Annals of Clinical Pharmacology & Toxicology

ISSN: 2642-9233

Review Article

Toxic Concentration Levels of Therapeutic Agents: Analytical Methods of Measurements in Biological Fluids

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Abstract

Drug over dosage generally results in adverse effects (toxicity). Administration of the drug antagonist assists in reversing the adverse effects associated with the drug toxicity. Therapeutic blood or plasma concentration levels may be exceeded in the elderly, in individuals with reduced liver functions, in patients who raise the drug(s) doses as a result of tolerance and during chronic therapy. Toxic symptoms may develop at lower drug concentrations in susceptible individuals as well as the existence of significant overlap between therapeutic and potentially toxic ranges.

The objective of the study was to present analytical methods used in the determination of toxic drug concentrations in biological fluids.

Relevant information was obtained from published works in scientific journals, official and other pharmaceutical books. Academic institution library as well as the internet websites offered assistance in the information gathering.

Adverse effects (indication of drug toxicity) are said to occur when the blood drug concentration level exceeds the maximum therapeutic concentration level. Titrimetric, spectroscopic, electrochemical, electrophoretic, chromatographic and immunological methods etc. have been used to determine toxic drug concentrations in biological fluids. Of all the methods, chromatographic methods were often the choice of the analysts. With the chromatographic methods, hyphenation technique was the technique of interest. Sample preparation methods such as extraction, protein precipitation and enzymatic hydrolysis were found to be the usual procedures employed to free the drug(s) from interfering materials in biological fluids.

In conclusion, a number of analytical methods were found to be available in determining toxic drug(s) concentrations in biological fluids however the method of choice depended on the sample matrix, physicochemical properties of the drug and most importantly on the accuracy, reproducibility, sensitivity and selectivity of the analytical method.

Keywords: Therapeutic agents; Toxicity; Biological fluids; Analytical methods

Introduction

According to World Health Organization (WHO), a drug is a substance or mixture of substances employed in diagnosis, treatment, mitigation or prevention of disease; restoring, correcting/modifying the organic functions in man or animal. These substances potential activities interfere with biological processes of the host or extraneous etiological agents and hence can be toxic. Toxic effect of drug is one that occurs by direct action upon the cells and produces tissue damage [1]. This occurs when blood drug concentration (following administration of therapeutically effective doses) exceeds maximum effective concentration required to achieve the pharmacological action. Toxicity under consideration therefore is defined as the manifestation of the adverse effects of drug(s) administered therapeutically or in the course of diagnostic analysis and not toxicity arising from accidental drug overdoses. This toxicity is considered to be responsible for the attrition of some drug candidates and contribute maximally to the high cost of drug development, particularly if unrecognized during clinical trials or post-marketing [2].

Drug toxicity can be (i) mechanism-based (on-target) toxicity-resulting from interaction of a drug with the same target that produces the desired pharmacological activity. The on-target toxicity concept is not a competitive inhibition between the efficacious and the toxic effects of the drug. Typical example is the statins that exhibit adverse effects (hypercholesterolemia) by inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase in the liver or muscle (the targets) [3,4]; (ii) off-target toxicity-drug binding to an alternate target. Typical example is terfenadine, which binds to the H_1-receptor (producing the desired antihistaminic response) as well as to the hERG channel and hence causing arrhythmias. (iii) immune hypersensitivity-drug-induced allergic reactions. The concept is based on drug (or its metabolite) reaction with proteins in the body (as haptens) to induce antibodies and immune responses [5]. Typical examples are penicillins, aminoglycosides (iv) bioactivation/covalent modification-involves reactive metabolites modifying proteins they interact with hence leading to toxicity (v) idiosyncratic responses-deal with specific individual adverse reaction and is rare [6,7].

Allergy in responses to drugs (drug hypersensitivity reactions) can be classified into: Type I (IgE-mediated) which is an immune

Citation: Chika J Mbah. Toxic Concentration Levels of Therapeutic Agents: Analytical Methods of Measurements in Biological Fluids. Ann Clin Pharmacol Toxicol. 2020;2(1):1014.

Publisher Name: Medtext Publications LLC

Manuscript compiled: Aug 04th, 2020

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reaction involving drug-IgE complex binding to mast cells with release of histamine and other inflammatory mediators. Such reactions clinically manifest as angioedema, bronchospasm, diarrhea, pruritus, urticaria, vomiting etc. and can occur minutes to hours after drug administration.

Type II (cytotoxic) involves specific IgG or IgM antibodies directed at drug-hapten coated cells and clinically manifest as hemolyticanemia, neutropenia, thrombocytopenia and its occurrence due vary.

Type III (immune complex) is tissue deposition of drug-antibody complexes with complement activation and inflammation, clinically manifesting as arthralgias, fever, glomerulonephritis, lymphadenopathy, rash, serum sickness, urticaria, vasculitis etc. and can occur one to three weeks following drug administration.

Type IV (delayed, cell-mediated) entails interaction of drug molecules to T cells resulting in the release of cytokine and inflammatory mediators and clinically manifest as allergic contact dermatitis, exanthematous or morbilliform eruption, maculopapular lesions (with distribution on the fingers, toes or soles), eczematous rash, scaly lesion etc. occurring two to seven days after cutaneous drug administration [8-10].

Drug toxicity can be affected by a number of factors and they include: (i) drug factors- dosage form, dose, route of administration etc. (ii) human factors- age, weight, time of sampling, method of analysis, presence of metabolites etc. (iii) pathological factors-disease state, body water, genetic disorders, anatomical abnormalities etc., (iv) pharmacological/biochemical factors- gastrointestinal absorption, antagonistic or synergistic actions of other drugs, induction or inhibition of microsomal enzymes, tissue binding, detoxification rate, elimination rate (excretion), tolerance etc [11,12].

Target body tissues /organs or systems mostly affected by drug toxicity include cardiovascular system, central and peripheral nervous system, reproductive system, liver, blood. Others affected are digestive system, lung, kidney, muscle, retina etc.

In the present study, attempts will be made to present various analytical methods that could be used to determine drugs in biological fluids at their toxic concentration levels. Drug metabolites are not considered in this study although these analytical methods could also be used for their determinations.

Biological fluids

Biological fluids (intracellular and extracellular) are very essential to life and help maintain body homeostasis. The biological fluids of interest in this study are extracellular fluids namely blood (whole blood, serum or plasma); urine; Cerebrospinal Fluid (CSF); saliva; amniotic fluid; ocular fluid; pleural fluid (from the sac surrounding the lungs); pericardial fluid (from the sac surrounding the heart); peritoneal fluid (also called ascitic fluid; from the abdomen) and synovial fluid (fluid that is found in joint cavities). Any of these fluids could be analyzed for parent drug(s) concentration levels however, blood and urine are mostly the fluids of choice [13,14].

Blood (or plasma or serum): Blood, the most commonly used biological fluid for testing drug toxicity consists a fluid portion called plasma (contains the dissolved ions and molecules) and a cellular portion (the red blood cells, white blood cells and platelets). Most drugs are found in the plasma. The plasma is obtained by removing the cells through centrifugation of the blood sample containing an additive (called an anticoagulant) that prevents the blood from clotting. However, centrifugation of the blood sample containing no additive gives a resultant liquid above the cells and clot called serum. Serum contains all the components of plasma except the clotting proteins. Plasma or serums are the preferred biological fluids of choice in toxicological analysis.

Urine: Urine is another fluid commonly used for drug testing. It is usual the recommended sample matrix for detecting drug use and abuse, evaluate kidney function, waste products excreted by the kidneys, metabolites cleared quickly from the bloodstream and accumulate in the urine such as drugs of abuse. Urine is the preferred specimen to be analyzed when the drug is present at very low concentration in blood. Urine is relatively easy to collect and different types of urine samples, representing collection at different times of day and for different durations of time, are used for laboratory analyses. Preservative is often added to urine sample not to be tested immediately to reduce bacterial metabolism or to prevent chemical decomposition of the drug(s) of interest. Some of the common urine preservatives include potassium phosphate, benzoic acid, sodium bicarbonate, acetic acid, hydrochloric acid and boric acid.

Prior to the identification and determination of the test drug, the sample is prepared by separating the drug from the biological fluid in which it is suspended by protein precipitation, liquid–liquid extraction, or solid phase extraction [15,16].

Sample preparation

Liquid-liquid extraction: Some of the organic solvents used in this type of extraction are dichloromethane, ethyl acetate, ethyl ether, hexane-dichloromethane, n-butyl chloride-ethyl acetate etc. It is important that the dug sample is spiked with an internal standard before the extraction process.

Solid phase extraction: The sorbent materials used include diatomaceous earth, bonded phase silica or polymeric materials. The column is usually conditioned with methanol, distilled water prior to introduction the sample matrix. Elution of drug of interest could be accomplished with ethyl acetate or other organic solvents of choice.

Protein precipitation: In plasma or serum samples, protein precipitation is best achieved by using organic solvents (acetone, acetonitrile, methanol or mixture of acetonitrile and methanol); acids (formic acid, perchloric acid, trichloroacetic acid); inorganic salts (ammonium sulfate, sodium sulfate, zinc sulfate). However, in urine samples, acid hydrolysis (for basic drugs) or base hydrolysis (for acidic drugs) is carried out to ensure that the drug(s) of interest is freely solvated. The urine sample can also be enzymatically hydrolyzed to free bound drug(s).

The enzymatic hydrolysis is often preferred over acid or base hydrolysis because it avoids cleavage or rearrangement of drug structure in extreme acidic or basic conditions.

At the completion of sample preparation, the drug in the biological fluid can be qualitatively and quantitatively analyzed.

Analytical methods: Analysis of drugs (inorganic, organic or mixture of both) involved in toxicity, usually entails qualitative identification and quantitative determination. The qualitative identification tests include (i) color test (ii) wavelength of maximum absorption, (iii) vibration frequency (iv) chemical shift, (v) mass to

charge ratio, (vi) retardation factor and (vii) retention time etc. The results obtained are usually compared with that of reference drugs [17].

Quantitative analysis provides the determination of the toxic concentration level of the drug.

Inorganic or organometallic drugs: Analytical methods such as atomic absorption spectroscopy (flame atomic absorption spectroscopy or graphite furnace atomic absorption spectroscopy), atomic emission spectroscopy etc. are often used for inorganic or organometallic drugs [18,19]. In atomic absorption, the atoms of the drug in ground state absorb energy in the form of light of a specific wavelength and are elevated to an excited state. The relationship between the amount of light absorbed and the concentration of reference drug can be used to determine toxic concentration of the drug in biological fluid. The light source is a Hollow Cathode Lamp (HCL) or an Electrodeless Discharge Lamp (EDL). In atomic emission spectroscopy, the optical emission from excited atoms of the drug is used to determine the toxic drug concentration in biological fluid. The measured optical emission intensity is usually compared to the emission intensity of standard of known concentration. Atomization of the drug is by an electric arc (4000-5000 degree K) or a high voltage spark or flame (1700-3200 degree K) or an argon plasma - Inductively-Coupled Plasma (ICP) or Direct-Current Plasma (DCP) or Microwave-Induced Plasma (MIP) or laser-induced plasma (4000-6000 degree K). Atomic emission spectroscopy is preferred over atomic absorption spectroscopy since all atoms in drug sample are excited simultaneously and can be detected simultaneously. Hyphenated systems such as gas chromatography-atomic absorption spectrometry (GC-AAS), Gas Chromatography-Atomic Emission Spectrometry (GC-AES), ion chromatography-Inductively Coupled Plasma-Atomic Emission Spectrometry (IC-ICP-AES) and ion chromatography-Inductively Coupled Plasma-Atomic Emission Spectrometry (IC-ICP-AES) are currently preferred methods used to determine these inorganic ions at toxic concentration levels in biological fluids [20,21].

Organic drugs: Analytical methods such as titrimetry, spectroscopy, electrochemical, capillary electrophoresis, chromatography and immunological assays are mostly used to quantify organic drugs at their toxic concentration levels in biological fluids. Immunoassay is bioanalytical method in which the quantification of the drug depends on the reaction of an antigen (drug) and an antibody. Immunoassay can be Radioimmunoassay (RIA) [22], Enzyme Immunoassay (EIA), Fluorescent Polarization Immunoassays, (FPIA) [23] and Enzyme Linked Immunosorbent Assay (ELISA) [24]. Although these immunological techniques appear to be very sensitive and useful for the analysis of a large number of drug samples, their disadvantages such as the large number of steps involved, the need to develop antisera and/or monoclonal antibodies, and the length of time to obtain final quantitative results limit their applications.

Titrimetry (aqueous or non-aqueous) is a rapid, easy to perform, cost-effective method and requires simple instrumentation. However, lack of selectivity and sensitivity make the technique falling into the least choice [25]. Typical examples of methods that fall under this category are amperometric, potentiometric, coulometric and polarographic titrations etc.

The spectroscopic methods (ultraviolet/visible, infrared, nuclear magnetic resonance, mass spectrometry, luminescence etc.) have available instruments, simple procedures, excellent precision and accuracy hence are analytical methods of interest. Furthermore, derivative spectrophotometry (unlike direct UV/VIS spectrophotometry) has offered an alternative approach to the enhancement of sensitivity and specificity in mixture analysis with spectral overlapping [26,27]. Of all the spectroscopic methods, luminescence (fluorescence) method has been widely used in biological fluid analysis because it has high selectivity, low detection limits and toxic drug concentrations ranging from ng/ml to pg/ml can be analyzed [28-31]. However, the use of luminescence technique has become limited because of light absorption and scattering observed with the technique and only certain classes of compounds exhibit fluorescence.

The electrochemical methods (amperometry, conductometry, coulometry, electrogravimetry, polarography, potentiometry, voltammetry) are considered an alternative to spectrometry and even chromatography because of minimal sample pre-treatment, reasonable speed, good sensitivity and selectivity, wide applicability, and low cost of instrumentation [32,33]. However, lack of analysis of related substances, as is observed with chromatographic and electrophoretic techniques limit the use of these techniques in the analysis of drug toxic concentrations in biological fluids.

The capillary electrophoresis (non-aqueous capillary electrophoresis, capillary isotachophoresis, capillary electrochromatography, immunoaffinity capillary electrophoresis, microemulsion electrokinetic chromatography) is an efficient separation technique. The technique has automated and simple instrumentation, low sample and solvent consumption, low analysis time, efficient separation which allows difficult separations and different means of detection (light emitting diode, fluorescence, chemiluminescence, contactless conductivity and mass spectrometry detectors) [34,35]. All these features give versatility and sensitivity to the analytical technique. However, drawbacks such as lack of usefulness as a preparative separation tool and low detection limit (due to the shorter path length of the flow cell) restraint the use of the analytical technique in analyzing toxic concentration levels of drugs in biological fluids.

The chromatographic methods (thin-layer chromatography, high performance thin-layer chromatography, gas chromatography, high or ultra-performance liquid chromatography) are excellent separation methods. Thin-layer chromatography (or high performance thin-layer chromatography) is a simple, rapid, moderately sensitive and inexpensive technique used for qualitative and quantitative analysis of a wide range of metal-organic and organic drugs [36,37]. Densitometric scanning is used in high performance thin-layer chromatography for quantification, hence can be very useful in analyzing toxic drug concentration levels in biological fluids [38,39].

Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC) is an accurate, reproducible, selective, sensitive, quantitative, and versatile technique for the analysis of complex mixtures [40,41]. GC technique is limited to volatile and thermally stable compounds, molecules that may undergo derivatization reactions to give thermally stable products.

Although chromatographic methods have been very versatile to determine most drugs at their toxic concentration levels in biological fluids, the extreme low concentration levels of some of the drugs in biological fluids have necessitated the combination of various

chromatographic and spectroscopic techniques. Such combinations are called hyphenated techniques. Hyphenation system provides best identification and quantification of the drug, excellent selectivity and sensitivity, accurate and rapid analysis, best sample throughput and best degree of automation. In hyphenation, chromatographic methods are largely used for separation while the interfaced spectroscopic methods are mostly for detection [42,43].

Some of the hyphenated systems are Gas Chromatography-Atomic Absorption Spectrometry (GC-AAS), Gas Chromatography-Atomic Emission Spectrometry (GC-AES), Gas Chromatography-Mass Spectrometry (GC-MS), [44,45]; Gas Chromatography-Inductively Coupled Plasma-Mass Spectrometry (GC-ICP-MS) [46]. Others include High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) [47], High Performance Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (HPLC-MS-MS) [48], High Performance Liquid Chromatography-Nuclear Magnetic Resonance (HPLC-NMR) [49]; high performance liquid chromatography-inductively coupled plasma-mass spectrometry [50]; Gas Chromatography-Infrared (GC-IR) [51]; Gas Chromatography-- Nuclear Magnetic Resonance (GC-NMR) [52]; Gas Chromatography--Mass Spectrometry-Mass Spectrometry (GC-MS-MS); Capillary Electrophoresis-Mass Spectrometry (CE-MS) [53,54], Eletrochemistry-Mass Spectrometry (EC-MS) [55]; Ion Chromatography-Mass Spectrometry (IC-MS) [56,57]; Ion Chromatography-Inductively Coupled Plasma-Mass Spectrometry (IC-ICP-MS) and Ion Chromatography-Inductively Coupled Plasma-Atomic Emission Spectrometry (IC-ICP-AES).

In general, the choice of an analytical method to be used in the analysis of toxic drug concentrations in biological fluids may depend on type of sample matrix to be analyzed, the physicochemical properties of the drug(s), number and chemical structures of the drug, range of concentrations to be measured, stability of the drug(s), context and objective of the analysis (clinical or forensic) and the degree of experience of the analyst.

The Table 1 provides the therapeutic and toxic concentration levels of some the drugs analyzed by the analytical methods.

The data in the Table show that the toxic concentration levels of some of the drugs fall within the detection limits of common analytical methods found in most analytical laboratories, indicating the feasibility of their quantification in biological fluid(s) provided that the components of biological fluid do not interfere with the drug(s) of interest. The Table also shows that some of the drugs often times occur at very low concentrations, therefore calling for the use of hyphenated sensitive and selective analytical methods.

Conclusion

Drug toxicity is said to occur when the blood drug concentration level exceeds the maximum therapeutic concentration level. Although they are numerous analytical methods to qualitatively identify and quantitatively determine concentration of drug(s) at toxic levels in terms of accuracy, precision, specificity, selectivity and sensitivity, certain factors such as chemical, human, pathological and pharmacological/biochemical factors that might affect analysis must be considered. Chromatographic methods (hyphenated or non-hyphenated) are the preferred analytical methods when compared to other methods in terms of sensitivity, selectivity, accuracy, reproducibility, small sample volumes, fast analysis and high separation efficiency etc.

Of the hyphenated techniques, Gas Chromatography-Inductively Coupled Plasma-Mass Spectrometry (GC-ICP-MS) seems to be the most effective and efficient technique for inorganic/organometallic drugs whereas High Performance Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (HPLC-MS-MS) is the technique of choice for organic drugs.

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Table 1: Therapeutic and toxic concentration levels of drugs [11,57,58].

Drug Category	Drug	Therapeutic Conc. (µg/ml)	Toxic Conc. (µg/ml)
Cardiovascular Drugs	Atenolol	0.1-1	2-3
	Captopril	0.05-0.5	5-6
	Digitoxin	0.01-0.025	0.03
	Digoxin	0.0005-0.0008	0.0025-0.003
	Ergotamine	0.00036-0.00042	0.00082
	Isradipine	0.0005-0.002	0.01
	Labetalol	0.03-0.18	1
	Lisinopril	0.02-0.07	0.5
	Methyldopa	1-5	9
	Nifedipine	0.025-0.15	0.15-0.2
Central nervous Drugs	Carbidopa	0.02-0.2	0.4
	Diltiazem	0.03-0.25	0.8-1
	Fluoxetine	0.12-0.5	1
	Fluvoxamine	0.06-.23	0.5-1.8
	Haloperidol	0.005-0.02	0.05-0.5
	Imipramine	0.05-0.35	0.5-1
	Levodopa	0.3-2	5
	Lorazepam	0.08-0.25	0.3-0.5
	Mirtazepine	0.03-0.3	1.2-2.3
	Meprobamate	5-10	10-25
Neurologic Drugs	Carbamazepine	2-8	10
	Ibuprofen	15-30	200
	Indomethacin	0.3-3	4-5
	Lamotrigine	1-14	20-30
	Mefenamic acid	2-20	25
	Morphine	0.01-0.1	0.1
	Phenobarbital	10-30	30-40
	Phenytoin	5-20	20-25
	Piroxicam	2-6	14
	Vigabatrin	12-5	20
Gastrointestinal Drugs	Atropine	0.002-0.05	0.03-0.1
	Cimetidine	0.25-3	30-50
	Dicyclomine	0.1	0.2
	Diphenhydramine	0.05-1	1-4
	Famotidine	0.02-0.2	0.4
	Metroclopramide	0.05-0.15	0.2
	Metronidazole	3-2020	200
	Prochlorperazine	0.01-0.05	0.2-0.3
	Promethazine	0.05-0.4	1-2
	Rivastigmine	0.008-0.02	0.04
Antibiotics	Amikacin	10.12	30
	Chloramphenicol	5-15	15
	Chlorotetracycline	1-10	30
	Ciprofloxacin	2.5-4	11.5
	Doxycycline	1-10	30
	Gentamycin	2-10	12
	Kanamycin	1-25	25-30
	Pefloxacin	1-10	25
	Tetracycline	1-10	30
	Tobramycin	4-10	12-15

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