Featured Articles

Compact Mass Detector for Drug Discovery Research

—Chromaster 5610 Mass Detector—

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OVERVIEW: Mass spectrometers are widely used in a variety of different scientific fields, including synthetic organic chemistry and biochemistry. Hitachi High-Tech Science commercialized the Chromaster 5610 MS Detector, a mass detector that is easy for a wide range of users to use, and is based on a new concept that is different from that of large mass spectrometers. This article describes examples of how this analyzer is used in the field of drug discovery research.

INTRODUCTION

MASS spectrometry (MS) is a technique for measuring the masses of the constituent molecules of a sample by ionizing them in the presence of a high voltage. It is widely used in a variety of different scientific fields, including synthetic organic chemistry and biochemistry, with the mass information being used to identify sample constituents, analyze their structure, and quantify them.

In the pharmaceutical sector in particular, mass spectrometry is used in all stages from drug discovery research to clinical testing and production management. While this mainly involves structural analysis using a high-resolution mass spectrometer or quantitative analysis using a high-sensitivity mass spectrometer, it cannot be said to satisfy all requirements because of the instrument's high cost and difficulty of operation, meaning it requires specialist operators. There is also growing demand for simple mass spectrometers that are easy to use even by operators without experience, primarily in fields such as synthetic research.

DEVELOPMENT OF A COMPACT MASS DETECTOR

Although a powerful analytical tool in the scientific field, the use of mass spectrometers is not widespread. In the high-performance liquid chromatography (HPLC) market, mass spectrometry was only recently added in the Japanese Pharmacopoeia as a testing method, and there is growing demand for mass spectrometry even among general HPLC users, primarily in the field of pharmaceutical manufacturing. In the HPLC market, however, less than 20% of the more than 200,000 liquid chromatography (LC) systems installed worldwide are connected to a mass spectrometer, and there are high barriers to the adoption of mass spectrometers by HPLC users. Compared to optical detectors for HPLC, the rate of adoption is still very low.

Major reasons for this low rate of adoption include the installation requirements, price, maintenance, and ease-of-operation of mass spectrometers. The Chromaster 5610 MS Detector has enhancements intended to deal with these issues, which act as barriers to adoption by HPLC users, and was developed with the aim of providing new users with a low-cost mass detector that is simple to use (see Fig. 1).

(1) Compact design

The Chromaster 5610 MS Detector has a similar installation footprint to a Chromaster HPLC system, with space being saved by the development of compact and accurate ion optics and the use of a small vacuum pump.

(2) Elimination of special installation requirements

When the Chromaster 5610 MS Detector is used as a liquid chromatography/mass spectrometry (LC/MS) system, the solvent (mobile phase) injected in the stage prior to the mass detector is split by a ratio of between 1/100 and 1/250 and introduced at a rate of only a few microliters per minute. This significantly reduces the amount of solvent that escapes into the environment. It also significantly reduces use of the nitrogen gas required during ionization compared to a conventional mass spectrometer, which makes it possible to use a simpler nitrogen gas supply system. Similarly, rather than the 200-V power supply required

LC: liquid chromatography MS: mass spectrometry
HPLC: high-performance liquid chromatography

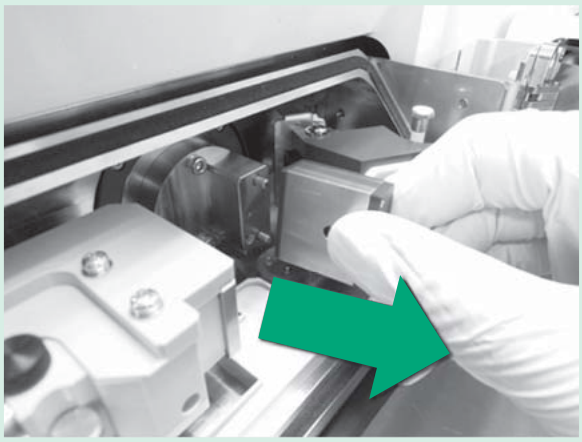
Fig. 1—Chromaster LC/MS System.
The Chromaster LC/MS system combines a Chromaster HPLC system (left) and a Chromaster 5610 MS Detector (right) to enable more reliable analysis.

by a conventional mass spectrometer, the Chromaster 5610 MS Detector can operate on a 100-V supply.

(3) Easier maintenance

Whereas maintenance work cannot typically be performed on a mass spectrometer without releasing the vacuum, the Chromaster 5610 MS Detector uses an atmospheric-pressure ion filter (AIF) as the ion optics filter in its ion injector. Accordingly, routine maintenance involves simply removing, cleaning, and reinserting this filter, without any need to release the vacuum (see Fig. 2). As the ion trajectory makes a right-angle turn inside the AIF, this serves to minimize neutral molecules and contaminating ions from the ion injector. Next, an axial shift chamber at the entrance to the downstream vacuum stage further reduces any remaining contaminating ions, preventing soiling of the electrodes in the interior of the vacuum region.

The aim with the Chromaster 5610 MS Detector is to open up new markets beyond those for large mass spectrometers, with the small size and elimination of installation restrictions making it easier to install the instrument at HPLC laboratories that otherwise lack the right conditions for installing an a mass spectrometer, and by reducing user workload by making maintenance easier.



AIF: atmospheric-pressure ion filter

Fig. 2—Removal of AIF.
Routine maintenance involves merely removing, cleaning, and reinserting the AIF (center).

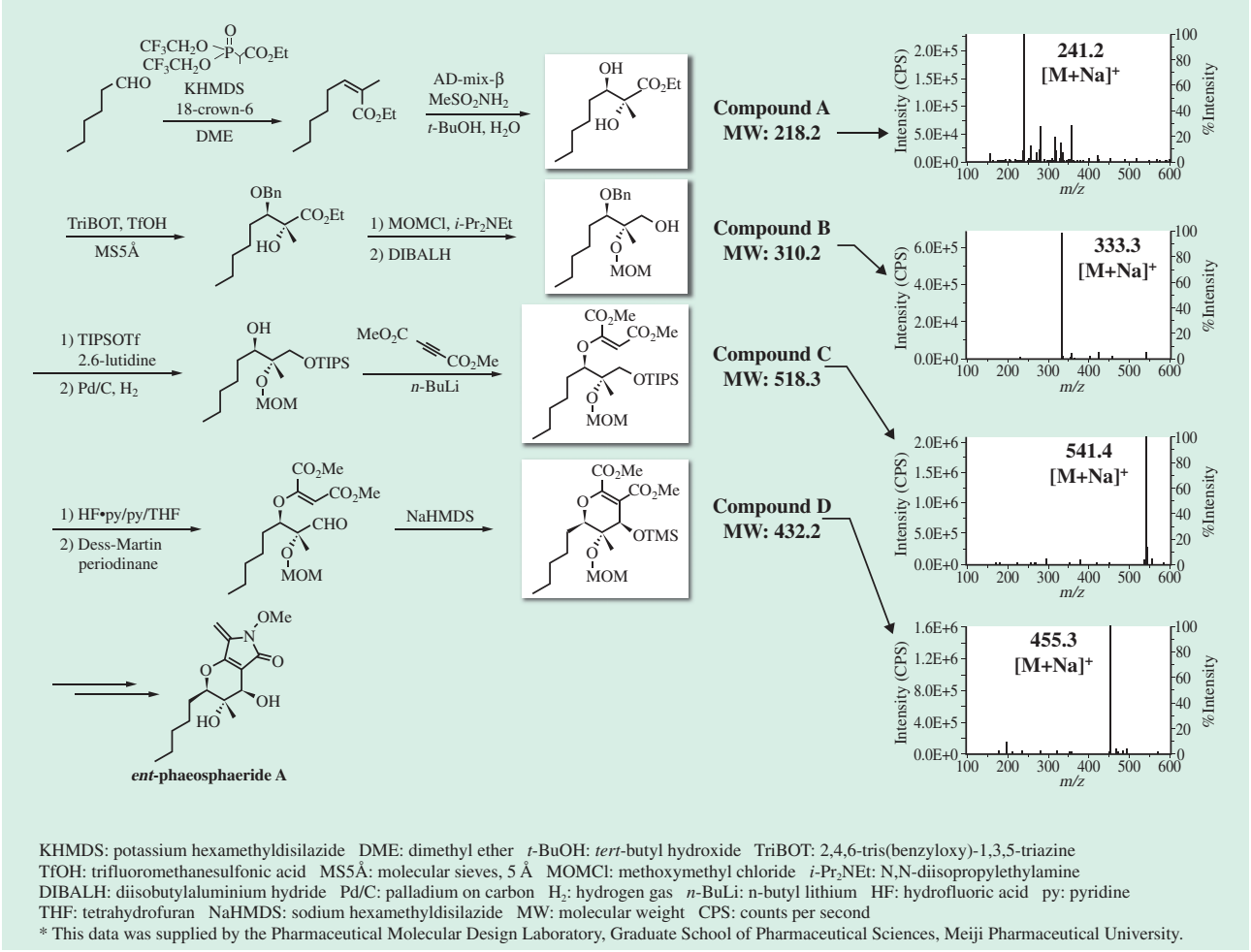
APPLICATIONS IN DRUG DISCOVERY RESEARCH

This section describes the three different configurations in which the Chromaster 5610 MS Detector can be used: as a conventional standalone mass detector used for mass spectrometry (using the direct infusion method), for LC/MS, and for the recently developed thin-layer chromatography-mass spectrometry (TLC-MS) method. It also presents examples from the drug discovery research sector of each of these uses.

Determining Mass Information for Intermediate Products of Natural Product Synthesis[1]

In the total synthesis of natural compounds selected as drug discovery targets, it is necessary to confirm that the desired compounds are being produced at each step of the reaction. The analysis of intermediate reaction products is particularly important in the case of natural compounds with complex structures for which synthesis is also complex. In this case, the Chromaster 5610 MS Detector was used to perform mass spectrometry on four intermediate compounds produced during the total synthesis of phaeosphaeride A, a naturally-occurring compound with anticarcinogenic properties (see Fig. 3).

Table 1 lists the mass spectrometry conditions. The compounds A to D shown in Fig. 3 were each diluted to 10 ppm with methanol and analyzed by the Chromaster 5610 MS Detector using the direct infusion method. In each case, the results indicated the



KHMDS: potassium hexamethyldisilazide DME: dimethyl ether *t*-BuOH: *tert*-butyl hydroxide TriBOT: 2,4,6-tris(benzyloxy)-1,3,5-triazine
TfOH: trifluoromethanesulfonic acid MS5Å: molecular sieves, 5 Å MOMCl: methoxymethyl chloride *i*-Pr_2NEt: N,N-diisopropylethylamine
DIBALH: diisobutylaluminium hydride Pd/C: palladium on carbon H_2: hydrogen gas *n*-BuLi: n-butyl lithium HF: hydrofluoric acid py: pyridine
THF: tetrahydrofuran NaHMDS: sodium hexamethyldisilazide MW: molecular weight CPS: counts per second
* This data was supplied by the Pharmaceutical Molecular Design Laboratory, Graduate School of Pharmaceutical Sciences, Meiji Pharmaceutical University.

Fig. 3—Scheme for Total Synthesis of ent-phaeosphaeride A (left) and Mass Spectra of Intermediate Products A to D (right).
The synthesis of natural compounds involves a large number of reaction steps. Whether or not each step of the synthesis reaction is proceeding correctly can be determined by using a mass detector to analyze the composition of intermediate products.

presence of sodium adduct ions ([M+Na]$^+$). As direct infusion is a very simple technique, involving the injection of the sample using a syringe, it can obtain mass information about the compounds quickly.

TABLE 1. Conditions for Analyzing Intermediate Products of *ent*-phaeosphaeride A Synthesis (Direct Infusion Method)
The table lists the conditions for analyzing the intermediate products of ent-phaeosphaeride A synthesis (by direct infusion).

Ionization method	ESI
Polarity	Positive
Ionization voltage	2,200 V
Measurement mode	Scan
Gas flow rate	0.5 L/min
IS/AIF temperature	70°C/120°C
Syringe pump flow rate	2 µL/min

ESI: electrospray ionization IS: ion source

LC/MS Analysis of Microbial Culture Fluid

HPLC separates the constituents of a sample by utilizing the differences in the affinities (holding force) of their stationary and mobile phases, and detects these using the appropriate detector for the properties of each constituent. While ultraviolet (UV) detectors are widely used, qualitative analysis of the material in this example is performed mainly by holding time, with quantitative analysis being performed by peak intensity and area. Although a diode array detector (DAD) works on the same principle as a UV detector, it can collect three-dimensional chromatogram data due to its ability to obtain spectrum information as well as time axis and peak intensity.

A mass detector can obtain this three-dimensional chromatogram data, made up of time axis, peak intensity, and mass spectrum, through its use in tandem with HPLC (see Fig. 4).

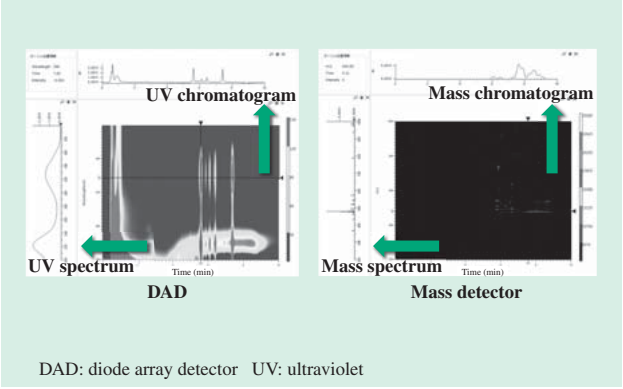


Fig. 4—Contour Maps Showing DAD and Mass Spectrometry Data from Analysis of Microbial Culture Fluid.
Presenting the data as contour lines enables the three-dimensional display of time axis, peak intensity, and mass information, providing a comprehensive overview of the results. This function, which is used routinely for DAD data, is also available for mass spectrometry data on the Chromaster 5610.

Using a DAD only, it is not possible to perform an analysis of compounds without UV absorption in the case of samples that contain a large number of different components, such as a mixture of different compounds in solution. It can also be more difficult to identify the composition because the DAD output is easily influenced by the mobile phase or differences in the concentration of the compounds in solution. When using a mass detector, on the other hand, there are also cases when it is difficult to detect components that are not easily ionized and to identify compounds that have the same mass. The following describes an example analysis that uses two detectors with different characteristics to overcome these problems.

The screening of biologically active compounds found naturally in sources such as microorganisms and plants is a traditional method for discovering lead compounds with the potential to become targets for drug discovery. Even now, however, when high-throughput screening is becoming a mainstream technique, its importance is being reappraised[(2)], with high-throughput screening meaning the artificial synthesis of large numbers of different types of new compounds and the isolation of their active components. The example described here involves screening microbial culture fluid for valuable compounds using an LC/MS system that combines a Chromaster 5610 MS Detector and a DAD. Tables 2 and 3 list the analysis conditions.

As shown in Fig. 4, the mass spectrometry data from the Chromaster 5610 is presented using contour lines in the same way as the DAD measurements. While the resulting data is very complicated in the case of samples, such as microbial culture fluid, that contain large numbers of compounds, presenting it in three-dimensional form helps visualize the change in each measurement.

Fig. 5 shows the UV spectra and mass spectra for peaks (1) to (3) detected at a UV wavelength of 324 nm. Whereas the UV spectra show three similar patterns, the mass spectra are completely different, indicating that the three peaks represent different compounds. While the components in this case are suited to mass spectrometry, UV analysis works better for compounds that are not easily ionized. In this way, samples containing a large number of different components can be analyzed by making complementary use of the UV and mass spectrum data.

Mass Spectrometry Using TLC-MS Interface

TLC is a type of chromatography that works by placing spots of a mixture on a glass or other plate coated with a thin film of an adsorbent such as silica gel or alumina and then allowing a solvent to act on it. It is widely used in synthetic research and other research as a simple, low-cost method for separating and analyzing the components of a mixture.

The use of mass spectrometry to identify the components of a sample separated by TLC used to take a lot of effort, involving (1) scraping the separated

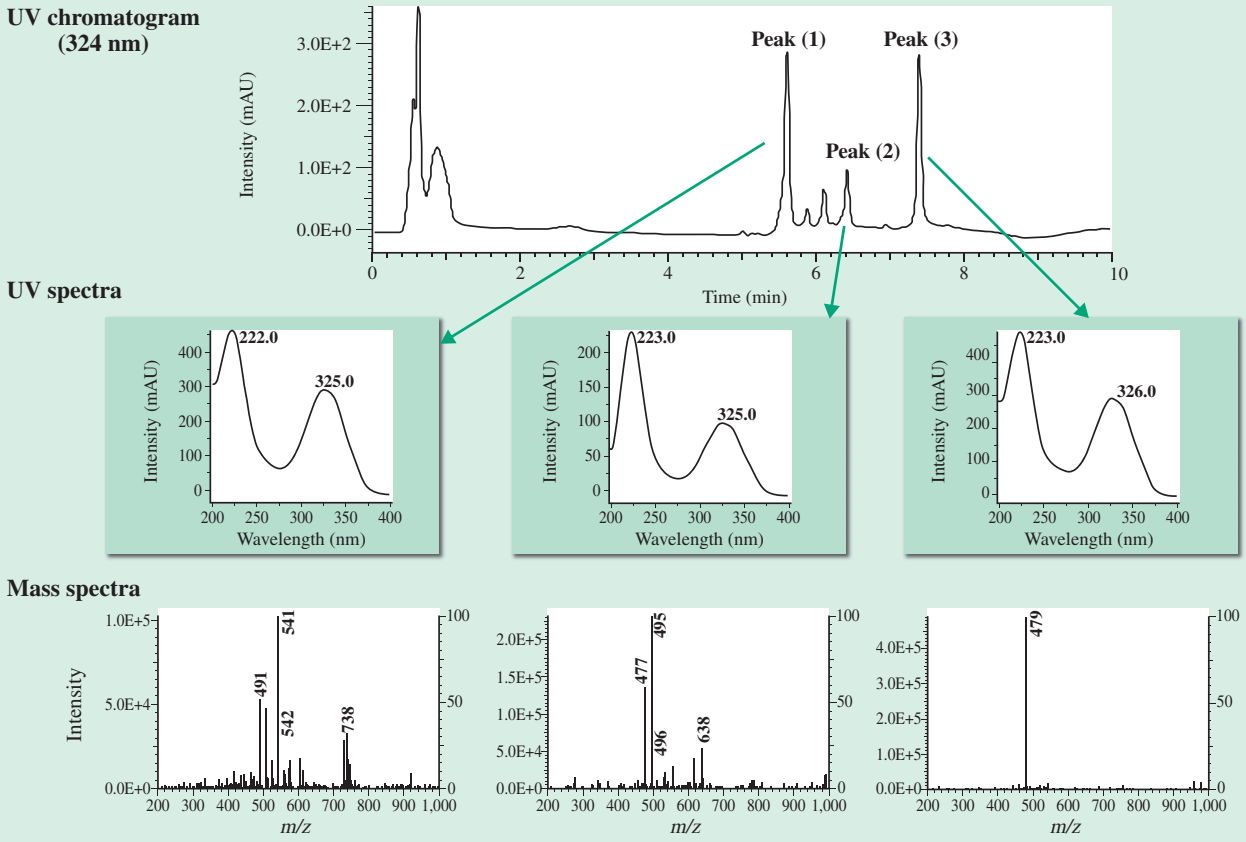
TABLE 2. Experimental Conditions of MS Detector for Analysis of Microbial Culture Fluid (LC/MS Analysis)
The table lists the experimental conditions of an MS detector for an analysis of microbial culture fluid (LC/MS analysis).

Ionization method	ESI
Polarity	Positive
Ionization voltage	2,700 V
Measurement mode	Scan: (*m/z* 200-400)

TABLE 3. Experimental Conditions of HPLC for Analysis of Microbial Culture Fluid (LC/MS Analysis)
The table lists the experimental conditions of HPLC for an analysis of microbial culture fluid (LC/MS analysis).

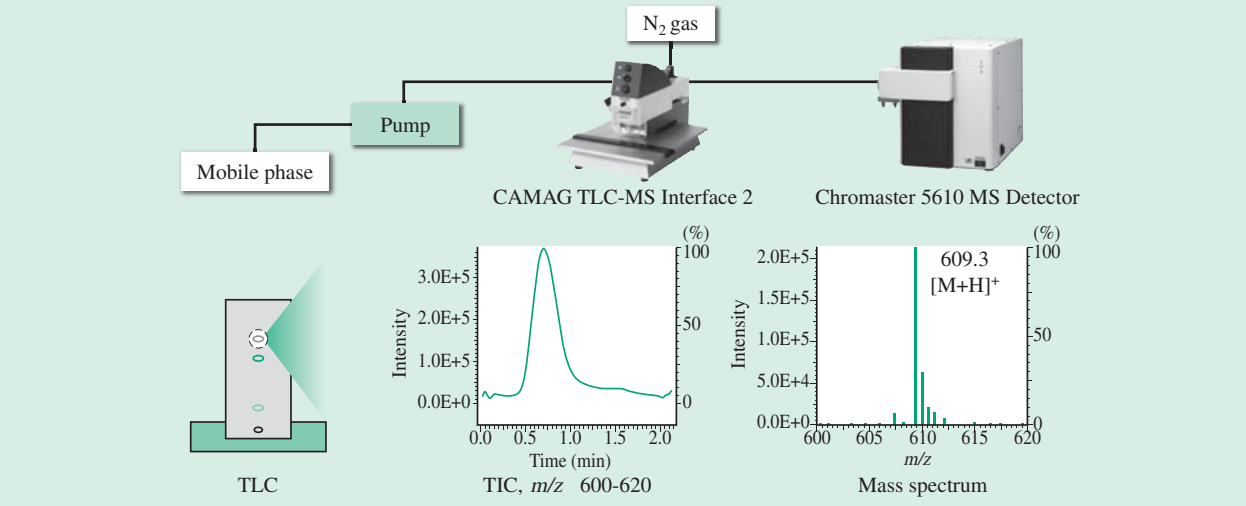
Column	LaChromUltra II (1.9 µm) 2.0 mm I.D.×50 mm
Mobile phase	A: 0.1% HCOOH in H_2O (v/v) B: 0.1% HCOOH in CH_3CN (v/v) %B=20 (0-0.5 min)-100 (3-5 min)-20 (5.1-10 min)
Flow rate	0.2 mL/min (split ratio: 1:50)
Injection amount	20 µL

I.D.: internal diameter v/v: volume per volume



mAU: absorbance unit
* This data was obtained with the cooperation of the Laboratory of Microbial Chemistry, Graduate School of Pharmaceutical Sciences, Kitasato University.

Fig. 5—Identification of Composition of Peaks Separated from Microbial Culture Fluid.
Identification can be difficult, as in this example, because the UV spectrum is easily influenced by the mobile phase and the compounds in the solution. In such cases, the reliability of identification can be improved by also obtaining the mass spectrum to clarify the differences between components.



N_2: nitrogen TLC: thin-layer chromatography TIC: total ion chromatogram

Fig. 6—System Configuration of Online TLC-MS System.
Mass information can be obtained in only one or two minutes by extracting the material directly from the spots on the TLC plate and performing online injection into the mass detector.

TABLE 4. Experimental Conditions of MS Detector for TLC-MS Analysis
The table lists the experimental conditions of an MS detector for TLC-MS analysis.

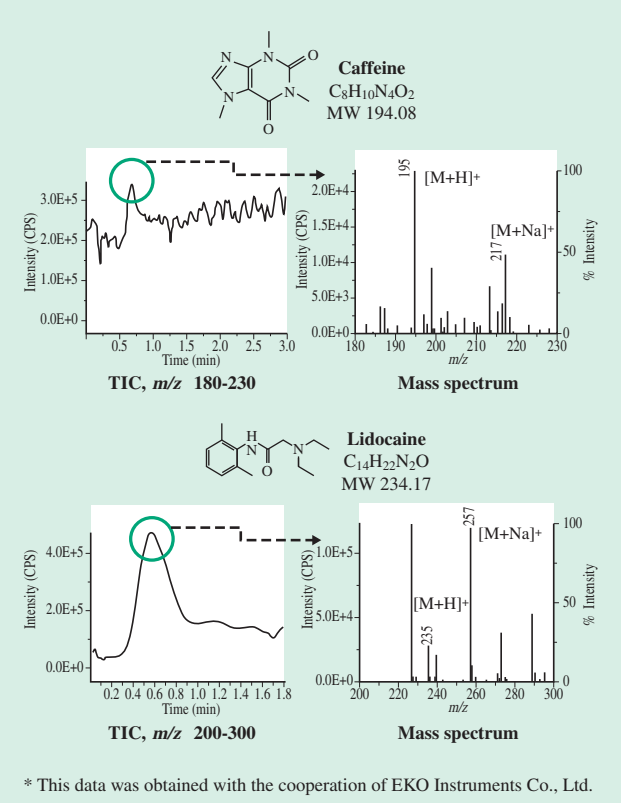
Ionization method	ESI
Polarity	Positive
Ionization voltage	2,600 V
Measurement mode	Scan

TABLE 5. Pump Settings for TLC-MS Analysis
The table lists the pump settings for TLC-MS analysis.

Mobile phase	Methanol
Flow rate	0.1 mL/min (split ratio: 1:50)

spots, (2) extracting them, and (3) analyzing them in a mass spectrometer. Recent years, however, have seen the availability of techniques for extracting the spots directly from the plate followed by online injection into the mass spectrometer (as shown Fig. 6), which have significantly simplified composition analysis. In this example, online TLC-MS analysis is performed by a CAMAG TLC-MS Interface 2 (CAMAG) and Chromaster 5610 MS Detector. Tables 4 and 5 list the analysis conditions.

Fig. 7 shows mass spectrometry data for caffeine and lidocaine obtained by TLC. Here, 2 µL each of caffeine and lidocaine in methanol solution (equivalent to 200 ng) was separated on a TLC plate and the components were extracted using a solvent (methanol) delivered via a pump by applying an extraction piston to the spots. The extracted fluid was transferred immediately to the Chromaster 5610 MS Detector and the mass data shown in Fig. 7 was obtained in one to two minutes. In this way, components separated using TLC can be identified quickly.



* This data was obtained with the cooperation of EKO Instruments Co., Ltd.

Fig. 7—Analyses of Caffeine (top) and Lidocaine (bottom) Using TLC-MS System.
Caffeine and lidocaine equivalent to 200 ng can be detected by extracting the material directly from the spots on the TLC plate.

CONCLUSIONS

This article has described three example applications for the compact Chromaster 5610 MS Detector: the analysis of the intermediate products of synthesis using the direct infusion method, the screening of microbial culture fluid using LC/MS, and the analysis of a mixture using TLC-MS.

There remains considerable unmet demand for this type of laboratory-level analysis using a simple mass detector and Hitachi High-Tech Science Corporation intends to continue supplying solutions to satisfy customer needs.

ACKNOWLEDGMENTS

Research data from the Pharmaceutical Molecular Design Laboratory of the Graduate School of Pharmaceutical Sciences at Meiji Pharmaceutical University and the Laboratory of Microbial Chemistry of the Graduate School of Pharmaceutical Sciences, Kitasato University School of Pharmacy was used in preparing this article. The authors would like to take this opportunity to express their deep gratitude.

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