Resolving Erroneous Reports in Toxicology and Therapeutic Drug Monitoring

A Comprehensive Guide

Amitava Dasgupta

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Preface

Therapeutic drug monitoring and testing for drugs of abuse are important clinical laboratory tests that have a significant impact on patient safety and patient management. Physicians rely on serum or whole blood levels of a therapeutic drug for dosage adjustment and also to interpret significant drug–drug interactions. Therefore a falsely elevated or falsely lowered drug concentration due to the presence of an interfering substance in the specimen has a very serious impact on patient safety. The interference can be a false positive or a false negative. A clinician usually questions the validity of a test result if the concentration of a therapeutic drug is unexpectedly high. However, negative interference has more serious clinical consequences because it occurs infrequently compared with positive interference, and a clinician may simply increase the dosage of the medication without recognizing that the drug concentration may be falsely low due to the presence of an interfering substance in the specimen. There are reports in the literature of severe digoxin toxicity due to increased digoxin dosage based on reports of low digoxin concentration due to negative interference (see Chapter 12). Drugs of abuse testing using immunoassays is subjected to interference. Many over-the-counter cold and cough medications containing ephedrine or pseudoephedrine may cause a false-positive amphetamine immunoassay test result due to cross-reactivity with antibodies used in these immunoassays. A false-positive phencyclidine test result due to the presence of dextromethorphan, a common ingredient in many over-the-counter cold medications, is well documented in the literature. Such false-positive test results are not of concern for workplace drug testing because positive immunoassay test results are always confirmed by a chromatographic method, most commonly gas chromatography combined with mass spectrometry (GC/MS). However, for medical drug testing where

GC/MS confirmation is not available, a physician may falsely accuse a patient regarding his or her drug abuse based on a false-positive immunoassay result, although in reality the patient is not a drug abuser. This may hamper physician–patient trust or may cause mental agony to the patient. A false-positive blood alcohol result using an enzymatic alcohol assay has a similar consequence. A false-positive blood alcohol level measured by breath analyzer may have a serious legal impact because a driver may be falsely accused of driving under the influence of alcohol (see Chapter 7 for a detail discussion on this topic).

This book provides a comprehensive guide for laboratory professionals and clinicians regarding the sources of errors in therapeutic drug monitoring and drugs of abuse testing and how to resolve such errors and identify discordant specimens. Error-free laboratory results are essential for patient safety. Because herbal medicines are widely used by the general population, drug–herb interactions are discussed. For example, warfarin is known to interact pharmacokinetically and pharmacodynamically with many drugs; Chapter 9 discusses many clinically significant interactions of warfarin with herbal supplements. Chapter 16 is devoted to other important drug–herb interactions where an apparent erroneous result in therapeutic drug monitoring is due not to the presence of an interfering substance but to clinically important drug–herb interactions. Clinical laboratory testing is also helpful in the diagnosis of certain plant poisoning and toxicities from the use of certain herbal supplements (Chapter 10).

This book is intended as a practical guide for laboratory professionals and clinicians who deal regularly with erroneous results in therapeutic drug monitoring and drugs of abuse testing. I hope this book will help them become more aware of such sources of errors and empower them to eliminate such errors when feasible.

I would like to thank Robert L. Hunter, chairman of the Pathology and Laboratory Medicine Department at the University of Texas–Houston Medical School, for his support when I worked on the project. I also thank Alice Wells for critically reading the entire manuscript and making helpful suggestions. Last but not least I thank my wife, Alice, for tolerating my long hours spent on writing the book on weekdays and weekends. Finally, readers will be the judge of the final success of this book. If they find this book useful, that will be my best reward for writing it.

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Chapter 1

An Introduction to Tests Performed in Toxicology Laboratories

1.1. INTRODUCTION

In general therapeutic drug monitoring, urine toxicology drug screens, analysis of blood alcohol and volatiles as well as emergency toxicology drug screenings are commonly offered tests in a toxicology laboratory. Certain drugs with a narrow therapeutic range require routine monitoring, and in general serum or plasma is the preferred specimen. However, certain immunosuppressant drugs such as cyclosporine, tacrolimus, sirolimus, and everolimus are monitored in whole blood, although another immunosuppressant, mycophenolic acid, is monitored in serum or plasma. Drug screening for a patient with a suspected drug overdose is more commonly performed using urine specimens, but blood and gastric fluid specimens are also analyzed for the screening of drugs in case of a suspected recent overdose. In addition, blood alcohol analysis is also commonly conducted in toxicology laboratories because alcohol use alone may cause life-threatening intoxication. In addition, many abusers of illicit drugs also consume alcohol at the same time to achieve euphoria. Bogstrand et al reported that psychoactive substances were found in approximately 50% of the patients admitted to the hospital within 12 hours of injury. Of a total of 1272 patients studied (510 women and 762 men), 38% of the women and 48% of the men had a positive blood sample of a psychoactive drug on admission. Alcohol was the most prevalent substance; 27% of patients had a positive blood alcohol test. Cannabis was the most prevalent illicit drug (6.2%); diazepam was the

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most common drug, detected in 7.4% of patients. The authors concluded that alcohol was the most common substance found in these patients and was particularly related to violence, whereas medicinal drugs were most prevalent in accidents at home (1). Alcohol is also a risk factor for injury in adolescents. Injured adolescents are more likely to visit the emergency department with an alcohol-related event during the early hours of the morning (2). Multiple abused drugs are also encountered in severely intoxicated patients and individuals who die from a drug overdose. Dickson et al reported a case of a 22-year-old white man who died from a drug overdose. Routine toxicological analysis detected morphine in the decedent's blood (0.06 mg/mL). In his urine specimen, 6-monoacetyl morphine (a marker compound for heroin abuse), morphine, codeine, doxylamine, and mephedrone were confirmed (3). In addition to poisoning due to alcohol, an overdose with various drugs may provoke a visit to the emergency department. Both salicylate and acetaminophen are commonly encountered drug in poisoned patients, and such drug levels are often screened in a toxicology laboratory using serum or plasma specimens.

1.2. ACETAMINOPHEN AND SALICYLATE ASSAYS

Acetaminophen (paracetamol) overdose, both intentional and accidental, remains a significant public health concern. In one report, the authors calculated that from 2000 to 2006, an age-adjusted rate of hospitalization related to acetaminophen was 13.9 per 100,000 population in the United States. Most acetaminophen overdoses were intentional (4). Acetaminophen can also cause liver toxicity. Because acetaminophen is a component of many medications, both prescription and over the counter, unintentional overdose can occur. Concurrent use of alcohol may also potentiate hepatoxicity of acetaminophen (5). Chronic alcohol abusers are also at an increased risk of acetaminophen-induced hepatotoxicity even after therapeutic use (6).

Salicylate poisoning is also common, and an adult can die from it. In 2005, according to the Toxic Exposure Survey from the American Association of Poison Control Center's National Poisoning and Exposure Database, there were more than 20,000 reported exposures from salicylate, and 64% of these patients were treated in a health care facility. It was considered that 50% of all exposures were intentional, and 60 patients died from a salicylate overdose (7). Galbois et al reported the case of a 74-year-old schizophrenic patient who died of salicylate poisoning; his blood salicylate level was 876 mg/L (87.6 mg/dL, a very toxic level) (8). Salsalate is a nonsteroidal anti-inflammatory drug that is mostly metabolized to two molecules of salicylic acid. However, approximately 7–10% of the drug is not hydrolyzed to salicylic acid and can be recovered in the urine either as the unchanged drug or as glucuronide conjugate (9). Delayed salicylate toxicity without early manifestation may occur

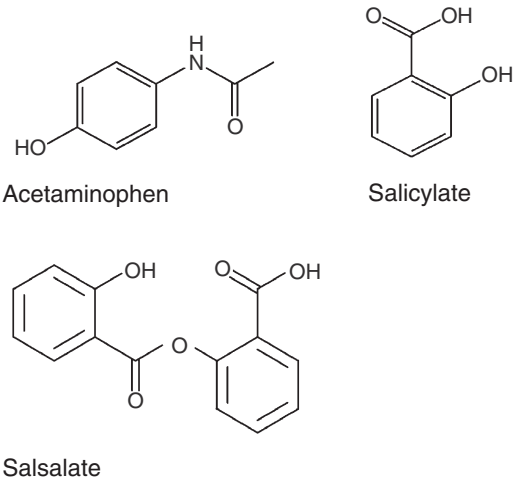

Figure 1.1. Chemical structures of acetaminophen, salicylate, and salsalate.

after overdose with both salicylate and salsalate. Chemical structures of salicylate, salsalate, and acetaminophen are given in Figure 1.1.

Case Study

A 14-year-old girl ingested 120 tablets of 81 mg aspirin extended release and 6 tablets of ciprofloxacin 2 hours prior to arrival at the emergency department. Upon arrival she denied nausea, shortness of breath, diaphoresis, or abdominal pain. Activated charcoal 50 g with sorbitol was administered orally for decontamination. No acetaminophen or ethanol was detected in her blood. In addition, a urine drug of abuse screen was also negative. The first salicylate blood level was 1 mg/dL (therapeutic: 10–20 mg/dL) drawn 4 hours after ingestion, but the salicylate level was elevated to 13 mg/dL 6 hours after ingestion, and the patient remained asymptomatic. The patient remained asymptomatic until 35 hours after exposure when she developed dizziness, tinnitus, and epigastric discomfort, and her blood salicylate concentration was elevated to 46 mg/dL. A second dose of 50 g of activated charcoal was administered along with bicarbonate infusion. She did not develop any renal failure, and after an observation period she was discharged to a psychiatric facility. The cause of delayed salicylate toxicity was unclear. Possibilities include delayed absorption due to enteric-coated or extended-release formulation, or the formation of bezoars (aggregates of drug that form a soft mass with limited surface area exposed to gastric fluid). The interior portion of the drug mass has mostly undissolved drugs. Other than salicylate, a variety of medications may form such bezoars (10).

Case Study

A 31-year-old man with a history of depression, posttraumatic stress disorder, and prior attempted suicide was discovered by his neighbor in the morning with greatly reduced consciousness. He was transferred to a hospital by the emergency medical team, and he admitted that he had attempted suicide the previous night by overdose but did not disclose the medication taken. Thirty-six new and old pill bottles were found on the scene that included acetaminophen, hydrocodone with acetaminophen, hydroxyzine, ibuprofen, lorazepam, magnesium oxide, morphine, oxycodone, paroxetine, ranitidine, salsalate, temazepam, tramadol, venlafaxine, and zolpidem. On arrival at the hospital, his blood pressure was 162/92 mm Hg, pulse 100 bpm, respiratory rate 14/min, and 98% oxygen saturation at room air. Toxicological investigation revealed a serum salicylate level of 29.2 mg/dL, and a urine drug screen was positive for benzodiazepines and cannabinoids. After 3 hours, the patient's level of consciousness and respiratory rate both decreased, and an arterial blood gas showed a pH of 7.31, a pCO_2 of 48, and pO_2 of 111. He was intubated 5.5 hours after admission due to apnea, and sodium bicarbonate was administered intravenously. In addition, two doses of activated charcoal (50-g dose) were administered by nasogastric tube. Salicylate concentration then peaked to 55 mg/dL just over 8 hours after presentation to the emergency department. Later his salicylate blood level declined, and sodium bicarbonate therapy was discontinued. Unfortunately, his blood salicylate level increased again later, peaking at 61.7 mg/dL 67 hours after presentation. The patient was later extubated and kept on a psychiatric hold with a one-to-one sitter. Salicylate ingestions are known to demonstrate unusual toxicokinetics and absorption patterns during overdose, and in this case a return to a toxic salicylate level was observed after apparent resolution of toxicity (11).

Acetaminophen and salicylate in serum, plasma, or urine can be measured by commercially available assays that may be either based on colorimetric principle or are immunoassays. These assays can be run on various automated analyzers. In addition, chromatographic methods such as high-performance liquid chromatography or gas chromatography can also be used for the determination of both acetaminophen and salicylate in various biological matrixes. Gaspari and Locatelli described a simple high-performance liquid chromatographic determination of both salicylate and acetaminophen in plasma after liquid-liquid extraction with hexane and ultraviolet detection at 228 nm (12). Miceli et al also described a liquid chromatographic method for the determination of salicylate and acetaminophen in human plasma using 8-chlorotheophylline as the internal standard (13). However, chromatographic procedures are labor intensive, and in toxicology laboratories, various automated assays are commonly used for routine determination of both salicylate and acetaminophen.

Unfortunately, these automated assays are subjected to interferences, and the presence of high bilirubin in serum or plasma may affect both the acetaminophen and salicylate assays. Stewart and Watson reviewed various methods available for the estimation of salicylate and acetaminophen in serum, plasma, and urine (14).

A false-positive acetaminophen level due to hyperbilirubinemia has been reported. In one report, the authors observed false-positive acetaminophen levels in two patients who had high bilirubin concentrations (25.5 mg/dL and 40.1 mg/dL, respectively) in their sera using the GDS Diagnostics enzymatic acetaminophen assay (GDS Diagnostics, Elkhart, IN). However, enzyme-multiplied immunoassay technique (EMIT) (Syva, Palo Alto, CA), acetaminophen assay, and gas chromatography/ mass spectrometric (GC/MS) assay did not reveal the presence of acetaminophen. The GDS assay utilizes an enzyme (n-arylacylamidase) to convert acetaminophen into para-aminophenol and acetate. Then p-aminophenol reacts with ortho-cresol in the presence of periodate to form the chromophore indophenol, which has a strong absorption spectra at 615 nm. The EMIT assay utilizes an antibody that recognizes acetaminophen. Although the mechanism of interference with the GDS enzymatic assay is unknown, the authors speculated that bilirubin may form a complex with ortho-cresol (15). Polson et al concluded that false-positive acetaminophen test results may occur when bilirubin concentration is above 10 mg/dL, leading to potential clinical errors especially with enzymatic-colorimetric assays (16). Significant positive bias of bilirubin in the Trinder reaction-based salicylate methods (color complex formed due to reaction of salicylate with ferric ions) on automated analyzers has been reported. However, such interference can be eliminated by using the fluorescence polarization immunoassay for salicylate using the AxSYM analyzer (Abbott Laboratories, Abbott Park, IL) (17). Broughton et al also described interference of bilirubin on a salicylate assay performed using the Olympus automated analyzer (18). Mitochondrial acetoacetyl-CoA thiolase deficiency is a rare metabolic disorder causing acute episodes of severe ketosis and acidosis. Tilbrook reported false-positive salicylate in an 18-month-old boy who presented to the hospital with severe acidosis. The authors concluded that false-positive salicylate using the Trinder reagent was due to the interference of a high level of acetoacetate in the specimen that interfered with the assay (19). However, immunoassays for salicylate manufactured by various diagnostic companies are free from such interferences.

Case Study

A 31-year-old woman was admitted to the hospital for abdominal pain, decreased appetite, malaise, confusion, and tea-colored urine. Investigation showed acute liver failure characterized by high bilirubin (70.7 mg/dL), alanine aminotransferase 6170 U/L, aspartate aminotransferase 5080 U/L, lactate dehydrogenase 6830 U/L, and alkaline phosphatase 150 U/L. Plasma

acetaminophen concentration of 121 μmol/L (therapeutic up to 100 μmol/L) resulted in suspicion of an acetaminophen overdose as the probable cause of liver failure because serological tests for hepatitis A and B were negative. However, her plasma acetaminophen level remained elevated even on day 3 (104 μmol/L) raising the suspicion of bilirubin interference in acetaminophen measurement because the acetaminophen assay on the Vitros analyzer (Johnson & Johnson, Rochester, NY) is based on the enzymatic conversion of acetaminophen to para-aminophenol and subsequent reaction with ortho-cresol to form the blue-colored complex indophenol, which is measured by change in absorption at 600 nm. High bilirubin interferes with the assay. When the authors measured acetaminophen concentration using protein-free ultrafiltrate, which is free from protein-bound bilirubin, the acetaminophen concentration was below the detection limit of the assay, indicating no acetaminophen was present in the plasma. When acetaminophen was remeasured using a chromatographic method, no acetaminophen level was detected, further establishing that the initial high acetaminophen result was a false-positive result due to the interference of bilirubin with the acetaminophen assay (20).

1.3. ANALYSIS OF ALCOHOL

Alcohol is a major cause of motor vehicle accidents, and such victims are treated in the emergency department of hospitals. Blood alcohol testing is a routine and widely ordered test in a toxicology laboratory. Blood alcohol can be measured by either an enzymatic method or by gas chromatography. Although enzymatic methods can be automated and are often applied for measuring blood alcohol in busy toxicology laboratories, these methods also suffer from interferences, especially if both high lactate and lactate dehydrogenase are present in the specimen. However, gas chromatographic methods are free from such interferences, and such methods should be used for legal alcohol determination. This important topic is discussed in detail in Chapter 7. In addition, gas chromatographic methods are capable of analyzing other volatile compounds such as methanol, isopropyl alcohol, acetone, ethylene glycol, and related volatile compounds along with alcohol (ethyl alcohol) simultaneously.

1.4. THERAPEUTIC DRUG MONITORING

The International Association for Therapeutic Drug Monitoring and Clinical Toxicology adopted the following statement to describe therapeutic drug monitoring, “Therapeutic drug monitoring is defined as the measurement made in the laboratory of a parameter that, with appropriate interpretation, will directly influence prescribing procedures. Commonly, the measurement is

in a biological matrix of a prescribed xenobiotic, but it may also be of an endogenous compound prescribed as a replacement therapy in an individual who is physiologically or pathologically deficient in that compound" (21). Therapeutic drug monitoring has been used in clinical practice since the 1970s with the goal of personalizing the dosage of a drug for maximum efficacy and minimal toxicity. Usually therapeutic drug monitoring is necessary for a drug with a narrow therapeutic window, and only a small fraction of all drugs available require therapeutic drug monitoring. In general, therapeutic drug monitoring is not needed for any over-the-counter drug because these drugs usually have a wider margin of safety. However, the intentional or accidental overdose of over-the counter medications such as salicylate or acetaminophen is troublesome because such an overdose may even be fatal.

A drug may be administered to a patient via various routes including oral, rectal, intravenous, intramuscular, transdermal, or through sublingual application. Each route of administration has its advantages and disadvantages. For example, the oral route of administration is easiest for a patient, but the drug may suffer low bioavailability due to first-pass metabolism or intake of food or the bioavailability may be higher if the patient consumes alcohol. Moreover, a peak drug level may be achieved after a long delay. In contrast, peak concentration can be achieved rapidly if the drug is administered intravenously or intramuscularly, but that route of administration may result in patient discomfort. Rapid absorption of a drug can be achieved by sublingual application, but the drug may undergo first-pass metabolism thus reducing the efficacy of the drug. Usually a drug is poorly absorbed after transdermal application, and absorption may also be low after rectal application of a drug. In addition, most drugs that require therapeutic drug monitoring are delivered orally except for vancomycin and aminoglycoside. Criteria for drugs to be a candidate for therapeutic drug monitoring are the following:

1. Narrow therapeutic range where the dose of a drug that produces the desired therapeutic concentrations is also closer to the dose that may also cause toxic serum concentration. Serious toxicity may be encountered if the drug is not monitored.
2. There is an unpredictable relationship between dose and clinical outcome but a predictable relation between serum or whole blood drug level and clinical efficacy as well as toxicity. Significant changes in metabolism due to genetic makeup, age, sex, or disease for these drugs are responsible for the poor relation between the dosage and the drug level in the blood.
3. Drugs that demonstrate nonlinear pharmacokinetic parameters are also candidates for therapeutic drug monitoring.
4. Toxicity of a drug may lead to hospitalization, irreversible organ damage, and even death; for example, vancomycin may cause irreversible ototoxicity.

1.4.1. Drugs Requiring Therapeutic Drug Monitoring

Most drugs monitored in clinical laboratories are administered to patients with chronic diseases. These drugs are often used as a prophylactic agent to prevent reoccurrence of symptoms. For example, phenytoin is used to prevent certain types of convulsions in patients. Patient compliance is a major issue for successful drug therapy, and often patients do not take drugs as recommended, especially when they are dealing with a chronic illness. Gillisen reported that in patients with asthma, the adherence rates to medications are sometimes below 50% (22). Patsalos et al concluded that therapeutic drug monitoring of anticonvulsant drugs is beneficial to assess compliance especially in patients with uncontrolled seizures and breakthrough seizures (23). The cure rate for acute lymphoblastic leukemia (ALL) may exceed 85%, but up to 3 years of maintenance therapy with weekly methotrexate and daily 6-mercaptopurine is needed. Therefore, compliance with therapy is essential for the cure of ALL. In one report, the authors compared direct structured interview, the search of lack of compliance documented in the clinical record, and therapeutic drug monitoring of methotrexate to investigate compliance with therapy among children receiving such treatment. In 5 of 49 interviews, at least an episode of noncompliance was observed; searching clinical records revealed 8 of 49 patients skipped taking medication, and therapeutic drug monitoring revealed that 14 of 49 children had no detectable level of methotrexate in serum, indicating noncompliance. The authors concluded that face-to-face interview and clinical file notes are unreliable to determine patient noncompliance, and therapeutic drug monitoring is the best way to identify noncompliant patients (24). To attain optimal efficacy, a drug must reach the optimal level in the blood. Other than noncompliance, the patient may experience a lower level of drug than expected based on dosage due to reduced absorption or ultrarapid metabolism of the drug due to genetic makeup. Therefore, when a person reaches a steady state, routine therapeutic drug monitoring is also helpful to assess the reasons for an altered drug response (25). In addition to genetic factors, clinically significant drug–drug as well as drug–herb interactions may also significantly alter the drug level in the blood. For example, if quinidine is added to the drug regime of a patient taking digoxin, reduction in digoxin dosage is necessary because an increase in serum digoxin concentration occurs in 90% of patients after initiation of quinidine therapy, which causes a reduction in the renal clearance of digoxin (26).

Usually anticonvulsants, cardioactive drugs, immunosuppressants, antiasthmatic drugs, antidepressants, antiretroviral drugs, antineoplastic drugs, and antibiotics with narrow therapeutic windows such as vancomycin and aminoglycosides are monitored. In most instances, trough blood level (15–30 minutes prior to the next dose) is the preferred specimen for therapeutic drug monitoring except for certain antibiotics (vancomycin and aminoglycosides) where both peak and trough drug levels are monitored. Vancomycin and aminoglycoside can produce serious nephrotoxicity and ototoxicity. Peak serum concentrations for aminoglycosides such as amikacin and kanamycin above

32–34 μg/mL are associated with a higher risk of nephrotoxicity and ototoxicity (27). Vancomycin also has a low therapeutic index, and both nephrotoxicity and ototoxicity can be encountered in patients undergoing vancomycin therapy (28). Therefore, it is also necessary to monitor both peak and trough concentration of vancomycin. Ranges for peak concentrations of 20–40 μg/mL have been widely reported (29). The recommended trough concentration is 5–10 μg/mL because trough concentrations above 10 μg/mL are associated with an increased risk of nephrotoxicity (30,31).

Commonly monitored drugs include classical anticonvulsants such as phenytoin, valproic acid, carbamazepine, and phenobarbital, although certain newer anticonvulsant such as lamotrigine may benefit from routine therapeutic drug monitoring. In addition, certain cardioactive drugs such as digoxin, procainamide, quinidine, lidocaine, and so on, require routine monitoring. All immunosuppressants also need therapeutic drug monitoring. Commonly monitored drugs are listed in Table 1.1. There are also less frequently monitored drugs that may benefit from therapeutic drug monitoring, for example various antiretroviral drugs including protease inhibitors that are used in treating patients with acquired immunodeficiency virus (AIDS). These less frequently monitored drugs are listed in Table 1.2. However, the list of drugs that may benefit from therapeutic drug monitoring is increasing steadily as more publications indicate the benefits of therapeutic drug monitoring in certain drugs including common drugs in a selected patient population. Gerin et al concluded that routine therapeutic drug monitoring of voriconazole is potentially helpful in infants and children even if voriconazole is administered intravenously (32). Pea et al demonstrated that therapeutic drug monitoring in optimizing drug exposure with high-dose daptomycin plus continuous infusion of meropenem in patients with severe cellulitis, morbid obesity, and changing renal function is highly beneficial (33).

1.4.2. Effect of Gender and Pathophysiology on Drugs Disposition and Utility of Therapeutic Drug Monitoring

There are some differences between males and females for the metabolism of certain drugs. In addition, pregnancy can also significantly alter metabolism as well as the protein binding of certain strongly protein-bound drugs. Many disease conditions such as uremia, liver disease, cardiovascular disease, thyroid dysfunction, and other pathological conditions may also affect drug disposition. Elderly people are more susceptible to drug toxicity than younger adult, whereas neonates may also have a reduced capacity to metabolize drugs. Therapeutic drug monitoring is very helpful for the proper dosage adjustments for these patients.

1.4.2.1. Effect of Sex on Drug Disposition. Men and women may show both pharmacokinetic and pharmacodynamic differences in response to a particular drug therapy. There are anatomical differences between male and female. In general, males have a higher bodyweight, greater body surface area,

TABLE 1.1. Commonly Monitored Therapeutic Drugs

Drug	Specimen Requirement	Therapeutic Range (Trough)
Anticonvulsants		
Carbamazepine	Serum or plasma	4–12 μg/mL
Ethosuximide	Serum or plasma	40–75 μg/mL
Methsuximide	Serum or plasma	10–40 μg/mL
Phenytoin	Serum or plasma	10–20 μg/mL
Phenobarbital	Serum or plasma	15–40 μg/mL
Primidone	Serum or plasma	5–12 μg/mL
Valproic acid	Serum or plasma	50–100 μg/mL
Cardioactive Drugs		
Digoxin	Serum or plasma	0.8–2.0 ng/mL
Lidocaine	Serum or plasma	1.5–5.0 μg/mL
Procainamide and	Serum or plasma	4–10 μg/mL
N-acetylprocainamide		4–8 μg/mL
Quinidine	Serum or plasma	2–5 μg/mL
Antiasthmatics		
Theophylline	Serum or plasma	10–20 μg/mL
Caffeine	Serum or plasma	5–15 μg/mL
Antidepressants		
Amitriptyline and Nortriptyline	Serum or plasma	120–250 ng/mL (Amitriptyline + Nortriptyline) 50–150 ng/mL (Nortriptyline alone)
Clomipramine	Serum or plasma	150–450 ng/mL
Doxepin and Nordoxepin	Serum or plasma	150–250 ng/mL (Doxepin and Nordoxepin)
Imipramine and Desipramine	Serum or plasma	150–250 ng/mL (Imipramine and Desipramine)\
Lithium	Serum or plasma	0.8–1.2 mEq/L
Antibiotics		
Amikacin	Serum or plasma	<5 μg/mL (trough) 15–25 μg/mL (peak)
Gentamicin	Serum or plasma	1–2 μg/mL (trough) 4–8 μg/mL (peak)
Tobramycin	Serum or plasma	1–2 μg/mL (trough) 4–8 μg/mL (peak)
Vancomycin	Serum or plasma	5–15 μg/mL (trough) 30–40 μg (peak)
Immunosuppressants		
Cyclosporine	Whole blood (EDTA)	>150–350 ng/mL
Tacrolimus	Whole blood (EDTA)	5–15 ng/mL
Mycophenolic acid	Serum or plasma	1–3.5 μg/mL
Everolimus	Whole blood (EDTA)	3–8 ng/mL
Sirolimus	Whole blood (EDTA)	4–12 ng/mL

TABLE 1.2. Less Commonly Monitored Drugs

Drug	Specimen Requirement	Therapeutic Range (Trough)
Anticonvulsants		
Carbamazepine, 10, 11-epoxide (active metabolite of carbamazepine)	Serum or plasma	0.4–4 μg/mL
Clonazepam	Serum or plasma	10–50 ng/mL
Gabapentin	Serum or plasma	2–12 μg/mL
Lamotrigine	Serum or plasma	1–4 μg/mL
Zonisamide	Serum or plasma	10–40 μg/mL
Cardioactive Drugs		
Amiodarone	Serum or plasma	1.0–2.5 μg/mL
Flecainide	Serum or plasma	0.2–1.0 μg/mL
Mexiletine	Serum or plasma	0.5–2.0 μg/mL
Tocainide	Serum or plasma	5–12 μg/mL
Verapamil	Serum or plasma	50–200 ng/mL
Antidepressants		
Fluoxetine and Norfluoxetine	Serum or plasma	300–1000 ng/mL
Paroxetine	Serum or plasma	20–200 ng/mL
Sertraline	Serum or plasma	30–200 ng/mL
Haloperidol	Serum or plasma	2–15 ng/mL
Antiretroviral Agents		
Amprenavir	Serum	150–400 ng/mL
Atazanavir	Serum	100 ng/mL
Indinavir	Serum	80–120 ng/mL
Lopinavir	Serum	700 ng/mL
Nelfinavir	Serum	700–1000 ng/mL
Saquinavir	Serum	100–250 ng/mL
Nevirapine	Serum	150–400 ng/mL
Efavirenz	Serum	100 ng/mL

and total water content (both extracellular and intracellular) than females, causing difference between volumes of distribution of certain drugs, especially lipophilic drugs, between males and females. Although absorption of a drug is not different between men and women, the absorption rate may be slightly slower in females. Hepatic metabolism of drugs by phase I (via CYP1A2, CYP2D6, and CYP2E1) and phase II (by glucuronyl transferase, methyltransferases, and dehydrogenases) reactions appear to be faster in males than females, although metabolisms of drugs by CYP2C9, CYP2C19, and N-acetyltransferase or clearance of drugs that are substrates for P-glycoprotein appear to be similar in both males and females (34). Women also have high levels of sex hormones and may also take oral contraceptives. Managing women with antiepileptic drugs is a challenge because in general estrogen is

a proconvulsant and progesterone is an anticonvulsant. Hormonal contraceptives usually contain progesterone alone or in combination with estrogen (natural or synthetic). Both natural and synthetic estrogens and progesterones (including common ethinyl estradiol) are metabolized by CYP3A4. The antiepileptic drugs such as phenobarbital, primidone, carbamazepine, oxcarbazepine, and phenytoin induce the cytochrome P450 system and may cause higher clearance of oral contraceptives, resulting in lack of contraception. Hormonal contraceptives can also interact with antiepileptic drugs. Ethinyl estradiol in combination with other components of oral contraceptive preparations can reduce the serum lamotrigine level by 50% (35). Soldin and Mattison reviewed sex differences in pharmacokinetics and pharmacodynamics. In general there are differences in pharmacokinetic parameters between men and women for many drugs including acebutolol, cefotaxime, clozapine, metronidazole, ofloxacin, valproic acid, and verapamil. In addition, pregnancy also significantly affect disposition of certain drugs (36). Gender differences in the pharmacokinetics of common drugs are listed in Table 1.3. Women are also more susceptible to adverse effects of certain drugs than men. Women are at increased risk of QT prolongation with certain antiarrhythmic drugs compared with men even at the same levels of serum drug concentrations. In contrast, certain psychotropic drugs such as chlorpromazine, fluspirilene, and various antipsychotic drugs appear to be more effective in women than men for the same dosage (37).

Epidemiologic surveys have indicated that between one third and two thirds of all pregnant women take at least one medication during pregnancy. Drug therapy in pregnant women usually focuses on safety of the drug on the fetus (tetragonic effect of drug), and therapeutic drug monitoring during pregnancy aims to improve individual dosage improvement, taking into account pregnancy-related changes in drug disposition (38). In general, the bioavailability of a drug is not significantly altered during pregnancy, but increased plasma volume and changes in protein binding may alter the volume of distribution of many drugs. The renal excretion of unchanged drugs is increased

TABLE 1.3. Gender Differences in Pharmacokinetics of Some Representative Drugs

Drug	Comments
Atenolol	Higher clearance in men than women, so greater reduction in blood in women than men
Ciprofloxacin	Clearance is lower in women
Clozapine	For similar dose, serum concentration higher in women than men
Diazepam	Plasma protein binding decreases during pregnancy increasing pharmacologically active free fraction
Erythromycin	Oral bioavailability may decrease in pregnant women
Lithium	Clearance increases during pregnancy
Ofloxacin	Clearance in lower in women compared with men
Phenobarbital	Clearance increases during pregnancy
Quinidine	Reduced plasma protein binding during pregnancy
Valproic acid	Plasma protein binding decreases during pregnancy

in pregnancy. In addition, metabolism of drugs catalyzed by isoenzymes of cytochrome P450 (CYP3A4, CYP2D6, and CYP2C9) and uridine diphosphate glucuronosyltransferase (UGT1A4 and UFT2B7) are increased in pregnancy. Therefore dosages of drugs that are metabolized by these routes may need to be increased during pregnancy to avoid loss of efficacy. In contrast, activities of some isoenzymes (CYP1A2 and CYP2C19) are reduced in pregnancy. Therefore, dosage reduction may be needed for drugs that are metabolized via these isoenzymes (39).

Significant increases in clearance of lamotrigine have been reported in pregnancy. Apparent clearance seems to increase steadily during pregnancy until it peaks approximately at week 32 when 330% increases in clearance from baseline values can be observed (40). Lower serum concentrations of lithium have been reported in pregnancy that may be related to an increase in the glomerular filtration rate (GFR) in pregnancy. Altered pharmacokinetics of ampicillin can be observed in pregnancy where serum concentrations may be lower by 50% in pregnant women compared with nonpregnant women due to altered pharmacokinetics. Faster elimination of phenoxymethylpenicillin (Penicillin V) in pregnant women has also been demonstrated (38). In general, dosage adjustments are required for anticonvulsants, lithium, digoxin, certain β-blockers, ampicillin, cefuroxime, and certain antidepressants in pregnant women. In addition, for certain drugs such as tetracycline, antithyroid medications, coumarin anticoagulants, aspirin, indomethacin, opioids, barbiturates, and phenothiazine unwanted effects in the fetus may occur despite careful adjustment of maternal dosage (41,42).

1.4.2.2. Renal Disease and Drug Disposition. Renal disease causes impairment in the clearance of many drugs by the kidney. Correlations have been established between creatinine clearance and the clearance of digoxin, lithium, procainamide, aminoglycoside, and several other drugs, but creatinine clearance does not always predict the renal clearance of a particular drug. In addition, elderly patients may have unrecognized renal impairment, and caution should be exercised when medications are prescribed for elderly patients. Serum creatinine remains normal until the GFR has fallen by at least 50%. Nearly half of the older patients have normal serum creatinine but reduced creatinine clearance. Dose adjustments based on renal function is recommended for many medications in elderly patients even for medications that exhibit large therapeutic windows (43). Renal disease also causes impairment of drug protein binding because uremic toxins compete with drugs for binding to albumin. Such interaction leads to increases in the concentration of pharmacologically active free drug concentration, which is clinically more important for strongly protein-bound drugs. Measuring free drug concentrations of strongly protein-bound anticonvulsant drugs such as phenytoin, carbamazepine, and valproic acid is recommended in uremic patients to avoid drug toxicity (44). Clinical utility of monitoring free anticonvulsants in uremic patients is discussed in detail in Chapter 13.

1.4.2.3. Liver Disease and Drug Disposition. Liver dysfunctions not only reduces clearance of a drug metabolized through hepatic enzymes or biliary mechanism, but they also affect plasm protein binding due to reduced synthesis of albumin and other drug-binding proteins. Mild to moderate hepatic disease may cause an unpredictable effect on drug clearance. Portal-systemic shunting present in patients with advanced liver cirrhosis may significantly reduce first-pass metabolism of high extraction drugs, thus increasing concentrations of such drugs in the blood and increasing the chance of drug overdose and toxicity (45). In addition, activities of several isoenzymes of cytochrome P450 enzymes (CYP1A1, CYP2C19, and CYP3A4/5) are reduced in liver dysfunction while activities of other isoenzymes such as CYP2D6, CYP2C9, and CYP2E1 may not be affected significantly. Therefore, drugs that are metabolized by CYP1A1, CYP3A4/5, and CYP2C19 may show increased blood levels in patients with hepatic dysfunction requiring dosage adjustment to avoid toxicity (46). Although the phase I reaction involving cytochrome P450 enzymes may be impaired in liver disease, the phase II reaction (glucuronidation) seems to be affected to a lesser extent, although both phase I and phase II reactions in drug metabolism are substantially impaired in patients with advanced cirrhosis. At this point there is no universally accepted endogenous marker to access hepatic impairment, and the semiquantitative Child-Pugh score is frequently used to determine the severity of hepatic dysfunction and thus dosage adjustments, although there are limitations to this approach (45). Nonalcoholic fatty liver disease is the most common chronic liver disease. This type of liver disease also affects activities of drug-metabolizing enzymes in the liver with the potential to produce adverse drug reactions from the standard dosage (47).

Mild to moderate hepatitis infection may also alter the clearance of drugs. Trotter et al reported that total mean tacrolimus dose in year 1 after transplant was lower by 39% in patients with hepatitis C compared with patients with no hepatitis C infection. The most likely explanation for these findings is decreased hepatic clearance of tacrolimus caused by mild hepatic injury from recurrent hepatitis C virus (48). Zimmermann et al reported that oral dose clearance of sirolimus (rapamycin) was significantly decreased in subjects with mild to moderate hepatic impairment compared with controls, and the authors stressed the need for careful monitoring of trough whole blood sirolimus concentrations in renal transplant recipients exhibiting mild to moderate hepatic impairment (49). Wyles and Gerber reviewed the effect of hepatitis with hepatic dysfunction on antiretroviral therapy especially highly active antiretroviral therapy (HAART) in patients with AIDS and commented that the dosage of the protease inhibitors indinavir, lopinavir, ritonavir, amprenavir, and atazanavir may require reduction in patients with liver disease, although hepatic dysfunction does not affect pharmacokinetics of nucleoside reverse transcriptase inhibitors because these drugs are not metabolized by liver enzymes (50).

Hypoalbuminemia is often observed in patients with hepatic dysfunction, thus impairing protein binding of many drugs. Because free (unbound) drugs

are responsible for pharmacological action, careful monitoring of free concentrations of strongly albumin-bound antiepileptic drugs such as phenytoin, carbamazepine, and valproic acid is recommended in patients with hepatic dysfunction to avoid drug toxicity. See Chapter 13 for a more in-depth discussion on this topic.

1.4.2.4. Effect of Cardiovascular Disease on Drug Disposition. Cardiac failure is often associated with disturbances in cardiac output, influencing the extent and pattern of tissue perfusion, sodium and water metabolism, and gastrointestinal motility that may affect absorption and disposition of many drugs. Hepatic elimination of drugs via oxidative phase I metabolism is also impaired in patients with congestive heart failure due to decreased blood supply in the liver (51). Theophylline metabolism, which is largely independent of hepatic blood flow, is reduced in patients with severe cardiac failure and dose reduction is needed. Digoxin clearance is also decreased. The quinidine plasma level may also be high in these patients due to the lower volume of distribution (52). Therefore therapeutic drug monitoring is crucial in avoiding drug toxicity in these patients. Physiological changes in critically ill patients can significantly affect the pharmacokinetics of many drugs. These changes include absorption, distribution, metabolism, and excretion of drugs in critically ill patients. Understanding these changes in pharmacokinetic parameters is essential for optimizing drug therapy in critically ill patients. Moreover, usually free fractions of strongly protein-bound drugs are elevated in critically ill patients due to low serum albumin concentrations, which are addressed in Chapter 13.

1.4.2.5. Altered Pharmacokinetics in Neonates, Children, and the Elderly. In the fetus, CYP3A7 is the major hepatic cytochrome P450 enzyme with CYP3A5 also present in significant levels in half of the children. However, in adults, CYP3A4 is the major functional cytochrome P450 enzyme responsible for metabolism of many drugs. CYP1A1 is also present during organogenesis, and CYP2E1 may be present in some second trimester fetuses. After birth, hepatic CYP2D6, CYP2C8/9, and CYP2C18/19 are activated. CYP1A2 becomes active during the fourth to fifth months (53). In general, the decreased capacity of the neonatal liver to metabolize drugs may prolong action of drugs such as phenobarbital, theophylline, and phenytoin. However, age is not considered to have a major influence on the absorption of drugs from the gut except for the first few weeks of life when absorption steps may be less efficient. Neonates and infants have an increased total body water to body fat ratio compared with adults, whereas the reverse is observed in the elderly. These factors may affect the volume of distribution of drugs depending on their lipophilic character. Moreover, altered plasma binding of drugs may be observed in both neonates and some elderly due to low albumin, thus increasing the fraction of free drug. In general, drug metabolizing capacity by the liver enzymes is reduced in newborns, particularly in premature infants, but increases

rapidly during the first few weeks and months of life to reach values that are generally higher than adult metabolizing rates. In contrast, the efficiency of cytochrome P450 enzymes declines with old age. Renal function at the time of birth is reduced by more than 50% of adult value but then increases rapidly in the first 2–3 years of life. Renal function then starts declining with old age. Oral clearance of lamotrigine, topiramate, levetiracetam, oxcarbazepine, gabapentin, tiagabine, zonisamide, vigabatrin, and felbamate is significantly higher (20–120%) in children compared with adults depending on the drug and the age distribution of the population. However, clearance of these drugs is reduced (10–50%) in the elderly population compared with middle-aged adults (54). Therefore, physiological differences between children and adult result in altered pharmacokinetics and drug effects. In neonates and infants decreased weight-adjusted doses are recommended because of reduced protein binding, renal excretion, and metabolism. However, children older than 1 year require significantly higher weight-corrected doses on many drugs compared with adults that are eliminated by CYP1A2, CYP2C9, and CYP3A4, although weight-corrected doses for drugs eliminated by renal excretion or metabolism via CYP2C19, CYP2D6, and N-acetyltransferase and UDP glucuronosyltransferase in children are similar to adults (55). Premature infants may also metabolize a drug different than adult. For example, only unchanged theophylline and caffeine are found in the urine of premature neonates, indicating the oxidative pathways for theophylline metabolism. In contrast, in children and adults, 3-methylxanthine and 1,3-dimethyl uric acid are the major metabolites of theophylline recovered in the urine (56).

1.4.2.6. Thyroid Dysfunction and Drug Disposition. Patients with thyroid disease may have an altered drug disposition because thyroxine is a potent activator of the cytochrome P450 enzyme system. Therefore, lower levels of drugs may result from high thyroxine levels due to the induction of the hepatic oxidative metabolism pathway. In contrast, hypothyroidism is associated with the inhibition of hepatic oxidative metabolism of many drugs. Hypothyroidism also affects the metabolism of immunosuppressants. Haas et al reported a case where a patient developed hypothyroidism 6 months after single lung transplant and was admitted to the hospital for anuric renal failure. The patient showed a toxic blood level of tacrolimus that was resolved with the initiation of thyroxine replacement therapy and a dose reduction of tacrolimus (57). Therefore, therapeutic drug monitoring of immunosuppressants can aid in avoiding such drug toxicity. Amiodarone is a potent antiarrhythmic drug associated with thyroid dysfunction because due to high iodine content amiodarone inhibits 5-deiodinase activity. Although most patients treated with amiodarone remain euthyroid, amiodarone-induced thyrotoxicosis or amiodarone-induced hypothyroidism may occur depending on the iodine status of the patient as well as the history of prior thyroid disease. Screening of thyroid disease before amiodarone therapy and periodic monitoring of thyroid functions are recommended for patients treated with amiodarone (58).

1.5. ANALYTICAL METHODS USED FOR THERAPEUTIC DRUG MONITORING

Immunoassays are commonly used for routine therapeutic drug monitoring in clinical laboratories. However, commercially available assays are available for roughly 25 different drugs that are commonly monitored in clinical laboratories. For less commonly monitored drugs, chromatographic methods such as gas chromatography, GC/MS, liquid chromatography combined with ultraviolet or fluorescence detection, or liquid chromatography combined with tandem mass spectrometry can be used. Application of gas chromatography for therapeutic drug monitoring is only applicable for drugs that are relatively volatile. For nonvolatile drugs, for example immunosuppressants, only liquid chromatographic techniques can be used. In general, liquid chromatography combined with tandem mass spectrometry can offer best specificity and lower limits of detection for a drug than other methods.

1.5.1. Various Formats of Immunoassays Used for Therapeutic Drug Monitoring

Many therapeutic drugs are measured in blood by immunoassay methods using automated analyzers, and in most immunoassay methods serum or plasma can be used directly without any pretreatment. The assays require very small amounts of sample (mostly less than 100 μL). With respect to assay design, there are two formats of immunoassays: competition and immunometric (commonly referred as "sandwich"). Competition immunoassays are desirable for analytes with small molecular weight such as most therapeutic drugs. In contrast, sandwich immunoassays are mostly used for analytes with larger molecular weight, such as proteins where two different specific antibodies are used. In the competitive immunoassay format, analyte molecules in the specimen, for example a particular drug, compete with analyte (or its analogs), labeled with a suitable tag provided in the reagent, for a limited number of binding sites in the analyte-specific antibody (also provided in the reagent). Thus, in these types of assays, the higher the analyte concentration in the sample, the less of the labeled antigen can bind to the antibody to form the conjugate, or if small amounts of drugs molecules are present, then more labeled antigens can bind to the antibody. If the bound label provides the signal, which in turn is used to calculate the analyte concentration in the sample, the analyte concentration in the specimen is inversely proportional to the signal produced. If the free label provides the signal then the signal produced is proportional to the analyte concentration. The signal is mostly optical: absorbance, fluorescence, or chemiluminescence.

There are several variations in this basic format of a competitive immunoassay such as homogeneous or heterogeneous format. In the homogeneous format, the bound label has different properties than the free-labeled antigen, and no separation of bound versus free labeled antigen is needed. For

example, in the fluorescent polarization immunoassay, the free label has different Brownian motion than when the relatively small molecular weight labeled antigen is complexed with a large antibody. Therefore, only when a labeled antigen is bound to the antibody, signal is generated. However, in the EMIT method, the enzyme used for labeling the antigen is glucose 6-phosphate-dehydrogenase, which is active in the free form but inactive when bound to the antibody. The active enzyme during reaction with the substrate also reduces the cofactor NAD to NADH, and the absorbance is monitored at 340 nm. Therefore, only free-labeled antigen can generate the signal. In the cloned enzyme donor immunoassay method, two genetically engineered inactive fragments of the enzyme β-galactosidase are coupled to the antigen and the antibody reagents. When they combine, the active enzyme is produced and the substrate, a chromogenic galactoside derivative, produces the assay signal.

In heterogeneous immunoassays, format bound label is separated from the unbound labels before measuring the signal. The separation is often done magnetically, where the reagent analyte (or its analog) is provided as coupled to paramagnetic particles (PMPs), and the antibody is labeled. Conversely, the antibody may be also provided as conjugated to the PMP, and the reagent analyte may carry the label. After separation and wash, the bound label is reacted with other reagents to generate the signal. This is the mechanism in many chemiluminescent immunoassays, where the label may be a small molecule that generates chemiluminescent signal (59). The label also may be an enzyme (enzyme-linked immunosorbent assay) that generates a chemiluminescent, fluorometric, or colorimetric signal. In older immunoassay formats, the labels used to be radioactive (radioimmunoassay, or RIA). But because of safety and waste disposal issues, RIA is rarely used today. Another type of heterogeneous immunoassay uses polystyrene particles. If these particles are micro-sizes, that type of assay is called microparticle-enhanced immunoassay. Microparticle enzyme immunoassay is also used for the analysis of drugs in biological matrix.

Antibody used in the immunoassay may be polyclonal or monoclonal. Usually polyclonal antibodies are generated using an animal, and for a small molecular weight analyte such as a therapeutic drug, it is most commonly injected as a conjugate of a large protein in the animal to generate the antibody. Since many clones of the antibodies specific for the analyte are generated, these antibodies are called polyclonal and are less specific than newer techniques that generate monoclonal antibody. To generate monoclonal antibody, a mast cell of the animal can be selected as producing the optimum antibody, and then it can be fused to an immortal cell. The resulting tumor cell grows uncontrollably, producing only the single clone of the desired antibody. Such antibodies may be grown in live animals or cell culture. Sometimes, instead of using the whole antibody, fragments of the antibody, generated by digestion of the antibody with peptidases, for example, Fab, Fab′ (or their

dimeric complexes) are used as a reagent. Even though the immunoassay methods are now widely used, there are few limitations of this technique. Antibody specificity is the major concern of an immunoassay, which is discussed in various chapters throughout the book.

1.5.2. Chromatographic Methods for Therapeutic Drug Monitoring

Chromatographic techniques used for therapeutic drug monitoring can be broadly classified as gas chromatography and liquid chromatography. Gas-liquid chromatography, also commonly referred to as gas chromatography (GC), is a separation technique first described in 1952 by James and Martin. In most GC columns, the stationary phase is a liquid and the mobile phase is an inert gas. In general the stationary phase has a low vapor pressure so that at column temperature it can be considered nonvolatile. Introduction of the capillary column significantly improves resolution of peaks in GC analysis. Depending on the stationary phase composition, a GC column may have low, intermediate, or high polarity. The sensitivity and specificity of GC analysis depends on the choice of detector. Mass spectrometry (MS) can be used in combination with a gas chromatograph where MS is capable of producing a mass spectrum of any compound coming out of the column of gas chromatograph. The nitrogen phosphorus detector is specific for nitrogen- and phosphorus-containing compounds and is very sensitive. An electron capture detector can detect any halogen-containing compounds. Flame ionization and thermal conductivity detectors are also used in gas chromatography. However, GC combined with MS has the best sensitivity and specificity over other detectors used along with gas chromatography. Although only nonpolar volatile compounds with low molecular weights can be analyzed by GC, if a relatively polar compound with small molecular weight (usually less than 500) can be chemically converted to a nonpolar relatively volatile compound through a process called derivatization, it can also be analyzed by GC.

Application of GC as a separation technique is limited to volatile molecules, but high performance liquid chromatography (HPLC) can be used for separation of both polar and nonpolar molecules. Usually derivatization is not necessary for HPLC analysis. HPLC is based on the principle of liquid–liquid chromatography where both the mobile phase and stationary phase are liquid. In normal liquid chromatography, the stationary phase is polar such as a silica gel column and the mobile phase (eluting solvent) is nonpolar. In reverse phase chromatography, the stationary phase is nonpolar, most commonly derivatized silica, and the mobile phase is polar. Detectors used in the HPLC method include ultraviolet detector, fluorescence detector, conductivity detector, refractive index detector as well as MS. Ultraviolet detection is commonly used in clinical laboratories, although other detection techniques such as the fluorescence technique and electrochemical detection technique may also be used. Mass spectrum is usually considered a molecular fingerprint of a

compound. Mass spectrometer used in gas chromatography is usually an electron ionization or chemical ionization mass spectrometric detector. Electron ionization (EI) at 70 eV produces reproducible mass spectrum, which is a common ion source used in GC/MS analysis of several therapeutic drugs with relatively low molecular weight (usually less than 500). It is relatively easy to combine a mass spectrometer with a gas chromatograph because a high-efficiency pump can remove the carrier gas in the gas chromatography, which is an inert gas such as helium. However, combining HPLC with a mass spectrometer is more complex because the liquid mobile phase is not compatible with a mass spectrometer, which usually works in the vacuum. However, there are technologies available for combining liquid chromatography with mass spectrometry but only moving belt and particle beam interfaces are compatible with EI (60). The electrospray interface is very common in the HPLC/MS analyzer used in clinical laboratories. The electrospray interface produces singly or multiple charged ions directly from a solvent system by creating a fine spray of highly charged droplets in the presence of a strong electric field with assistance from heat or from pneumatics. In this process nonvolatile and polar compound can be ionized. The atmospheric pressure chemical ionization interface produces sample ions by charge transfer from reagent ions. The reagent ions are produced from the solvent vapor of the mobile phase.

Chromatographic methods are used for the therapeutic drug monitoring of drugs where there is no commercially available immunoassay, for example antiretroviral and other drugs used in HAART for AIDS patients, some newer anticonvulsants, several anticancer drugs other than methotrexate, and various antidepressants. Yadav et al described a liquid chromatography combined with tandem mass spectrometric method for determining concentrations of the antiretroviral drugs tenofovir, emtricitabine, and lamivudine in human plasma (61). D'Avolio et al described a liquid chromatography combined with mass spectrometric method simultaneous quantification of 14 antiretroviral agents in the peripheral blood mononuclear cell of patients infected with the human immunodeficiency virus optimized using corpuscular volume evaluation (62). Although immunoassays are available for the routine monitoring of certain immunosuppressants such as cyclosporine, tacrolimus, sirolimus, everolimus, and mycophenolic acid, these assays suffer from many limitations, and HPLC combined with tandem mass spectrometry can be used for therapeutic monitoring of these drugs. See Chapter 15 for a more detailed discussion on this topic. Taylor et al commented that liquid chromatography combined with tandem mass spectrometry is the preferable method for therapeutic drug monitoring of immunosuppressants and antiretroviral drugs (63). Although there are immunoassays for determining total tricyclic antidepressant concentration in serum or plasma, chromatographic methods are preferred because commercially available immunoassay cross-reacts with all tricyclic antidepressants and their metabolites as well as other drugs, for example carbamazepine, hydroxyzine, and cetirizine (64,65). See Chapter 14 for a more detailed discussion on this topic.