SR 2D micro-XRF imaging on Entire Rat Thin Sections in view of a Pharmacokinetic Study of a new Bromine containing Drug against Tuberculosis

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Tuberculosis (TB) is a common and often deadly infectious disease caused by various strains of mycobacteria, usually Mycobacterium tuberculosis in humans. Because of population growth, the absolute number of new cases is still increasing. Multi-drug-resistant tuberculosis (MDR-TB) is defined as resistance to the two most effective first-line TB drugs and is a public health issue in many developing countries, as treatment is longer and requires more expensive drugs. Recently, a new experimental diarylquinoline anti-tuberculosis drug was discovered at Janssen Pharmaceutica, referred to as TMC207 and shown in Fig. 1. Before TMC207 can be made commercially available, one of the necessary investigations is a thorough pharmacokinetic study to investigate its absorption, distribution, metabolism and excretion (ADME) [1].

In order to investigate the distribution of TMC207, the ^{14}C labelled compound TMC207 was administered to rat samples which were then killed by inhalation of anaesthetic after 1/4/8h respectively. Animals were immediately frozen (hexane/CO_2; -78°C) and embedded in carboxymethyl cellulose (CMC). Subsequently, thin sections at a position of interest are obtained by microtomy which are freeze-dried afterwards. Finally, the thin sections are exposed to phosphor-imaging plates (IP's), after which the tissue concentrations are analyzed as shown in Fig. 2 and quantified.

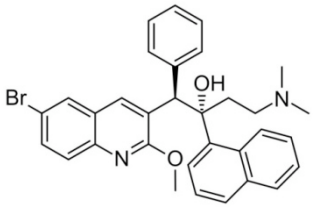

Figure 1: TMC207.

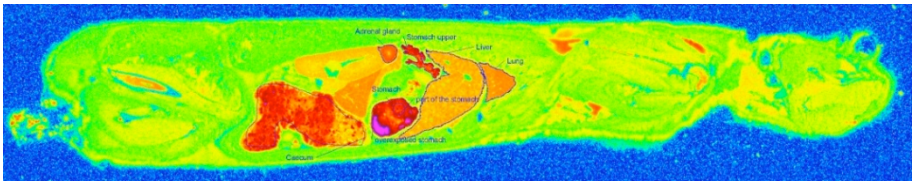

Figure 2: Radioluminography (RLG) of rat thin section.

Interestingly, TMC207 contains a single Br atom (Fig. 1), potentially allowing its trace level imaging by means of synchrotron radiation micro X-ray Fluorescence (SR micro-XRF) without using radiolabelled compounds. Therefore, the previously described rat thin sections were subjected to micro-XRF analysis at the hard X-ray microprobe Beamline L. A multilayer monochromator was used to obtain an excitation energy of 15 keV, optimally exciting the Br-K edge. The thin sections were mounted on a cardboard frame which was then attached to a high-speed motor stage with a large travel range as shown in Fig. 3. A polycapillary (PC) half lens (XOS, USA) at non-optimal working distance delivered a beam size of 90 µm (V) x 300 µm (H) FWHM possessing a higher flux density compared to a collimated beam of these dimensions, improving the scanning time for the same detection limit considerably. The rat thin sections were scanned in a vertical continuous scanning mode (500 µm step size, LT=1.5s/point), compensating for the smaller beam size in the vertical dimension. A Silicon Drift Detector (SDD, VORTEX) with 80 mm² active area was used for collecting the XRF point spectra which were subsequently processed with the AXIL software. An estimation of the detection limits was performed using NIST SRM 1577B (Bovine Liver). Elemental distributions for Cl, K, Fe and Zn respectively within an entire rat thin section are shown in Fig. 4. Corresponding detection limits for these elements (LT=1.5s) were estimated to be 130, 25, 0.9 and 0.4 ppm respectively.

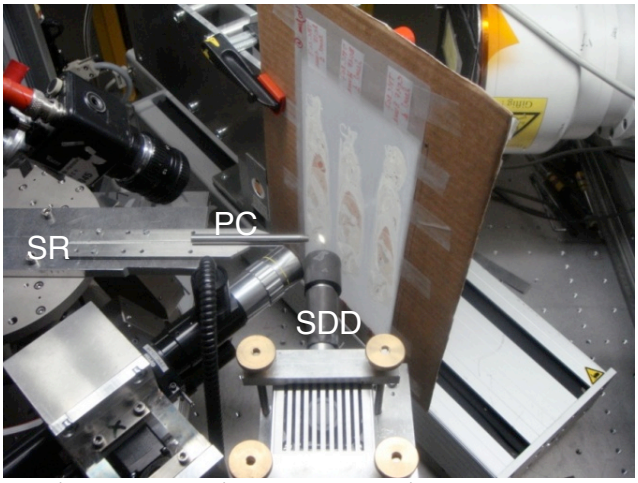

Figure 3: SR micro-XRF scanning set-up.

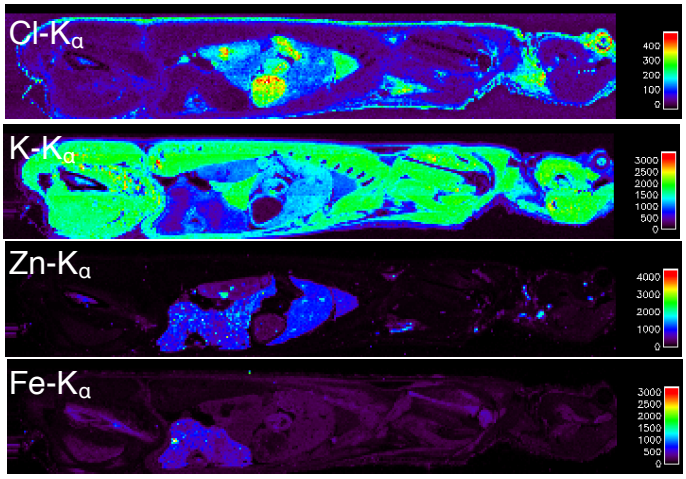

Figure 4: 2D micro-XRF results on rat thin sections.

Due to 1) the low concentration of Br in the sample (~0.3 ppm) 2) the high concentration of Br in the CMC support and 3) the higher detection limit (~1 ppm) corresponding to the scanning time of 1.5s/point, an elemental distribution of high quality of this element could unfortunately not be obtained. However, several line scans (10 points, LT=50s, range=2mm) were performed upon tissues of interest (lung, liver, exposed/upper stomach, adrenal glad). This resulted in XRF sumspectra of the different tissues as shown in Fig. 5. Moreover, a better detection limit for Br was obtained (MDL ~ 55 ppb), which resulted in a clearly distinguishable Br content between the different tissues. Principal component analysis was performed on the normalised sum spectra of which the results are shown in Fig. 6. A clear decreasing trend of the Br concentration can be observed from the stomach (point 3/5/6) towards the lung tissue (point 1) and the liver tissue /adrenal gland (point 2/4) respectively. These results are perfectly matching with results obtained from ^{14}C labelling followed by radioluminography (Fig. 2) and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS), indicating the interesting alternative of the SR based micro-XRF technique for spatially resolved pharmacokinetic studies of heteroatom containing drugs in an essentially non-destructive manner.

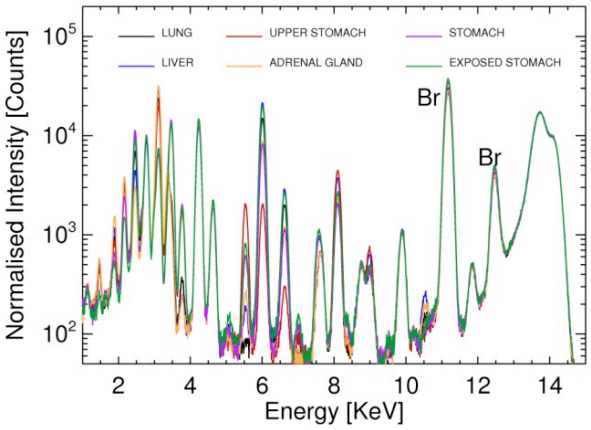

Figure 5: Sum spectra of XRF line scans.

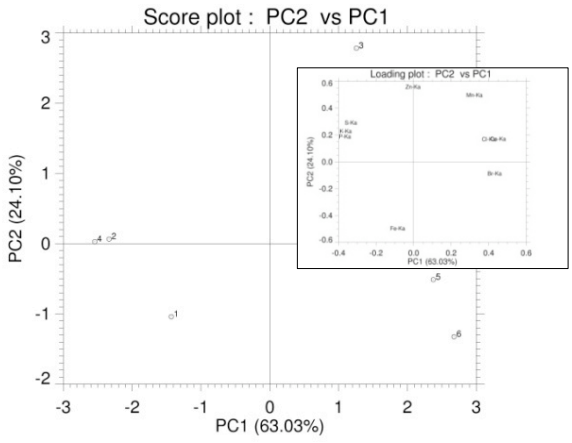

Figure 6: PCA analysis of XRF sum spectra.

References

[1] K. Andries et al., Science **307**, 223-227 (2005)