstrates that a suite of instrumentation enables detection of all target compounds, and therefore, a multiplatform MSI strategy is required for effective pharmaceutical research and development (R&D).

MATERIALS AND METHODS

Materials and Reagents. Analytical grade acetonitrile, methanol, and formic acid were obtained from Fisher Scientific (Loughborough, Leicestershire, U.K.). 2-Methylbutane was obtained from Sigma-Aldrich (Poole, Dorset, U.K.). Test compounds were obtained in house from AstraZeneca compound management group (Macclesfield, Cheshire, U.K.) with the exception of moxifloxacin which was purchased from Sigma-Aldrich (Poole, Dorset, U.K.). MALDI-MS grade α -cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma-Aldrich (Poole, Dorset, U.K.).

Animals. Adult male Hans Wistar rats (approximate weight 260 g) were obtained from Charles River Laboratories

(Margate, Kent, U.K.) and were acclimatized on site for a minimum of 3 days prior to dosing. Compounds administered by oral gavage and intravenous bolus injection (via the tail vein) were formulated in 5% dimethyl sulfoxide/95% (30% w/v Captisol in water). Control animals were dosed with vehicle via the same administration route in each arm of the study.

Dosing and Scheduling. An initial pilot study using intravenous administration consisted of the following: one animal was dosed with vehicle, one animal was dosed discretely with haloperidol (2 mg/kg), and two animals were cassette-dosed (2 mg/kg/compound, haloperidol, bupropion, midazolam, and clozapine). All intravenously dosed animals were euthanized at 15 min post dose.

A more extensive study using oral administration consisted of the following: one animal was dosed with vehicle, two animals were dosed discretely with moxifloxacin (25 mg/kg), two animals were dosed discretely with olanzapine (10 mg/kg), and three animals were cassette-dosed (moxifloxacin, olanzapine, erlotinib, and terfenadine at 25, 10, 10, and 25 mg/kg, respectively). Blood samples were taken at 0.5, 1, 2, 3, 4, and 5 h post dose via the tail vein (0.2 mL) and 6 h via cardiac puncture (0.5 mL); the blood was spun at 4500g for 2 min to yield plasma (approximately 0.1 mL) which was subsequently stored at $-20\ ^\circ\text{C}$ prior to analysis. Animals were euthanized at either 2 or 6 h post dose.

All tissue dissection was performed by trained AstraZeneca staff (project license 40/3484, procedure number 10). Tissues (brain, kidneys, lungs, and liver) were snap-frozen in 2-methylbutane on dry ice, subsequent transfer of tissues was done on dry ice, and samples were stored at $-80\ ^\circ\text{C}$ until tissue processing.

Tissue Processing. Tissue sections were cut at a thickness of $14\ \mu\text{m}$ and thaw-mounted onto indium tin oxide (ITO)-coated MALDI target slides (Bruker Daltonics, Bremen, Germany) or standard glass microscope slides (VWR, Leicestershire, U.K.). Sections were taken at approximately equal depth from all organs to allow visualization of similar structures between samples. Organ tissue sections from cassette-dosed, discrete-dosed, and vehicle control animals were mounted adjacent on the same slides to minimize variability caused through variations in matrix application or when analyzing separate MALDI targets. Tissue sections were analyzed randomly and nonsequentially to limit the risk of any observed variation in relative abundance being as a result in loss of analyzer sensitivity during the course of the analysis. Mounted tissue sections were stored at $-80\ ^\circ\text{C}$ until required.

MALDI Matrix Application. Thaw-mounted tissue sections were dried in a stream of nitrogen when removed from $-80\ ^\circ\text{C}$ storage. Optical images were taken using a standard flat bed scanner (Seiko Epson, Negano, Japan) prior to MALDI matrix application. Matrix coating was applied as previously described for the analysis of small molecules using a pneumatic TLC sprayer (Sigma-Aldrich).¹⁹ Either 15 mL of CHCA (10 mg/mL, 50/50 v/v acetonitrile/water, 0.1% TFA) or DHB (70 mg/mL, 50/50 v/v acetonitrile/water, 0.1% TFA) was applied. Following matrix application all subsequent transportation was performed with samples sealed in container to limit effects of light and humidity on sample and matrix. For high spatial resolution, tissue sections were transferred from $-80\ ^\circ\text{C}$ to a desiccator and dried for about 30 min prior to matrix coating. An optical image of the slide was acquired using a flatbed scanner (Epson perfection V500). The tissue was coated with DHB at a lower concentration of 35 mg/mL (50/50 v/v

methanol/water, 0.2% TFA) to reflect the more direct application of matrix and to prevent system blockages when using the TM-Sprayer tissue MALDI sample preparation system (HTX Technologies, LCC, Carrboro, NC, U.S.A.). A flow rate of 80 $\mu\text{L}/\text{min}$, nitrogen pressure 8 psi, and a spray nozzle temperature of 95 $^{\circ}\text{C}$ were used for two passes and 90 $^{\circ}\text{C}$ for a subsequent four passes of the tissue.

MALDI Mass Spectrometry Imaging. MALDI MSI was initially performed using a MALDI q-TOF MS (MALDI SYNAPT G2 HDMS, Waters Corporation, U.K.). The region of interest selected for imaging was defined using HDI Imaging software (version 1.2, Waters Corporation, U.K.), where the spatial resolution was also set (100 μm). Positive ion data was acquired with the mass spectrometer set to sensitivity mode over the range of m/z 100–1200 with 300 laser shots per raster position using a 1 kHz laser. Optimization of the mass spectrometer was achieved by tuning acquisition settings while collecting data from a manually deposited control spot of the target compounds (0.5 μL of drug standard solution at approximately 2 μM manually spotted onto the ITO target with equal volume of matrix in solution). Raw data was converted to image files via processing through Mass Lynx (version 4.1, Waters Corporation, U.K.), then viewed in HDI Imaging software (version 1.2, Waters Corporation, U.K.). Data was normalized by total ion current, and subsequent image analysis was performed. Mass filter windows were selected with a precision of ± 0.04 Da.

High mass resolution data were acquired on a 12 T SolariX FTICR (Bruker Daltonics, Billerica, MA) running SolariX control v1.5.0 build 103. Ions were excited (frequency chirp 48–500 kHz at 100 steps of 25 μs) for 0.8 s to yield broadband 2 Mword time-domain data. Each mass analysis was the sum of 400 random laser sample positions across tissue. Fast Fourier transforms and subsequent analyses were performed using DataAnalysis v 4.1 build 362.7 and Fleximaging v4.0 build 32 (Bruker Daltonics, Billerica, MA).

High spatial resolution MSI experiments were carried out in positive reflectron mode over a mass range of m/z 150–900 using the UltrafleXtreme MALDI-TOF/TOF MS (Bruker Daltonics) equipped with a 2 kHz, smartbeam-II Nd:YAG laser. Data was collected at a spatial resolution of 15 μm , summing up 500 laser shots/raster position. FlexImaging 3.0 (Bruker Daltonics) was used for data analysis, normalization, and molecular image extraction typically using mass selection window of ± 0.05 Da.

DESI Mass Spectrometry Imaging. DESI MSI analysis was performed using an Exactive mass spectrometer (Thermo Fisher Scientific Inc., Bremen, Germany) operated in positive ion mode. The mass spectrometer was equipped with a custom-built automated DESI ion source. The mass resolution used for all measurements was set to 100 000. Mass spectra were collected in the mass range of m/z = 150–1300. Methanol/water (95:5 v/v) was used as the electrospray solvent at a flow rate of 2.5 $\mu\text{L}/\text{min}$. Nebulizing nitrogen was used as gas at a pressure of 10 bar. The height distance between the DESI sprayer and the sample surface was set to 2 mm with the distance between the sprayer and the inlet capillary set to 14 mm. The distance between the sample surface and the inlet capillary of the mass spectrometer was <1 mm. The angle between the sprayer tip and the sample surface was set at 80 $^{\circ}$. For line scan experiments, the parameters described above were used with a scan speed of 150 $\mu\text{m}/\text{s}$. Imaging experiments were conducted using 150 μm spatial resolution. Image analysis

consisted of individual horizontal line scans combined into imzML format using the imzML converter V.1.1.4.5 (www.maldi-msi.org). All images were created using 0.01 Da bin size and were normalized to the total ion count (TIC) to compensate for signal instabilities.

LESA Mass Spectrometry Imaging. LESA MSI was performed using LESA-MS/MS on a Triversa Nanomate chip-based electrospray ionization system (Advion, Ithaca, NY, U.S.A.) coupled to a QTRAP 5500 (AB Sciex, Framingham, MA, U.S.A.) mass spectrometer that was operated in positive ion multiple reaction monitoring (MRM) mode. The LESA sampling method consisted of aspiration of a 0.9 μL volume of extraction solution (acetonitrile/water/formic acid 60/40/0.1 v/v/v). An amount of 0.5 μL of the solution was then dispensed at a height of 0.4 mm above the tissue with a 1 s postdispense delay time; a liquid microjunction between the pipette tip and the sample was maintained throughout the procedure. An amount of 1.1 μL of sample was reaspirated into the pipet tip prior to infusion via the Nanomate chip for tandem mass spectrometry (MS/MS) analysis. Relative abundance was determined between samples by comparison of MRM transition intensity at m/z 326 > 291.2, 262.2 > 188.1, 327.1 > 269.9, and 376.1 > 165.1 for midazolam, bufuralol, clozapine, and haloperidol (intravenously dosed cassette), respectively, and 402.1 > 261.1, 313.1 > 256.1, 394.1 > 278.0, and 472.2 > 436.2 for moxifloxacin, olanzapine, erlotinib, and terfenadine (orally dosed cassette). LESA-MS/MS data was processed using a purpose-built software package capable of extracting relative abundance values from Analyst 6.1 (ABSciex, Framingham, MA, U.S.A.).

LESA-MS/MS images were created using in-house-developed software capable of color grading ion intensities acquired from each individual LESA spot in a heat map configuration.

PK Bioanalysis. Details of the preparation of bioanalytical stock solutions and the bioanalytical method used can be found in the Supporting Information.

■ RESULTS AND DISCUSSION

Obtaining knowledge of the abundance and spatial distribution of compounds in target tissue can be extremely valuable in drug discovery or during a toxicological investigation. An understanding of the distribution profile of candidate drugs can be fed back into project design/make/test cycles, allowing researchers to refine the properties of chemical series early in the drug discovery process. In order to use such distribution information effectively the analysis techniques need to be high throughput while not compromising data quality. This study explores cassette dosing as a viable method of increasing throughput and decreasing the numbers of animals used in distribution studies using mass spectrometry imaging. Data quality was validated by comparing compounds dosed using the cassette approach with compounds that are dosed orally and intravenously as discrete formulations.

Technical replicates were not performed due to instrument time constraints, number of samples involved, and the cost of LESA consumables (specifically nanoESI emitters). However, reproducibility of MSI strategies have previously been reported and therefore were not considered to be required for these experiments where relative abundances were measured. If absolute quantitation was performed we would recommend technical replicate analysis to be performed.

Test Substance Selection. Four compounds were selected for a pilot intravenous cassette dosing experiment. Haloperidol,

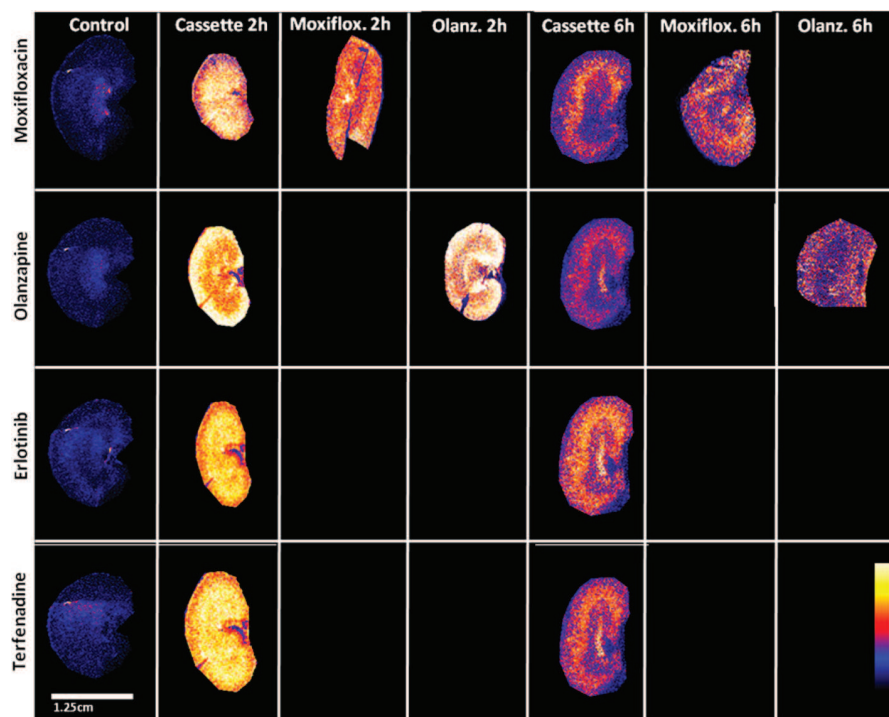


Figure 2. MSI abundance distributions of cassette- and discrete-dosed compounds in rat kidney sections ($14\ \mu\text{m}$) at 2 and 6 h post dose. Moxifloxacin and olanzapine distributions are comparable at the two time points validating the cassette approach.

a dopamine D_2 antagonist, bufuralol, a β -adrenoceptor antagonist, and midazolam, a GABA antagonist, were chosen at random; no data about prior MSI analysis has been published to date. Clozapine, a serotonin antagonist that has been used in several in-house studies and is known to be detectable by MALDI MSI, was added to the intravenous cassette to mitigate the risk of failing to detect all compounds in the pilot study by our primary analytical technique.

A further four compounds were selected for a more extensive oral cassette dosing experiment. Terfenadine, an H_1 receptor antagonist, has been analyzed by MSI following a 50 mg/kg dose (single oral dose) using a hybrid quadrupole–time-of-flight (QqTOF, QStar Pulsar, Applied Biosystems)²⁰ and at the same dose by LESA profiling (QTRAP 5500, AB Sciex).¹³ Erlotinib, a tyrosine kinase inhibitor, was detected using a hybrid quadrupole–time-of-flight (QqTOF, QStar XL, AB/MDS Sciex))²¹ following 5 mg/kg oral dose. Olanzapine, a dopamine D_2 antagonist, was orally dosed at 8 mg/kg and detected using a MALDI time-of-flight (Autoflex, Bruker).²² The final compound in the cassette was moxifloxacin, a DNA gyrase inhibitor, detected on a FlashQuant QTRAP (AB Sciex) following oral dose at 25 mg/kg.²³

Intravenous (iv) Dosing. An initial intravenously dosed pilot study consisting of cassette-dosed, discrete-dosed, and vehicle-dosed animals was performed in order to confirm the proof of principle that cassette dosing and discrete dosing show a similar relationship in terms of relative distribution across different tissues. Animals were euthanized at 15 min post dose, with brain, kidney, liver, and lung samples dissected and snap-frozen in 2-methylbutane postmortem.

Distribution of Intravenously Dosed Compounds by MALDI MSI. Brain, kidney, liver, and lung sections ($14\ \mu\text{m}$) were thaw-mounted onto ITO-coated microscope slides. Sections were arranged, as stated in the Materials and Methods

section, to minimize intra-analysis variability caused by matrix applications and interanalysis variability. Typically three brain sections representing cassette-dosed, discrete-dosed, and control were mounted on the same slide. Positive ion MALDI MSI (MALDI Synapt Q-TOF, Waters, Manchester, U.K.) was employed to analyze brain and liver sections. DHB and CHCA were used as standard MALDI matrixes with limited success. Haloperidol, bufuralol, and midazolam were not detected with either matrix from either tissue type even though initial ionization testing in the absence of tissue had been positive using both matrixes (data not shown). This poor response could be due to various factors such as ionization suppression from endogenous components and salts in the tissue and could be rectified by using an alternative MALDI matrix, solvent system, or by carefully optimized slide washing of the tissue prior to matrix application²⁴ and analysis. Clozapine was detected on both tissue types using CHCA as the MALDI matrix; the relative distribution of clozapine at 15 min post dose in a sagittal brain section can be seen in Figure 1, normalized by total ion count, at a spatial resolution of $100\ \mu\text{m}$.

Additional MSI Analysis by LESA-MS/MS and DESI-MS. The failure of MALDI MSI to detect several of the intravenously dosed compounds presented an opportunity to highlight the value of orthogonal analysis by other techniques. Figure 1 shows images of intravenously dosed sagittal brain sections analyzed by LESA-MS/MS ($1\ \text{mm}$ spatial resolution) and coronal brain sections analyzed by DESI-MS ($150\ \mu\text{m}$ spatial resolution). LESA-MS/MS detected all of the compounds in the sagittal brain sections. Relative distribution of the compounds was largely around the frontal cortex with little distribution into the rear left and right hemispheres of the cerebrum. Cassette-dosed and discrete-dosed comparisons of haloperidol in the brain sections by LESA-MS/MS (Figure 1) highlighted some differences in distribution. This is due to the

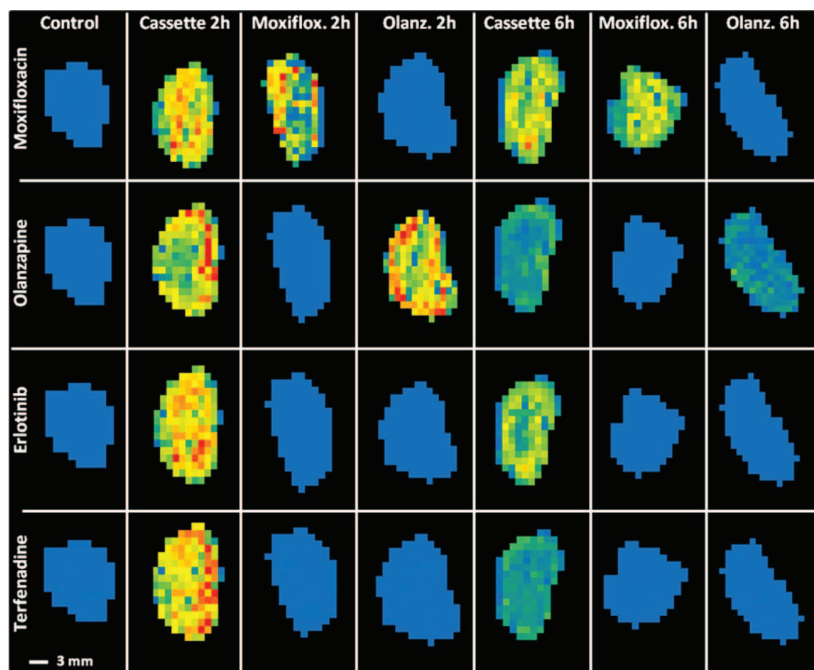


Figure 3. LESA-MS/MS abundance distributions of cassette- and discrete-dosed compounds in rat kidney sections ($14\ \mu\text{m}$) at 2 and 6 h post dose. This data mirrors the MALDI MSI data showing comparable distributions of moxifloxacin and olanzapine dosed discretely and by the cassette approach.

in-house imaging program applying a rainbow scale to a narrow drug intensity window across the two sections; the actual distribution was relatively homogeneous throughout the tissue. Coronal brain tissue sections ($14\ \mu\text{m}$) were thaw-mounted onto standard glass slides for accurate mass DESI-MS analysis. Due to the requirement to place DESI-MS samples onto nonconductive glass slides, additional tissue sections were cut by cryostat and thaw-mounted. It was necessary to use a brain from a different animal (dosed and sacrificed under same conditions) as the first brain fractured during the remounting process. While this prevents intersample consistency it does allow the coronal DESI analysis to show symmetry in detected analytes and endogenous compounds. Detection of haloperidol, bupropion, and clozapine at (mass \pm ppm) 376.1468 ± 1.6217 , 262.1795 ± 2.4945 , and 327.1366 ± 1.5254 Da, respectively, was achieved (Figure 1). Midazolam could not be detected with sufficient mass accuracy (326.0850 Da) from the tissue sections using DESI-MS.

Oral (po) Dosing. A larger, more comprehensive study was designed to compare cassette dosing to discrete dosing after oral administration. Plasma analysis by LC-MS/MS revealed that cassette- and discrete-dosed animals were exposed to the test substances in all instances. Comparative plasma concentrations post dose for moxifloxacin and olanzapine were equivalent in cassette-dosed versus discrete-dosed animals (Figure S-1, Supporting Information), indicating that plasma exposures were not adversely affected by drug–drug interactions. PK parameters for each compound are listed in Table S-1, Supporting Information. The parameters exhibit reproducibility between orally administered cassette- and discrete-dosed moxifloxacin and olanzapine and provide an initial indication that the cassette dosing approach is valid.

Distribution of Orally Dosed Compounds by MALDI MSI. Brain, kidney, liver, and lung tissue sections ($14\ \mu\text{m}$) were

thaw-mounted onto ITO-coated microscope slides and were arranged in a similar order as the intravenously dosed sections to maintain a consistent approach; where possible five sections were mounted per slide, representing cassette dose 2 and 6 h, discrete dose 2 and 6 h, and control tissue. The mass spectrometer source was rigorously cleaned after every two analytical runs to limit the risk of any observed variation in relative abundance being as a result in loss of analyzer sensitivity during the course of the analysis. Positive ion MALDI MSI (MALDI Synapt Q-TOF, Waters, Manchester, U.K.) was employed to analyze the kidney sections and produced high-quality MS images (Figure 2), normalized by total ion count, at a spatial resolution of $100\ \mu\text{m}$ and signal thresholding consistent across samples. The spatial distribution and relative abundance of the test substances at the 2 h time point were homogeneous throughout the kidney tissue. Moxifloxacin and olanzapine distributions were relatively consistent between cassette- and discrete-dosed animals, with some minor differences which could be attributed to pharmacokinetic interanimal variability (supported by the differences in plasma PK levels, Supporting Information Table S-1) or the depth at which the section was cut from the kidney sample. At the 6 h time point the relative abundance of the test substances in the cassette-dosed kidneys was approximately 6-fold lower in abundance than the 2 h time point for moxifloxacin. A reduction was also detected for olanzapine (30-fold lower), erlotinib (8-fold reduced), and terfenadine (3-fold diminished). This drop in relative abundance reflects the decrease in concentrations observed in the plasma samples between the two time points but was not directly comparable in terms of the drop in response in tissue versus the drop in concentration in plasma. Erlotinib and terfenadine were distributed in both the cortex and medulla of the kidney, with greater intensity in the medullary region,

whereas moxifloxacin and olanzapine were more localized in the medulla of the kidney. The consistent relative abundance between the images for moxifloxacin and olanzapine at each time point for cassette and discrete dosing provides further validation of the cassette dosing approach. Liver and brain sections were also analyzed by MALDI MSI to confirm detection of the test substances in the various tissue types (data not shown).

Analysis of Orally Dosed Tissues by LESA-MS/MS. An advantage of tissue sectioning is that, from each sample, multiple, nearly identical tissue sections can be taken, allowing analysis such as LESA-MS/MS and DESI-MS to be performed in addition to MALDI MSI. LESA-MS/MS is based upon nanoelectrospray ionization, a proven, robust technique that is capable of ionizing a wide variety of different chemical species. The combination of the ionization technique with sensitive tandem mass spectrometry offers a complementary method of analysis to MALDI MSI. MALDI by contrast is a much more complex process, subject to subtle changes in ablation, ionization, and sensitivity due to matrix choice, matrix crystal size, preparation conditions, and endogenous interferences from material in the tissue such as salts. These limitations of the technique lead to a lower than ideal success rate when working in a high-throughput environment. LESA-MS/MS and other direct ionization techniques such as DESI-MS provide a way of bridging that gap, enabling MSI scientists to pass on meaningful distribution data to project teams. Kidney sections were analyzed by LESA-MS/MS (QTRAP 5500, ABSciex, Framingham, MA, U.S.A.) to generate low spatial resolution (1 mm) mass spectrometry images. Figure 3 displays the images from the LESA-MS/MS analysis of the orally cassette-dosed and discrete-dosed sections. The images are generated using in-house-developed software that creates a heat map of the mean ion intensity data from each LESA extraction (~125 extractions per kidney section). The LESA imaging data correlate well with the observations of the MALDI MSI data, showing a clear difference in the relative abundance of the test substances at 2 and 6 h post dose and good reproducibility in terms of spatial distribution between cassette- and discrete-dosed compounds.

High-Resolution MSI. Lung sections were analyzed by high mass resolution MS (12 T Solarix FTICR, Bruker Daltonics, Billerica, MA, U.S.A.) for confirmation of the presence of the test substances. Sample spectra are shown in Figure S-2, Supporting Information; the presence of moxifloxacin, olanzapine, erlotinib, and terfenadine at m/z 402.1824, 313.1481, 394.1761, and 472.3210 is confirmed by accurate mass and comparison of each compound with a vehicle-dosed sample.

High spatial resolution MSI is a powerful tool that allows near-cellular resolution. The higher spatial resolution images can be used to identify regions of tissue structure within organ tissue sections. Figure 4 shows a high spatial resolution image (20 μm , UltrafleXtreme MALDI-TOF/TOF MS, Bruker Daltonics) of dosed lung section. Olanzapine (m/z 313), represented in green, is distributed throughout the lung tissue, while a blood vessel can be clearly seen in the optical image and is reflected in the MSI image as an area of high heme abundance (m/z 616). High spatial resolution MSI can highlight distribution of test substances into localized areas of tissue to demonstrate drug presence at the site of action and could be expanded further to confirm target engagement via changes in endogenous disease markers.

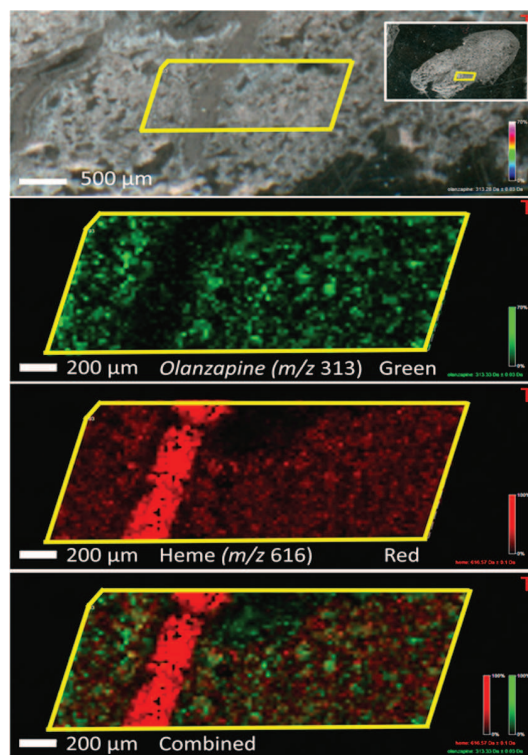


Figure 4. High spatial resolution MSI (15 μm) of orally cassette-dosed lung tissue showing an optical image describing the region of interest and MSI images of olanzapine (green) and heme (red). A blood vessel can be clearly seen with the compound being homogeneously distributed over the rest of the tissue, highlighting the power of high-resolution MSI.

CONCLUSIONS

Cassette dosing is a widely adopted practice within pharmaceutical discovery research for plasma PK analysis. Here we have demonstrated for the first time its utility in PK tissue distribution studies using mass spectrometry imaging techniques. Cassette dosing fundamentally increases throughput, in this case by 4-fold, greatly reducing what has traditionally been an analytical bottleneck. The combination of cassette-dosed PK and drug distribution studies has many advantages; ethically the technique reduces the number of animals used, typically by around 75%; the combination also leads to a wealth of pharmacokinetic and distribution data being available at a much earlier stage of drug discovery and can lead to a much greater confidence that compounds are present at the pharmacological site of action. It is also possible to measure and monitor compound abundance target tissue in response to dose or time.

Mass spectrometry imaging covers a variety of relatively new and exciting technologies that can be used to study the regional abundance of xenobiotics within tissues, while also simultaneously measuring endogenous molecules. A single technique is yet to emerge that can successfully analyze the full scope of chemistries encountered in drug discovery, at sufficient sensitivity and with high spatial resolution. MALDI MSI was successfully used to analyze orally cassette-dosed tissue sections at 100 μm and at higher spatial resolution (15 μm). LESA-MS/MS was validated against the MALDI MSI data. The success of MALDI MSI to analyze the compounds in the oral cassette-dosed study was contrasted against an intravenously dosed

cassette study in which the technique had less success. LESA-MS/MS and DESI-MS were used to profile the relative distribution of the intravenously dosed compounds, at lower spatial resolution, but with a higher success rate.

The cassette dosing strategy has been shown to be a successful approach to obtain early, combined pharmacokinetic and distribution data. Mass spectrometric imaging techniques have an increasingly important role to play in pharmaceutical R&D, with techniques being developed to provide more quantitative data and the expansion of MALDI MSI, in particular, into areas of targeted and untargeted metabolomics.

■ ASSOCIATED CONTENT

■ Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: john.swales@astrazeneca.com. Phone: +44 0 7818 523 771.

Notes

The authors declare no competing financial interest.

■ REFERENCES

- (1) Bass, A. S.; Cartwright, M. E.; Mahon, C.; Morrison, R.; Snyder, R.; McNamara, P.; Bradley, P.; Zhou, Y.; Hunter, J. *J. Pharmacol. Toxicol. Methods* **2009**, *60*, 69–78.
- (2) Berman, J.; Halm, K.; Adkison, K.; Shaffer, J. *J. Med. Chem.* **1997**, *40*, 827–829.
- (3) Alien, M. C.; Shah, T. S.; Day, W. W. *Pharm. Res.* **1998**, *15*, 93–97.
- (4) Olah, T. V.; McLoughlin, D. A.; Gilbert, J. D. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 17–23.
- (5) Frick, L. W.; Adkison, K. K.; Wells-Knecht, K. J.; Woollard, P.; Highton, D. M. *Pharm. Sci. Technol. Today* **1998**, *1*, 12–18.
- (6) Manitpisitkul, P.; White, R. E. *Drug Discovery Today* **2004**, *9*, 652–658.
- (7) White, R. E.; Manitpisitkul, P. *Drug Metab. Dispos.* **2001**, *29*, 957–966.
- (8) Swales, J. G.; Wilkinson, G.; Gallagher, R.; Hammond, C.; O'Donnell, C.; Peter, R. *Int. J. High Throughput Screening* **2010**, *1*, 1–14.
- (9) Madden, S.; Patterson, A.; Stevenson, K. *Drug Metab. Rev.* **2012**, *44*, 63–63.
- (10) Stoeckli, M.; Chaurand, P.; Hallahan, D. E.; Caprioli, R. M. *Nat. Med.* **2001**, *7*, 493–496.
- (11) Solon, E. G.; Schweitzer, A.; Stoeckli, M.; Prideaux, B. *AAPS J.* **2010**, *12*, 11–26.
- (12) Stegemann, C.; Drozdov, I.; Shalhoub, J.; Humphries, J.; Ladroue, C.; Didangelos, A.; Baumert, M.; Allen, M.; Davies, A. H.; Monaco, C. *Circ.: Cardiovasc. Genet.* **2011**, *4*, 232–242.
- (13) Eikel, D.; Vavrek, M.; Smith, S.; Bason, C.; Yeh, S.; Korfmacher, W. A.; Henion, J. D. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 3587–3596.
- (14) Parson, W. B.; Koeniger, S. L.; Johnson, R. W.; Erickson, J.; Tian, Y.; Stedman, C.; Schwartz, A.; Tarcsa, E.; Cole, R.; Van Berkel, G. J. *J. Mass Spectrom.* **2012**, *47*, 1420–1428.
- (15) Blatherwick, E. Q.; Van Berkel, G. J.; Pickup, K.; Johansson, M. K.; Beaudoin, M.; Cole, R. O.; Day, J. M.; Iverson, S.; Wilson, I. D.; Scrivens, J. H. *Xenobiotica* **2011**, *41*, 720–734.
- (16) Goodwin, R. J.; Pitt, A. R. *Bioanalysis* **2010**, *2*, 279–293.
- (17) Goodwin, R. J. *J. Proteomics* **2012**, *75*, 4893–4911.
- (18) Sugiura, Y.; Setou, M. *J. Neuroimmune Pharmacol.* **2010**, *5*, 31–43.
- (19) Goodwin, R. J.; Dungworth, J. C.; Cobb, S. R.; Pitt, A. R. *Proteomics* **2008**, *8*, 3801–3808.
- (20) Hsieh, Y.; Chen, J.; Knemeyer, I.; Crossman, L.; Korfmacher, W. A. *Drug Metab. Lett.* **2008**, *2*, 1–4.
- (21) Signor, L.; Varesio, E.; Staack, R. F.; Starke, V.; Richter, W. F.; Hopfgartner, G. *J. Mass Spectrom.* **2007**, *42*, 900–909.
- (22) Hamm, G.; Bonnel, D.; Legouffe, R.; Pamelard, F.; Delbos, J.; Bouzom, F.; Stauber, J. *J. Proteomics* **2012**, *75*, 4952–4961.
- (23) Prideaux, B.; Dartois, V.; Staab, D.; Weiner, D. M.; Goh, A.; Via, L. E.; Barry, C. E., III; Stoeckli, M. *Anal. Chem.* **2011**, *83*, 2112–2118.
- (24) Shariatgorji, M.; Källback, P.; Gustavsson, L.; Schintu, N.; Svenningsson, P.; Goodwin, R. J.; Andren, P. E. *Anal. Chem.* **2012**, *84*, 4603–4607.
- (25) Campbell, D. I.; Ferreira, C. R.; Eberlin, L. S.; Cooks, R. G. *Anal. Bioanal. Chem.* **2012**, *404*, 389–398.