



Analytics



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Summary

Having reliable data available when you need them is a key success factor at every phase of the drug discovery and drug development process.

Across Barriers has the analytical expertise and state-of-the-art technology to provide its clients with in vitro cell and tissue analyses, physicochemical profiles, and other pharmaceutical analytical support services. When it comes to solving complex analytical problems, Across Barriers provides its customers in the chemical, cosmetic, and pharmaceutical industries with a suite of services ranging from sample preparation to method development and validation. It goes without saying that international regulatory standards are taken into account. By

offering access to high-quality resources and services, Across Barriers can also provide customers with support for routine problems and applications or whenever there are personnel shortages or time constraints.

Introduction

HPLC analysis has become a routine technique in the analytical separation of active ingredients and for assays. HPLC methodology now covers a broad range of techniques including the detection of substances at trace levels. The full power of an HPLC system is best tapped by combining it with an advanced detection system such as a mass spectrometer. Across Barriers offers a full set of advanced analytical tools ranging from classical pharmacopoeia procedures using HPLC-UV to high-throughput methods for routine assays using LC-MS and LC-MS/MS techniques. The coupling of different chromatographic techniques is a particularly effective means of improving the significance and reliability of chromatographic data.

Analytical systems

The following analytical methods are available:

- UV/VIS
- Fluorescence measurements
- Scintillation measurements
- Thin-layer chromatography
- High-pressure liquid chromatography

Coupled analytical systems

In order to address complex questions particularly in the area of bioanalytics, Across Barriers makes use of the following coupled techniques:

- HPLC-UV
- HPLC-PDA (photodiode array)
- HPLC-PDA-FD (fluorescence detection)
- HPLC-EC
- HPLC-PDA-MS
- HPLC-UV-MS/MS

All equipment used is in a validated state in accordance with GLP guidelines. The techniques are regularly tested and revalidated on the basis of standard operating procedures.

Method development / Validation

Depending on the specific problems to be solved, analytical methods are developed and validations performed on the basis of the latest ICH guidelines. Development work and subsequent validation is conducted in close collaboration with our customers. A comprehensive validation report covering the test procedures developed is also produced.

The development process covers methods of identification, purity assessment and assays. The following items can be tested as part of the validation process:

- Specificity
- Selectivity
- Precision
- Linearity
- Linear range
- Limits of detection
- Limits of quantitation
- Robustness



Fig. 1: Pictures taken at Across Barriers' laboratories

Automated method optimization and quantification using LC-MS and LC-MS/MS

When analyzing large numbers of samples, the analysis cycle should be kept as short as possible so that significant results can be obtained as early as possible. Reducing the separation time in a liquid chromatographic analysis using a classical detection method (such as UV) can, however, cause a deterioration in selectivity. When attempting to quantify active ingredients in biological samples, with their frequently complex matrices, selectivity is an essential requirement of the assay method. One technique that offers short analysis times, selectivity, and sensitivity is LC-MS/MS. Across Barriers is not only equipped with this technology, but also has staff experienced in its use. Measurement runs can be automated yielding optimized analyses at a high level of precision.

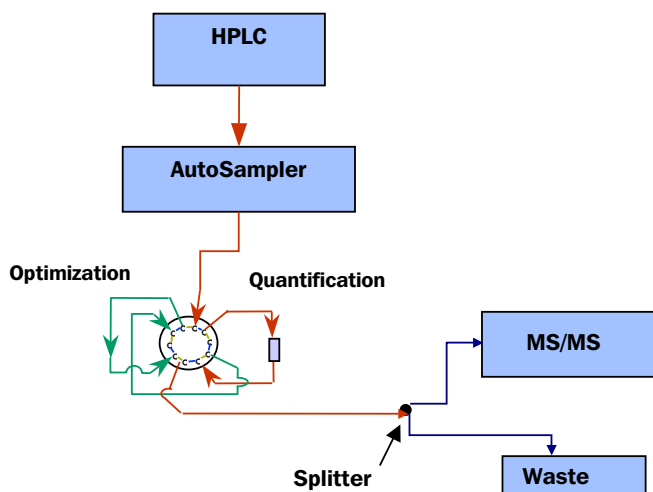


Fig. 2: Schematic diagram of an HT-LC-MS/MS system with column switching.

LC-MS/MS is the analytical method of choice when it comes to high-throughput screening assays to characterize new drug candidates for in vitro studies such as Caco-2 or dermal systems.

Calibration of an automated quantitative determination of propranolol

Propranolol is an excellent candidate for carrying out transport studies on Caco-2 cells or for studying metabolism by liver microsomes. When determining the stability of a particular test substance to microsomes, the concentration of the analyte must be typically less than 1 μM to prevent inhibition of the microsomal enzymes. In such cases, quantitative determination of the substance under test requires the use of a sensitive measurement technique like LC-MS/MS.

Compound name : L0470
 Correlation coefficient : r = 0.999986, r² = 0.999972
 Calibration curve : 16520.1 * x + -8.31296
 Response type : External Std, Area
 Curve type : Linear, Origin : Include Weighting : 1/x, Axis labels : None

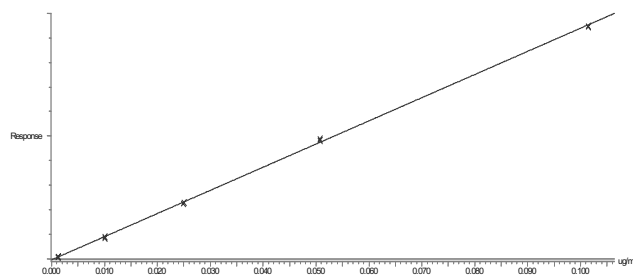


Fig. 3: Calibration line for propranolol from 0.001 μg/mL to 0.1 μg/mL in KRB buffer (Caco-2 trial).

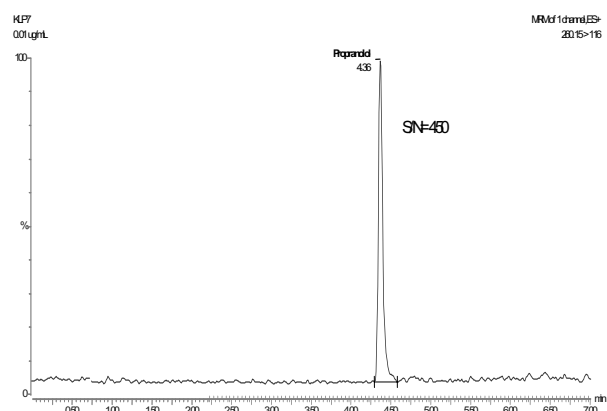


Fig. 4: Electrospray-LC-MS/MS chromatogram of propranolol from 0.01 μg/mL in KRB buffer (Caco-2 trial), MRM mode m/z = 260 > 116.

Despite the complex matrix, LC-MS/MS provides an accurate quantitative determination of propranolol over a wide concentration range (see Fig. 3 and Fig. 4).

Example: High-throughput LC-MS detection of furosemide

Sample turn-around times can be significantly shortened as the example of an LC-MS analysis of furosemide in Fig. 5 shows.

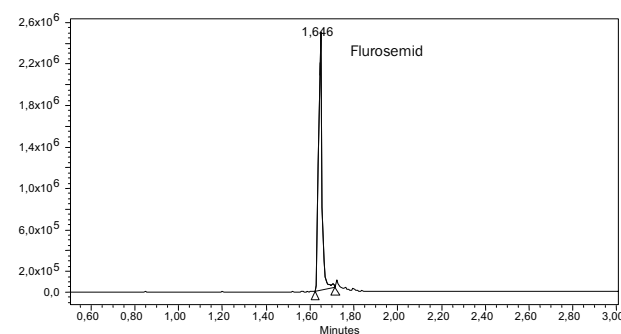


Fig. 5: Electrospray LC-MS chromatogram of 50 μM furosemide in KRB buffer m/z= 329 ESI negative.

Electrospray-ionization tandem mass spectrometry enables the selective trace-level determination of analytes in complex sample mixtures while keeping analysis cycles short.

Routine use of LC-MS/MS or LC-MS

The optimized analytical procedures shown above allow in vitro testing to be carried out efficiently and economically. In vitro testing includes measurement of ADMET parameters such as absorption and metabolism. To classify permeability, measure penetration, or elucidate the transport mechanism involved (e.g. p-glycoprotein-mediated efflux system), the following cell models are used:

- Dermal systems: human and porcine skin, reconstructed skin models, other animal skins
- Gastrointestinal systems: Caco-2 cells, excised porcine intestinal tissue
- Cerebral systems (blood-brain barrier): primary porcine brain capillary endothelial cells
- Respiratory systems
- Buccal and nasal tissue models

These techniques are also used to address classical pharmaceutical and chemical problems such as assays, purity determinations, or establishing the structural identity of contaminants. Another area of application is chemical stability testing when profiling active ingredients or when conducting product stability tests, especially in the cases where separation and quantification are difficult.

In the field of bioanalytics, where high sample throughput rates and high sensitivity are required, the coupled techniques HPLC-MS and HPLC-MS/MS are the methods of choice. These techniques are used particularly for assays of body-fluid and tissue samples, for determining the stability of biological matrices such as serum, plasma, gastric or intestinal juice, or for examining liver or microsome stability.

Sample preparation

In the analysis of highly complex matrices, such as creams, ointments, or whole blood samples, sample preparation is often necessary in order to separate the active ingredient from the matrix. This can be done relatively simply using liquid-liquid extraction or solid-phase extraction (SPE) methods. Sample preparation techniques are thus an integral part of the Across Barriers portfolio. During the extraction of matrix-contaminated samples, the active ingredient to be determined becomes highly diluted. In such cases, the extracts are transferred to a vacuum concentrator (SpeedVac) where they are concentrated by gently evaporating the solvent. An advantage of the SpeedVac method is that it effectively eliminates the problem of sample “carry over”, which can arise if the conventional technique of blowing off the solvent in a nitrogen gas stream is used.