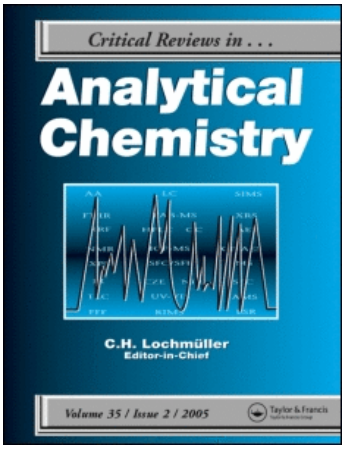

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The Use of LC-MS in Studies of Migration from Food Contact Materials: A Review of Present Applications and Future Possibilities

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ABSTRACT: Chromatographers rightly regard Mass Spectrometry (MS) as more than just a detection method. It is, in fact, another separation technique, and it is orthogonal to high performance liquid chromatography (HPLC), that is, it relies on a different physical property of the analyte to effect separation. Although HPLC relies on the analyte affinity for a stationary phase, MS relies on the mass-to-charge ratio (*m/z*) of ions derived from the compounds of interest. Liquid chromatography coupled to mass spectrometry (LC-MS) has become an indispensable tool for problem solving in virtually all analytical fields requiring "information-rich" chemical analysis. In the next decade, the LC-MS instrument market is expected to grow at more than twice the rate of the broader instrument market and will probably surpass gas chromatography–mass spectrometry (GC-MS) as the leader of the so-called hyphenated techniques. The aim of this review is to facilitate the growth, efficiency, and discussion of the use of LC-MS in studies of chemical migration from food packaging materials and other materials intended to come into contact with food.

KEY WORDS: LC-MS, food contact materials, food packaging, chemical migration.

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GLOSSARY

AC	Atomic composition
APCI	Atmospheric Pressure Chemical Ionization
API	Atmospheric pressure ionization
BADGE	Bisphenol A diglycidyl ether
BFDGE	Bisphenol F diglycidyl ether
CE	Capillary electrophoresis
CF	Continuous flow
CF-FAB	Continuous flow fast atom bombardment
CI	Chemical ionization
CID & CIF	Collision induced dissociation and fragmentation
CoE	Council of Europe
DLI	Direct liquid introduction
EI	Electron impact ionization
ES, ESI	Electrospray ionization
EC	European Commission
EEC	European Economic Community
EU	European Union

FAB	Fast atom bombardment
FCM	Food contact materials (to contain food and beverages, often used synonymously with ‘packaging’)
FD	Field desorption
FDA	Food and Drug Administration (USA)
FI	Field ionization
FT	Fourier transform
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
HR	High resolution
ICP	Inductively coupled plasma
ISP	Ionspray
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
MB	Moving belt
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
m/z	Mass to charge ratio
PB	Particle beam
PEG	Polyethylene glycol
PET	Polyethylene terephthalate
ppm	Parts per million (1E-6, μg/g)
ppb	Parts per billion (1E-9, ng/g)
ppt	Parts per trillion (1E-12, pg/g)
PSP	Plasmaspray
PY	Pyrolysis
Q	Quadrupole
R	Resolution
RCF	Regenerated cellulose film
Rf	Radio frequency
SCF	Scientific Committee for Food
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SIM	Selected ion monitoring
SIMS	Secondary ion mass spectrometry
SIR	Selected ion recording
TAG	Triacylglycerol
TIC	Total ion current
TOF	Time of flight
TSP	Thermospray
UV	Ultraviolet
m-XDA	*m*-Xylenediamine

I. BACKGROUND TO CHEMICAL MIGRATION AND THE ANALYTICAL NEEDS

Food contact materials (FCMs) may be defined as any materials intended to come into contact with food in the supply chain from source to consumer (note that the term ‘food’ is used throughout this review to include beverages also). The most important types of FCMs are packaging materials, but others constitute objects used during food manufacture and transport, such as cooking utensils, storage vessels, conveyor belts, tubing, and food preparation surfaces. FCMs may broadly be divided into nine main categories.

- plastics and coatings;
- metals and alloys;
- glass and ceramics;
- paper and board;
- rubber and elastomers;
- regenerated cellulose film (RCF);

- wood and cork;
- textiles;
- paraffin and micro-crystalline waxes.

Because by definition FCMs come into contact with food, it is possible for their chemical constituents to migrate into the food with which they come in contact, that is, for there to be "mass transfer from an external source into food by submicroscopic processes".[1] Any chemical migration into food is important because it can affect:

1. Food safety — substances used to prepare FCMs can be harmful if ingested in large enough quantities; and
2. Food quality — migrating substances can impart taint or odor to the food and reduce its appeal for the consumer.

Migration from FCMs is not negligible. For some food-packaging combinations the concentration of migrants in the food can approach that of substances used as direct food additives, such as colors and preservatives, at tens of ppm.

In one of the first comprehensive schemes regulating the use of FCMs, the US Food and Drug Administration (FDA) issued legal provisions for plastics during the 1950s. This initiative was closely followed by German and Italian migration regulations that likewise focused mainly on plastics. French, Dutch, and Belgian authorities later issued similar laws. In 1972 the Commission of the European Communities (the Commission) drew up a broad program of action designed to harmonize existing laws. Its Framework Directive set out the guiding principles, listing the materials to be regulated and defining the procedures and criteria to be used in adopting specific regulations for each type of material (Table 1).

It is beyond the scope of this review article to discuss all national and international regulations concerning chemical migration from FCMs; this field has been reviewed fully by Katan et al.[2] However, a brief discussion of European legislation is pertinent because (1) similar principles exist in other economic areas of the world, and (2) the legal regulations determine the nature of the problems that the analytical chemist must address.

Framework Directive 89/109/EEC[3] states that materials and articles should not transfer their constituents to food in quantities that could endanger human health or bring about unacceptable changes in the food. Specific Directives have been issued to deal with particular materials such as ceramics, regenerated cellulose, and plastics. Monomers, other starting substances, and some additives allowed in food contact plastics are published in a so-called positive list of permitted ingredients. Directive 90/128/EEC[4] and its amendments[5,6,7,8] place restrictions in the form of specific migration limits on a large number of substances used. These limits are the maximum permitted concentrations of the substances that migrate into food. The exact numerical limits are determined from toxicological evaluation of each substance. In the longer term there are plans to supplement the directive on plastics and regulate technological adjuvants, dyes, inks, adhesives, coatings, paper, and board.

Because foods themselves are complex, and also to allow for testing for the general case, four food simulants ('model foods') are specified for testing plastics for migration.[9] These are distilled water, 3% (w/v) acetic acid, 10% (v/v) ethanol, and rectified olive oil, which are intended to mimic aqueous, acidic, alcoholic, and fatty foods, respectively. Other test simulants can be used under certain circumstances, including 95% (v/v) ethanol and isooctane.

The above regulations set the scene for the analytical chemist who has to test for migration. There are several hundred chemicals that could in principle migrate from a wide range of materials. The matrix to be tested could be simulants or the actual food or beverage itself. The level of interest could be in the ppm range, for example, for additives used to make plastics or paper, down to ppb levels or lower for the most noxious monomers.

The methodology for analysis and testing of FCMs is still being developed. Implementation of the Directives carries an obligation for the Member States to carry out the necessary tests to ensure that FCMs comply with their requirements. This task often falls on the official food control laboratories, but the packaging industry also has

TABLE 1
European Directives Already Adopted in the Area of FCMs

Food Contact Material (FCMs)	Directive
Framework Directive	89/109/EEC
Symbol	80/590/EEC
Regenerated cellulose (RCF)	93/10/EEC
Regenerated cellulose (1st amendment)	93/111/EC
Release of N-nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers	93/11/EEC
Ceramics	84/500/EEC
Plastics (Base Directive: monomers)	90/128/EEC
Plastics (1st amendment: monomers)	92/39/EEC
Plastics (2nd amendment: monomers)	93/9/EEC
Plastics (3rd amendment: monomers)	95/3/EEC
Plastics (4th amendment: monomers)	96/11/EC
Plastics (basic rules for migration tests)	82/711/EEC
Plastics (1st amendment of basic rules)	93/8/EEC
Plastics (2nd amendment of basic rules)	97/48/EC
Plastics (List of simulants)	85/572/EEC
Plastics (1st, incomplete list of additives)	95/3/EC
Vinyl chloride	78/142/EEC
Method of determining VC in PVC	80/766/EEC
Method of determining VC in foods	81/432/EEC

a responsibility to ensure that FCMs are manufactured in accordance with good manufacturing practice[10] and comply with the relevant legislation.

Given the plethora of substances that need to be tested for in foods and food simulants, there is a great need for rapid, reliable, and robust techniques. Volatile and semivolatile substances can usually be determined by gas chromatography-mass spectrometry (GC-MS), which is now commonly found in the workplace. However, recent technological advances in the area of liquid chromatography–mass spectrometry (LC-MS) mean that this technique is now becoming affordable and more widely used in the analytical laboratory. This review covers the development of LC-MS instrumentation and methodology for studies of chemical migration.

II. TYPES OF LC-MS CURRENTLY AVAILABLE

The combination of the separating potential of liquid chromatography and the analyzing power of mass spectrometry makes LC-MS a highly useful tool for analytical chemists. Due to its high selectivity and sensitivity, it is finding increasing use in the analysis of a wide range of substances in complex mixtures. The major challenge in coupling of LC with MS is posed by the fact that gas-phase ions must be produced in order to obtain a mass spectrum. The many types of LC-MS interface currently available have been reviewed extensively by Ashcroft[11] and De Hoffman et al.[12] They include[13] continuous flow fast-atom bombardment (CF-FAB), moving-belt (MB), chemical ionization (CI), thermospray (TSP), particle beam (PB), ion spray (ISP), electrospray (ES), atmospheric

pressure chemical ionization (APCI), inductively coupled plasma (ICP), and plasmaspray (PSP) interfaces. Some of these coupling methods, such as MB or PB, are based on the selective vaporization of the LC solvent before it enters the MS source. Other methods, such as direct liquid introduction (DLI)[14] or CF-FAB, rely on limiting the flow of liquid into the interface to a rate that can be directly handled by the source and its associated vacuum pumps. Finally, certain LC-MS coupling methods, such as TSP, nebulizer-assisted ES and APCI, can tolerate typical LC flow rates of about 1 ml/min, which favors sensitivity. Classic ES can accept flow rates from nl/min up to 0.2 ml/min. As ES is more dependent on concentration than on the total quantity injected, there is little advantage in using it at high flow rates, other than ease of coupling to standard bore HPLC columns. Figure 1 shows the application range of different chromatography–MS coupling methods (except DLI and CF-FAB) as a function of the mass and nature of the analyte. Table 2 lists the commonly used ionization methods, together with the type of analyte information generated, complementary mass spectrometer(s), and the general analyte classes to which these ionization methods can be applied.[1,13,15,16]

LC mobile phases containing inorganic mineral acids, nonvolatile buffers and high levels of additives (>100 m*M*) are generally not recommended for LC-MS because they can deposit on the ion source. With these exceptions, most LC-MS systems are compatible with a wide range of aqueous and organic solvents and mixtures thereof, and also with volatile pH control agents such as buffers (e.g., ammonium acetate), acids (e.g., formic, acetic and trifluoroacetic), and bases (e.g., trialkylamines and ammonia). An understanding of the effect that an LC mobile phase can have on ionization helps in the selection of an appropriate mobile phase during method development.[17,18] We detail below the principles of a number of LC-MS interfaces that have been or may be used for the analysis of FCMs.

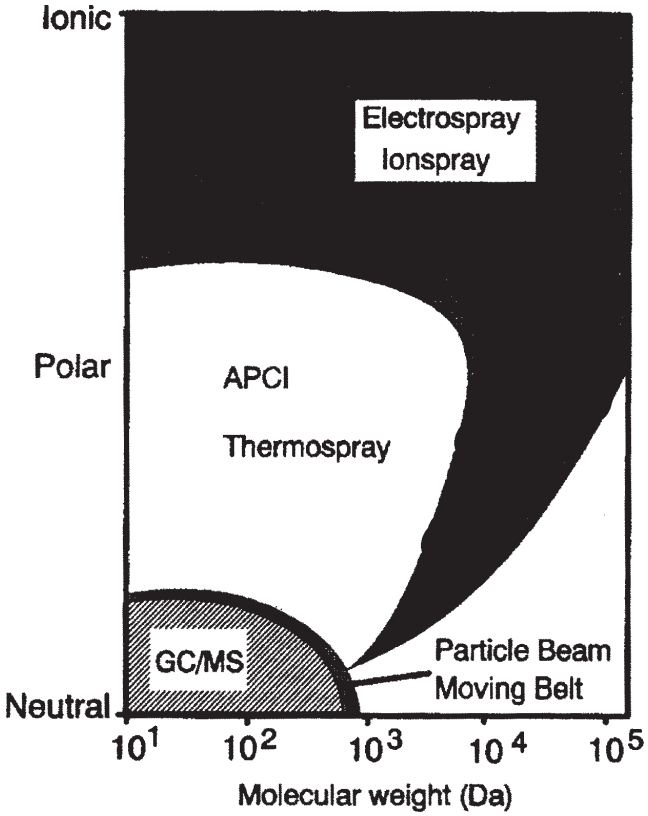


FIGURE 1. Application ranges of the different chromatography–MS coupling methods (except DLI and CF-FAB).

TABLE 2
Ionisation Methods and Their Compatibility with Different Types of LC-MS

Ionisation method	Interface required	Principal ions detected	Sample class (approx. Mwt limit)	Optimum flow rates (min-max)	Detection level range	Notes
EI	Particle Beam	$M^{+\cdot}$ and some fragment ions	non-polar and some polar organic substances ≤ *ca.* 1000 da	0.5 to 2 ml/min	ppm	higher flow rates only with a low aqueous content mobile phase
ES	Electrospray	$[M+H]^+$, $[M+nH]^{n+}$, $[M+matrix]^+$, $[M-H]^-$, $[M-nH]^{n-}$	polar organics, proteins, biopolymers, organometallics ≤ *ca.* 200,000 da	30 nl/min to 1 ml/min	ppb	wide range of solvents acceptable - probably the most universal technique
APCI	Atmospheric Pressure Chemical Ionisation	$[M+H]^+$, $[M+matrix]^+$, $[M-H]^-$	polar and some non-polar organic substances ≤ *ca.* 1000 da	0.2 to 2 ml/min	≤ ppb	wide range of solvents acceptable
FAB/FIB/LSIMS	Continuous Flow FAB	$[M+H]^+$, $[M+matrix]^+$, $[M-H]^-$	polar organic, proteins, organometallics ≤ *ca.* 10,000 da	1 to 10 µl/min	ppm	needs a FAB matrix present throughout the chromatographic run or added post-column
TSP	Thermospray	$[M+H]^+$, $[M+NH_4]^+$, $[M+matrix]^+$, $[M-H]^-$	Polar and some non-polar organic substances ≤ *ca.* 1000 da	0.5 to 2 ml/min	ppb	wide range of solvents acceptable - needs either an electrolyte present, *e.g.* NH_4OAc, or a discharge electrode

A. Direct Liquid Introduction (DLI)

By far the simplest approach to LC-MS is the direct liquid introduction approach pioneered by Tal'Rose et al.[19] and Baldwin and McLafferty.[20] DLI interfaces are designed to be compatible with the direct probe inlet systems of most commercial GC-MS systems. A large number of such devices have been described in the literature, but the common thread to all has been the 3 to 5 μm opening in the probe tip projecting into the MS source, which creates a fine liquid jet. The DLI probe is usually water-cooled to prevent fluctuations in the ion current generated by the jet. Typical flow rates range from 5 to 15 μl/min. DLI is often used to couple supercritical fluid chromatography (SFC) with MS because it permits the entire flow to enter the MS source. This flow can easily be accommodated because the supercritical mobile phase (usually CO_2) then decompresses to a gas and is readily pumped out of the system.[21]

B. Moving-Belt (MB)

This interface was first demonstrated by Scot et al.[22] who used a moving wire to carry the solvent/analyte into the MS source. In more recent designs the eluent from the chromatography column is deposited on a moving belt made of Kapton, a DuPont polyimide that has good mechanical properties above 400°C and is chemically inert. The belt enters an initial vacuum lock where it is heated by infrared radiation to remove most of the LC solvent (Figure 2). It then enters the source, where it is flash-heated to evaporate the analytes. It leaves the source through another vacuum lock and is finally heated to a high temperature to clean it. At the lock outlet the belt has completed the loop and comes back, hopefully clean, to the LC deposition station.

C. Thermospray (TSP) and Plasmaspray (PSP)

The LC-MS interfaces described above do not perform well with typical LC flow rates (*i.e.*, 1 to 2 ml/min) or with substances of low volatility or poor thermal stability. The development of TSP by Blakely and Vestal[23] helped provide a solution to both these problems. Although the popularity of TSP and PSP has waned significantly in recent years, owing to the introduction of more robust atmospheric pressure ionization (API) techniques, many laboratories continue to use TSP and PSP with success. TSP is a soft ionization technique and produces predominantly $[M+H]^+$ or $[M-H]^-$ ions, sometimes together with smaller fragments. TSP is best suited to the analysis of organic substances of less than 1000 Da that exhibit some degree of polarity. It involves the

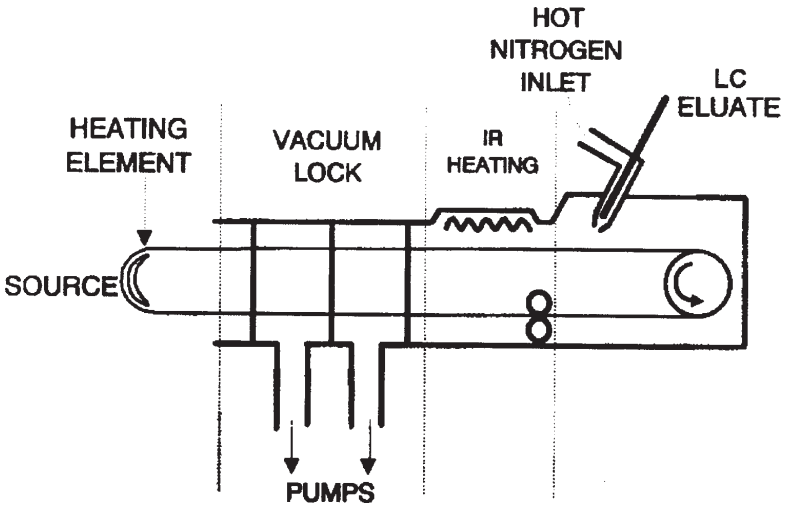


FIGURE 2. Diagram of a typical moving belt interface.

introduction into the ion source of a relatively high flow of solvent (0.2 to 2 ml/min). A typical TSP interface is shown in Figure 3. The solvent is pumped down the resistively-heated capillary tube or vaporizer (~100 μm in diameter) inside the probe, the tip of which is situated in the source. The source is heated to prevent condensation of solvent, and the temperature of the capillary is adjusted until the solvent is vaporized. This generates a jet of vapor that contains small, electrically charged droplets if the solvent is at least partially aqueous and contains an electrolyte. The presence of the electrolyte (typically 50 to 100 m*M* ammonium acetate) is important, as without it the ion yield, and hence sensitivity, is quite low. The droplets continue to vaporize and shrink in volume as they travel through the source. Eventually they are sufficiently small for free ions to be expelled from their surface. Positive or negative ions are generated depending on the gas phase acidity or basicity of the solvent and solutes. Ions leave the source through a sampling cone that has an orifice in the center, and this process is encouraged by an ion repeller that is situated directly opposite the sampling cone. The ions are then analyzed by the mass spectrometer. A high voltage applied to the repeller will tend to induce fragmentation of the analyte ions.

If an electrolyte cannot be used for a particular analysis (e.g., in normal-phase separations), then an electrical discharge in the source may be employed to induce ionization. Most TSP sources are fitted with such a discharge needle, and when this is used the technique is known as plasmaspray (PSP). In reality, however, the ionization process in many analyses is a mixture of TSP and PSP. This is especially true in separations employing reversed-phase HPLC and gradient elution: in the early stages, in which the LC mobile phase has a high water content, TSP is used; later, when the aqueous content of the eluent has diminished and the TSP ionization process begins to fail, the discharge pin is switched on to give PSP ionization.

D. Continuous Flow Fast Atom Bombardment Ionization (CF-FAB)

The CF-FAB coupling invented by Caprioli et al.[24,25] consists simply of a capillary chromatography column coupled to the end of a FAB nozzle by a capillary passing through the sample introduction nozzle (Figure 4). Glycerol (1 to 5%) is added to the LC solvent, which flows at 1 to 5 μl/min. The solvent readily evaporates, leaving the analytes at the nozzle surface mixed with glycerol, which serves as a FAB matrix.

E. Particle Beam (PB)

Uniquely for LC-MS interfaces, the PB interface gives electron-impact spectra and so offers a high degree of fragmentation, structural information, and library search compatibility. This makes it a highly desirable method of ionization, although the difficulties in interfacing an ionization method based on the use of a fragile filament in a high vacuum with a chromatographic technique eluting approx. 1 ml/min are evident.

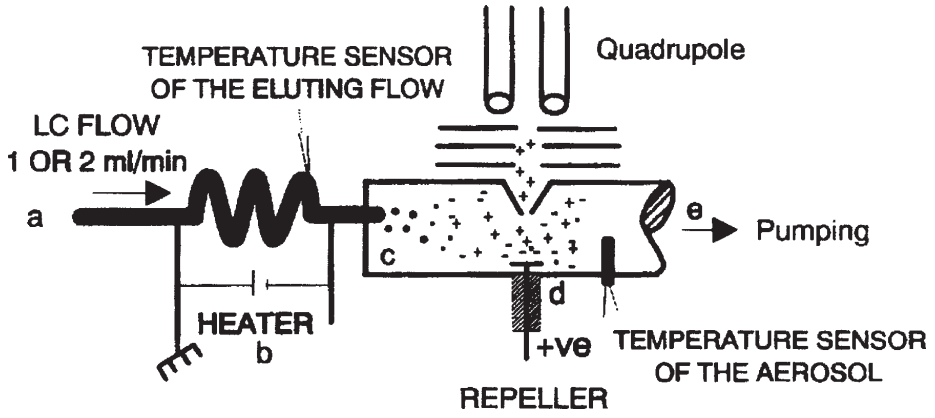


FIGURE 3. Diagram of a typical thermospray/plasmaspray interface.

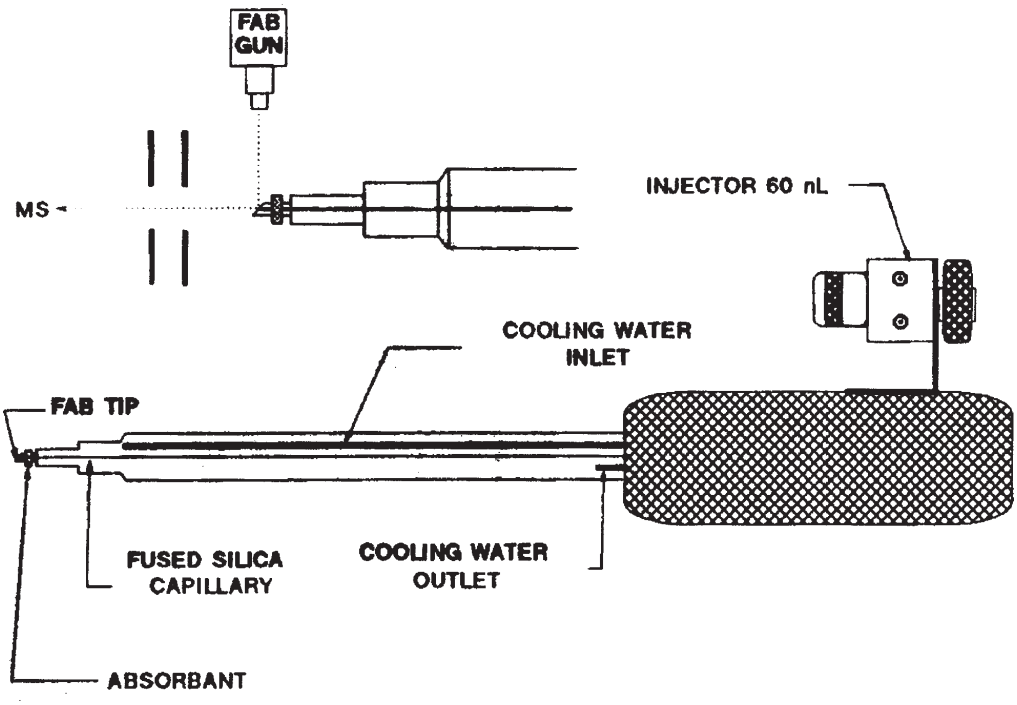


FIGURE 4. Diagram of a typical continuous flow fast atom bombardment ionization interface.

The pioneer workers in this field[26] christened the technique MAGIC-LC-MS (monodisperse aerosol generation interface for combining liquid chromatography with mass spectrometry), but the name particle beam (PB) is now generally used. The LC eluate enters a chamber heated at between 40 and 50°C, where it is nebulized by helium gas into a fine spray of droplets from which the volatile solvent molecules evaporate more readily than the solute molecules (Figure 5). The solute molecules tend to remain as particles that then pass through a nozzle into a vacuum chamber, where further solvent molecules are removed. The desolvated solute molecules arrive in the source as a spray of uncharged particles and are there ionized by conventional EI or CI methods. Different PB interfaces have variations on the chambers described here, but the basic principle is the same. PB interfaces generally cope with flow rates of between 0.5 and 2 ml/min, and like most interfaces are more tolerant of normal phase (organic) solvents than reversed-phase (aqueous) solvents. PB ionization is not suitable for very volatile analytes, which tend to be pumped away along with the solvent, or for thermally labile substances (because the source is held at temperatures in the range 200 to 250°C). The PB is the only form of LC-MS interface that yields spectra that can be compared for matches with proprietary software libraries (*e.g.*, the NIST and Wiley libraries) to assist substance identification. However, poor overall sensitivity has led to it being largely superseded by the API-based techniques ES and APCI).

F. Electrospray (ES)

ES ionization is widely used on quadrupole and magnetic sector mass spectrometers, and now also on time-of-flight (TOF) instruments. As with all API techniques, the formation of ions takes place outside the MS vacuum system (Figure 6). In the ES interface ions are evaporated from a droplet into the gas phase.[11,27] The analyte solution is introduced into the ionization source through a stainless steel capillary (75 to 100 μm internal diameter) that runs through a mass spectrometer probe. Flow rates can range from 30 nl/min to 1 ml/min, but are typically between 5 and 300 μl/min. The lowest concentration producing a full spectrum depends on the propensity of the analyte to ES ionization, but is typically 1 to 50 ng/μL for analytes of up to 1000 Da and 1 to 20

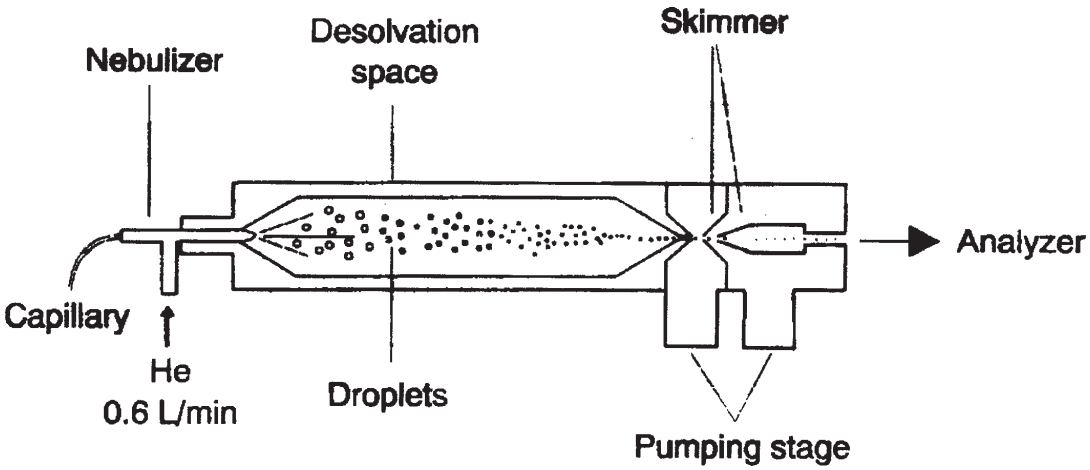


FIGURE 5. Diagram of a typical particle-beam interface.

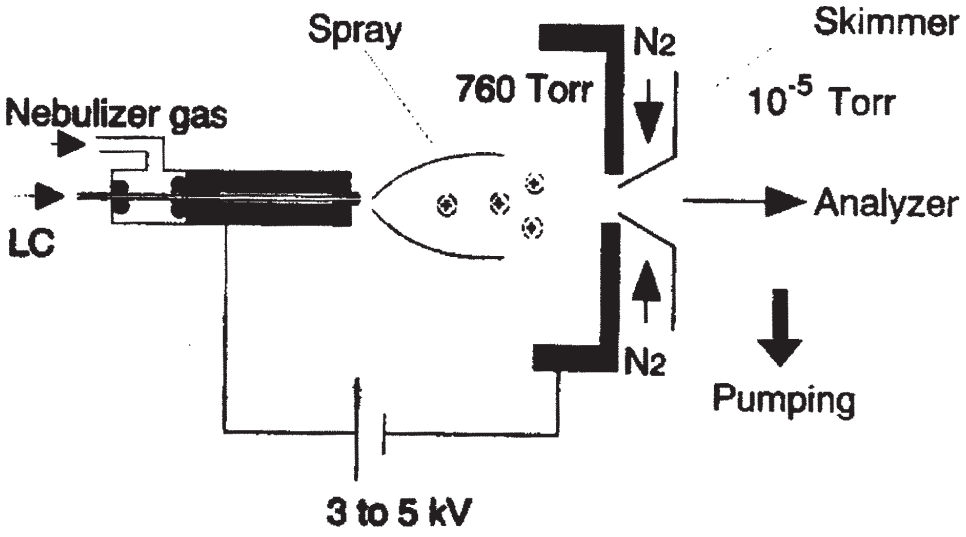


FIGURE 6. Diagram of a typical nebulizer-assisted electrospray interface.

pmol/μL for analytes of higher molecular mass. A voltage of 3 to 4 kV is applied to the tip of the capillary, and this strong electric field disperses the LC eluate into a highly charged aerosol. The ES process is often aided by a nebulizing gas flowing down the probe coaxially around the outside of the capillary (nebulizer-assisted ES). This gas (usually nitrogen) helps to direct the spray emerging from the capillary tip. The charged droplets diminish in size due to evaporation, assisted by a flow of warm drying gas (again N_2) that passes across the front of the source. Eventually, analyte ions free of solvent are released from the droplets. Some of these ions pass through a sampling cone or orifice into a pumped intermediate vacuum region (approx. 1 mbar pressure), and then through a small hole, the skimmer, into the analyzer of the mass spectrometer. The analyzer is under high vacuum. The skimmer acts as a momentum separator: the heavier analyte ions pass through, while the lighter solvent and gas molecules are pumped away in the intermediate vacuum stage.

Both ES and APCI (Section II.G) are 'soft' ionization techniques causing very little fragmentation, which can be a disadvantage for identification of unknown substances. Fragmentation can sometimes be induced by applying a voltage to the sampling cone: this causes the extracted ions to accelerate and resulting collisions between the accelerated ions and residual solvent and gas molecules may be of sufficient energy to cause fragmentation of the ions (collisionally induced dissociation, CID).

G. Atmospheric Pressure Chemical Ionization (APCI)

APCI has some similarities to ES ionization.[28] The source is a modified ES source and so the extraction of ions into the mass spectrometer is much the same. The ionization process itself, however, is quite different. With APCI no high voltage is applied to the probe tip, and so nebulization and ionization are independent (Figure 7). The solute and solvent, flowing at between 200 μl/min and 2 ml/min, elute from a capillary that is surrounded by a co-axial flow of nebulizing gas (usually N_2) and sometimes by a second, outer coaxial flow of 'sheath gas' (again usually N_2). The capillary and the gas(es) are contained in the triaxial probe that is heated at temperatures of up to 700°C depending on the type of analyte under investigation. The source is typically heated to between 120 and 180°C. The combination of nebulizer gas and heat converts the solvent flow into an aerosol, which then begins to evaporate rapidly. Inside the heated source is a corona discharge needle held at a voltage of 2.5 to 3 kV, and this is responsible for ionizing the solvent molecules. In the atmospheric pressure region around the corona pin, a combination of collisions and charge transfer reactions generates a chemical ionization reagent gas plasma. Any analyte molecules that elute and pass through this region can be ionized by proton transfer to produce $[M+H]^+$ or $[M-H]^-$ ions. APCI tolerates a variety of HPLC solvents from fully aqueous (with or without buffers) to fully organic.

H. Modified ES Probe for Capillary Electrophoresis–Mass Spectrometry (CE-MS)

A modified ES probe is the most common way to interface CE with MS. Two types of interface are used for this coupling: the triaxial probe (Figure 8) or alternatively the liquid junction interface. The triaxial probe is the more common.[29] It involves inserting the outlet electrode of the electrophoresis capillary directly into the ionization source of the MS. The rate at which solvent emerges from the CE system is typically just a few nl/min, and so a solvent make-up is required to bring the total solvent flow into the recommended ES range. Within the triaxial probe, the separation capillary is surrounded by another capillary containing the solvent make-up, and then finally the nebulizing gas (N_2) flows around these two capillaries. The solvent make-up flow also serves to make electrical contact between the CE buffer and the probe tip.

I. Tandem Mass Spectrometry and MS^n

The operation of two mass analyzers in series is known as tandem mass spectrometry (MS/MS,

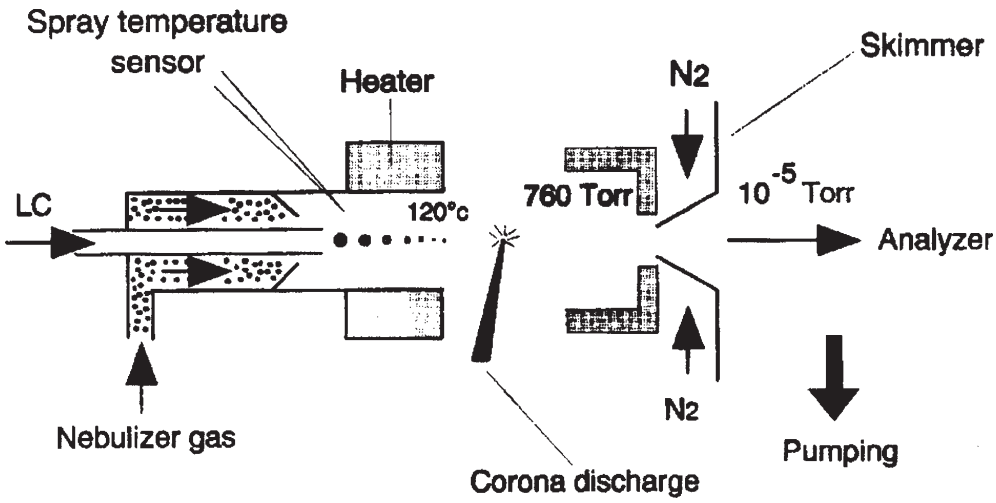


FIGURE 7. Diagram of a typical atmospheric pressure chemical ionization interface.

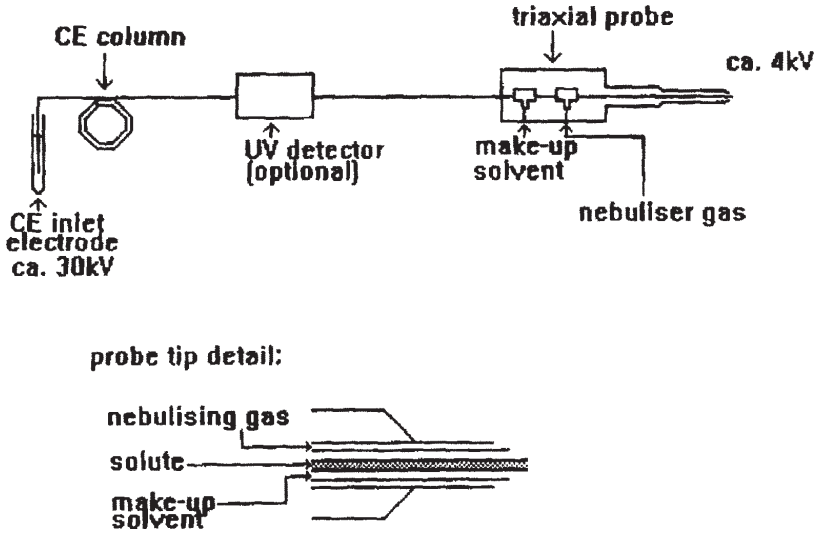


FIGURE 8. Diagram of a typical CE-MS interface.

or MS^2). The use of LC-MS/MS is fast becoming widespread in analytical laboratories because it yields structural information additional to that obtained from the sensitive but 'soft' API techniques. Most commercial tandem mass spectrometers have a collision cell in between two mass analysers. MS/MS systems in which the collision cell and mass analyzers are all quadrupoles are often referred to as 'triple quad' systems. Often the collision cell is simply an Rf-only quadrupole. An inert gas such as argon or helium is usually added to the collision cell, where, with sufficient energy, it bombards the trapped sample ions and causes additional fragmentation to occur by CID.

Several types of MS/MS experiments can be undertaken on triple-quad systems (Figure 9).

1. Product ion scan (daughter scan) experiments consist of selecting a precursor ion (or parent ion) and determining all of the product ions (daughter ions) resulting from CID. If a reactive gas is used in the collision cell, collision-activated reactions (CARs) may be observed. When only fragment ions are produced, this scan mode is also referred to as 'fragment ion scan'.
2. Precursor ion scan (parent scan) experiments consist of choosing a product ion (or daughter ion) and determining its precursor ions (or parent ions).
3. Neutral loss scan experiments consist of detecting all the fragmentations leading to the loss of a fragment. Perhaps the most simple and specific assignments that can be made in the spectrum are for the small neutral species lost in the formation of the fragment ions of highest mass in the spectrum, especially those formed directly from the molecular ion. For example, important ions at masses $[M-1]^+$, $[M-15]^+$, $[M-18]^+$, and $[M-20]^+$ almost always represent the losses of H, CH_3, H_2O, and HF, respectively, from the molecular ion. With a reactive gas, adduct ions can sometimes also be observed.

In theory, any number of analyzers can be used in series. If n analyzers are used, the technique is termed MS^n. However, in practice the

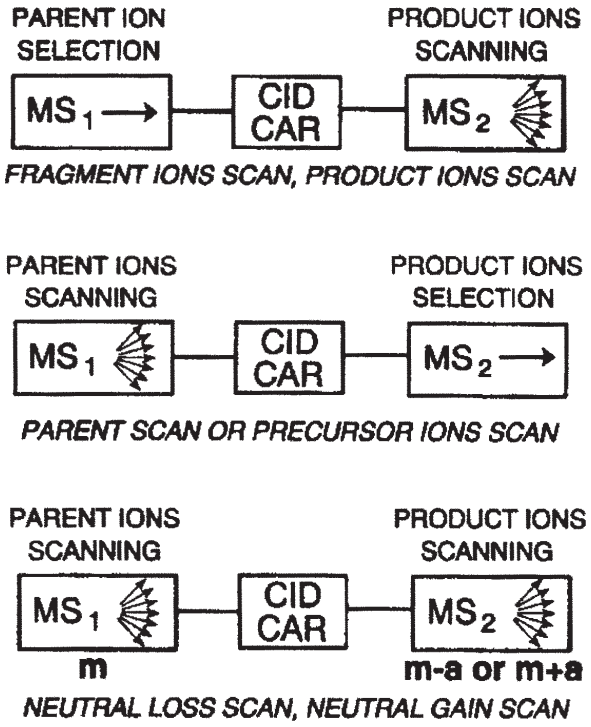


FIGURE 9. Main experimental modes in tandem mass spectrometry (MS/MS).

maximum is 3 or 4 analyzers in the case of beam instruments.

With the advent of more reliable ion-trapping technology, LC-MS^n can be successfully undertaken for n up to about 9. All mass analysis and CID/CAR processes occur in a single ion trap source. However, these instruments only allow product ion scans.

J. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

In the analysis of organometallic compounds methods based on GC require them to be derivatized so as to confer thermal stability, whereas this is unnecessary with liquid chromatography separations. However, the LC separation systems developed require the use of an organic modifier (methanol or acetonitrile), which is not compatible with the argon plasma used in ICP-MS. Therefore, to couple the LC separation to the ICP-MS, the interface between them has to perform two major functions. First, it must reduce the loading of organic modifier to the plasma so that the plasma is not extinguished. This is accomplished by using a silvered spray chamber cooled to between –15 and –20°C which condenses out the modifier before it reaches the plasma. Second, oxygen is added to the sample aerosol. This reduces deposition of carbon on the sampling cone by the formation of carbon dioxide, which in turn stops the sampler orifice from becoming blocked. A blocked sample cone orifice reduces the quantity of material that can enter the mass spectrometer and adversely affects the detection limit.

III. APPLICATIONS OF LC-MS TO THE ANALYSIS OF FOOD CONTACT MATERIALS

GC-MS is widely regarded as the method of choice to analyze for volatile and semivolatile substances in- and migrating from- FCMs. Some polymer stabilizer additives can be analyzed by GC-MS or by direct introduction into the mass spectrometer. High-molecular-weight polymer binder systems, because of their low volatility, must be degraded prior to conventional mass spectrometric analysis; another option would be LC-MS. Soft ionization methods, which afford only molecular weight information, are often used to characterize low-molecular-weight oligomers, cross-linkers, and functionalized monomers.[30]

Krost[31] has investigated the use of LC-MB-MS to identify selected members of various chemical classes. Among the chemical groups examined were benzidines, nitrosamines, anilines, nitroaromatics, dinitroaromatics, hydrazines, amides, phenylenediamines, organophosphites, acrylates, pyridines, phthalates, nitrophenols, halogenated triazines, pesticides, halogenated pyridines, and alkyl tins. Selective ion monitoring (SIM) was used for detection in all cases because of a lack of sensitivity in the full-scan mode. The limit of detection (LoD) was approximately 10 ng of substance. Substances of molecular weight exceeding 250 Da generally induced a lower response. Presumably, some lower-molecular-weight substances within given groups exhibit low sensitivity because of volatilization losses in the inlet.

API interfaces are now the most usual in LC-MS systems. ES and related methods, including APCI with a heated nebulizer, all began as specialized ionization techniques, which became much more widely accepted when combined with tandem mass spectrometry. Today, both APCI and ES are widely used for quantitative and qualitative LC-MS and LC-MS/MS analyses.

Some authors use mass spectrometers equipped with ES or APCI interfaces in order to produce molecular ions for substance identification without prior chromatographic separation. In this way different combinations of antioxidants have been identified correctly in test solutions and in solutions obtained in migration tests of commercial plastics. Analysis time was less than one-tenth of that required for conventional HPLC analysis of the migrants.[32]

In this section we examine the published applications of LC-MS to the analysis of food contact materials. In particular, we focus on plastics starting substances, because these have been the analytes in most method development work.

A. Plastics Additives

LC-MS has been used in series with UV detection to analyze antioxidants and UV light stabilizers extracted from plastics.[33] Nanogram quantities of the additives were detectable by LC-MB-MS.

A strategy employing MS/MS and HPLC for analysis of additives such as antioxidants in polymers has been described by Egsgaard et al.[34] Integrated LC-MS would undoubtedly further facilitate this type of analysis, at least in the final stages. However, in the initial stages, in which the class of analyte remains unknown, a two-step approach involving direct screening analysis by MS/MS followed by targeted HPLC analysis appears to be advantageous.

LC-MS has become invaluable for identifying benzothiazole derivatives used as accelerators in the vulcanization of rubber. Niessen et al.[35] performed LC-MS analysis under isocratic conditions using various interfaces: MB in both EI and positive-ion CI modes, TSP in both buffer ionization and discharge-on modes, and PB in EI mode. For further structure elucidation, they performed MS/MS in combination with TSP, and also acquired GC-MS data. Their work may be compared with the use of direct MS techniques on the same type of rubber accelerators by Lattimer.[36]

One of the authors laboratories has developed a large-volume injection LC-APCI-MS method for the analysis of vulcanization accelerator residues in aqueous food and drink (unpublished work). The substances measured are 2,2′-dithiobis(benzothiazole) and *N*-cyclohexyl-2-benzothiazole sulfenamide, along with the degradation products mercaptobenzothiazole and benzothiazole. Full-scan mass spectra for each substance are shown in Figure 10. Using SIM, the LoD in food and beverages was 10 ppb. The advantage of the method over conventional GC-MS or LC-UV methods[37] is that sample preparation is minimal: with the sensitivity and specificity afforded by LC-APCI-MS, and only a simple dilution or extraction with solvent is required.

The transformation products of the phenolic antioxidants Irganox 1330[38] and Irganox 1010[39] in food contact polyolefins subjected to electron-beam irradiation have been analyzed by particle beam LC-MS. The principal radiolysis products arose through cleavage of C-O bonds leading to subunit losses. The formation of quinone methides was observed for Irganox 1330, but was not a major feature of the radiolytic reactions of Irganox 1010.

Organotins are used as stabilizers in polyvinyl chloride, as catalysts in the production of polyurethane foam, as biocides in antifouling paints, and as preservatives (fungicides, bactericides, insecticides) for wood. The identification of organotins is complicated by the fact that, depending of the alkyl chain size and the number of substituents, they can range in type from hydrophobic to polar and even to nonvolatile and ionic. Dauchy et al.[40,41] have separated trace amounts of mono-, di-, and tributyltins using LC-ICP-MS.

Di-alkyl phthalate esters are used frequently as plasticizers in tubing, containers, label adhesives, and a wide array of materials that come into contact with food, pharmaceutical products, or physiological fluids. Due to their widespread use and moderate resistance to degradation, they are virtually ubiquitous in the environment and consequently in foodstuffs. Their degradation products, the mono-alkyl phthalate esters, are also commonly found. The primary objective of a study by Baker[42] was to develop a thermospray LC-MS method for the detection and characterization of both mono- and di-alkyl phthalates. It was found that all the mono- and di-alkyl esters produced strong $[M+H]^+$ pseudomolecular ions (the base peak of the spectrum in most cases). Using SIM at *m/z* 149, all the mono-esters were easily detected as individual chromatographic peaks, while none of the di-alkyl esters produced an *m/z* 149 response.

B. Plastics Monomers and Oligomers

Jones et al.[43] have investigated the transport properties of an LC-PB-MS interface using oligomers of polystyrene as test substances. Polystyrene was chosen because it is available as a well-defined series of oligomers spanning the mass range of the spectrometer, and because it is known to undergo thermal degradation in a conventional solids insertion probe inlet. The results indicated

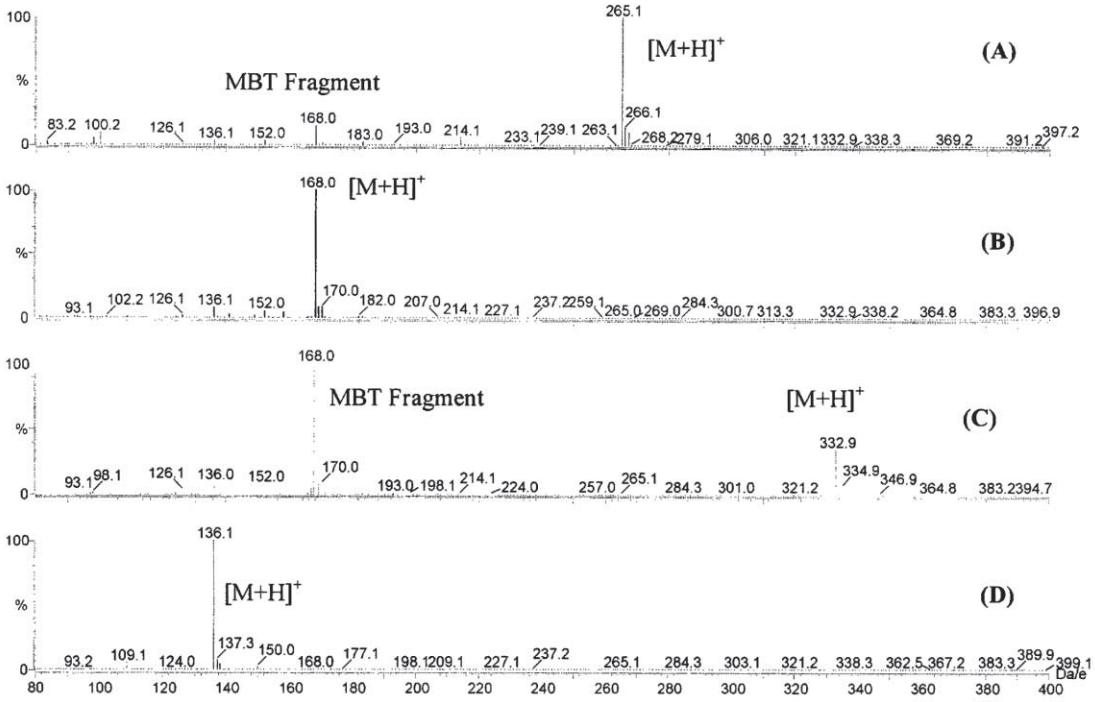


FIGURE 10. APCI (+ive) full scan mass spectra of (A) *N*-cyclohexyl-2-benzothiazole sulfenamide, (B) mercaptobenzothiazole, (C) 2,2′-dithiobis(benzothiazole), and (D) benzothiazole.

that intact styrene oligomers with molecular weights up to approximately 2000 Da were quantitatively transferred through the interface and into the mass spectrometer ion source. Using electron impact ionization, the authors observed molecular ions for oligomers up to n = 18 at *m/z* 1930, well beyond the heaviest molecular ion reported as having been detected using a solids insertion probe.

Milon[44] extracted the polyethylene terephthalate (PET) film from a food package that contained a microwave susceptor material and analyzed the extract by plasmaspray LC-MS using acetonitrile with formic acid as the LC mobile phase and negative-ion mode MS. This researcher observed that an unusually high probe tip temperature of 370°C was required, and even then could confirm only the presence of the cyclic trimer and tetramer. Guarini et al.[45] used negative-ion LC-TSP-MS to examine depolymerization reaction mixtures obtained by glycolysis of PET. Sensitivity was improved by reacting the terminal hydroxyl groups with perfluoroanhydrides, and oligomers up to the linear tetramer were observed. Barnes et al.[46] observed cyclic PET oligomers up to the heptamer, plus a minor series of related cyclic oligomers, by optimization of LC-APCI-MS conditions. Harrison et al.[47] reported the field-desorption mass spectra and LC-MS spectra of cyclic PET oligomers $[CO.C_6H_4.CO.O.CH_2.CH_2.O]_x$ (x = 3-8) obtained by a polymer-supported reaction; in the presence of traces of ammonium ions APCI produced intense ammoniated molecular ions. Additional structural information was afforded by MS/MS spectra obtained by fragmenting the ammoniated cyclic PET oligomers (x = 3-6) with 30 eV argon in the collision cell of a triple quadrupole.

Auriola et al.[48] have developed an LC-TSP-MS method for simultaneous determination of polyethylene glycols (PEGs) in the 238 to 986 molecular weight range. The method proved to be suitable for trans-membrane permeability studies performed with an *in vitro* apparatus. The quantitation limit for PEG 600 was 60 ng per injection. This method may be compared with (more laborious and less discriminating) pure HPLC techniques.[49,50]

A method for simultaneous extraction of residual bisphenol A diglycidyl ether (BADGE) and *m*-xylenediamine (*m*-XDA) from cured epoxy res-

ins has been developed and applied to resins with precure compositions of 1:1, 1:2, and 1:3 BADGE/*m*-XDA equivalent ratios. After extraction of the epoxy-amine formulation in refluxing 1:3 chloroform/methanol for 10 h and derivatization of the *m*-XDA with fluorescamine on a precolumn, the residual starting substances were determined by reversed-phase HPLC with fluorescence detection.[51] However, the confirmation of m-XDA/fluorescamine derivatization products was performed by reversed-phase LC-TSP-MS.[52] In a related study, impurities and hydrolysis products in commercial BADGE were characterized using reversed-phase LC-TSP-MS.[53] The degradation products of bisphenol F diglycidyl ether (BFDGE) in water-based food simulants have also been identified by LC-TSP-MS.[54] BADGE and BFDGE subsequently have been determined in varnish extracts by positive ion LC-MS with ES and APCI interfaces.[55]

(Methoxymethyl)melamine resins are complex mixtures of melamines with different degrees of hydroxymethylation and methoxymethylation. They have extensive industrial applications. Their characterization is difficult because of their complex composition. Longordo et al.[56] isolated monomer, oligomer, and polymer fractions of a resin by high-performance size exclusion chromatography (HPSEC), and determined the molecular weights of components within each HPSEC fraction by FAB-MS. They then used this information to assign tentative structures to all major peaks in HPLC. LC-TSP-MS has provided a simpler way to identify LC-separated components; 20 monomeric and 13 dimeric substances being identified in a study by Chang.[57] In similar work, Nielen and Van de Ven[58] applied three complementary techniques to characterize a complex (methoxymethyl)melamine resin: gradient reversed-phase LC-MS, SEC-MS, and CE-MS. The LC-MS system used an APCI interface and the spectra showed characteristic fragmentation patterns and detailed structural information of the monomeric components. Up to 18 individual monomers and 7 dimers were identified. Likewise, Marcelli et al.[59] analyzed resins of this type by LC-MS with ES ionization and ion trap mass spectrometry.

A number of polyimides and poly(amic acids) have been studied using GC-MS and LC-PB-MS techniques following hydrolysis by NH_4OH.[60] With direct injection of the hydrolysate, GC-MS was unable to determine either the amine or acid portion of these polymers. The amine component of the polymers was readily detected in ether extracts, but the acid component remained undetected. In contrast, LC-PB-MS was able to identify and measure both the amine and acid monomeric units in the raw hydrolysate.

Composites used in dentistry for cavity filling, cementation, and sealing have also been analyzed by GC-MS and LC-PB-MS.[61] Monomers based on bisphenol A and urethane, co-monomers based on diols and methacrylates, diverse additives (polymerization initiators, photoinitiators, co-initiators, photostabilisers, inhibitors, plasticizers, catalysts, etc.), and contaminants introduced during manufacturing processes were all identified, including toxicologically hazardous substances.

Difuranic diamine dihydrochlorides are popular intermediates for the preparation of polyurethane derivatives. The presence of the two reactive functions allows these molecules to act as cross-linking agents in the synthesis of polymeric macromolecules. Their fragmentation pathways under ES-MS/MS with low-energy collisional activation have been elucidated, and product-ion and precursor-ion MS/MS scans for selected intermediate ions formed during cone-voltage-fragmentation of the ionized species have provided an explanation of their fragmentation behavior.[62]

Begley et al.[63] have developed a method for determining the residual oligomers in nylon food packaging. In addition, the method was adapted to quantify oligomers migrating to a liquid food simulant (oil) during oven cooking conditions. The oligomers were identified by LC-MS and MS/MS. Solutions for MS/MS were prepared by collecting oligomer peaks separated by HPLC.

When polyesters are analyzed by LC-ES-MS, singly and doubly charged ions are produced and oligomeric species can be clearly identified.[64] The presence of alkali metal salts and the solvent system have been found to have marked effects on ES-MS spectra. Under optimal conditions this enables identification of the range and relative abundance of chemical structures in the polyester, and hence average molecular weights, polydispersity, the distribution of acid and alcohol

end-groups, overall monomer proportions, average functionality per molecule, and average frequency of branching per molecule. The identity of the ES-MS peaks was confirmed by tandem mass spectrometry.

Pattanaargsorn et al.[65] have shown that LC-APCI-MS can be used to analyze nonionic surfactants under conditions minimizing fragmentation of the analyte molecules. They simultaneously determined the oligomer distributions of alkylphenol polyethoxylates and fatty alcohol polyethoxylates with respect to both the degree of ethoxylation and alkyl chain length. In addition, their method was simultaneously able to detect contaminating polyglycol ether and characterize its molecular mass distribution.

The classic method for determining the molecular weight distributions of polymers is SEC. Combining SEC with on-line spectroscopic detection can provide insight into chemical composition and molecular parameters. LC-ES-MS is compatible with the SEC conditions used in routine analysis of synthetic oligomers and polymers, and the coupling allows accurate molecular weight calibration, investigation of chemical composition, and complex mixture analysis. The fact that ES promotes the formation of multiple charged molecular ions is an advantage for the analysis of single analytes or relatively simple mixtures, as quite large molecules can be analyzed within the domain of conventional mass analyzers such as quadrupoles, but higher-molecular-weight polydisperse analytes, such as synthetic polymers, have ES mass spectra with broad, unresolved "bands" due to overlapping charge envelopes. Prior SEC provides a solution to this problem by resolving the overlapping charge envelopes. SEC ES-MS may not be a method of choice for analysis of non-polar oligomeric mixtures, because the prerequisite of the ionization technique is the presence or formation of ions in solution. However, non-polar analytes may be amenable to SEC-PB-MS.[66] Coupling SEC to Fourier transform mass spectrometry (FTMS) addresses some of the limitations inherent in using a quadrupole mass filter, such as limited resolving power and mass range. SEC/ES-FTMS is an extremely powerful tool for polymer characterization.[67]

Van der Doelen et al.[68] have compared HPLC-MS and HPLC-MS/MS data for fresh resins and aged dammar and mastic varnishes. The fragmentation behavior of triterpenoids under APCI was studied by comparing mass spectra obtained with APCI-MS and APCI-MS/MS with GC-EI-MS data for the same substances, because fragmentation patterns are better understood under electron impact conditions.

C. Contaminants in Plastics

To reduce packaging waste, the use of recycled plastics for food packaging and the re-use (refilling) of food packages is being encouraged. Any sorbed substances that are not removed by cleaning procedures for plastic containers before refilling may migrate into the packed product, resulting in off-flavors or even toxicity. LC-MS has proved to be a powerful technique for monitoring the quality and safety of recycled and reused food-contact materials. Potential contaminants that have been determined by LC-MS include surfactants,[69] mutagenic and carcinogenic heterocyclic amines,[70] radical adducts,[71] sulphonic acids,[72] chemical warfare agents,[73] fullerenes,[74] neutral pesticides, and herbicides.[75] Phenolic substances and acidic herbicides can be determined in a single run.[76]

Characteristic mass spectral data will be needed more than ever in this area to aid in elucidating the identity of completely unexpected (and hence initially unknown) substances. The interface of choice for coupling liquid chromatography with mass spectrometry is then the particle beam (PB), although its relatively low sensitivity may be a problem and recourse to concentration procedures is likely to be needed.[77]

D. Toxicological Studies

Phenylglycidyl ether (PGE) is an epoxide that is used in the production of epoxy resins and that shows *in vitro* mutagenicity against mammalian cells. Interaction of this xenobiotic with DNA leads to adducts that may play an important role in the misreplication of DNA and tumor forma-

tion. Lemière et al.[78] have discussed LC-ES-MS and LC-MS/MS of the PGE adducts.

IV. PROBLEMS AND SOLUTIONS IN THE FIELD OF MIGRATION FROM FOOD CONTACT MATERIALS: OPPORTUNITIES FOR LC-MS

Compared with gas chromatography, liquid chromatography has the following advantages: the need for complex and time-consuming sample clean-up is reduced, which minimizes possible sources of error and increases absolute recoveries; and there is no need to use "appropriate" internal standards to correct for poor recovery due to injection discrimination. There are also many food contact compounds, particularly those that have high molecular weight or polar functional groups, which cannot be readily analyzed by GC because they are not sufficiently volatile or tail badly or are thermally labile and decompose at the temperature required for GC. Liquid chromatography is preferable to gas chromatography when dealing with polar substances, high-molecular-weight substances or thermally unstable analytes.

A. Analysis of Polar Food Simulants

Three of the four food simulants (distilled water, 10% *v/v* aqueous ethanol and 3% *w/v* aqueous acetic acid) that model the food classes aqueous, alcoholic, and acidic, respectively, are not well suited for direct injection into a gas chromatograph but are more suited to liquid chromatographic analysis, especially with on-line preconcentration. Moreover, a solid-phase extraction (SPE) cartridge can be switched into the mobile phase flow path of the HPLC system so that purified analytes elute from the SPE column into the analytical column, thus eliminating off-line sample transfers including eluate collection, evaporation, reconstitution, and injection. This on-line SPE approach offers ruggedness, excellent precision, and high sample throughput. These SPE-HPLC methods are fast, consistent, and can be run automatically. There is also the chance of interfacing solid-phase microextraction (SPME) to the HPLC system. Anyway, SPE is the area of sample preparation that has seen the greatest number of innovative sorbents. It is also the technique most amenable to high-throughput automation. In fact, SPE can be so well integrated into the concept of LC-MS that in many automated applications, no clear distinction exists between SPE and LC. Table 3 summarizes the general characteristics of the most used LC-MS sample preparation techniques. So far, however, there have been no reports of integrated SPE or SPME-HPLC-MS applied in studies of migration from FCMs.

B. Analysis of Polar Food Contact Materials

Within the EU the analytical focus has hitherto been on constituents of rather nonpolar plastics (polyethylene, PE; polypropylene, PP; polyvinylchloride, PVC; etc.). Now interest is turning to more polar plastics, such as polyamides (PA), polyurethanes, and polycarbonates, and to paper and board packaging. Substances that may migrate from these materials are likely to be amenable to GC-MS analysis only after derivatization, which has a number of disadvantages:

- the derivatizing agent may be difficult to remove and interfere in the analysis, which is particularly disadvantageous when the purity of a compound is being measured;
- the derivatization conditions may cause unintended chemical changes; and
- the derivatization step increases analysis time.

For quantitative accuracy in a derivatization procedure, it is recommended that the derivatization reaction be taken to completion, and that a similarly reactive internal standard be used for quantitative determination. LC-MS may prove to be the method of choice in this area.

C. Analysis for Plastic Additives

Regulations on the use of BADGE within the EU now stipulate that 'total' BADGE concentrations should be determined, including the hy-

TABLE 3
Summary of LC-MS Sample Preparation Types

Sample Preparation Technique	Advantages	Disadvantages
Liquid-liquid extraction	Excellent selectivity; very clean extracts; trace enrichment possible.	Difficult to automate; complex method development; may have recovery problems; solvent disposal issues.
Ultrafiltration	Fast, simple; band-pass filtration with dual-stage filters.	Limited clean-up – molecular weight filter only; cannot concentrate sample; finite filter capacity.
Inmunoaffinity	Excellent clean-up and selectivity; automatable; trace enrichment.	Few commercial columns; custom columns expensive and time consuming.
Column switching LC-LC	Highly automatable with standard HPLC hardware; broad array of available column phases; very reproducible; high recoveries; high degree of trace enrichment.	Complex HPLC hardware; potential method development complications; expensive; column lifetime questions with dirty samples.
Solid-phase extraction	Many choices of media and phases, including cartidges, columns and discs; highly automatable, including automation of method development; matches well with 96-well plate format; uses less sample, solvent and time compared with LC; good trace enrichment; many good method development resources now available.	Clean-up and selectivity may be limited; expensive hardware for automation; some method development may be complex.

drolysis products and chlorinated transformation products. Philo et al.[79] reported an LC-APCI-MS method for the determination of BADGE and its hydrolysis products in the four EU food simulants. Work is needed to extend LC-MS to provide robust methods for the analysis of a wide variety of epoxides and epoxide reaction products in foodstuffs.

Other plastics additives, such as dialkylphthalate esters, diethylhexyl adipate (DEHA), epoxidized soya bean oil (ESBO) and polymeric plasticizers, can at present only be analyzed after chemical breakdown to smaller units. Of particular interest is ESBO, which may be used as a plasticizer — to increase film flexibility and provide 'cling' — but its primary function is as a heat stabilizer to protect the plastic during processing. Materials such as poly(vinyl chloride), poly(vinylidene chloride), and polystyrene frequently contain ESBO as an additive at levels ranging from 0.1 to 27%.[80] Classically, ESBO is determined by GC-FID or by GC-MS following a time-consuming extraction and transmethylation procedure.[81] Figure 11 shows a chromatogram produced by a preliminary LC-APCI-MS procedure for determination of ESBO in olive oil. Under the conditions of this procedure (reversed-phase, with a propionitrile/hexane gradient) the polar ESBO elutes earlier than the more lipophilic triglycerides of olive oil. Optimization should lead to a simpler, more rapid method for ESBO analysis than the GC procedures.

D. Analysis for Paper Chemicals

Paper and board materials are often used in contact with dry or frozen foods and only to a lesser extent are they used in direct contact with moist or fatty foods. With the main exception of contaminants that may be introduced by the use of

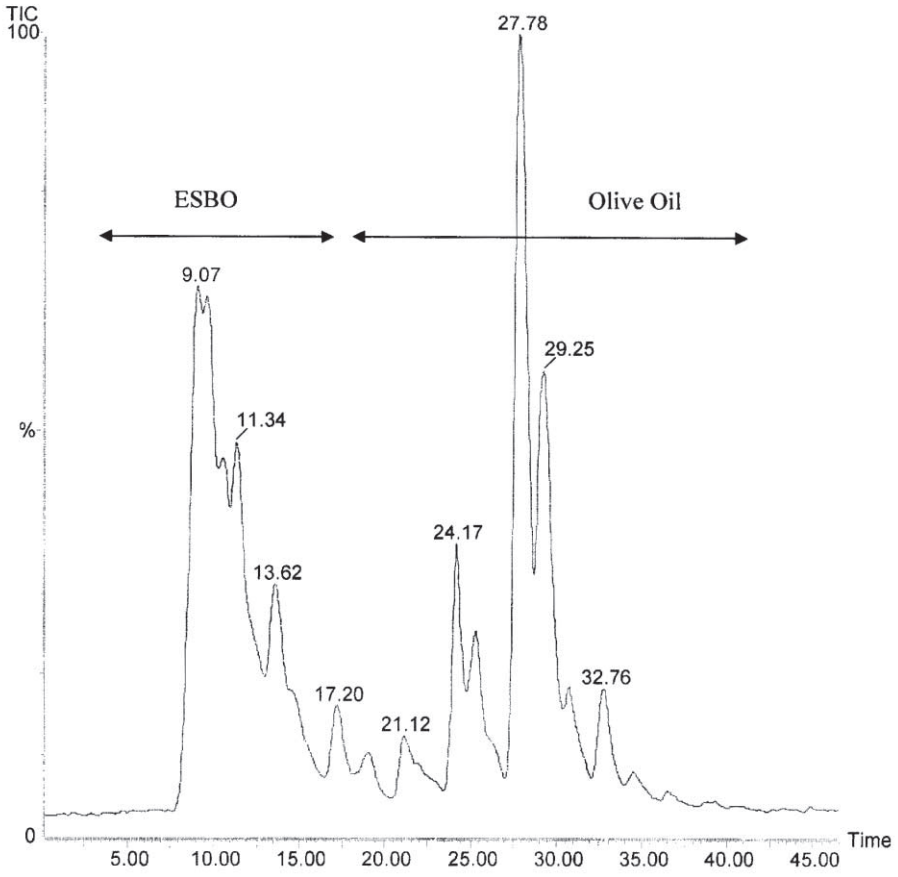


FIGURE 11. LC-APCI-MS analysis of olive oil mixed with epoxidized sobean oil (50% ESBO in olive oil).

recycled fibers, the potential for migration of paper-making chemicals seems to be rather low. Chemicals used in paper-making tend to be water soluble and derivatization is often necessary for GC analysis. Examples are anionic stilbene optical brighteners, biocides, wet-strength agents, and retention aids. The development of LC-MS methods for such chemicals therefore would be welcome.

E. Analysis for High Molecular Weight Oligomers

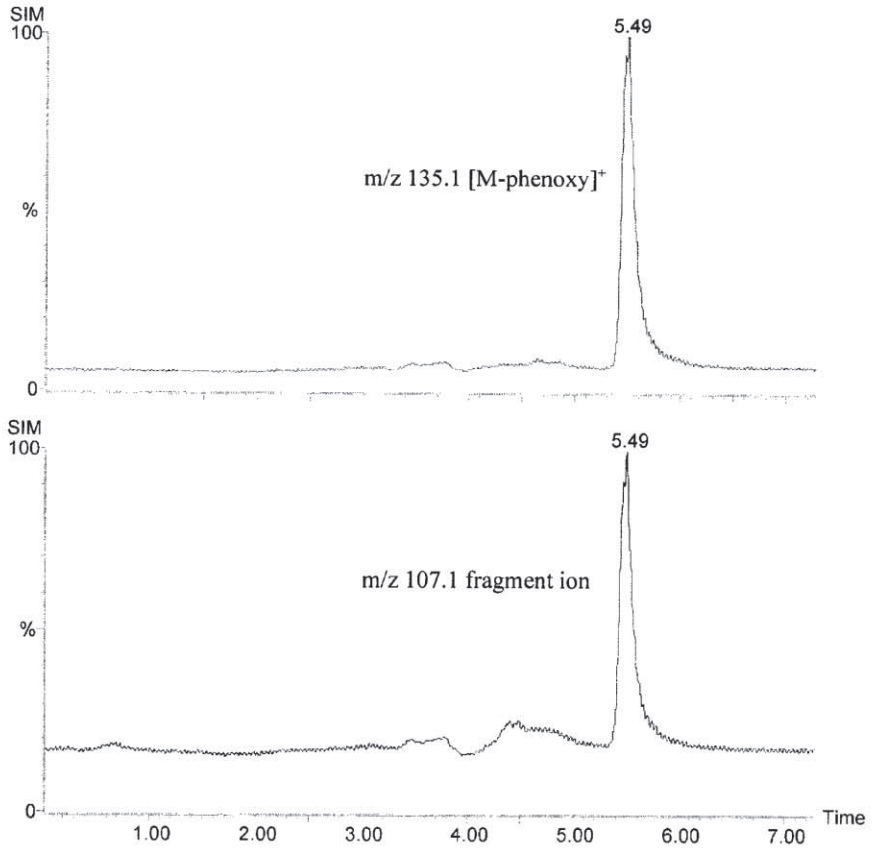
Oligomers from plastics and coatings currently attract great interest, and LC-MS has much to offer in this area. One new and interesting separation technique is gradient polymer elution chromatography (GPEC), in which polymer samples are progressively dissolved in a solvent gradient and separated on the LC system. If a mobile phase in which the polymer is poorly soluble is used at the outset, the polymer sample is deposited at the head of the LC precolumn; because the solvent strength increases during the gradient, the components of the polymer sample dissolve and are transferred to the LC column, where they are separated further prior to detection. The elution is mainly influenced by solubility, interaction with the stationary phase, and molecular weight. The advantage of the technique is that sample preparation is reduced, and that no degradation or discrimination can occur during extraction. Some interesting examples of characterization of the chemical (micro)structure of polymers by this technique have been reported in the literature. The technique coupled to MS to give molecular weight information may prove useful in determining and characterizing the fraction of plastics oligomers and polymeric additives. This information is needed in toxicological evaluations because it is conventionally assumed that there is a cut-off of molecular weight about 1000 Da, which is the limit for absorption of substances from the gastrointestinal tract.

F. Analysis of High-Consumption Liquid Food Products

Chemicals that may migrate into beverages are of special interest to a surveillance laboratory. This is because the consumption of beverages is higher than of solid foods, and so the potential exposure to migrating chemicals is higher from beverages than from foods. The largely aqueous nature of beverages presents a problem in sample concentration and presentation in GC methods of analysis, whereas an aqueous sample is well suited for direct injection into a reversed-phase LC-MS system. Aqueous samples also offer the further advantage of possibilities for on-line concentration to get superior levels of detection. For example, in the authors laboratory a direct LC-MS approach has been used in the analysis for bisphenol A and for mercaptobenzothiazole in beverages. Bisphenol A is used to manufacture coatings on the inside of cans used for certain canned foods, and to make polycarbonate FCMs (e.g., feeding bottles). BPA in beverages and in liquid infant formula can be analyzed with minimal sample preparation (usually simple dilution/precipitation with acetonitrile) and then direct analysis by LC-MS. Figure 12 shows a typical chromatogram obtained using large-volume injection LC-APCI-MS. Ionization in the APCI-positive ion mode may not be optimal, and further developments require examination of other, hopefully more sensitive ionization techniques.

G. Analysis for Biomarkers

A traditional way to estimate consumer exposure to chemicals in foods, be they migrants from packaging, contaminants from other sources, or direct food additives, is to analyze a very wide range of foodstuffs to determine the concentrations of the chemicals of interest in each, and then to calculate total intake on the basis of dietary habits, brand loyalty, etc. An attractive alternative approach is to measure so-called biomarkers of exposure. An example of this approach is an investigation of our exposure to phthalate esters[82] that in the past have been used extensively as industrial chemicals and that are quite ubiquitous in the environment and therefore in some foods. The principal metabolites of these phthalates are the monoesters[83] (Figure 13). Phthalates labeled with ^{13}C or ^{2}H have been used in volunteer studies to determine the rate of urinary excretion and



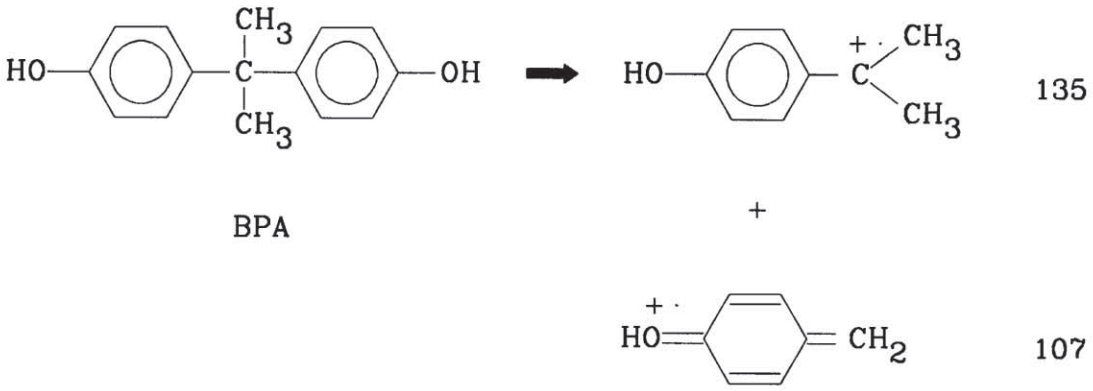


FIGURE 12. Typical LC-APCI-MS chromatograms of Bisphenol A (BPA, 50 ng/ml, 400 µl injection).

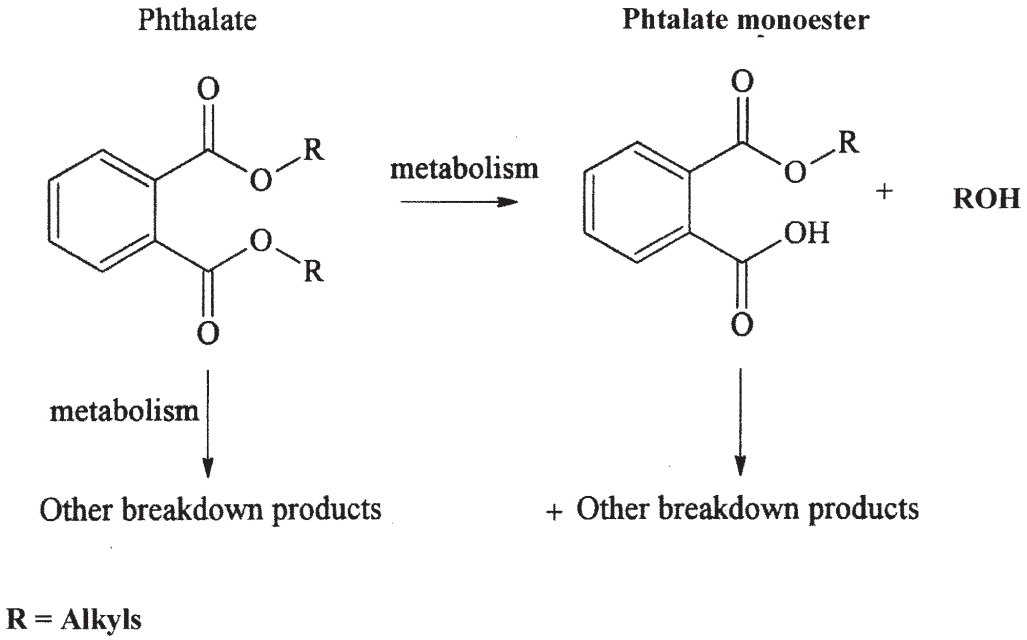


FIGURE 13. Conversion of phthalate esters to phtalate monoesters.

yield of the labeled monoester metabolites. LC-MS proved to be invaluable for this approach, because it made it possible to determine both parent substances and metabolites without prior derivatization.[84] Having established the relationship between dose and excretion, LC-MS is currently being used to analyze urine samples from more than 300 volunteers to determine their exposure to phthalates in their diet and from other sources.

H. LC-ICP-MS for Organometallics

Measurement of the total level of a metal in a sample matrix reveals very little, if anything, about its possible mobility, toxicity, or biochemical function. To provide answers to these questions, it is necessary to determine the actual chemical form, or speciation, of the element under investigation. Metal speciation is "...the qualitative identification and the quantitative determination of the individual chemical forms that comprise the total concentration of a given trace element in a sample".[85] This illustrates the important chararacteristics of metal speciation, namely, the structural identification of the metal species of interest, its accurate measurement in the presence of other interferring compounds, and the fact that the sum concentration of the metal species present equals the total concentration of the metal.

Organometallic compounds arise when a metal forms a covalent bond with a carbon atom. They occur with many different elements, but particularly mercury, arsenic, lead, tin and other heavy elements. This class of metal compound has very important consequences in terms of toxicity effects; for example, methylmercury chloride is approximately 10 times more toxic than inorganic mercury chloride, but much less toxic than dimethylmercury. Organometallic compounds also behave differently to inorganic forms in terms of mobility; some organometallic forms are more likely to penetrate lipid membranes and accumulate in tissue. A recent example of this is the identification of the active ingredient of antifouling coatings, tributyltin (TBT), in human blood and liver samples.[86]

Numerous analytical procedures have been used for the analysis of trace elemental speciation in complex matrices. However, state-of-the-art techniques are based on coupling powerful separation technology (gas chromatography (GC), capillary electrophoresis (CE), supercritical fluid chromatography (SFC), and high-performance liquid chromatography (HPLC)) to sensititive element-specific detectors (atomic absorption spectroscopy and in-

ductively coupled plasma mass spectrometry [ICP-MS]). In some instances where hydride or cold vapor generation is possible it is used to improve the detection limit by a factor of approximately 15; this also has the added bonus of removing the matrix from the analysis because the sample is detected as a gas rather than solution. The requirements of the detection system are for a low limit of detection because the levels of metal species present are much lower than the total metal content of the sample. Detection of the metal-containing species is made easier when the technique used is specific to the element of interest and this can also reduce the degree of sample preparation that is necessary. It is possible to quantify organometallic compounds down to the low ng/g level using ICP-MS and confirm their identity by APCI-MS, thus providing greater validation of the method.[87]

I. Applications in the Overall Migration (OM) Test

At present, testing plastics for OM analysis[88] into olive oil food simulant relies on the correction of observed OM value for reverse migration of olive oil into the plastic itself. Quantitation of olive oil in the plastic is typically undertaken by GC-FID following transmethylation of the triacylglycerols into individual fatty acid methyl esters (FAMEs). Although analysis of FAMEs is relatively straightforward, it is rather labor intensive. Neff and Byrdwell,[90] and Byrdwell and Emken[91] have used LC-APCI-MS for the analysis of a selection of vegetable oils. The spectra obtained displayed a protonated molecular ion $[M+H]^+$, and abundant diglyceride ions $[M-RCO_2]^+$. Mottram and Evershed[92] have also developed LC-APCI-MS methodology for the analysis of triacylglycerols; these authors' spectra displayed protonated molecular ions $[M+H]^+$, which increased in intensity with the degree of unsaturation of the triacylglycerol, $[M-RCO_2]^+$ ions caused by loss of fatty acid moieties, and in some cases $[RCO]^+$ ions derived from the fatty acid moieties themselves. The relative intensities of the $[M-RCO_2]^+$ ions provide information on the positions of the fatty acids in individual TAG species. Figure 14 shows a typical LC-APCI-MS TIC chromatogram for olive oil. Each peak corresponds to an individual triacylgycerol substance present in the oil. For example, the peak at 26.82 min corresponds to the triacylglycerol OOO (i.e., glycerol substituted at each of the three hydroxy groups with oleic acid). While olive oil concentrations are more difficult to determine by LC-APCI-MS, this type of determination does give the analyst more information concerning which acylglycerols may be absorbed by the plastic during migration testing, which could in theory give information about the crystalline state of the plastic and a more detailed understanding of the importance of swelling and leeching effects that can accelerate migration.

V. PRESENT LIMITATIONS OF LC-MS AND POSSIBLE SOLUTIONS

Although API methods (APCI and ES) are the most widely used forms of ionization for LC-MS (especially in the pharmaceutical and biological areas), there still remains a place for other ionization methods (such as PB, CF-FAB, or TSP) for certain applications. Some of the problems detected with the different LC-MS ionization methods are currently being carefully investigated.

A. Inhomogeneity of Nebulization

API sources are not as well behaved as could be desired. Although interfacing liquid flows with an atmospheric pressure source provides some practical advantages over interfacing to a low-pressure source, the challenges involved in taking ions or molecules from within a liquid at atmospheric pressure into a vacuum of 10^{-5} torr have still not been perfectly overcome.[93] Spraying the liquid into an ion source causes problems due to turbulence, variations in the efficiency of solvent vaporization, and contamination of sources with buffers. All these effects are sources of noise, instability, and variability that reduce signal-to-noise ratio. The basic problem is inhomogeneity. Although the analyte in the liquid may be well

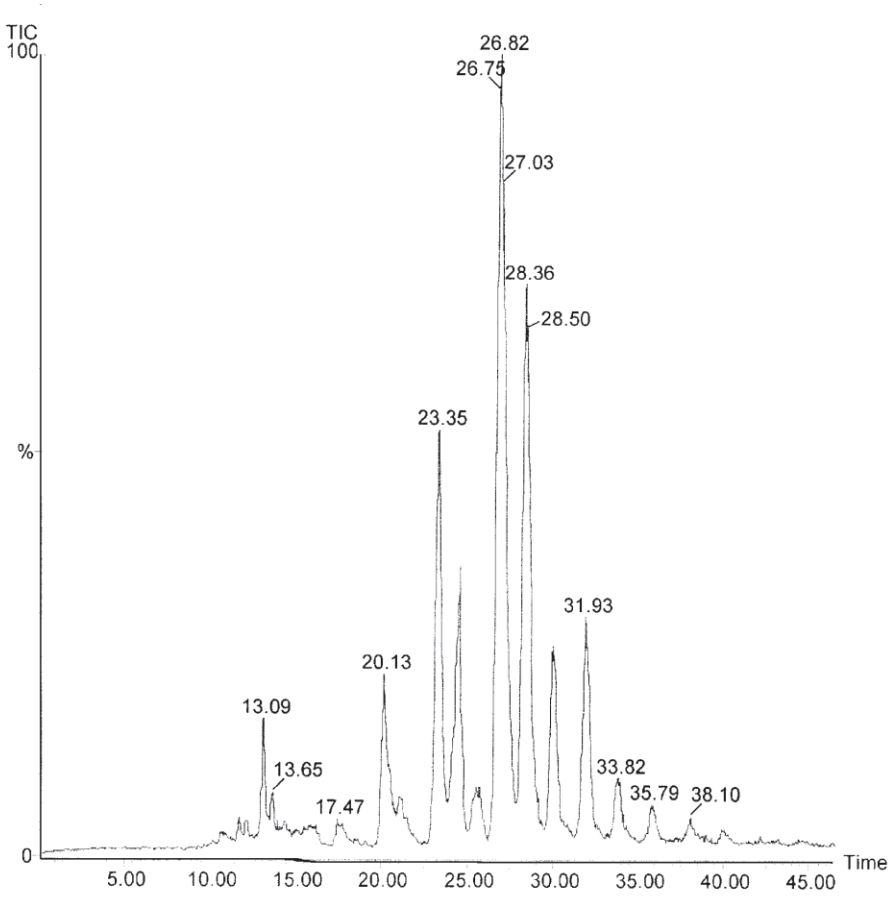


FIGURE 14. LC-APCI-MS chromatogram of olive oil (5% in THF).

mixed, the action of separating the analyte from the solvent creates inhomogeneity by generating an environment of solvent (gas), ions, liquid droplets, and solid particles with wide variations in the local concentration of analyte ions. This can result in a noisy signal (compared to the signal from an electron impact ion source, for example) and difficulty in tuning. The droplet size distribution, droplet charge distribution, solvent and buffer concentrations, reagent ion concentration, and local gas velocity can all affect sensitivity and noise level. These factors nevertheless are being improved.

B. Interferences in Atmospheric-Pressure Ionization

Components of the sample matrix or the chromatographic mobile phase can have an adverse effect on the results from an LC-atmospheric-pressure ionization-MS analysis. These adverse effects can be broadly categorized as spectral interference, system compromise, adduct formation, and ion suppression.

Spectral interference: Ions that appear at the same or nearly the same *m/z* value as the component of interest can cause spectral interference. Molecules giving similar *m/z* value can generally be separated by the chromatographic process before they enter the mass spectrometer. In situations in which it is impossible to do this, analysts can use selected reaction monitoring with an MS-MS system to make a clear distinction among the components. Finally, newer LC-MS systems that use TOF mass analyzers are generally able to distinguish much better than unit mass resolution and therefore can pull apart spectral peaks that overlap in lower-resolution systems.

System compromise: Mobile-phase components that degrade the performance of the LC-MS system, generally through precipitation in the atmospheric-pressure ionization interface, can compromise the system. The components of the mo-

bile phase that are likely to precipitate in the atmospheric-pressure ionization interface are generally buffer salts. Substituting volatile buffers or simply adjusting the pH with a volatile acid or base will solve this problem. Sample preparation techniques may be necessary before chromatography to eliminate sample matrix elements that can precipitate.

Adduct formation: Adduction of another ion with the component of interest shifts the *m/z* value at which the component of interest appears in the spectrum. Adduct ions such as sodium, potassium, and ammonium can be picked up from the sample itself, from reagents used, or the container holding the sample. Adduct formation in MS has often been used to improve signals, especially for macromolecules. However, uncontrolled adduct formation is generally undesirable and requires specific sample preparation procedures to reduce or eliminate it.

Ion suppression: Ion suppression is the result of components that suppress the ionization of or compete in the ionization process with the component of interest. Ion suppression is the most critical of these interferences because it is often the most difficult to determine. It has been demonstrated that even components of the sample that do not appear in the mass spectrum can cause ion suppression.[94] In food samples, natural variation in endogenous compound concentrations from one sample to another can cause varying levels of ion suppression. This variation in turn contributes to unacceptable variability in the signal response for the compounds of interest. In principle, the solution to this problem is either better clean-up of the sample or better chromatography conditions. If neither is possible, one must resort to calibrating instrumental response by the standards addition procedure.

The most common ions formed in API-MS are protonated molecules, symbolized $[M+H]^+$. Similarly, deprotonated molecules, $[M-H]^-$, appear in negative-ion operation. These molecules are formed through ion evaporation in electrospray (ES) and through gas-phase chemical ionization in atmospheric-pressure chemical ionization (APCI). Understanding these reactions is the basis for understanding the origin of ion-suppression effects. In positive-ion operation, the gas-phase ion-molecule reactions will cause the formation of the weakest acid, that is, the weakest proton donor.[95] For example, in the APCI analysis of the amine $R\text{-}NH_2$, water is a stronger proton donor than the amine and therefore readily gives up its proton from H_3O^+ to form the $R\text{-}NH_3^+$ ion. However, if we introduce a large amount of another compound that can form an even weaker acid than the analyte (for example, R_3N), then the gas-phase reaction will form R_3NH^+, which is a weaker acid. The $R\text{-}NH_2$ analyte will not be ionized or will be poorly ionized and therefore not seen at significant levels in the mass spectrum. The same type of proton transfer reaction can occur in ES interfaces. As an analogous model for negative ion operation, the weakest proton acceptor (weakest base) is formed. See Figure 15 for a summary of the relative proton affinity of common solvents and analyte functional groups.

Another type of ion suppression is thought to occur when very strong ion pairs are formed and not broken apart by the conditions in the API interface. Ion-pairing agents of various types can contribute to ion suppression; therefore, analysts should avoid their use in LC-MS where possible.

C. Solvent-Dependent Spectra and Compatibility with Spectral Libraries

The continued popularity of LC-PB-MS is due to its ability to yield either classic EI spectra that can be sought in spectral libraries, or solvent-independent CI spectra. Its primary limitations[96] are limited sensitivity (which makes it unsuitable for trace analysis), dependence of quantitative performance on the HPLC conditions (particularly for aqueous mobile phases), and limited linearity (particularly at low concentrations). Further fundamental studies on nebulization, desolvation, and momentum separation are required in order to improve the performance of second- and third-generation PB designs. Ultratrace analysis may prove better suited to APCI and ES interfaces, but excellent sensitivity (in the ppb or ppt range) has been achieved with PB systems by careful choice and optimization of

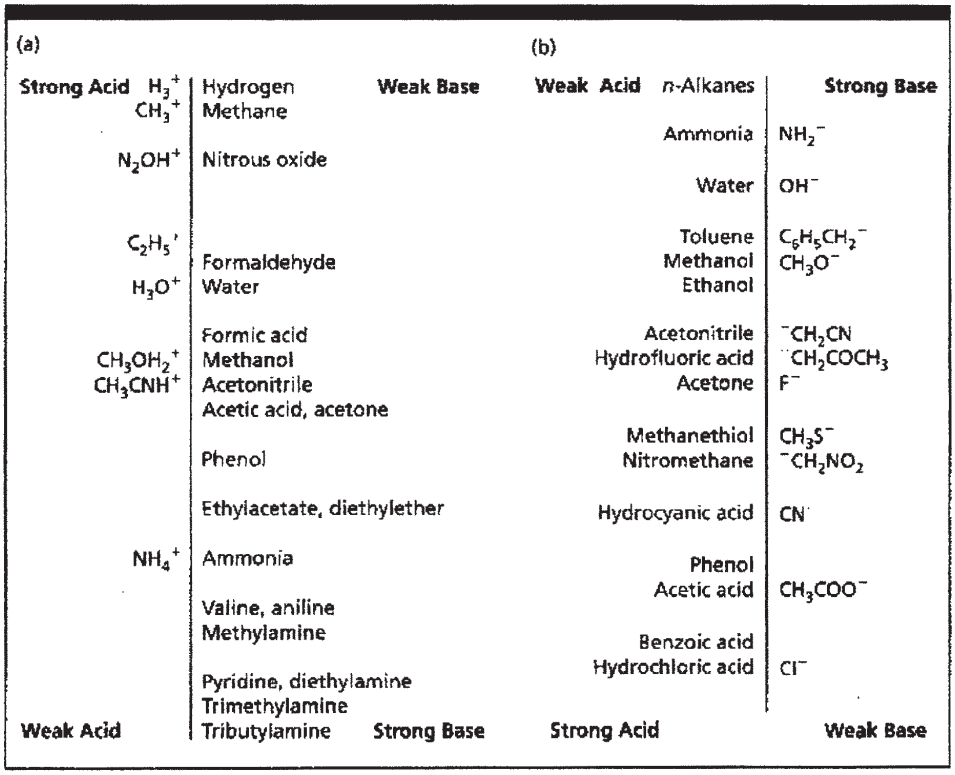


FIGURE 15. Charts of HPLC reagents and analytes and their resulting ions in the gas phase of an API interface in (a) positive and (b) negative ion modes.

sample work-up procedures, HPLC conditions, carrier addition, interface parameters, and mass spectrometric conditions.[97]

VI. FUTURE PROSPECTS

Robust and affordable API-based LC-MS systems are now becoming common throughout the world. The potential uses of LC-MS in the field of food contact material analysis are innumerable, and the technique will flourish in the EU when attention is given to the analysis of nonvolatile substances (e.g., additives, oligomers, polymers, paper and board, etc.). For most laboratories, the analysis of residues in organic extracts of food packaging or in aqueous or fatty food simulants can be performed by LC-MS. Exceptionally, direct MS analysis of both types of extract might be possible without matrix interference problems.[98,99,100,101] The analysis of residues in organic food packaging extracts can in some cases be undertaken by SFC-MS,[102,103,104] or if possible directly by on-line SFE-SFC-MS;[105,106] and the analysis of residues in aqueous food simulants, by CE-MS. In the case of better-equipped laboratories, analysis of polymer additives, monomers, and oligomers could be performed by surface techniques based on laser mass spectrometry.[107]

There are movements within the EU to standardize acceptance criteria for MS analyses. In the near future, the one or two ions generated by standard API instruments will no longer be sufficient to confirm the presence of an analyte. It is probable that at least five ions will be required for each substance. Because reanalysis on GC or LC columns of different polarity is time-consuming, it will become necessary to use MS/MS (or perhaps LC-PB-MS).

VII. CONCLUSION

LC-MS is a powerful tool that will be used increasingly in all areas of analytical chemistry. To optimize the data obtained from these analyses, analysts must understand and deal with mass spectral interferences that come from the sample itself, the mobile phase, or the environment. For-

tunately, analysts today have a wide variety of sample preparation strategies to help them deal with these issues.

Newer, more efficient, and more highly automated sample preparation procedures for LC-MS are the topics of vigorous research. As instruments based on these new automated procedures come to market, the distinction between the part of the analysis called chromatographic separation and the part called sample preparation will become less important. Instead they will be replaced with a concept of complete LC-MS system integration. SPE will continue to grow in importance because its similarity to HPLC and because of the very high degree of automation possible with the technique. Paralleling this development will be continued miniaturization of the entire analytical system, including sample preparation. Techniques such as SPME and even microdialysis and nano-LC lend themselves very well to the instrument-on-a-chip concept in which a micro autoinjector will be integrated with a sample preparation technology, chromatographic column, and MS capillary interface in a single micromachined device that attaches directly to an API-MS instrument optimised for submicroliter flow rates and sample volumes.

In conclusion, the use of LC-MS is fast becoming indispensable in quality control and research laboratories concerned with the quality and safety of food contact materials.

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