

Drug distribution imaging of rabbit whole-eye section by MALDI mass spectrometry [↗](#)

Version 2

PLOS One

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ABSTRACT

Matrix-assisted laser desorption/ionization–imaging mass spectrometry (MALDI-IMS) evaluates drug distribution in biological samples. However, there have been few investigations of drug distribution in ocular tissues, including whole-eye segments. In the present study, we explored the spatial distribution of atropine in a whole-eye segment using MALDI-IMS. Atropine is a reversible muscarinic receptor used to treat various diseases. However, its distribution in ocular tissues remains unknown. A 1% atropine solution was administered to a rabbit and after 30 min, its eye was enucleated, sectioned, and analyzed by MALDI-IMS. Atropine accumulated primarily in the tear menisci but was found at substantially lower concentrations in the tissue surrounding the conjunctival sacs. Relative differences in atropine levels between the anterior and posterior regions provided insights into the post-instillation behavior of atropine. Atropine signal intensities differed among corneal layers and between the superior and inferior eyeball regions. Differences in signal intensity among tissues indicated that the drug migrated to the posterior regions via a periocular scleral route. This information is useful for atropine delivery and indicates that MALDI-IMS is effective for revealing drug distribution in whole-eye sections.

EXTERNAL LINK

<https://doi.org/10.1371/journal.pone.0211376>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Mori N, Mochizuki T, Yamazaki F, Takei S, Mano H, Matsugi T, Setou M (2019) MALDI imaging mass spectrometry revealed atropine distribution in the ocular tissues and its transit from anterior to posterior regions in the whole-eye of rabbit after topical administration. PLoS ONE 14(1): e0211376. doi: [10.1371/journal.pone.0211376](https://doi.org/10.1371/journal.pone.0211376)

PROTOCOL STATUS

Working

MATERIALS

NAME	CATALOG #	VENDOR
Acetonitrile	012-19851	FUJIFILM Wako Pure Chemical Corporation
Trifluoroacetic Acid	206-10731	FUJIFILM Wako Pure Chemical Corporation
Ultrapure Water	214-01301	FUJIFILM Wako Pure Chemical Corporation
2-Methylbutane	166-00615	FUJIFILM Wako Pure Chemical Corporation
Carboxymethyl Cellulose Sodium Salt	039-01335	FUJIFILM Wako Pure Chemical Corporation
Acetone	00310-95	Nacalai Tesque
Hematoxylin	View	Sakura Finetek
Eosin	View	Sakura Finetek

NAME ▾	CATALOG # ▾	VENDOR ▾
Atropine Sulfate Hydrate	011-23731	FUJIFILM Wako Pure Chemical Corporation
α -Cyano-4-hydroxycinnamic acid	201344	

Enucleation of eyeball from rabbit

- 1 Immediately after euthanizing the animal, its eyeball is excised, washed in saline, and enucleated. Excess saline solution is absorbed by blotting with a paper towel.

NOTE

Wash out ophthalmic solution by washing the eyeball in saline to prevent contamination.

Preparation of frozen tissue block

- 2 The enucleated eyeball is flash-frozen by immersing it in isopentane cooled with dry ice.

NOTE

If a crack appears on the eyeball, freeze it on pulverized dry ice prior to immersing it in isopentane.

The eyeball frozen in dry ice-cooled isopentane is embedded in pre-cooled 2% carboxymethylcellulose (CMC) solution, and stored at -80°C until sectioning.

Sectioning

- 3 The frozen tissue block is fixed on a stage using optimal cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan) and equilibrated at -20°C on a cryostat (Leica CM1950, Leica Biosystems, Nussloch, Germany). Tissues are sagittally sectioned into $10\text{-}\mu\text{m}$ thick slices and applied to the surface of an 20Ω indium/tin oxide-coated glass slide (Matsunami Glass Ind., Ltd., Osaka, Japan).

NOTE

It is recommended to switch the razor blade regularly to avoid contamination.

If the slide is stored, it is placed into a 50 mL tube containing silica gel to avoid condensation. Each slide is dried in a desiccator at room temperature before matrix coating.

Matrix coating

- 4 Coating is performed using an automated MALDI matrix deposition system, the TMSprayer (HTx Technologies, Chapel Hill, NC, USA).

NOTE

Matrix vapor deposition sometimes make the eyeball section cracked. Spray coating is better for eyeball section.

CHCA (5 mg mL^{-1}) in 50% v/v acetonitrile with 0.1% v/v trifluoroacetic acid is sprayed onto the tissue sections using the TMSprayer.

NOTE

Matrix vapor deposition can occasionally create cracks in the eyeball section. Therefore, spray-coating is the preferred method for eyeball sections.

It might be needed to optimize the matrix compound (DHB, CHCA, 9-AA), its concentration, and the solvent.

It might be needed to optimize the matrix coating program (spray speed and spray flow) for each compound, tissue or matrix solvent.

If tissue staining is needed, the matrix is washed off in 100% acetone and the tissue is stained with hematoxylin-eosin (H&E).

MALDI-IMS analysis

5 Optical images are acquired using an automated image scanner (NanoZoomer Slide Scanner 2.0-HT; Hamamatsu Photonics, Shizuoka, Japan).

Mass spectra are acquired with a Solarix FT-ICR mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) and a 7.0-T superconducting magnet in positive ion mode.

Before analysis, the instrument is calibrated with sodium formate.

NOTE

Mass spectrometry calibration is performed according to the instrument instructions.

The laser spot size is set to medium focus for low spatial resolution and to small focus for high spatial resolution. Its dimensions are ~50 μm and ~25 μm , respectively.

Atropine and the CHCA matrix ion are initially detected in the corneal region. The laser intensity is optimized and fixed at the start of each run.

NOTE

Laser power settings are optimized on the tissue region where the drug concentration is high enough to achieve a high sensitivity for detecting the target drug and matrix ion.

The mass range is set to m/z 200-310.

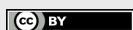
NOTE

Mass range should be set individually for each target compound.

Because far more CHCA sodium adducts (observed m/z 212.031814) are produced than CHCA proton adducts (theoretical m/z 190.05), the former was set as the lock mass.

NOTE

The lock mass setting is required because mass accuracy can easily deviate.



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