Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

Chapter 15

State of the Art Mass Spectrometric and Chromatographic Techniques for Drug Analysis

Katerina Mastovska

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038

In recent years, mass spectrometric (MS) and chromatographic instrumentation and techniques have scored dramatic developments, resulting in the introduction of many useful tools for analysis of both small and large drug molecules. This chapter describes state of the art MS and chromatographic techniques that can be used in the analysis of drugs in various applications, including characterization of controlled-release drug delivery systems. In applications that require minimum sample preparation, direct MS detection of drug molecules can be performed using novel atmospheric pressure ionization techniques, such as desorption electrospray ionization or direct analysis in real time. Visual information about distribution of drugs in various materials or tissues can be obtained through imaging MS, mainly using secondary ion MS or matrix assisted laser desorption/ionization. In liquid and gas chromatography combined with MS (LC- and GC-MS), drug analysis can be speeded up using various fast chromatographic techniques that are becoming practical due to the introduction of modern LC, GC, and MS instruments.

U.S. government work. Published 2008 American Chemical Society.

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

Mass spectrometry (MS) has become the analytical technique of choice in modern laboratories performing drug analysis. In complex samples, such as biological fluids or tissues, the combination of MS with a chromatographic separation provides enhanced selectivity, which is invaluable for detection of trace level components. In recent years, MS and chromatographic instrumentation and techniques have scored dramatic developments, resulting in the introduction of many useful tools for analysis of both small and large drug molecules. This chapter describes state of the art MS and chromatographic techniques that can be used in the analysis of drugs in various applications, including characterization of controlled-release drug delivery systems. The chapter discusses mainly novel approaches that require minimum sample preparation, provide high speed/high throughput analysis, and/or improve the qualitative or quantitative aspects of the analytical process.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

Mass Spectrometry without Chromatographic Separation

The basic MS process involves ionization of sample molecules, followed by separation of ionized molecules and their fragments based on their mass-to-charge ratio (*m/z*) and detection (counting) of the ions, which results in a mass spectrum (a snapshot of ion intensities plotted against their *m/z*) showing the mass distribution of the ions produced from the sample. MS offers both qualitative and quantitative information; it can detect compounds, elucidate their structures, and determine their concentrations.

Direct MS measurements enable rapid analysis, especially when the analytes can be sampled directly on the sample surface at atmospheric pressure, which can be done using some recently introduced ionization techniques, such as desorption electrospray ionization (DESI) or direct analysis in real time (DART). To compensate for the lack of compound separation prior to the MS step in complex samples, MS without a chromatographic separation usually employs a highly selective MS technique, such as high-resolution, accurate mass, and/or mass-selective fragmentation (multi-stage MS^n; usually a tandem MS, MS/MS) measurements. The selectivity of MS analysis can be also greatly enhanced by using a new technology called high-field asymmetric waveform ion mobility spectrometry (FAIMS) that can separate ions prior to their introduction into the vacuum chamber of an MS instrument.

Recent developments in ionization techniques, mass analyzers, and also data processing technology have opened doors to applications that can advance a wide array of scientific disciplines. For instance, imaging MS can provide visual information about distribution of compounds in various materials, such as drugs in tissues or delivery systems, which is invaluable to scientists performing medical, material and/or drug development research.

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

Novel Ionization Techniques for Direct Sampling of Drugs on Surfaces

In the history of MS development, the most significant breakthrough in drug analysis can be probably attributed to the introduction of two ionization techniques in the late 1980s: (i) matrix assisted laser desorption ionization (MALDI), which is applicable to the analysis of solid materials *(1)* and (ii) electrospray ionization (ESI), which is used for the analysis of solutions *(2)*. This breakthrough was well recognized in 2002, when John B. Fenn and Koichi Tanaka received the Nobel Prize in chemistry for their work on ESI and MALDI, respectively. The introduction of ESI started the development of modern liquid chromatography-mass spectrometry (LC-MS) instrumentation, which revolutionized the analysis of polar compounds and became an indispensable tool in the analysis of small and large drug molecules.

MALDI belongs to a family of desorption ionization techniques, which desorb and ionize molecules from a surface of a condensed-phase sample by impacting it with projectiles (see Figure 1), such as photons in laser desorption (including MALDI), translationally excited atoms (in fast atom bombardment, FAB), or energetic ions (in secondary ion mass spectrometry, SIMS). MALDI requires the sample to be mixed with an excessive amount of a UV-absorbing matrix compound, which is ionized by the laser and then ionizes sample molecules by proton transfer.

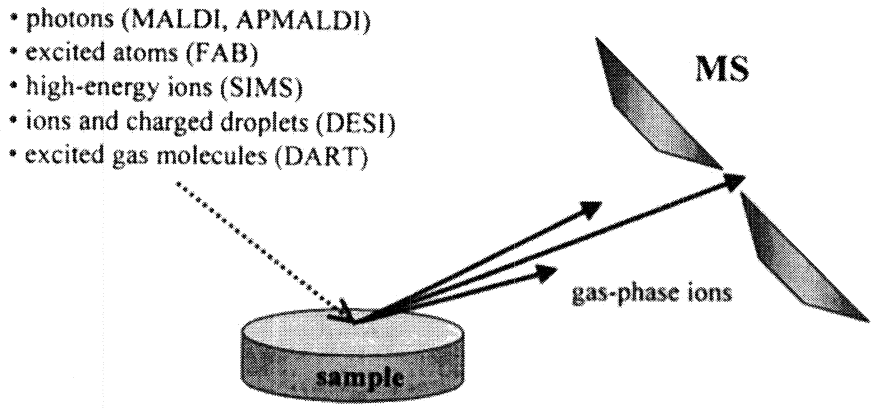

Figure 1. Illustration of the process involved in MS ionization methods for direct sampling on surfaces of condensed-phase samples in vacuum (MALDI, FAB, SIMS) or at atmospheric pressure (APMALDI, DESI, DART). See the text for the explanation of the acronyms.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

The desorption/ionization process in MALDI, FAB, or SIMS takes place in vacuum, thus the sample is not accessible during the analysis. Certain applications, especially those in the bioanalytical field, would benefit from direct sampling at ambient conditions, which would allow full access to the sample for observation and additional physical processing during the analysis.

Atmospheric pressure MALDI (APMALDI), first described in 2000 *(3, 4)*, was the first ionization technique in which a condensed-phase sample could be examined at atmospheric pressure. However, APMALDI still requires sample dilution/coating with a UV-absorbing matrix compound. Furthermore, the source must be enclosed to protect the operator from potential exposure to laser radiation. Recently introduced ionization methods DESI *(5)* and DART *(6)* (reported in 2004 and 2005, respectively) do not pose these limitations. They require essentially no sample preparation and allow full access to the sample while mass spectra are being recorded.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

Desorption Electrospray Ionization (DESI)

DESI is a soft ionization technique that uses gas-phase solvent ions and charged microdroplets of electrosprayed solvent interacting with a condensed phase sample to yield gas-phase ions under ambient conditions *(5, 7)*. Several possible ionization mechanisms have been suggested, including chemical sputtering involving gas-phase ions generated by electrospray ionization (ESI) and subsequent charge transfer between these primary ions and sample molecules on the surface. Also, multiply charged solvent droplets probably impact the surface and dissolve sample molecules, resulting in the formation of charged droplets carrying the sample molecules (ESI-like droplet pick-up mechanism), which then leads to formation of gas-phase sample ions through mechanisms similar to normal ESI.

DESI enables rapid, high-throughput detection of analytes present under ambient conditions on a variety of surfaces positioned between the solvent spray and the MS source. DESI is applicable to small organic molecules as well as to proteins and other biological macromolecules. DESI applications described for drug analysis include direct detection of various drugs in tablets *(8-12)*, ointments applied to the surface of a special holder or a cardboard piece *(8-10)*, liquid pharmaceutical preparations *(10)*, gel formulations applied to the surface of human skin *(10)*, biological fluids (urine, blood, or plasma) absorbed onto a filter paper or dried on other appropriate surface *(7, 9, 10)*, or pharmaceutical samples (components of an over-the-counter pain medication) separated on thin-layer chromatography plates *(13)*.

Desorption atmospheric pressure chemical ionization (DAPCI) *(14)* was developed as an alternative approach for compounds with a lower ionization

efficiency in DESI, such as for weakly polar corticosteroids *(9, 10)*. DAPCI employs gaseous ions of volatile compounds (such as toluene or methanol) produced by corona-discharge ionization of vapors carried by a high-velocity nitrogen jet *(14)*. A similar technique, called atmospheric-pressure solids analysis probe (ASAP), was recently reported for a rapid analysis of volatile and semi-volatile liquid or solid materials *(15)*. In ASAP, the sample is vaporized in the hot nitrogen gas stream followed by ionization of the vapors by corona discharge.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

Direct Analysis in Real Time (DART)

DART is a new ion source for rapid, non-contact analysis at atmospheric pressure. In DART, the sample surface is exposed to a stream of excited gas, such as metastable helium atoms or nitrogen molecules, which are produced in a discharge chamber of the DART source *(6)*. Several ionization mechanisms have been postulated, including Penning ionization by energy transfer from an excited atom or molecule of energy greater than the ionization energy of the sample molecules. When helium is used, the excited helium neutrals primarily react with atmospheric water to produce hydronium ions, which transfer protons to the sample molecules. As opposed to DESI, DART does not use any solvent, but its applicability is probably limited to smaller organic molecules.

DART has been tested in a wide variety of applications *(6, 10, 16)*, including drug analysis in tablets, capsules, ointments, liquid pharmaceutical preparations, biological fluids (urine, blood, and saliva) or, in the case of illicit drugs, on currency, clothes, glassware or in alcoholic beverages. In the case of fluids, the sample was absorbed onto a filter paper *(10)* or a glass rod was simply dipped into the sample *(6)* and placed in front of DART ionizing beam (the latter procedure has already been automated).

Imaging Mass Spectrometry

Imaging MS produces molecular images of samples through ionization from a clearly identified point on a flat sample and performing a raster of the sample by moving the point of ionization over the sample surface. The collected data (positional data and *m/z* intensities) are converted into images that show distribution of targeted compounds (based on their specific *m/z*) in tissues or in various other materials.

Imaging MS requires direct sampling of the compounds from precisely defined points on the sample surface with an adequate spatial resolution. Spatial resolution refers to the smallest distance between two points that can be clearly

distinguished. The most important parameter determining spatial resolution is the size of the focused spot from which the ions are emitted *(17)*.

Two desorption/ionization methods have been mostly employed for MS imaging: (i) SIMS for small (<1000 Da) molecules and (ii) MALDI for larger (<100 000 Da) molecules *(17)*. DESI also has already been tested for this kind of application *(7, 18)*, achieving a spot size of 50 μm. In SIMS, the sample surface is bombarded by a high-energy ion (*e.g.* Ar^+, Cs^+, or Ga^+) beam, which can be focused to spot sizes as small as 10 nm in diameter, although spot sizes of 0.1-30 μm are usually used *(17, 19)*. In MALDI, laser beams can be focused to about 1 μm spots, but much larger spot sizes (5-100 μm in diameter) are more common in practice *(17)*.

A time-of-flight (TOF) mass analyzer is typically used in MS imaging applications both in combination with SIMS or MALDI. TOF is a non-scanning instrument, which provides full spectrum data at fast acquisition rates and often high-resolution and high-accuracy MS measurements. A tandem MS instrument, such as an ion trap or triple quadrupole, also offers enhanced selectivity (as compared to a single-stage MS instruments) and may serve as a lower-cost alternative to a high-resolution TOF.

TOF-SIMS imaging has been employed for the mapping of sample surfaces in a variety of applications *(19)*, including the characterization of drug delivery systems *(20)*. The entire cross-section of a pellet, bead, or capsule containing an active drug encapsulated in a multi-layer coating can be studied to provide information about the drug distribution and the chemical composition and morphology of the coating layers *(21)*. Similarly, TOF-SIMS can be used for imaging of active molecules stamped onto polymer surfaces *(22, 23)*. This kind of characterization can support development of effective drug delivery systems, especially in the case of controlled-release systems, as well as serve for defending against patent infringement and counterfeiting.

MALDI-TOF is particularly useful for imaging of larger molecules, such as peptides and proteins, but smaller molecules can be also analyzed by this technique. Drug researchers are mainly interested in distribution of investigated drugs in target tissues and in tissue response to the presence of the drug, which can be characterized by the production of a specific peptide or protein.

Figure 2 illustrates the basic process involved in an imaging MALDI-TOF MS analysis of biological tissues *(24)*. The sample surface (in this case a rat brain section) is coated with a suitable matrix compound (*e.g.* sinapic acid) that assists in ionization of sample molecules desorbed by a laser. The figure shows an MS spectrum obtained from one, precisely located spot on the sample surface. The peaks in the spectrum represent various compounds and their relative concentrations. Combining these MS data from all the spots, molecular images of different compounds (characterized by a specific *m/z*) can be obtained as demonstrated for the three selected peaks.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

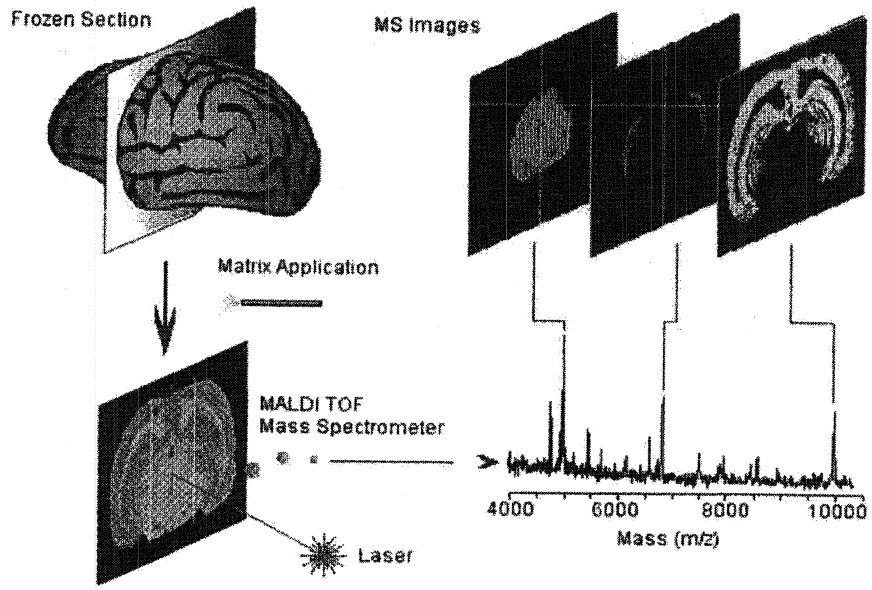

Figure 2. Schematics of the imaging MS process using MALDI-TOF MS (Reproduced with permission from reference 24. Copyright 2001 Nature Publishing Group.)

Mass Spectrometry Combined with High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)

FAIMS is a relatively new technology for separation of gas-phase ions at atmospheric pressure *(25, 26)*. In FAIMS, a mixture of ions is introduced between two metal plates, to which an appropriate voltage is applied, causing some ion types to drift and hit the metal plates while other types of ions remain between the plates and can reach MS for further separation and detection. The ion drift towards a plate is caused by the difference in ion mobility in strong and weak electric fields, which are applied using a high frequency asymmetric waveform characterized by a significant difference in voltage in the positive and negative polarities of the waveform.

For example, this waveform can be a square wave, in which a high positive voltage is applied for a short time and a low negative voltage is applied for a longer time as shown in Figure 3. The strong field is provided by the application of the peak voltage of the waveform called the dispersion voltage (DV). The weak field of opposite polarity is applied for a correspondingly longer time.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

When the ion is driven by a strong electric field, the collision of the ion with a bath gas (*e.g.* nitrogen) is more energetic than in the case of a stationary ion. This may increase or decrease ion mobility relative to the mobility of a weak field. The change in mobility is both ion and bath gas dependent. Figure 3 illustrates the motion of an ion with a higher mobility in the strong field.

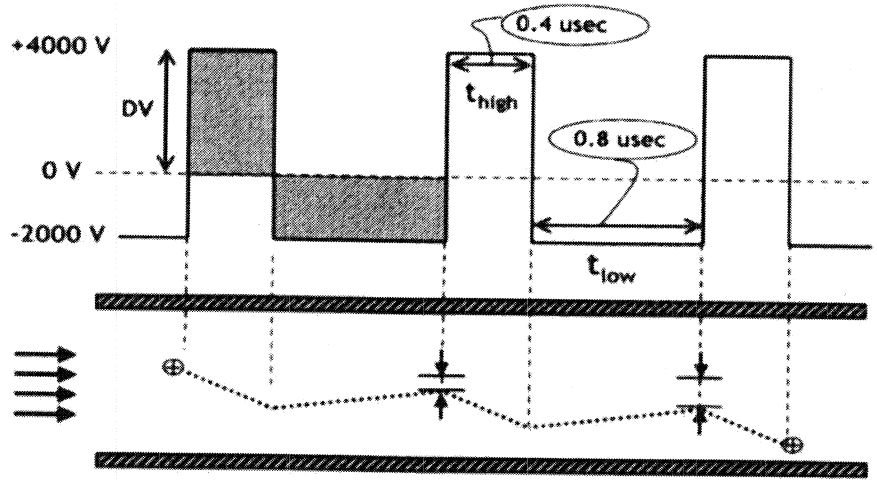

Figure 3. Illustration of the ion motion between FAIMS electrodes for an ion with increasing mobility in strong electric fields. (Reproduced with permission from reference 26. Copyright 2004 Elsevier.)

To stop the ion drift, a small dc voltage of appropriate magnitude and polarity can be applied to either of the plates. This voltage, called the compensation voltage (CV), enables transmission of ions through the FAIMS device, for which the CV is the right voltage to balance the drift caused by the application of the asymmetric waveform of a given DV.

As a result, FAIMS can greatly enhance selectivity by separating analyte ions from chemical background noise using CVs characteristic for given analytes. Also, cylindrical geometry FAIMS can focus and trap ions to provide an increase in sensitivity. These two factors can greatly improve analyte detection in complex matrices by direct MS analysis (or in combination with LC-MS), which was for example demonstrated in the analysis of various drugs in urine *(27)*.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

Liquid Chromatography-Mass Spectrometry

In recent years, LC-MS has become a widely used, reliable technique that has been utilized in numerous applications *(28)*. Most modern, commercially available LC-MS instruments employ atmospheric pressure ionization (API), mainly ESI or atmospheric pressure chemical ionization (APCI).

ESI enables analysis of polar compounds, while APCI serves as an alternative for less polar, more volatile molecules that are not as sensitive with ESI. As opposed to APCI, ESI can produce multiply charged ions, thus reduce *m/z* ratio for large molecules and enable their analysis using conventional MS instruments. Recently introduced atmospheric pressure photoionization (APPI) provides an option for even less polar, weakly or non-ionized compounds, such as steroids *(29)*.

API techniques are soft ionization methods, producing predominantly protonated or deprotonated molecular ions (without or with adducts), which provide limited information about the analyte structure. This disadvantage is in practice usually overcome by using MS^n (mostly MS/MS) or high-resolution, accurate-mass MS that improve detection selectivity and enable further characterization for identification/confirmation purposes. A wide range of tandem MS instruments became available for LC-MS in the past years, including triple quadrupoles (usually the best choice for quantitative analysis), traditional and linear ion traps, or combinations of a quadrupole with a TOF MS. Also, several vendors offer high-resolution, accurate-mass benchtop TOF MS instruments.

The capability of tandem MS or accurate mass measurements to identify a truly unknown compound are, however, far inferior to those of a GC-MS with electron ionization (EI). EI spectra have characteristic fragmentation patterns, which can be interpreted to elucidate compound structures or searched against a database (MS library). Although some MS/MS or accurate-mass libraries exist for LC-API-MS, they can hardly be compared to extensive, (practically) instrument-independent EI spectra libraries.

Particle beam LC-MS can provide EI spectra but this technique became almost forgotten because of its rather low sensitivity and limited applicability compared to LC-MS with ESI or APCI. Recently reported LC-MS with EI of cold molecules in supersonic molecular beams (SMB) has a potential to provide an invaluable identification tool for a wide range of small organic molecules *(30)*, but this technique has not been commercialized yet. SMB-MS has been successfully combined with GC to offer many unique features as discussed in the section on GC-MS.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

The most prominent trend, observed basically in any analytical field, is the effort to speed the analysis and provide high throughput measurements, which are very useful in pharmaceutical applications (*e.g.* in drug discovery, development, or quality control). In fast LC-MS, the LC part dictates the speed, while the MS must keep up with it, which mainly means that the MS instrument should provide fast data acquisition rates to detect rapidly eluting peaks from the LC column.

In addition to employing shorter and narrower (packed or capillary) columns, two main fast LC approaches are currently investigated and start being applied in practice: the use of columns with small particle sizes (< 2 μm) and column operation at elevated temperatures.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

Fast Liquid Chromatography using Small Particle Sizes

According to the chromatographic theory, as the particle size decreases, the separation efficiency increases because the number of theoretical plates is inversely proportional to the particle size, and so is the optimum linear velocity of the mobile phase. Thus, columns with smaller particle sizes can provide an increase in speed due to higher optimum flow rates. Although this theory has been well known for decades, the practical application of this approach has been difficult for several reasons.

For one, the operation of small particle size columns at high flow rates creates high back pressures (often exceeding 10,000 psi), requiring special instrumentation that enables injection of samples and reproducible mobile phase pumping at these pressures. Moreover, the entire LC system must have very low extra-column volumes to preserve the separation efficiency. Another challenge involves the design and development of < 2 μm particles and their packing into reproducible and rugged columns. Also, the samples must be well-filtered to prevent clogging of the frits holding the particles in the column.

Recently, two manufacturers introduced columns with smaller particles (1.7 and 1.8 μm) together with compatible LC-MS systems, providing adequate pressure limits, injection systems, low extra-column volumes, as well as fast MS detection for narrow peaks. One manufacturer termed the technology as ultra performance liquid chromatography (UPLC) *(31)*, whereas the other describes the columns as suitable for rapid resolution LC.

The UPLC term and technology is spreading throughout the scientific community, which can be demonstrated by a number of papers evaluating this approach in various applications, including metabolite profiling in urine or serum *(32-35)*, drug development studies *(36)*, forensic drug analysis *(37)* or multiresidue analysis of veterinary drugs in milk and eggs *(38)*.

High-Temperature Liquid Chromatography

The application of elevated temperatures in high-temperature LC (HTLC) decreases viscosity of the mobile phase and increases analyte diffusivity, which leads to lower back pressures and increased mass transfer, respectively *(39)*. As a result, LC columns can be operated at higher flow rates, reducing the analysis time without making significant sacrifices in the separation efficiency. Moreover, elevated temperatures can be employed in the combination with small particle size columns, thus providing an additional gain in speed or separation efficiency, while keeping the backpressure within reasonable limits. Also, temperature programming of the column can change separation selectivity, which may improve analyte separation or serve as an alternative to solvent gradient.

Another attractive aspect involves the use of superheated water (at 100-200°C) as a potential replacement for medium-polarity mobile phases (such as acetonitrile-water mixtures) in reversed-phase LC *(40, 41)*. This would reduce solvent and waste costs and simplify system operation. Under high-temperature conditions, water behaves as a moderate polarity solvent because its dielectric constant decreases from about 80 to about 35 over the range of 25 to 200°C *(42)*, while it retains an appreciable density (> 0.85 g/mL), cohesive energy, and hydrogen bonding potential *(43)*.

Until recently, the application of HTLC was rather difficult in practice due to instrument and column limitations. Instrumentation for HTLC is now available, which allows operation at temperatures up to 200°C with mobile phase preheating to eliminate thermal mismatch *(44)*. The thermal mismatch occurs when the cool mobile phase enters the heated column and warms up faster along the walls, leading to band broadening due faster flow of the mobile phase along the column wall than in the center *(45)*.

Another practical concern involves the stability of the stationary phase at the elevated temperatures. Conventional, reversed-phase LC columns with silica-based stationary phases are stable at temperatures up to 90°C (50-60°C limit is more common). Stationary phases with higher temperature stability are based mainly on zirconium oxide, although graphitized carbon or rigid polystyrene-divinylbenzene phases can also tolerated elevated temperatures *(44)*.

With the recent advancements in column technology and instrumentation, HTLC may soon become a practical tool, especially in the combination with narrower columns and/or columns with small particle sizes and in applications that can tolerate higher temperature with respect to the analyte stability.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

Gas Chromatography-Mass Spectrometry

GC-MS is a suitable technique for thermally stable compounds that can be readily volatilized. Most drugs are rather polar molecules, thus require derivatization prior to the GC-MS analysis. The availability and affordability of LC-MS instruments have led to the replacement of GC-MS in most drug analyses in modern laboratories. However, GC-MS with derivatization may still be employed for confirmation purposes, providing orthogonal selectivity to LC-MS. Also, certain coumpounds, such as steroids, do not provide sufficient sensitivity in common LC-API-MS techniques, thus GC-MS with derivatization remains a viable approach in these cases *(46)*.

The derivatization procedure can be automated using, *e.g.* in-vial derivatization, on-fibre derivatization *(47, 48)* or derivatization performed directly in the GC inlet. The latter approach can be done using the direct sample introduction (DSI) technique *(49)*. In DSI, a liquid or solid sample is placed in a disposable microvial, to which a derivatization solution can be added *(50)*. After this step, the microvial is introduced into the injection port using a manual probe or more recently using an autosampler. In the automated version, the liquid sample and/or derivatization solutions are injected into the microvial placed in a liner *(51)*, which is then inserted into the inlet (or a thermodesorption unit attached to the inlet).

Some fast GC-MS techniques may permit analysis of less volatile and thermally stabile analytes even without derivatization *(52)*. Supersonic molecular beam GC-MS (GC-SMB-MS) is of a particular interest in this respect. In GC-SMB-MS, organic molecules pass through a small opening (about 0.1 mm i.d.) placed between the GC outlet and the MS, form an SMB by co-expansion with a carrier gas into vacuum and are vibrationally supercooled in the process *(53)*. As a result, an enhanced molecular ion occurs practically for all molecules, increasing the MS detection selectivity in EI. SMB-MS still provides typical EI fragmentation patterns, thus enabling conventional MS library searching, but with a higher confidence in correct compound identification due to the presence of a prominent molecular ion.

SMB-MS can handle very high flow rates (up to 240 ml/min with the prototype instrument), enabling very fast analyses, especially when combined with a short megabore column. In addition to an increase in speed, the fast flow rates and other unique features of GC-SMB-MS also enable analysis of thermally labile and low-volatility analytes, such as large polycyclic aromatic hydrocarbons or drug molecules (see Figure 4), thereby extending the scope of GC-MS to compounds currently done only by liquid chromatography *(54, 55)*. However, the lack of commercial availability is currently a severe limitation in the use of the GC-SMB-MS approach.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

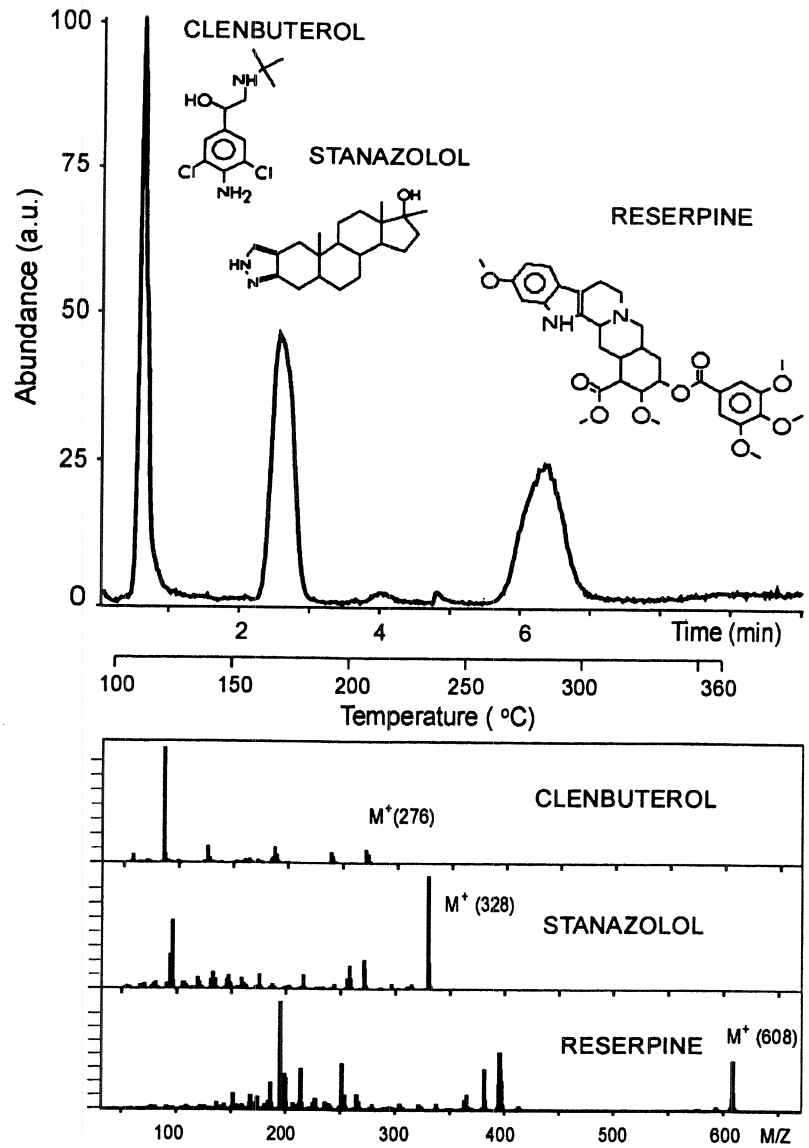

Figure 4. GC-SMB-MS analysis of thermally labile drugs without derivatization. (Reproduced with permission from reference 55. Copyright 2003 Elsevier.)

References

1. Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299-2301.
2. Fenn, J. B. Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64-71.
3. Laiko, V. V.; Baldwin, M. A.; Burlingame, A. L. *Anal. Chem.* **2000**, *72*, 652-657.
4. Laiko, V. V.; Moyer, S. C.; Cotter, R. J. *Anal. Chem.* **2000**, *72*, 5239-5243.
5. Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471-473.
6. Cody, R. B.; Laramee, J. A.; Durst, H. D. Anal. Chem. **2005**, *77*, 2297-2302.
7. Takats, Z.; Wiseman, J.M.; Cooks, R.G. *J. Mass Spectrom.* **2005**, *40*, 1261-1275.
8. Chen, H.; Talaty, N.N.; Takats, Z.; Cooks, G.R. *Anal. Chem.* **2005**, *77*, 6915-6927.
9. Williams, J.P.; Scrivens, J.H. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3643-3650.
10. Williams, J.P.; Patel V.J.; Holland, R.; Scrivens, J.H. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1447-1456.
11. Rodriguez-Cruz, S. E. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 53-60.
12. Leuthold, L. A.; Mandscheff, J. F.; Fathi, M.; Giroud, C.; Augsburger, M.; Varesio, E.; Hopfgartner, G. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 103-110.
13. Van Berkel, G. J.; Ford, M. J.; Deibel, M. A. *Anal. Chem.* **2005**, *77*, 1207-1215.
14. Takats, Z.; Cotte-Rodriguez, I.; Talaty, N. N.; Chen, H. W.; Cooks, R. G. *Chem. Commun.* **2005**, *15*, 1950-1952.
15. McEwen, C. N.; McKay, R. G.; Larsen, B. S. *Anal. Chem.* **2005**, *77*, 7826-7831.
16. http://www.jeol.com/ms_/msprods/accutof_dart.html
17. Todd P. J.; Schaaff, T. G.; Chaurand, P.; Caprioli, R. M. *J. Mass Spectrom.* **2001**, *36*, 355-369.
18. Wiseman, J. M.; Puolitaival S. M.; Takats, Z.; Cooks, R. G.; Caprioli, R. M. *Angew. Chem. Int. Ed.* **2005**, *44*, 7094-7097.
19. Belu, A. M.; Graham, D. J.; Castner, D. G. *Biomaterials* **2003**, *24*, 3635-3653.
20. Davies, M. C.; Shakesheff, K. M.; Roberts, C. J.; Tendler, S. J. B.; Bryan, S. R.; Patel, N. In *The Encyclopedia of Drug Delivery*; Mathiowitz, E., Ed.; Wiley: New York, NY, 1999; 269-277.
21. Belu, A. M.; Davies, M. C.; Newton J. M.; Patel, N. *Anal. Chem.* **2000**, *72*, 5625-5638.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

In New Delivery Systems for Controlled Drug Release from Naturally Occurring Materials; Parris, N., et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2008.

22. Yang, Z.; Belu, A. M.; Liebmann-Vinson, A.; Sugg, H.; Chilkoti, A. M. *Langmuir* **2000**, *16*, 7482-7492.
23. Hyun, J.; Zhu, Y.; Liebmann-Vinson, A.; Thomas, P.; Beebe, J. *Langmuir* **2001**, *17*, 6358-6367.
24. Stoeckli, M.; Chaurand, P.; Hallahan, D. E.; Caprioli, R. M. *Nature Medicine* **2001**, *7*, 493-496.
25. Guevremont, R.; Purves, R. W. *Rev. Sci. Instrum.* **1999**, *70*, 1370-1383.
26. Guevremont, R. *J. Chromatogr. A* **2004**, *1058*, 3-19.
27. McCooeye, M. A.; Mester, Z.; Ells, B.; Barnett, D. A.; Purves, R. W.; Guevremont, R. *Anal. Chem.* **2002**, *74*, 3071-3075.
28. Niessen, W. M. A. *J. Chromatogr. A* **2003**, *1000*, 413-436.
29. Robb, D. B.; Covey, T. R.; Bruins, A. P. *Anal. Chem.* **2000**, *72*, 3653-3659.
30. Granot, O.; Amirav, A. *Int. J. Mass Spectrom.* **2005**, *244*, 15-28.
31. Swartz, M. E. *J. Liq. Chrom.* **2005**, *28*, 1253-1263.
32. Wilson, I. D.; Nicholson, J. K.; Castro-Perez, J.; Granger, J. H.; Johnson, K. A.; Smith, B. W.; Plumb, R. S. *J. Proteome Res.* **2005**, *4*, 591-598.
33. Crockford, D. J.; Holmes, E.; Lindon, J. C.; Plumb, R. S.; Zirah, S.; Bruce, S. J.; Rainville, P.; Stumpf, C. L.; Nicholson, J. K. *Anal Chem.* **2006**, *78*, 363-371.
34. O'Connor, D.; Mortishire-Smith, R.; Morrison, D.; Davies, A.; Dominguez, M. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 851-857.
35. Nordstrom, A.; O'Maille, G.; Qin, C.; Siuzdak, G. *Anal Chem.* **2006**, *78*, 3289-3295.
36. Wren, S. A.; Tchelitcheff, P. *J. Chromatogr A.* **2006**, *1119*, 140-146.
37. Apollonio, L. G.; Pianca, D. J.; Whittall, I. R.; Maher, W. A.; Kyd, J. M. *J Chromatogr. B* **2006**, *836*, 111-115.
38. Cui, X.; Shao, B.; Zhao, R.; Yang, Y.; Hu, J.; Tu, X. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2355-2364.
39. Greibrokk, T.; Andersen, T. *J. Chromatogr. A* **2003**, *1000*, 743-755.
40. Fields, S. M.; Ye, C. Q.; Zhang, D. D.; Branch, B. R.; Zhang, X. J.; Okafo, N. *J. Chromatogr. A* **2001**, *913*, 197-204.
41. Coym, J. W.; Dorsey, J. G. *J. Chromatogr. A* **2004**, *1035*, 23-29.
42. Yang, Y.; Belghazi, M.; Lagadec, A.; Miller, D. J.; Hawthorne, S. B. *J. Chromatogr. A* **1998**, *810*, 149-159.
43. Pawlowski, T. M.; Poole, C. F. *Anal. Commun.* **1999**, *36*, 71-75.
44. Marin, S. J.; Jones, B. A.; Felix, W. D.; Clark, J. *J. Chromatogr. A* **2004**, *1030*, 255-262.
45. Mao, M.; Carr, P. *Anal. Chem.* **2001**, *73*, 4478-4485.
46. Van Thuyne, W.; Delbeke, F.T. *Biomed Chromatogr.* **2004**, *18*, 155-159.
47. Rodríguez, I.; Carpinteiro, J.; Quintana, J. B.; Carro, A. M.; Lorenzo, R. A; Cela, R. *J. Chromatogr. A* **2004**, *1030*, 255-262.
48. Chia, K.-J.; Huang, S.-D. *Anal. Chim. Acta* **2005**, *539*, 49-54.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015

49. Amirav, A.; Dagan, S. *Eur. Mass Spectrom.* **1997**, *3*, 105-111.
50. Ding, W. H.; Liu, C. H.; Yeh, S. P. *J. Chromatogr. A* **2000**, *896*, 111-116.
51. Cajka, T.; Mastovska, K; Lehotay, S. J.; Hajslova, J. *J. Sep. Sci.* **2005**, *28*, 1048-1060.
52. Mastovska, K; Lehotay, S. J. *J. Chromatogr. A* **2003**, *1000*, 153-180.
53. Amirav, A.; Gordin, A.; Tzanani, N. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 811-820.
54. Dagan, S.; Amirav, A. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 737-752
55. Fialkov, A.B.; Gordin, A.; Amirav, A. *J. Chromatogr. A* **2003**, *991*, 217-240.

Downloaded by KUNGLIGA TEKNISKA HOGSKOLAN on April 17, 2013 | http://pubs.acs.org
Publication Date: November 7, 2008 | doi: 10.1021/bk-2008-0992.ch015