

## Introduction

Mass Spectrometry Imaging (MSI) and Quantitative Whole Body Autoradiography (QWBA) are related techniques to assess the distribution of drug related material. While QWBA is the gold standard for quantification in biological tissues the technique does not allow individual components to be identified. MSI has long been used to provide molecular distribution information in a specific mass range and has recently shown capabilities in quantifying material in biological tissues (QMSI). This study demonstrates the QMSI capabilities as a complementary approach with QWBA to follow chloroquine and one of its metabolites (desethyl-chloroquine) at different time points following a single oral administration of <sup>3</sup>H-Chloroquine to Long-Evans rats.

## Experimental

Long-Evans rats were administered a single dose of 30 mg/kg of <sup>3</sup>H-Chloroquine (300 $\mu$ Ci/kg). They were euthanized under deep anesthesia (isoflurane) at 4, 24, 72, 168, and 336 hours post-dose and carcasses were immediately frozen in a hexane/dry ice bath for approximately 8 minutes, embedded in chilled carboxymethylcellulose and frozen into blocks for analysis. Sections were collected onto adhesive tape for QWBA and QMSI analysis in the sagittal plane at 20  $\mu$ m thickness. QWBA images were obtained following exposure to phosphorimaging screens and scanned using a GE Typhoon scanner. For QMSI, MALDI matrix spiked with a known concentration of stable labeled chloroquine-d4 and its metabolite desethyl-chloroquine-d4 were sprayed onto the slide to normalize the data by evaluating the extinction of the compounds into the different organs. Analyses were performed by a 7T MALDI-FTICR in positive mode in the head region of the animal targeting the eye and in the mid whole-body region for other organs of interest. Distribution and quantification were performed with Quantinetix<sup>TM</sup> software.

### Whole-body mid region

In the whole-body mid region, both drug parent and metabolite were detected in various tissues from the prepared T4h sections (Figure 2). The two molecules were mainly co-localized within the different tissues analyzed and the quantitative distribution data obtained by QMSI matched QWBA parent-equivalent concentration data. The parent drug data from QMSI within the stomach contents was compared directly to the QWBA data since the bulk of the contents would be parent drug at T4h; whereas the liver can provide a good understanding of drug metabolite distribution.

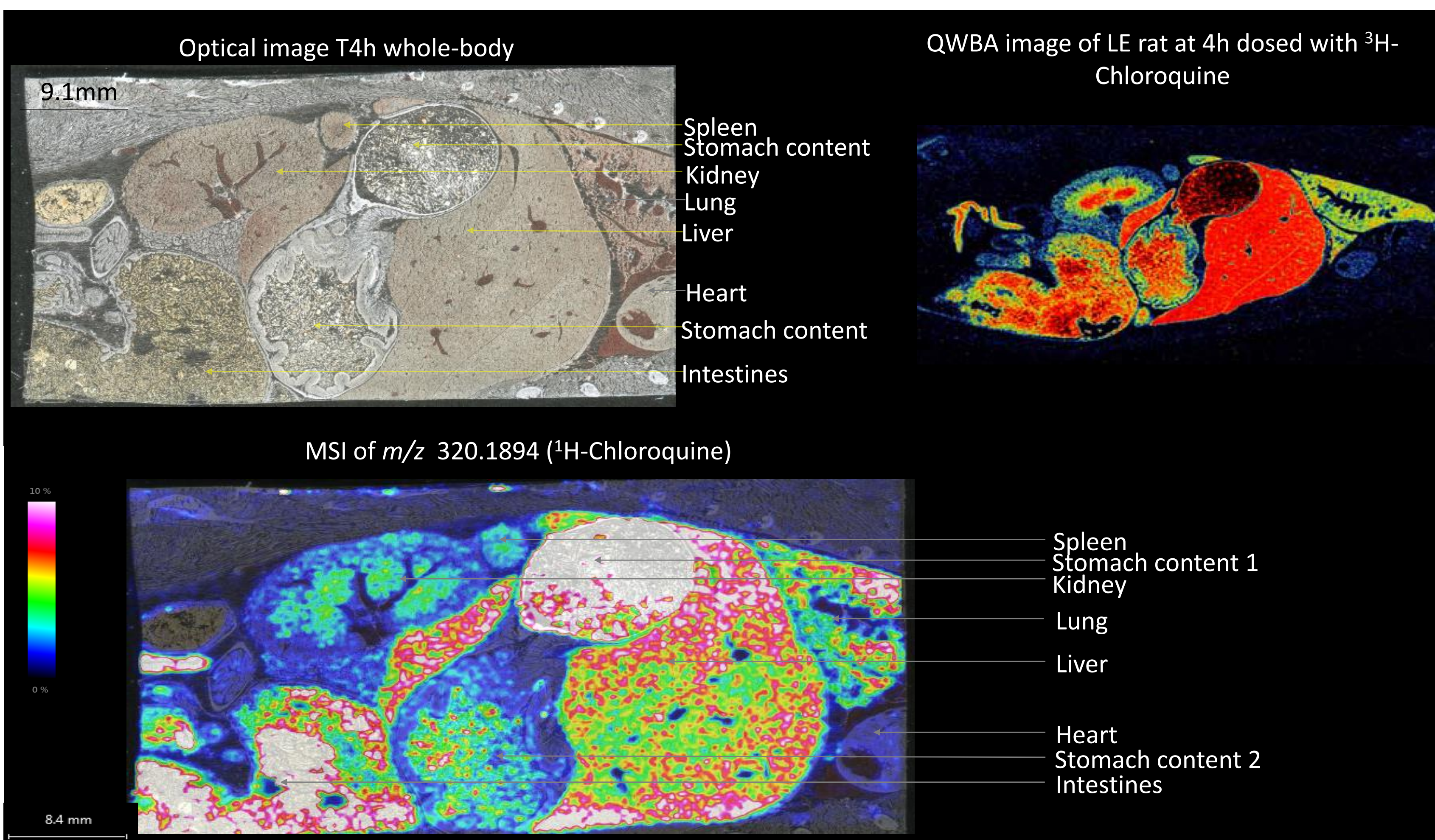


Figure 2: MSI vs WBA in the whole-body region at T4h

**Quantification results**  
A calibration range was prepared on a liver tissue section by spotting a mixture of the parent and its metabolite before analysis by MSI (Figure 3). This calibration curve allowed the limit of detection (LOD) and lower limit of quantification (LLOQ) of the two compounds to be determined. Tissue Extinction Coefficients were calculated with the deuterated forms sprayed with the matrix to evaluate the suppression effects of the different organs (other than liver) of the dosed tissue. The quantification was processed with Quantinetix<sup>TM</sup> software for both <sup>1</sup>H-chloroquine and <sup>1</sup>H-desethyl-chloroquine taking into account the direct absolute quantification of the liver and the different TEC values for other organs. Results of the absolute quantification are summarized in the Table 1.

Figure 3: Calibration curve on a Liver section of <sup>1</sup>H-Chloroquine

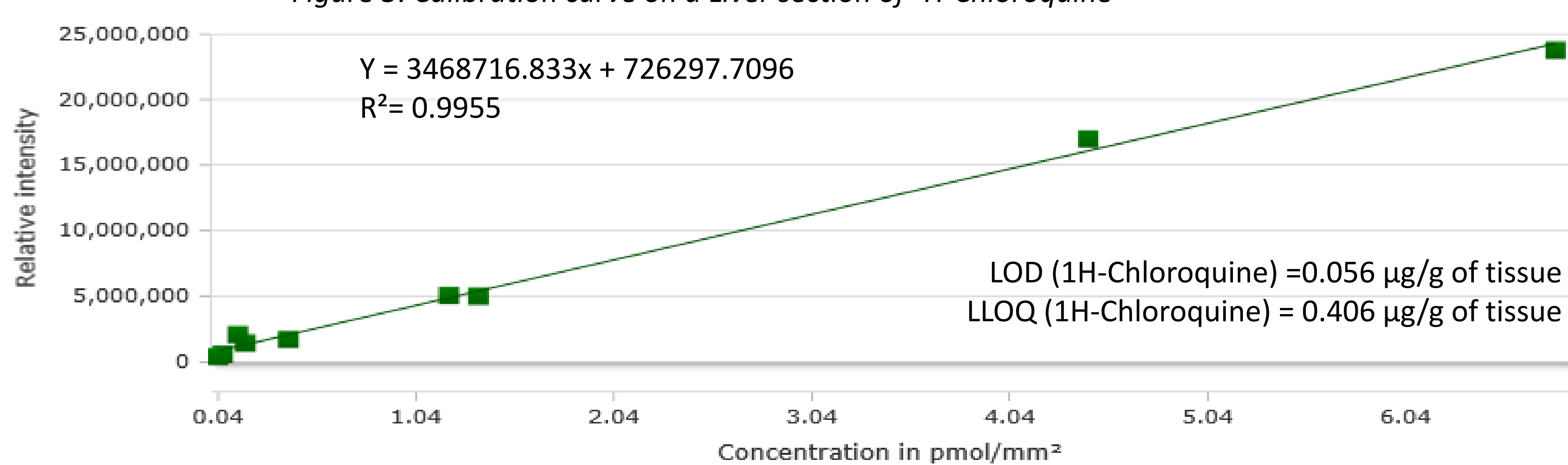


Table 1:  
QMSI vs  
QWBA

| Organ           | QMSI (µg/g of tissue)      |                                     |            | TOTAL QWBA (µg eq/g of tissue) |
|-----------------|----------------------------|-------------------------------------|------------|--------------------------------|
|                 | <sup>1</sup> H-Chloroquine | <sup>1</sup> H-Desethyl Chloroquine | TOTAL QMSI |                                |
| Liver           | 111.4                      | 3.1                                 | 114.5      | 103.0                          |
| Stomach         | 293.8*                     | BLOQ                                | 293.8      | 174.0                          |
| Kidney, Cortex  | 13.5                       | BLOQ                                | 13.5       | 17.6                           |
| Kidney, Medulla | 25.2                       | BLOQ                                | 25.2       | 29.6                           |
| Kidney          | 17.7                       | BLOQ                                | 17.7       | 23.3                           |
| Lung            | 26.3                       | 0.7                                 | 27.0       | 41.7                           |
| Uveal tract     | 0.6                        | BLOD                                | 0.6        | 26.0                           |
| Harderian gland | 0.6                        | BLOD                                | 0.6        | 9.6                            |
| Lens            | BLOD                       | BLOD                                | BLOD       | 0.9                            |
| Skin            | BLOQ                       | BLOD                                | BLOQ       | 3.0                            |

A correlation between QMSI and QWBA was observed by totaling the parent + metabolite concentrations with the exception of the uveal tract and Harderian gland for which a ratio 10:1 QWBA:QMSI was observed. This difference appears to be due to the limited region of interest scanned by MSI as compared to the broader region of interest observed in the QWBA image (Figure 1). The low levels observable in the uveal tract is most likely related to melanin binding of chloroquine and insufficient extraction by the matrix solvent. The high concentration of the drug in the stomach was limited in the QMSI evaluation due to the potential of saturation of the signal, crystallization differences, or suppression effects impacting the linearity of the method. Another representation to correlate the quantification data of WBA (µg equivalents/g of tissue) with MSI is presented in the Table 2 and Figure 4 where the liver was normalized to 1 (100%) and all the organs were compared to this level. Figure 4 highlights the closeness of the results between the two methods for relative quantification purposes.

| Organ            | Relative QWBA (µg Equivalents [3H]Chloroquine/g of tissue) | Relative QMSI (µg [1H]Chloroquine/g of tissue) | Relative QMSI (µg [1H]Desethyl Chloroquine/g of tissue) |
|------------------|--|--|---|
| Liver            | 1.00   | 1.00   | 1.00  |
| Kidney, Cortex   | 0.17   | 0.16   | 0.57  |
| Kidney, Medulla  | 0.29   | 0.26   | 0.50  |
| Kidney           | 0.23   | 0.20   | 0.53  |
| Stomach*         | 1.69   | 2.91   | BLOD  |
| Lung             | 0.40   | 0.28   | 0.60  |
| Uveal tract*     | 0.25   | 0.02   | BLOD  |
| Lens*            | 0.01   | 0.00   | BLOD  |
| Harderian gland* | 0.09   | 0.02   | BLOD  |
| Skin*            | 0.03   | 0.01   | BLOD  |

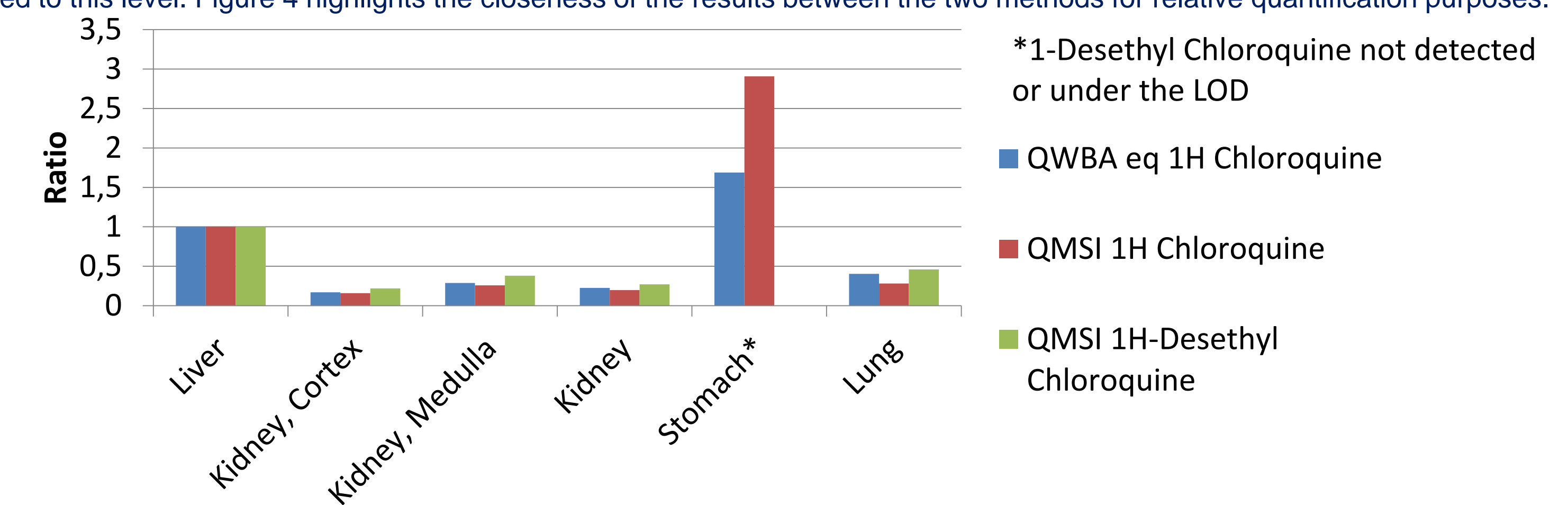


Figure 4: Relative quantification in whole-body region by QMSI and QWBA (Reference organ: Liver)

## Conclusions

- High sensitivity and linearity of QWBA methodology was combined with high resolution mass spectrometry and specificity of the MALDI to discriminate the chloroquine from its metabolite and enable the quantifications of each compound.
- High correlation was found between the two methods in the distributions and the normalized quantifications.
- Limits of the QMSI were illustrated in the low and high limits of detection compared to the QWBA because of the restricted range of linearity.