# Label-free drug uptake analysis of whole cells by MALDI-TOF mass spectrometry using the transport protein OATP2B1 as an example

In this application note, we demonstrate a MALDI MS cellular drug uptake and inhibition assay [1]. It was conducted in HEK293 cells overexpressing the transport protein OATP2B1.


## Abstract

Estrone-3-Sulfate (E3S) is a substrate for this transporter and its uptake was investigated in a pilot screen with 294 drugs with potential OATP2B1 uptake inhibiting properties. The use of the rapifleX mass spectrometer combined with MPP software led to a measurement time of ~5 min per 384 well target plate enabling MALDI MS-based screening and determination of precise IC50 values in concentration-response experiments.


## Introduction

Transport proteins have gained interest in the last years due to their involvement in human diseases and drug-drug interactions (DDI). The ageing population makes the concomitant treatment with various drugs more likely, which could lead to an accumulation of DDI effects. Therefore, it is of great importance to increase the research on transport proteins and their inhibition. The status quo in this field of research is


*Keywords: rapifleX, MALDI Pharma Pulse, MALDI compound screening, cell-based assay, concentration-response, cellular drug uptake*


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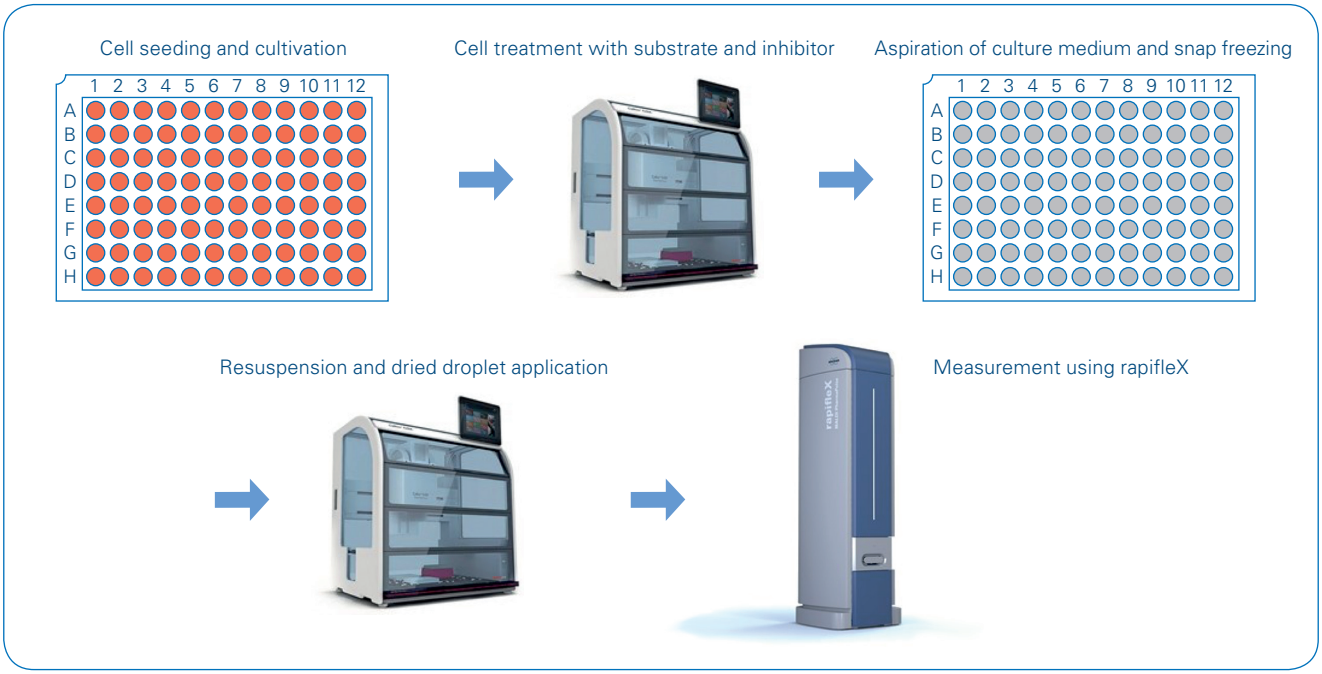


*Figure 1: Workflow of compound screen.*

the use of radioactive- and fluorescence-based readouts that are associated with several pitfalls. Negative aspects about radiolabelled readouts are the waste management and exposure to working personnel, whereas fluorescence-based readouts are prone to false negatives and positives due to photonic artefacts like autofluorescence and quenching. Both of the methods rely on the identification of inhibitors through the use of labelled substrates. Matrix-assisted laser desorption/ionisation (MALDI) represents an excellent label-free alternative. Due to its speed and possibility of automation, it has become a powerful tool in compound screening already. The introduction of MALDI MS toward high throughput screening took place in 2016 followed by several approaches relying on biochemical interactions [2, 3]. Cell-based approaches nevertheless are still underrepresented. Whole cell MALDI MS analysis has gained more and more attention in the last years, since it showed the possibility to classify bacteria, identify apoptosis markers, detect concentration-dependent responses leading to $IC_{50}$ determinations and the identification of fatty acid synthase inhibitors [4]. Here, we describe an automated MALDI MS compound screen for the identification of inhibitors of the uptake of estrone-3-sulfate (E3S) through the transport protein OATP2B1. It was conducted on the rapifleX mass spectrometer and measured with the use of MALDI Pharma Pulse (MPP) software [1].

## Method

HEK293 cells overexpressing OATP2B1 or vector control were cultured in DMEM high glucose supplemented with 10% FCS, 1% PEN/STREP and 1.6% geneticine. For the compound screen, the cells were seeded in poly-L-lysine coated 96-well plates at a density of $0.5*10^6$ cells/mL. Directly after seeding the cells out, they were incubated with 5 mM sodium butyrate to increase the expression of the transporter. After 24 h, the medium was changed to serum-free medium and incubated for 1 h. Then, compound treatment was performed using the CyBio FeliX pipetting platform (Analytik Jena). Cells were preincubated with test substances (10 $\mu$M)/ DMSO for 10 min followed by the addition of 10 $\mu$M E3S for 2 min. Cells were harvested by aspirating the liquid, washing once with ice cold PBS and freezing the plate in liquid nitrogen. The plate was stored at -80°C until the measurement. The plates were resuspended with water and 0.3 $\mu$M deuterated internal standard of E3S (D4-E3S). An automated dried droplet application was done using 2.5 mg/mL Ph-CCA-$NH_2$ in 70% acetonitrile on the CyBio FeliX. The 384-well MALDI target plate was then measured with a rapifleX using the MALDI Pharma Pulse software (MPP). Measurement was done in reflector negative ion mode using a mass range of 300-900 *m/z*. Internal linear calibration was performed on E3S and D4-E3S. A positive (E3S+4 $\mu$M erlotinib as standard inhibitor) and negative (DMSO+E3S) control were applied on each target plate and $pIC_{50}$ values were calculated using the GraphPad Prism software.

## Results

294 drugs of a compound set were screened regarding their ability to inhibit the uptake of E3S through OATP2B1. The compound set consisted of the top marketed drugs of the year 2017 and other compounds known as substrates or inhibitors of OATP2B1. The method was optimised on the detection of E3S in HEK293 cells resulting in the usage of Ph-CCA-$NH_2$ as a matrix and automated dried droplet application of it using the CyBio FeliX. The single point screen was conducted in two biological replicates. The positive control (E3S+erlotinib) was considered 100% inhibition and the negative control (E3S+DMSO) was considered 0% inhibition. Compounds that led to a % inhibition value ≥50 were calculated as hits and further processed in $pIC_{50}$ determination. Of the 294 used compounds, there were 67 compounds verified as a hit during $pIC_{50}$ determination. 14 of those had a $pIC_{50}$ ≥6 marking rather potent inhibitors.

The intra-assay comparability was acceptable. Testing of 6 biological replicates for the determination of the $pIC_{50}$ led to a mean CV% value below 10 and a mean standard deviation of 0.3 speaking for a robust and reproducible assay. Comparison of the dataset with a fluorescence-based assay [5] revealed several overlapping inhibitors. Especially a comparison with a similar dataset measured with a radiolabelled assay revealed an overlap of 83% regarding the identification of inhibitors [6]. The developed assay therefore is comparable to the literature and proved the robustness of cell-based MALDI MS assays.

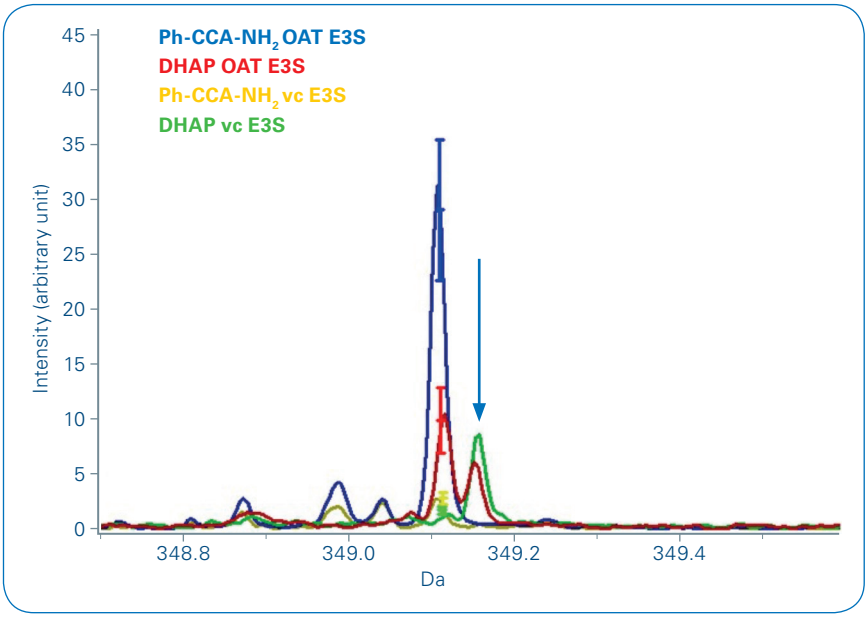


*Figure 2: No peaks interfering with the analyte peak in case of Ph-CCA-NH2 matrix application in comparison with DHAP matrix*

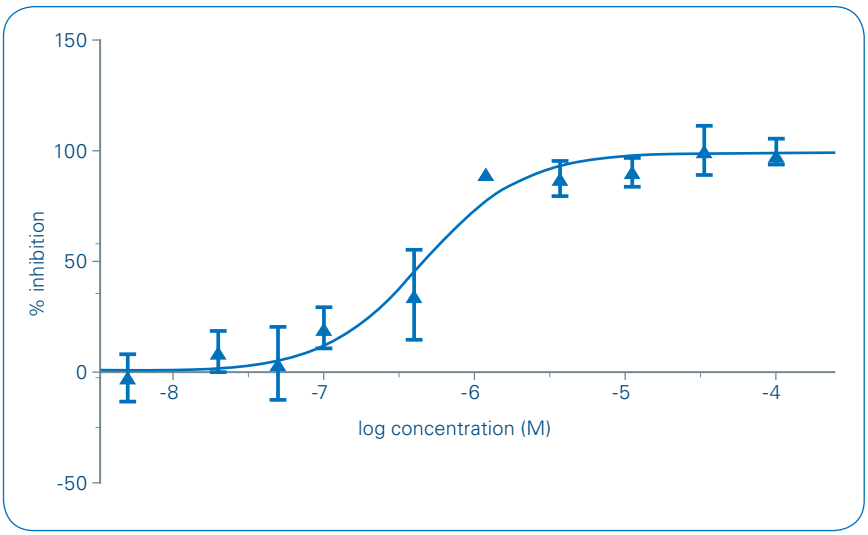


*Figure 3: pIC50 curve of Atorvastatin.*

## Conclusion

- rapifleX MALDI MS cell assays are capable of the analysis of drug uptake into cells
- Possible drug-drug interactions can be identified in a screening-approach based on MALDI-MS
- The combination of a liquid handling system together with the MALDI MS enables compound screening on a high throughput basis.

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**References**

[1] Unger MS, Schumacher L, Enzlein T, Weigt D, Zamek-Gliszczynski MJ, Schwab M, Nies AT, Drewes G, Schulz S, Reinhard FBM, Hopf C (2020). *Direct Automated MALDI Mass Spectrometry Analysis of Cellular Transporter Function: Inhibition of OATP2B1 Uptake by 294 Drugs*. Analytical chemistry, **92**: 11851-11859.

[2] Beeman K, Baumgartner J, Laubenheimer M, Hergesell K, Hoffmann M, Pehl U, Fischer F, Pieck JC (2017). *Integration of an In Situ MALDI-Based High-Throughput Screening Process: A Case Study with Receptor Tyrosine Kinase c-MET. SLAS discovery* : advancing life sciences R & D, **22**: 1203-1210.

[3] Simon RP, Winter M, Kleiner C, Ries R, Schnapp G, Heimann A, Li J, Zuvela-Jelask, L, Bretschneider T, Luippold AH, Reindl W, Bischoff D, Buttner FH (2019). *MALDI-TOF Mass Spectrometry-Based High-Throughput Screening for Inhibitors of the Cytosolic DNA Sensor cGAS. SLAS discovery* : advancing life sciences R & D: 2472555219880185.

[4] Weigt D, Parrish CA, Krueger JA, Oleykowski CA, Rendina AR, Hopf C (2019). *Mechanistic MALDI-TOF Cell-Based Assay for the Discovery of Potent and Specific Fatty Acid Synthase Inhibitors*. Cell chemical biology, **26**: 1322-1331.e1324.

[5] Unger MS, Mudunuru J, Schwab M, Hopf C, Drewes G, Nies AT, Zamek-Gliszczynski MJ, Reinhard FBM (2020). *Clinically Relevant OATP2B1 Inhibitors in Marketed Drug Space*. Molecular pharmaceutics.

[6] Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E, Lai Y, Haglund U, Artursson P (2012). *Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug-drug interactions*. Journal of medicinal chemistry, **55**: 4740-4763.


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