A good connection

Integrated in an online system: sample preparation and HPLC-MS analysis of biological samples

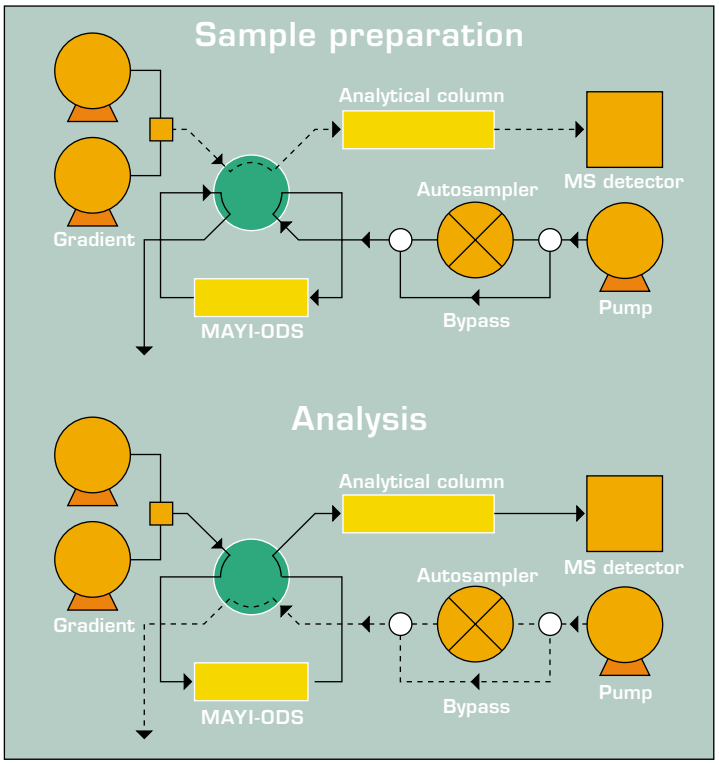


Figure 1: Flow diagram of the Co-Sense BA

HPLC and LC/MS techniques are used in many application areas for the analysis of pharmaceutical active compounds in biological samples. Mass spectrometric detectors are steadily gaining importance based on their high selectivity and sensitivity.

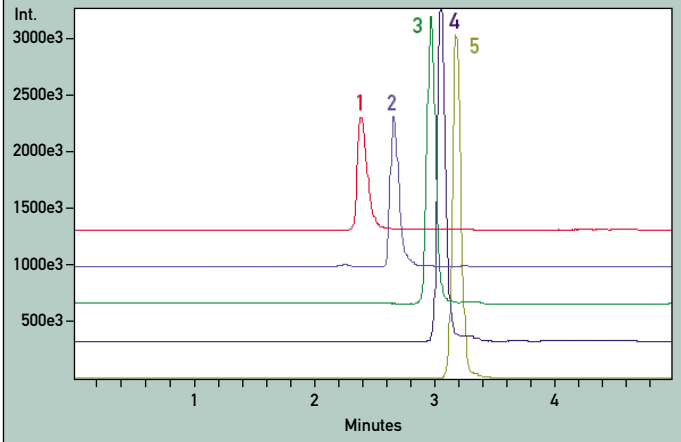
For the analysis of biological samples it is, however, extremely important to remove undesirable contaminations such as proteins and salts, from the matrix. A careful and often complex sample preparation procedure is therefore the most crucial aspect of a sensitive and robust analysis.

Up to now, the common sample preparation protocols for the chromatographic analysis of biological samples (blood plasma, serum or urine) described below, could only be automated to a limited extent, such as protein precipitation with subsequent solid-phase extraction of the supernatant. Sample preparation is therefore a time consuming and complex procedure and is often subjected to numerous possible sources of error. For an optimal result, the sample should be injected directly into an instrument where sample preparation and chromatographic analysis are integrated in an online system. Shimadzu's Co-Sense BA system now enables fully automated analysis of biological samples.

The column is part of the integrated process

The heart of the system is a special chromatographic column (MAYI-ODS) for the extraction of biological samples. A column switching system combines sample preparation and chromatographic analysis within an integrated analytical process.

The MAYI-ODS column is used simultaneously for the removal of macromolecules and for solid-



Chromatographic conditions:
Sample preparation:
injection volume: 10 µL
Column: Shim-pack MAYI-ODS
Mobile phase: 10 mM ammonium acetate/acetonitrile (95/5)
Flow rate: 3 mL/min at 45 °C

Analysis:
Column: Shim-pack GVP-ODS
Gradient: ammonium acetate (A)/ acetonitrile (B) from 5 % B up to 90 % B in 3 minutes, flow rate 0.8 mL/min at 45 °C, split 1:3 (MS: waste), + 4.5 kV ESI positive

The mass traces of the following compounds are shown:
1: Metoprolol m/z 268 · **2:** Propranolol m/z 260 · **3:** Lidocaine m/z 235 · **4:** Dibucaine m/z 344
5: Bupivacaine m/z 289

Figure 2: HPLC-MS analysis of rat serum, spiked with 5 active compounds (each 1 µg/L). The total analysis cycle including extraction and gradient analysis takes 5 minutes

phase extraction of the target molecules. The column is packed with relatively large particles whose inner pore surfaces are chemically modified with C18 groups. The outer particle surface is coated with a polar polymer layer which prevents proteins and salts from entering the pores. Online dilution of the samples with buffer (and, if necessary, a small volume of organic solvent) before they are injected onto the MAYI-ODS column, assures that even compounds with a high degree of protein binding are collected and can be concentrated. Interfering proteins are efficiently removed.

Via column-switching the analytes, which were concentrated on the precolumn, can be eluted onto the analytical column using a binary gradient system, where they are separated. A diagram of the switching system is shown in Figure 1.

An example of an analysis of several betablockers and local anaesthetics is shown in Figure 2.

When using the fast SIL-HT autosampler, the total analysis time of the entire process can be reduced to less than 5 minutes. This high-performance autosampler features, in addition to an injection speed of only 15 seconds for a standard injection, a special needle coating which minimised sample carry-over even for strongly adsorbing compounds.

Figure 3 shows the long-term stability of the MAYI-ODS sample preparation column. A comparison of the results from the first analysis with the chromatograms after 100, 200 and 300 runs on the same column shows the excellent reproducibility and stability of the system.

The wide application area of the Co-Sense BA makes this an attractive system for many types of applications in clinical chemistry.

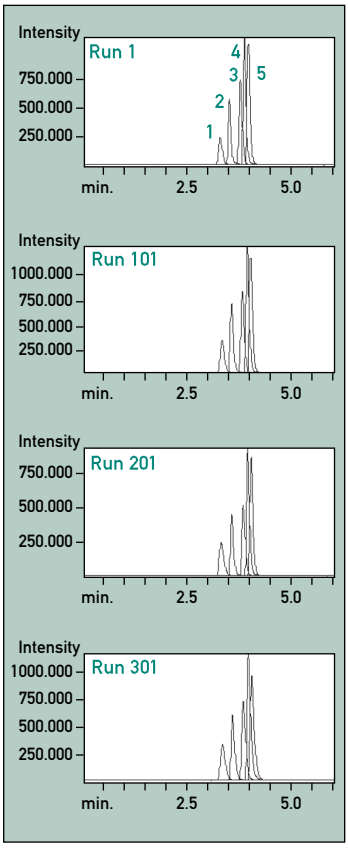


Figure 3: SIM chromatogram after 100, 200 and 300 analyses.

1: Metoprolol
2: Propranolol
3: Lidocaine
4: Dibucaine
5: Bupivacaine

Injection volume: 50 µL

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