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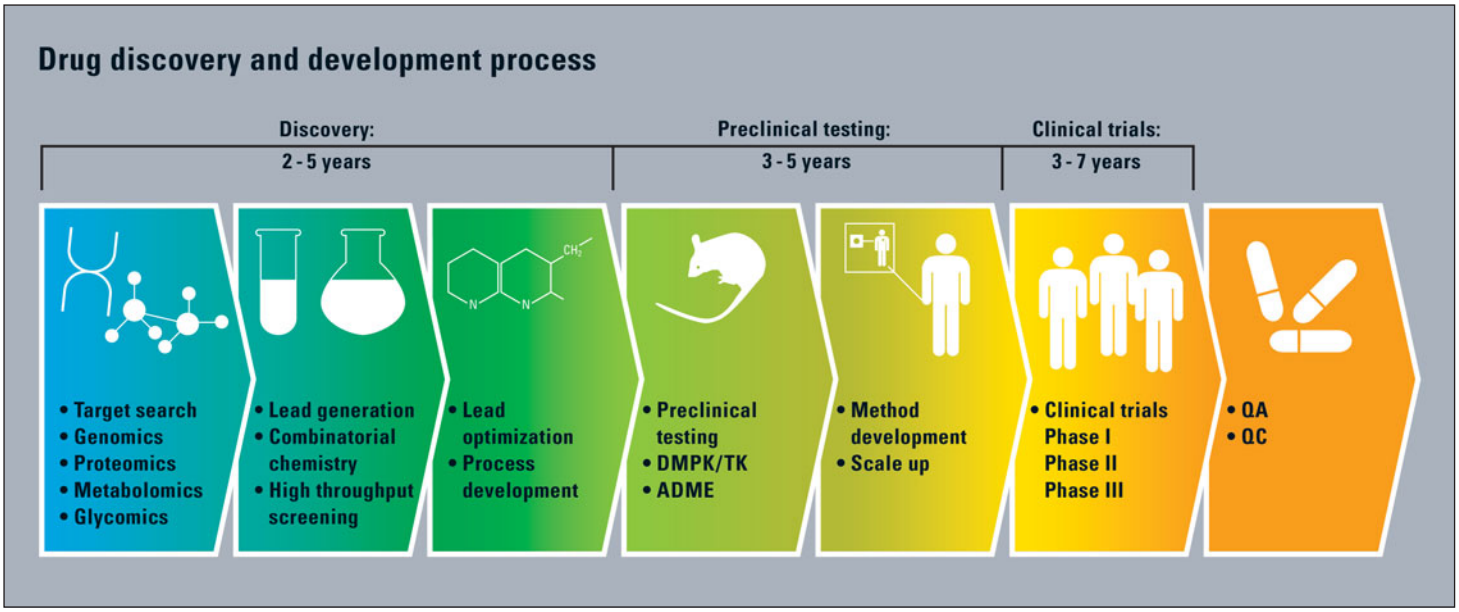


Preparative LC and LCMS

Preparative LC as well as the combination of preparative LC with MS detection is used in several steps in drug development.

A preparative system can be used to recover high purity components from some types of liquid extract obtained either from a synthesis reaction or from a natural substance by separating and purifying the target compounds. Obtaining these target compounds at high purity enables their structural analysis, permitting evaluation and analysis of their various functions while allowing their subsequent processing to be conducted more reliably. Since a preparative system must separate the target components from the coexisting substances in a sample, the basic system configuration comprises of solvent delivery pumps, sample injector, column and detector, as in a typical LC system. Additional is a mechanism for collecting fractions consisting of the target substances, thereby completing the basic configuration of a preparative system.

Efficient collection of target compounds is key

The objectives of a typical LC system are to conduct quantitative and qualitative analysis, but a preparative system is used more for so-called "pretreatment" with the objective of actually obtaining the necessary compounds for evaluation and analysis, as well as for subsequent processing. It is therefore important that target compounds are obtained quickly and at high purity. Just as with a typical pretreatment process, productivity is the key point in a preparative system. To ensure that the intended evaluation and analysis operations can be started as soon as possible following the preparative process to achieve the highest possible productivity, an appropriate system should be able to separate the quantity of compounds and integrate a detection principle that can most effectively ensure collection of the all possible target compounds.

Figure 1: Preparative LC systems with automatic sample injection and fraction collection

What is the optimal detector?

Most simple solutions are based on UV detectors (ideally multi-wavelength or photo diode array). If the target compound or the coexisting impurities in the sample solvent have no optical absorbance, detectors based on principles other than optical absorbance detection such as ELSD (evaporative light scattering detector) and MS (mass spectrometer) can be used as supplementary detectors to improve purification efficiency, thereby improving productivity.

Since ELSD and MS detectors rely in principle on nebulization of the column eluate inside the detector, the target substances emerging from these detectors cannot be recovered. To resolve this problem, most of the column eluate is directed to the fraction collector, and the remaining micro volume is introduced into the ELSD or MS. To accomplish this, an active APV splitter (Automated Proportioning Valve) is used to split the flow line. In addition, if the volume of eluate to be introduced into the ELSD or MS detector is ultra small, a make-up pump is used to augment the mobile phase flow and maintain steady detection by the ELSD or MS.

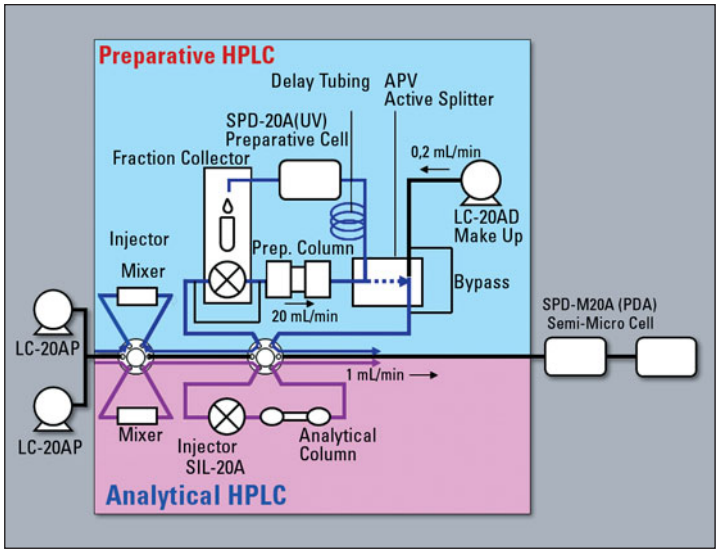


Figure 3: Configuration schema of prep LCMS system

Determination of the optimum preparative system

Once the total amount of fraction necessary for evaluation and analysis of the target compounds and subsequent processing is calculated and the detector has been selected, the next step is to determine what scale of fractionation is required. The amount of sample that can be injected at one time (load with respect to column) to maximize productivity must also be determined. Once the maximum sample injection volume is known, the number of required fractionation runs can be calculated based on the target fraction quantity. This allows determination of the optimum preparative system based on time (total time to collect the required fraction) and cost (purchase price of preparative system and column, and solvent expense). This type of examination can be conducted efficiently by first using a general purpose LC (conventional LC).

If the concentration of the fractionation target compound in the sample is known, the absolute injection quantity of the fractionation target compound can be obtained from this concentration and the sample injection volume.

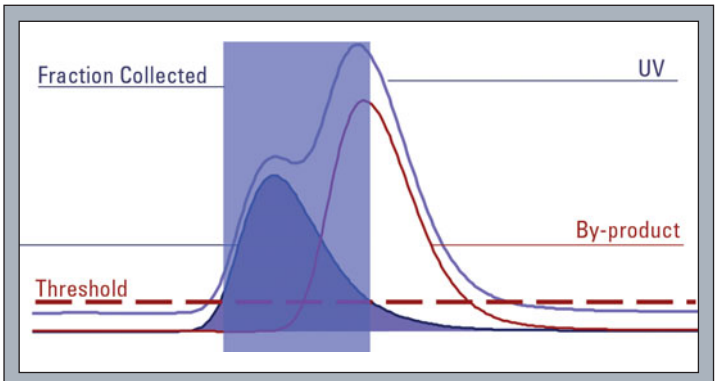


Figure 4: Fraction tiggered by MS only

When an UV trace is used to trigger fractions and both slope and threshold are triggered, the inflection in the UV trace is detected where the by-product starts to elute and the fraction tube being collected to is automatically changed.

The same amount of fraction is still collected but this time into two tubes. The first tube contains the pure target product. Therefore purifi-cation has been achieved.

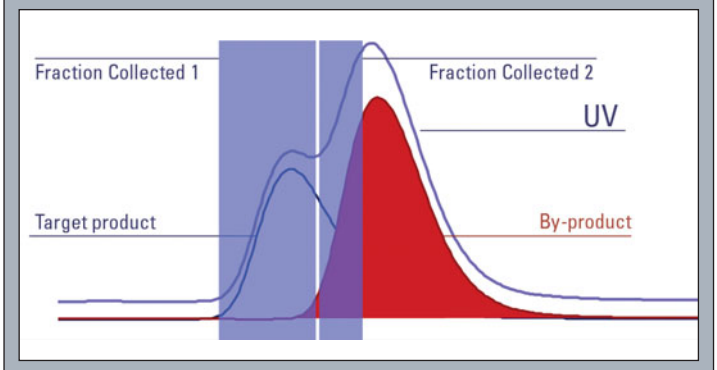


Figure 5: Fraction tiggered by combination UV and MS signal

With MS, only a specific product can be targeted. In order to trigger a fraction on the target product the trace for target product ions m/z is extracted and the collection of fractions using this single mass's trace is triggered. Co-eluting substances may also be present. Therefore, with a partial co-elution the target product continues to collect although the by-product has started to elute. This leads to contamination of the target product's fraction with the by-product. Purification has therefore not been achieved.

It is recommended that the triggering option for the UV trace be set to slope due to the excellent peak shape achieved by the UV detector; the MS should trigger on an intensity threshold since the MS only considers the target ion and is very sensitive. The AND logic is used to combine the two triggers i.e., both the MS and UV trace must trigger together for the fraction to be collected. This enables narrowing the collection down to the product of interest, reducing fraction width and therefore helping to remove the problem of contamination from other products eluting close to the product of interest. Combining the power of slope and threshold along with the MS and UV trace both being considered, a target compound can be isolated even in a complex mixture with many products eluting close to each other.

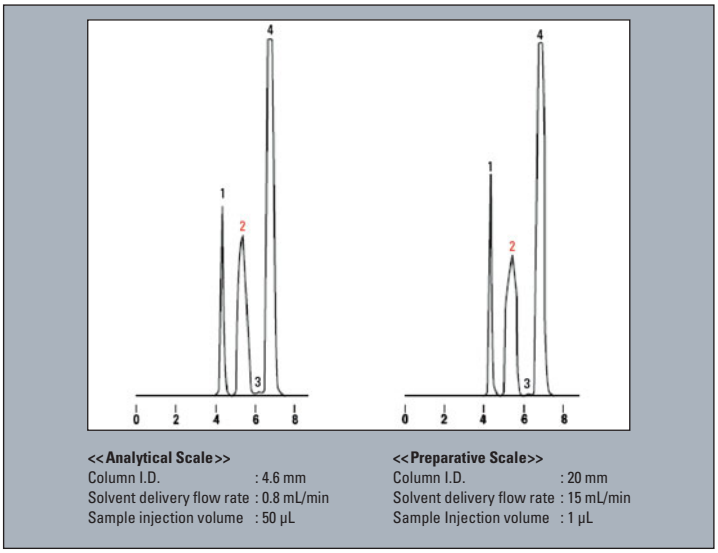


Figure 6: Example for scale-up

To efficiently use the preparative LCMS for fractionation – Mass Directed Purification signals from UV detector as well as m/z values obtained with the LCMS are applied. The following example (see box on page 4) shows the advantage of combining both signals.

Transfer analytical condition and separation to preparative scale

After examining conventional-size column conditions, the next step is to scale up from analysis to preparative level. To smoothen this transition, a preparative column is selected using the same packing material as in the analytical column. With the same packing material, the mobile phase flow rate and sample injection volume need only be increased according to the analytical column and preparative column cross-sectional area ratio to obtain nearly the same chromatographic pattern (see table 1).

An example in which the scale-up was conducted using the same packing material is shown in figure 6. Here, the 4.6 mm I.D. column used for the analytical scale was exchanged with 20 mm I.D. column for the preparative scale. Since the cross-sectional area of the 20 mm I.D. column is about 19 times that of the 4.6 mm I.D. column, the solvent delivery flow rate was scaled up from 0.8 mL/min to 15 mL/min, while the sample injection volume was scaled up from 50 µL to 1 mL.

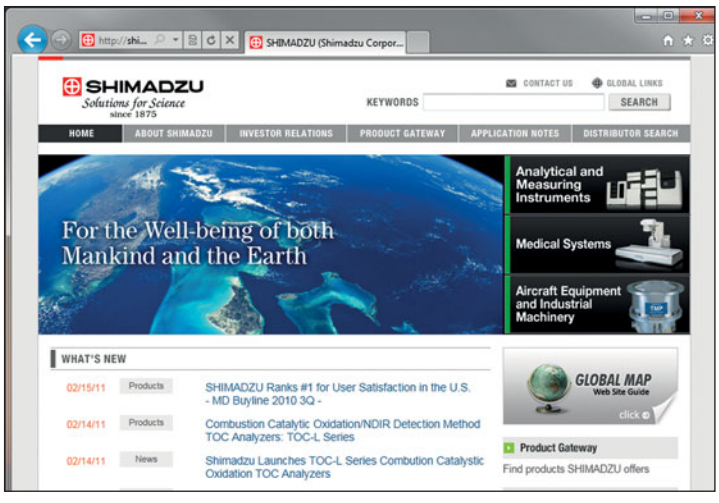


Figure 7: Website Shimadzu (Internet Explorer)

As a result, nearly equivalent chromatographic patterns were obtained.

At this point, further handling of the fractionated compounds usually determines the next steps.
If the target purity is not achieved because of insufficient separation, the sample injection volume and column length etc. must be reexamined. The same applies with gradient conditions.

If high sensitivity is required while using a small injection volume in this purity confirmation analysis, the usage of an analytical system for verification rather than a preparative system is suitable.

These reprocessing steps are closely related to easy-to-use software to provide a fast overview of the results.

Open Solution software

There is a growing demand for multifunctional software that can be used to set conditions and perform high-level analysis in an easy and intuitive way. But, what would be the ideal software which could easily be used by anyone? From the view of day-to-day operations, access to the internet is meanwhile considered as essential to communicate by e-mail and to access readily available web-based information.

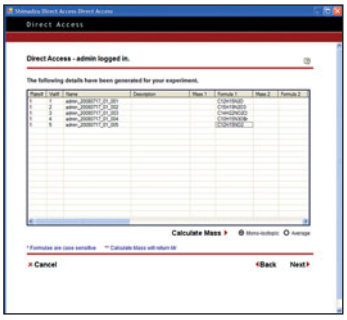
If this effortless Internet Explorer could be used for LC and LC/MS analysis, a reduction or even elimination of the cost of training to impart the expertise for software operation could be avoided. Furthermore, there would be no need for software licensing for each PC or user, thereby reducing even further the overall costs associated with analysis related operations.

Might it be possible to use Internet Explorer for LC and LC/MS analysis?

At this time, there is probably a small advantage to be gained by running the currently used LC and LC/MS software in their current states under the Internet Explorer environment, since the software operations would remain basically unchanged. In general, the following criteria would need to be satisfied for Internet Explorer to be effectively employed for controlling analysis: the ability to

(1) construct an open access environment
(2) display sufficient information in the Internet Explorer window.
(3) output information in printed as well as electronic format (PDF output) Note 1.

Once these problems are resolved, analysis using Internet Explorer will become a reality. Furthermore, since Open Solution runs only on the PC that is directly connected to the analytical instrument, it can also run in stand alone mode, so any future lab network construction could be accomplished without wasting past investments.



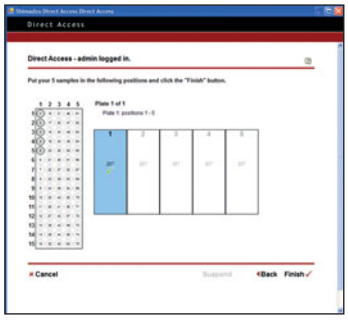


Figure 8: Open Solutions – How to setup samples for analysis

Column				
Internal Diameter (mm)	Cross Sectional Area (mm^2)	Cross-Sectional Area Based on '1' on 4.6 mm ID cross sectional area	Flow rate based on '1' as 4.6 mm ID flow rate (mL/min)	Injection volume based on '10' as 4.6 mm ID injection volume (µL)
4.60	17	1	1	10
20	314	19	19	190
50	1963	115	115	1150

Table 1: Relationship between Column Cross-sectional area and Flow rate / Injection volume (out of Technical Report 31)

A true open-access environment

The ability to construct an open access environment within the Internet Explorer environment, as indicated above, is critical. "Open access" refers to access by whoever, whenever, wherever, with respect to any data, and for whatever reason, while satisfying a given set of security conditions. The possibilities become greatly extended if this open access environment is achieved using an Ethernet network, as this overcomes positional and geographical restrictions to allow information sharing from any PC on the network. This defines a true open access environment.

Open Solution is a newly developed software application which achieves this open access environment.

Open Solution is a software enabling control of the Shimadzu high performance liquid chromatograph *prominence* and the ultra high performance liquid chromatograph (*Nexera* and *prominence* XR), in addition to the LCMS-2020 mass spectrometer – not through direct operation of their control software, but via open access.

Open Solution utilizes the Internet Explorer, thereby eliminating both the need to install special software on every PC terminal and the requirement for additional software licenses. By simply installing Open Solution on the PC directly connected to the analytical instrument, operations such as viewing analysis results and outputting reports can be conducted from any PC on the network.

Open Solution pushes the limit

Elimination of every unnecessary mouse click is a wish shared by all lab personnel. Analysts believe, correctly, that their job is to conduct analysis and not operate instruments. There is justifiable frustration with the complicated PC operations which actually impede the efficiency with which analysis might otherwise be conducted.

Open Solution pushes the limit to achieve a simplified level of PC operation. From login to analysis start, the process is reduced to a level equivalent to operating a simple lab device such as a pH meter or electronic balance. The PC operations are reduced to One-Two-Three: (Step 1) Log in / (Step 2) Specify number of vials, and set vials in place / (Step 3) Start.

The rest can be left to the instrument. The analysis may even be completed by the time the analyst returns to the office.

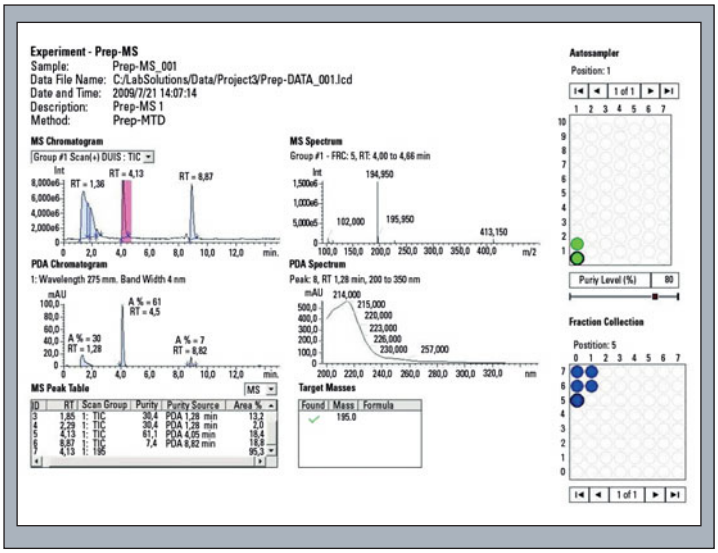


Figure 9: Display of the Analysis results with Open Solution

Figure 10: Peak purity analysis

In addition to simplifying PC operation to the greatest extent possible, the ease with which analysis results can be checked is also important to increase analysis throughput. Since analysis results are displayed in the Internet Explorer window when using Open Solution, the information to be displayed can be limited to the minimum required without performing complicated operations.

The information in this window is organized in a simple, easy-to-understand manner. For example, the sample rack and loaded vials are displayed graphically, so a quick glance allows a comparison of the samples (vials) with the analysis results. The window layout can of course be changed to suit individual preferences (see figure 9 and 10).

Moreover, when outputting the analysis results, the information displayed in this window can be printed and outputted easily in a predefined format.

The ability to perform these operations from any PC on the network is certainly one of the big advantages of open access.

Ease of data retrieval is also important for improving analysis throughput. Where data from a previous analysis is required for verification of suitability of conditions in the current analysis, this data may be located among huge amounts of analysis data. The time needed to retrieve this data may counterbalance any time benefit achieved up to then.

Hyperlink function for high-speed access to analysis results

In such situations, the Windows search function would typically be used, but this may be very time consuming depending on the file structure and other factors. Open Solution addresses this limitation using the hyperlink function to achieve high-speed access to analysis results. For example, an e-mail message is generated and transmitted to inform that an analysis is complete, and the URL specifying the location of the stored results is included. Just clicking on the URL hyperlink will display the analysis results in the Internet Explorer window. Furthermore, by clicking on a vial in the sample rack diagram, the display switches to the analysis results obtained from that sample. In this way, the analysis results obtained from another vial in the same rack can also be viewed instantly.

Open Solution supports *prominence* / *Nexera* system configurations including the pumps, autosampler, column oven and all detectors, and because of this supports measurement using multiple detectors simultaneously. Furthermore, rather than just a single system, multiple systems can be used in the open access environment provided by Open Solution. If collective data management is also required, use of a server PC is supported.

Open Solution provides an open access environment

Open Solution can effectively fill the requirements of a preparative LC or preparative LC/MS open access environment. In the case of LC/MS, for example, the mass of a target compound can be used to obtain a purity fraction higher than possible with a UV detector, but the seamless synchronization of the LC/MS and fraction collector provided with Open Solution allows fractions to be easily verified and reliably collected at very high speed. As described above, there are great advantages to be realized in an open access system comprising a closed network in a laboratory that is linked to an open network in a separate office environment. The introduction of Open Solution provides an open access environment which can be utilized to its maximum to achieve great efficiencies in LC and LC/MS analysis in the analytical laboratory.

Remark

Further information can be found in technical reports No. 29 (Open Solution: Open Access Environment by Web Browser) and 31 Basic techniques of preparative Liquid Chromatography and Apporaches to Efficency Improvement (1)

Together we are strong

The demand to identify and characterize unknown substances occurs in many application fields in the pharmaceutical industry, for example analysis of impurities of synthesized compounds, degradation products in stability studies, metabolite identification to detect expected and unexpected metabolites and metabolite profiling experiment to search for differences in mass profiles in larger sample sets and their identification. In general, identification is an important part, because further actions are based on this identification. The ability to deliver an answer in a reasonable time or to efficiently support the decision process for further charac-terization steps defines the strength of a total solution.

LCMS-IT-TOF with its many unique software options is one of such powerful solutions.

Combining two different mass spectrometric tools (ion trap and TOF in one instrument) results in capabilities beyond any single mass analyzing technology (Figure 1). Ion trap mass spectrometry allows target molecules to be selected and fragmented easily (MS^n; MS/MS, MS/MS/MS, MS/MS/MS/MS…) while time-of-flight MS provides high mass resolution and accurate mass determination.

The LCMS-IT-TOF can be equipped with different ionization sources (ESI, APCI, APPI, ESI/APCI combi source as well as a nano spray source) and can easily be combined with fast chromatographic separation delivering the required results with 100 msec polarity switching time in one run. In general, it delivers a mass accuracy of around 3 ppm externally calibrated, independent of the MS modus. A flight tube constantly kept at 40° Celsius supports mass stability even during analysis of larger sample set studies.

The easy and reliable generation of high quality data is an important issue for identifying unknown compounds. However, the software tools supporting interpretation of the data are equally of importance.

In addition to the fragment information, the elemental composition and the isotopic pattern of a compound are important information for the characterization of an unknown substance. Recent publications have highlighted that mass accuracy, while extremely important, is not sufficient for confident compound confirmation and identification of unknowns. Accurate isotopic abundances of measured ions are critical as well. The relative isotope abundance error has to be below 5 % for effective molecular formula generation for unknowns, helping to eliminate a large percentage of possible compounds.

"Formula Predictor" software

To determine the elemental composition of an unknown sample, Shimadzu's *Formula Predictor* software uses all generated accurate masses of the compound, including the parent mass as well as generated fragment masses from the MS^n experiments to calculate and validate the total elemental composition of the molecule of interest, and generates a candidate list. Additionally, the software uses isotope pattern information to compare the measured sample with the theoretical pattern of potential candidates which either increases the probability score or excludes certain candidates from the ranking. This dramatically decreases the number of false positive candidates and makes determination much easier for the operator. Shimadzu holds the patent for using the fragment

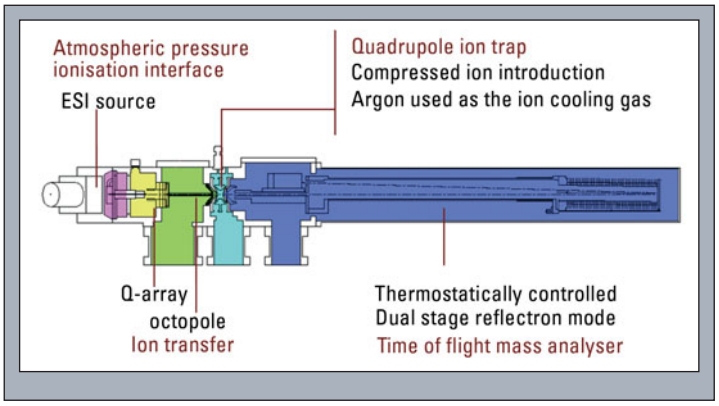


Figure 1: Schematic of the LCMS-IT-TOF

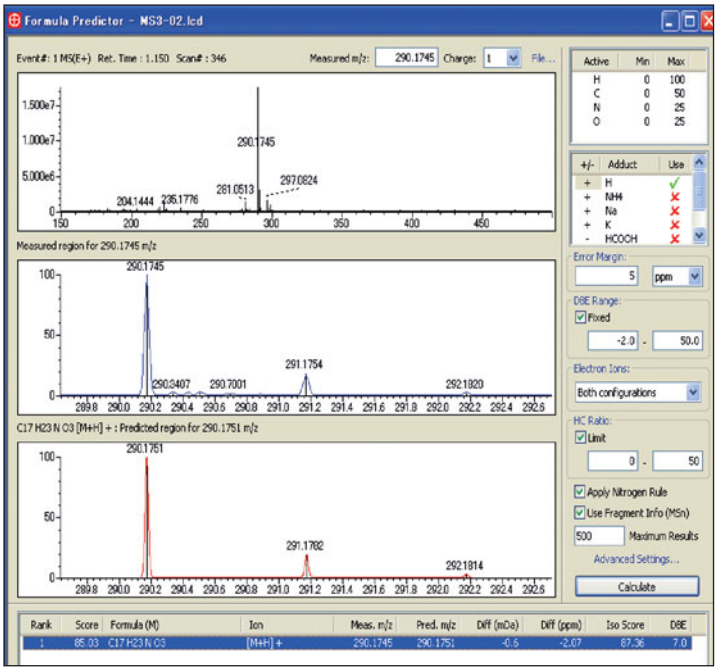


Figure 2: Formula Predictor software window showing one hit after using MS3 spectra

information of MS^n experiments to predict the elemental composition of the molecular ion.

MetID Solution identifies metabolites in time-series analyses of bio fluids

The MetID Solution is used in the field of toxicity assessment/toxicology, where pharmaceutical companies test the toxicity of potential drug candidates; it is applied in the field of functional genomics where it determines the phenotype caused by a genetic manipulation, such as gene deletion or insertion. The MetID Solution software combines three different approaches to determine expected as well as unexpected metabolites.

First Workflow – Traditional Peak Picking

One or more time-series analyte files (i.e. subject's biofluid at intervals after taking drug) are screened against a control file (i.e. subject's biofluid just after taking drug). The common peaks are excluded, the remaining peaks contain metabolites.

There are published biotransformations for which parent drug functional groups can be metabolized (added to or replaced by) in the body. These peaks are called expected transformations and can be screened for by generating accurate mass XIC's (Extracted Ion Chromatogram) using the metabolized formula.

Remaining peaks that cannot be categorized are called unexpected and are manually checked for metabolic likelihood. This can be laborious, but the Formula Predictor software reduces the effort.

Second workflow – Isotope Labelling

To aid confirmation of metabolites, discovery labs can label the parent using rare isotopes (which may or not be radioactive).

MetID automatically handles labelling in the Peak Picking workflow and can also use peaks in a radiolabel detector chromatogram.

Additionally, it can synthesize an Isotope Filtered Chromatogram from the MS chromatogram to locate these labelled metabolites (which helps to locate compounds containing distinctive isotope patterns such as halogens; schematic shown in figure 3).

Third workflow – PLS regression analysis (Partial least square)

With the LCMS-IT-TOF the constant mass accuracy and resolution of MS^n data can be utilized to first build a 'fingerprint' of the parent compound or drug (Y) and then acquire MS^n data for each peak during analysis to get metabolite 'fingerprints' (X's).

Partial least squares regression scores show how similar each X is to Y and since metabolites share functional groups with the parent, many unexpected peaks can be screened rapidly for potential metabolites and also to find new biotransformations.

Profiling Solution software

The comparison of large data sets and the detection of difference is the strength of the Profiling Solution software package. Fast and reliable, it uses the generated chromatographic and MS data of multiple sample measurements to recognize a difference (Figure 5).

The software supports and offers the following steps:

- In a patented way, it aligns multiple data sets using a unique spectral binning technique and generates an aligned data array using spectrum data (mass resolution independent) and chromatogram data for multiple data streams (supports polarity switching within a run)
- Integrated filters for pooled QC analysis (filtering out idiosyncratic ion behaviour based on peak area, retention time)
- Integrated statistical tools

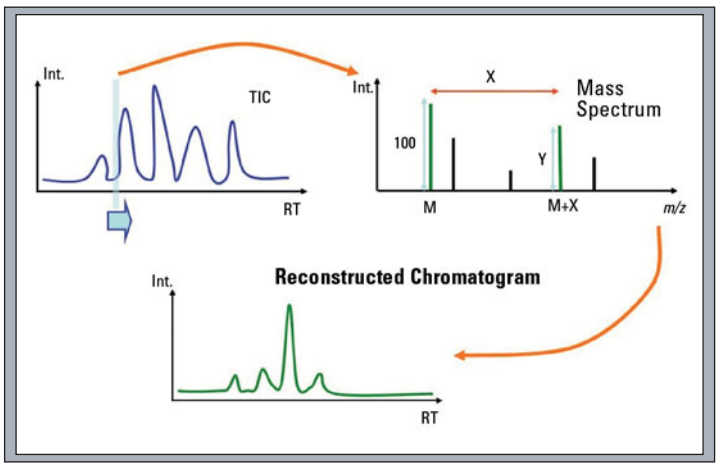


Figure 3: Schematic of the Isotope Filtering Chromatogram procedure

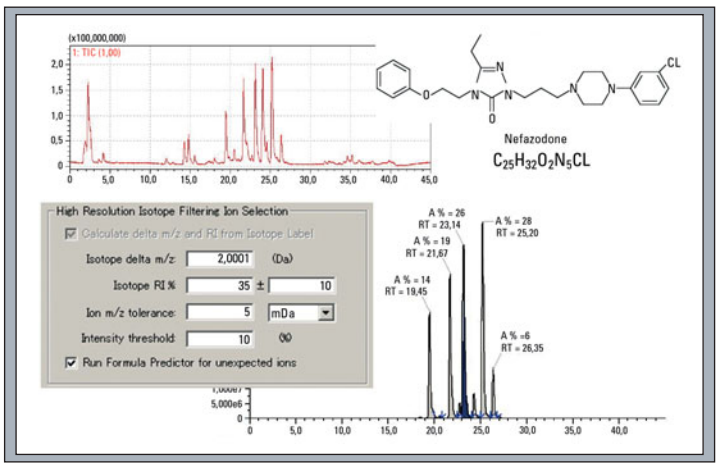


Figure 4: Real example of extracted isotope filtered chromatogram of Nefazodone, based on the chlorine isotope distribution

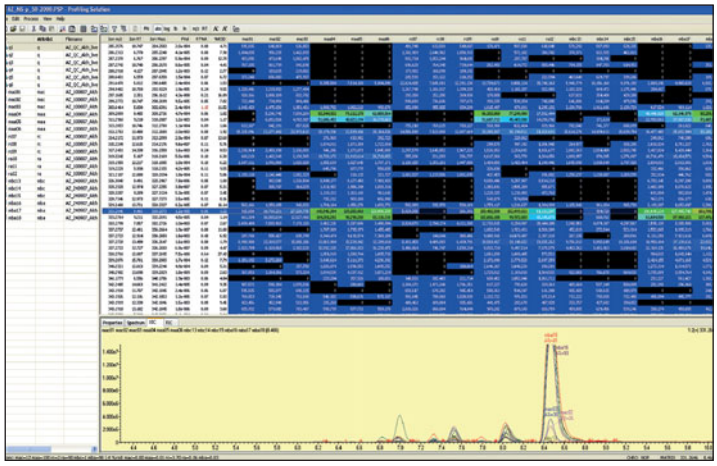


Figure 5: Array of different samples where significant variations are highlighted

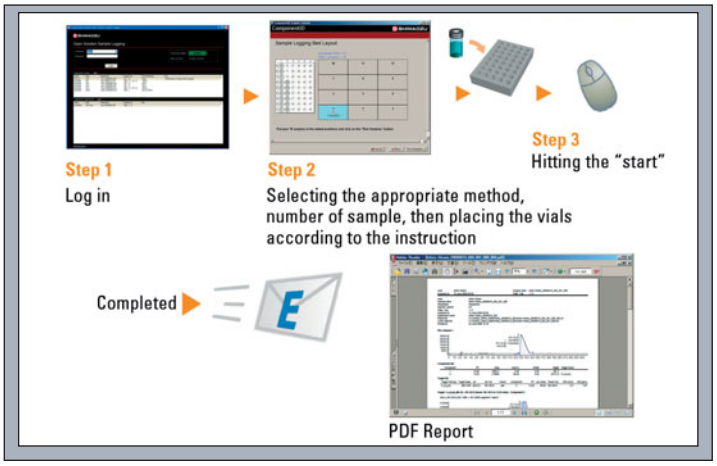


Figure 6a: Workflow to generate accurate mass data in an open access environment

(p-value, normalisation, de-isotoping)
- Export to statistical packages (SIMCA-P, MatLab as a text file)
- Hot links to web based data search engines (LipidMaps, KEGG, Metlin, ChemSpider)

Open Solution ComponentID

In many synthesis laboratories chemists get access to a single quadrupole system using "Open Access" software. However, in order to get an accurate mass measurement for a target compound the mass spectrometry department in general have to analyze the samples. This can create a big overhead for this mass spectrometry service departments. Open Solution Component ID is a software tool to automate the formula prediction workflow (Figure 6a). Its combination with the reliability of the LCMS-IT-TOF makes it to a powerful solution for molecular weight confirmation and impurities profiling.

ComponentID provides a simple and easy to understand user interface for logging samples (and running analyses) and printing reports of results using the LCMS-IT-TOF (Figure 6b). This makes it easy for synthesis researchers and other laboratory users to analyze their own samples. It provides an effective tool for customers, such as those described below, that like to use the instrument in a manner generally referred to as "open access."

- Customers who intend to share a single LCMS-IT-TOF system among many researchers, so each can confirm the molecular weight of synthesized compounds or confirm their purity level or contaminants
- Users that want to utilize formula prediction software to confirm synthesis and improve the accuracy of such determinations
- Users with their desks located away from the instruments, which involves time-consuming movement before they can view the results.

ComponentID offers the following features to address the customers' needs described above.

1. After logging in to the software, it takes only five steps to start an analysis: enter the number of samples, select a method, enter the target chemical composition, place the samples in the autosampler, and click the [Start] button. A report of the results can be received via email. Training is not therefore necessary for general users. LC/MS system administration and method development is performed by the system administrator. The sample logging function is the same as that used in the highly acclaimed Open Solution and PsiPort software for LCMS-2020/LCMS-2010 series systems.
2. MS^n accurate mass measurement results from the LCMS-IT-TOF system are utilized to accurately predict the composition of synthesized substances and impurities. These results are then output automatically in a report.
3. ComponentID supports high-throughput LC/MS analysis using *prominence* systems, and also supports flow injection analysis which does not use a column. Using flow injection allows the processing of a larger number of samples in a short time.
4. Data entry time can be reduced by importing a list of sample information, such as the composition of synthesized compounds, from an Excel spreadsheet or directly copying and pasting from Excel. This is also convenient for synthetic substances in plate format.
5. On completion of an analysis, obtained results are printed as a PDF file, attached to an email and sent to a pre-specified e-mail address. The layout of the output can be customized by the system administrator, so the PDF file can include not only formula prediction software results, but also MS chromatograms, spectra, LC chromatograms or other information desired by the user.
6. In addition, ComponentID includes many other functions developed from actual operation of OpenSolution and PsiPort systems, such as a desktop lock feature, overnight analysis feature and priority settings.

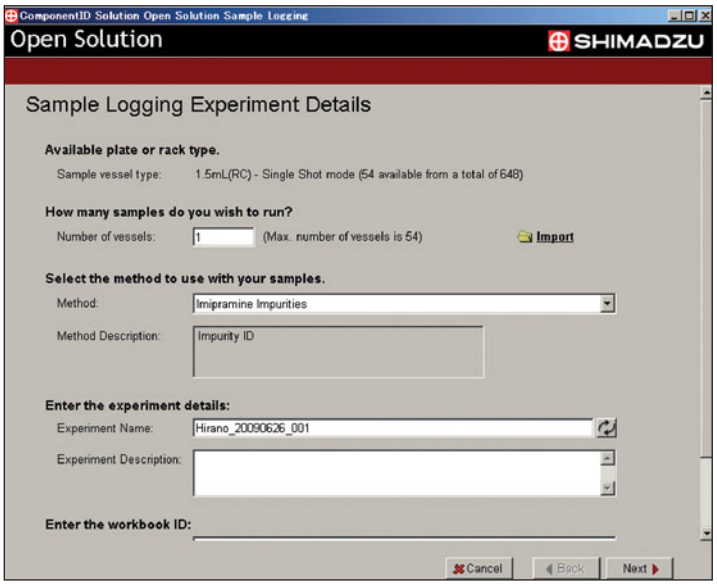


Figure 6b: Login Window of ComponentID Solution

From Crude to Powder

Solid-Phase Trapping as a new approach to shorten and improve purification process

Figure 1: Vials with powder after pruification and solvent evaporation

Pharmaceutical research and drug development are time-consuming processes. After target finding and assay development it may take 10 - 15 years until a new drug can be released. Development costs often reach 500 - 1000 million Euro. New and more efficient technologies in drug discovery can help to accelerate the development process and decrease the costs.

During the drug discovery process, auto purification systems utilizing preparative LC/MS have been utilized widely for purifying newly synthesized compounds ahead of screening. Reversed-phase gradient HPLC methods are often used as 'generic' or 'universal' methods for a wide range of compounds due to their adaptability and scalability in addition to ease-of-use.

Fractionation and evaporation determine the speed of the purification process

For further structural identification and more detailed testing it is required, which needs fractioning the compounds and transfering them from solution into solid stage. Fractionation and evaporation are key steps after the purification as they determine the speed of the whole process and the quality of the sample for subsequent analytical methods.

Typically, the evaporation is seen as the bottleneck, as soft drying and solvent evaporation take a long time compared to the relatively fast separation. This process is even more problematic when mobile phase additives such as TFA (Trifluoroacetic Acid) remain and may cause false results in screening. Eliminating or shortening the evaporation process is desired by many researchers for improving the throughput and quality of the auto purification process.

Modern ways of purification (e.g. LC/MS) inevitably produce 'solutions' of pure sample (normally solid) in a liquid (mobile phase). Recovery of these solids is generally attempted by evaporative removal of the liquid (N_2 blow-down, vacuum centrifuge or a combination of both). This process is generic but unfortunately has some potentially 'unmeasured' associated errors.

Any substance which is not sufficiently volatile/evaporable in the mobile phase/background will concentrate together with the sample, leading to loss of mass-purity and potential for significant sample contamination. This can lead to 'weighing errors' if weighing is the quantification method, which propagate into preparation of a known-concentration solution. Furthermore, common mobile phase additives such as Formic acid/TFA/TEA etc. can form salts with specific chemical functionalities leading to salt form/stoichiometry issues. In general, HPLC or HPLC-MS based techniques are increasingly the pharmaceutical industry's method of choice in order to obtain very high purity compounds.

Trapping concept as an alternative to evaporation

An alternative to evaporation utilizes a trapping concept whereby fractions are transferred onto a trap column, washed and preconcentrated and in a second step extracted from the trap as powder. The eluate from the preparative separation is transferred to the trapping module and flushed onto a trap column. During the transfer the 'sample' can be diluted, while modifiers can be applied to ensure that either a salt form or the free base is trapped.

Why 'Trapping'?

Trapping technology offers several advantages:

- removing chromatographic fraction volume scale dependence
- removal of certain salt forms (e.g. TFA)
- removing (large volume) evaporation as a standard approach.

One removal process for all, independent of sample trapping method

Trapping allows as an alternative means the selective removal of just the sample onto a solid support, enabling everything else to pass through or be washed out. The salt form of the sample can then be manipulated prior to recovery in a convenient solvent system (i.e. one that is very easy to remove). This trapping task can be performed on-the-fly as part of

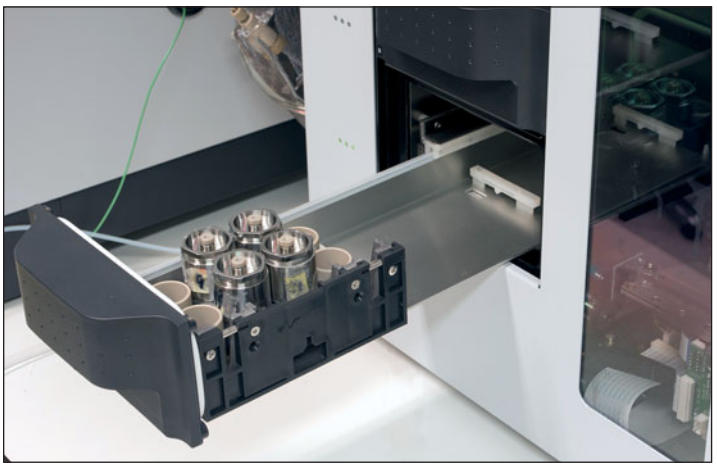


Figure 2: Rack Changer P with tray for trap cartridge and vials

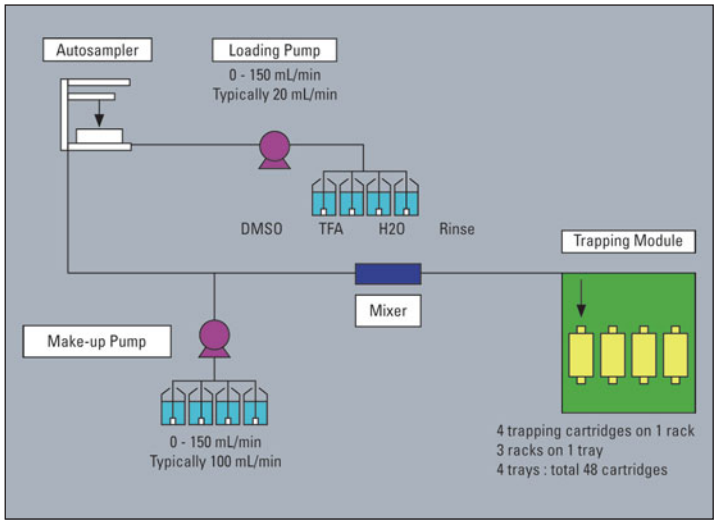


Figure 3: Schematic of the flow line to the trapping systems / Trapping Module

your purification process as well as from already collected fractions.

How does trapping actually work?

Trapping works by transferring pure compound 'fraction' (obtained from Mass directed auto-preparation or similar), with addition of a diluent, onto a trap (20 x 30 mm dimension) which contains a specific resin based on PS-DVB (polystyrene-divinylbenzene). The compound is immobilized while the background solvent/additives etc. are not. The compound can be converted to the free base on-trap.

Compound recovery

The trap can then be transferred to a recovery module where the compound can be retrieved as a very concentrated solution in a volatile organic solvent, or directly as the dry, powdered material.

Technically, the trap column is flushed in the opposite direction using a non-water miscible solvent which washes out the water from the trap column. The compound is released with this solvent (dichloromethane being used in practice at many sites). The dissolved compound is sprayed with nitrogen into a pre-heated vial and transferred back to the solid stage, remaining as powder in the vial.

The recovery module is available as manual dual channel as well as six channel parallel fully automated version.

It is important for subsequent analysis steps, that the trapped molecule is of high quality, meaning:

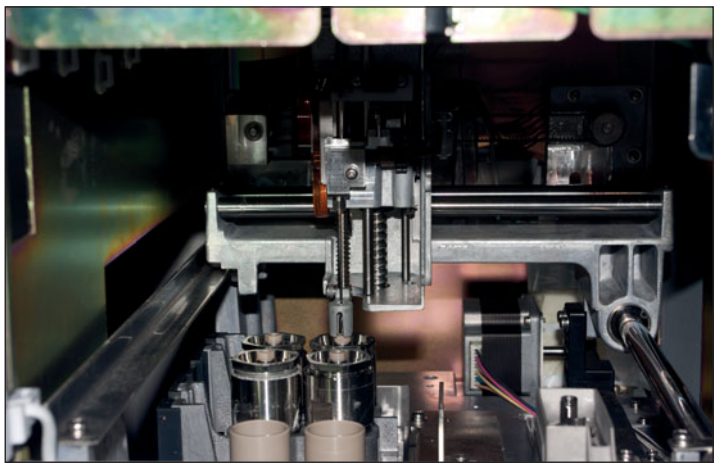


Figure 5: Trapping System with Needle in Trap position (fraction load on cartridge 4)

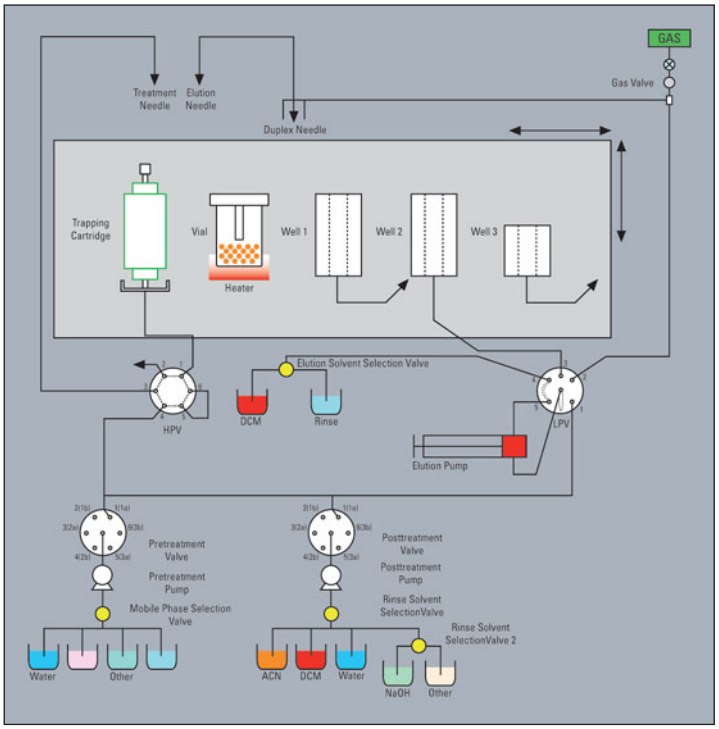


Figure 4: Schematic of the recovery module

- high chemical purity (very low levels of structurally related contaminants) giving confident specificity
- high 'weight purity' (no detector-invisible or non-structurally related process contaminants or solvents) giving confident potency

Ideally, 1 mg of compound weight contains 1 mg of compound (100 % weight purity).

Accurate weights mean accurate concentrations. This in turn means testing and ranking compounds correctly. Generally, storing compounds as dry solids gives the highest confidence that compound integrity is maintained.

Sample and Workspace Management

Each user (individual chemist) can submit his/her own fractions manage their own traps with ultimately dry recovered samples by management of their own workspace. This takes the form of a set of up to four traps and corresponding recovery (haystack) vials contained within an MTP-footprint workspace (see below).

Instrumentation

Task-specific instrumentation developed by Shimadzu performs trapping and recovery operations in dedicated hardware modules. This enables automation of the whole process detailed in an Open Access (OA) mode with a dedicated software wizard. Ultimately, the system can be combined with the LC-MS front-end to function as a complete 'crude-to-pure' system purifying up to 150 mg mass of sample in a single trap.

Summary

A new compound isolation and handling process based on 'Solid Phase Trapping' has been developed providing a new and much improved workflow to deliver pure, largely dry compounds. The offline, two step process will be possible in new, purpose designed instrumentation. Ultimately, this can be combined with the purification system to form a full on-line system.

Bringing light into darkne

The analytical method development section in CMC (Chemistry, Manufacturing and Control) sections of pharmaceutical companies constitute part of the pharmaceutical developmental process that deals specifically with the physical nature of a drug substance and the drug product, for example how it is made, and the control of the manufacturing process to provide a reliable and reproducible product. HPLC systems in these areas are utilized for the specification tests of active pharmaceutical ingredients (API) to ensure the quality of the product as well as the level of impurities.

Traditionally, non-volatile mobile phases containing phosphate buffer solutions have been used in the HPLC test methods for QA/QC. When LC/MS analysis is performed, it is necessary to switch from a mobile phase containing non-volatile additives to a completely volatile mobile phase suitable for atmospheric pressure ionization techniques.

This alteration in analytical conditions may cause changes in the elution order of analytes as well as introduce the possibility of obscuring impurities due to their close proximity to the chromatographic peaks of the major constituents. The change in method conditions requires attention to detail and a great deal of effort by the user. Furthermore, recent modifications in regulation of impurities along with globalization of the supply chain have led to even greater demand for impurity identification. Of course, this causes a bottleneck due to the necessity to modify HPLC conditions conforming to the existing validated test methods, and the need to satisfy demands from the manufacturing section for identification of impurities. Against this backdrop, it is no wonder that a new analytical system is eagerly anticipated.

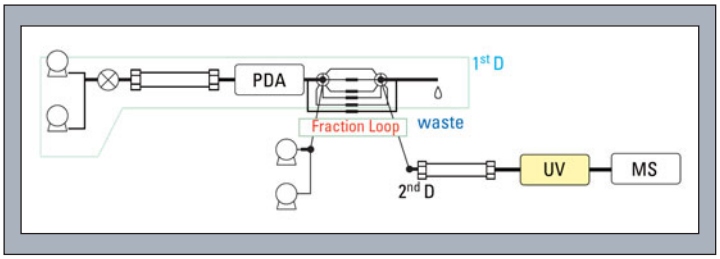


Figure 1: Basic flow diagram of Trap free 2D LCMS impurity system

To address the demand for LC/MS analysis allowing utilization of existing non-volatile mobile phase analytical conditions, Shimadzu has developed a new kind of setup, working independently of a trapping column approach. The advantage of simplifying the flow path is that the time and effort required for optimizing the conditions can be shortened. This overall analysis of the system led to the construction of the Co-Sense LC/MS system with a modified flow path.

Desalting is executed using a divert valve installed just before the MS inlet. The addition of the UV detector to the second dimension greatly facilitates the detection of impurities. In an actual sample workflow, both impurity data and the corresponding blank data are acquired, and the impurity peaks are identified through comparison of the respective chromatograms. Peak isolation and fractionation is conducted applying multiple valves in specific combinations. Creating the peak isolation program is easy using a provided macro program. The macro automatically specifies the best valve sequence, demanding only that the user adds the retention times of the first dimension separation. To make operation even easier, a separate macro-program which automates the construction of a batch schedule for acquiring multiple impurity and blank data, is also provided. The individually collected impurities are then analyzed with the LCMS-IT-TOF. In a very fast and efficient way fragment spectra can be generated and used with its accurate mass to determine the elemental composition of impurities. Additional software packages, like MS fragmenter from ACD labs, can use this accurate mass

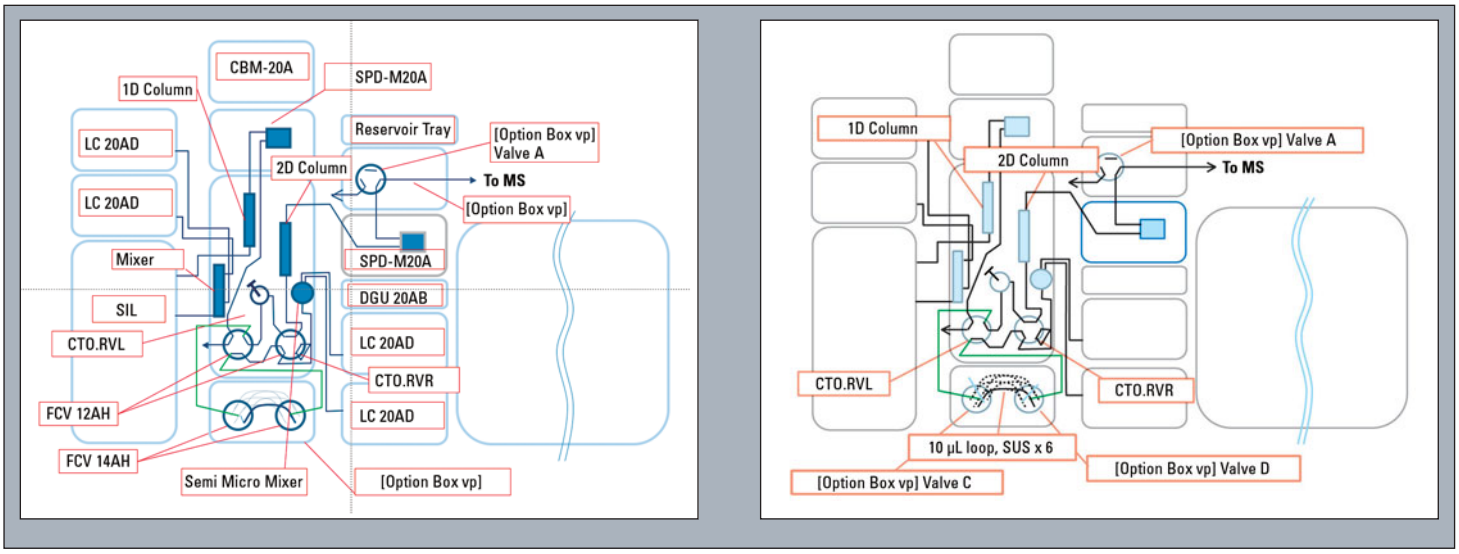


Figure 2: Used HPLC modules and system flow diagram

ess 2-D LC and LCMS-IT-TOF – Better drug impurity ID efficiency

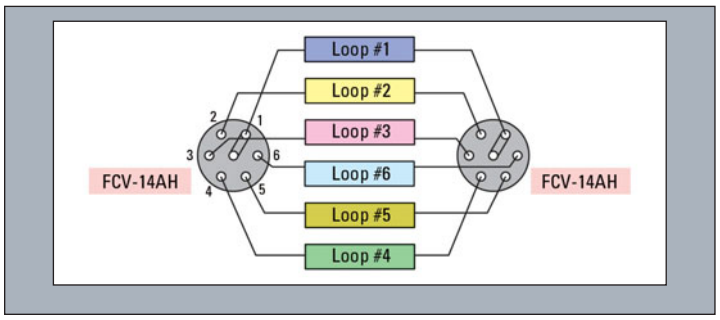


Figure 3: Fractionating loops available in 5 µL, 10 µL, 20 µL and 50 µL

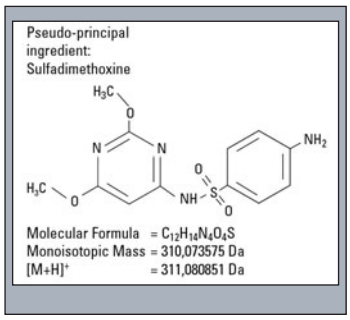


Figure 4: Structure of Sulfadimethoxine

fragment pattern information and search for homologies between the impurity and the main compound.

Analysis example

A sample consisting of sulfadimethoxine (Figure 4) as the principle compound, and four sulfa drugs having a similar structure to serve as impurities is used for this example (Figure 5). The impurities were each mixed with the principle compound at percentages of 0.1 % relative to the principle compound which had a concentration of 500 µg/L. The impurities were selected and fractionated into the loops based on their elution times (Figure 6). The structures of the respective substances are shown below, and the measurement results are shown in figure 7. All of the "impurities" were confirmed in the results.

Analytical conditions LC first dimension

Column: Shim-pack VP-ODS, 150 mm L. x 4.6 mm I.D., 5 µm
Mobile phase: 0.01 mol/L phosphate buffer, solution (pH 2.6), methanol mixture (7:3)
Mobile phase flow rate: 1 mL/min
Column temperature: 40 °C
Sample injection volume: 10 µL
PDA detection wavelength: 200 - 350 nm (270 nm monitored)

Analytical conditions LC second dimension

Column: Shim-pack XR-ODS 75 mm L. x 2.0 mm I.D., 2.2 µm
Mobile phase A: Shim-pack XR-ODS 75 mm L. x 2.0mm I.D., 2.2 µm
Mobile phase B: Methanol
Mobile phase ratio: 10 % B (0 min) -50 % B (10 min) -10 % B (10.01 - 20 min)
Mobile phase flow rate: 0.3 mL/min
Column temperature: 40 °C
Sample injection volume: 0.3 mL/min
UV detection wavelength: 270 nm

MS conditions

Ionization mode: ESI+
Nebulizer gas flow rate: 1.5 L/min
Drying gas pressure: 0.15 MPa
Impressed voltage: 4.5 kV
CDL temperature: 200 °C
BH temperature: 200 °C
Scan range: m/z 100 - 1000

Utilizing the data browser

The data browser provided in LCMSsolution software offers additional functionality enhancing the efficiency of impurity analysis-related tasks. The layout template for picturing data can be customized and saved beforehand. As shown in figure 8, the stored layout was constructed so that the upper tier is reserved for displaying the UV chromatogram and MS spectrum of impurity-related data (2-D LC), and the lower tier for showing the UV chromatogram and MS spectrum of the blank data corresponding to the impurity peak (2-D LC). In this case, the Unk-1 (sulfamerazine) data and the respective blank data are presented using the "drag and drop" function from the data explorer for display in the data browser.

The differences in retention times between those obtained with the UV detector and those obtained with MS were synchronized using the retention time correction function provided in the browser. The retention times are stored in the method file, and since this retention time averaging function can be set up beforehand to be ⏵

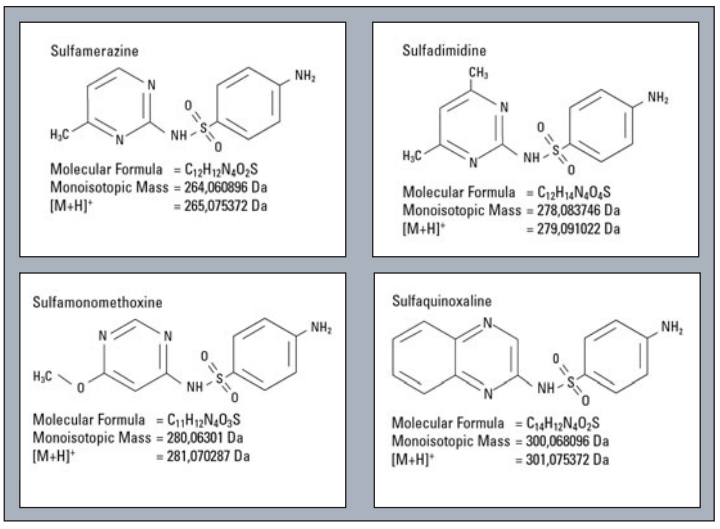


Figure 5: Compounds of similar structure like Sulfadimethoxine serving as impurities for this example

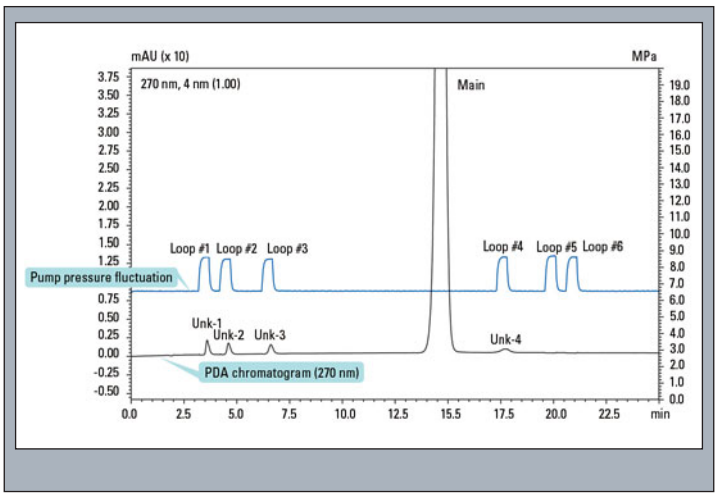


Figure 6: First Dimension PDA Chromatogram: The blue coloured trace displays the system pressure and the black coloured trace the UV 270 nm signal. These correlate perfectly, indicating the elution peaks are reliable fractioned in the loop.

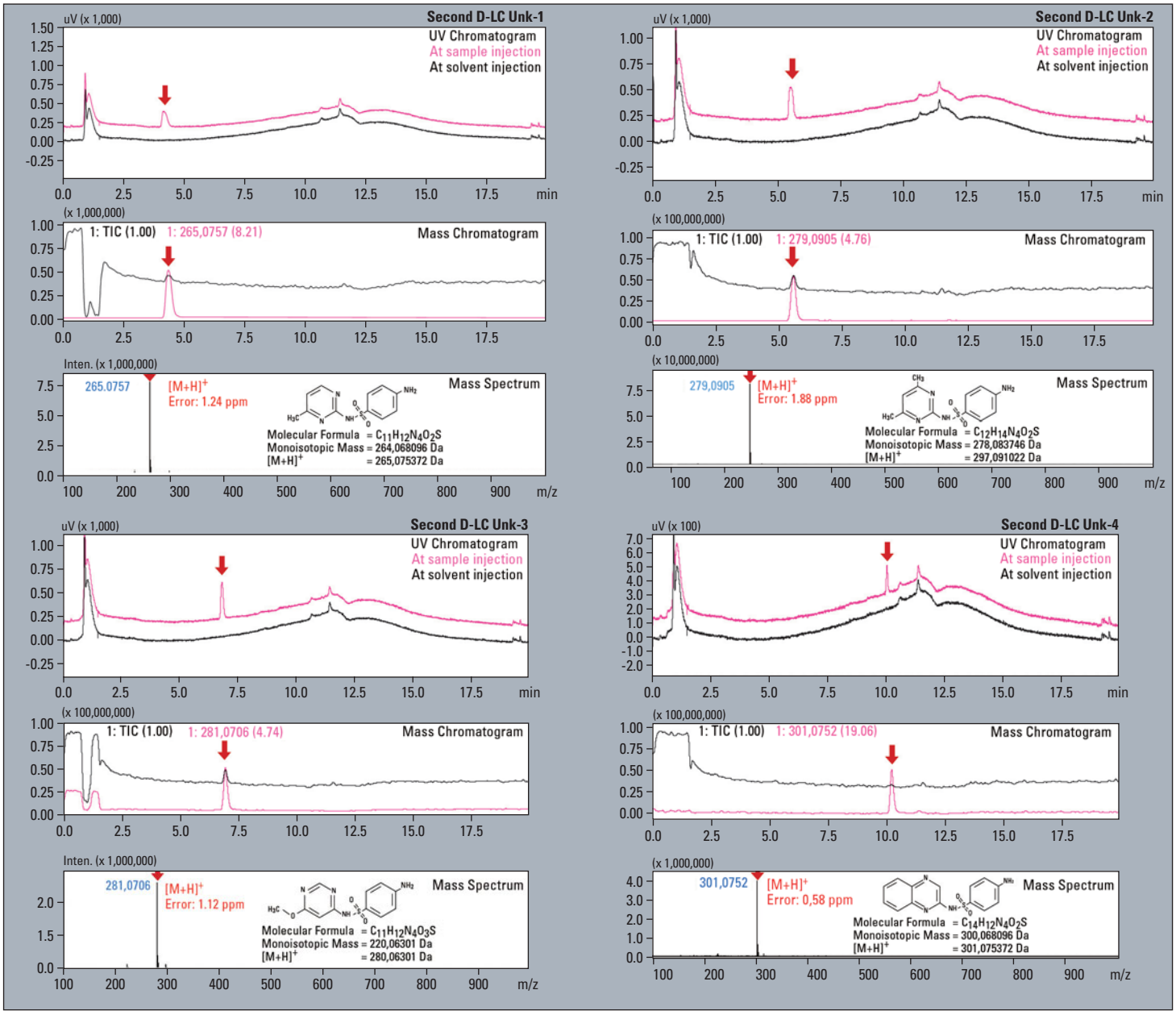


Figure 7: UV Chromatograms, MS Chromatograms and MS spectra of four fractionated impurities

executed automatically, it does not need to be executed manually each time a new set of data is displayed.

Furthermore, utilization of this function permits automatic detection of the impurity peak from the UV chromatogram and the corresponding display of the MS spectrum. In addition, since all of the data files are processed automatically, identification of the impurity on the UV chromatogram allows not only synchronous display of the corresponding impurity spectrum, but also synchronous presentation of the blank MS spectrum as well. The actual operation is conducted using just the mouse to drag from the start of the impurity peak in the impurity UV chromatogram to the end of the peak. Upon releasing the mouse button, the averaged corresponding impurity spectrum and blank spectrum will be shown.

When using the two dimensional LC system introduced here, repeatability of retention time in the first dimension is extremely important because impurity peak fractionation is conducted within specific time-controlled intervals. In other words, this technique cannot be applied unless retention times are consistent.

However, it is reasonable to assume that the repeatability of retention times has already been determined since retention time repeatability is fundamental to the development of a specific test method. If the separation technique for the first dimension separation is already established, measurement can be conducted without re-evaluation of the technique introduced here, eliminating the need for LC/MS measurement using complex volatility conditions that would have been required otherwise. The ability to confirm impurities through comparison with blank data should contribute greatly to the efficiency of CMC impurity identification.

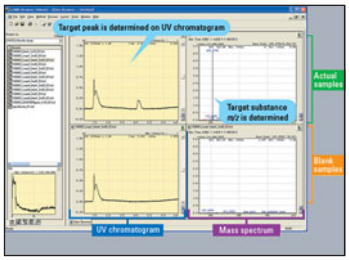


Figure 8: Unk-1 and Blank Data Displayed in Data Browser

Future-proof analysis – UHPLC 3.0

Nexera perfectly serves all needs of the Pharmaceutical Industry

Figure 1: *Nexera* System with Rackchanger II for large sample capacity

No matter whether Drug Discovery, Pre-Clinical and Clinical Studies, Active Ingredients or Finished Products – HPLC is the technique of choice in the Pharmaceutical Industry. Depending on the stage, e.g. drug development or production of finished pharmaceuticals, there are many demands on HPLC system properties.

Nowadays, screening for active substances as well as the chemical optimization is unthinkable without the use of LCMS. There are different needs and priorities for the MS detectors applied in this stage, but the requirements for front-end HPLCs are more or less the same for all. The systems are expected to perform high resolution analysis at high speed with zero carry over. Furthermore, they should be capable of handling various plate and vial formats as well as large sample quantities. The systems need to perform consistently with a minimum of variations from system to system so that poor precision does not obstruct data pooling later on. Since sample amounts are often limited, there is a high demand for systems dealing with smallest sample quantities while still providing high sensitivity measurements.

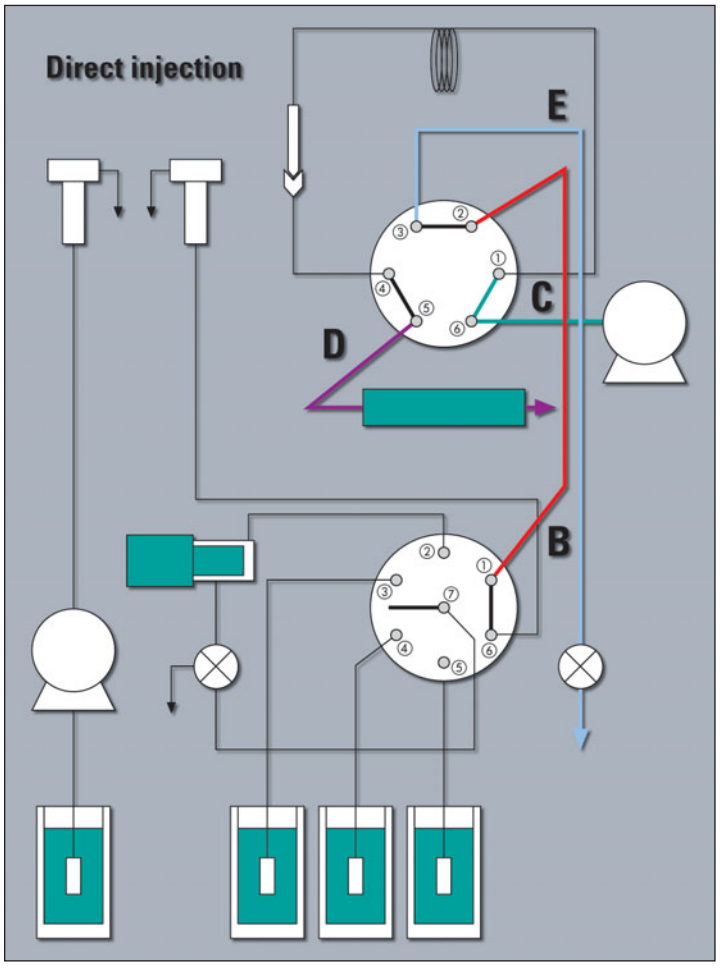
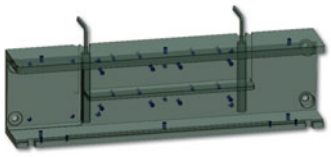


Figure 2: SIL-30AC Flow Line DIIMS. Sample suction line is part of the flow line and the metering pump is isolated via a low pressure valve.

At a later stage, testing for identification, quantification/impurity, stability/content uniformity, dissolution as well as cleaning validation requires different and additional options. HPLC determinations in cleaning validation demand high sensitivity and universal detection using large sample quantities at low concentrations, while HPLC determinations in dissolution testing require specific accurate detection of large and small quantities within a limited time. Quality Control usually calls for precise and robust HPLC systems. High sensitivity is important for stability and impurity testing, since degradation products usually appear in much smaller concentrations.

High system precision enables early trend recognition and intervention before out-of-specification results, thereby offering major advantages over medium-precision HPLC systems.

Whether in drug detection or quality control, the choice of dedicated HPLC systems should not be limited by the software platform controlling the systems and processing the data.



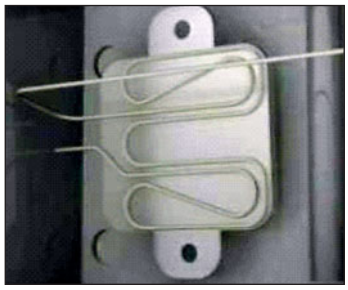


Figure 3: Inside view of CTO-30A. First figure: Heating block and separated heating cartridges. Second figure: Low volume low/dispersion pre-heater. Third figure: Column compartment.

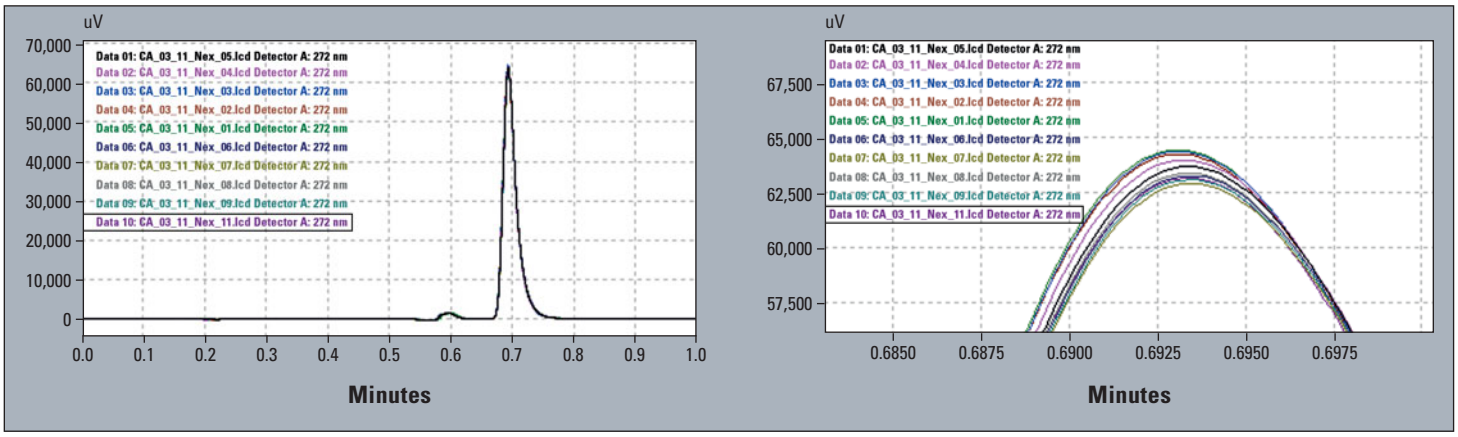


Figure 4: Chromatogram Overlay of 10 subsequent runs (Right Part: Zoomed Peaks)

In development of the *Nexera* UHPLC system, Shimadzu not only focussed on high throughput, high resolution capable of handling modern sub 2 µm particle columns with system pressures over the 1,000 bar limit of most current UHPLC systems. Shimadzu also aimed at a versatile system with almost zero carryover which could be utilized in any area based on its comprehensive pressure flow range and scalable injection volume range. Shimadzu's *Nexera* provides a flexible all-round HPLC system with a precision and robustness suited for daily routine analysis, as well as groundbreaking high speed and high resolution separations. *Nexera* can be controlled by the LabSolutions software and other software platforms such as Waters Empower™, Dionex Chromeleon™ and AB Sciex Analyst™.

Nexera is a modular and flexible UHPLC system consisting of

- LC-30AD high precision solvent delivery pumps
- fast and flexible SIL-30AC autosampler with lowest carryover marketwide
- Rackchanger II for extra-large sample quantities
- standard column thermostat handling column switching valves, mixing reactors and large columns as well as the new dedicated high temperature column thermostat allowing column heating up to 150 °C.

With the low dispersion semi-micro flow cells and the 100 Hz sampling rate, Shimadzu HPLC detectors are fit for high speed/high resolution separations.

In the following text, the main *Nexera* modules are described in more detail.

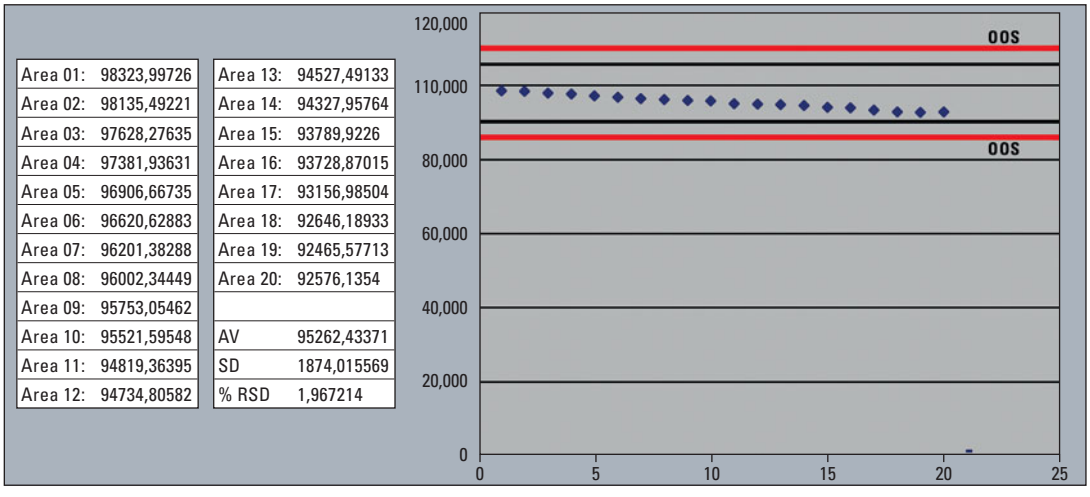
The LC-30AD solvent delivery module is a high resolution micro volume dual piston pump bringing new performance in precision and accuracy to modern solvent delivery pumps. Its flow rate range covers 0.0001 mL/min to 10 mL/min with a pressure range up to 130 MPa. The LC-30AD allows setting up of binary or ternary high pressure gradient systems as well as quaternary low pressure gradient systems.

Two types of multilayer solvent blending reactors (Shimadzu patent) allow solvent mixing with minimized system dwell volumes. Depending on requirements, 180 µL or 20 µL reactors can be utilized.

In terms of carryover and reproducibility, the autosampler SIL-30AC exceeds the performance of any other HPLC autosampler on the market. For critical samples and matrixes, carry-over can be eliminated by the use of three rinsing solvents (plus one external needle wash solvent) in combination with various rinse modes before and after sample aspiration.

In addition to the well-established and precise DIIMS technique (Direct Injection with Isolated Metering System), the autosampler also offers fixed-loop injection for ultra-fast separation with minimized peak width and gradient delay. An overlapping injection function is standard with *Nexera* enabling maximum reduction in cycle time.

For high-temperature LC analysis up to 150 °C, *Nexera* offers efficient pre-heating for solvents to enable stable and reliable conditions. The advanced Intelligent Heat Balancer (IHB) minimizes band-broadening during high-temperature analysis through use of independently controlled heat zones within the column compartment, ensuring accurate column temperature regardless of flow rate. The post-column cooler can reduce detector noise when equipped for high-temperature analysis.



Area 01:	98323,99726	Area 13:	94527,49133
Area 02:	98135,49221	Area 14:	94327,95764
Area 03:	97628,27635	Area 15:	93789,9226
Area 04:	97381,93631	Area 16:	93728,87015
Area 05:	96906,66735	Area 17:	93156,98504
Area 06:	96620,62883	Area 18:	92646,18933
Area 07:	96201,38288	Area 19:	92465,57713
Area 08:	96002,34449	Area 20:	92576,1354
Area 09:	95753,05462		
Area 10:	95521,59548	AV	95262,43371
Area 11:	94819,36395	SD	1874,015569
Area 12:	94734,80582	% RSD	1,967214

Figure 5: Trend Plot of Peak Areas

Why select a system exceeding requirements?

The following example shows a series of runs to determine the content of an active ingredient. The User Requirement Specification for Peak Area Precision

(injection volume = 5 µL) is ≤ 2 % RSD.

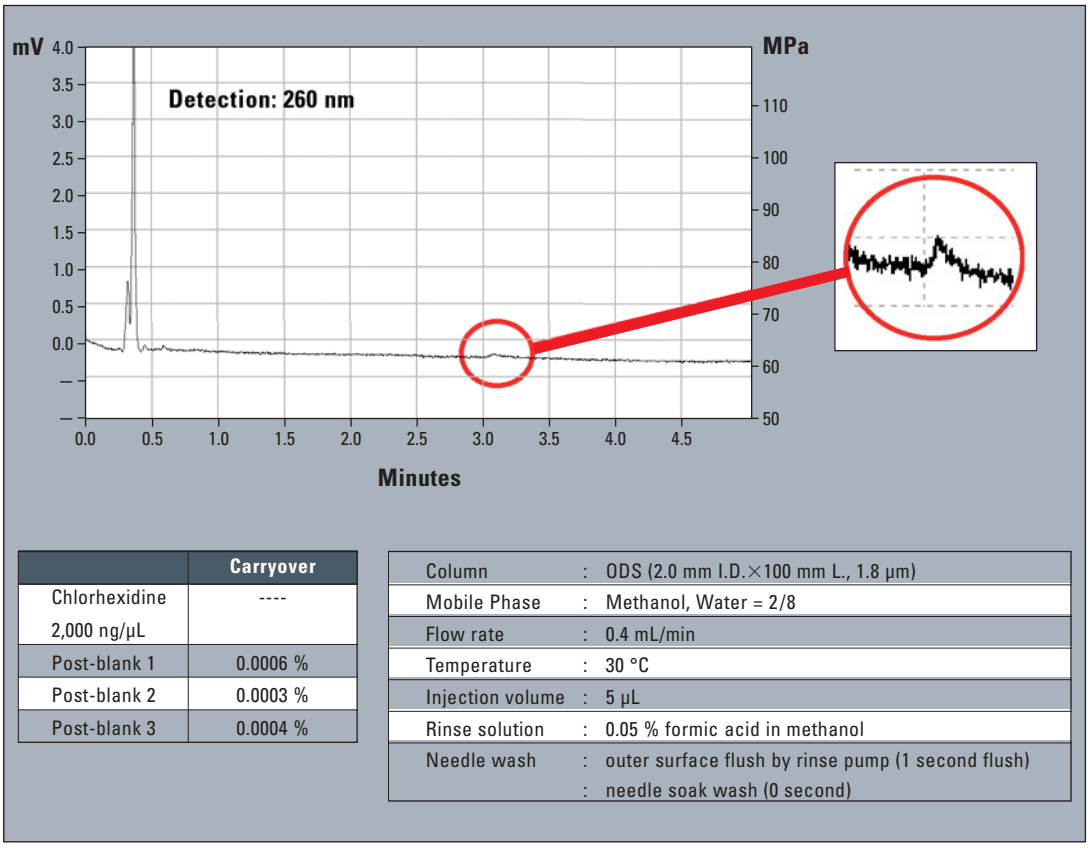
The overlay of the chromatograms (Figure 4) shows good precision, but in the zoomed view a clear trend towards decreasing peak area is apparent. The % RSD for peak area precision is within the limit of 2 % RSD, but the trend (Figure 5) indicates a problem. It is therefore possible to intervene and avoid out-of-specification results.

Carryover works against productivity:

Particularly in drug discovery, sample carryover may cause delays and additional costs, since false positive candidates pass through the entire process. With DIIMS (Direct Injection with Isolated Metering System) construction and well selected materials of the SIL-30AC autosampler, carryover is reduced to a minimum. By applying special rinse modes sample carryover can be reduced to an undetectable amount. The figure below shows 0.0006 % carryover for a Chlorhexidine injection.

By applying additional rinsing steps, carryover can be eliminated completely as shown in figure 7.

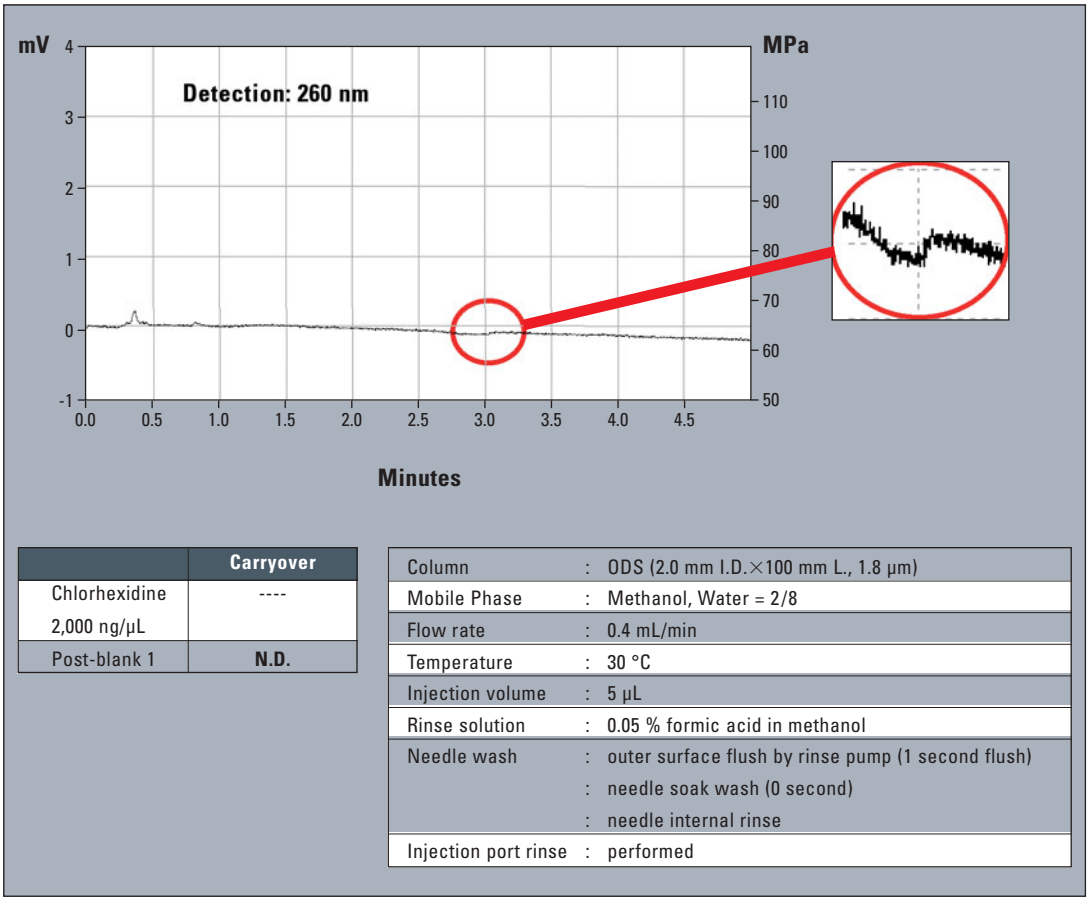
These are just a few examples demonstrating the advantages of a high end UHPLC system. The *Nexera* system provides the features and flexibility to cope with all demands expected of a modern UHPLC used in the Pharmaceutical Industry. In the final analysis, system reliability and long maintenance interval defines the real value in daily routine use. This was taken into account when designing the *Nexera*. The result is a reliable, robust and precise future-proof UHPLC system.



	Carryover
Chlorhexidine 2,000 ng/µL	----
Post-blank 1	0.0006 %
Post-blank 2	0.0003 %
Post-blank 3	0.0004 %

Column	:	ODS (2.0 mm I.D.×100 mm L., 1.8 µm)
Mobile Phase	:	Methanol, Water = 2/8
Flow rate	:	0.4 mL/min
Temperature	:	30 °C
Injection volume	:	5 µL
Rinse solution	:	0.05 % formic acid in methanol
Needle wash	:	outer surface flush by rinse pump (1 second flush)
	:	needle soak wash (0 second)

Figure 6: Carryover of chlorohexidine without injection port rinse, needle internal rinse



	Carryover
Chlorhexidine 2,000 ng/µL	----
Post-blank 1	N.D.

Column	:	ODS (2.0 mm I.D.×100 mm L., 1.8 µm)
Mobile Phase	:	Methanol, Water = 2/8
Flow rate	:	0.4 mL/min
Temperature	:	30 °C
Injection volume	:	5 µL
Rinse solution	:	0.05 % formic acid in methanol
Needle wash	:	outer surface flush by rinse pump (1 second flush)
	:	needle soak wash (0 second)
	:	needle internal rinse
Injection port rinse	:	performed

Figure 7: Carryover of chlorohexidine with injection port rinse, needle internal and outer surface rinse

From powder to pill – quality cont

Active agent analysis using FTIR spectroscopy

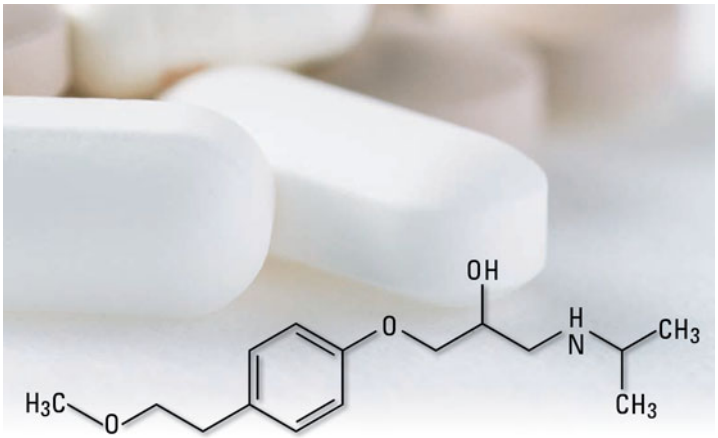


Figure 1/2: A tablet with beta blocker based on Metoprolol succinate with 47.5 mg active agent out of 447.5 mg total weight / Structure of Metropolol

In analytical instrumentation, FTIR measurement is the only technique providing non-destructive analysis of substances. It identifies powder, solid or liquid samples within shortest time, needing just milligrams of materials.

FTIR spectroscopy's advantage is the ability to verify substances within 1 minute. Proof of identity is guided by documents prepared by the pharmaceutical industry as well as the national or European Pharmacopoeia. The European Pharmacopoeia set up a range of comments for each of Pharmacopoeia's relevant substances. The same set of rules helps in its physical observations in qualification of the instruments. Some of the pharmaceuticals prepared for the export business are regulated by USP or FDA rules. The task of a modern FTIR is to be equipped with a validation concept which accounts for all cases.

More and more, a concept of easy-to-use and result-oriented analysis is required. A selection of functions is therefore needed which will select the requested comparison analysis based on Pharmacopoeia. A comparison can be:

- a simple visual comparison
- search in a library
- purity check
- comparison of dedicated signals
- contaminant analysis.

The cycle of measurement

The highly accurate measurement technique should still be fast. FTIR analysis with non-destructive approach supports these important aspects. A cycle of measurement can be described easily:

- take the sample
- place it in the measurement window
- press the sample (solids only)
- measure
- remove and clean the instrument's window.

A time frame of one minute is a rough approximation for this handling cycle. Such simple and quick solutions must be supported by accessories of the same class, such as single reflection accessories enabling a simple quality control from raw material up to the final product, e.g. a tablet. In case of very hard substances, a diamond-based version of single reflection supports the application, pressing the substance onto a measurement window. A soft sample gives easy access to the IR spectrum because of a good contact between sample and window. In case of hard materials, more force is needed to establish a good contact.

The measurement technique is ATR (attenuated horizontal reflection). Depending on the optical elements used the analysis beam will penetrate the sample surface by approximately 2 µm. In the case of ready-to-use pills, the surface cover can be hard and blocks the pharmaceutical agent below. Such pills need only a cut. Capsules have to be opened, whereas film-styled surfaces can be cut. For example, capsules made for liquids can be cut and the liquid transferred onto the measurement window.

Active agent analysis with FTIR

A different aspect in pharmaceutical analysis is the packaging of the goods. It can be the pill capsule made from gelatin, sugar, and starch or it can be the blister carrying the pills and providing easy handling for the end-user. These blisters, metal foils as well as metal and plastic tubes for paste and creams can be analyzed with FTIR.

One sample of analysis will be presented here: the control of Metoprolol succinate, an active agent reducing blood pressure. Identification was carried out using FTIR-Spectroscopy.

Next to the active agent, other substances are contained in a tablet (Figure 1), determining color or contributing to stability. These fillers and accompanying materials including dye enable a regulated reception of the active agent (dissolution) by the human organism.

High blood pressure is a creeping illness that can occur slowly over the years. It can cause heart disease, circulation problems and strokes. Pharmaceutical active agents based on Metoprolol represent an effective means to reduce

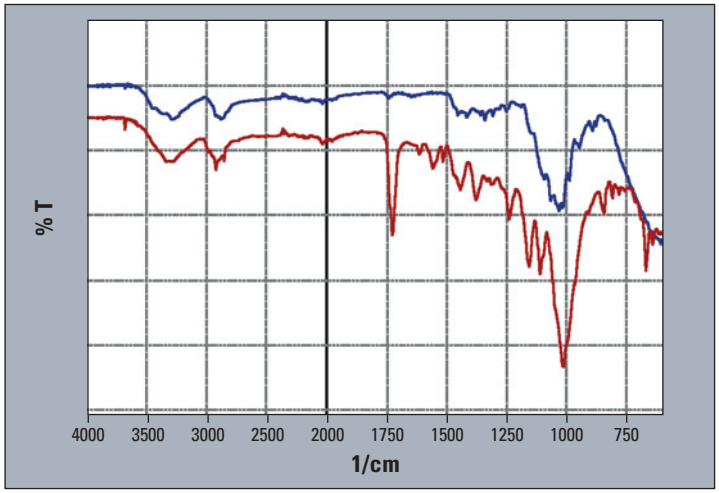


Figure 2: IR-spectrum of the surface measurement of the tablet using a single reflection unit (blue) and IR-reflection-spectrum of aparticle from the inside of the tablet (red)

trol of pharmaceutical goods

high blood pressure. Common variants are Metoprolol tartrate and Metoprolol succinate. Tartrate and succinate are salts belonging to the class of bicarbonic acids, derived respectively from wine acid and amber acid. In the Pharmacopoeia, both materials are contained.

Composition declared

Active agent: Metoprolol succinate (PH. Eur) of beta blocker 47.5 mg per tablet
Contents: Cellulose (E460), Polyvinyl pyrrolidone, glucose, Lactose-Monohydrate, Macrogol 4000 (Polyethylenglycol), magnesium stearate (PH. Eur), corn starch, Polyacrylate, Silicon dioxide, Sucrose, talcum, titanium dioxide as a dye (E171)

The active agent Metroprolol is described as follows: (Metoprolol) 2 'HO_2C-CH_2-CH_2-CO_2H, sum formula $C_{34}H_{56}N_2O_{10}$; molecular weight of the racemic mixture Mr = 653 g/mol. The active agent makes up approx. 10 % of the total weight of the tablet, which should be detectable in the complex mixture.

Measurement

- The tablet was first tested in its as-is state.
- A single-reflection-ATR-unit was used.
- The IR-spectrum of the surfaces measured determined starch and other filling materials (Figure 2).

The tablet was then divided in two, and a cross-section was taken. This powder granular material was then measured (Figure 2, red spectrum).

When comparing the spectra, clear differences are apparent. The surface spectrum (penetration depth of the light beam approx. 2 µm) of the filler material highlights a strong signal around 1,100 cm^{-1}, covering many of the components in the contents list. Starch, cellulose and sugar (in the form of Lactose and Sucrose) influence this signal.

When observing the second spectrum in figure 2, filler materials with their infrared signals are present, but the spectrum is located around some dissolved signals. Interestingly, a secondary Amino molecular group appears which is

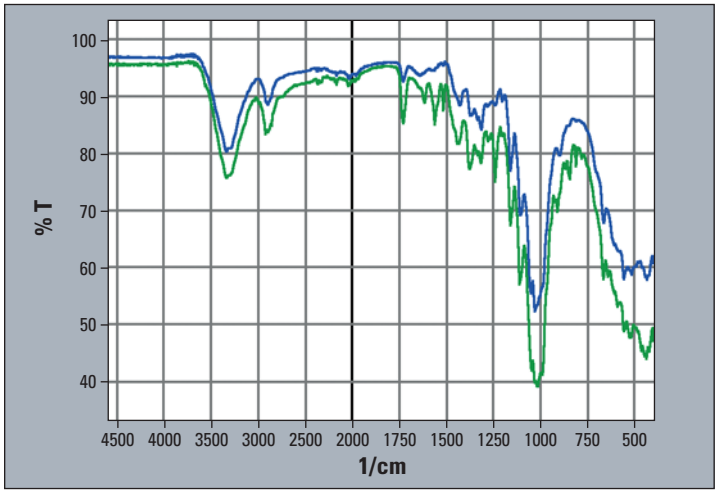


Figure 3: Infrared spectra of tablets with low and high dosage agent (blue = low, green = high dosage)

Wavenumbers [cm^{-1}]	Vibration	Material
1014.56	-C-C-	Filler and active agent
1049.28		
1070.49		
1111.00	C-O-C asy. Stretch	Metoprolol
1157.29	-NH	+ Succinate
1240.23	Ether C-O Stretch	Metoprolol
1332.81		Metoprolol
1444.68	C = C Stretch Ring, aldehyde	
1516.05	C = C Stretch Ring	Metoprolol
1558.48	NH secund. Amine	
1614.42	C = C Stretch Ring	
1726.29	-C = O Aldehyd	Succinate
1977.04	Combination band	
2029.11	Combination bands + NH fine structure	
2916.37	-CH asy. Stretch influences through -O-CH_3	
3332.99	-OH	
3398.57	-NH secund. Amine	

Table 1: Coordination of the vibration signals

from the active agent Metoprolol; as do oxygen atoms in the form of Ethoxy-, Phenoxy- or Hydroxy-groups, as well as a 6-atom ring molecule.

For filler materials, interpretation of the signal in the IR-spectrum is more complex than with analysis of the pure materials. However, some significant signal vibrations can be recognized unambiguously: Metroprolol and Succinate (Table 1). The vibrations representing Succinate could not be found in the spectra of the filler materials or in the spectra of the pure materials of Metoprolol and Metoprolol tartrate (Sadtler Crime/Forensic Libraries).

An intensive CO-signal volume is noticeable in 1,726 cm^{-1}, that could be identified as Succinate. Furthermore, a signal doublet was found similar to Succinate in 1,111 and 1,157 cm^{-1} with a difference of 46 cm^{-1}, corresponding to a doublet of the amber acid (1,084 and 1,132, with a difference of 48 cm^{-1}). The signal deviation can be explained by the different molecule interactions in the salt in comparison to the acid (approx. 20 cm^{-1}).

IR-spectra of tablets with 47.5 mg and 95 mg are shown in figure 3. The active agent's increase in concentration can be recognized by a reduction in the single signal heights.

Instruments

FTIR-instrument: IRprestige-21
Software: IRsolution including search function
Accessories: DuraSamplIRII
Libraries: Shimadzu Pharmaceutical, Sadtler

Accessory

A single-reflection-ATR-unit was used. The measurement was carried out by simple dropping and pressing of the sample.

Method transfer and validation ac
Design requirements DryLab®2010 and *Nexer*

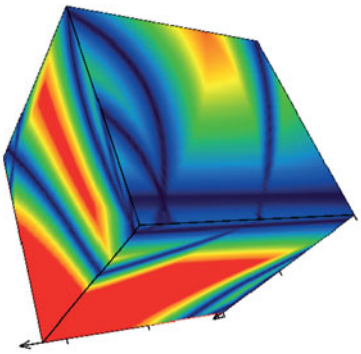


Fig. 1: Cube generate by DryLab2010 – the read area describes the robust area of the method

In 2004, the FDA issued the PAT initiative (process analytical technology), intending to change development processes also in the pharmaceutical industry. Nowadays, the Quality by Design concept as a logical continuation is discussed and influences current and future actions. Guidelines issued by ICH (International Conference on Harmonization) and other regulatory authorities request the application of QbD principles.

The complexity of parameters in a HPLC method makes it very difficult to change and modify some of the parameters once this method is validated. Adapting a separation from conventional to ultra-high pressure LC and the related effect are difficult to describe and predict. DryLab2010 is a valuable tool to simplify this process, based on designed experiments to predict method changing effect and describe the 'Design Space.'

Shimadzu and the Molnar Institute in Berlin cooperated in applying well-known DryLab2010 software with the new generation of UHPLC instruments.

With Drylab2010 it is now possible to simultaneously optimize several measured experimental parameters (tG, T, pH or ternary composition of eluent B) with six other derived factors (column length, -ID, dp, flowrate, dwell volume, extra column volume) to further define the Design Space of a separation. DryLab2010 uses two-dimensional tG-T resolution maps [2]. By applying three of them, three-dimensional resolution spaces can be created in which the combined influence of three parameters can be assessed and optimized.

DryLab applies QbD principle since 20 years with a systematic evaluation of parameters influencing method performance (selectivity, resolution).

DryLab simplifies and speeds up the process of developing excellent chromatographic separations by enabling users to model changes in separation conditions using their personal computer. The time-consuming laboratory runs that are typically required to achieve a satisfactory separation or development of a complete method are replaced with instantly generated chromatograms corresponding to conditions that can be selected individually.

DryLab is useful in almost all chromatography applications in the lab. The software helps to

- quickly find a good set of separation conditions
- develop complete method in minimal time
- evaluate method robustness
- shorten run times
- transfer methods to better and more modern instruments such as Shimadzu *Nexera*
- easily carry out validation of your methods
- find the best separation conditions for any component in a mixture
- fine tune or troubleshoot existing methods
- make the method fit to its purpose
- adjust methods for ageing columns.

The Cube

The cube represents over a million simulated chromatograms and evaluates three parameters simultaneously. It finds the best separation with a mouse click and helps to find the optimum solvent composition (e.g. Ratio between Acetonitril / Methanol). The necessary input data for these 3D models is depicted in figure 4: three tG-T models at three different values of a third parameter are needed.

Figure 2: Shimadzu *Nexera* with LCMS 2020

Design of Experiments

Preliminary evaluation determined that the most influential experimental variables on critical resolution (separation between critical peak pair) were

- the gradient time (tG)
- the temperature (T)
- the pH value
- the ternary eluent composition.

Overnight, these experiments can be run automatically and unattended using the Shimadzu LabSolution software.

Over 30 years of solvophobic theory [1] makes the continuous change in reversed-phase chromatography selectivity understandable. Retention forces are primarily based on the highly organized water structure and on the desire of water to reduce the cavity interfaces towards nonpolar molecules. Lipophobicity of water can be reduced by dilution with the strong eluent: MeOH or ACN.

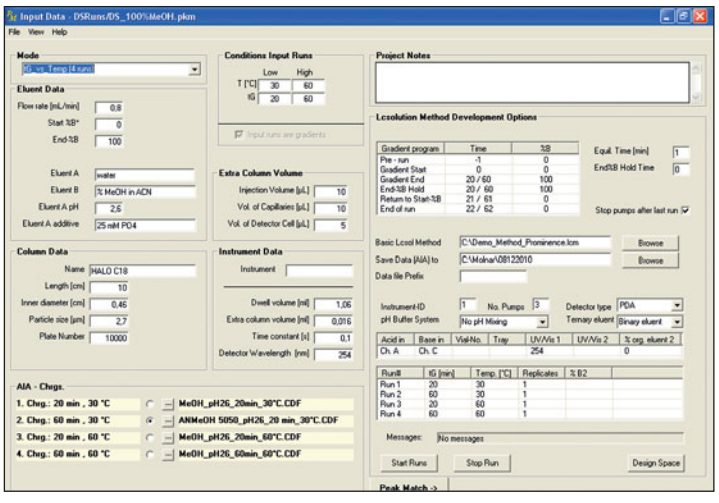


Figure 3: Interface of Peak Match with Shimadzu's LabSolution software parameters

cording to Quality by

ra – a powerful combination

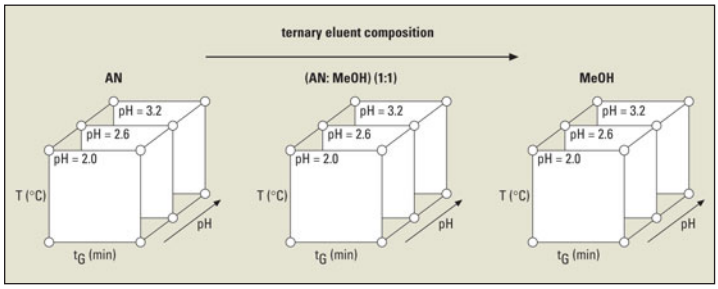


Figure 4: Experimental design for a three-dimensional HPLC method optimization

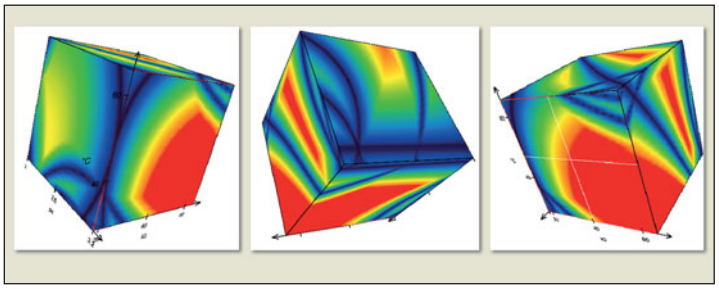


Figure 5: Design Space Visualization with DryLab®2010. Each point in these 3D resolution spaces corresponds to a highly accurate chromatogram

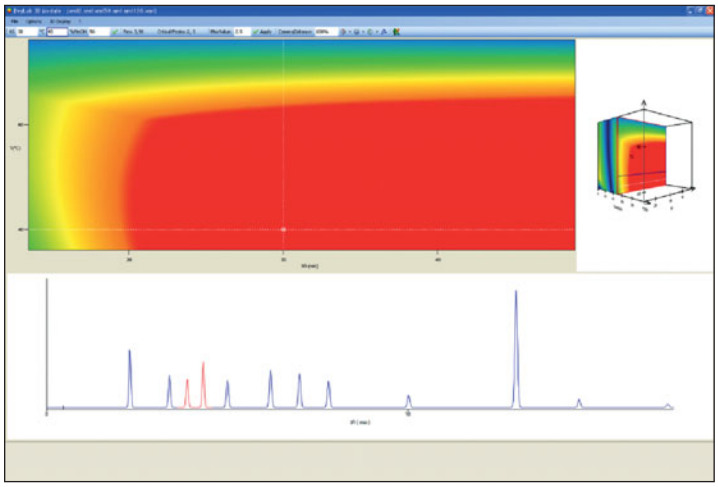


Figure 6: DryLab 3D model. The best chromatogram by one mouse click.

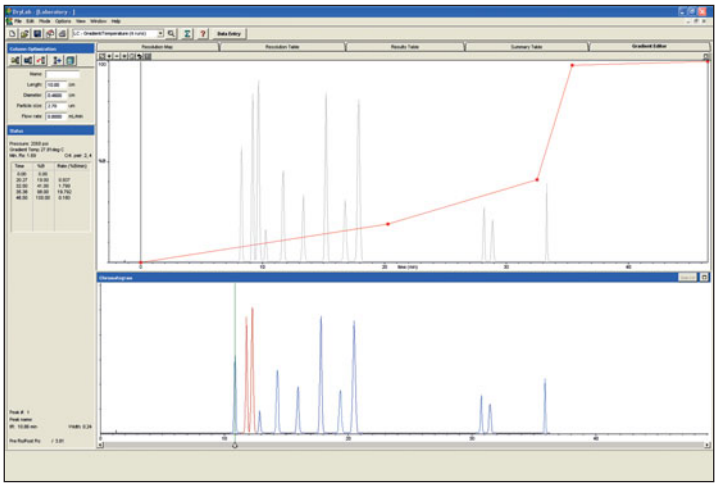


Figure 7: Gradient Editor

The following example of an optimization of a separation of sulfonamides and additional drugs underlines the power of the DryLab2010 and *Nexera* combination.

Sulfonamides are the basis of several groups of drugs. The original antibacterial sulfonamides are synthetic antimicrobial agents containing the sulfonamide group.

Standard configuration, *prominence* with LPGE, column oven and PDA detector.

Chemicals:
A: Buffer 25 mM phosphoric acid
B: Acetonitril
C: Buffer 25 mM Sodium Dihydrogen Phosphate
D: Methanol
Column: Gemini-NX® Phenomenex (150 x 4.6 mm, 3 µm)
Flow rate: 1.00 mL
Wavelength: 280 nm
Temperature: 40 °Celsius

Test mixture including following substances (see table 1).

Optimization Step with DryLab 2010 3D Cube

Optimization on *Nexera* and Shim-Pack XR column with PDA detector

Flow rate: 1.30 mL/min
Temperature: 48 °Celsius
Wavelength: 280 nm
Column: Shim-pack XR-ODS III (75 x 2 x 1.6 µ)
Buffer A: 0.05 mol/L, pH = 2.3 Potassium dihydrogen phosphate (KH_2PO_4)
Buffer B: 50 % Methanol 50 % Acetonitril

Conclusion

With the support of the Molnar Institute and the DryLab 2010 software the group of drugs was separated from starting conditions with a conventional

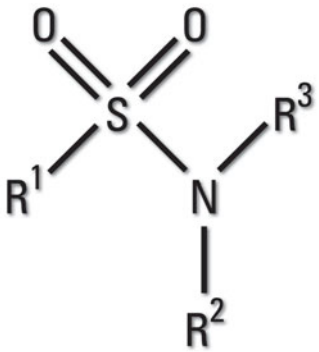


Figure 8: Sulfonamide group

Peak	Name	Formula
1	Acetaminophene	$C_8H_9NO_2$
2	Sulfathiazole	$C_9H_9N_3O_2S_2$
3	Caffeine	$C_8H_{10}N_4O_2$
4	Sulfadimidine	$C_{12}H_{14}N_4O_2S$
5	Sulfamerazine	$C_{11}H_{12}N_4O_2S$
6	Sulfamethoxypyridazine	$C_{11}H_{12}N_4O_3S$
7	Sulfamethoxazaole	$C_{10}H_{11}N_3O_3S$
8	Sulfafurazole	$C_{14}H_{13}N_3O_3S$
9	Sulfaquinoxaline	$C_{14}H_{12}N_4O_2S$
10	Propyhenazone	$C_{14}H_{18}N_2O$
11	MPPH (5-(p-Methylphenyl)-5-phenylhydantoin)	$C_{16}H_{14}N_2O_2$
12	Oxazepam (N-Ethyl)	$C_{15}H_{11}ClN_2O_2$

Table 1: List of compounds

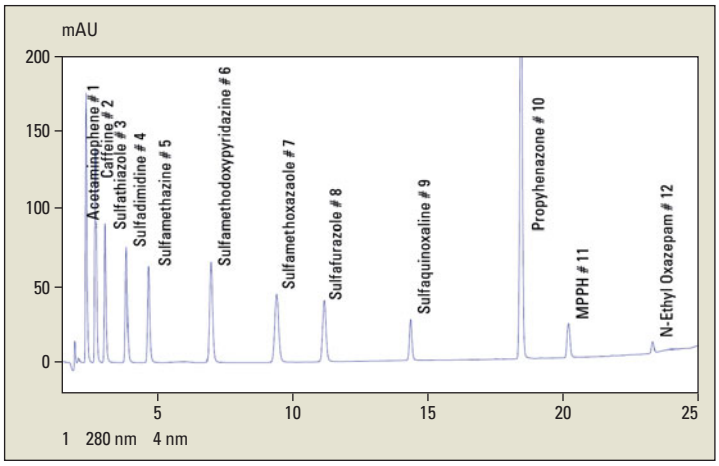


Figure 9: Chromatogram of scouting run

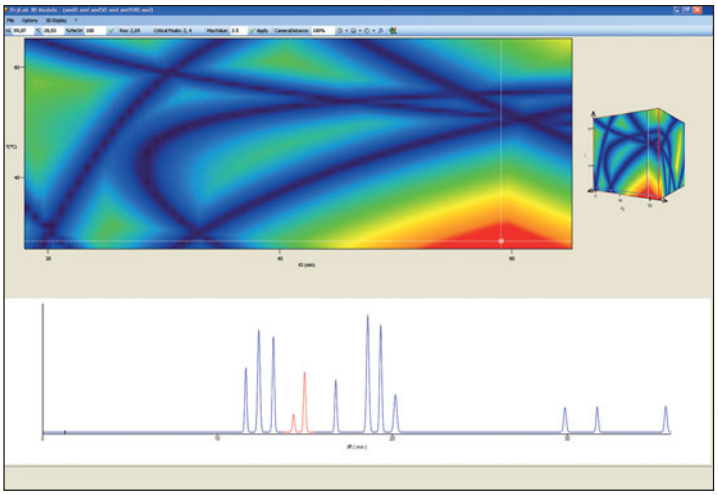


Figure 10: 3D Cube from DryLab®2010

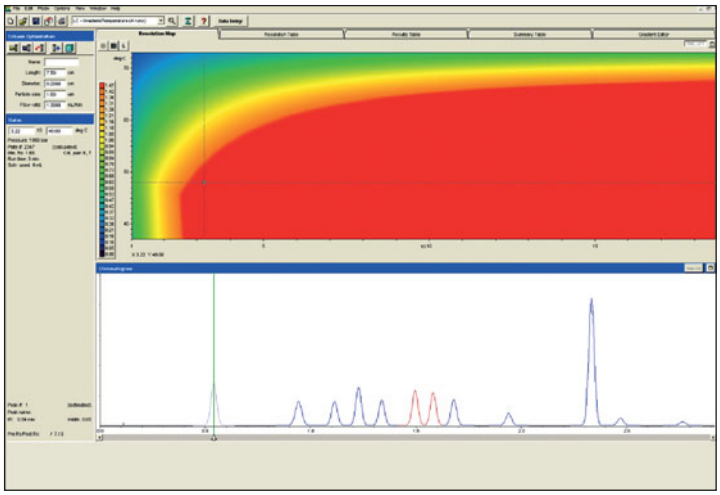


Figure 11: Prediction and Analytical Conditions with Shimadzu *Nexera* UHPLC

HPLC and a runtime of more than 30 min by switching to a modern column, here the Shim-pack XR ODS III (75 x 2 x 1.6 µ) and the UHPLC System *Nexera*, with binary gradient to a runtime of less than three minutes. The critical peak pair (peak 6 and peak 7) has a resolution of 1.65; the pressure on the system is 1089 bar.

Quality by Design

The Quality by Design (QbD) concept was outlined by quality expert Joseph Juran (1904-2008), stating that quality can be planned, and that most quality problems relate to the way it was planned originally.

The ideal state for pharmaceutical manufacturing in the 21st century mean:

- understanding the product and its production processes
- understanding the methodologies (HPLC, etc.) which generate data to support the product quality.

[1] Solvophobic Theory Cs. Horvath, W. Melander, I.Molnàr J. Chromatogr. 125 (1976) 129.

[2] Aspects of the "Design Space" in high pressure liquid chromato-graphy method development; I.Molnàr; H.-J. Rieger, K. E: Monks J. Chromatogr. A, 1217 (2010) 3193-3200

Molnár-Institute for applied chromatography

Founded in 1981, the Institute brings 30 years of experience in high performance liquid chromatography (HPLC). Dr. Imre Molnár, former coworker of Prof. Csaba Horváth from Yale and of Lloyd Snyder and John Dolan of LCResources USA, is a well known expert in HPLC method development.

The Molnár-Institute plays a small but essential role in the improvement of worldwide healthcare, ensuring safe products in pharmaceutical, life science- and food industries, while supporting research and development at universities. The Molnár-Institute continuously serves companies all over the world in successfully designing and shaping HPLC method conditions. By offering software solutions, courses and development services, the Molnár-Institute defines its mission in applying modern software tools such as DryLab2010 to increase efficiency in HPLC laborato-ries and to help to find the best separation as soon as possible.

Speed beyond comparison

High speed drug screening and quantification

Nexera / LCMS-8030 a perfect combination of high resolution UHPLC with MS/MS detection

In the different phases of drug development, metabolites need to be screened on a regular basis. In the early stage where the number of samples screened is still relatively low, the focus is mainly on sensitivity. However, it shifts in general to higher throughput with the demand for moderate sensitivity at a later stage.

In order to be able to fulfill this demand, the analytical solution needs to be reliable on the HPLC as well on the MS whereas a one hand supplier solution has a number of advantages. Fast separation, resulting in narrow peak width, requires the acquisition of enough data points over a peak to be able to generate accurate information for quantification as well as generating product ion scans for further confirmation. In addition to the required fast scan speed the polarity switching is an important feature as well to shorten run time without sacrificing the level of information generated. The demand of low dwell and pause time may be important if a large number of MRM transitions (Multiple Reaction Monitoring) are relevant to screen. In this context, a low cross talk between the different MRMs is important to avoid contamination between the different identification steps as well as an error in the quantification.

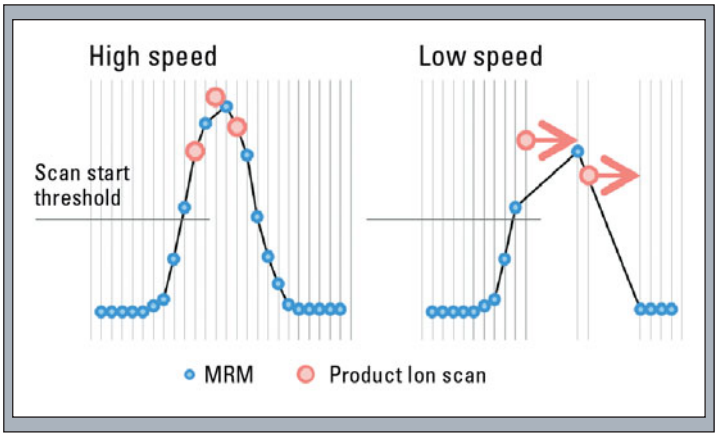


Figure 1: The fast scan speed enables generation of product ion scans without the risk of losing too many data points.

Highest speed in its class

The LCMS-8030 has the highest speed in its class, even in Multiple Reaction Monitoring mode for the triple quadrupole LC/MS/MS. In combination with the high performance *Nexera* UHPLC system with the lowest carryover market-wide and fastest auto sampler, speed can be reached which is beyond comparison.

In order to minimize cross talk in the collision cell which is a bottleneck in high-speed analysis using triple quadrupole LC/MS/MS, Shimadzu has developed its unique UFsweeper™ collision cell (patent pending). As a result, the LCMS-8030 can obtain stable, highly-reliable data even during ultra fast measurement.

Compound	MW	Detection	
		Positive	Negative
Atenolol	266.16	Yes	No
Procaine	236.15	Yes	No
Lidocaine	234.17	Yes	No
Atropine	289.17	Yes	No
Yohimbine	354.19	Yes	Yes
Chlorpheniramine	274.12	Yes	No
Propranolol	259.16	Yes	No
Alprenolol	249.17	Yes	No
Tetracaine	264.18	Yes	No
Diphenhydramine	255.16	Yes	No
Doxepin	279.16	Yes	No
Desipramine	266.18	Yes	No
Imipramine	280.19	Yes	No
Nortriptyline	263.17	Yes	No
Amitriptyline	277.18	Yes	No
Verapamil	454.28	Yes	No
Carbamazepine	236.09	Yes	No
Isopropylantipyrine	230.14	Yes	No
Alprazolam	308.08	Yes	No
Triazolam	342.04	Yes	No
Cilostazol	369.22	Yes	Yes
Nifedipine	346.12	Yes	Yes
Diazepam	284.07	Yes	No
Warfarin	308.10	Yes	Yes
Chloramphenicol	322.01	No	Yes
Nitrendipine	360.13	No	Yes

Table 1 : List of screened compounds

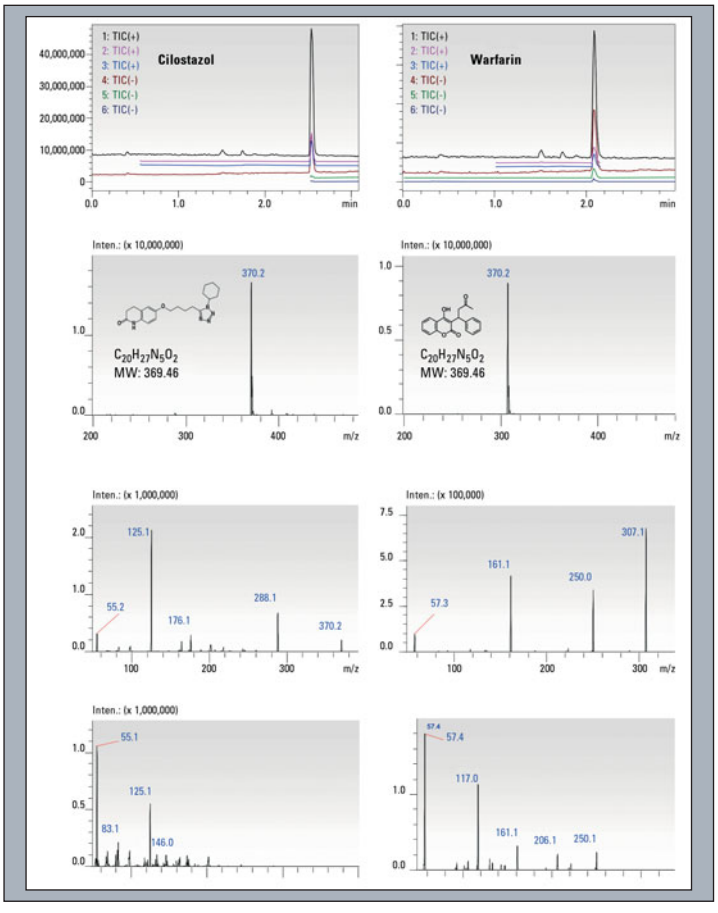


Figure 3: Analytical results (Cilostazol, Warfarin)

Here up to 500 channels/sec are possible with minimum dwell time of 1 msec as well as a pause time of 1 msec respectively.

Best conditions for a certain compound are defined within 10 minutes

The LCMS-8030 system enables the operator to optimize instrument parameters such as mass accuracy of the precursor ion and fragment ions as well as collision energy for compounds of interest by flow injection. The instrument uses multiple injections of a standard to optimize the individual instrument parameters so that in less than 10 min the best conditions for a certain compound are defined. This process can be set up easily for overnight runs so that larger sample sets can also be conveniently used for optimization.

26 pharmaceutical compounds were analyzed using Shimadzu's Synchronized Survey Scan (SSS) shown in figure 1. In this mode, full scan measurement is rapidly followed by automated product ion scanning. High-speed polarity switching (15 msec) and rapid scan rates (15,000 u/sec) allow multiple collision energies to be employed for unknowns even with narrow peak widths. This enables molecular weight confirmation from the Q3 scan data and also generates structural fragmentation information from the same peak.

A total of 26 pharmaceutical compounds were evaluated. Figure 3 shows the analytical result for Cilostazol and Warfarin. As illustrated in table 1, all compounds were detected in either positive mode, negative mode or both, demonstrating the LCMS-8030's effectiveness for drug discovery and synthesis confirmation.

Chromatographic condition

Column: Shim-pack XR-ODSII (2.0 mm I.D. x 50 mm L.)
Mobile phase A: 5 mmol/L ammonium formate-water
Mobile phase B: acetonitrile
Gradient program: 5 %B (0 min) –> 95 %B (3-3.50 min) –> 5 % B (3.51 - 5 min)

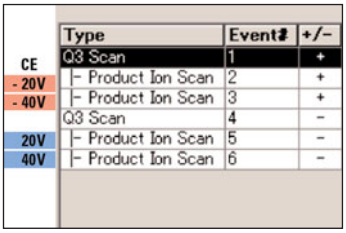


Figure 2: MS method using **S**ynchronized **S**urvey **S**can function

Flow rate: 0.3 mL/min
Injection volume: 5 µL
CDL temperature: 250 °C
Block heater temperature: 400 °C

Nebulizing gas flow: 1.5 L/min
Drying gas flow: 10 L/min
Column temperature: 40 °C
Collision energy: pos -20V, -40V; neg 20V, 40V
Q1/Q3 resolution: Unit/Unit
Scan type: Q3 scan – product ion scan
Scan range: m/z 200 - 500
Ionization mode: ESI positive and ESI negative
Scan speed: 3333 u/sec

The *Nexera*/LCMS-8030 triple quadrupole solution enables setting up of screening experiments in a very efficient way and stands out due to its highest flexibility in pressure range, flow rate and scan speed paired with ease of use and maintenance.

Residual Solvents

U.S. Pharmacopoeia – revised General Chapter Monograph <467>

Figure 1: GC-2014 and HT200H

Satisfy new method requirements using the Shimadzu GC-2014 and HT200H

Residual solvents are trace-level chemical residues in drug substances and drug products that are byproducts of manufacturing, or that form during packaging and storage. It is the drug manufacturers' responsibility to ensure that these residues are removed, or are present only in limited concentrations.

The United States Pharmacopeia (USP) recently revised General Chapter <467> on residual solvent analysis by adopting the International Committee on Harmonization (ICH) Q3C guidelines. The new revisions also include analytical methods for the identification, control and quantification of residual solvents that are adopted from the European Pharmacopoeia (EP). This revision, effective July 1, 2008, replaces previous methods and significantly increases the requirements with which a pharmaceutical company must comply in order to demonstrate that all drug products (not just new) are compliant with Chapter <467> limits. The change increases the number of solvents requiring testing from seven to fiftynine.

While most companies have extensive data on the solvents used in the manufacturing of their API, the information regarding solvents present in the excipients is usually much lower. The drug product manufacturer is responsible for control of the limit of solvents.

Testing is only required for those solvents used in the manufacturing or purification process of drug substances, excipients or products. This allows each company to determine which solvents it uses in production and to develop testing procedures addressing their individual needs.

Solvents and health risks

Solvents have been classified into three main categories based on their potential health risks:

Class 1: Solvents should not be used due to unacceptable toxicities or deleterious environmental effects.

Class 2: Solvents should be limited because of inherent toxicities.

Class 3: Solvents may be regarded as less toxic and of lower risk to human health.

Method overview

The USP has provided a method for the identification, control and quantification of residual solvents. For solvents of Class 1 and 2, the method calls for a gas chromatograph analysis with flame ionization detection (FID) and a headspace injection from either water or organic diluent. The monograph has suggested two procedures for qualitative analysis:

Procedure A specifies a G43 (Zebron ZB-624 or equivalent) phase and **Procedure B** specifies a G16 (Zebron ZB-WAXplus or equivalent) phase. **Procedure C** is for quantitative analysis. Procedure A should be used first.

If a compound above the specified concentration limit is determined, then Procedure B should be used to confirm its identity. Since there are known co-elutions on both phases, the orthogonal selectivity ensures that co-elutions on one phase will be resolved on the other. Neither of the procedures is quantitative, so to determine the concentration, the monograph specifies Procedure C, utilizing whichever phase gives the fewest co-elutions.

Procedure A: G43 (6 %-cyanopropy -94 % dimethylpolysiloxane)
Procedure B: G16 (polyethylene glycol)
Procedure C: G43 or G16 depending on the procedure with the fewest co-elutions

Class 3 solvents may be determined by <731> Loss on Drying unless the level is expected to be > 5000 ppm or 50 mg. If the loss on drying is > 0.5 %, then a water determination should be performed using <921> Water Determination.

USP monograph <467> allows the use of alternate methodologies as long as they have been

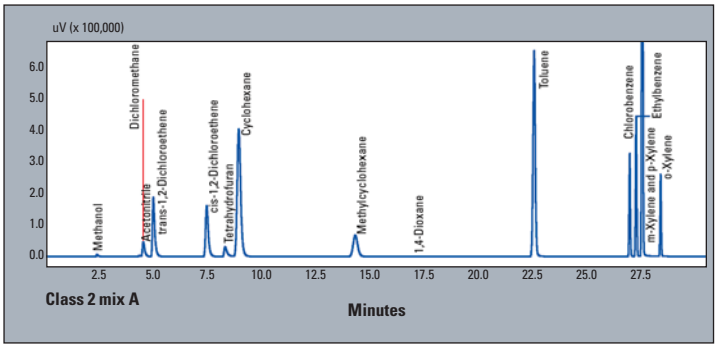


Figure 2: USP Method <467> Procedure A: Class 2 mix A for water soluble compounds

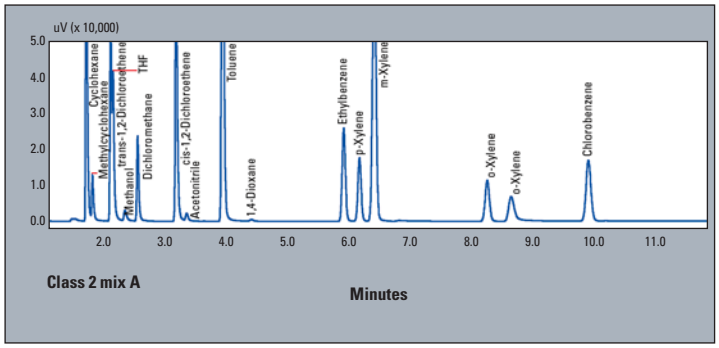


Figure 3: USP Method <467> Procedure B: Class 2 mix A for water soluble compounds

validated appropriately. However, only the results obtained by the procedures given in the general chapter are conclusive. So, the results from the alternate method will have to be compared to the monograph before being acceptable to the Food and Drug Administration (FDA).

Instrumentation

All procedures were performed using a Shimadzu GC-2010, an HT200H headspace autosampler (Figure 1) and Phenomenex GC columns.

The Shimadzu GC-2010 offers high-end GC performance ready for all analytical tasks including full fast GC functionality with filter time constant and sampling frequency of 4 ms and 250 Hz respectively for all GC detectors.

The HT200H headspace autosampler has been specially designed to meet the needs of General Chapter <467>. The sensitivity and reproducibility have been exhaustively evaluated at multiple locations around the world to ensure consistent method. The sampler has also demonstrated performance characteristics for drug substances, excipients and drug products. All show extremely consistent data.

Procedure A – Identification (Class 1 and 2 Solvents)

- System suitability requirements: signal-to-noise ratio of 1,1,1-trichloroethane > 5
- signal-to-noise ratio of each peak of Class 1 solvent should be > 3
- resolution between acetonitrile and methylene chloride > 1.0
- At the concentration limits specified by the monograph, signal-to-noise ratio for 1,1,1-trichloroethane was 59.9; and all other compounds exceeded 3. Resolution between acetonitrile and methylene chloride was 1.71 (Figure 2).

Conditions

Gas chromatograph: Shimadzu GC-2010
Injection: Split 5:1 @ 140 °C, 1 mL
Carrier gas: Helium @ 35 cm/s (constant linear velocity)
Oven program: 40 °C for 20 min to 240 °C @ 10 °C/min for 20 min
Detector: FID @ 250 °C
HT200H headspace: 60 minute conditioning at 80 °C, syringe at 85 °C, 0.5 min on/off shaker, 1 min flush
Column: Zebron ZB-624, 30 m x 0.32 mm x 1.8 µm

Procedure B – Confirmation (Class 2 and 3 Solvents)

- System suitability requirements: signal-to-noise ratio of benzene > 5
- signal-to-noise ratio of each peak of Class 1 solvent should be > 3
- resolution between acetonitrile and trichlorethylene chloride > 1.0
- At the concentration limits specified by the monograph, signal-to-noise ratio for benzene was 104.2; and all other compounds exceeded 3. Resolution between acetonitrile and trichloroethylene chloride was 1.52 (Figure 3).

Conditions

Gas chromatograph: Shimadzu GC-2010
Injection: Split 5:1 @ 140 °C, 1 mL
Carrier gas: Helium @ 35 cm/s (constant linear velocity)
Oven program: 50 °C hold 20 min to 165 °C @ 6 °C/min hold 20 min
Detector: FID @ 250 °C
HT200H headspace: 60 minute conditioning at 80 °C, syringe at 85 °C, 0.5 min on/off shaker, 1 min flush
Column: Zebron ZB-WAXplus, 30 m x 0.32 mm x 0.25 µm

Conditions

All pharmaceutical companies need to determine as soon as possible how the changes to General Chapter <467> will impact their testing procedures. The more excipients and excipient vendors a company uses, the more effort it will require to demonstrate compliance with the new methodology.

The new USP regulations are aimed at improving patient safety and will need to be implemented for all products, whether existing or new. Although the USP has provided a testing method that can be used to identify and quantitate most Class 1 and 2 solvents, the method can be improved based on each companies needs.

However, if a company changes any of the procedures specified by <467>, the new method will need to be fully validated. Only those solvents used in the manufacturing process must be tested in the final dosage form.

For the best solution, each company must consider the number of samples, analysis time, method validation, accuracy, precision and cost of equipment. Once method performance has been achieved, it is also important to consider whether the method can be transferred to other manufacturing facilities. Do they have the knowledge and instrumentation to implement the method?

The changes to the <467> monograph have applied since July 1, 2008, and it is essential to formulate a strategy now for compliance. During the process, many questions and concerns will undoubtedly arise. To ensure the USP addresses as many of these concerns as possible in the new method, an open dialog between industry and the USP is criticial.

The combination of a Shimadzu GC-2010 with an HT200H headspace autosampler is a cost-effective solution to meet the needs of static headspace injection with GC analysis.

Extrusion testing made easy

Testing machine for blister packaging

EZ-Test universal testing machine.

Tablets, capsules, sera, ointments, aerosols – pharmaceutical products must be thoroughly and securely packaged in order to prevent damage during the route from production to patient. Depending on the dosage form, packagings are manufactured from various materials, equipped with specific functionalities and are available in various forms.

The packagings are regularly tested. One of these tests measures the press-through strength of blister contents, which can include for instance tablets.

During the test, the sample is pushed through the packaging using a constant force or speed. The packaging lies on a smooth, flat surface containing a hole through which the sample can be pushed. Whether or not the packaging film ruptures evenly is tested. At the same time it can be checked whether the blister packaging can withstand the various storage and transport conditions undamaged. The test also determines whether the foil or the packaging ruptures, in order to avoid possible cut injuries.

Two force values are determined during this test. The first maximum value is the force needed to rupture the packaging film. The second, increasing value indicates the force needed to push the tablet out of the packaging. This corresponds effectively with the tear propagation test on the film.

Shimadzu's universal testing machines are highly suitable for these extrusion tests. Based on their compact dimensions and straightforward exchange of load cells and accessories, testing machines in the EZ-Test model series are particularly versatile and convenient.

A distinct feature is the comprehensive Trapezium-X software that enables exceptionally flexible data reporting and features a multitude of measuring modes. An intuitive user-friendly method assistant supports the selection of parameters. Versatile statistical functions as well as control card systems complete the new Trapezium-X software.

Quick Setting Panel | Show Batch Results | Show single result

	Name	Max_Force	Max_Stroke	Energy1
	Parameter	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Area
	Pass/Fail			
	Unit	N	mm	J
1 2	Print	☑	☑	☑
	10mg _ 1	☑ 22,2300	1,00100	0,02347
	10mg _ 2	☑ 23,1300	0,87567	0,02370
	10mg _ 3	☑ 20,1175	0,83300	0,02233
	10mg _ 4	☑ 20,2450	0,91667	0,02380

Figure 2: Force-distance diagram of an extrusion test of a blister packaging

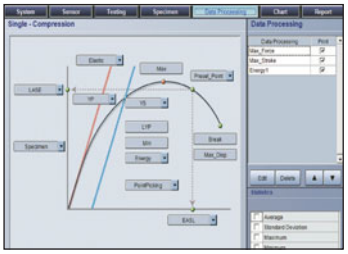


Figure 2: Trapezium-X software

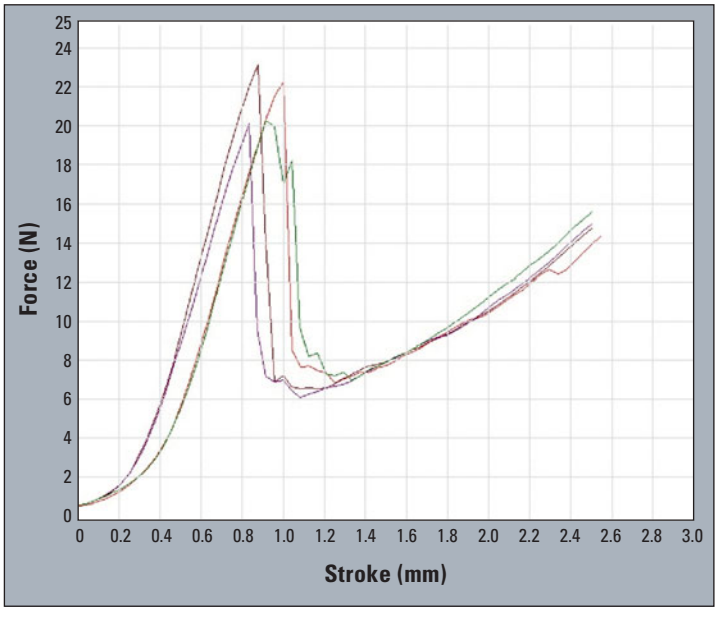


Figure 1: Force-stroke diagram of an extrusion test of a blister packaging

Big potential with protein based drugs

Quality control of recombinant proteins by N-terminal Edman sequencing

Shimadzu has more than 20 years of experience in the field of Edman sequencing and has recently released the latest product PPSQ 31/33A for the European market

The future of pharmacy will be a special category of therapeutics and diagnostics referred to as protein-based drugs. To date, modern medicine has relied heavily on synthetically or chemically produced drugs to treat and prevent ailments and diseases.

However, developments in molecular biology have led to an increase in knowledge of biological systems and their interactions. For example, scientists now know more about the sources of many diseases and how the human body fights them. They now focus on using the body's own tools for developing therapeutics that mimic the actions of the body's arsenal. Today, advances in biotechnology have led to increased use of living organisms and biological substances in the production of protein drugs for human health care. Specifically, the use of recombinant DNA (rDNA) technology has enabled the production of large quantities of protein drugs.

Today, several types of protein based-drugs are available, for example:

1) Cytokines
These drugs regulate the immune system. They are proteins which activate the immune system cells to carry out different immune functions.

2) Hormones
Protein drugs that regulate functions in the body. As drugs, these proteins can be used to elevate levels of specific hormones, such as estrogen during menopause or growth deficiency. They can also be used to treat certain diseases such as diabetes, or conditions such as infertility.

3) Clotting factors
Proteins that regulate the clotting of blood. These drugs are used to treat blood clotting disorders such as hemophilia.

4) Vaccines
Proteins that stimulate the immune system to produce specific antibodies used to prevent or treat diseases.

5) Monoclonal Antibodies
Proteins that mark a specific foreign material (such as cancer cells, disease-causing bacteria or viruses) for removal or destruction by other components of the immune system. These are also used as effective diagnostic tools for many specific genetic diseases and other conditions such as pregnancy.

The first substance of this kind approved for therapeutic use was biosynthetic 'human' insulin made via recombinant DNA technology.

Edman degradation for sequencing amino acids

Protein production and process optimization needs good quality control in order to avoid conditions producing truncated versions of the protein or having a higher level of impurities in the sample. The method of choice is still the N-terminal sequencing method or so-called Edman Sequencing.

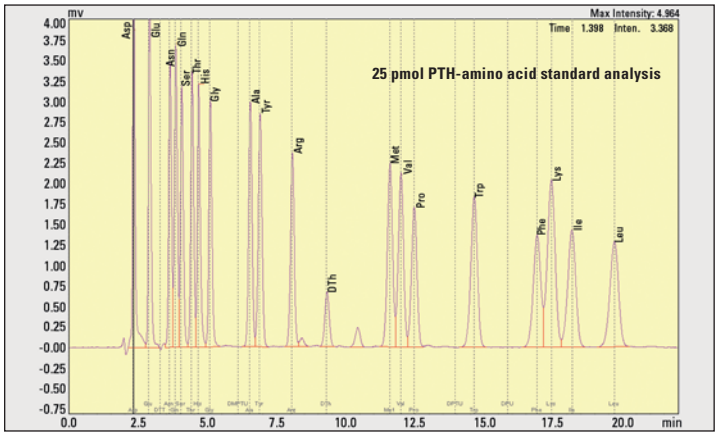
Edman degradation, developed by Pehr Edman, is a method of sequencing amino acids in a peptide or protein. In this method, the amino-terminal residue is labeled and cleaved from the peptide without disrupting other peptide bonds between other amino acid residues. Phenyl isothiocyanate is reacted with uncharged terminal amino group under mildly alkaline conditions to form a phenylthiocarbamoyl derivative. This derivative of the terminal amino acid is then cleaved under acidic conditions as a thiazolinone derivative. The thiazolinone amino acid is then selectively extracted into an organic solvent and treated with acid to form the more stable phenylthiohydantoin (PTH)-amino acid derivative that can be identified using chromatography and comparison with standards. This procedure can be repeated to identify the next amino acid.

Although the method was established more than 40 years ago it is still used throughout different stages of drug discovery or to demonstrate comparability and consistency between batches for release during manufacturing. In Europe, different regulations of the European Medicines Agency (EMEA) need to be followed to characterize protein-based products, e.g.

- Guideline on Development, Production, Characterization and Specifications for Monoclonal

PTH-amino acid	Elution time		
	Day 1	Day 7	Day 15
Asp	2.45 min	2.44 min	2.44 min
Glu	3.14	3.15	3.15
Asn	3.84	3.83	3.83
Gln	4.06	4.05	4.05
Ser	4.25	4.25	4.25
Thr	4.61	4.60	4.60
His	4.90	4.89	4.90
Gly	5.26	5.26	5.26
Ala	6.65	6.65	6.66
Tyr	7.04	7.04	7.05
Arg	7.66	7.65	7.65
Met	11.44	11.45	11.47
Val	11.79	11.79	11.82
Pro	12.22	12.22	12.25
Trp	14.43	14.44	14.49
Phe	16.49	16.50	16.55
Lys	17.03	17.04	17.11
Ile	17.58	17.59	17.64
Leu	18.99	19.01	19.06

Table 1: Reproducibility of retention time of the different amino acids in comparison to 1/7/15 days



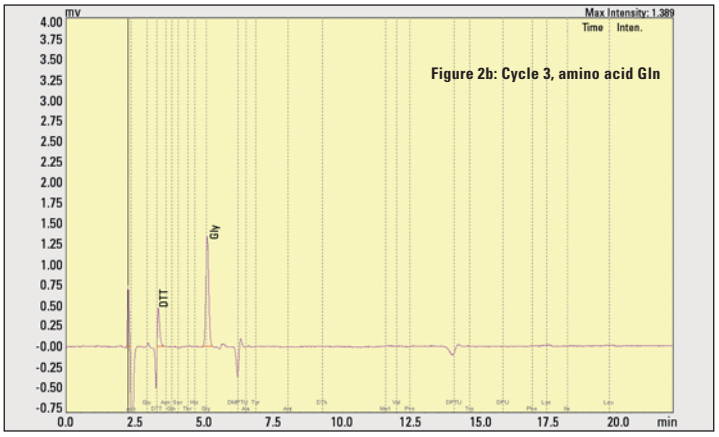
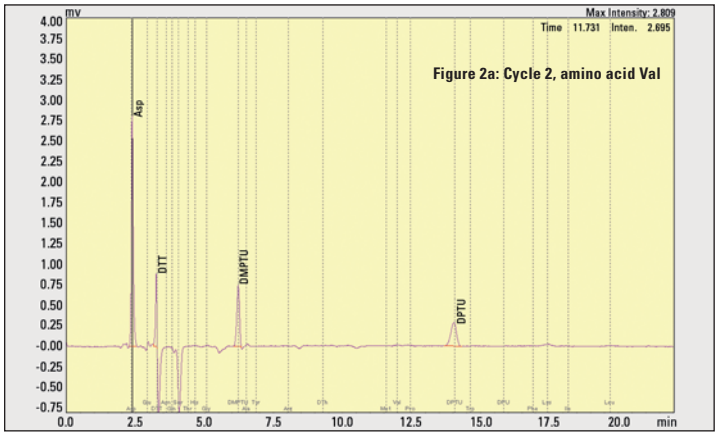


Figure 1: Chromatogram of standard amino acids



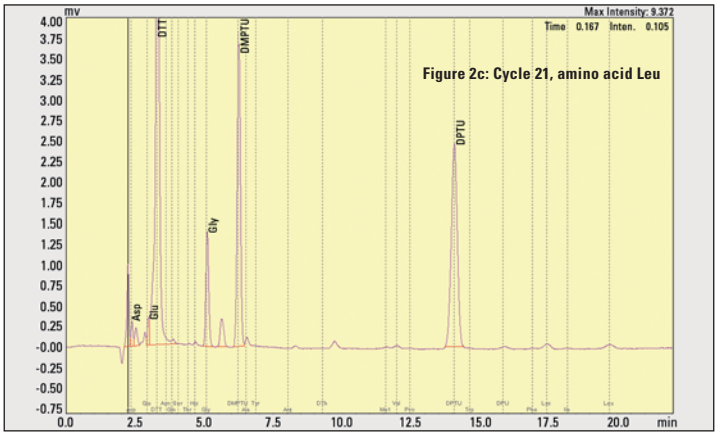


Figure 2a, 2b, 2c: Results of amino acids eluting in different cleavage cycles. The negative signal peaks result from the automatic substraction function, helping to identify rising signals in the next cycle.

Antibodies and Related Products. (EMEA/CHMP/BWP/157653/2007)

- Guideline on similar biological medicinal products (EMEA/CHMP/437/04)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substances: Quality Issues (EMEA/CHMP/BWP/49348/2005)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substances: Non-clinical and Clinical Issues (EMEA/CHMP/BMWP/42832/2005).

Analysis efficiency with single- and triple-reactor type sequencers

The robustness of the Edman sequencing method in routine environments such as quality control, the possibility to correctly determine isobaric amino acids (Leu / Ile) and acquiring of quantitative information from the product as well as from possible impurities makes it still the method of choice. Even where mass spectrometry has replaced Edman degradation in the field of protein identification, it cannot reliably answer all questions and is more difficult to interpret. Edman degradation is therefore also used for discovery of *de novo* sequencing of new novel proteins or peptides.

The PPSQ 31A/33A was released in Europe in 2009, based on the well-established *prominence* HPLC modules. The PPSQ 31 A is a single-reactor type and the PPSQ 33A is a triple-reactor type permitting sequential automated analysis of three samples to enhance analysis efficiency.

PPSQ series protein sequencers achieve baseline stability and allow high-sensitivity analysis of PTH-amino acids by separating them isocratically. Isocratic sequence analysis provides more stable retention times (Table 1). Peaks detected in previous cycles can then be cancelled using substation chromatogram processing, making it easier for users to identify sequences. Performing PTH-amino acid analysis in isocratic mode makes it possible for laboratories to reduce liquid waste and running costs. Reagents and separation column are available from Wako and enable further reductions in operating costs (www.wako-chemicals.de).

New software significantly improves data analysis

In addition, the newly developed sequencer software provides important chromatogram reprocessing for determination of amino-acid sequences. This software processes multiple chromatograms aggregated by sample, thereby significantly improving data analysis functionality. Furthermore, by adopting the latest HPLC analysis system, noise levels are reduced, enabling high sensitivity detection of PTH-amino acids.

PPSQ's capability was demonstrated impressively in the analysis of an unknown protein sample, which was correctly sequenced up to 58 cycles starting with approx. 200 pmol sample and up to 40 cycles with approx. 40 pmol sample material.

Analysis of residual catalysts in pharmaceuticals

ICP-OES technology quantifies 14 elements according to EMEA guidelines

Today, the determination of toxic inorganic analytes (such as metal residues in pharmaceutical products) is a typical method for highly advanced instruments, such as inductively coupled plasma optical emission spectrometers (ICP-OES). This technology enables extremely accurate, quantifiable and reproducible analyses, and a high sample throughput.

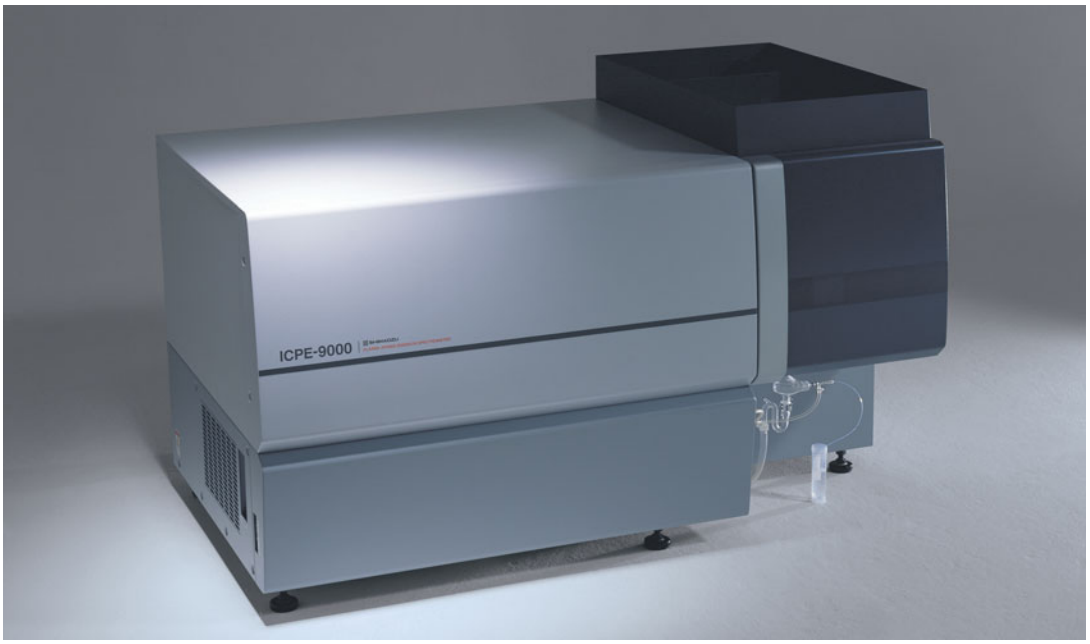


Figure 1: ICPE-9000 simultaneous inductively coupled plasma optical emission spectrometer with CCD detector

Metal residues in pharmaceutical substances or drug products may originate from several sources, e.g. metal catalysts and metal reagents used during synthesis of the active pharmaceutical substance and the excipients, manufacturing equipment and piping, bulk packaging, the environment, cleaning solvents etc. Since metal residues do not provide any therapeutic benefit to the patient, and product risk should commensurate with the level of product benefit, the specification of a pharmaceutical substance or the drug product may need to include a limit and validated method for metal residues to guarantee acceptable product quality.

Dedicated guidelines for residues of metal catalysts

For this reason, the European Medicines Agency (EMEA), a London located decentralized agency of the European Union, has been defining dedicated guidelines, such as the guideline on specification limits for residues of metal catalysts or metal reagents in pharmaceuticals (EMEA/CHMP/SWP/4446/2000). The objective of this guideline is to recommend maximum acceptable concentration limits of metal residues arising from the use of metal catalysts or metal reagents in the synthesis of drug substances and excipients.

Since the use of these metals is restricted to defined chemical reactions, limitation of their residues in pharmaceutical substances will normally be sufficient. Limitation of these metal residues in the final drug product will normally not be necessary.

The concentration limits in this guideline are based on safety criteria and assure an adequate quality of the pharmaceutical substance and the drug product. It is therefore not considered appropriate to expect that the pharmaceutical industry tightens the concentration limits in the regulatory dossier based on GMP, process capabilities or any other quality criteria. Since the origin of metal residues is irrelevant regarding their potential toxic effects, the concentration limits in this guideline are in principle also applicable to residues from sources other than catalysts and reagents.

However, for these other sources adoption of a concentration limit and a validated method in the specification is only necessary in the very exceptional cases where these residues are known to be insufficiently limited by GMP, GDP or any other relevant provision. Pharmaceutical companies are not expected to perform extensive tests on metal residue findings of unknown sources in order to comply with this guideline. They may rely on general information from trustworthy suppliers.

The metals currently included in this guideline are listed in table 1. The guideline may be updated to include other metal residues in due course. Any interested party can submit relevant safety data. The classification and concentration limits of the currently included metals may also change when new safety data becomes available.

EMEA guidelines

Table 1 shows the permissible amounts and concentrations according to the guidelines issued by the EMEA on February 21, 2008. Based on risk to human

health, these guidelines classified 14 types of metals into three classes. Class 1 was further divided into three sub-categories due to concerns over potential carcinogenic or other serious toxicity issues in humans. The permissible limits adressed the three administration pathways of oral, parenteral or inhalation exposure for each class. Permissible levels were based on the Guideline for Residual Solvents (ICH Q_3C) 4. As the maximum permitted value for pharmaceuticals, the Permitted Daily Exposure (PDE, units µg/day) was used. The permissible concentration values were based on computing of the PDE, assuming a 10 g/day dosage of the pharmaceutical. Where daily dosage exceeds 10 g, the permissible value was computed based upon content of the active ingredients in the pharmaceutical.

Classification	Oral Exposure		Parenteral Exposure		Inhalation Exposure
	PDE (µg/day)	Conc. (ppm)	PDE (µg/day)	Conc. (ppm)	PDE (µg/day)
Class 1A: Pt, Pd	100	10	10	1	Pt: 70
Class 1B: Ir, Rh, Ru, Os	100	10	10	1	
Class 1C: Mo, Ni, Cr, V Metals of significant safety concern	250	25	25	2.5	Ni: 100, Cr (VI): 10
Class 2: Cu, Mn Metals with low safety concern	2,500	250	250	25	
Class 3: Fe, Zn Metals with minimal safety concern	13,000	1,300	13,000	130	

Table 1: Class Exposure and Concentration Limits for Individual Metal Catalysts and Metal Reagents (PDE: permitted daily exposure)

Experimental work

The 14 elements of the EMEA guidelines were measured as residual metal catalysts in the pharmaceutical substances. Reagents commercially available for research purposes were used as the samples. For convenience in these tests, an organic solvent containing little contamination was selected for pretreatment of the test samples, rather than resorting to acid digestion (the choice in this case being DMSO due to its high solubility).

0.5 g of each test sample was weighed and dissolved in DMSO to bring the volume to 5 mL (a 10-fold dilution). At this time, yttrium (Y) was added as an internal standard element to produce a Y concentration of 0.1 µg/mL, and a standard sample of tosufloxacin tosilate was added to a dilution solution to obtain a tosufloxacin tosilate concentration of 1 µg/mL. This was used for addition and for recovery testing. The solutions used to make the calibration curves were standard single-element solutions diluted with DMSO solvent. In preparing these calibration solutions, Y was also added as an internal standard element to produce a Y concentration of 0.1 µg/mL.

1 µg/mL concentration solutions were used to prepare calibration curves and changes over time and were measured continuously over two hours. Good results were obtained; the RSD (relative standard deviation) over the two hours of measurement was less than 1 % for all elements. Furthermore, the addition and recovery samples to which the tosufloxacin tosilate was added were subjected to repeated testing over a three-day period in a reproducibility test. The results are shown in table 2.

Element	1st Day	2nd Day	3rd Day	RSD (%)
Cr	1.00	1.00	1.02	1.18
CU	0.99	1.00	0.99	0.46
Fe	1.42	1.43	1.43	0.19
Lr	0.98	0.98	0.97	0.60
Mn	0.99	0.99	1.00	0.89
Mo	1.01	1.01	1.01	0.26
Ni	0.99	0.99	0.99	0.24
Os	1.00	1.00	1.01	0.66
Pd	0.98	0.98	0.99	0.85
Pt	1.00	1.00	1.02	0.96
Rh	0.98	0.97	0.97	0.55
Ru	0.98	0.98	1.00	1.35
V	1.00	1.00	1.00	0.23
Zn	0.99	0.98	0.97	0.87

Table 2: Reproducibility Results over 3 Days (units µg/mL)

In these reproducibility tests, a calibration curve was prepared each time in performing the quantitative analysis. Very good reproducibility was obtained with each element, with the 3-day RSD being about 1 %.

Summary

In this paper, an overview of the ICP optical emission spectrometry was introduced and examples were presented using the ICPE-9000 to quantify 14 elements in pharmaceutical substances identified under EMEA guidelines, as a method for the analysis of residual catalysts in pharmaceuticals. The Japanese Pharmacopoeia (15th Revision) currently lists only the colorimetric method, a test method for heavy metals, and atomic absorption spectrometry as analytical methods for metals in pharmaceuticals. No other metal analysis methods have been adopted.

However, when reviewing the testing for metals in pharmaceuticals that has been conducted on a global scale in recent years, it is certain that the ICP analytical method will be essential for the analysis of pharmaceuticals. As demonstrated in this introduction, the ICP optical emission spectrometry is an ideal method for analyzing residual catalysts in pharmaceuticals.

References:

Director of the Evaluation and Licensing Division, the Pharmaceutical and Food Safety Bureau (Iyaku Shokuhinkyoku Shinsa Kanrika), the Ministry of Health, Labour and Welfare: Revised Guidelines on Impurities in New Drug Substances, No. 1216001, issued by the Evaluation and Licensing Division, the Pharmaceutical and Food Safety Bureau (Iyaku Shokuhinkyoku Shinsa Kanrika) December 16, 2002.

Committee for Medical Products for Human Use (CHMP): Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagent (Doc. Ref. EMEA/CHMP/SWP/4446/2000). London (February 21, 2008)

USP Ad Hoc Advisory Panel on Inorganic Impurities and Heavy Metals and USP Staff: "General Chapter on Inorganic Impurities: Heavy Metals," stimuli to the revision process, Pharmacopeia Forum 34 (5), 1345-1348 (Sept.-Oct. 2008)

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: ICH Harmonised Tripartite Guideline, Impurities: Guideline for Residual Solvents Q3C(R3). Step 4 version

How cleaning validation p

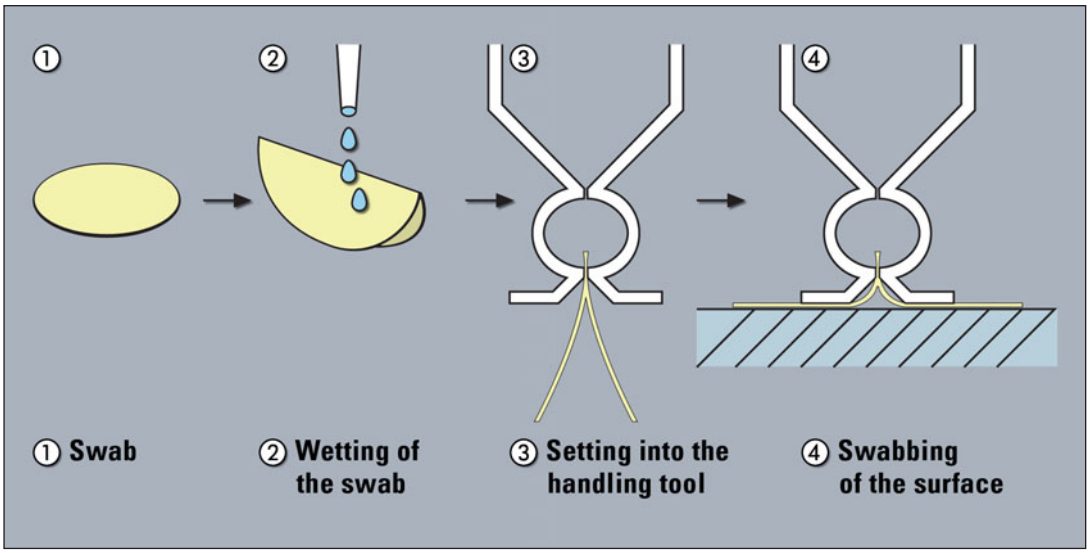


Figure 1: Sampling with swabs

Cleanliness is an important issue in the production of any type of drug, since highest purity and careful handling of substances and active substances are essential in the pharmaceutical industry. The basic request is the effective removal of residual products incurred during production. A clean plant reduces contamination of the medicine. When using a batch process, subsequent cleaning is particularly important due to the production of several drugs in the same plant. Contamination by the previous product must be prevented. Cleaning validation ensures the efficiency of the cleaning process.

Cleaning validation is not a new issue. In 1963, the Good Manufacturing Practice (GMP) already declared:

„Equipment shall be maintained in a clean and orderly manner" (GMP Reg../FDA Part 133.4). A similar section on equipment cleaning was included in the 1978 CGMP regulations.

The implication of cleaning validation has lately been increasingly significant since the pharmaceutical industry develops more and more active substances which are effective even in low concentrations. Proof of contamination needs to be validated by analytic methods which are sensitive enough to define accepted limits of residues. A typical criterion is 10 mg/L or 1/1,000 of therapeutical doses of the medication.

Based on the large variety of pharmaceutical drugs, different analytical methods are applied in the analysis of residues. In particular, HPLC and TOC analysis have been proven to demonstrate the effectiveness of the rinsing process.

Cleaning process

Selection of the right cleaning process is the decisive factor in effectively cleaning the plant. Two processes are normally used.

1. CIP (Clean In Place)
The cleaning is done automatically without dismounting the plant. In this case, the plant has to have a CIP-design (rinsing system with recycling possibility and no dead volumes) which usually requires a high investment.

On the other hand, optimizing of time, temperature, cleaning agent and solvents is possible, resulting in very effective cleaning. The automatic rinsing enables a standardized and validated procedure.

2. COP (Cleaning Out of Place)
With COP, the complete plant needs to be dismantled and all components cleaned individually. This procedure requires more time and staff resources. Due to the individual cleaning, standardization and validation is more difficult but low investment and the opportunity to inspect all components visually are among the benefits.

Sampling and analytics

Depending on the cleaning process, several facilities can be used for sampling to check the cleaning efficiency. The easiest and fastest way is the analysis of the final rinse solution. Depending on the solvent used, subsequent analysis can be carried out with HPLC or TOC.

The swab method is applied to test the surface of the production container. In this way, areas can be evaluated, which are difficult to clean but reasonably accessible and the level of contamination of residues per given surface can be determined. In addition, residues that are "dried out" or insoluble can be sampled by physical removal. Traditionally, the swabs are subsequently extracted and the resulting solution is further analyzed. However, use of a TOC-solid module with glass-

revents contamination

fibre swabs free of any carbon enables direct analysis of the swabs.

TOC-Analysis

Total Organic Carbon measures the carbon content of organic matter present in different matrices. The carbon content of the sample is oxidized to carbon dioxide and detected with a NDIR-detector. Water samples can be measured easily and very quickly. (Typical measurement time is four minutes).

In cleaning validation, TOC-values cover contamination of the pre-product and the remaining rinsing solution. If this result is below the defined limit, further analysis is unnecessary. However, if the result exceeds the limit, HPLC-analysis is needed to identify the source of contamination.

Shimadzu's modular TOC-L series simplifies TOC-analysis, regardless of how samples of final rinse or swab method are measured. The TOC-L is based on the proven technologies of catalytic combustion (680 °C) and NDIR-detection. In this way, it is possible to detect low molecular mass acids and invisible particles. Due to its measuring range of up to 30,000 mg/L (detection limit: 4 µg/L), the instrument is not only suitable for the low range but also for analysis of highly contaminated samples, for example during the cleaning validation process.

TNM (Total Nitrogen Measurement) is an analytical technique used for the determination of total water-borne nitrogen (TN) by means of oxidative combustion chemiluminescence. Both combustion tube and oxidation catalyst are shared with TOC (Total Organic Carbon) analysis. The well-known TOC applications used for Cleaning Validation purposes are therefore complemented by the determination of nitrogen in the related samples. This enhances the analytical information obtained from the cleaning validation samples, in particular proteins and amino acids since nitrogen is an essential part of these compounds.

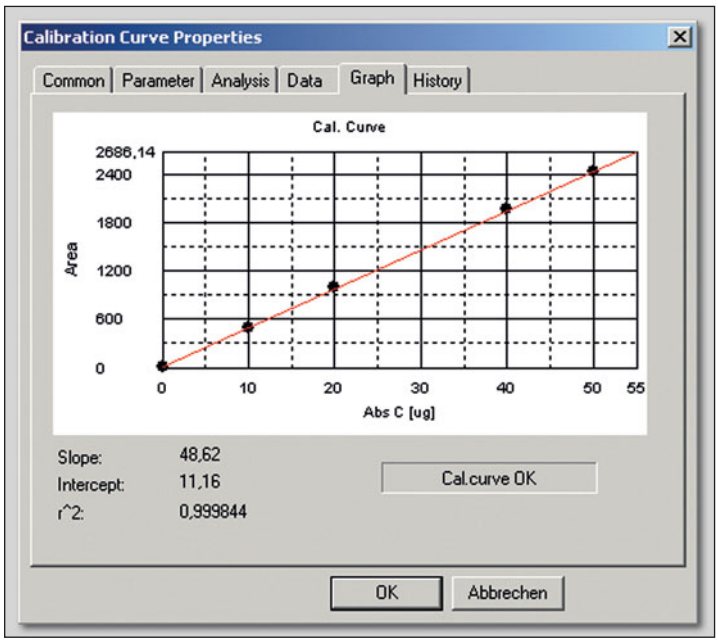


Figure 2: Calibration curve of TOC-solid module up to 50 µg

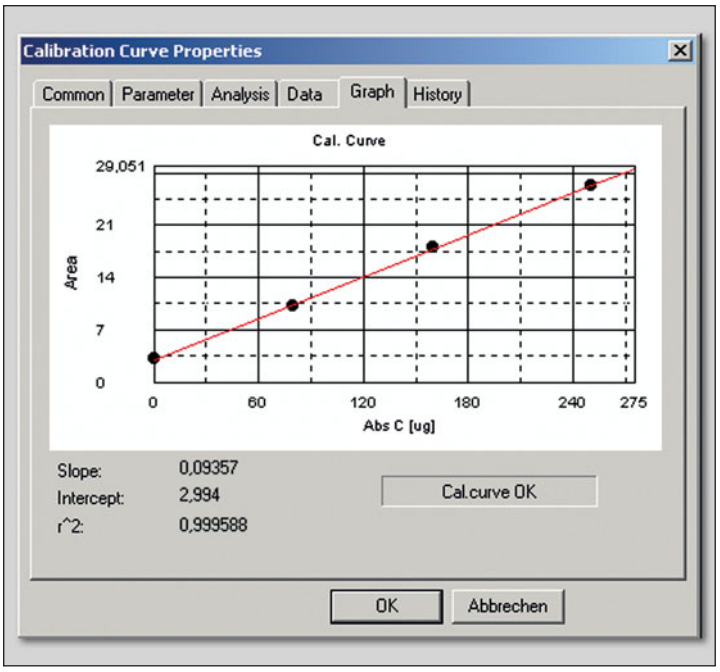


Figure 3: Calibration curve of TOC-solid module up to 250 µg/L

Since the number of insoluble compounds in the pharmaceutical industry is increasing, direct determination of swabs with the TOC-solid module has been gaining more significance. In this case, a carbon-free glass-fibre swab is wetted with purified water and the defined surface is swiped according to a prescribed procedure. The swab is then folded and placed in a clean ceramic boat and inserted in the TOC-solid module. The system configuration and calibration curve are selected depending on the expected concentration or the defined limiting value. The calculated carbon content now corresponds directly to the area of the swabbed surface.

Conclusion

Cleaning validation is an essential part of good manufacturing practice, avoiding cross contamination in the pharmaceutical production process. Fundamental components of cleaning validation are the analytical methods applied. Regardless of the type of cleaning procedure, Shimadzu's instrumentation and software supports the examination of cleaning validation samples with TOC. Specific software tools help to perform the partly complex analysis and support documentation and archiving of the analysis results.

Determination of sodium, potassium and calcium

Analysis of Na, K and Ca with flame atomic absorption spectrometry in microsampling mode

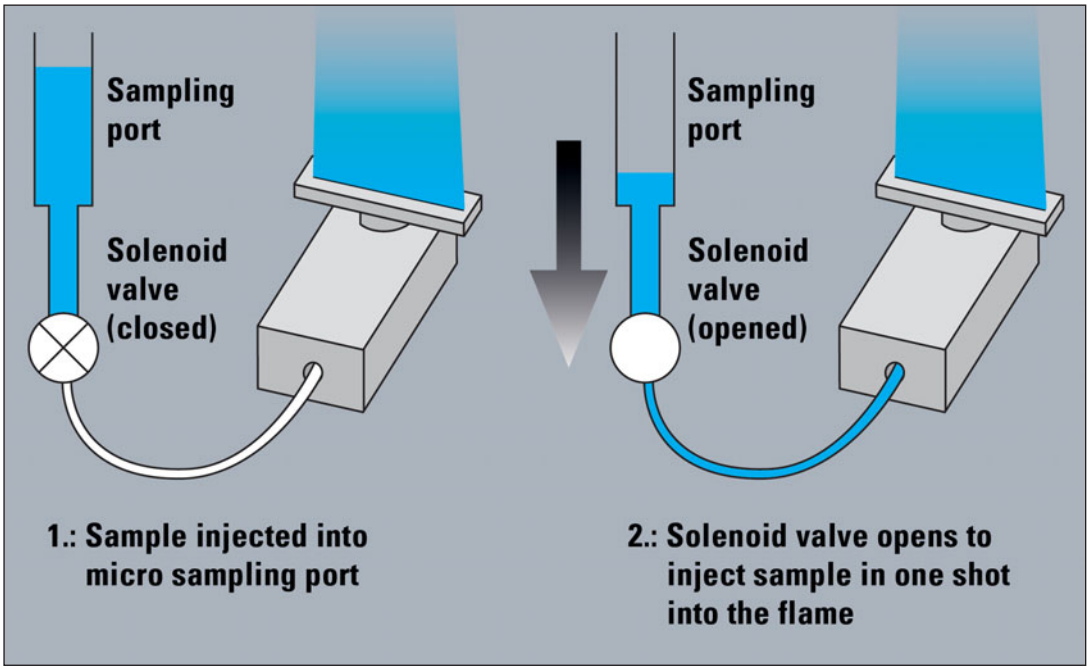


Figure 1: Microsampling method on AA-7000F

Determination of elements in pharmaceutical products such as crystalloid solutions typically applies atomic absorption spectrophotometers such as AA-7000. Concentrations of sodium (Na), potassium (K) and calcium (Ca) are the same as in the body fluids/extracellular fluids and need to be monitored carefully.

Crystalloid solutions are rapidly excreted by the kidneys and appear virtually unchanged. Furthermore, the solutions are suitable for use in dissolution and parenteral administration of drugs. Upon administration the crystalloid solutions are rapidly eliminated. They have therefore proven to be the solutions of choice for administration of drugs. Table 1 shows typical element concentrations of crystalloid solutions.

The AA-7000 combined with the ASC-7000 sample preparation station enables the automated flame micro sampling method (Figure 1). In this method, flame atomic absorption analysis is conducted with small sample volumes (2 - 90 µL), while in the conventional flame method (hereafter "flame continuous method"), the sample is aspirated continuously with a flow rate of approximately 8 mL/min and larger sample volumes are needed for aspiration.

The right choice: the flame micro sampling method

When compared with the flame continuous method, flame micro sampling has several advantages: the analysis is possible with a small amount of sample, and when the autosampler is used, automatic dilution of the sample and automatic addition of buffer solutions are possible in order to compensate for interferences. Moreover, since only a small amount of sample is introduced, the flame micro sampling method is effective for analysis of high matrix samples which may cause clogging of the burner in the flame continuous method.

The method is the right choice for determination of alkaline and alkaline earth elements in crystalloid solutions.

Sodium, potassium, and calcium belong to the essential mineral substances in the human organism. These elements are influential in the generation of enzymes and hormones, control osmotic pressure in tissues and body fluids and are important for the exchange procedures in the cell membranes.[1]

Particularly during surgery, severe loss of extracellular fluids (interstitial fluid and blood) is critical and must be compensated by crystalloid solutions having a similar composition to the extracellular fluids. Blood losses may also be compensated by these solutions provided the quantity of the loss is not too critical.

Electrolyte concentration in mmol / L			
Sodium	Potassium	Calcium	Chloride
147	4	2.3	155.5

Table 1: Typical example of a full electrolyte solution/crystalloid solution

Reliable determination of elements in high matrix samples

Control of Na, K, and Ca in crystalloid solutions according to the European Pharmacopoeia has been performed with the Shimadzu atomic absorption spectrophotometer AA-7000 in a fully automatic multi element sequence. The blank, standards and the test samples have been analyzed in the direct calibration method.

All solutions were placed in the autosampler ASC-7000 and mixed automatically with the corresponding reagents necessary to achieve reliable results. In the case of sodium and potassium 40 µL of CsCl-solution (12.65 g CsCl + 50 mL HCl (d = 1.16) filled up to 500 mL volume with H_2O) was added for a 400 µL mixing volume of standard and sample solution, homogenized before injection to the flame. In the case of calcium, a La_2O_3-solution (5.875 g La_2O_3 + 50 mL HCl (d = 1.12) filled up to 250 mL volume with H_2O) was used.

Instrumental parameters and measuring conditions were set element-specific from the system software. These conditions are automatically set for each element including optimized burner height and gas flow rates and are listed in table 2.

Instrument	AA-7000		
Autosampler	**ASC-7000 incl. microsampling kit**		
Measurement element	Na	K	Ca
Wavelength	589.0 nm	766.5 nm	422.7 nm
Slit width	0.2 nm	0.7 nm	0.7 nm
Lamp current	8/600 mA	8/600 mA	10 mA
Backgroundcorrection	SR	SR	D_2
Flame type	Air-C_2-H_2	Air-C_2-H_2	Air-C_2-H_2
Gas flow rate	1.8 l/min	2.0 l/min	2.0 l/min

Table 2: Instrument and Analytical Conditions

Under these conditions a series of crystalloid solutions has been analyzed to demonstrate that the AA-7000 is an effective tool for the reliable determination of elements in high matrix samples during routine analysis.

[1] Mineralstoffe und Spurenelemente, Leitfaden für die ärztliche Praxis, Bertelsmann, 1992

Quality control of special pharmaceutical organics

Identification with UV spectroscopy

The European Pharmacopoeia guideline for instruments details adjustment parameters and promotes a common understanding of their performance. Up to nine checks are done to calibrate and validate an instrument.

The most important requirement of scanning UV instruments is their capability to perform 1 nm resolution. For the standard requirements of the Pharmacopoeia an instrument such as the Shimadzu UV-1800 is a good solution. This double beam spectrophotometer is equipped with a slit of 1 nm. The proof of performance is the signal resolution of a mixture prepared from Hexane and Toluene. The instrument applies all Shimadzu and Pharmacopoeia validation criteria. It automatically integrates validation in one level and automated tests with certified standards in another level.

UV-1800

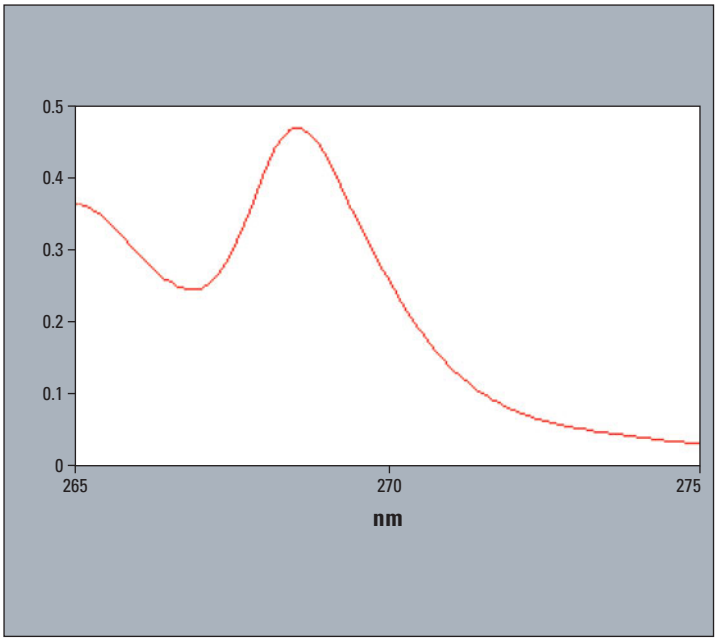


Figure 1: Graph-hexane/toluene resolution

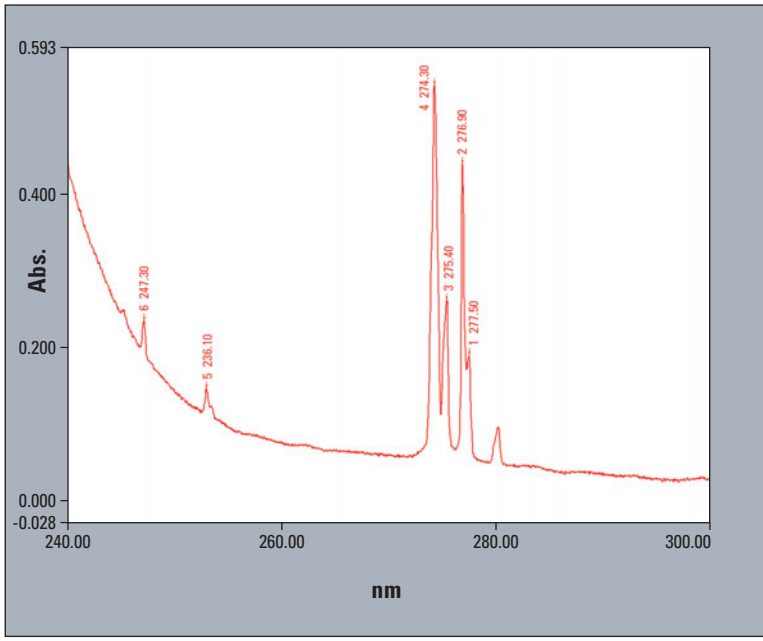


Figure 2 and 2a: Spectra of Gadodiamide in the UV-range with automated peak detection; the spectra were measured with different resolution; the red spectrum was with

The UV-1800 is a recommended system because of its stability in baseline and the accuracy of each data point over the complete measurement range.

This system can be used for simple quality control of pharmaceutical substances according to the requirements of EP, USP and FDA.

Some applications needing much higher resolution are also of interest. In these cases a high-performing instrument is required, in particular when the analysis shall be performed with a resolution of lower than 1nm.

An example of such a high-resolution request is the measurement of Gadodiamide in UV range in combination with a Shimadzu UV-2550 spectrophotometer.

The analysis

Gadodiamide (known under the brand name of Omniscan, for

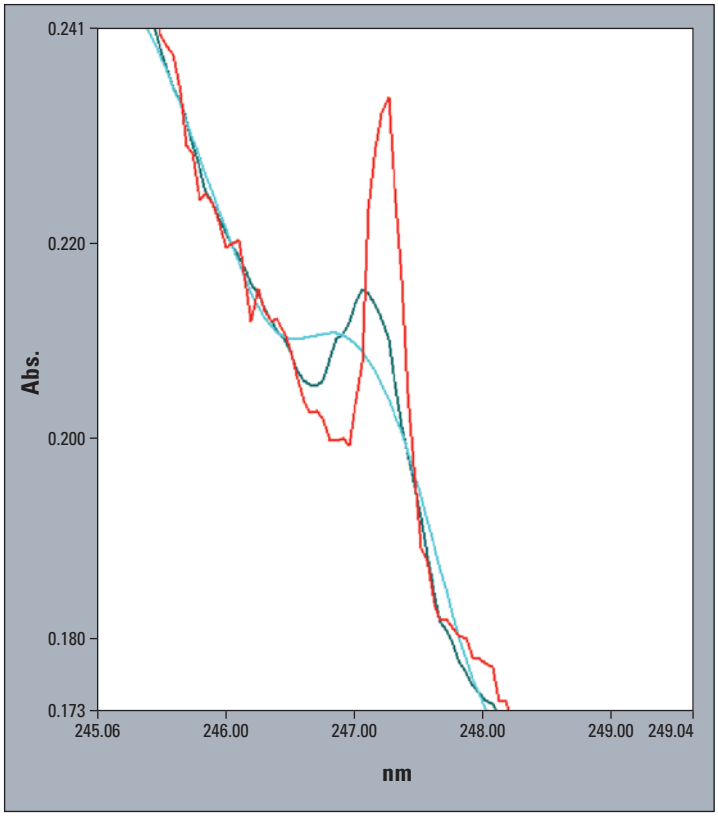
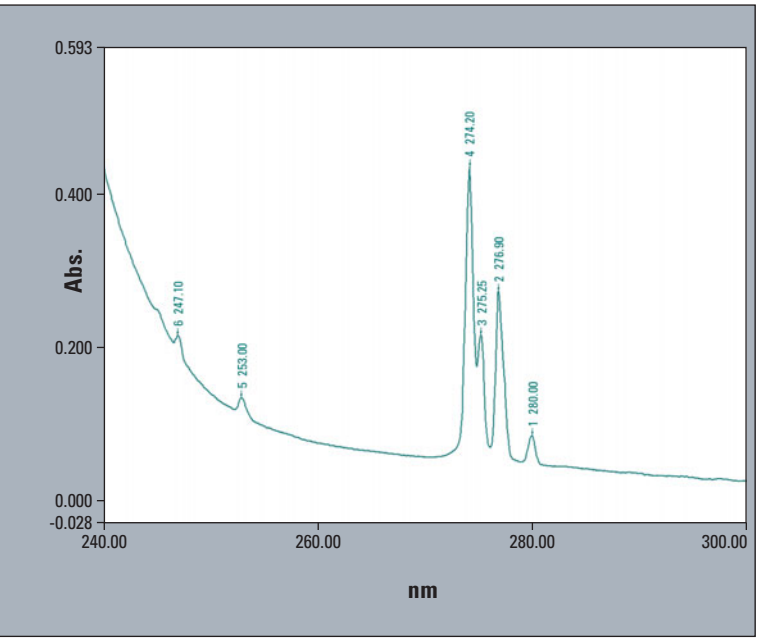


Figure 3: Zoom into the region of interest. The dark green line has 0.5 nm resolution and the red one 0.2 nm slit selection. The signal developed by the 0.2 nm slit is much higher and sharper. Identification of the signal is much more accurate. The light green spectrum has 1 nm resolution, showing the difficulty of detection.



0.2 nm resolution and the green spectrum with 0.5 nm

example) is used as a magnetic resonance (MR) contrast medium. Gadodiamide is used as an indicator for the neuro part or whole body of a scan. From a chemical point of view, this substance is a chelate complex which is a molecular structure of negatively charged groups surrounding a metallic ion. In this case, it is the Gd (Gadolinium) ion. The substance is also explained in the following formula as Gd-DTPA-BMA. The characteristic is a linear chelate, uncharged and with low osmolarity.

For the identification high resolution is needed for the signal shape of position 247.3 and 253.1 nm.

In figure 2 the spectrum with 0.2 nm is shown on the left side, and the same substance with 0.5nm resolution is shown on the right side. Automatic determination of peak position can verify both signals of interest only with higher resolution out of the high basic absorption at the area from 260 to 240 nm. The signal at 247 nm disappears into a shoulder when the measurement is done with 1 nm resolution. An automated detection is impossible and manual detection becomes difficult. It will result in time consuming activities of spectrum manipulation.

The UV-2550 instrument was applied. The spectra generated were measured in the absorbance mode.

Following parameters were investigated under high resolution measurement with the UV-2550:

Measurement Properties

Wavelength Range:
240 to 300 nm
Scan Speed: Very Slow
Sampling Interval: 0.1 nm
Scan Mode: Single
Path Length: 10 mm; 1 cm standard quartz cell

Synonyms	Omniscan
Molecular weight	576.68036
Molecular formula	$C_{16}H_{29}GdN_5O_8$
Classifications	known drug
	FDA approved drug
	bioactive

Table 1: First characteristics of Gadodiamide*

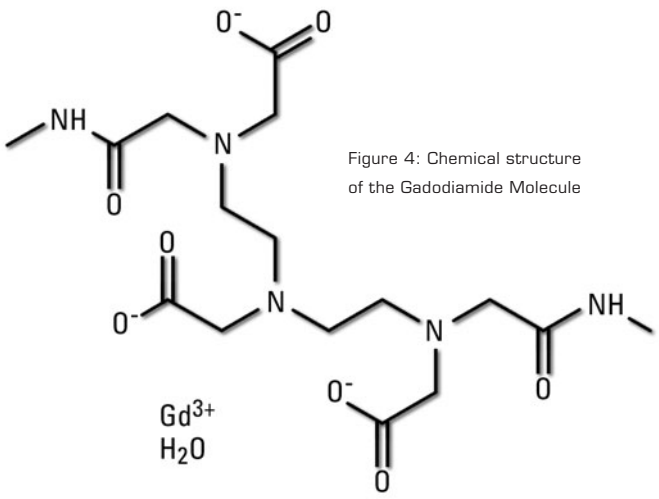


Figure 4: Chemical structure of the Gadodiamide Molecule

Instrument Properties

Instrument Type: UV-2550
Measuring Mode: Absorbance
Slit Width: 0.2 nm
Light Source Change Wavelength: 360 nm
S/R Exchange: Normal

*Sources:
1: http://chembank.med.harvard.edu
2: Wikipedia

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Electronic version

Quality control of ultra pu pharmaceutical manufact

Two TOC instruments offering different oxidation met

TOC-L mit ASI-L

Ultra pure water is one of the most widely used reagents in industry and its quality is therefore of utmost importance in all industrial processes. Quality control has, for many years, been carried out and documented via conductivity measurements, which provide an assessment of the concentration of all inorganic species present in water. This detection method does not take organic pollutants into account as they typically do not contribute to conductivity. However, organic pollutants can tremendously influence further industrial processes and it has become increasingly more important to include quantitative determination of all organic species in quality control of water samples.

The TOC value (Total Organic Carbon) can be used as sum parameter for organic compounds. Similar to conductivity signals that are composed of ionic compounds, the TOC value is a measure of the contribution of many organic compounds present in a water sample.

Different areas of application therefore require different grades of pure water. The European Pharmacopoeia (EP) defines several grades of quality including 'Purified Water', 'Highly Purified Water' and 'Water for Injection.'

Clarifying the Terminology

Water for Injection is ultra-pure water used in the preparation of injection solutions. Ultra-pure water is produced via distillation. Its TOC content may not exceed 0.5 mg/L (water for injection in bulk).

Highly Purified Water is sterile water for the production of drugs not requiring 'Water for Injection.' It is often used for the final rinse during cleaning as well. Highly purified water is produced via reversed osmosis. Its TOC content may not exceed 0.5 mg/L. However, the United States Pharmacopoeia does not apply this classification.

Purified Water is used for the production of drugs not requiring a separate standard. Organic content is determined via TOC value (0.5 mg/L) or the permanganate test for purified water in bulk.

TOC determination according to EP 2.2.44

The EP 2.2.44 guidelines do not prescribe any particular oxidation technique for TOC determination. But the TOC systems must differentiate between inorganic and organic carbon. This can be utilized via removal of the inorganic carbon species (NPOC method), or a separate determination (difference method). The limit of detection for TOC should be at least 0.05 mg carbon/L. Applicability of the method must be determined via a system suitability test.

To carry out a system suitability test, a standard solution consisting of sucrose with a carbon content of 0.5 mg/L is prepared as well as a control solution of 1,4-benzoquinone with the same carbon content. The blank water reference solution (ultra-pure water) used for this purpose may not exceed a TOC content of 0.1 mg/L. For the system suitability test, all solutions including the blank water reference are subsequently meas-

re water in uring

thods

ured and the resulting signals are recorded.

Blank water reference: r_w
Standard solution (sucrose): r_s
Control solution (benzoquinone): r_{ss}

The signal of the blank water reference is subtracted from the response signals of both standard solutions. Then, the recovery of the benzoquinone standard is subsequently calculated with respect to the sucrose standard.

Recovery in %:

$$\frac{r_{ss} - r_w}{r_s - r_w} \cdot 100$$

Results between 85 - 115 % are acceptable. The ultra-pure water sample corresponds to the guidelines when its response signal (r_u) does not exceed $r_s - r_w$.

TOC determination in ultra-pure water

Two oxidation methods are routinely being used in TOC analysis:

1. Catalytic combustion where carbon compounds are converted into CO_2 using a catalyst under high temperatures with subsequent detection of the CO_2 using a NDIR detector.
2. Wet chemical oxidation which uses a combination of UV irradiation and persulphate for oxidation.

Both methods can be applied for ultra-pure water TOC determination. Which of the two methods will offer the best solution for different applications can not be answered in general, but requires closer examination of the particular application.

TOC series

Shimadzu's TOC series covers all water quality analysis requirements. Two systems are particularly suitable for ultra-pure water TOC analysis. Both offer a wide measuring range of 0.5 µg/L up to 25,000 mg/L and support a broad range of applications – from ultra-pure water up to highly polluted wastewater (or cleaning validation). Both systems are available as a PC as well as a standalone version and are easily adjustable to any laboratory environment.

The two TOC instruments for ultra-pure water analysis differ in their methodology. The TOC-$V_{WP/WS}$ uses wet-chemical oxidation whereas the TOC-L uses the catalytic oxidation method at 680 °C.

TOC-L: Oxidation via catalytic combustion

The TOC-L applies the proven catalytic oxidation at 680 °C. The integrated ISP sample preparation unit (an 8-position switching valve with syringe and sparging gas connection) considerably reduces analysis time and complexity, as dilution, acidification and sparging are fully automated by the instrument. Automatic dilution increases the measuring range up to 25,000 ppm (detection limit: 4 µg/L).

In addition, the combustion technique can be used in combination with the TNM-1 module, whereby a single injection is sufficient for simultaneous TOC/TN_b (total bound nitrogen) determination. This is carried out in accordance with EN guidelines for TN_b determination via chemiluminescence detection.

In this case catalytic combustion happens at 720 °C. Simultaneous TOC/TN_b determination is highly suitable for cleaning validation, as this makes differential determination between cleaning agent and product possible.

Wet-chemical oxidation using the TOC-V_{WP}

The key technique of the TOC-V_{WP} analyzer is the powerful oxidation via the combination of sodium persulphate and UV-oxidation at 80 °C.

A persulphate solution is needed for the determination. Therefore, it is important that this solution does not contain any contaminants affecting the measuring value negatively. The TOC-V_{WP} contains an automatic reagent preparation function that eliminates possible contamination of the persulphate solution in order to assure that the average TOC value truly originates from the sample – and not from the reagent solution used.

Combined with the large injection volume (up to 20.4 mL) and the highly sensitive NDIR detector, this leads to an extremely low detection limit (0.5 µg/L) and excellent reproducibility in the lower ppb range. The TOC-$V_{WP/WS}$ is therefore highly suitable for TOC determination in the ultra-trace range.

Conclusions

Both types of instruments with their different oxidation methods can be used for TOC determination according to EP 2.2.44 guidelines. The advantage of the combustion method is its high oxidation potential, especially for samples containing particulate matter. Moreover, simultaneous TOC/TN_b measurements can be carried out using this instrument.

The advantage of wet-chemical oxidation is its high injection volume, which leads to higher sensitivity and therefore enables high precision measurements in the lower ppb range.

How heavy metals can migrate into pharmaceuticals

EDX series detects elements from C to U – carbon and uranium

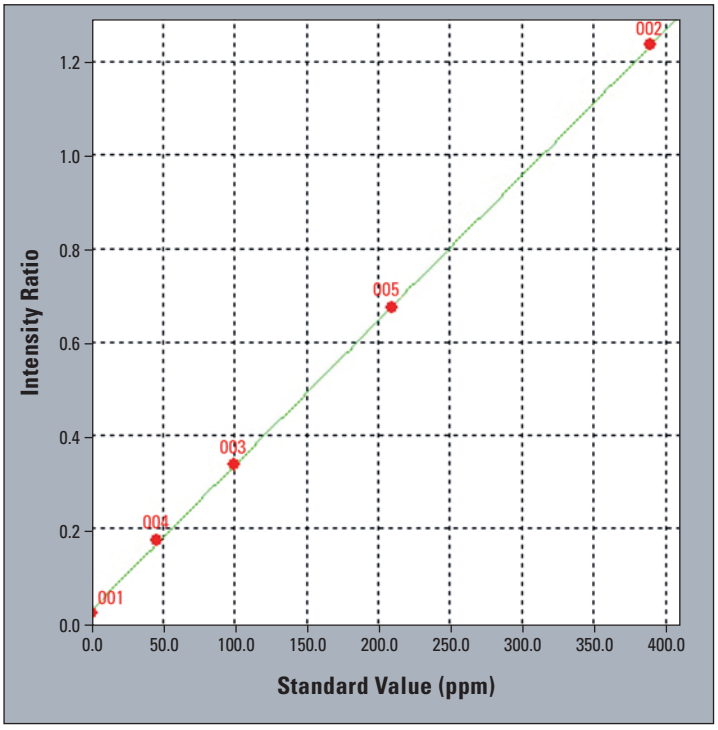

Figure 1: Measurement of a PET bottle containing around 250 ppm of Sb

Pharmaceutical substances can be toxic and dangerous to the human health if not manufactured with proper care. All substances from starting materials to finished products are therefore strictly regulated. In general, this covers proof of product stability, drug-release profiles and examination of the active pharmaceutical ingredients (API). Furthermore, product safety including sterility and absence of non-proprietary materials, e.g. heavy-metals is determined.

One of the analytical tests in the pharmaceutical industry is the quantification of heavy metals or inorganics in all substances and materials in a pharmaceutical product. For example, toxic heavy metals such as cadmium (Cd), mercury (Hg) and lead (Pb) are checked normally. It is also common practice to measure catalysts such as palladium (Pd) and platinum (Pt). Throughout the manufacturing processes, there are many potential sources of contamination. In addition to testing the raw materials it is therefore essential to also measure all finished products and in some cases intermediates to guarantee compliance with the appropriate regulations.

Contamination by the packaging

But even when the product is produced without contamination, the final product can contain heavy metals. Contamination can be caused by the packaging material, for example by PET bottles containing antimony (Sb) as a catalyst. Residues of antimony remain in the bottle and can, under certain conditions, migrate into the pharmaceutical product. It is therefore logical to control heavy metal content in the packaging material.

Is there evidence that antimony migrates into the product?

Unfortunately, yes. Scientists at the University of Copenhagen, Denmark, recently studied antimony levels in 42 juice drinks and found concentrations above EU limits for drinking water in eight of them.

Freshly produced juice drinks appear to be free from increased antimony content. The acid in the juice seems to enhance the migration process of antimony from the bottle into the liquid contained. PET bottles are used not only for juice drinks, but also for pharmaceutical products.

This discovery is of concern since antimony is a known toxic element. In small doses, it can cause headaches, dizziness and depression. Larger doses can lead to violent and frequent vomiting, and even to death within a few days.

Shimadzu's EDX series of x-ray fluorescence spectrometers has been designed for the analysis of a wide variety of elements from carbon (C) to uranium (U), in concentrations from 100 % down to a few ppm. The instruments measure the content of heavy metals in pharmaceuticals products and their packaging materials. To demonstrate the performance of the EDX instrument a calibration was made using plastic standards ranging from 50 ppm to 400 ppm.

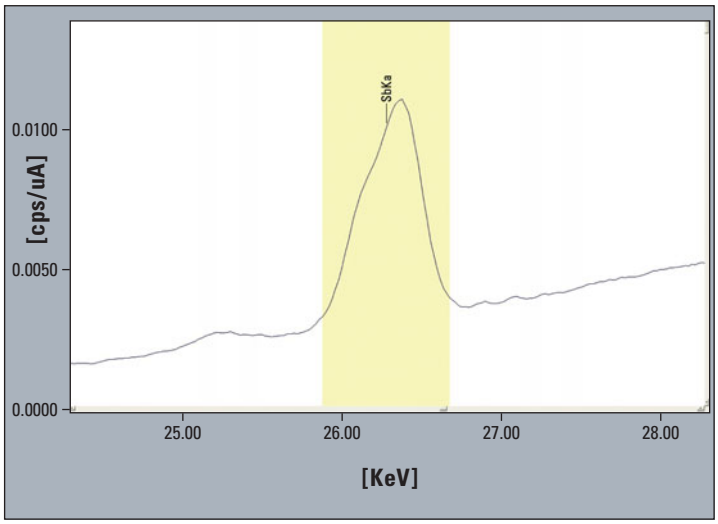

Figure 2: EDX measurements are a great tool for analyzing pharmaceutical products and their packaging materials in a fast and cost-efficient way