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TRACE AND RESIDUE ANALYSIS

Trace analysis is the detection of minute quantities of organic and inorganic materials. The definition of trace analysis continues to evolve. In the 1960s, it implied determinations under 0.01% of a sample. As of the mid-1990s, trace analysis is generally recognized as those determinations that represent around 0.0001%, ie, at the parts per million (ppm) level, where 1 ppm is equivalent to 1 μg/g (1). Ultratrace analysis, ie, determination below trace analysis, corresponds to levels below ppm or $<\mu$g/g (2–4). Residue analysis is the analysis of material left from an operation, ie, residual. Examples are solvents left in pharmaceuticals (qv) or pesticides (qv) left in fruits. The nature of the sample and the type of analysis to be performed, dictate methodology used. Analyses performed at or below ppm level, or those analyses where the actual analyte is at low micrograms level, are discussed herein, as are a variety of methodologies and some criteria for selection.

There are numerous applications for trace or ultratrace analyses in the chemical process industry. The following two examples highlight the need for such analyses. Although much controversy still surrounds the nature of its toxicity and possible safe levels, dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)) is frequently described as the worst poison known. It has been found to cause abortion in monkeys, even at a level of 200 parts per trillion (ppt) (5). Allowing for a hundredfold margin of safety for human exposure, the safe food level for TCDD would have to be less than 2 ppt. Polychlorinated biphenyls (PCBs) at 0.43 parts per billion (ppb) in water have been found to weaken the backbones of trout by interfering in collagen synthesis (6). The analysis of fish backbones from such water revealed excess calcium levels and a deficiency in collagen and phosphorus. The fish were also deficient in vitamin C, a cofactor in collagen synthesis. Thus it was concluded that the trout used vitamin C for detoxification of PCBs instead of for skeletal development.

The U.S. FDA monitors foods for half of the approximately 300 pesticides having official EPA tolerances as well as a number of other pesticides that have no official tolerances. Multiresidue methods, most of which are based on chromatography protocols, are employed (7). Not all pesticides are monitored on all foods and sampling (qv) is purposely biased to catch possible problems. The overall incidence of illegal pesticide residue is, however, quite small: 1% for domestic surveillance samples and 3% for imported foods. The methods employed can usually quantify residues present at 0.01 ppm. Quantitation limits range from 0.005 to 1 ppm.

Detectability limits are generally either not given in the scientific literature, or when limits are given, the units vary (8). It has been recommended (1) that grams/grams be used as the unit for data of ultratrace analyses. This would permit comparison of various detectabilities and allow easy calculation into ppm, ppb, etc. When this information is provided in molar units, the molecular weight should also be given. Moreover, the following important analytical parameters have been recommended to be reported for each method: amount present in grams in the original sample (APIOS) per mL or g; minimum amount detected (MAD) in g; minimum amount quantitated (MAQ) in g.

2 TRACE AND RESIDUE ANALYSIS

1. Frontiers of Low Level Detection

Extremely low level detection work is being performed in analytical chemistry laboratories. Detection of rhodamine 6G at 50 yoctomole (50×10^{-24} mol) has been reported using a sheath flow cuvette for fluorescence detection following capillary electrophoresis (9). This represents 30 molecules of rhodamine, a highly fluorescent molecule (see Electroseparations, electrophoresis; Spectroscopy, optical).

Claims of single molecule detection in liquid samples have been made by combining the high sensitivity of laser-induced fluorescence (lif) and the spatial localization and imaging capabilities of optical microscopy (qv) (10). This technique combines confocal microscopy, diffraction-limited laser excitation, and a high efficiency detector. The probe volume is defined latitudinally by optical diffraction and longitudinally by spherical aberration. Using an unlimited excitation throughout and a low background level, this technique allows fluorescence detection of single rhodamine molecules at a signal-to-noise (S/N) ratio of approximately 10 in 1 ms. The use of confocal fluorescence microscopy can be extended to individual, fluorescently tagged biomolecules, including deoxynucleotides, whether single-stranded primers or double-stranded deoxyribonucleic acid (DNA).

Analysis of single mammalian cells by capillary electrophoresis has been reported using on-column derivatization and laser-induced fluorescence detection (11). Dopamine and five amino acids were determined in individual rat pheochromocytoma cells after on-column derivatization.

Radioactive tracers (qv) are powerful tools for trace detection. A method of labeling proteins using ^{99m}Tc has been described. The immunoreactivity of monoclonal antibodies after radiolabeling was demonstrated by radioimmunoimaging of thrombi using a ^{99m}Tc-labeled antifibrin monoclonal antibody (12). Radiotracer imaging agents have been used for mapping sympathetic nerves of the heart (13). The radioiodination of analogues of a calichemicin constituent have been employed as a possible brain-imaging agent (14) (see Medical imaging technology).

2. Samples

2.1. Sampling

A sample used for trace or ultratrace analysis should always be representative of the bulk material. The principal considerations are determination of population or the whole from which the sample is to be drawn, procurement of a valid gross sample, and reduction of the gross sample to a suitable sample for analysis (15) (see Sampling).

The analytical uncertainty should be reduced to one-third or less of sampling uncertainty (16). Poor results obtained because of reagent contamination, operator errors in procedure or data handling, biased methods, and so on, can be controlled by proper use of blanks, standards, and reference samples.

2.2. Sample Preparation

Sample contamination must be prevented throughout the sampling procedures. Factors that can influence sampling of an analyte include impinged material, residual solvents, sample preserving method, analyte absorption, and potential contamination from the environment. The contamination from sample holders or loss to them have to be considered. No significant changes should occur in the sample when it is being held for analysis. Sample stabilization generally includes storage at low temperatures; however, any stabilization step should be validated. A review of sample composition and properties is advised. This would include number of compounds present, chemical structures (functionality) of compounds, molecular weights of compounds, pK_a values of compounds, uv spectra of compounds, nature of sample matrix (solvent, fillers, etc), concentration range of compounds in samples of interest, and sample solubility. These properties provide the bases for the

Table 1. Samples Analyzed by Solid-Phase Extraction

Sample	Matrix	Solid-phase column	Detectability, ng/mL	Ref.
3-methoxy-4-hydroxyphenyl glycol	plasma	alumina	1–10	17
oxytetracycline	fish tissue	C-8	5–10[a]	18
doxefazepam	plasma	C-18	0.1[b]	19
cortisol	urine	C-18		20
basic drugs	plasma	CN	therapeutic levels	21
Δ^9-tetrahydrocannabinol	plasma	C-18	2, 100 pg	22
ibuprofen	plasma	C-2	1.3[b]	23
ranitidine	plasma, other fluids	CN	2	24
chlorpromazine and metabolites	plasma	C-8		25
cyclotrimethylenetri-nitramine	biological fluids	C-18		26
sotalol	plasma, urine	C-8	10	27
cyclosporin A	serum, urine	cyanopropyl		28
cyclosporine	blood	C-18	10	29
growth factors	urine	C-1	200–1400-fold enrichment	30
carbamazine and metabolites	plasma	C-18	50	31

[a]Value is in ng/g.
[b]Value is in μm/mL.

selection of an extraction solvent, or a disposable cartridge for sample-extract cleanup such as Supelclean, Quick-Sep, Sep-Pak, or Bond-Elut can be used (2). A detailed discussion of sample preparation methods is available (2). Table 1 lists some samples analyzed by solid-phase extractions from different matrices.

Frequently, preconcentration of an analyte is necessary because the detector used for quantitation may not have the necessary detectability, selectivity, or freedom from matrix interferences (32). Significant sample losses can occur during this step because of very small volume losses to glass walls of the recovery containers, pipets, and other glassware.

2.2.1. Solid-Phase Microextraction

Solid-phase microextraction (SPME), used as a sample introduction technique for high speed gc, utilizes small-diameter fused-silica fibers coated with polymeric stationary phase for sample extraction and concentration (33). The trapped analyte can be liberated by thermal desorption. By using a specially designed dedicated injector, the desorption process can be shortened to a fraction of a second, producing an injection band narrow enough for high speed gc. A modified system has been investigated for the analysis of volatile compounds listed in EPA Method 624. Separation of all 28 compounds by ion trap mass spectrometric detector is achieved in less than 150 seconds.

SPME has been utilized for determination of pollutants in aqueous solution by the adsorption of analyte onto stationary-phase coated fused-silica fibers, followed by thermal desorption in the injection system of a capillary gas chromatograph (34). Full automation can be achieved using an autosampler. Fiber coated with 7- and 100-μm film thickness and a nitrogen–phosphorus flame thermionic detector were used to evaluate the adsorption and desorption of four *s*-triazines. The gc peaks resulting from desorption of fibers were shown to be comparable to those obtained using manual injection. The 7-μm fiber, designed for the analysis of semivolatile analytes, was used to investigate the effect of desorption temperature and on-column focusing temperature on peak response. The desorption temperature was found to be noncritical. An optimum focusing temperature of 40°C was used. Evaluation of 100-μm film fiber demonstrated its potential to adsorb greater quantities of analyte from solution. An absorption time of 15 minutes gave an equilibrium distribution of the solutes between the stationary and liquid phases. For the thicker film fiber the effectiveness of the desorption process was reduced at temperatures below 140°C. The linear dynamic range of the technique was evaluated over three

orders of magnitude. To enhance method sensitivity, the fiber was used to extract 0.1 ppb solution of herbicide by repeatedly adsorbing and desorbing from the same solution and focusing the combined solutes at the front of the analytical column prior to elution and analysis.

2.2.2. Supercritical Fluid Extraction

Polycyclic aromatic hydrocarbons (PAHs) have been extracted from contaminated land samples by supercritical fluid extraction (SFE) with both pure and modified carbon dioxide (35) (see Supercritical fluids). An experimental design approach, based on central composite design, was used to determine which SFE variable affects the total recovery of 16 PAHs. Four parameters were chosen for evaluation: pressure, temperature, extraction time, and percentage of methanol modifier addition. Accessible levels of each parameter were dependent on instrumental constraints. A statistical treatment of the results indicated that extraction time and percentage of modifier addition were the only variables to affect PAH recovery significantly. The levels of these variables were set at the maximum values while the pressure and temperature were maintained at their midpoint value in design. These conditions were used in a repeatability study ($n = 7$), which extracted an average of 458.0 mg/kg total PAHs from the contaminated land sample with a relative standard deviation (RSD) of 3.1%. Sequential extractions on three of these samples, using identical operating conditions, did not show the presence of PAHs. The results were compared with the Soxhlet extraction and microwave-assisted (MAE) extraction of the sample, which recovered an average of 297.4 (RSD 10.0%) and 422.9 (RSD 2.4%) mg/kg, respectively.

Removing an analyte from a matrix using supercritical fluid extraction (SFE) requires knowledge about the solubility of the solute, the rate of transfer of the solute from the solid to the solvent phase, and interaction of the solvent phase with the matrix (36). These factors collectively control the effectiveness of the SFE process, if not of the extraction process in general. The range of samples for which SFE has been applied continues to broaden. Applications have been in the environment, food, and polymers (37).

2.2.3. Microwave-Assisted Extraction

Sample preparation techniques that prevent or minimize pollution in analytical laboratories, improve target analyte recoveries, and reduce sample preparation costs were evaluated with regard to the microwave-assisted extraction (MAE) procedure for 187 compounds and four Aroclors listed in EPA Methods 8250, 8081, and 8141A (38) (see Microwave technology). The results indicate that most of these compounds can be recovered in good yields from the matrices investigated. For example, recoveries ranged from 80 to 120% for 79 of the 95 compounds listed in Method 8250; 38 of the 45 organochlorine pesticides listed in Method 8081; and 34 of 47 organophosphorus pesticides listed in Method 8141A. When recoveries from freshly spiked oil samples were compared with those of aged samples, it was found that recoveries usually decreased in the aged samples. There was more spread in recoveries with increased aging time. For 15 compounds in a reference soil, the recoveries of 14 compounds by MAE were equal to or better than recoveries obtained by Soxhlet extraction (naphthalene was an exception). For selected organochlorine pesticides, recoveries from spiked oil samples were at least 7% higher for MAE than for either Soxhlet or sonication extraction.

Comparative studies were performed to evaluate microwave digestion with conventional sample destruction procedures. These included the analysis of shellfish, meats, rocks, and soils. Generally, comparable accuracy at much shorter digestion time was found for the MAE vs the classical digestion method (39).

2.3. Sample Cleanup

The recoveries from a quick cleanup method for waste solvents based on sample filtration through a Florisil and sodium sulfate column are given in Table 2 (40). This method offers an alternative for analysts who need to confirm the presence or absence of pesticides or PCBs.

Synthetic organic chemicals have been isolated by either resin adsorption or direct methylene chloride liquid–liquid extraction. Analyses for 48 distinct chemical entities in river water from a river located in

Table 2. Florisil Filtration Recovery Efficiency[a]

Compound	Sample concentration, ppm	Recovery, %[b]	Standard deviation
aldrin	2	93	9.5
BHC	2	86	6.1
lindane	2	92	13.5
chlordane	2	79	4.7
DDD	2	69	16.0
DDE	2	92	11.0
DDT	2	89	7.2
dieldrin	2	88	3.5
endosulfan	2	91	7.6
endrin	2	98	7.6
heptachor	2	97	2.9
toxaphene	3	90	16.5
Arochlor 1016	20	93	2.0
Arochlor 1221	20	95	6.4
Arochlor 1232	20	100	8.2
Arochlor 1242	20	93	8.3
Arochlor 1248	10	95	9.1
Arochlor 1254	10	86	9.7
Arochlor 1260	10	87	12.2

[a]Ref. 40.
[b]Average recovery of triplicate analysis.

Table 3. Ultratrace Analyses Methods[a]

Method	Minimum amount detected, g
mass spectrometry	
electron impact	10^{-12}
spark source	10^{-13}
ion scattering	10^{-15}
flame emission spectrometry	10^{-12}
liquid chromatography	
ultraviolet detection	10^{-11}
fluorescence detection	10^{-12}
gas chromatography	
flame ionization	10^{-12} to 10^{-14}
electron capture	10^{-13}
combination techniques	
liquid chromatography/mass spectrometry	10^{-12}
gas chromatography/mass spectrometry	10^{-12}
electron capture (negative)/ionization mass	10^{-15}

[a]Ref. 3.

North Carolina's Piedmont area were carried out. The river was sampled at three locations several times during a 13-month period (41). Most frequently included among the 48 chemicals found were atrazine, methyl atraton (triazine herbicides), dimethyl dioxane, 1,2,4-trichlorobenzene, tributylphosphate, triethylphosphate, trimethylindolinone, and tris(chloropropyl) phosphate. Many of these chemicals are indigenous to industrial and agricultural activities in Piedmont. The concentrations were in the ng/L to mg/L range.

3. Method Validation

Statistically designed studies should be performed to determine accuracy, precision, and selectivity of the methodology used for trace or ultratrace analyses. The reliability requirements for these studies are that the data generated withstand interlaboratory comparisons.

The following principles should be used to establish a valid analytical method (42).

(1) A specific detailed description and protocol should be written (standard operating procedure (SOP)).
(2) Each step in the method should be investigated to determine the extent to which environmental, matrix, material, or procedural variables, from time of collection of material until the time of analysis and including the time of analysis, may affect the estimation of analyte in the matrix. Variability of the matrix owing to its physiological nature should be considered.
(3) A method should be validated for its intended use with an acceptable protocol. All experiments conducted to make claims or draw conclusions about the validation of the method should be documented in a method validation report.
(4) Wherever possible, the same matrix should be used for validation purposes. The stability of the analyte in the matrix during the collection process and the sample-storage period should be assessed, preferably before sample analysis. Accuracy, precision, reproducibility, response function, and specificity of the method, with respect to endogenous substances, metabolites, and known degradation products, should be established with reference to the biological matrix. With regard to specificity, there should be evidence that the substance being quantitated is the intended analyte.
(5) The concentration range over which the analyte will be determined must be defined in the method, on the basis of actual standard samples over the range (standard curve).
(6) It is necessary to use a sufficient number of standards to adequately define the relationship between concentration and response.
(7) Determination of accuracy and precision should be made by analysis of replicate sets of analyte samples of known concentration from equivalent matrix. At least three concentrations representing the entire range of the calibration should be studied: one near the minimum (MAQ), one near the middle, and one near the upper limit of the standard curve.

4. Methodologies

The commonly used methods for ultratrace analyses together with the accepted detection limits are given in Table 3. Mass spectrometry (qv), which provides coverage for all elements at sensitivities of 10^{-12} to 10^{-15} g, is a sensitive detector for organic molecules and fragments. This technique is generally used in combination with gas or liquid chromatography for ultratrace analysis (see Analytical methods, hyphenated instruments). Derivatization chromatography can further help improve selectivity and detectability of a number of compounds (3).

4.1. Atomic Absorption/Emission Spectrometry

Atomic absorption or emission spectrometric methods are commonly used for inorganic elements in a variety of matrices. The general principles and applications have been reviewed (43). Flame-emission spectrometry allows detection at low levels (10^{-12} g). It has been claimed that flame methods give better reproducibility than electrical excitation methods, owing to better control of several variables involved in flame excitation.

Table 4. Elemental Detection Limits by Flame Emission Spectrometry[a]

Elements	Emission lines, nm	Atomizer		
		Nebulizer flame, ng/mL	Rod-in-flame, pg	Particles-in-flame, pg
Ag	328.1	3	5	0.2
Ba	553.6	1	3	0.2
Ca	422.7	1	10	1
Cr	425.4	2	2	0.2
Cs	852.1	5×10^{-3}	0.03	10^{-4}
Cu	327.4	1	3	0.2
Eu	459.4	0.3	1	0.1
Ga	417.2	2	3	0.2
In	451.1	0.5	3	0.2
K	766.5	0.1	1	0.01
Li	670.8	10^{-4}	0.003	10^{-4}
Mg	285.2	3	10	1
Mn	403.1	1	1	0.05
Na	589.0	0.1	1	0.01
Rb	794.8	0.005	0.03	0.001
Sr	460.7	0.03	0.1	0.01
Tl	535.0	1	10	0.5
Yb	398.8	0.3	1	0.1

[a]Ref. 3.

Detection limits for selected elements by flame-emission spectrometry given in Table 4. Inductively coupled plasma emission spectrometry may also be employed.

4.2. Neutron Activation Analysis

A radiochemical neutron activation analysis technique for determination of 26 elements, including the emitting elements Th and U and Cu, Fe, K, Na, Ni, and Zn, has been developed (44). The radiochemical separation was performed by anion exchange on Dowex 1×8 column from HF and HF–NH_4F medium, leading to selective removal of the matrix-produced radionuclides ^{46}Sc, ^{47}Sc, ^{48}Sc, and nearly selective isolation of ^{239}Np and ^{233}Pa, the indicator radionuclides of U and Th, respectively. For K, Na, Th, and U, a limit of detection of 30, 0.05, 0.03, and 0.07 ng/g, respectively, was achieved. For the other elements, the detection limits were between 0.002 ng/g for Ir and 45 ng/g for Zr.

4.3. Thin-Layer Chromatography

The most commonly used approach in thin-layer chromatography (tlc) entails separations on a silica (qv) gel plate where the silica gel is coated as a thin layer on a glass plate. The plate is developed using the mobile phase of choice after a sample has been applied to the starting line of the plate. Quantification is achieved directly by scanning the plate or indirectly by scraping and eluting the sample. A tlc assay, performed for the determination of rifampicin and its degradation products in drug-excipients interaction studies (45), involved a mobile phase consisting of chloroform–methanol–water (80:20:2.5). The peaks were quantified by densitometric evaluation of the chromatograms. The method gave a limit of detection of 10 ng per band and good precision and linearity in the range of 50–3000 ng per band for rifampicin, 3-formylrifamycin SV, rifampicin *N*-oxide, and 25-desacetylrifampicin, and 100–350 ng per band of rifampicin quinone.

A rapid tlc immunoaffinity chromatographic method has been reported for quantitation in serum of an acute phase reactant, C-reactive protein (CRP), which can differentiate between viral and bacterial infections

Table 5. Solid-Phase Automated Immunoassay Analyzers[a]

Instrument	Manufacturer	Tests/h	Phase/Sepn	Detection[b]	Analytes/run
ACS-180	Ciba-Corning	90–180	magnetic	chemi	13
Access	Sanofi Diagnostics	100	magnetic	chemi	24
Affinity	Becton-Dickinson	20–30	coated cuvet	color	[c]
AIA-600	Tosoh-Medics	60	magnetic	fluor	[c]
AIA1200DX	Tosoh Medics	120	magnetic	fluor	21
Cobas Core	Roche Diagnostics	100–150	magnetic	color	10
ES300	Boehringer Man.	120	coated tube	color	12
Immulite	Diagnostic Products	120	centrifugation	chemi	12
Immuno 1	Miles/Technicon	120	magnetic	color	16
IMX	Abbot Diagnostics	32–48	glass fiber	fluor	1
Luminomaster	Sankyo Co.	120	coated tube	chemi	20
Opus	PB Diagnostics	50–190	dry reagent	fluor	[c]
Opus Magnum	PB Diagnostics	50–190	dry reagent	fluor	>20
Radius	Bio-Rad	80–125	coated well	color	12
SRI	Serono-Baker	31–60	magnetic	color	[c]
Stratus Intelect	Baxter Diagnostics	45–72	radial	fluor	1
System 7000	Biotrol	100	magnetic	color	20
Vidas	Bio Merieux	36–45	coated tube	fluor	[c]
Vista	Syva	38–76	magnetic	fluor	15

[a]Ref. 96.
[b]Methods: chemi = chemiluminescence; color = colorimetry; and fluor = fluorescence.
[c]Manual loading.

Table 6. Thermal Ionization ms

Sample	Elements	Reference
precipitates	Fe, Mn, Zn, Cu, Ni, Pb	110
aerosol samples	Cd, Cu, Pb, Zn	111
lake sediments	$^{240}Pu/^{239}Pu$	112
marine sediments, tissues	Cu, Zn, Cd, Pb	113
aerosol particulates	Cr, Fe, Ni, Cu, Zn, Cd, Ti, Pb	114
marine sediments SRM 1941	S	115
reference materials	Tl	116
surface waters	Cu, Zn, Cd, Pb	117

Table 7. Detection Techniques for the Explosives[a]

Technique	Type of explosive	Basis of detection
	Radiation measurements	
nmr, esr	organic	structural information
x-ray absorption	inorganic, organic	heavy metals, density
x-ray emission	inorganic	heavy metals
x-ray diffraction	solid, organic/inorganic	crystallinity
γ-ray absorption	organic nitro, inorganic	nitrogen density
thermal neutron activation	organic nitro, inorganic	nitrogen density
fast neutron activation	organic nitro, inorganic	C, O, N densities
	Vapor measurements	
ms	organic	molecular structure
gc/ec	organic	chromatographic properties
gc/chemluminescence	organic, nitro	chromatographic properties

[a]Ref. 123.

(46). The analysis is based on the sandwich assay format using monoclonal antibodies directed against two sites of CRP (see Immunoassays). One of the antibodies is covalently bound to defined zones on a thin-layer immunoaffinity chromatography membrane, while the other antibody is covalently bound to deeply dyed blue latex particles. After incubation (CRP sample and latex particles), the CRP–latex immunocomplex is allowed to migrate along the immunoaffinity chromatography membrane. In the presence of antigen, a sandwich is formed between the CRP–latex immunocomplex and membrane-bound antibodies, resulting in the appearance of blue lines on the membrane. Antibody immobilization on the tlc membrane is made with a redesigned piezoelectric-driven ink-jet printer. The time required for analysis is less than 10 minutes. Quantitation is achieved either by counting the lines visually, using scanning reflectrometry, or using a modified bar-code reader. The limit of detection was estimated to be in the low femtomolar range by visual detection.

A number of compounds have been quantified by tlc or high performance thin-layer chromatography (hptlc) using absorption or fluorescence scanning densitometry (47). An example of trace determination relates gentiopicroside in various biological matrices with 40 ng/spot sensitivity by scanning at 270 nm (48). Using hptlc gave more consistent data and better precision when compared to hplc and a commercial elisa kit for determination of aflatoxins in peanut butter (49). Hptlc was combined with a new immunoblot approach called elisagram, to detect and quantify zearalenone and aflatoxin families at the picogram level (50). Aflatoxins and trichothecenes were separated and identified in submicrogram quantity by 2-D tlc/FAB-ms (51). Vitamin B_1 was quantified in pharmaceutical products by silica gel hptlc involving post-chromatography derivatization with potassium hexacyanoferrate(III)–sodium hydroxide reagent (500 pg/spot sensitivity) and fluorodensitometry (52).

4.4. Gas Chromatography

Gas chromatography is a technique utilized for separating volatile substances (or those that can be made volatile) between two phases, one of which is a gas. Purge-and-trap methods are frequently used for trace analysis. Various detectors have been employed in trace analysis, the most commonly used being flame ionization and electron capture detectors.

On-column gas chromatographic detection of nicotine at low picogram levels has been reported. Nicotine is first subjected to chemical derivatization with heptafluorobutyric anhydride in the presence of pyridine (53). The high yield reaction results in the opening of the *N*-methylpyrrolidine ring of nicotine to concomitant formation of a highly electrophilic *N*,*O*-diheptafluorobutyryl derivative. After the extraction of nicotine derivative into isooctane, it is subjected to splitless capillary gas chromatographic analysis using a ^{61}Ni electron-capture detector and moderately polar fused-silica capillary column. The nicotine derivative can be detected on-column at levels below 5 pg.

Gas chromatography/tandem mass spectrometry (gc/ms/ms) using selected reaction monitoring was applied to the analysis of urinary metabolites of sulfur mustard, derived from lyase pathway and from hydrolysis (54). In the case of lyase metabolites, a limit of detection of 0.1 ng/mL was obtained, compared to 2–5 ng/mL using single-stage gc/ms and selected ion monitoring. The gc/ms/ms methodology was less useful when applied to the analysis of thiodiglycol bis(pentafluorobenzoate) using negative ion chemical ionization although selected reaction chromatograms were cleaner than selected ion chromatograms. The advantage of using gc/ms/ms was demonstrated by the detection of low levels of -lyase metabolites in the urine of casualties who had been exposed to sulfur mustard.

The composition of technical DDT was investigated using achiral and chiral high resolution gas chromatography (hrgc) and electron-ionization mass spectrometry (ei/ms). 2,4′-DDT and 2,4′-DDD, two important components of technical DDT, were enantiomerically resolved by chiral hrgc using silylated β-cyclodextrin and by chiral hplc with permethylated γ-cyclodextrin as chiral selectors (55) (see Chiral separations (Supplement)). The (+)- and (−)-enantiomers were assigned by chiral hplc using chiroptical measurements. Enantiopure isolates were then used to identify these enantiomers in chiral hrgc analyses. Previous data indicated (+)- and

(−)-2,4′-DDT to have (*S*)- and (*R*)-configuration, respectively, but the absolute configurations for (+)- and (−)-2,4-DDD were hitherto unknown. These have been assigned via the reductive dechlorination of individual 2,4′-DDT enantiomers, which proceeded stereoselectively to the corresponding 2,4-DDD enantiomers. The results showed (+)- and (−)-2,4′-DDD to have (*R*)- and (*S*)-configurations, respectively. The enantiomers of 2,4′-DDD thus have reverse signs of rotation for polarized light compared to the 2,4′-DDT enantiomers with the same configuration. The method should be useful for the analysis of environmental and biological samples.

4.5. High Pressure Liquid Chromatography

High pressure liquid chromatography (hplc), frequently referred to as simply lc or as high performance liquid chromatography, is used in virtually all fields of chemistry. Nonvolatile or thermally labile compounds are best separated by hplc. Although techniques such as adsorption and ion-exchange chromatography have been used, the technique of choice is reversed-phase liquid chromatography (rplc). In rplc the stationary phase is nonpolar and the mobile phase is polar and its polarity can be suitably changed.

In hplc, detection and quantitation have been limited by availability of detectors. Using a uv detector set at 254 nm, the lower limit of detection is 3.5×10^{-11} g/mL for a compound such as phenanthrene. A fluorescence detector can increase the detectability to 8×10^{-12} g/mL. The same order of detectability can be achieved using amperometric, electron-capture, or photoionization detectors.

Hplc is capable of routine determination at the nanogram range (3). Using special techniques it is possible to perform analysis even when only a few nanograms of the analyte is available. Detection limits of a picogram or less have been demonstrated with the state-of-the-art capabilities. Achieving these low limits depends on the equipment, chromatographic conditions, special techniques, and the individual sample. The use of special techniques such as on-line derivatization assumes a great significance when only a small quantity of sample is available, since this includes sample enrichment as a means to improve detectability. To optimize detectability at trace or ultratrace levels, the user needs to have a thorough understanding of separation processes and various factors that affect them (56).

Equipment can play a significant role. Peak broadening arises from dispersion and mixing phenomena that occur in the injector, column connecting tubes, and detector cell, as well as from electronic constraints that govern the response speed of the detector and recorder. The quality of an instrument may therefore be judged by its ability to minimize extra-column band broadening and reproduce retention volumes. The constancy of retention volumes is primarily a function of solvent delivery system. The independent factors that contribute to extra-column band broadening can be treated as additive in their second moments or variances (2), according to the following relationship:

Total variance measured from chromatogram = column variance + variance due to instrument volumes + variance due to electronic response time

The assumption that these individual contributions are independent of one another may not be true in practice. For an accurate calculation of instrument variance, it may be necessary to couple some of the individual contributions.

For an analyte of molecular weight 5000 and good chromatographic conditions, most photometric detectors can be expected to provide detection limits of 2–5 ng. Improvement into the mid-picogram or lower range normally requires the use of more sensitive detection means such as fluorescence or electrochemical detectors.

A study was conducted to measure the concentration of D-fenfluramine HCl (desired product) and L-fenfluramine HCl (enantiomeric impurity) in the final pharmaceutical product, in the possible presence of its isomeric variants (57). Sensitivity, stability, and specificity were enhanced by derivatizing the analyte with 3,5-dinitrophenylisocyanate using a Pirkle chiral recognition approach. Analysis of the calibration curve data and quality assurance samples showed an overall assay precision of 1.78 and 2.52%, for D-fenfluramine HCl and L-fenfluramine, with an overall intra-assay precision of 4.75 and 3.67%, respectively. The minimum quantitation

limit was 50 ng/mL, having a minimum signal-to-noise ratio of 10, with relative standard deviations of 2.39 and 3.62% for D-fenfluramine and L-fenfluramine.

A reversed-phase isocratic hplc method has been developed for the determination of AG-331, a novel thymidylate synthase inhibitor, in human serum and urine (58). The method involves a solid-phase extraction from C-18 cartridges without addition of an internal standard. The methanol eluent is evaporated under nitrogen at 40°C, and reconstituted in mobile phase, acetonitrile–water (35:65) containing 25 m*M* ammonium phosphate. Separation of AG-331 was obtained on a C-18 column at a flow rate of 1 mL/min. Chromatographic signals were monitored by a photodiode array detector at a primary wavelength of 457 nm with a bandwidth of 4.8 mm. Standard curves are linear in the range of 22–175 ng/mL in plasma and 44–2175 ng/mL in urine, respectively. The extraction recovery ranged from 92.9 to 102.4%. Intraday coefficient of variation was less than 9.5%, and interday coefficient of variation was less than 14.3% for AG-331 concentration of 44 ng/mL. This method can be used to characterize the pharmacokinetics of AG-331 in cancer patients as part of ongoing phase I trials.

An hplc assay was developed suitable for the analysis of enantiomers of ketoprofen (KT), a 2-arylpropionic acid nonsteroidal antiinflammatory drug (NSAID), in plasma and urine (59). Following the addition of racemic fenprofen as internal standard (IS), plasma containing the KT enantiomers and IS was extracted by liquid–liquid extraction at an acidic pH. After evaporation of the organic layer, the drug and IS were reconstituted in the mobile phase and injected onto the hplc column. The enantiomers were separated at ambient temperature on a commercially available 250×4.6 mm amylose carbamate-packed chiral column (chiral AD) with hexane–isopropyl alcohol–trifluoroacetic acid (80:19.9:0.1) as the mobile phase pumped at 1.0 mL/min. The enantiomers of KT were quantified by uv detection with the wavelength set at 254 nm. The assay allows direct quantitation of KT enantiomers in clinical studies in human plasma and urine after administration of therapeutic doses.

Post-column in-line photochemical derivatization permits fluorescence detection of the common aflatoxins B1, B2, G1, and G2 (60). Chromatographic evidence indicates that photolysis causes the hydration of the nonfluorescent B1 and G1 components to B2a and G2a components, respectively. Analysis of naturally contaminated corn samples show no interfering peaks and permits the determination of 1 and 0.25 ppb for B1 and B2, respectively.

Derivatization is useful for detection of compounds such as amino acids and amines that lack easily detectable groups. For similar reasons, saccharides, as a class of compound, elicit much interest. Two derivatization schemes have been reported using benzamide (61) and FMOC–hydrazine (62) to produce fluorescent products.

An on-line concentration, isolation, and liquid chromatographic separation method for the analysis of trace organics in natural waters has been described (63). Concentration and isolation are accomplished with two precolumns connected in series: the first acts as a filter for removal of interferences; the second actually concentrates target solutes. The technique is applicable even if no selective sorbent is available for the specific analyte of interest. Detection limits of less than 0.1 ppb were achieved for polar herbicides (qv) in the chlorotriazine and phenylurea classes. A novel method for determination of tetracyclines in animal tissues and fluids was developed with sample extraction and cleanup based on tendency of tetracyclines to chelate with divalent metal ions (64). The metal chelate affinity precolumn was connected on-line to reversed-phase hplc column, and detection limits for several different tetracyclines in a variety of matrices were in the 10–50 ppb range.

A new cyanide dye for derivatizing thiols has been reported (65). This thiol label can be used with a visible diode laser and provide a detection limit of $8 \times 10^{-6}\ M$ of the tested thiol. A highly sensitive laser-induced fluorescence detector for analysis of biogenic amines has been developed that employs a He–Cd laser (66). The amines are derivatized by naphthalenedicarboxaldehyde in the presence of cyanide ion to produce a cyanobenz[f]isoindole which absorbs radiation at the output of He–Cd laser (441.6 nm). Optimization of the detection system yielded a detection limit of $2 \times 10^{-12}\ M$.

Primary and secondary alcohols can be tagged using a fluorescence derivative (67). The detection limit for 1-propanol was 70 fmol for a 10-mL injection volume. Disodium EDTA and calcium chloride have been used

as fluorescence intensity for determination of tetracycline antibiotics (68). The largest fluorescence increasing reagents for tetracycline was produced in a mobile phase when concentrations of EDTA and $CaCl_2$ were 25 and 35 m*M*, respectively, and pH was 6.5. The detection limit of the method ranged from 49 to 190 pg for three different tetracycline compounds. A post-column on-line immunochemical detection system was utilized for a very selective and sensitive method for determination of digoxin and digoxigenin (69). Fluorecine-labeled antibodies are used to target the chosen analytes, and the fluorescence detection system provides detection limits of 200 and 50 fmol, respectively, for digoxin and digoxigenin.

The limits of lifetime detection and resolution in on-the-flight fluorescence lifetime detection in hplc were evaluated for simple, binary systems of polycyclic hydrocarbons (70). Peak homogeneity owing to coelution was clearly indicated for two compounds having fluorescence lifetime ratios as small as 1.2 and the individual peaks could be recovered using predetermined lifetimes of the compounds. Limits of lifetime detection were determined to be 6 and 0.3 pmol for benzofluoranthene and benzo[k]fluoranthene, respectively.

A protein-binding assay (BA) coupled with hplc provided a highly sensitive post-column reaction detection system for the biologically important molecule biotin and its derivative biocytin, biotin ethylenediamine, 6-(biotinoylamino) caproic acid, and 6-(biotinoylamino)caproic acid hydrazide (71). This detection system is selective for the biotin moiety and responds only to the class of compounds that contain biotin in their molecules. In this assay a conjugate of streptavidin with fluorescamine isothiocyanate (streptavidin–FITC) was employed. Upon binding of the analyte (biotin or biotin derivative) to streptavidin–FITC, an enhancement in fluorescence intensity results. This enhancement in fluorescence intensity can be directly related to the concentration of the analyte and thus serves as the analytical signal. The hplc/BA system is more sensitive and selective than either the BA or hplc alone. With the described system, the detection limits for biotin and biocytin were found to be 97 and 149 pg, respectively.

An ion chromatographic system that included column switching and gradient analysis was used for the determination of cations such as Na^+, Ca^{2+}, Mg^{2+}, K^+, and NH^+_4 and anions such as Cl^-, NO^-_2, NO^-_3, and SO^{2-}_4 in fog water samples (72). Ion-exchange chromatography compares very well with more generally used spectroscopic techniques for cation determinations. Determination limits range from 6 μg/L for Na^+ to 40 μg/L for K^+. Analysis of natural fog water samples can be performed at cost and time savings as compared to other techniques. Ion chromatography with self-regenerating suppression and conductivity detection was used for the simultaneous determination of Na^+, NH^+_4, K^+, Mg^{2+}, and Ca^{2+} in melted snow samples from high alpine sites (73). In order to determine low concentrations of winter snow samples (<2 g/kg), preconcentration of the sample was applied, resulting in better detection limits of 0.1–0.4 g/kg, depending on the cation.

4.6. Liquid Chromatography/Mass Spectrometry

Increased use of liquid chromatography/mass spectrometry (lc/ms) for structural identification and trace analysis has become apparent. Thermospray lc/ms has been used to identify by-products in phenyl isocyanate precolumn derivatization reactions (74). Five compounds resulting from the reaction of phenylisocyanate and the reaction medium were identified: two from a reaction between phenyl isocyanate and methanol, two from the reaction between phenyl isocyanate and water, and one from the polymerization of phenyl isocyanate. There were also two reports of derivatization to enhance either the response or structural information from thermospray lc/ms for linoleic acid lipoxygenase metabolites (75) and for cortisol (76).

A method for lc/ms of carotenoids has been reported that uses a C-30 reversed-phase column and a gradient solvent system containing methanol–methyl *tert*-butyl ether–ammonium acetate at a flow rate of 1.0 mL/min (77). The entire hplc column effluent passes through a photodiode array absorbance detector and then into the electrospray lc/ms interface without solvent splitting. In this way maximum sensitivity is achieved for both the diode array detector, which records the uv/visible spectra of each carotenoid, and mass spectrometer, which measures the molecular ion of each carotenoid. Molecular ions without evidence of any fragmentation were observed in the electrospray mass spectra of both xanthophylls and carotenes. In order to enhance the formation

of molecular ions, solution-phase carotenoids oxidation was carried out by means of post-column addition of halogenated solvents to the hplc effluent. Several different halogenated solvents were evaluated, including chloroform, 2,2,3,3,4,4,4-heptafluoro-1-butanol, 2,2,3,3,4,4,4-heptafluorobutyric acid, 1,1,1,1,3,3,3-hexafluoro-2-propanol, and trifluoroacetic acid. Among these halogenated solvents, 2,2,3,3,4,4,4-heptafluor-1-butanol at a concentration of 0.1% was found to produce the best combination of carotenoid molecular ion abundance and reproducibility. The limits of detection for lutein and β-carotene were between 1 and 2 pmol each, which was a hundredfold lower than the detection limit of the photodiode array detector signal.

The development of methods of analysis of triazines and their hydroxy metabolites in humic soil samples with combined chromatographic and ms techniques has been described (78). A two-way approach was used for separating interfering humic substances and for performing structural elucidation of the herbicide traces. Humic samples were extracted by supercritical fluid extraction and analyzed by both hplc/particle beam ms and a new ms/ms method. The new ms/ms unit was of the tandem sector field-time-of-flight/ms type.

Liquid chromatography/thermospray mass spectrometric characterization of chemical adducts of DNA formed during *in vitro* reaction has been proposed as an analytical technique to detect and identify those contaminants in aqueous environmental samples which have a propensity to be genotoxic, ie, to covalently bond to DNA (79). The approach for direct acting chemicals includes the *in vitro* incubation of DNA with contaminated aqueous samples at 37°C, pH 7.0 for 0.5–6 h, followed by enzymatic hydrolysis of the DNA to deoxynucleosides and lc/ms of the resulting solution. A series of allylic reagents was used to model reactive electrophiles in synthetic aqueous samples to demonstrate that adduct formation was linear with both contaminant concentration and electrophilic activity potential. The characterization can also estimate the proportion of bonding to different sites on a base, for instance, the ratio of oxygen- vs nitrogen-bonding products, which is an important parameter in assessing the genotoxicology of chemicals. Electrospray lc/ms interface has been used to study various positive ions, including NH^+_4, Na^+, K^+, Cs^+, and Ca^+, and the protonated BH^+ ions of 30 organic nitrogen bases (B) (80). Detection limits in the subfemtomole to attomole range were achieved. The sensitivity for organic bases is pH-dependent and increase as the concentration of BH in solution increases. On-line hplc, electrospray ionization (es), and ms have been applied to the separation and identification of brevotoxins associated with red tide algae (81). Brevotoxins, toxic polyethers produced by the marine dinoflagellate *Gymnodinium breve*, are responsible for fish kills, and pose certain health risks to humans. The lc/ms method employs reversed-phase microbore hplc on a C-18 column with a mobile phase consisting of 85:15 methanol–water, a flow rate of 8 μL/min, and a post-column split ratio of 3:1 (uv absorbance detector/ms). A brevotoxin culture sample was found to contain at least six components, including two well-separated peaks corresponding to brevotoxin PbTx- and PbTx-1, as well as unknown compounds, including one with a molecular mass of 899 DA (possibly an isomer of PbTx-9). The brevotoxin molecules exhibited a high tendency to bind to alkali cations in the positive ion es/ms mode. For standard PbTX-9, PbTx-2, and PbTx-1, brevotoxins analyzed on the lc/ms system, the detection limits (employing ms scans of 100 m/z units) were determined to be less than 600 fmol, 1 pmol, and 50 fmol, respectively (S/N = 3).

Using capillary hplc, femtomole amounts of recombinant DNA-derived human growth hormone (rhgh) have been successfully detected from solutions at nanomolar concentrations (82). A sample of rhgh that was recovered from rat serum was analyzed by capillary reversed-phase hplc, using both acidic- and neutral-pH mobile phases, as well as by capillary ion-exchange chromatography. Submicrogram amounts of rhgh were also analyzed by tryptic mapping, using capillary hplc, and the resulting peptides were identified by capillary lc/ms.

4.7. Capillary Electrophoresis

Capillary electrophoresis (ce) is an analytical technique that can achieve rapid high resolution separation of water-soluble components present in small sample volumes. The separations are generally based on the principle of electrically driven ions in solution. Selectivity can be varied by the alteration of pH, ionic strength,

electrolyte composition, or by incorporation of additives. Typical examples of additives include organic solvents, surfactants (qv), and complexation agents (see Chelating agents).

The inorganic composition of rat airway surface has been analyzed by ce (83). The pulmonary airways are covered by a layer of airway surface fluid (ASF), which is typically $<30\ \mu$m in thickness. ASF composition is an important factor in the pathogenesis of several lung diseases, including cystic fibrosis. Because of the very small volume of ASF, it is difficult to determine its composition, particularly for inorganic ions, since sampling by lavage is not suitable. Using nanoliter injected volumes, capillary electrophoresis is ideally suited to ASF analysis. A novel technique has been developed using separate sampling and injection capillaries whereby microliter volumes of ASF (typically 100 nL) can be collected from airways and then analyzed by ce. Cations (Na^+, K^+, $Ca^+{}_2$, $Mg^+{}_2$), and anions including Cl^- are quantitated (RSD $<10\%$) using indirect uv detection. In healthy rat lungs, ASF was found to be hypotonic, consistent with observations made in human airways.

A newer precolumn reagent for amino acid determination, 2-(9-anthryl)ethyl chloroformate (AEOC), was introduced to obtain higher sensitivity in two capillary separation techniques, lc and ce (84). Replacement of the chromophore in the (9-fluorenyl)-methyl chloroformate (FMOC) reagent by anthracene resulted in a reagent having very high molar absorptivity (ϵ_{256} = 180,000 L/(mol·cm)) permitting AEOC-tagged species to be detected at nanomolar levels using a uv absorbance detection in standard 50 mm ID fused-silica capillaries. Weaker absorption bands match the uv argon laser lines of 351 and 368 nm, which allows for convenient lif detection. In this mode picomolar limits of detection are obtained.

Two different mixtures of peptides and alkaloids (qv) have been analyzed by ce/uv/ms using sims to determine whether this technique can detect trace impurities in mixtures (85). The first mixture consisted of two bioactive peptide analogues, which included Lys-bradykinin (kallidin) and Met-Lys-bradykinin. The presence of 0.1% Lys-bradykinin was detected by sim ce/ms but not by ce/uv at 0.1% level as it migrated from the capillary column prior to the main component, Met-Lys-bradykinin. The second mixture consisted of two antibacterial alkaloids, berberine and palmitine. The presence of 0.15% palmitine was detected by ce/uv and sim ce/ms at 0.15% level as it migrated from the capillary column, following the main component berberine. This technique can provide a complementary technique for trace components in such sample mixtures.

Determination of catecholamines by capillary zone electrophoresis using laser-induced fluorescence detection was performed on low concentration samples derivatized with naphthalene-2,3-dicarboxaldehyde to give highly fluorescent compounds (86). When the borate concentration in the derivatization medium was decreased from 130 to 13 mM, sensitivity for noradrenaline and dopamine was greatly enhanced, whereas resolution between the two compounds decreased. At 50 mM borate concentration, optimal resolution having high separation efficiency (3.1 million theoretical plates/m for dopamine) was achieved. The injection of 2.4 nL of a noradrenaline and dopamine solution derivatized at $10^{-9}\ M$ produced peaks with signal-to-noise ratio of 8:1 and 3:1, respectively, corresponding to 1.8 amol of each catecholamine. This method was used to determine noradrenaline in brain extracellular fluid: a peak corresponding to a basal level of $5 \times 10^{-9}\ M$ of endogenous noradrenaline was observed in microdialysates from the medial frontal cortex of the rat, and its nature was confirmed by electrophoretic and pharmacological validations.

Methylmalonic acid (MMA) in serum is an established marker of cobalamine deficiency. MMA and other short-chain dicarboxylic acids react with L-pyrenyldiazomethane to form stable, highly fluorescent L-pyrenylmethyl monoesters (87). These esters have been analyzed in human blood by ce combined with lif detection. To miminize solute adsorption to the capillary wall, they were coated with polyacrylamide, and hydroxypropyl methylcellulose and dimethylformamide were used as buffer additives to achieve reliable separations. Separation was performed in tris-citrate buffer, pH 6.4, under reversed polarity conditions. The assay was linear for serum MMA concentrations in the range of 0.1–200 μmol/L.

A novel interface to connect a ce system with an inductively coupled plasma mass spectrometric (icpms) detector has been developed (88). The interface was built using a direct injection nebulizer (din) system. The ce/din/icpms system was evaluated using samples containing selected alkali, alkaline earths, and heavy-metal

ions, as well as selenium (Se(IV) and Se(VI)), and various inorganic and organic arsenic species. The preliminary results show that the system can be used to determine metal species at ppt to ppb level.

Electrochromatography (ec) has been utilized to separate mixtures of 16 different polycyclic aromatic hydrocarbons (PAHs). Fused-silica capillary columns ranging in size from 50 to 150 mm ID were packed (20–40 cm sections) with 3 mm octadecylsilica particles (89). A potential of 15–30 kV was applied across the 30–50 cm total length capillary column to generate electro-osmotic flow that carries the PAHs through the stationary phase. An intracavity-doubled argon ion laser operating at 257 nm was used to detect PAHs by lif. The limits of detection for individual PAHs range between 10^{-17} and 10^{-20} mol.

4.8. Supercritical Fluid Chromatography

Supercritical fluid chromatography (sfc) combines the advantages of gc and hplc in that it allows the use of gc-type detectors when supercritical fluids are used instead of the solvents normally used in hplc. Carbon dioxide, *n*-petane, and ammonia are common supercritical fluids (qv). For example, carbon dioxide (qv) employed at 7.38 MPa (72.9 atm) and 31.3°C has a density of 448 g/mL.

Derivatization of primary and secondary amines using 9-fluorenylmethyl chloroformate to form a nonpolar, uv-absorbing derivative has been reported (90, 91). Amphetamine and catecholamine were used as probes to evaluate this procedure. The derivatives were well behaved and allowed separation in a short time.

The analysis of mefloquine in blood, using packed-column sfc, a mobile phase consisting of *n*-pentane modified with 1% methanol and 0.15% *n*-butylamine, and electron capture detection has been reported (92). The method compares favorably to a previously published hplc-based procedure having a detection limit of 7.5 ng/mL in 0.1 mL blood sample.

The sfe of chlorpyrifos methyl from wheat followed by on-line lc/gc/ecd has been investigated (93). Extraction profiles were generated to determine the maximum analyte recovery and the minimum extraction time. Using pure CO_2, a 65% recovery of chlorpyrifos methyl spiked onto wheat at 50 ppb was reported. When 2% methanol was added to the CO_2, the recovery from a one gram sample averaged 97.8% ($n = 10$, 4.0% RSD).

4.9. Immunoassays

Immunoassays (qv) may be simply defined as analytical techniques that use antibodies or antibody-related reagents for selective determination of sample components (94). These make up some of the most powerful and widespread techniques used in clinical chemistry. The main advantages of immunoassays are high selectivity, low limits of detection, and adaptibility for use in detecting most compounds of clinical interest. Because of their high selectivity, immunoassays can often be used even for complex samples such as urine or blood, with little or no sample preparation.

A two-site immunometric assay of undecapeptide substance P (SP) has been developed. This assay is based on the use of two different antibodies specifically directed against the N- and C-terminal parts of the peptide (95). Affinity-purified polyclonal antibodies raised against the six amino-terminal residues of the molecule were used as capture antibodies. A monoclonal antibody directed against the carboxy terminal part of substance P (SP), covalently coupled to the enzyme acetylcholinesterase, was used as the tracer antibody. The assay is very sensitive, having a detection limit close to 3 pg/mL. The assay is fully specific for SP because cross-reactivity coefficients between 0.01% were observed with other tachykinins, SP derivatives, and SP fragments. The assay can be used to measure the SP content of rat brain extracts.

A number of solid-phase automated immunoassay analyzers have been used for performing immunoassays. Table 5 (96) provides useful information on maximum tests that can be run per hour, as well as the maximum number of analytes per sample. A number of immunoassay methods have been found useful for environmental analysis (see Automated instrumentation).

5. Applications

Trace or ultratrace and residue analyses are widely used throughout chemical technology. Areas of environmental investigations, explosives, food, pharmaceuticals, and biotechnology rely particularly on these methodologies.

5.1. Environment

Detection of environmental degradation products of nerve agents directly from the surface of plant leaves using static secondary ion mass spectrometry (sims) has been demonstrated (97). Pinacolylmethylphosphonic acid (PMPA), isopropylmethylphosphonic acid (IMPA), and ethylmethylphosphonic acid (EMPA) were spiked from aqueous samples onto philodendron leaves prior to analysis by static sims. The minimum detection limits on philodendron leaves were estimated to be between 40 and 0.4 ng/mm^2 for PMPA and IMPA and between 40 and 4 ng/mm^2 for EMPA. Sims analyses of IMPA adsorbed on 10 different crop leaves were also performed in order to investigate general applicability of static sims for detection of alkylmethylphosphoric acids (AMPAs) on a wide variety of leaves. Interference owing to organophosphorous pesticides were investigated as well as the effect of AMPA solution pH. The results suggest that static sims is a promising approach for rapid screening of organic surface contaminants on plant leaves.

The development of new immunosorbents for the selective solid-phase extraction of phenylurea and triazine herbicides from environmental waters has been presented (98). Silica-based sorbents were shown to be more effective than rigid hydrophilic polymers for covalent coupling of antibodies because no nonspecific interactions occurred between analytes and silica matrix. Off-line enrichment using a cartridge shows that immunosorbents can trap several analytes in the same group (9 from a mixture containing 13 phenylurea herbicides; 6 from a mixture containing 9 triazine herbicides). A stepwise elution using increasing amounts of methanol showed that the analytes had different affinities for the immunosorbents. High selectivity was illustrated by analyzing Seine River samples spiked with mixtures of phenylurea or triazine herbicides. Isoproturon was easily detected in surface waters at the range of 0.1 μg/L level with no cleanup.

A new chemical sensor based on surface transverse device has been developed (99) (see Sensors). It resembles a surface acoustic wave sensor with the addition of a metal grating between the tranducer and a different crystal orientation. This sensor operates at 250 mHz and is ideally suited to measurements of surface-attached mass under fluid immersion. By immobilizing atrazine to the surface of the sensor device, the detection of atrazine in the range of 0.06 ppb to 10 ppm was demonstrated.

Hplc and high flow pneumatically assisted electrospray mass spectrometry using negative ionization was used for determination of several acidic herbicides (qv) (100). To achieve good separation, acidification of hplc eluent and subsequent post-column addition of a neutralization buffer are needed to avoid signal suppression. A combination of an on-line automated liquid extraction step using OSP-2 autosampler containing C-18 cartridges can be used for the trace determination of herbicides in environmental waters. Only 50 mL of water are required and the limit of detection is between 0.01 and 0.03 μg/L.

The monocarboxylic dicarboxylic metabolites of the herbicide Dacthal were quantitatively determined in groundwater samples by concentrating the analytes onto strong anion exchange disks (101) (see Groundwater monitoring). The metabolites are then eluted and derivatized to their ethyl esters. The concentrations of monocarboxylic metabolites ranged from 0.02 to 1.04 μg/L in a survey of groundwater wells from eastern Oregon, whereas the concentration of the dicarboxylic acid metabolites ranged from 0.60 to 18.70 μg/L.

Dissolved nitrite in natural waters was determined at subnanomolar levels by derivatization with 2,4-dinitro-phenylhydrazine followed by hplc (102). The method has a detection limit of 0.1 nM, with an average precision of 4% RSD at ambient natural water concentrations. Irgasan 300 ((2,4-dichlorophenoxy)-phenol) has been determined in the wastewater of a slaughterhouse, using gc/ecd after derivatization with diazomethane (103). The detection limit was found to be 0.2 ng/L.

Simultaneous quantification of the herbicides atrazine, simazine, terbutylazine, propazine, and prometryne and their principal metabolites has been reported in natural waters at 3–1500 ng/L concentration (104). The compounds were enriched on graphitized carbon black and analyzed with hplc and a diode array uv detector.

A multiresidue analytical method based on solid-phase extraction enrichment combined with ce has been reported to isolate, recover, and quantitate three sulfonylurea herbicides (chlorsulfuron, chlorimuron, and metasulfuron) from soil samples (105). Optimization for ce separation was achieved using an overlapping resolution map scheme. The recovery of each herbicide was $>80\%$ and the limit of detection was 10 ppb (see Soil chemistry of pesticides).

High efficiency denuders that concentrate atmospheric SO_2 were coupled to an ion chromatograph to yield detection limits on the order of 0.5 ppt (106). A newer approach has been introduced for the quantitative collection of aerosol particles to the submicrometer size (107). When interfaced to an inexpensive ion chromatograph for downstream analysis, the detection limit of the overall system for particulate sulfate, nitrite, and nitrate are 2.2, 0.6, and 5.1 ng/m^3, respectively, for an 8-min sample. A two-stage membrane sampling system coupled with an ion trap spectrometer has been utilized for the direct analysis of volatile compounds in air, with quantitation limits to low ppt levels (108). Toluene, carbon tetrachloride, tricholoroethane, and benzene were used in these studies. The measurement of nitrogen dioxide at ppb level in a liquid film droplet has been described (109) (see Air pollution). A number of elements in environmental samples have been determined by thermal ionization ms (Table 6). The detection limit for Pu was as low as 4 fg.

Some of the methods used for determination of organic pollutants in the environment follow (118). The most notable are polyaromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs).

Analytes	Methods
n-alkanes	gc/fid
VOCs	prefractionation and gc
PAHs	sample clean up and gc
bromoform and other bromine compounds	gc for flame-retardant compounds in air
nitrodibenzopyranone	isomeric separations based on gc
PCDDs and PCDFs	gc/ms
PAH	on-line lc/gc for PAH in air
chlorinated PAH	lc/gc/ms
nitrated PAH	hplc with electrochemical detection
azaarenes	column chromatography, cleanup, and tlc
toluene diisocyanate	micro lc
dimethyl sulfoxide or sulfone	atmospheric pressure chemical ionization (APCI) ms
VOCs	ion trap monitoring
volatile chlorine compounds	fiber-optic emission sensor-based on AA of chlorine
formaldehyde	monitoring tape: hydroxylamine sulfate, methyl yellow
methyl nitrite	nitrogen oxide-indicating tubes with ir detection
VOCs	ir-based methods for on-site analysis
isocyanate species	chemiluminescent techniques
benzene	photoionization detector
hydrazine	coulometric methods
nitrobenzene	piezoelectric sensors
fluorocarbon	metal oxide sensors
perchloroethylene	quartz balance and calorimetric transducer

A variety of organic and inorganic analytes have been analyzed in air and in water. The organic compounds in air include the following (119):

Analyte	Detection
nonmethane HC	gc
polar and nonpolar HC	gc/fid
alkadienes, alkenes	gc/pid and fid
benzene, toluene	differential optical absorption spectroscopy
organobromines	gc/ms
halocarbons	gc/ecd
glycol ethers	multidimensional gc/ms
organic acids	gc/fid or -npd
carboxylic acids	ion chromatography
formaldehyde	lc
carbonyl compounds	lc/fluorescence
PAH, nitro-PAH	gc/hrms
polyhalogenated dioxins and furans	gc/hrms
aliphatic amines	gc-*N*-selective; lc/fluorescence
monoethanolamine	gc/tsd
aliphatic polyamines	lc/fluorescence
alkyl nitrates, halocarbons	gc/ecd
nitrofluoranthene	lc/fluorescence
dimethyl sulfate	gc/flame photometric

The inorganic compounds in air include the following (120):

Analyte	Method
mercury	AA; ligand exchange
phosphine, arsine	sensor conductivity, potentiometric
plutonium 239	α-spectra
lead	AA; x-ray fluorescence
indium	epithermal neutron
Zn, Cd, Pb, Cu	anodic stripping voltammetry
trace metals	AA (flame, furnace, and electrothermal)
chlorine, bromine, bromide ion mobility	ion chromatography
sulfur	scanning electron micoscopy
hydrogen peroxide	chemiluminescence

In water the inorganic analytes include the following (121):

Analyte	Method
Al	spectrofluorometric, neutron activation
As	hplc coupled to icp/aes
Be	AAS
Co, Mo, V	icp/aes
Cu, Pb	potentiometric
ClO_2	ion chromatography
Ge	preconcentration
Pb	reaction followed by spectrophotometry
trace metals	preconcentrated as various complexes
U, Th, Po, Ra	α-spectroscopy and liquid scintillation

Organic compounds in water include the following (122):

Sample/technique	Method
aromatic hydrocarbons in presence of OH-containing species	electrochemiluminescent sensing
coated fibers for chlorinated HC	evanescent wave spectroscopy
PAHs	fluorescence
17 pesticides	atmospheric ionization
benzene, chlorobenzene, and dichloroethane	direct insertion membrane
ms/ms for chlorodinitrophenol isomers	electrospray ms
ms/ms used for pesticides and dyes	electrospray ion trap ms
amines	electrospray ion mobility
surfactants, atrazine	fast-atom bombardment
phenylurea pesticides, acidic, basic and neutral pesticides; C-18 disks used for isolation	particle beam ms

5.2. Explosives

Explosives can be detected using either radiation- or vapor-based detection. The aim of both methods is to respond specifically to the properties of the energetic material that distinguish it from harmless material of similar composition. A summary of techniques used is given in Table 7. These techniques are useful for detecting organic as well as inorganic explosives (see Explosives and propellants).

Procedures for trapping accelerant vapors in the headspace of a closed container on charcoal that is either encased in a porous pouch or impregnated into a flexible membrane have been described (124). Trace amounts of explosive compounds can be trapped from hplc effluents onto a porous polymer microcolumn for confirmatory gc examination (125).

An enzyme-linked immunosorbent assay (elisa) has been developed for the detection of residues on hands. As little as 50 pg of TNT can be detected (126). Liquid chromatography/thermospray negative-ion tandem ms has been successfully used to detect picogram levels of explosives in post-blast debris (127).

5.3. Food

Laws and regulations controlling contamination of food were once the province of religious organizations. As far back as the Dark Ages, government set standards. Significant changes have occurred only relatively recently, since the time analytical chemists could characterize most of the substances that comprise food and thus more effectively control contaminants in it. The reliability of data regarding medicated feeds (see Feeds and feed

additives), dairy products (see Milk and milk products), seafood, meat products (qv), fruits and vegetables, and beverages has been reviewed (2).

The development of analytical strategies for the regulatory control of drug residues in food-producing animals has also been reviewed (128). Because of the complexity of biological matrices such as eggs (qv), milk, meat, and drug feeds, well-designed off-line or on-line sample treatment procedures are essential.

Methylmercury in fish was extracted and cleaned using column chromatography and then determined using flameless atomic absorption (129). The method gave a reproducibility of 18.2% (RSD) at 0.1 ppm level of Hg. Many trace elements, particularly the heavy metals, can be determined in food. For example, electrothermal atomic absorption has been applied for the determination of Cd, Co, and Pb in foods (130). Electrothermal AA has been compared to flame AA spectrophotometry for determination of cobalt (131). Electrothermal AA was more accurate, however, flame AA was more precise. The detection limits were 1.8 and 2.27 ppb by the electrothermal flame methods, respectively. A rapid and sensitive electrothermal AA spectrophotometric method using a combination of microwave digestion and palladium as a stabilizer was used for detecting Cd and Pb at sub ppm levels in vegetables and protein in foodstuffs (132).

The efficient recovery of volatile nitrosamines from frankfurters, followed by gc with chemiluminescence detection, has been described (133). Recoveries ranged from 84.3 to 104.8% for samples spiked at the 20 ppb level. Methods for herbicide residues and other contaminants that may also relate to food have been discussed. Inorganic elements in food can be determined by atomic absorption (AA) methods. These methods have been extensively reviewed. Table 8 lists methods for the analysis of elements in foods (134).

5.4. Pharmaceuticals

Examples of trace and ultratrace analyses of various drugs and pharmaceuticals have been provided throughout. The purity of the active ingredient, its content and availability in dosage form, therapeutic blood levels, delivery to target areas, elimination (urine, feces, and metabolites), and toxicity are always of importance.

5.4.1. Pharmaceutical Purity

A safety profile of a generic drug can differ from that of the brand-name product because different impurities may be present in each of the drugs (154). Impurities can arise out of the manufacturing processes and may be responsible for adverse interactions that can occur. For example, serious adverse reactions (Lyell syndrome) were observed upon the use of isoxicam in 1985. These seemed to have resulted from trace elements of a manufacturing by-product that was within the manufacturing quality control specifications.

The subject of impurity analysis of pharmaceutical compounds has been insufficiently addressed in the scientific literature (3, 4). Many monographs in the *United States Pharmacopeia* have nonspecific assay methods. An attempt has been made to address this problem by focusing on specific methodologies and delineating origination and concentration of impurities found in pharmaceutical compounds (2). A capsule review of methodologies used for the following classes of compounds is available (2, 3).

alkaloids (qv)	antibiotics (qv)
antineoplastic agents	amines (qv)
carboxylic acids	carbonyl compounds
local anesthetics	hydroxy compounds
steroids (qv)	prostaglandins (qv)
vitamins (qv)	sulfonamides (see Antibacterial agents, synthetic)
analgesics (see Analgesics, antipyretics, and antiinflammatory agents)	amino acids/peptides
	antidepressants, tranquilizers (see Psychopharmacological agents)

Chiral separations have become of significant importance because the optical isomer of an active component can be considered an impurity. Optical isomers can have potentially different therapeutic or toxicological activities. The pharmaceutical literature is trying to address the issues pertaining to these compounds (155). Frequently separations can be accomplished by glc, hplc, or ce. For example, separation of $R(+)$ and $S(-)$ pindolol was accomplished on a reversed-phase cellulose-based chiral column with fluorescence emission (156). The limits of detection were 1.2 ng/mL of $R(+)$ and 4.3 ng/mL of $S(-)$ pindolol in serum, and 21 and 76 ng/mL in urine, respectively.

5.5. Biotechnology

Particular attention must be paid to the detection of DNA in all finished biotechnology products because of the possibility that such DNA could be incorporated into the human genome and thus become a potential oncogene. The absence of DNA at the picogram-per-dose level should be demonstrated in order to assure the safety of biotechnology products (157).

The isolation and purification of DNA and ribonucleic acid (RNA) restriction fragments are of great importance in the area of molecular biology (158). These fragments are the product of site-specific digestion of large pieces of DNA and RNA with enzymes called restriction endonucleases. The fragments may range in size from a few base pairs to tens of thousands of base pairs. An ion-exchange column can provide DNA and RNA separations within one hour, giving resolution equivalent to that obtained with gel electrophoresis. Nucleic acid fragments are then visualized, using on-line uv detection and sample loading from 500 ng to 50 mg.

Molecular biologists are utilizing hplc for characterization and purification of proteins (qv), peptides, and antibodies (159). In fact, rplc is rapidly becoming the method of choice for resolving complex peptide mixtures from protein cleavage reactions (for example, peptide mapping of CNBr and tryptic digests), discrimination of homologous proteins from different species, and separation of synthetic diastereioisomeric peptides.

An electrospray ionization source interfaced to an ion trap storage/reflectron time-of-flight mass spectrometer was used as a sensitive detector for microbore hplc (160). Using the total ion-storage capabilities of the trap over a broad mass range, total ion chromatograms of tryptic digest bovine cytochrome c and bovine B-casein are obtained, following hplc separations with samples in the low picomole range.

Amino acids (qv) and peptides from both standard solutions and biological samples derivatized with 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde at a low sample concentration to form highly fluorescent isoindoles gave minimum detectabilities using ce and laser-induced fluorescence detection in the low attomole range (161). Indirect fluorescence detection was used to monitor subfemtomolar components in tryptic digest by ce (162). Dideoxycytidine chain-terminated fragments were separated in gel-filled capillaries (163) and a post-column laser-induced fluorescence detector provided a mass detection limit below attomole range for fluorescamine-labeled DNA fragments.

5.6. Miscellaneous

Trace analyses have been performed for a variety of other materials. Table 9 lists some uses of electrothermal atomic absorption spectrometry (etaas) for determination of trace amounts of elements in a variety of matrices. The applications of icp/ms to geological and biological materials include the following (165):

Sample	Analyte
muscovites, granites	rare-earth elements
rocks, minerals	rare-earth elements
geological reference materials	Os, Ir, Pt, Au
coals	PGE
U.S. Geological Survey reference standard	28 elements
calcites	La, Ce, Eu, other REEs
high purity quartz sand	14 elements
electronic-grade quartz	U, Th, others
automotive catalysts	Pd, Rh, Ce, Ni, Fe, Ba
hplc components	Fe, Cr, Ni, Cd, Mn, Mo, Zn, Pb
blood serum	various elements
blood plasma	B
urine	Sb, Hg, Cd
rat hair	^{63}Cu, ^{65}Cu
animal tissue	Pt
brain tissue	39 elements
liver tissue	Th
dietary intake/uptake	Zn ratios/Sr ratios
milk powder	I
soil ingestion studies	Al, Ba, Mn, Si, Ti, V, Y, Zn
bone ash	U, Th

Thermal neutron activation analysis has been used for archeological samples, such as amber, coins, ceramics, and glass; biological samples; and forensic samples (see Forensic chemistry); as well as human tissues, including bile, blood, bone, teeth, and urine; laboratory animals; geological samples, such as meteorites and ores; and a variety of industrial products (166).

Table 8. Analysis of Elements in Foods[a,b]

Element	Food	Reference
	Icp atomic emission spectrometry	
As, Pb, Cd	seafood	136
various elements	tea	(137, 138)
Al	canned cherries	139
Fe, Cu, Zn	tinned mussels	140
Al, Ba, Mg, Mn	tea leaves	141
various elements	honey	142
Co, Cr, Cu, Fe, Pb, Zn	Brazilian beer	143
Ca, P, Mg, Fe, Cu, Zn	maternal milk	144
	Icp mass spectrometry	
As, Cd, Pb	seafood	145
various elements	maternal milk	146
various elements	foods	147
	Neutron activation analysis	
minor and trace	agricultural products	148
Mo	edible oils, margarine	149
I	human, animal, commercial milk	150
I	dates	151
As, Hg, Se, Zn	seafood	152
H, B, Cl, K, Na, S, Ca, Cd	orange juice	152
various	tea	153

[a] Refs. 134 and 135.
[b] Icp = inductively coupled plasma.

A discussion of methods and applications for trace analysis of cosmetics is available (167). Analyses of elements from Al to Zn by a variety of methods has also been described recently (168). Detection techniques for some of the elements of interest follow:

Analyte	Technique
Al	solid-phase spectrofluorometry
As	fluorescence
B	azomethine method
Be	photothermal spectrometry, fluorescence
Cd	fluorescence, neutron activation
Cl	on-wafer fabricated sensor
Cn	AAS
Cs	flame emission
Cu	energy-dispersive x-ray fluorescence; ion selective electrode
Fe	hplc
Hg	icpms, AAS, fluorescence, photoacoustic spectrometry
In	neutron activation
Li	neutron activation
Mg	amperometry
Mn	fluorescence, galvanostatic stripping
Os	kinetic spectrophotometry
Pb	anodic stripping, AA
Po	α-spectrometry
^{226}Ra	Cerenkov counting
Sb	neutron activation, AA
Se	gc/ec, spectrophotometry
Tb	fluorescence
^{230}Th	α-spectrometry
Zn	fluorescence

Table 9. Etaas Analysis of Solids and Slurries[a]

Matrix	Element	Comment
airborne aerosols	Pb	Zeeman BC
aluminum alloys	Ga	graphite powder
biological materials	Pd, Pb, Zn, Mn, Cu	D2 BC, autoprobe
bird feathers	Cd, Pb	Zeeman BC
nuclear waste	Mo, Ru, Rh, Pd	graphite modifier
urinary calculi	Cd, PB, Cr, Ni, Hg	Zeeman BC
rocks, minerals	alkali metals	FAES
soils	Pb	fast temperature program

[a]Ref. 164.

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